CRISPR/Cas9: A revolutionary genome editing tool for human cancers treatment

Fatima Akram, PhD¹, Ikram ul Haq, PhD¹,², Sania Sahreen, MPhil¹, Narmeen Nasir, BS¹, Waqas Naseem, BS¹, Memoona Imitaz, BS¹ and Amna Aqeel, MS¹

Abstract
Cancer is a genetic disease stemming from genetic and epigenetic mutations and is the second most common cause of death across the globe. Clustered regularly interspaced short palindromic repeats (CRISPR) is an emerging gene-editing tool, acting as a defense system in bacteria and archaea. CRISPR/Cas9 technology holds immense potential in cancer diagnosis and treatment and has been utilized to develop cancer disease models such as medulloblastoma and glioblastoma mice models. In diagnostics, CRISPR can be used to quickly and efficiently detect genes involved in various cancer development, proliferation, metastasis, and drug resistance. CRISPR/Cas9 mediated cancer immunotherapy is a well-known treatment option after surgery, chemotherapy, and radiation therapy. It has marked a turning point in cancer treatment. However, despite its advantages and tremendous potential, there are many challenges such as off-target effects, editing efficiency of CRISPR/Cas9, efficient delivery of CRISPR/Cas9 components into the target cells and tissues, and low efficiency of HDR, which are some of the main issues and need further research and development for completely clinical application of this novel gene editing tool. Here, we present a CRISPR/Cas9 mediated cancer treatment method, its role and applications in various cancer treatments, its challenges, and possible solution to counter these challenges.

Keywords
CRISPR/Cas9, cancer, diagnosis, gene editing, immunotherapy

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Introduction
Cancer, a complex disease, is the second largest cause of death across the globe. Primarily, cancer is a genome ailment, feeding off mutations in DNA that consequently result in activating oncogenes and inactivating tumor suppressors, along with dysregulating the epigenome, which regulates the normal expression of genes. Additionally, cancer can also be defined as the disease of a cell resulting from the changes in the structure of the cell, metabolism, and motility to permit growth in bleak and unreceptive conditions. It eventually becomes a sickness for the organism, absorbing normal cell types, tissue functions and outmaneuvering the host’s defense mechanisms.¹ With the development of high throughput sequencing technologies, a myriad of genes coupled with the initiation and progression of cancer have been identified during the past 2 decades.²³ Despite the thrilling accomplishments in cancer therapeutics which include surgery, targeted biotherapy, chemotherapy, and radiotherapy, increased rates of postoperative relapse, resistance to chemotherapy/radiation along with detrimental off-target effects continue to be a hurdle in life span and standard life quality of a cancer patient.⁴ For the development of effective and efficient treatment options aiming to enhance the denouement for millions of people diagnosed with cancer annually, it is critical to understand how genetic alterations, cellular adaptations, and modifications in the microenvironment of tumor influence the initiation, development, and treatment response of certain malignancies.⁵

¹ Institute of Industrial Biotechnology, Government College University, Lahore, Pakistan
² Pakistan Academy of Sciences, Islamabad, Pakistan

Corresponding Author:
Fatima Akram, Institute of Industrial Biotechnology, Government College University, Lahore 54000, Pakistan.
Emails: fatima_iib@yahoo.com; fatimaakram@gcu.edu.pk

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In recent years, noteworthy advances in biotechnology have been made, and the field of genetic engineering is now progressing at an exponential rate, offering countless benefits. Genome editing tools have transformed biological and genetic research and exploration through their groundbreaking ability to squarely amend, alter and reconfigure the genomes of living organisms. In current times, diverse genome manipulating techniques have been utilized to study simple, intricate, and raveled genomes.6,7 Since the introduction of genome editing technologies in the 1990s, various strategies for targeted genome editing have been devised. One important use of gene-editing technology is genome modification, which comprises gene inactivation, insertion of a new sequence, and/or rectification of mutant regions with proper nucleotide sequences.8 To modify genes, many approaches are used, including zinc-finger endonucleases (ZFNs),9 transcription activator-like effector nucleases (TALENs),10 and the clustered regularly interspaced short palindromic repeats (CRISPR) associated nuclease system.

CRISPR, a dominant and powerful gene-editing technique, emerged in 1986 and quickly rose to prominence as the century’s most important genetic tool outweighing the previous methods owing to its outstanding advantages such as simplicity, cost-effectiveness, speed, and high efficiency.11,12 The CRISPR/Cas system was initially found in Escherichia coli, but it is now known to be prevalent across a vast population of prokaryotic species.13,14 In bacteria and archaea, it is a sequence-specific adaptive immune system that provides resistance to viruses, phages, and other genetic materials.15 Apart from its role in antiviral immunity, CRISPR serves as a barricade against horizontal gene transfer mechanisms and therefore plays an imperative role in sustaining the integrity of the genome.16

The CRISPR/Cas9 framework is a hereditary defense system in bacteria and archaea that utilizes RNA-directed nucleases to cleave targeted DNA.17,18 CRISPR locus is an assembly of short direct repeats spaced by spacer sequences.19 The repeats within a particular locus are identical in sequence and length. The spacers are likewise uniform in length, although their sequence composition varies greatly. Repeats in various species range from 21 to 47 bp, with an average of 32 bp, while spacers range from 20 to 72 bp. Although related species may share identical repeat sequences, the overall sequence diversity of both spacers and repeats in archaea and bacteria is quite high.20 This system is consisting of DNA endonuclease (Cas9) and single guide RNA (sgRNA). The Cas9/sgRNA complex binds specifically to target dsDNA which has a complementary sequence match to 5' end of sgRNA (match of first 17 to 20 nt of sgRNA) and also has a PAM area (protospacer adjacent motif). The sgRNA guides the Cas9 endonuclease to break the DNA strands at the desired target site.21 Cas9, DNA nuclease with two active domains, cleave the target DNA strands at 3 nt upstream of PAM and produce a double-stranded break (DSB). DSBs are repaired through a host natural repair mechanism, i.e., NHEJ method (nonhomologous end joining) or HDR method (homologous direct repair pathway). CRISPR/Cas9 mediated cleavage is not always specific and sometimes may result in unwanted binding of Cas9 to target DNA producing nonspecific cleavage, and giving-off target effects. CRISPR/Cas9 target efficiency and subsequent cleavage specificity depend on gRNA and PAM location to the target loci. Off targets effects and strategies to avoid them have been discussed in CRISPR/Cas9 challenges section.22,23 To minimize the off-target effects, tools to design desirable sgRNA have been provided in Table 1.

CRISPR/Cas9 technology offers a unique and striking future in laboratory cancer research, with the possibility to generate

### Table 1. List of Tools to Design sgRNA and Their Applications in sgRNA Designing.

| Name                  | Web address                                      | Applications                                      | References |
|-----------------------|--------------------------------------------------|--------------------------------------------------|------------|
| CHOPCHOP              | https://chopchop.fas.harvard.edu/                | Off-target prediction and PCR assay design        | 24         |
| sgRNA designer        | http://www.broadinstitute.org/maipublic/analysis-tools/sgrna-design | Efficacy prediction                              | 25         |
| CRISPR ERA            | http://crispr-era.stanford.edu/                  | Multiple purposes: knock-out, knock-in, CRISPR, and gene activation | 26         |
| ZiFiT Target          | http://zifitpartners.org/ZiFiT/                  | Flexible target definition                       | 27         |
| WU-CRISPR             | http://crispr.wustl.edu/                         | Efficacy prediction and off-target prediction     | 28         |
| CCTop                 | http://crispr.cos.uni-heidelberg.de/             | Off-target prediction and flexible target site definition | 29         |
| E-CRISP               | http://www.e-crisp.org/E-CRISP/                  | Design for multiple purposes                      | 30         |
| CasOT                 | http://zendb.zfgenetics.org/casot/               | Off-target prediction and local installation      | 31         |
| CRISPR seek           | http://www.bioconductor.org/packages/release/bioc/html/CRISPRseek.html | Off-target prediction and allele-specific sgRNA design | 32         |
| Cas                   | http://www.genome.net/cas-offfinder/             | Off-target prediction                            | 33         |
| Cas9 design           | http://cas9.cbi.pku.edu.cn/index.jsp              | SNP matching to target sites                      | 34         |
superior disease models for cancer, therapeutic targets, and the identification and exploration of treatment resistance. It is an affordable technology offering a high level of flexibility, potency, and ease to use in laboratory research. CRISPR/Cas9 has effectively been exploited to develop cancer disease models, by inactivating multiple tumor suppressor genes such as Nf1, P53, Pch1, and Pten in mouse brain which has resulted in the creation of medulloblastoma and glioblastoma disease models. General overview of cancer modeling with CRISPR/Cas9 technology is given in Figure 1.

Current status of CRISPR/Cas9 in Cancer Treatment

CRISPR/Cas9 Mediated CAR-T Centered Cancer Treatment

CAR-T cells are T lymphocytes (human immune cells) that have been genetically modified to express a chimeric antigen receptor (CAR). Chimeric antigen receptor, commonly known as CAR, is a synthetic receptor that can recognize specific antigens. It consists of an antibody-driven target-binding extracellular domain, a hinge region, a trans-membrane domain, and an intracellular signaling moiety that can activate T-cells. A CAR-targeted antigen can be recognized by the CAR-bearing altered T-cells, this consequently provokes the proliferation of T-cells, manufacturing of cytokine, and critical and targeted cytotoxicity versus tumor cells. The performance of CARs is determined by several factors. A few of these include the position of the target cell surface antigen as well as careful adjustment of intracellular and extracellular domains, including the nonfunctional linking transmembrane domain and hinges.

CAR-T-cell-based clinical trials for cancer treatment have shown promise in eliciting long-term full remissions in patients with a range of hematologic and solid malignancies, including relapsed/refractory acute lymphoblastic leukemia (ALL) and multiple myeloma, with response rates ranging from 80% to 100%. CAR-T cell therapy for hematological malignancies, particularly B-cell malignancies, has a high success rate. However, their application in treating solid tumors has not been successful yet. One of the barriers to its extensive use is CAR-T cell dysfunction, counting both the senescence and exhaustion of the cells limiting their effectiveness. Other barriers are the higher prevalence of toxicities, such as cytokine release syndrome, the possibility of graft versus host disease. Currently, only five CAR-T products have been approved, for hematologic malignancies, by the United States Food and Drug Administration (FDA) which are (i) Abecma (idecabtagene vicleucel) for patients with relapsed or refractory (R/R) myeloma, (ii) Brexynzi (lisocabtagene maraleucel) for R/R large B-cell lymphoma patients, (iii) Tecartus (brexucabtagene autoleucel) for R/R mantle-cell lymphoma patients, (iv) Kymriah (tisagenlecleucel) for patients especially peds and young adults suffering with CD19 + R/R B-cell, (v) Yescarta (axicabtagene ciloleucel) specific for refractory large B-cell lymphoma patients.

A big restrictive factor in CAR-T cell therapy for cancer treatment is that the patients must wait for long periods before the treatment can be applied since every step of harvesting, engineering, expanding, and transfusing cells is tailored to each patient and takes time. Moreover, this procedure requires logistical and scientific support. CRISPR/Cas9-mediated genetic manipulation has the potential to increase the efficacy of immunotherapy by generating a universal “off-the-shelf” cellular product or modifying immune cells for overcoming resistance to hematological or solid malignancies. The idea of universal allogeneic CAR-T products eliminates the requirement of harvesting, modifying, expanding, and transfusing T-cells from each patient, conserving resources and time. By altering allogeneic (healthy donor) T-cells to express the CAR by knocking-in and then knocking-out the genes involved in immune recognition of these nonself cells, the cells can be effectively transferred into patients without the danger of immunological rejection. It may also be used to precisely alter some cytokine genes for boosting the production of cytokine and immune cell reaction, preventing T-cell depletion and autoimmune responses correlated with other techniques of cytokine increase like viral transduction. To ensure long-lasting engagement of T-cells, the CRISPR/Cas9 system can be employed for knocking out checkpoint inhibitor genes, for instance, the genes that code for CTLA-4 and PD-I. The CRISPR-manipulated cells have the ability to be mass-produced, lyophilized, and kept at medical centers across the country, alleviating the need of transporting living cells for every patient from the manufacturing sites. The precise insertion of CAR into the exact position in the genome of the T-cells to ensure its sufficient expression can be facilitated by CRISPR.

CRISPR/Cas9 Mediated Adoptive T-Cell Centered Cancer Treatment

In this therapy, the T-cells of patients are modified to express specific TCRs that can recognize antigens released by tumor cells and trigger T-cells to kill the tumor cells. Tumor-infiltrating lymphocyte (TIL) treatment and chimeric antigen receptor T-cell (CAR-T-cell) therapy are the two forms of adoptive cell therapy. ACT for cancer is a prime focus for the gene-editing trials. During adoptive T-cell therapy, a biopsy is performed, and tumor cell are subjected to whole-exome sequencing and RNA sequencing. Sequencing results along with bioinformatic prediction algorithms help in identifying immunogenic neoantigens. T-cells specific for these immunogenic neoantigens are isolated from the patient (either from peripheral blood mononuclear cells [PBMCs] or tumor-infiltrating lymphocytes (TILs) of a patient). Later, T-cell receptors (TCRs) are sequenced and electroporated into the same patient’s PBMCs along with Cas9 and gRNA. This results in the production of neoantigen-specific T-cells.
which can be expanded and introduced back into the same patient for cancer therapy.\textsuperscript{54}

In 2016, researchers from the Chinese PLA General Hospital started the first CRISPR phase I clinical research in humans by infusing PD-1-KO essential T-cells into patients with stage IV metastatic nonsmall cell cellular breakdown in the lungs (NCT02793856). The clinical productivity of the treatment was not resolved thus the viability of this study is questionable.\textsuperscript{55} Three further stage I clinical investigations are being led to assess PD-1-KO essential T-cells in stage IV bladder disease (NCT02863913), hormone-resistant prostate malignant growth (NCT02867345), and metastatic renal cell carcinoma (NCT02867332), starting around the same time. Be that as it may, later every one of these was removed due to lack of funding, which resulted in the studies being withdrawn. Although TILs have shown viability in metastatic diseases, especially melanoma,\textsuperscript{56} and have shown similarly more particularity and effectiveness than essential altered T-cells, hardly any preliminaries are following hereditarily adjusted TILs.

Two clinical preliminaries are, as of now, in progress utilizing CRISPR/Cas9 to disturb CISH in TILs taken from gastrointestinal growth areas (NCT04089891 and NCT04426669). The silencer of cytokine signaling (SOCS) protein, cytokine-induced SH2 (CISH), is a part of the SOCS family, CISH protein is expanded in CD8+ T-cells in light of TCR initiation, subsequently hindering T-cells hostile to growth action. \textit{In-vivo} examinations have shown how CISH cancellation causes TIL development, capacity, and cytokine discharge, as well as growth backslide.\textsuperscript{57} Currently, mispairing of $\alpha$ and $\beta$ chains of the therapeutic TCR complex is a main problem with ACT. This results in lessened effectuality for engaging TCR with the target antigen. Moreover, PD-1 expression on T-cells has a negative regulatory effect, which lowers the antigen reaction and, thus, the effectiveness of T-cell-mediated tumor killing.\textsuperscript{58}

**Role of CRISPR/Cas9 in Cancer Diagnosis and Treatment**

**For Diagnosis**

Cancer may be detected and treated earlier, which reduces the risk of mortality and improves patients’ quality of life following the treatment. Many cancer detection techniques are extensively employed, however, they ought to be improved in terms of sensitivity, specificity, and speed. As a result, for cancer prevention identifying vulnerable genes by genetic diagnosis is critical.\textsuperscript{59} CRISPR has remarkable potential to be used in diagnostics where it can detect genes involved in various cancer developments, their proliferation, and metastasis or in drug resistance. Detection of cancer-specific sequence changes can be done with the help of CRISPR-mediated enzymatic digestion which works as a diagnostic tool. CRISPR can detect microsatellites (a diagnostic marker in various cancers), which are made of short tandem repeats (STRs) through detecting these STRs. There are four Cas systems, i.e., Cas9, Cas12, Cas13, and Cas14, which are currently being used in CRISPR-based molecular diagnostics. These four CAS systems use different techniques for diagnosis such as Cas9 uses CRISPR-Chip and CRISDA, Cas12 uses DETECTR, HOLMES, and SHERLOCKv2, Cas13 uses SHERLOCK, and SHERLOCKv2 while Cas14 uses Cas14 DETECTR. In the processes of DNA recognition, shearing, and degradation, Cas12a works as a corresponding protein of Cas13a. Based on Cas12a, RPA was introduced as a form of new nucleic acid detection technique named DETECTR which compared with

\[ \text{Figure 1. Overview of cancer modeling with CRISPR/Cas9 technology.}^{37} \]
SHERLOCK can eliminate transcription of amplified DNA products into RNA step\(^1\). Researchers have also used Cas13a system as a basis and introduced RPA (temperature amplification of DNA technology) to create SHERLOCK (specific high sensitivity enzymatic reporter UnLOCKing). It has a site-specific amplification principle such as in the case of DNA; it will be followed by amplification through RPA and in the case of RNA detection; it will be amplified by RT-RPA. Target RNA transcribed from amplified DNA products is detected by Cas13a-crRNA and reporter RNA. This technique has its advantage as it is highly specific, sensitive in nature, and has many uses in CRISPR-based diagnostics. Its high sensitivity allows it to do single-base resolution based on its crRNA design. It has its applications in the detection of specific nucleic acid molecules, pathogen identification, detection of drug-resistant genes, liquid biopsy, virus and its subtype detection and differentiation, and cancer mutation analysis.\(^6\)\(^0\) Similar to this, Cas12-DETECTR biopsy, virus and its subtype detection and differentiation, and SHERLOCK and DETECTR and that is it need no amplification, another diagnostic technique that has a big advantage over amplification, it will be amplified by RT-RPA. Target RNA transcribed from amplified DNA products is detected by Cas13a-crRNA and reporter RNA. This technique has its advantage as it is highly specific, sensitive in nature, and has many uses in CRISPR-based diagnostics. Its high sensitivity allows it to do single-base resolution based on its crRNA design. It has its applications in the detection of specific nucleic acid molecules, pathogen identification, detection of drug-resistant genes, liquid biopsy, virus and its subtype detection and differentiation, and cancer mutation analysis.\(^6\)\(^0\) Similar to this, Cas12-DETECTR was designed due to four characteristics of Cas14: (1) small size of Cas14 makes its large-scale production easy; (2) target DNA is ssDNA; (3) it has no PAM sequence and thus any site sequence in the desired DNA can be targeted; (4) can detect DNA through a single base resolution. It has its applications in fast and efficient cancer and another disease diagnosis. SNP genotyping analysis method was also made possible to establish due to the properties of Cas14.\(^6\)\(^1\)

Other CRISPR-based diagnostic techniques for cancer detection include improved SHERLOCK, which is used in fast liquid biopsies of cancer patient. It can detect very small amounts of viral RNA \(\approx 2\) am.\(^6\)\(^2\) HOLMES is another CRISPR-based diagnostic tool based on the Cas12a system and PCR. It is fast, highly specific, simple, low-cost, sensitive, and needs no professional equipment for its working. It has its applications in the detection of cancer mutations, SNPs, and pathogens.\(^6\)\(^3\) CRISPR-Chip is another diagnostic technique that has a big advantage over SHERLOCK and DETECTR and that is it need no amplification of tested DNA.\(^6\)\(^0\) Cas12 and Cas13 detect nucleic acids through SHERLOCK (specific high-sensitivity enzymatic reporter unlocking) and DETECTR (DNA endonuclease-targeted CRISPR trans reporter) for an easy, affordable diagnosis of SARS-CoV-2 infection. Currently, CRISPR-DS is being clinically evaluated for p53 mutation detection in ovarian tumors. Thus, it can be freely said that CRISPR has its advantages in diagnosis as a personalized, sensitive, and safe monitoring system for cancer patients.\(^1\)\(^-\)\(^6\)\(^0\)

MicroRNAs (miRNAs) have a promising role in cancer pathologies by repression of protein-coding oncogenes or by impeding the expression of tumor suppressors. It can aid in the detection of and therapy for cancer. miRNAs are composed of a set of short, noncoding proteins, and small RNA molecules (20-24 nt in length). These RNA molecules are made from an endogenous transcript which contains a hairpin local structure, with the help of RNase-III-type enzyme Dicer. CRISPR/Cas9 technique targets the terminal loop or 50 regions of pre-RNA and thus suppressed the expression of miRNA. CRISPR/Cas9 targets desired DNA/ hsa-miR-17 gene with the help of sgRNA which binds with the target DNA at upstream of PAM (5’-NGG-3’). Cas9 nuclelease introduces DSB in targeted DNA at Drosha or Dicer processing sites which are 3 bp upstream of PAM. This DSB is repaired by host own repair mechanisms. CRISPR/Cas9 with the help of sgRNA can also alter miRNA biogenesis process through intentional targeting of sequences either within or adjacent to Dicer and Drosha repairing sites in the secondary stem-loop structure of primary miRNAs. Mechanism of CRISPR/Cas9 mediated miRNA has been given in Figure 2.\(^6\)\(^4\)

Functional genome screening techniques based on CRISPR system might show variations in the expression of a gene after medication and pinpoint genes linked to drug resistance thus adding novel biomarkers for precision therapy and offering an additional understanding of cancer development.\(^6\)\(^5\) One promising example includes the screening of a gene related to cancer metastasis.\(^6\)\(^6\) Where after mice were infected with lung cancer metastasis, genome-scale CRISPR knockout (mGeCKO) sgRNA library was created, and transduced cells were implanted into immuno-compromised mice. Six weeks later, mice with lung cancer metastasis were chosen for sequencing of the enhanced sgRNA. Finally, several candidate genes interrelated to lung metastasis were identified and verified, these included \(\text{Pien}, \text{mir-152,} \text{mir-345}\), along with many novel genes such as \(\text{Nf2, Trim72, and Fga}\).

**CRISPR/Cas9 for Cancer Treatment**

There are many types of cancer treatment methods, all have their own pros and cons. The type of treatment method one received mostly depends on cancer type, and its cancer progress stage. More often than not, combinations of treatment methods are suggested. Some of the most common cancer treatment methods are (1) surgery (to remove cancerous body tissue/part); (2) chemotherapy (drugs are prescribed to kill cancer cells); (3) hormone therapy (effective on hormones released during a certain type of cancer such as breast and prostate cancer); (4) Hypothermia (heat up to 113°F is provided to kill or stop cancer cell with little to no damage to normal cells); (5) Radiation therapy where radiations are used to treat cancer, etc.\(^7\)\(^0\) Among all these methods, CRISPR/Cas-mediated genome editing offers vast promise in cancer therapy, to make quick progress in fundamental oncology research. Tumorigenesis is a multistep process in which cancer cells communicate with the host immune system.\(^7\)\(^1\) Cancer immunotherapy is a well-known treatment option after surgery, chemotherapy, and radiation therapy. Adoptive T-cell immunotherapy, specifically chimeric antigen receptor (CAR) T-cell therapy, has marked a turning point in cancer treatment.\(^7\)\(^2\)

In the United States in 2019, the first clinical trial for the treatment of cancer using CRISPR was studied at The University of Pennsylvania, funded by the National Cancer Institute. In this type of immunotherapy, the immune cells of patients are genetically engineered to detect and destroy tumors in a better way. The treatment involves the introduction of four genetic changes into the T-cells. These are immune cells
capable of battling and killing cancerous cell. First, a claw-like protein is provided to T-cells by the addition of a synthetic gene. This claw-like protein commonly known as a receptor recognizes NY-ESO-1, a chemical found on certain cancer cells. Subsequently, three genes are deleted with the help of the CRISPR system. Among these three, two have the ability to interact with the NY-ESO-1 receptor and one gene is capable to hinder the ability of cells to fight malignancy. It results in the abundant production of NYCE T-cells which are then injected into patients. In the long run, it is one of the precise and safe strategies to kill cancer cells. It also helps in improving the efficacy of T-cells. A diagrammatic representation of the study performed at The University of Pennsylvania for the treatment of cancer using CRISPR Cas9 in the human body is illustrated in Figure 3.

According to studies conducted by Edward Stadtmauer, an improved response was shown by NY-ESO-1–directed T-cells, and that too with minimal toxicity. Edward and his colleagues also tried to observe whether the function of T-cells is improved with the deletion of three genes using CRISPR. The trials proved that the treatment so done employing CRISPR is harmless. The treatment was provided to three patients out of which two had severe multiple myeloma and one had metastatic sarcoma. All three patients had malignancies that contained the T-cell therapy’s target (NY-ESO-1). This therapy was proven to be safe on the basis of the preliminary data. According to the researchers, some side effects occurred, but they were most likely caused by chemotherapy in patients before injecting them with NYCE cells. Furthermore, there was no evidence of an immune response to the CRISPR-edited cells. List of ongoing CRISPR/Cas9 mediated cancer treatment clinical trials have been provided in Table 2.

**Role of CRISPR/Cas9 in Human Cancers**

Cancer is the second most common cause of death across the world, representing around 8.8 million passing in 2015. Throughout the following 20 years, the quantity of new cases is anticipated to ascend by almost 70%
internationally. Current cancer-fighting methods are insufficient, and scientists are continually on the lookout for new technology that can help. CRISPR/Cas9 system offers new promise in this area. CRISPR technology allows humans to rewrite their genetic code. CRISPR induction is easier and faster than previous technologies, and it will likely speed up gene-editing processes all around the world. It’s been utilized in a variety of in-vivo and in-vitro malignant tumor models up to this point. Oncologists are especially excited about CRISPR’s viability, precision, and potential in malignant tumor research. CRISPR/Cas9 innovation has been utilized by researchers from one side of the planet to the other to address malignant tumor treatment from many examination perspectives.

**Colorectal Cancer**

Colorectal malignant growth (CRC) is a type of cancer that occurs in colon or rectum. To investigate the function of potential colorectal cancer-associated genes, in-vivo studies using genetically modified mouse models (GEMMs) are performed. In GEMMs, the ApC and Trp53 tumor suppressor genes in colon epithelial cells were base edited by CRISPR/Cas9 and through orthotopic transplantation of ApC-edited colon organoids to produce tumor in mice. The study was successful in activation of an oncogene within the model mice. The study helped in cancer-associated genes characterization and to understand tumor progression and metastasis. This gene editing technique can be utilized in numerous model-based cancer studies to investigate genes responsible for a certain cancer type and the associated changes due to its manifestation in the human body.

**Breast Cancer**

Bosom disease (BC) is another tumor that exterminates women all around the globe. TNBC has the most exceedingly terrible forecast of all the BC subtypes on the grounds that it needs articulation of the estrogen receptor, progesterone receptor85, and HER2/neu tyrosine kinase receptor. The CRISPR/Cas9 technology was utilized to restrict cancer advancement and lung metastasis by reducing Cripto-1, an early-stage undeveloped cell marker of cancer stem-like cells. In this review, Cripto-1 was found to be a potential therapeutic target for TNBC. The Brahma (BRM) and Brahma-related gene 1 (BRG1) are both overexpressed in essential BCs. BRM and BRG1 work as ATPases inside mammalian SWI/SNF complexes and are important for their functioning. The CRISPR/Cas9 gene editing machinery was used to take out the BRG1 or BRM genes, revealing that these two genes play an important role in BC cell multiplication. Therefore, both BRG1 and BRM could be utilized to treat BC.

One more type of human BC is intrusive lobular carcinoma (ILC). The deficiency of cell grip protein and methylation of the CDH1 gene advertiser are normal elements of this disease. This recently made innovation can be utilized to examine potential cancer silencer genes embroiled in ILC in-vivo rapidly. Comparable frameworks could be utilized to make new
in-vivo models for the ID and treatment of different BC subtypes.\textsuperscript{82} Table 3 shows targeted genes and their functions in various cancer types.

**Table 3.** Targeted Genes and Their Functions in Various Cancer Types.

| Cancer Type                     | Gene                      | Function                                  | Delivery Method | Sponsor |
|---------------------------------|---------------------------|-------------------------------------------|-----------------|---------|
| Ovarian Cancer                  | Epithelial-to-mesenchymal translation (EMT) | During cancer metastasis, epithelial cells lose their affiliations, and gene expression is changed. This shift is accomplished by different master regulators, including Snail1, TWIST, and zinc-finger E-box confining (ZEB). The Snail1 factor was taken out using CRISPR/Cas9 advancement, revealing that the actin cytoskeleton is changed when Snail1 is deleted. This approach was also used to analyze the components of Snail1 and to decrease it in human ovarian illness RMG-1 cells.\textsuperscript{103} The CRISPR/Cas9 innovation can be utilized to study the genetic reason for chemoresistance in epithelial ovarian disease. Chemoresistance... |

Data extracted from https://clinicaltrials.gov/76
was reversed when the ovarian malignant growth biomarker HE4 was altered.\textsuperscript{104} \textit{In-vitro}, taking out \textit{LY75} diminished cancer cell rearrangement and obtrusiveness, and \textit{in-vivo}, taking out \textit{LY75} brought down the metastatic capability of EOC cell lines.\textsuperscript{105} The ovarian carcinoma immuno-reactive \textit{antigen area containing 1 (OCIAD1)} gene was additionally taken out using CRISPR/Cas9 in a BJNhem20-OCIAD1-CRISPR-39 line.\textsuperscript{106} This \textit{ex-vivo} study, as well as other CRISPR/Cas9-based research, prepares for future ovarian disease treatment.\textsuperscript{107} More generally, CRISPR/Cas9 has been broadly utilized in disease research, for certain promising discoveries.

**Table 3. Gene of Targets, and Their Function in Various Kinds of Cancer Treatment.**

| Cancer’s types          | Targeted gene | Gene functions of gene                                      | Experimental system used | References |
|-------------------------|---------------|-------------------------------------------------------------|--------------------------|------------|
| Lung cancer             | FGFR          | Decrease EMT-linked drug resistance                          | Mesenchymal cell lines   | 82         |
|                         | NOP10         | Decrease growth of cell, migration, colony and invasion     | NSCLC cell lines         | 83         |
|                         | STK11         | Enhance growth of tumor, resilience to therapies and cause   | LUAD models              | 84         |
|                         | KEAP1         | early death                                                 |                          |            |
|                         | IGF1R         | Promote MET gene and get erlotinib resistance               | HCC827 NSCLC cells       | 85         |
| Breast cancer           | MIE1I         | Lower the risk of Disease Development and metastasis        | MDA-MB-231 cells         | 86         |
|                         | NATI          | Reduced pyrimidine production and fatty acid β-oxidation    | MDA-MB-231 cells         | 87         |
|                         | PARP1         | Chemotherapy’s efficacy is improved by reducing proliferation, migration, and viability | NBC cell lines, MDA-MB-231, MDA-MB-436 | 88         |
| Colorectal cancer       | EPHA1         | Boost cell spread and adherence, and increase cell growth   | HRT18 cells              | 89         |
|                         | LGALS2        | Increases cell growth                                        | Mice                     | 90         |
|                         | ATF3          | Decrease cell invasion and proliferation.                   | HCT116, RKO cells        | 91         |
| Ovarian cancer          | ZNF587B       | Overcome cisplatin resistance                               | A2780, SKOV3 cell lines  | 92         |
|                         | BIRC5         | inhibiting epithelial to mesenchymal transition            | SKOV3, OVCAR3 cells      | 93         |
| Gastric cancer          | PDEF          | Inhibits migration and invasion                             | SGC, AGS cells           | 94         |
|                         | SST           | Ameliorates cell migration and attack                        | BGC823 Cell              | 95         |
|                         | TFF1          | Promotes tumorigenesis                                     | Mice                     | 96         |
| Pancreatic cancer       | HIF-1α        | Inhibit cells metastasis                                    | BxPC-3 cell lines        | 97         |
|                         | PSMA6         | Prompt cells apoptosis                                      | HPNE, HPAF-II, AsPC-1, Mia PaCa-2 cell lines | 98         |
| Cervical cancer         | Trio          | Decline cells movement and attack                            | Caski, HeLa cell lines   | 99         |
|                         | cREL          | Diminished cell multiplication                               | HeLa Kyoto cells         | 100        |

**Lung Cancer**

Cellular breakdown in the lungs is the most terrible cancer among different tumors occurring in both industrialized and underdeveloped countries, including China, United States, and countries in Europe.\textsuperscript{108,109} Cellular breakdown in the lungs is brought about by various genes and signaling pathways.\textsuperscript{110} It has been a subject of broad clinical exploration and dynamic gene modifications therapy is considered as disease quality treatment.\textsuperscript{111} It has been a subject of broad clinical exploration. Compared with other gene editing technologies like ZFNs\textsuperscript{112} and transcription activator-like effector nucleases (TALENs),\textsuperscript{113} CRISPR/Cas9 framework is a much more desirable method with its high target specificity, ease of use, and thus is being used more recently to study lung cancer, its prognosis, and treatment.\textsuperscript{35–114} CRISPR-based approach can be used to investigate lung cancer in individual patients, obstacles in anti-cancer medication efficacy, and/or to treat genetic causes of cancer before it progresses any further.\textsuperscript{115} The applications of CRISPR/Cas9 in studies on the treatment of lung cancer is given in Figure 4.

**Bone Cancer**

In children and adolescents, osteosarcoma (OS) is a highly vascular and particularly destructive tumor.\textsuperscript{117,118} Distant metastases (25%–30\%)\textsuperscript{119} and pathologic fracture due to bone loss\textsuperscript{120} are the most common consequences. The most common metastatic location of OS is the lung.\textsuperscript{121} Surgery in combination with chemotherapy is the standard treatment for OS.\textsuperscript{117,121,122} Patients with OS who have metastasis, on the other hand, have a much worse prognosis.\textsuperscript{123,124} As a result, developing novel therapeutic techniques for these people is critical. The angiogenic agent VEGFA (vascular endothelial growth factor A) is an inducer of angiogenesis and lymphangiogenesis.\textsuperscript{125} Studies reveal that VEGF’s effect on cancer development is not restricted to angiogenesis.\textsuperscript{126} In light of hypoxia, VEGFA is delivered to the cancer microenvironment.\textsuperscript{117} From one perspective, malignant growth cells’ paracrine VEGFA advances angiogenesis through endothelial cells, which convey oxygen and supplements. VEGFA, then again, goes about as an auto-crine growth cell endurance factor.\textsuperscript{127} VEGFA is presently known to be profoundly transformed in OS cells and to be firmly related to an unfortunate disease in patients.\textsuperscript{128} VEGFA suppression repressed OS development, metastasis, and 72 angiogenesis,\textsuperscript{129–131} inferring that VEGFA is a possible therapeutic option for OS.
Prostate Cancer
Prostate cancer (PCa) is treated primarily by inhibiting androgen receptor (AR) signaling, although the disease eventually advances to castration-resistant prostate cancer (CRPC). Patients with CRPC have seen a significant improvement in survival thanks to next-generation AR signaling inhibitors, but resistance is still an issue.132–134 Regardless of the way, genomic studies have recognized hereditary causes (ETS combinations, CDKN2A misfortune, PTEN, RB1, and SPOP changes, among others) and subatomic subtypes of PCa for designated treatment,135–137 some of the inherited anomalies found in patients’ of prostate cancer have shown promise as suitable drug targets. Thus, getting a more profound understanding of PCa’s crucial conditions might prompt more sensible restorative methodologies. In contrast to shRNA-or siRNA-based hereditary reliance testing, CRISPR/Cas9 innovation limits adverse consequences, is efficient, and recognizes more positive benefits.138,139 CRISPR/Cas9 framework has been effective in recognizing genes important for malignant tumor cell endurance as forthcoming targets,140,141 yet benefits of combining it with existing therapeutics have gotten less consideration. A summary of the genes targeted for the treatment of different cells or/tissues in various human cancers is provided in Table 4.

Challenges of Using CRISPR in Cancer Treatment
CRISPR/Cas9 has in no time evolved as a productive genome editing technology in only a couple of years. It has enormous potential in cancer therapy; however, there are still many challenges in its full clinical applications.
Off-Target Effects

The first challenge in CRISPR-based cancer therapy is off-target effects. In therapeutics, every small and low off-target editing is detrimental thus they should be controlled and identified accurately. Off-target DSB can cause small- and large-scale indels, inversions, and translocations at the nontarget sites. Through whole genome sequencing, large-scale off-target mutations can be detected. However, it is very difficult both technically and economically to do the same for small-scale off target mutations as whole genome sequencing is unable to distinguish between SNP and small genetic alterations. A better, practical and cost-effective option for detection of small scale indels is through whole exome sequencing which focuses on off-target mutations occurring in coding region of genes. Various strategies have been developed to avoid or minimize off-target mutations such as (1) identification of a unique target sequence with very few homologous sequences in then whole genome; (2) high GC content up to 75% in target sequence; (3) use of more potent Cas9 variants to avoid off-target effects; (4) designing of more suitable, and powerful sgRNA, i.e., truncated, 17 to 18 nt long, stem loop 2 of sgRNA, etc. A combination of these approaches can be used to completely avoid off-target effects.

Editing Efficiency

Editing efficiency is another challenge in CRISPR Cas9 therapy in cancer patients. Unmodified cells tend to proliferate more quickly than modified cells and can lead to relapse, thus CRISPR-based therapies need very high editing efficiencies. Editing efficiency is based on (1) careful selection of target sites; (2) efficient and reliable delivery vectors; (3) potent Cas9 selection; (4) careful selection and designing of sgRNA. Further research insights are needed to get a higher genome editing efficiency.

Efficient Delivery of CRISPR/Cas Components

Other than off-target effects and editing efficiency, another challenge in CRISPR-based cancer treatment is the efficient delivery of CRISPR/Cas components at target sites. The most widely used vehicles for delivery are viral vectors and in particular adeno-associated virus vectors (AAVs). AAV is the most preferable choice because of its transient expression, impressive efficiency, and low cytotoxicity. AAV has a packaging obstacle that has just been solved with the help of Staphylococcus aureus (SaCas9) based split-Cas9 system or Streptococcus thermophiles (St1Cas9) based smaller Cas9 orthologs. There are

| Cancer type | Target gene | Treated tissues and cells | Knockout status | Role | References |
|-------------|-------------|---------------------------|----------------|------|------------|
| Colorectal cancer | ERO1α | HCT116 cells | Yes | Suppress cancer development | 141 |
| | | HCT116 cells | | Edit gene mutation | 142 |
| | ATF3 | HCT116 and RKO Cells | Yes | Inhibit tumor invasion and metastasis | 91 |
| | aTAT1 | HCT116 cells | Yes | Inhibit proliferation and invasion | 143 |
| | APC, P53, KRAS and SMAD4; APC and P53 | Human intestinal stem cells | Yes | Enhance tumor progression | 144 |
| Breast cancer | miR-27b | MCF7 cells | Yes | Tumor suppressor | 145 |
| | CXCR7 and CXCR4 | MDA-MB-231 cells | Yes | Suppress tumor growth | 146 |
| | TMEM106A | MDA-MB-231, MDA-MB-468 cells | Yes | Breast cancer metastasis suppressor | 147 |
| | OPN | MDA-MB-231 cells | Yes | Help overcome radioresistance in cancer treatment | 148 |
| Ovarian cancer | CXCR4 and CXCR7 | MDA-MB-231 cells | Yes | Slow down TNBC cancer growth | 149 |
| | Egfl6 | SKOV3 cells | Yes | Tumor angiogenesis | 150 |
| | Trp53 and Brc2 | HGSC Murine Models | Yes | Promotes tumor growth and invasion | 141 |
| | miR-21 | SKOV3 and OVCAR3 cells | Yes | Inhibits EMT in cancer cells | 151 |
| | ITK | SKOV3 cells | Yes | Possible cancer suppressor gene | 152 |
| | BIRC 5 | SKOV3 and OVCAR3 cells | Yes | Enhance cell sensitivity to Paclitaxel | 93 |
| Lung cancer | CD38 | A549 cells | Yes | Oncogene | 153 |
| | FAK | KRAS NSCLC cells | Yes | Oncogene | 154 |
| | Rsf1 | H460, H1299 cells | Yes | Oncogene | 155 |
| | EGFR (L858R) | H1650 cells | No | Oncogene | 156 |
| | Igf1r | HCC827 cells | Yes | Oncogene | 85 |
| | Got1 | A549 cells | Yes | Tumor | 157 |
| | Keap1 | Kras-driven mouse model | Yes | Tumor suppressor | 158 |
| | miR-1504 | A549 cells | Yes | Tumor suppressor | 159 |
some other nonviral delivery methods aimed to enhance cell membrane permeability and target specificity of CRISPR/Cas9, i.e., cationic lipids, electroporation-mediated gene transfer, nanoparticles, and cell-penetrating peptides (CPPs). In a study on adult mice, a combination of AAV encoding sgRNA, homologous template, and lipid nanoparticle-mediated delivery of Cas9 mRNA was successfully applied to activate gene repair in adult mice. This suggests that a careful combination of viral and nonviral delivery methods may be an alternative approach for cancer treatment in patients.

**Low Efficiency of HDR**

In CRISPR-based cancer therapies, the low efficiency of HDR is another issue on which further work is needed. Both NHEJ and HDR repair DSB however, NHEJ is more preferable than HDR. In some cases, HDR-based DSB repair is needed where low efficiency of HDR becomes a problem. One way to increase HDR efficiency is by inhibition of NHEJ pathway. Recently, in a study paired Cas9 nickase has been found to increase HDR efficiency by producing single-strand nicks.

**Future Perspectives and Conclusion**

CRISPR/Cas9 is a novel and effective way of genome editing that has lately outperformed other approaches due to its significant characteristics. This multifaceted tool, which is often described as an umbrella terminology, has transformed life sciences by enabling advancements in basic research for a wide range of applications. The CRISPR/Cas9 genome altering apparatus has colossal research and clinical possibilities in disease treatment. This straightforward and scalable approach has the potential to aid in the comprehension of cancer predisposition and metastatic pathways, along with the prediction of therapy response and drug tolerance. CRISPR is expected to be implemented eventually in clinics, permitting an extensive range of treatment opportunities for human diseases, particularly cancer. The ongoing effort in developing and revolutionizing new ways to deliver genome manipulating tools into cells, as well as refining their modification capabilities, will enable these technologies to be utilized in a number of therapeutic applications. The production of chimeric antigen receptor (CAR) T-cells that can identify specific antigens on cancer cells is a prominent application of the CRISPR technique. CRISPR/Cas9 can play a critical role in CAR-T cell research, which has surged in recent years. However, certain concerns including off-target effects, *in vivo* delivery, immunogenicity, and ethical considerations must be handled in order for it to be implemented successfully. Nevertheless, many clinical trials are being conducted to overcome these issues, and we anticipate gene-editing technology to play an important role in the future.

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**ORCID iD**

Fatima Akram https://orcid.org/0000-0001-8438-706X

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