Frontiers of CRISPR-Cas9 for Cancer Research and Therapy

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Abstract

In recent years, gene editing technologies have made significant progress in understanding gene function and regulation. The Clustered regularly interspaced short palindromic repeats-CRISPR associated protein 9 (CRISPR-Cas9) system has emerged as a versatile tool for gene editing and genome engineering. In the last few years, CRISPR-Cas9 technology has been widely applied to cancer research, mainly to understand the mechanisms of oncogenesis, drug-target identification, and the development of various cell-based therapies. When combined with genome sequence information, this technology has also shown promise to cure heritable genetic disorders. This review summarizes some of the recent developments and preclinical applications of CRISPR-Cas9 technology in cancer research and therapy. We will discuss how CRISPR based approaches have been used as a tool to identify cancer-specific vulnerabilities and potential applications in cancer therapy.

Keywords: CRISPR; Cas9; Cancer; Gene editing; Non-coding RNA.

Introduction

Cancer is one of the leading causes of death worldwide.1 It is a complex disease originating from various genetic alterations/epigenetic aberrations. Due to its mechanistic complexity, current cancer-specific therapies have certain limitations, emphasizing the need for alternative therapeutic approaches. The Cancer Genome Atlas has described over 15,000 tumors that have generated multi-dimensional data that illuminate the complexities of oncogenesis. Researchers have made a significant effort to comprehend the underlying biology of tumorigenesis, which has helped in the development of various chemotherapeutic agents, small molecule drugs, and antibodies. However, different cancer patients have vastly different genetic alterations/aberrations that can drastically alter tumor progression and susceptibility to therapy. As a result, there have been a significant thrusts and emphasis on identifying various genetic/epigenetic mutations, comprehending the genomic landscape, and developing more productive and safe therapeutic approaches for cancer.8,9 RNA interference (RNAi) and random mutagenesis were initially used to discover genetic alterations.8,9 Subsequently, the discovery of programmable nucleases such as zinc-finger nucleases (ZFNs) and Transcription activator-like effector nucleases (TALENs) provided an immense opportunity to study the function of genes in cancer. These genome editing technologies have enabled researchers to target the genome at specific sites to generate gene-knockouts.12,13 Both TALENs and ZFNs are engineered nucleases and are created by fusing DNA binding domains with a DNA cleavage domain. They can target and cleave specific DNA sequences, which are then repaired by the endogenous DNA repair machinery, thereby precisely altering DNA sequences in the complex genome.12,13 However, TALENs and ZFNs are considered to have low editing efficiency and are expensive tools. The recent identification of RNA-guided programmable nucleases of the bacterial adaptive immune system has shown tremendous success and proven to be a highly efficient tool for gene editing/genome engineering.13–17

The Clustered regularly interspaced short palindromic repeats-CRISPR associated protein 9 (CRISPR-Cas9) system of gene editing has gained significant attention in recent years for its precision and effectiveness in genome editing in several model systems and human cells. Not only that, CRISPR-Cas9 is also used to introduce transcriptional and epigenetic modifications in different mammalian systems. Direct somatic editing using CRISPR-Cas9 has transformed the druggable space by helping researchers to correct any altered genomic aberrations or modify regulatory elements and...
splicing patterns.\textsuperscript{18,19} The technological demonstration of CRISPR-Cas9 and the molecular basis of editing has been covered elsewhere.\textsuperscript{20–22} In short, a single guide RNA (sgRNA) directs Cas9 endonuclease to introduce a double-stranded break at a specific site in the genome.\textsuperscript{23} The endogenous DNA repair mechanisms later repair the genome using two different mechanisms: namely, the predominant error-prone non-homologous end joining\textsuperscript{24–26} and less frequent homology-directed repair (HDR).\textsuperscript{27–31} which requires a donor template. Non-homologous end joining has been used to disrupt the genome sequences to create deletion and insertions, while HDR has been used to alter specific regions of the genome using exogenous repair templates. In addition to this, a modified version of Cas9 protein, catalytically dead Cas9 (nuclease-dead Cas9), has been utilized to either activate or repress the target genes; these mechanisms are known as CRISPR activation and CRISPR interference respectively (Fig. 1).\textsuperscript{32–34}

In this review, we will discuss how CRISPR-Cas9 is revolutionizing cancer research and therapy. The role of CRISPR-Cas9 system in understanding cancer genomics, exploring non-coding regions, \textit{in vivo} gene editing, and the generation of novel organoid models will also be discussed. The recent advances in the delivery of CRISPR-Cas9 for \textit{in vivo} gene editing will be reviewed. Lastly, we will summarize the preclinical and clinical applications of CRISPR-Cas9 technology in cancer.

**CRISPR-Cas9 as a tool for target discovery in cancer**

CRISPR-Cas9 is a powerful tool to identify novel targets in cancer. Due to precise gene editing capabilities, CRISPR has become the tool of choice to study the function and regulation of specific genes. It has been used in high-throughput screening approaches, where CRISPR-Cas9 is used to generate a large number of genetic knockouts using which one can monitor whether there is a phenotypic effect due to knockout of a particular gene in a cell population. In cancer, this strategy has been used to perform high-throughput screens and look at gene-drug interactions when used in conjunction with small molecules.\textsuperscript{35–38} The recent advancement in next-generation DNA sequencing and genome-wide association studies has provided a more comprehensive understanding of variations in the human genome. This has unraveled thousands of single nucleotide polymorphisms and mutations attributed to various diseases and their predisposition. Several genomic repositories (such as The Cancer Genome Atlas, the Cancer Cell Line Encyclopedia or the Encyclopedia of DNA Elements)\textsuperscript{39,40} have been developed, containing a catalog of disease-specific variation at the genome level. This has also encouraged personalized medicine by integrating available patient data and genetic information. However, the hypothesis generated by these high-throughput genomics data requires thorough testing and validation using accurate genetic models to develop new treatment standards. It is often seen that mutations in genes with known functions do not always directly correlate with the disease phenotype. To understand such relationships, researchers often need a large number of matched patient and regular tissue samples, which can be difficult to obtain due to ethical concerns. The advent of CRISPR technology has helped researchers to generate isogenic human knockout/genetically modified cells to address this problem.\textsuperscript{41} Likewise, CRISPR technology has been used in many cell types, particularly cancer cell lines, primary cells, organoids, and induced pluripotent stem cells.\textsuperscript{41–44} One example where CRISPR is used in cancer biology is if a tumor-derived cell line with a genetic lesion is thought to be sensitized to a particular therapeutic drug, one can use CRISPR to test the hypothesis of synthetic lethality in the appropriate cell line.\textsuperscript{45–47}

Although this technique shows a lot of promise, the rates of CRISPR-mediated cell modification vary from cell type to cell type, especially HDR. Primary cells such as neurons and other non-mitotic cells are challenging to modify especially using HDR. The development of microhomology-mediated integration has shown promise to bypass HDR-mediated cell line modification.\textsuperscript{48–50} Furthermore, the discovery of newly engineered Cas enzymes has also enabled researchers to modify primary cells, alter the genome, and change specific bases. For example, the fusion of cytidine deaminases to Cas9 has enabled researchers to modify RNA-guided base editing,\textsuperscript{51,52} which has shown promise in editing a wide variety of cell types.

**CRISPR-Cas9 as a tool to investigate non-coding regulatory regions in cancer**

The majority of the human genome is comprised of non-protein coding regions that consist of various regulatory elements such as enhancers, silencers, and insulators. Due to the dearth of robust molecular biology tools, these regions have not been fully characterized. The importance of these non-coding regions is highlighted by the fact that dysregulation of these elements could contribute to oncogenesis.\textsuperscript{53} Therefore, a comprehensive understanding of these regulatory elements may help to understand the genomic landscape of cancer cells. Several studies have utilized CRISPR-Cas9 technology to interrogate the non-coding regions of the genome, in particular various cis-regulatory elements such as enhancers, super-enhancers, and trans-acting factors.

In one of the landmark studies, researchers utilized pooled CRISPR-Cas9 libraries to identify functional non-coding regions around \textit{CUL3}, \textit{NF1}, and \textit{NF2} genes, which have been previously shown to be responsible for promoting Vemurafenib resistance to melanoma cells harboring the Braf V600E mutation.\textsuperscript{54,55} Notably, the authors found significantly more regulatory regions around the \textit{CUL3} gene compared to the \textit{NF1} and \textit{NF2}. Furthermore, the authors used chromosome conformation capture studies to show a direct interaction between these non-coding regions to the 5' end of the \textit{CUL3} gene.\textsuperscript{54}

In another interesting study, researchers have performed CRISPR-Cas9 screens to identify several functional elements that regulate two transcription factors TP53 and ESR1, which play essential roles in tumor initiation and progression.\textsuperscript{56,57} These pooled CRISPR-Cas9 screens identified six different enhancer elements, one of which promoted the activation of the \textit{CDKN1A} gene during a cell cycle arrest program called oncogene-induced senescence, and the other one mediated the expression of the \textit{CCND1} gene in response to ERS1 signaling. The identification of these regulatory elements and their associated target genes could lead to tumor diagnostics and therapeutics.\textsuperscript{56}

The advent of CRISPR-Cas9 high-throughput screens has also provided researchers a versatile tool to interrogate the non-coding genome at high resolution, particularly the characterization of several non-coding RNAs in cancer and other diseases. Studies have utilized genome-wide deletion screens of long-noncoding RNA (lncRNAs) using paired sgRNAs to show that knockout of specific lncRNAs significantly altered cell proliferation eluding to their role in oncogenesis.\textsuperscript{58} Other studies have used CRISPR interference screens on various cell lines to study the effects of lncRNAs on cell viability.\textsuperscript{59} One of these studies identified 499 lncRNAs essential for cell proliferation. CRISPR-Cas9 has also been used
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Fig. 1. Schematic representation of CRISPR-Cas9-mediated genome editing. (a) The Cas9 nuclease is directed to the target DNA by complementary base pairing with its bound guide RNA in which the target site is followed by a PAM sequence (NAG, NAG). Cleavage of the dsDNA promotes either error-prone NHEJ or HDR. (b) Two guide RNAs (targeting two different sites, namely target A and target B) can be used simultaneously to remove a longer stretch of DNA and a new DNA can be inserted using a donor DNA using HDR. (c) Catalytically dead Cas9 (dCas9) can be tagged with transcription activators (or repressors) and fluorescent proteins (such as GFP) to regulate gene expression or visualization. CRISPR, clustered regularly interspaced short palindromic repeats; Cas9, CRISPR associated protein 9; dCas9, dead CRISPR associated protein 9; gRNA, guide RNA; mRNA, messenger RNA; dsDNA, double stranded deoxyribonucleic acid; PAM, protospacer adjacent motif; NHEJ, non-homologous end joining; HDR, homology directed repair; GFP, green fluorescent protein.

to visualize lncRNAs in scenarios where the conventional fluorescence in-situ hybridization approaches have failed. One study used catalytically dead Cas9-GFP-sgRNA fusion probes to visualize X-inactive specific transcript lncRNA which has a complex secondary structure and was highly condensed for the access of fluorescence in-situ hybridization probes. The authors used this modified Cas9 to efficiently visualize the X-inactive specific transcript lncRNA along with repressive H3K27me3 histone marks in female fibroblasts.60

Although the role of CRISPR-Cas9-mediated identification of lncRNAs shows promise, there is one caveat; since various lncRNAs have bidirectional promoters and are located near/within pro-
tein-coding genes, targeting them with CRISPR-Cas9 affects the expression of these genes. Therefore, more complex CRISPR-Cas9 approaches need to be developed to interrogate the molecular function of IncRNAs.

CRISPR-Cas9 as a tool to generate organoid models of cancer

In recent years, organoids have become popular in vitro models over two-dimensional cell cultures to study human diseases. Adult human stem cells are isolated and stimulated with specific growth factors to help them proliferate and subsequently differentiate into three-dimensional organoids.22 Traditionally, small molecule therapeutic assays were performed on two-dimensional cell cultures, but this did not mirror normal physiological conditions where cells are in a three-dimensional microenvironment. These organoids mimicked cell-matrix and cell-cell environments compared to cells in two-dimensional cultures which helped researchers to develop physiologically relevant in vitro systems for their studies.

The advent of CRISPR-Cas9 has aided researchers to generate tumorigenic organoid models to interrogate putative mechanisms of oncogenesis. The first proof of concept study for genome editing using CRISPR-Cas9 was carried out in intestinal stem cell organoids where researchers repaired the CFTR locus from a Cystic Fibrosis patient. A few years later, healthy human intestinal stem cell-derived organoids were transformed into their tumorigenic counterparts by introducing several tumor-inducing mutations using CRISPR-Cas9. The activation of oncogenes (such as PI3K and KRAS, etc.) and the disruption of tumor-suppressors (such as TP53, APC, SMAD4, etc.) using CRISPR-Cas9-mediated gene editing helped establish organoid models of cancer in vitro.42,44 Another group used CRISPR-Cas9 to delete an essential DNA repair gene called MLH1 from healthy human stem cell organoids to accurately model oncogenesis induced by replication errors and mismatch repair. Thus the use of CRISPR-Cas9 in generating various stem-cell derived organoid models can be used to mimic patient-derived samples for functional assays of small-molecule and other high-throughput screens.65

CRISPR-Cas9 as a tool to overcome drug resistance in cancer

With the development of CRISPR-Cas9, researchers can modify the genome to understand the role of specific genes and molecular mechanisms underlying drug resistance in different types of cancer such as breast cancer, lung cancer, liver cancers, and gliomas. Using a genome-wide CRISPR-Cas9 approach, researchers showed that deletion of transcriptional factors such as MED1, CREBBP, and EP300 increased the efficacy and synergy between two drugs, namely erlotinib (an epidermal growth factor receptor (EGFR) inhibitor) and TZH1 (a CDK7/12 inhibitor). These drugs are used in the treatment of EGFR-dependent lung adenocarcinoma. Another study used a combination of CRISPR, short-hairpin RNA (shRNA), and expression screen in non-small cell lung cancer cells to identify a protein called PBRM1 (a subunit of SWI/SNF complex) which attenuated the gefitinib mediated EGFR inhibition for prolonged cell survival.67

A genome-wide association study in women treated with selective estrogen modulators for breast cancer prevention identified a single nucleotide polymorphism (rs9940645) in a gene called ZNF423. Using CRISPR-Cas9 editing, the authors modified ZR75-1 cells to harbor the same variant. Studies on these cell lines showed that this variant containing the cell line was more sensitive to raloxifene, olaparib, and cisplatin.68 Another genome-wide CRISPR screen revealed the role of BAK in breast cancer. In this study, when breast cancer cells with high expression of MCL-1 were treated with S63845 (an MCL-1 inhibitor), a synergistic relationship with other drugs such as docetaxel, trastuzumab, and lapatinib which are used in the treatment of triple-negative breast cancer and HER2-amplified breast cancer was revealed. The CRISPR screen revealed that the deletion of BAK or double deletion of BAK and BOX showed resistance to S63845.69

Other genome-wide CRISPR-Cas9 screens have identified factors involved in drug resistance in liver cancers and glioblastoma. One screen identified a protein called SCOL1 (a protein involved in mitosis), the loss of which led to sorafenib resistance in hepatocellular carcinoma. Furthermore, it was shown that loss of SGOL1 from hepatocarcinoma cell lines decreased the cytotoxicity of sorafenib.70 CRISPR-Cas9-mediated knockout of a glioblastoma-associated oncogene called GLI1 showed increased apoptosis when combined with an antipsychotic drug Penfluridol.71 CRISPR-Cas9-mediated depletion of a centriole satellite protein PCM1 revealed that its removal inhibited glioblastoma cell proliferation and had increased sensitivity to temozolomide in patient-derived glioblastoma.72 Saber et al.73 and Liu et al.74 have reviewed these studies.

Delivery of CRISPR-Cas9 for in vivo editing

One of the significant challenges in CRISPR-mediated gene therapy is how editing components can be delivered into specific tissues in humans without leading to off-targets and activating the immune system. Broadly, there are two ways to deliver CRISPR-Cas9 for in vivo gene editing, namely the non-viral method and viral based method using adeno-associated virus (AAV).

Non-viral delivery of CRISPR-Cas9

Several non-viral methods of CRISPR-Cas9 delivery have been developed. One of the simplest delivery methods utilizes cell-penetrating peptides conjugated to sgRNA and Cas9 protein. It has been shown to edit HEK293T cells with an efficiency of 6.2% and can be a potential delivery approach for in vivo applications in future studies. Another delivery method is Lipid nanoparticles (LNPs) based. LNPs are produced using a combination of ionizable lipids, cholesterol, and PEGylated lipids. These LNPs are then used to deliver sgRNA and Cas9 to the mouse liver to target the transthyretin gene. This approach showed a more than 97% reduction in serum transthyretin levels.76 Polymer-based synthetic nanomaterials are also being developed for CRISPR-Cas9 delivery. Cationic polymers such as polyethyleneimine are conjugated with CRISPR-Cas9 vectors and injected directly into tumors in EGFP expressing mice and a significant drop in EGFP has been shown, exhibiting high knockout efficiency.77 Additionally, colloidal gold nanoparticles are also being used to successfully deliver gRNA-Cas9 ribonucleoproteins along with HDR template in mice to treat Duchenne muscular dystrophy with minimal off-target effects.78 Although all these approaches have shown promise, further research is required to improve the scalability and efficacy of these methods in preclinical and clinical studies.

AAV based delivery of CRISPR-Cas9

In the last decade, a lot of research has been undertaken to study...
and characterize AAVs. These studies have led to the discovery of multiple serotypes of AAVs with distinct tropisms to selectively target different tissues in humans for gene therapy. Therefore, AAVs have become a potent delivery tool for CRISPR-Cas9 to specific tissues for gene modification. For example, CRISPR-Cas9 harboring AAVs have been subretinally injected to knockdown the NLR gene in post-mitotic retinal photoreceptors to prevent retinal degeneration in mice and to the striatum to edit the huntingtin gene to alleviate Huntington’s disease. For systemic delivery, two crucial things need to be considered: (a) the serotype of the AAV and (b) tissue-specific promoters under which the Cas9 is cloned. Once these conditions are determined, the engineered virus can be used to deliver Cas9 and sgRNA for expression in specific tissues. This dual AAV system has been used to edit the ornithine transcarbamylase gene in mice liver and the dystrophin gene in the mouse model for Duchene muscular dystrophy and muscle stem cells in mice. Although the editing efficiency was between 10–70%, these experiments had significant phenotypic improvements in mice. Recent studies have also shown that although AAVs and Cas9 induce an immune response in mice, they do not inflict extensive cellular damage, making them potentially safer delivery methods over other delivery systems.

High titer AAV libraries have been used to target mouse tissues in vivo to generate tumors with several mutations in glioblastoma and liver tissue. This approach can help researchers robustly study the oncogenic role of individual mutations, helping develop patient-specific treatments with genetically matched tumors. This technique may also help in the development of tumor heterogeneity models, which has been recognized as an important contributor to drug resistance and cancer relapse. Additionally, this technique could be used to model, predict and investigate tumor clonal dynamics and eventually serve as a platform for novel cancer therapies.

One limitation of using AAV as a delivery tool is that AAVs have a limited cargo size, as two separate vectors are used for delivery, both directly to the target organ and systemically. Researchers are also using LNPs, cell penetrating peptides, and polymers-based synthetic nanomaterials to deliver CRISPR-Cas9 as well (discussed above). Recent studies have used lipid nanoparticles to efficiently deliver Cas9 into mouse liver to knockdown specific genes and can deliver donor templates for HDR-mediated gene editing. There are two significant advantages of using lipid nanoparticles as a method of delivery: (a) they were previously used to deliver siRNA and mRNA in clinical trials and (b) they can be industrially manufactured. Additional research is required to improve the efficiency of ex vivo and in vivo CRISPR-Cas9 editing, and new screens are being performed to gain new insights on cancer-associated genes.

Clinical trials using CRISPR-Cas9

The idea of somatic gene therapy has been proposed long before the advent of gene editing technology was discovered. Somatic gene therapy is described as introducing genetic material into somatic cells, which will then express that novel gene product. These cells can then be put back into patients for therapeutic purposes. Before the introduction of modern gene therapy techniques, these early trials have been mostly unsuccessful due to problems associated with the host immune response, gene silencing, and random mutagenesis. Modern techniques have allowed researchers to overcome these problems along with reliable and permanent modification of patient somatic cells.

The first clinical trials were performed using ZFNs to target the human immunodeficiency virus (HIV) receptor called CCR5. ZFNs were used to modify HIV-infected patient’s T-cells, which were then introduced back into the patients to promote resistance to HIV infection. This resulted in a significant reduction of viral particles compared to the control and was well tolerated by the patients. Although this treatment did not have a lasting effect, it ushered in an era for several other gene therapy clinical trials. The first CRISPR-Cas9 trial was performed on non-small cell lung cancer patient T-cells, ex vivo modified by CRISPR-Cas9 to knock out programmed cell-death protein (PD-1) (NCT02793856). PD-1 ligand (PD-L1) is highly expressed on various cancer cell types, and it binds PD-1 receptors on the activated T cells, which leads to the inhibition of the cytotoxic T cells. The PD-1/PD-L1 pathway thus represents an immune checkpoint mechanism exerted by tumor cells in response to endogenous immune anti-tumor activity. Neutralizing antibodies of PD-1 (or the PD-L1) have been recently used to treat different types of cancer. In this gene therapy approach, patient peripheral blood was collected and CRISPR-Cas9 was used to knock-out PD-1 ex vivo. After rigorous testing, the PD-1 knockout cells were introduced back into the patients. There are speculations as to whether the PD-1 modified T-cells are better compared to therapeutic antibodies against PD-L1 or PD-1 since the production of engineered T-cells is a costly and laborious process.

Another clinical trial in its Phase I/II has the PD-1 gene knocked out in an Epstein-Barr virus specific autologous T-cell for cancers positive for Epstein-Barr virus (NCT03044743). Researchers are now generating chimeric antigen receptor (CAR)-T cells with CRISPR-Cas9. Another preclinical study was conducted where CAR was delivered to the T-cell receptor α-chain loci using CRISPR-Cas9 that enhanced its tumor rejection capability compared to conventionally produced CAR-T cells in a mouse model. Multiple other clinical trials have also been registered in patients with other types of cancer such as renal, esophageal, and bladder cancer, some of which are phase II clinical trials (clinical-trials.gov) (Table 1).

Although a fair number of studies are ongoing with clinical trials using ex vivo genome editing, only two are being implemented for in vivo clinical trials. One trial (NCT03057912), which is still in the process of recruiting patients, involves the delivery of constructs targeting HPV16 and HPV18 using either TALENs or CRISPR-Cas9 using a gel that will be locally applied to the patient’s infected cervix. Further research is needed to improve the specificity and delivery of CRISPR-Cas9 to target specific tissues, and this will promote the development of CRISPR based clinical trials in the future.

Future perspectives

CRISPR/Cas9 technology has evolved as a versatile tool to manipulate and edit the genome and epigenome across a broad range of cell types and organisms. The applications of CRISPR/Cas9 in both basic and translational cancer research are beginning to unfold. In the future, applications of various CRISPR-based genome-wide screens may help to unravel novel genes, new biomarkers, and regulatory pathways that can be therapeutically targeted in cancer. Integrating the information from these screens with available data on the genetic and epigenetic landscape of various cancer types may help identify synthetic lethal interactions and discover novel drug targets. However, this will require considerable research to thoroughly dissect the biological mechanisms underlying...
a multitude of genetic/epigenetic interactions revealed by CRISPR screens. Furthermore, the ability of CRISPR/Cas9 to manipulate non-coding regions of the genome will augment the functional exploration of these less characterized features of the cancer genome. The precise engineering of pathogenic and driver mutations will enable researchers to gain deeper insights into the biological changes elicited by these mutations in cancer. Although there has been an increasing interest of the scientific community to examine and broaden the translational potential of CRISPR/Cas9 tools, translating these into the clinic successfully remains a challenge. Some of the major concerns are safety, including uncontrolled off-target effects.

Fig. 2. CRISPR-Cas9 is used to knockout PD-1 in T cells from patients. T-cells are isolated from the blood of a cancer patient. CRISPR-Cas9 ribonuclear proteins are electroporated into the T-cells and the TRAC, TRBC1, TRBC2, and PDCD1 loci are edited. The cells are then transduced with a lentiviral vector to express TCR-specific cancer testis antigens NY-ESO-1 and LAGE-1. These cells are then infused back into the patients and are safely monitored to check the safety and efficacy of treatment. T cell, thymus lymphocyte cell; PD-1, programmed cell death protein 1; TCR, T cells receptor; CRISPR, clustered regularly interspaced short palindromic repeats; Cas9, CRISPR associated protein 9; PAM, protospacer adjacent motif; NY-ESO-1, New York esophageal squamous cell carcinoma-1; TRAC, T cell receptor alpha constant; TRBC, T cell receptor beta constant; PDCD1, programmed cell death protein 1.

Table 1. A table summarizing the different clinical trials using CRISPR-Cas9 in progress

| Clinical trial identifier | Cancer type                             | Phase | Treatment strategy                                      |
|--------------------------|-----------------------------------------|-------|--------------------------------------------------------|
| NCT02867345              | Hormone refractory prostate cancer      | I     | PD-1 knockout T cells                                   |
| NCT02867332              | Renal cell carcinoma                    | I     | PD-1 knockout T cells                                   |
| NCT03081715              | Esophageal cancer                       | II    | PD-1 knockout T cells                                   |
| NCT02863913              | Bladder cancer                          | I     | PD-1 knockout T cells                                   |
| NCT02793856              | Non-small cell lung cancer              | I     | PD-1 knockout T cells                                   |
| NCT03044743              | EBV positive advanced stage malignancies| I/II  | PD-1 knockout EBV-CTL                                    |
| NCT03166878              | B cell lymphoma / leukemia              | I/II  | CRISPR-Cas9 edited CAR-T Cells Targeting CD19 and CD20 or CD22 |
| NCT03057912              | HPV related cervical intraepithelial neoplasia | I   | CRISPR-Cas9-sg HPV E6/E7 gel to disrupt HPV DNA |
| NCT03398967              | B cell lymphoma / leukemia              | I/II  | CRISPR-Cas9 edited CAR-T cells targeting CD19          |
| NCT03057912              | HPV related cervical intraepithelial neoplasia | I    | CRISPR-Cas9-sg HPV E6/E7 gel to disrupt HPV DNA |

NCT, national clinical trial; EBV, Epstein-Barr virus; HPV, human papillomavirus; PD-1, programmed cell death protein 1; EBV-CTL, Epstein-Barr virus (EBV) specific cytotoxic T cells; CAR-T, chimeric antigen receptor T cells; CD, cluster of differentiation; CRISPR, clustered regularly interspaced short palindromic repeats; Cas9, CRISPR associated protein 9.
effect, the immunogenicity of Cas nucleases, and the carcinogenic effect of CRISPR components, adverse immunological effects, and so forth. Many challenges associated with CRISPR technology still exist, particularly in clinical use. The future use of CRISPR/Cas9 technology will rely on its precision and efficacy and efforts to develop novel Cas9 variants with minimal off-target effects and enable precise editing of the genome. Furthermore, new advancements in the methods to deliver CRISPR components, improvements in viral and non-viral delivery methods will accelerate the in vivo application of the CRISPR-Cas9 system.

Conclusions

The CRISPR-Cas9 technology has evolved rapidly and has revolutionized the scientific field from basic research to clinical applications. Undoubtedly, this technology has provided many promising avenues for a greater understanding of cancer and treatments. Innovations in the CRISPR technology are likely to increase its precision, effectiveness, and safety for its future preclinical and clinical applications in cancer. In the next decade or so these technologies will play a pivotal role in identifying new drug targets, and developing dependable therapeutic strategies for several diseases, including cancer.

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Conflict of interest

The authors declare no conflict of interest.

Author contributions

Conceptualization (AB, SD, and SKM), original draft preparation (AB and SD), review and editing (AB, SD and SKM), visualization (AB and SD), supervision (AB, SD, and SKM). All authors have read and agreed to the published version of the manuscript.

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