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Review Article

A Pioneering Genome Editing Tool with Crispr-CAS9 Approach for Application in Cancer Therapy: Future Perspectives and Challenges

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Abstract

Cancer is a rapidly progressive condition characterized by a loss of control over cell division and mortality, DNA alterations, impaired healing process, and other causes. The cancerous cell environment is crucial in disease progression since it contains a variety of immunological cells, both innate and adaptive, that contribute to the onset of the disease. Aside from the medicines that have been discovered, the inadequate medical effects of tumors can be overcome in the future by expanding genetic toolboxes. Immunotherapy has resulted in the modern era of cancer therapy however; it is still in its early stages and must be monitored to avoid problems. Subsequent genomic research and engineering may hasten the development of sophisticated technology. Prior research showed that genetic and mutant genes are directly associated with the incident, development, and medical prospects of cancerous cells. The progress of innovative gene modification tools, particularly the globally recognized clustered regularly interspaced short palindromic repeats (CRISPR-Cas9) platform, showed considerable prospective in overcoming medical constraints. The present study demands to offer an extensive review of potential cancer targets for CRISPR-Cas9 and underscore the clinical evidence supporting its potential application in cancer treatment. Moreover, the findings offer an enlarged perspective of the CRISPR-Cas9 clinical relevance as well as its limits. As a result, the study intends to highlight a prospective and futuristic immunotherapeutic technique in conjunction with established treatment procedures.

Keywords: Cancer immunotherapy; WHO; FDA; EMA; CART; Agrobacterium

Introduction

As of February 2017, the World Health Organization (WHO) released a fact sheet indicating that cancer was a significant cause of global mortality, responsible for 8.8 million deaths in the year 2015. This alarming statistic reveals that approximately one in every six deaths worldwide was directly linked to cancer [1-3]. In

2019, it was anticipated that 140,690 incidences of cancer will be confirmed, with a significant portion of these patients expected to face the ongoing challenges of the disease throughout their lives [4,5]. Cancer is a significant proliferative disease that involves an impairment of development and apoptotic control, DNA disruption, and an improper repairing process. Because it comprises lymphocytes (B and T-cells), a tumor context serves a significant part in disease progression. The substances formed by tumor pro-inflammatory cells are the most critical molecules for developing an association between inflammatory reactions, innate/adaptive immune response, and cancer [6-9].

In 1909, Paul Ehrlich proposed the concept of tumor immunology and developed antibodies that had possible ability to combat cancerous cells [10,11]. In 1950s, Burnet and Thomas proposed autoimmune surveillance concept, arguing that the immune response destroys a risk cells at the primary site before they grow into apparent cancer cells [12]. In 2001, Robert D Schreiber and his colleagues coined the word immunoediting to describe the process by which tumors are determined [13]. Immunotherapy has shown positive response in a variety of clinical studies whereby diverse exogenous manipulated interferons, cytokines, interleukins, and antibodies are used to induce a higher immune reaction than traditional approaches [14]. Globally, various cancer treating therapies, including as radiotherapy, surgery, and chemotherapy are currently recognized and widely used [15-18]. Although, directed therapy, photothermal (PT) and photodynamic therapy (PDT) are all being researched [19].

Given the use of such traditional treatments as bridges, there is a gap in treatment possibilities for patients relapse-free lifespan [20]. So, there is a need for advances in cancer treatment to counteract these limitations. Immunotherapy has grown as a new approaches in the modern scenario, and different kinds of drugs are being developed as illustrated in Figure 1 [21]. Immunotherapy is used in conjunction with conventional drugs or with adjuvants to form neoadjuvant treatments [21], and may assists in avoiding the risk of tumor relapse among individuals with preliminary tumors [22]. Combination chemotherapy used when a number of medicines is given during adjuvant treatment, while hormonal therapy is known to be effective in instances of cellular cancers i.e. breast cancer [23]. Tumor genesis necessitates a number of genes and epigenetic modifications [24,25].

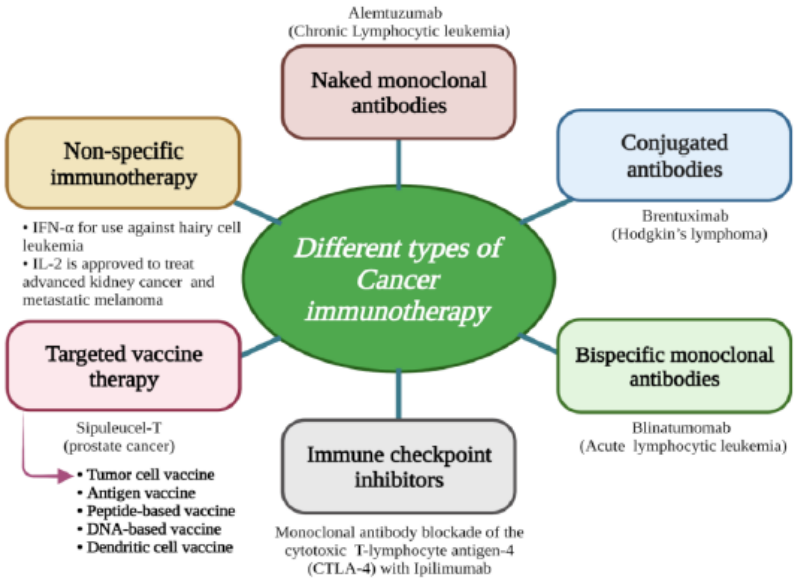

Figure 1: Various immunotherapeutic medicines are employed to treat different types of cancer.

The WHO has forecasted a persistent increase in cancer mortality rates for the foreseeable future. By the year 2030, it is projected that around 13.1 million deaths worldwide will be attributed to cancer [1,26-28]. In 2021, the American Cancer Society (ACS) confirmed about 1.9 million individuals are expected to be screened for melanoma. Tragically, it is anticipated that around 608,570 deaths will be attributed to melanoma during that period [29-31]. Traditional tumor therapy can result in radiation attacks, toxic substances, and other negative responses, which may result in death; consequently we must expand current therapies to address possible cancer response [32,33]. Tumor immunotherapy aims to prevent tumor cell proliferation and infiltration by activating the

immune cells [34]. The immune-mediated checkpoint inhibition (ICI), adaptive cellular therapy (ACT), cancer vaccines, oncolytic virus, dendritic cell (DC), and antibody-drug conjugate (ADC) therapies were all used for treating cancers. Furthermore, the usage of anti-CD19 chimeric antigen receptor (CAR) action proceeds a substantial progress on the cure of lymphatic lesions [35].

The FDA licensed anti-CD19 chimeric antigen receptor (CAR) T-cell therapies to serve treatment of both chronic B-cell-mediated malignancies and carcinomas in 2017, indicating a significant step forward in its curative potential [36,37]. Regina studied the progress of CAR-T therapy, including numerous approaches combining artificial genomes and multimodal ligands to combat antigenic evasion and influence the microenvironment of cancer cells [38,39]. Even so, immune treatment is generally limited for the preclinical phase due to immunosuppressant antagonists and cytokines release syndrome of CAR-T cells [40].

In 2018, James P. Allison was awarded the Nobel Prize in Physiology or Medicine for his pioneering discovery of cytotoxic T-lymphocyte-associated protein (CTLA-4), while Tasuku Honjo received the same prestigious award for his significant contribution in identifying programmed cell death protein 1 (PD-1) or programmed cell death protein ligand 1 (PD-L1) [41- 44]. Immunotherapy offers a variety of drawbacks, the risk of disrupting immunological equilibrium by inducing an allergic reaction that is ineffective against healthy tissue. Common adverse effects of drugs and other disorders include persistent irritation, diarrhoea, and itchy skin [45]. In cancer surveillance, tumor cell-derived chemicals cause reactions by combining receptors on antigen-presenting cells (APCs) or by a unique effector role aimed at removing developing cancerous cells [46]. Tumor-associated antigens (TAAs) assists host immunity in detecting and eliminating malignancies that invade normal tissue [47,48].

Despite conventional therapy, immunotherapy remodels the microenvironment of cancer with chemokines, cytokines, and lymphocytes, which may result in significant effects and prevent relapse [49,50]. For in vitro or in vivo studies, a clinical trial has proved the beneficial effects of tumor therapy. A proper therapy regimen is determined by the origin and stage of the cancer [51,52]. The FDA has approved a wide range of prescribed drugs for use in oncology therapy. Over the past 30 years, Interleukin 2 for kidney cancer, pioneering monoclonal antibodies (mAb) for B-cell tumors, and the prostate cancer vaccination based on DC, CAR-engineered methods for B-cell lymphoma, and PD-L1 inhibitors for cancer have all been recognized to be used to treat cancer [53]. In late-stage clinical trials, researchers continued to evaluate over forty antibodies in melanoma [54]. In 1988, Greg humanity approach contributed to the production of mAbs for the therapeutic use of various cancer [55]. The US FDA and the European Medicines Agency (EMA) obtained two vaccinations for late prostate tumors, Imylgic® (T-VEC) to accelerate cancer formation and Provenge® (Sipuleucel-T) to deliver the GM-CSF coding genes [56].

Currently, The FDA and EMA have authorized over 100 mAbs for therapeutic purpose of cancer and immunological and chronic inflammatory disorders [59,60]. Additionally, the FDA and EMA have both provided approval for a range of checkpoint antagonists, such as ipilimumab, pembrolizumab, nivolumab, atezolizumab, durvalumab, cemiplimab, as well as conventional chemotherapy agents like carboplatin, paclitaxel, and targeted therapy agents like bevacizumab and avelumab [61-63]. For a decade, immunoglobulins targeting immunoinhibitory molecules are frequently prescribed the therapeutic drugs [64]. Several antibodies and small compounds, notably; CD276, CD39, CD73, the A2A, and CD47 all are being tested in clinical studies to block immune regulatory receptors [65]. The US FDA has authorized Nivolumab, Pembrolizumab, and Cemiplimab antibodies as PD-1 agonists [66]. Many cancer treatment combos are now approved by the FDA, notably ipilimumab, pembrolizumab combined T-VEC, ipilimumab coupled with peptide vaccination, nivolumab with a peptide vaccine, pembrolizumab with virus vaccine, and ipilimumab with Sipuleucel-T in prostate cancer cases [58].

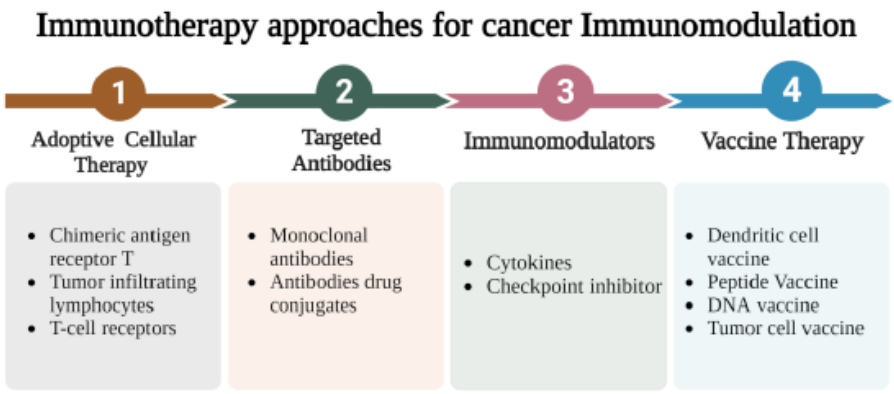

Figure 2: Methods of cancer therapy based on immunotherapy.

Tumor immunotherapy techniques are divided into four categories, as indicated in Figure 2: immune modulation, adoptive cellular treatment, targeted antibodies, and tumor vaccinations [57]. Furthermore, numerous drugs have an effect on immune regulation; the primary process relies on the activation of APCs to T lymphocytes, with the result that cancer cells are destroyed. The cytokines are the most often used as immune stimulation drugs, with many cytokine-based modulators approved for cancer therapy. For instance, Proleukin® (Aldesleukin) is a synthetic interleukin 2 generated by gene recombination for bladder cancer and melanoma treatment [67]. A CTLA-4 agonist Yervoy® (Ipilimumab) designed to suppress the expression of CTLA-4 on T lymphocytes, also employed to treat metastatic melanoma, rheumatoid arthritis (RA), and colitis with ulcers [68]. The Atezolizumab (Tecentriq®), a PD-L1 inhibitory agents used to treat lung and urothelial carcinoma [69].

Further studies for novel checkpoint inhibitors as are currently being planned. In 1-phase cases, antibodies targets to CD47, CD73, A2Ar, and TIM-3 proved to be effective in the solid tumor therapy [70]. Tumor-Infiltrating Lymphocyte (TIL) represents a form of immunotherapy that uses lymphocytes (T cells) found in the malignant tissue targeted, amplified, and re-infused back into the patient's body with a sufficient proportion. Given its obvious potential, the TIL method has a few drawbacks. For example, while T cells proliferate in vitro, this may not be the case in patients. A genetically engineered T cell receptor technique based on peripheral cell lines was developed to solve such issue [71,72]. Both TIL and TCR methods may be employed to target malignant cells exhibiting antigens. The T lymphocytes recognize malignancy that is major histocompatibility complex (MHC) independent in the CAR-T pathway. Example of personalized therapy in action, and the FDA and EMA confirmed the drugs Kymriah® and Yescarta® for myeloma treatment [73].

The mAbs are immunoglobulins (Ig) that usually have two Fab terminals that attaches to targets and an Fc terminus that binds to receptors on the exterior of lymphocyte [74-77]. The finding of antigens specific to tumors raised concerns regarding antibodies. As a result, Rituximab was the first mAb designed to target CD20 activity on B cells in non-Hodgkin lymphoma (NHL). Following this, anti-HER2 Trastuzumab and anti- VEGF Bevacizumab were approved in the therapy of breast cancer, as also anti-EGFR Cetuximab and anti-HER2 Trastuzumab for the cure of colorectal cancer [78,79]. In 2013, an antibody-drug conjugate called Kadcyla is originally authorized as the therapeutic drug of HER2-sensitive advanced cancers [80]. Active therapy has been shown by the use of vaccines in tumor immunotherapy [81]. The first commercial DC based vaccination licensed by the FDA was sipuleucel-T (Provenge) designed for prostatic cancer treatment [82], and Talimogene laherparepvec (T-VEC) vaccine serves for treating cancers [83,84]. Traditional therapy entails either providing a gene whose activity can disrupt the synthesis of tumor-promoting genes or inserting a functional gene to compensate for an inactive gene. As a result, greater genetic locus specificity is necessary to generate superior and more secure outcomes [85,86].

For over 20 years, tumor-suppressing genes, metabolism-related DNA, and markers for chemotherapy and radiation-resistant genes have been identified and altered by a combination of the CRISPR-Cas9 tool to suppress the onset and malignancy progression [87-90]. In addition, immunotherapy has marked the dawn of an era of progress in cancer care, it is effective only in certain types of tumors and is tolerated by a small percentage of cancer patients [91]. Technologies for editing genomes, such as zinc-finger endonucleases (ZFNs), transcription activator-like effector nucleases (TALENs), and the CRISPR-Cas9 approach are utilized to target, knockout, insert, and modify specific genes, aiming to eradicate cancer [92]. The ZFNs are hybridized that contain two dimer subunits (DNA binding and cleavage domain), a highly specialized pair of molecule scissors, and FokI nuclease from Flavobacterium Okanokotes fused into double stranded breaks (DSBs) that could be corrected through mechanism of DNA repairing.

The TALENs repeats produced by Xanthomonas bacteria are made up of a FokI endonuclease that fuses with a variable DNA-binding target to cause DSBs [93-96]. Because of its simplicity, feasibility, and potential diversity, CRISPR-Cas9 approach is preferred for modifying genes. As a result, CRISPR-Cas9 gene editing could offer a novel strategy for cancer immunotherapy [97-99]. Aside from the expanding area of the base and prime editing, the ability to create DSBs at unique and precise loci that affects the efficacy and feasibility of DNA modification [100]. As a result, impaired ends are fixed by either using non-homologous end joining (NHEJ) and homologous recombination repair (HDR) mechanism [97,101]. The current study emphasizes the implementation of CRISPR-associated approaches as a viable means of combating cancer. Moreover, we evaluate present challenges as well as future prospects. However, the potential for negative effects restricts its application in medicine, necessitating an effective ethical evaluation. The biological underpinnings of the CRISPR-cas9 technique are examined in this publication, along with the benefits and drawbacks of cancer research endeavors.

Principal action of the CRISPR-Cas9 method

The discovery of DNA modification approach in E. coli was originally documented in 1987. Subsequently, these distinct repetitive sequences were also observed in the genomes of various microbial and archaeal organisms [102-104]. In 2020, Emmanuelle Charpentier and Jennifer Doudna were awarded the Nobel Prize to honor their significant advances to the CRISPR-Cas9 technology. This prestigious recognition came shortly after the identification of the essential chemical modules of the CRISPR-Cas9 method. Their pioneering research upon DNA modification typically stated to as the genetic scissors, earned them a joint Nobel Award in Physiology and Medicine [104-107]. Until their function was unknown, when the spacer segments were found to comparable sequences found in bacteria, archaeal viruses, and plasmids. If these repeats are matches, the invading exogenous DNA cannot infect bacterial cells, showing that they serve as a bacterial defensive strategy [108]. As shown in Figure 3, the CRISPR-Cas9- mediated immune response occurs in three stages: acquisition, transcription, and interference

[109-111]. Bacteria have developed a method for collecting and integrating viral DNA snippets from invaders into their own genes, ending in the formation of CRISPR arrays. This unique mutation allows bacteria to anticipate viral interactions. In reply to an attack, bacteria use CRISPR array-derived fragments of RNA to execute a targeted attack on the viral genome, providing a defense mechanism against viral infections [112].

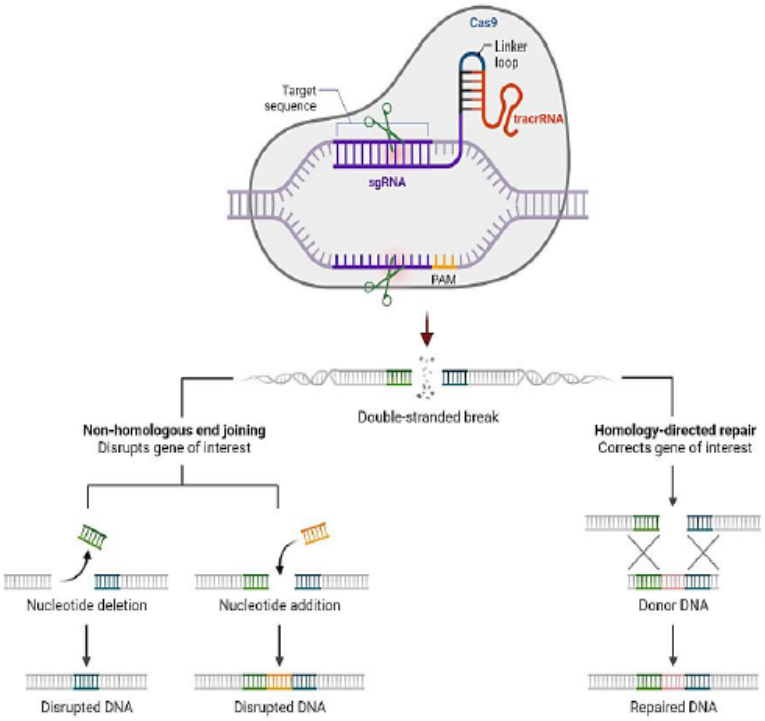

Figure 3: CRISPR-Cas9 mediated gene modification. The Cas9 binds to the sgRNA, resulting in DSBs in the PAM region. The NHEJ and HDR methods are able to repair DNA damage. The NHEJ mechanism inserts and ligates spontaneous insertions and deletions (InDels). The homologous genomic sequence serves as a blueprint to create repaired DNA during the HDR mechanism.

The microorganisms use Cas9 or a related enzyme to trim the genetic region, decreasing the virus survival and adverse activity. The bacterial CRISPR-Cas9 system consists of up of a pair of distinct segments of RNA called the mature RNA (crRNA) and the trans-activating crRNA (tracrRNA) [86,115,116]. This tracrRNA is combined with the crRNA, an active guide RNA (gRNA) is generated. The tracrRNA span contains 3 stem-loop chains and 1 anti-repetitive domain, while the crRNA just has guide and repeat segments. By combining amino acids with the nucleotide targeting region, the Watson and Crick guiding region creates the gRNA and DNA heteroduplex. Notably, the complex structure is directed to identify viral strands by the crRNA spacer segment rather than the tracrRNA component of the guide, with which Cas9 interfaces [115, 117, 118]. Indeed, crRNA and tracrRNAs collaborate for producing the Cas9- RNA protein complex, leading to DSBs in the viral genome. The main advantage is how the gRNA may be adjusted irrespective of the endonuclease, allowing CRISPR to be enhanced as a DNA editing approach with infinite targeted potential and significant precision [119-121]. Despite typically repeated sequences in DNA, nonrepeating sections of genetic material known as spacers from infectious viruses define CRISPR repeat clusters [122,123].

However, spacer repeats and protospacer-adjacent motif (PAM) series chosen by the gRNA are short chains of DNA (typically 2-6 bp) positioned 5′-NGG-3′ below from the targeted sites [124,125], resulting in blunt sequence ended. As seen in Figure 4, DSBs activate cellular repair pathways, most notably NHEJ or HDR repair [126,127]. The PAM binding cleaves the invading phage DNA, creating a DSB and interfering with phage expression and replication. The CRISPR method couples the Cas9 enzyme, with a sgRNA that functions similarly to the crRNA and tracrRNA tandem in microbes. While sgRNA is critical in determining nuclease selectivity and snipping activity [128]. Two nuclease domains are involved in the cleavage: the HNH domain (cleaves the desired strands), and the RuvC domain (divides the contrasting side). The Type-II attract the attention of researchers due to their small level of complexity [129,130]. In 2013, after Cas9 optimized to target human coding and nuclear localization signals were included,

CRISPR-Cas9 was initially employed among mammals and mouse embryonic cells [131,132]. Thus, CRISPR-Cas9 seemed as a novel dynamical technique for modifying genes since by changing only the crRNA pattern and tracrRNA and endonuclease, unaffected. This method avoids the challenging issue of protein production, resulting in affordable and faster. In mammals, DSBs are repaired by NHEJ after Cas9 PAM identification and fragmentation. This might lead to an apparent reading frameshift mutation and a decrease in the yield of the altered chromosomal region [86,133,134].

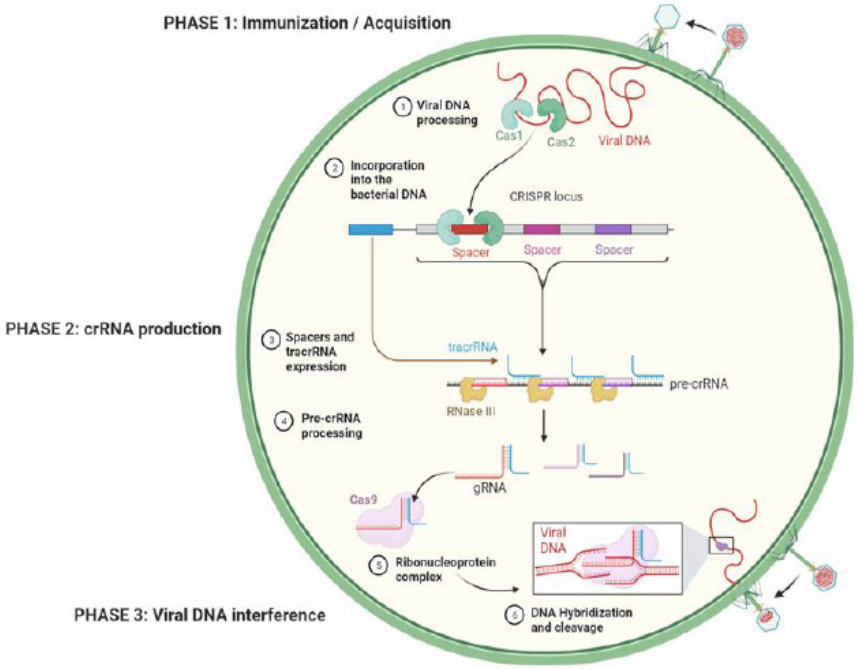

Figure 4: CRISPR/Cas microbial adaptive immunity comprises three phases: acquisition, crRNA production, and viral DNA interference.

Animal and Human biology of CRISPR-Cas9 methods

The CRISPR-Cas9 has enormous potential for treating and diagnosing cancer, involving 1) the adoption of CRISPR-Cas9-based screening tools SHERLOCK and DETECTR for tumor testing, 2) offering TCR knockout (KO) CAR-T cells, 3) KO of antagonistic receptors such as PD-1 and LAG-3 to boost tumor immunotherapy competence, 4) eradication of cancer-causing virus-like HPV, and 5) through eliciting genetic polymorphisms produce in vivo cancer models [139,140]. Moreover, it could be employed for producing and replicating alterations, by screening the growth of tumor formation in model animals [92]. The pups and hogs are included in the group of genome altering breeds for hereditary and medical studies owing to a scarcity of knowledge about embryological stem cells and the HR viability [141]. Over 25 studies are being carried out to look into the dependability and effectiveness of employing CRISPR-Cas9 for treating cancer, with significant advances in the CRISPR concept targeted at overcoming any difficulties that may hamper its clinical application [86]. As shown in Table 1, multiple studies are underway to examine the potency and anticancer effects of CRISPR-Cas9 in various cancers [86,142,143].

Table 1: Overview of cancer research studies involving CRISPR approach.

Pathological state	Identifier	Trails	CRISPR-modified therapeutic drug	References
Acute lymphocyticleukaemia of B lymphocytes	NCT04557436	Ph 1	Allogeneic transplantation of T cells PBLTT52CAR19	[144-146]
CD19⁺ carcinoma andleukaemia	NCT05037669	Ph 1	PACE CART19 allogeneic T cell transplant	[147-149]
Cancers of the intestinaltract	NCT04426669	Ph½	CISH CRISPR TILs transplanted autologously	[86,150]
HIV-infected patients withhema-tological cancers	NCT03164135	N/A	Allogeneic transplant of CRISPR/Cas9 CCR5 gene CD34+poietic progenitor and stem cells	[86,151,152]
Tumor neoplasm causedby the human papillomavirus	NCT03057912	Ph 1	Local direct application of HPV16 E6/E7T1 or CRISPR/Cas9-HPV18 E6/E7T2	[86,153]

Epstein-Barr virus (EBV)-associated malignancies	NCT03044743	Ph½	EBV-CTL self-transplantation of PD-1 deficient cells	[86,154,155]
Lung cancer	NCT02793856	Ph 1	PD-1 knockout T cell autologous transplantation	[156,157]
Renal cell carcinoma	NCT02867332	Ph 1	Autologous infusion of PD-1 mutant T cells	[49,158,159]
Prostate cancer	NCT02867345	Ph 1	Autologous infusion of PD-1 mutant T cells	[92,158,159]
Bladder cancer	NCT02863913	Ph 1	Autologous infusion of PD-1 mutant T cells	[49,160-163]
Hepatocellular carcinoma	NCT04417764	Ph 1	Autologous infusion of PD-1 mutant T cells	[164-166]
Esophageal cancer	NCT03081715	Ph 1	Autologous infusion of PD-1 mutant T cells	[86,143]
$CD19^+$ leukemia andlymphoma	NCT03166878	Ph½	Allogenic transplantation of UCART019	[86]
Leukemia	NCT03398967	Ph½	CAR-T cell allogenic transplants against CD19, CD20, or CD22	[86,167,168]
T lymphocyte or lymphoma with B cells	NCT04502446	Ph 1	Allogenic transplantation of CTX130	[147,169]
B-cell malignancies	NCT04035434	Ph 1	Allogenic transplantation of CTX110	[170]
Solid tumors	NCT03747965	Ph 1	Mesothelin-directed CAR-T cells	[171,172]
Renal cell tumor	NCT04438083	Ph 1	Allogenic transplantation of CTX130	[173,174]
Multiple myeloma	NCT04244656	Ph 1	Allogenic transplantation of CTX120	[175,176]
Solid tumors	NCT03545815	Ph 1	Anti-mesothelin CAR-T cells	[177,178]
Non-Hodgkin lymphoma	NCT04637763	Ph 1	$CD19^+$ relapsed or refractory cancer or anemia	[179,180]
Acute myeloid leukemia	NCT05066165	Ph½	CRISPR/Cas9-engineered autologous WT1-directed TCR Tcells	[181,182]
***Ph** = Phase				

Furthermore, there is significant promise in establishing inherited disorders across the model animals, and model simulation of polymorphism in CRISPR-Cas9 lineage, which could lead to advancements in DNA therapy and renewing healthcare [183]. The invention of tumor-specific toolkits boosts the potency of DNA therapy through the regulation of tumor inhibitor and oncogenic activity [184]. Human DNA editing using CRISPR-associated Cas9 mediators, as well as gene screens and immune-based therapeutic applications in disease biology [185,186]. The mutagenic method also enables for forecasting the markers sensitive to insignificant compounds, such as Schlafen Family Member 11 (SLFN11) that responds to poly-ADP ribose polymerase and serves against lung cancer diagnosis [187]. The study of polymorphism in EGFR-tyrosine antagonists resulted in the discovery of actual variants and the defeat of specific susceptibility [188]. Genetically altered mice got infected with adeno-associated virus vectors containing gRNAs targeted at the p53 and RB1 tumor inhibitor variants in small cell lung cancer (SCLC). The modeled species revealed clinical traits similar to human cancer, mouse genes suppressed by specific changes in P53 and RB1 was used as potential human target therapy [92].

Further, gRNAs were specifically created to target other genes, so as better comprehend their association with the disorder and pathology. Biological luminescence microscopy showed significant luciferase expression in gRNA-107 and 130 cells infected 6 months after tumor formation, indicating their significance in early cancer spread. As such, CRISPR was effective at replicating cancer in animal models and finding variants in novel traits that cause small lung cancer metastases [189]. In addition, GeCKO allowed the discovery of changes and the recognized the collaborative role of potential Tp53 and the KRAS cancer genes in KrasG12D rendered rat early fibroblasts, resulting in improved knowledge of early carcinomas in mice [190]. A human GeCKO library screened 6 potential cisplatin resistance genes for cervical cancerous cells, with identified genes (SULF1, ZNF587B), whose absence elevated cisplatin vulnerability [191]. Another study, scientists examined the FRK tumor gene significant in H1299 lung cancerous cell line, revealing that FRK deletion inhibited epidermal to mesenchyme shift cell growth and cancer formation [192]. The TP53KO cells found for diagnosis drugs and P53 tumor gene [141]. Because of its capacity to alter not only short segments of genes but also large sections in model organisms, the CRISPR-Cas9 strategy can be utilized to produce abnormal chromosomes [193]. Most of the aforementioned cases indicated that CRISPR- Cas9 potency in cancer modeling and in vitro research, as well as its potential applications for gene therapies. Though this strategy is still not widely used, the literature review is likely to increase attention to its worth for health care and may offer greater insight into the molecular etiology of a range of disorders. Thus, Table 1 displays instances of active clinical research including therapeutic drugs with a CRISPR-Cas9 component [86, 194].

Implications of CRISPR-Cas9 in infectious and inherited diseases

During the pathogenic activity, CRISPR-Cas9 has become potential to enhance the release of either DNA or RNA virus from infected cells. CRISPR-Cas9 offers effective genomic treatment for fighting mammalian infections by targeting several stages of the virus lifecycle [140]. To modify infectious viruses, efficient antiviral

approaches based on CRISPR-Cas9 were used, and known to be highly effective in fighting viruses that cause immune deficiency such as HIV, and hepatitis. The findings indicate a promising approach to viral manipulation and offer prospective responses for dealing with viral diseases [195,196]. DNA viruses such as Kaposi's sarcoma herpesvirus (KSHV), Epstein-Barr virus (EBV), human papillomavirus (HPV), hepatitis B virus (HBV), and Simian virus 40 (SV40) are frequently attributed for cancer development. Aside from these DNA viruses, certain RNA viruses, such as human T-lymphotropic virus-1 (HTLV-1) and hepatitis C virus (HCV), have been linked to cancer [197,198]. The CRISPR/Cas9 combined with additional therapies, including the NU7026 P mediator, can effectively eradicate the disease-causing HBV gene [199]. In this context, in vitro ablation of the HPV16-E7 CRISPR-Cas pathway raised cell death and delayed the formation of cervical carcinoma strains while having no consequence against HPV cells. The absence of E7 DNA improved the production of the tumor suppressor protein retinoblastoma, implying that E7 might be targeted for DNA editing methods for treating cervical carcinoma [200].

Furthermore, it was shown that integrating CRISPR-Cas9 with immune-regulating drugs, which are FDA authorized to enhance the cancer treating purposes [201,202]. Combining CRISPR-Cas9 enabled ablation of the HPV16 gene with a PD1 inhibitor enhanced survival rates and tumor progression. Furthermore, medication raised the level of APCs, CD8+ cytotoxic, and CD4+ T helper cells in the cancerous tissue, resulting in strong anticancer effects in mice [140]. CRISPR/Cas9 technology has demonstrated its capability in correcting defective genes associated with various diseases. Examples include the correction of genes like DMD in Duchene muscular dystrophy, CFTR in cystic fibrosis, factor IX in hemophilia B, and several others linked to conditions such as Dementia, Huntington's, and Parkinson's diseases, Tay-Sachs disease, and fragile X syndrome. This breakthrough technology holds immense potential for gene correction and treatment of these disorders [86,91,121,140,203]. Clinical trials are currently evaluating the delivery systems for CRISPR-Cas9 monitored by the transplantation of these cells to enhance their anti-tumor activity in patients. Chira and colleagues have provided comprehensive insights into CRISPR/Cas9 delivery methods in a book section, shedding light on this area of research [86].

According to Mehta, et al. [263], presume that CRISPR-Cas9 has significant inhibitory effects due to virus escape and rapid growth. To conquer such obstacles, the development of an effective, reliable, and widely accepted CRISPR/Cas13a technique is crucial. Recently, CRISPR/Cas13a exhibits the greatest scalable RNA targeted technology for RNA modifications and RNA virus targeting among the three Cas13 protein families [264]. The Cas13a protein are used for nucleic acid identification, RNA knockdown, and transcript tracking in human and plant cells [265]. By using basic [266] and multiplexed gene editing [267], CRISPR has raised plant disease resistance and also many other attributes such as yield [268], quality of crop [269] tolerance to biotic and abiotic stressors, and sperm sterility. Particularly, many multiplexed genome editing tools may alter many genes at the identical time are now available [270]. The CRISPR may offer remedies for COVID-19 patients alongside to having diagnostic significance. For SARS-CoV-2 DETECTR, a CRISPR Cas12-based test for the detection of coronavirus was recently developed.

The VI-D CRISPR-Cas13d variant derived from Ruminococcus flavefaciens was chosen for its tiny size, allowing for facile packaging in viral carriers, useful sensitivity, and robust catalytic action in human cells [113,271]. Despite the fact that SARS-CoV-2 has an extremely high mutation and recombination rate, this technique evolved to concurrently target multiple loci for RNA depletion, paving the door for an essential pan-coronavirus targeting approach. With this advancement, the CRISPR-Cas system might be re-implemented to serve its initial intent as a virus-fighting action to aid in the the outbreak response [272, 273].

Future Perspective

Over decades, CRISPR-Cas9 genetic modification was successfully entered the preclinical and clinical phases as a disease therapeutic strategy. As gene-editing methods evolved and new drug for diseases have been uncovered, clinical translation and practical research in the genomic area has grown. CRISPR-Cas9 is known to be highly effective not only in insects and plant life, but also in animals and human beings, and it has a promising future in cancer biology since it is a versatile, easy, convenient, and efficient technique. The method brings a novel approach to cancer treatment by enabling previously unattainable genomic modifications in target cells. A particular gene variation raised cancer migration, invasion, and angiogenesis, which may be dealt with through genetic modifications. The CRISPR-Cas system is now being used in vivo to combat cancer and immunological problems. Research studies to validate the effects of CRISPR- Cas9 are underway, with promising results. There were investigations with just a few of patients and minimal afterward, and further in-vivo studies are anticipated.

Meanwhile, continuous surveillance is required to validate the effects and identify any unanticipated problems. Expansion and refinement of Cas9-based modification may accelerate research toward medicinal uses and provide a number of therapy options for a variety of diseases, including cancer. Regardless of how CRISPR/Cas9 is employed, it shows promise for gene mutation-related cancer therapy; nevertheless, challenges such as off-target and ethics must be addressed. Scientists are bound to follow the global consensus and strive to positively develop society through technology.

Researchers have effectively created Cas9 variations that are specifically targeted to minimize off-target effects (OTEs) while keeping high editing efficacy. According to the theory, the high affinity between Cas9 nuclease and the specific DNA could contribute to OTEs. By adding mutations to four critical residues responsible for direct hydrogen linkage between Cas9 and the phosphate core of the target DNA, the SpCas9-HF1 showed no detectable off-target activity when compared to wild-type SpCas9. These Cas9 variant production advances have the prospective to improve the accuracy and security of CRISPR-based genetic

modification. The Cas9 enzyme has the ability to help manage the off-target features and undesired side effects of the CRISPR system. Researchers should look into a number of physical or chemical substances, such as tetracycline and doxycycline, as a potential method for developing Cas9 expression. Furthermore, a variety of online tools are accessible to assist researchers in creating sgRNA with low off-target effects in order to increase selectivity for human gene modification.

Currently, a few CRISPR-mediated gene modifications, such as shorter sgRNA, Cas9 nickase, and Fok1- dCas9 interaction, have shown promise in reducing off-target effects. The Cas9 massive size poses packing challenges, particularly in low immunogenic AAV vectors, which are commonly used in gene delivery. According to a new study, the extended attachment of Cas9 nuclease to DSBs restricts repair protein access to the target location, lowering repair efficiency. Nonetheless, the translocating RNA polymerase might be used to separate the template-bound Cas9 only if the DSBs are found in a precise direction by the RNA polymerase.

Furthermore, using the current CRISPR/Cas9 technology, some carcinogenic genomic variations, such as chromothripsis, aneuploidy, and LINE-1-mediated genome modification, remain difficult to cure. However, the mutagenesis efficiency of CRISPR/Cas9 should be boosted in the near future by creating more potent Cas9 and devising effective delivery systems with dominant sgRNA. In model organisms and humans, the CRISPR/Cas9 technology allows for precise editing of a target sequence for therapeutic research. It is also potentially possible to use it to treat viral, inherited, and malignant conditions. Recent research has shown that Cas9 ribonucleoproteins (RNPs) are increasingly used as a viable alternative to plasmid vectors for delivering the CRISPR reagent into target cells. This method offers notable advantages, including improved efficiency, a shorter duration of Cas9 activity, and the elimination of vector sequence integration. However, it is important to note that this delivery approach does not have a direct effect on addressing chromosomal rearrangements. While Cas9 RNPs provide benefits in terms of delivery and reducing integration concerns, addressing chromosomal rearrangements may require additional strategies in the context of CRISPR-based applications.

Concluding Remarks

It is concluded that new techniques for overcoming the gaps surrounding the grey areas in Immunotherapy can be developed with the intervention of cost-effective and promising therapies. Furthermore, as tactics and technologies evolve, immune cells can lead to the creation of cost-effective immunotherapeutic treatments, which can then be used to generate personalized medicine based on patients' tumor immune profiles. Despite the fact that adjuvant therapy and other immunizations are effective for the therapy of metastatic tumors, there is still a big opportunity to develop vaccines with minimal side effects. Aside from the traditional treatments of surgery, radiation, and chemotherapy, cancer immunotherapies are predicted to emerge as one of the most effective therapeutic choices available. This has also fueled conventional ways to boost the chance of long-term tumor decrease in cancer patients, leading to significant therapy alternatives.

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