International Journal of Health Sciences and Research
[Indexed, Peer-Reviewed Journal]
Volume 15; Issue: 7; July 2025
Website: www.ijhsr.org
Review Article *ISSN: 2249-9571*

Review of Current Applications of CRISPR Gene Editing Techniques in Breast Cancer Therapeutics Development

Yichen Zhang

Polygence Research Program, San Jose, USA.

DOI: https://doi.org/10.52403/ijhsr.20250745

ABSTRACT

CRISPR/Cas9 gene-editing technology has revolutionized biomedical research by enabling precise, efficient, and cost-effective genome manipulation. This paper focuses on the application of CRISPR/Cas9 in cancer research, with an emphasis on triple-negative breast cancer (TNBC), a particularly aggressive and treatment-resistant subtype of breast cancer that lacks expression of estrogen, progesterone, and HER2 receptors. Breast cancer is the most commonly diagnosed cancer among women worldwide, and TNBC represents one of its most challenging forms due to the absence of effective targeted therapies. While hormone receptor-positive and HER2-positive subtypes benefit from endocrine and targeted treatments, TNBC patients are often limited to chemotherapy, which is associated with high toxicity and limited efficacy. This review synthesizes recent CRISPR-based studies that identify oncogenes and tumor suppressors relevant to TNBC, explores advanced delivery systems such as non-viral nanoparticles, and evaluates the therapeutic promise of this technology. Despite current limitations—including off-target effects, delivery challenges, and ethical concerns—CRISPR/Cas9 offers a promising path toward developing personalized, gene-targeted treatments for TNBC and other hard-to-treat cancers.

Keywords: CRISPR/Cas9, gene editing, breast cancer, triple negative breast cancer

INTRODUCTION

Discovery of Clustered Interspaced Palindromic Repeats/CRISPR-associated protein 9 (CRISPR-Cas9)

Clustered Interspaced Palindromic Repeats/CRISPR-associated protein 9(CRISPR/Cas9) is a revolutionary gene-editing technology in biomedical research [1]. CRISPR/Cas9 system enables researchers to make permanent gene edits or modulate gene expression efficiently, cost-effectively, and precisely [1]. CRISPR/Cas9 was originally discovered in a bacterial genome where it protected against invading viruses [1]. The CRISPR/Cas9 system works in three major steps: recognition, cleavage, and repair [2]. First, a single guided RNA (sgRNA) directs the Cas9 endonuclease to recognize the target sequence by utilizing a 5'-CRISPR RNA (crRNA) complementary base pair component [2]. Then, the Cas9 nuclease makes a double-stranded break (DSB) at a site 3 base pairs upstream of the protospacer adjacent motif (PAM) [2]. Finally, host cellular machinery, like non-homologous end joining (NHEJ) and homology-directed repair (HDR), repairs the DSB [2] (Figure 1).

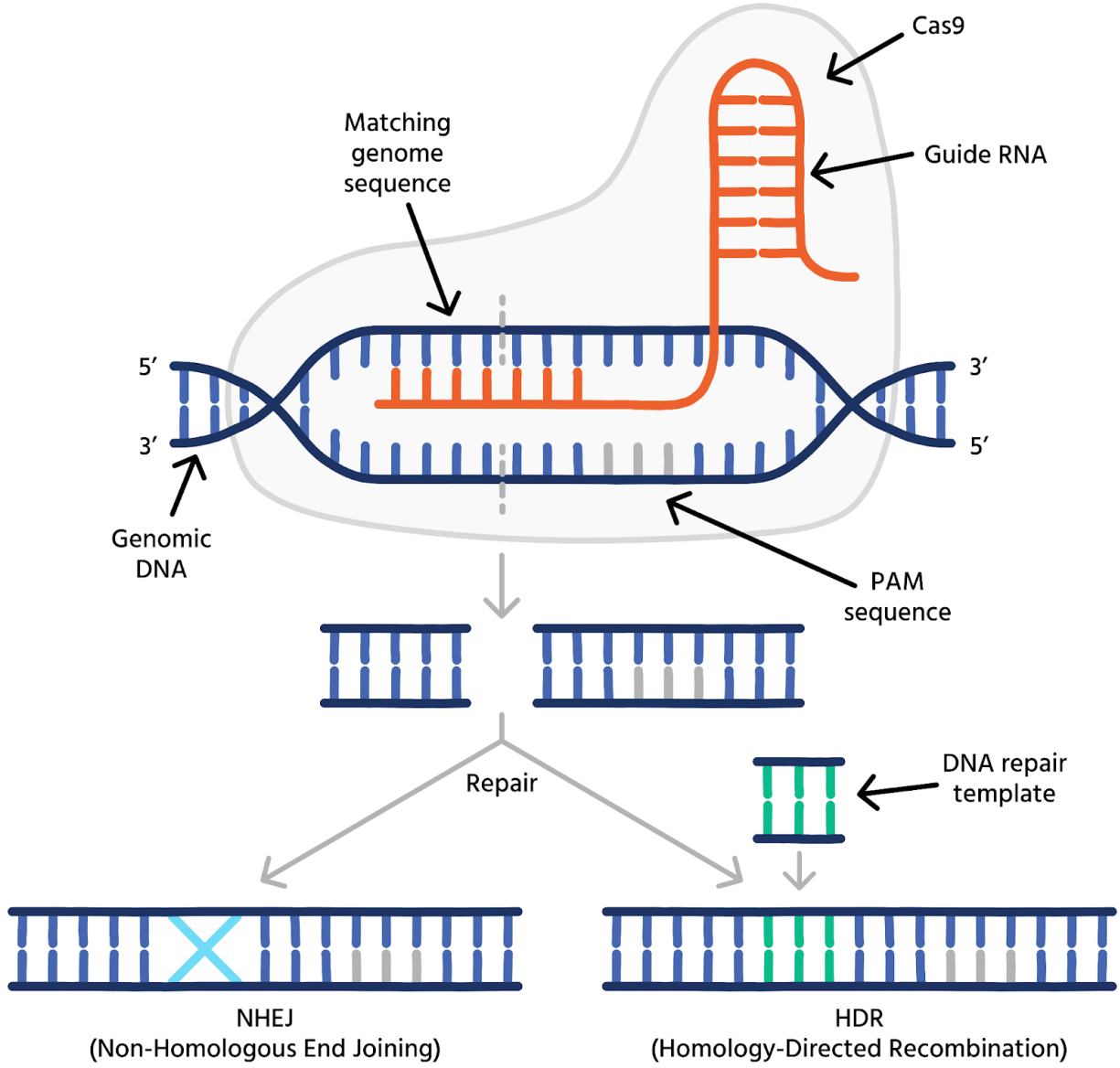

Figure 1. CRISPR/Cas9 gene editing mechanism. An overall schematic of CRISPR/Cas9 gene editing. Once a double-stranded DNA break (dsDNA break) is introduced by the CRISPR/Cas9 system, cells repair the dsDNA break by the homologous-directed repair (HDR) mechanism. [39]

Since its discovery, the CRISPR/Cas9 system has been widely used in various laboratory applications, including rapid generation of cellular and animal models, functional genomic screens, and live imaging of the cellular genome [1]. Before the discovery of CRISPR/Cas9, researchers used a variety of genome editing technologies, such as meganucleases, zinc-finger nucleases (ZFNs), and transcription activator-like effector nucleases (TALENS) that directly target DNA or RNA interference (RNAi) technologies that target RNA [3]. These technologies were limited due to incomplete gene silencing, off-target effects, and variability in efficiency [3]. RNAi, especially, relies on the selective degradation of mRNA, which is inefficient and inconsistent, leading to unreliable results [3]. Additionally, RNAi has higher off-target effects, an unintended targeting of genes other than the gene of interest [3].

In contrast, CRISPR/Cas9 provides more precise, efficient, and robust genome editing capabilities [4]. CRISPR/Cas9 can completely knock out genes by implementing double-stranded breaks in the DNA, allowing for a clearer functional analysis of genes in the genome [4]. CRISPR/Cas9 helped to create genome-

wide libraries using sgRNAs, which can help facilitate rapid and large-scale screenings for gene function, targets, and interactions, enhancing genomic research [4].

CRISPR/Cas9 gene editing technology is widely implemented in translational research on single-gene disorders, viral infections, and cancers [7]. For example, gene editing/gene therapy has been developed to treat HIV disease [7]. The two strategies for HIV treatment, targeting the provirus and targeting host genes that are crucial for the entrance of viruses into cells, are achieved by CRISPR/Cas9 techniques [7]. CRISPR/Cas9 was successfully employed to induce indels in the CCR5 gene (CCR5-null blood cells are resistant to HIV-1 entry) in donor-derived hematopoietic stem and progenitor cells (HSPCs), making it so that engineered HSPCs and allogeneic (taken from different individuals of the same species) transplantation in a patient with HIV infection could be considered a potential strategy for HIV infection and acute lymphoblastic leukemia [7]. CRISPR/Cas9 has also been utilized to develop gene therapies where we can repair defective DNA in mouse models of genetic disorders, like Duchenne muscular dystrophy [5]. Zuccaro et al. showed specific chromosome content could be manipulated using the CRISPR/Cas9 system in donor sperm models, suggesting its utility in gene correction in embryos. For example, a study used CRISPR/Cas9 to snip a mutation that causes hereditary blindness in a gene called EYS, and the results showed that some cells were able to repair the DNA, but half the embryos were unable to handle the break [6]. Regarding cancer research, CRISPR can be used to investigate the molecular mechanisms of tumor initiation, progression, and metastasis by efficiently mutating the gene(s) of interest. For example, Mao et al. knocked out the EZH2 gene using CRISPR/Cas9 and found that the knockout of the gene suppresses the proliferation and migration of triple-negative breast cancer cells, thereby uncovering potential therapeutic targets [8]. CRISPR-based therapies are also being developed to treat blood cancers such as leukemia and lymphoma [7]. CRISPR therapy was used in a non-small cell lung cancer (NSCLC) patient in 2016 who was injected with PD-1 altered T cells. Many other trials using CRISPR-based immunotherapies are ongoing to treat cancer [9].

An Overview of Breast Cancer in the United States

Breast cancer is the most common type of cancer in women globally [10]. In 2022, approximately 2.3 million individuals were newly diagnosed with breast cancer worldwide [40]. It is a highly heterogeneous cancer with distinct subtypes that can be further sub-classified based on the immunohistochemical expression of hormone receptors (HR): estrogen receptor-positive (ER+), progesterone receptor positive (PR+), human epidermal growth factor receptor positive (HER2+), and triple-negative (TNBC) which is characterized by the lack of expression of the above receptors [10]. The standard of care globally is surgical resection of breast cancer mass followed by radiotherapy (RT) or chemotherapy, as it can reduce the locoregional recurrence rates (LRR), which refers to cancer coming back at the same area it started or in nearby tissues and lymph nodes, and distant metastasis rates for the various types of cancers [11]. Additional treatments for breast cancer are more targeted, like hormone/endocrine therapy, targeted therapy, and immunotherapies [11].

Hormone therapy, otherwise known as endocrine therapy, is proven to improve the survival of patients with HR-positive breast cancer, such as ER+, PR+, and HER2+ subtypes [12]. It includes the use of antiestrogens and aromatase inhibitors, which are mainly used for ER-positive breast cancers [12]. Because ER-positive breast cancers are also PR-positive, treatments for ER can also be extended to

treat ER+/PR+ breast cancer [12]. However, long-term estrogen stimulation can have side effects, including osteoporosis, hot flashes, and muscle cramps. Additionally, they can have antagonist reactions to hormone treatment [12]. Targeted therapies target specific genes and molecules in breast cancer tumors to stop their progression and have been shown to significantly increase overall survival in patients who are resistant to endocrine therapy and are more effective than chemotherapy [12]. Immunotherapies are specifically used to treat TNBC as it lacks expression of ER, PR, and HER2, so TNBC patients do not benefit from endocrine therapy or HER2-targeted therapy [12]. Immunotherapy is a strategy for destroying abnormal cells using the patient's immune system to suppress tumor growth, and its treatments include immune checkpoint inhibitors, T-cell transfer therapy, monoclonal antibodies, treatment vaccines, and immune system modulators [12]. For breast cancer, doctors may also combine treatments to target multiple pathways in breast cancer tumors [12].

This kind of classification is needed to categorize patients who may benefit from targeted therapies, like hormone therapy and anti-HER2 therapy [10]. The major subtypes of breast cancers based on hormone receptor markers are Luminal types (A and B), HER2-positive types, and triple-negative breast cancer (TNBC) types [10]. Luminal-type tumors are the most common subtypes of breast cancer, with luminal A being the majority [13]. Luminal-type tumors express hormone receptors and can be subclassified into luminal A and luminal B [13]. Luminal A and luminal B tumors represent the ER-positive, PR-positive, HER2-negative and the ER-positive, PR-positive, HER2-positive tumors, respectively [13]. However, this is inaccurate because only a subset of luminal B tumors are HER2 positive [13]. Luminal tumors respond well to hormone therapy but poorly to conventional chemotherapy [13]. Because luminal A tumors have low recurrence scores, meaning that the chance of this cancer coming back is low and that chemotherapy may not be worth it, endocrine therapy can adequately treat them [13]. In contrast, luminal B tumors, with high Recurrence Scores (more proliferative), may benefit from the combined therapeutic strategy of chemotherapy and hormonal treatment [13]. Next, HER2 over-expression tumors refer to those with ER-, PR-, and HER2+ subgroups [13]. Even though they do have a poor prognosis, they are more sensitive to anthracycline and taxane-based neoadjuvant chemotherapy than luminal breast tumors [13]. The poor prognosis of this subtype derives from a higher risk of early relapse among those without complete eradication of tumor cells, and cancers of this subtype derive the most benefit from improvements in chemotherapy [13]. For HER2 overexpressing cancers, molecularly targeted agents like the anti-HER2 monoclonal antibody, trastuzumab, are available and are effective [13]. The basal subtype comprises ER-, PR-, and HER2- (triple negative) tumors with expression profiles mimicking that of the basal epithelial cells of other parts of the body and normal breast myoepithelial cells [13]. These tumors follow an aggressive clinical course and currently lack any form of standard targeted systemic therapy [13]. These aggressive tumors are not amenable to conventional targeted breast cancer therapies, leaving chemotherapy the only option as a therapy [13].

Although the therapies described above do work well for their respective subtypes of breast cancer, a problem that arises with these treatments is that they do not achieve adequate results when it comes to the TNBC subtype of breast cancer. CRISPR-Cas9 has great potential in discovering new anticancer therapies for TNBC. As of now, we do not have FDA-approved CRISPR-based anticancer therapies for solid tumors [14]. However, cancer researchers have adapted CRISPR-Cas9 techniques to research TNBC cells in determining the functions of various genes by knocking them out and identifying potential breast

cancer therapies [14]. For example, CRISPR is being used to genetically engineer Chimeric Antigen Receptor (CAR)-T cells for liquid tumors, also known as hematologic malignancies [14]. However, we have not yet shown the efficacy of CRISPR-based anticancer therapies for solid tumors [14].

MATERIALS & METHODS

To conduct a comprehensive literature review, relevant keywords were searched on our topic, including "breast cancer" and "CRISPR-Cas9" on the PubMed database, along with a filter to search for articles from the past ten years, and 706 articles from 2015 to 2025 were identified 706 articles. The selection of articles to review was narrowed down by excluding previously published review articles and including primary literature articles focusing on the utility of CRISPR/Cas9 in breast cancer research or optimization of CRISPR-Cas9 delivery systems. Additionally, Google Scholar was queried using the same keywords, but also added the keywords "colon cancer" and "GI cancer" to widen the search to see if CRISPR has been used in the research of other cancers, as well. In the end, we narrowed the total selection to 18 peer-reviewed manuscripts. Findings were summarized from the 18 peer-reviewed articles we selected in the results section.

RESULT

Current status of CRISPR/Cas9 use in triple-negative breast cancers (TNBC)

In regard to gene editing, CRISPR/Cas9 has been used widely in cancer research, especially in TNBC or Basal-like breast cancers, to find genes that could be potential targets for gene therapy, and usually, this is done through the knocking out of genes to determine their functions in cancer cells. Many oncogenes, mutated genes that can cause cancer, of breast cancer have been found using this method. EZH2, NOX4, CDH1, LINC0051, and CDK4 are all oncogenes found in breast cancers, with EZH2 being found in TNBC [8, 15-18]. To list a few, Mao et al. found that the knockout of EZH2 suppresses the proliferation and migration of TNBC cells in vitro, where its expression was decreased, and in vivo, where tumor growth decreased [8]. Al-Mulhim et al. found that targeting CDH1 using CRISPR/Cas9 activation plasmid and CDK11 using CRISPR/Cas9 knockout plasmid helped in controlling cancer progression, invasion, and metastasis in rats [15]. Ahmed et al. found that knocking out CDK4 in cells using CRISPR/Cas9 resulted in those cells having a decrease in cellular viability and a decreased ability to proliferate and migrate [16]. Azadbakht et al. used CRISPR/Cas9 to knock out LncRNA LINC00511, which suppressed malignant cell proliferation and invasion in vitro and increased apoptosis of tumor cells [17]. Javadi et al. utilized CRISPR/Cas9 techniques to knock out the NOX4 gene and found that it increased apoptosis rates in cancer cells and invoked the breast cancer cancer stem cell (CSC) trait [18]. Additionally, some tumor suppressor genes and other genes for breast cancer were found through his process as well. OBSCN is a tumor suppressor gene for breast cancer, and Guardia et al. found that by using CRISPR to activate the OBSCN promoter and the OBSCN-AS1 promoter, OBSCN and OBSCN-AS1 were both upregulated, which in turn suppresses breast cancer cell migration and invasion for TNBC [19]. They also found that OBSCN restoration suppresses breast cancer metastasis in vivo in TNBC as well [19]. Behbahani et al. used CRISPR/Cas9 to knock out OPN, a gene in breast cancer, which resulted in cells not being able to survive when they are irradiated [20]. They found that knocking out the gene and then giving radiation therapy gave better results in the MDA-MB-231 breast cancer cell line [20]. Furthermore, CRISPR isn't limited only to breast cancer research; it has also been used to knock out genes in GI and colon cancers [21-23]. These findings highlight the versatility of CRISPR/Cas9 in

identifying both oncogenes and tumor suppressors critical to breast cancer progression, especially in aggressive subtypes like TNBC. Overall, CRISPR-based gene editing holds great promise for uncovering therapeutic targets and enhancing treatment strategies across various cancer types.

Recent advances in CRISPR/Cas9 anticancer therapy delivery methods

Currently, a major limitation in using CRISPR as a cancer therapeutic is the targeted and efficient delivery of CRISPR to tumor cells in vivo [14]. For the CRISPR-Cas9 technique to be utilized as a therapy, the delivery of CRISPR needs to be optimized in vitro and then in vivo [14]. Current CRISPR system delivery methods can be divided into three main categories: physical delivery, viral delivery, and non-viral delivery [14]. Physical methods, such as microinjection (direct injection of CRISPR components into cells), electroporation (using electric pulses to open cell membranes), and hydrodynamic tail vein injection (high-pressure injection into veins), offer high efficiency but can cause cell damage, nonspecific effects, or operational challenges [14]. Viral vectors, like lentiviruses, adenoviruses, and adeno-associated viruses, deliver CRISPR components using engineered viruses, ensuring high editing efficiency but risking immune responses, oncogenic effects, and insertional mutations [14]. Non-viral methods, such as lipid-based nanoparticles, polymers, and cell-penetrating peptides, are safer and cost-effective but can face rapid clearance in vivo and require complex preparation processes [14]. Next, non-viral CRISPR vehicles include biosynthetic nanobubbles, DNA nanoparticle complexes or DNA nanosystems, nanoparticles derived from synthetic zwitterionic ionizable phospholipids, microneedles, and biomimetic nanocarriers [14]. Out of the three, the non-viral CRISPR delivery methods seem to be preferable to the others in vitro settings due to their high delivery and editing efficiencies, low cost, and safety profile [14].

Extensive research has been done on designing biosynthetic nanobubbles, which are nanometer-sized bubbles with proteinaceous shells that are like cell membranes, as a CRISPR delivery method. Gao et al. showed that the gene vectors (GVs) mediated gene transfection approach to deliver the CRISPR/Cas9 system for gene editing applications successfully edited the Cdh2 gene in the 4T1 cancer cells and in vivo, which reduced invasion and metastasis behavior [24].

Song et al. created a DNA/Upconversion nanoparticle complex, or a DNA/UCNP complex, which is a structure where a DNA molecule is attached to the surface of an upconversion nanoparticle to load Cas9 RNP, PP, and hemin [25]. They found that this helped overcome the hypoxia-associated photodynamic therapy (PDT) resistance, which consequently elevated reactive oxygen species (ROS) production, weakened the antioxidant mechanism of cancer cells, and disrupted ROS elimination through the disruption of the Nrf2 gene [25]. Promoting ROS elimination led to cell apoptosis [25]. The high gene editing efficiency and efficient PDT were achieved both in vitro and in vivo [25]. Also, Li et al. developed a proton-activatable DNA-based nanosystem, which is a nanostructured delivery system made from DNA that is designed to release its cargo (like a therapeutic molecule) only when exposed to acidic conditions (high proton concentration), typically found within tumors, allowing targeted delivery of drugs to specific sites in the body, to efficiently deliver Cas9/sgRNA RNP and DNAzyme for combined gene therapy [26]. They found that the biocompatibility and gene therapy effect were achieved, suggesting that the DNA-based nanosystem can be used to deliver other gene editing tools [26].

Moitra et al. derived a nanoparticle from synthetic zwitterionic ionizable phospholipids which are tiny particles, typically used for drug delivery, composed

of specially designed phospholipids that have a "zwitterionic" charge, meaning they possess both positive and negative charges within their molecular structure, and are also "ionizable," meaning their charge can change depending on the pH environment, allowing them to effectively encapsulate and deliver molecules like nucleic acids that were developed to target the POXC1 gene [27]. There was a reduction in tumor volume when injected with non-targeted and targeted CRISPR DNA-loaded nanoparticles [27].

Additionally, Huang et al. created a nanocarrier using cancer cell membranes which are tiny particles designed to deliver drugs directly to cancer cells by coating the nanoparticle surface with a membrane derived from cancer cells, allowing it to mimic the cancer cell's natural properties and effectively target tumors while evading the immune system to deliver photothermal agents and CRISPR/Cas9 for photothermal/gene therapy (PPT/GT) [28]. They found that it significantly enhanced therapeutic efficacy through photothermal therapy (PTT) treatment and gene editing of survivin-mediated downregulation of HSP70 [28]. Furthermore, Wang et al. created microneedles, which are tiny needles used to deliver drugs, that are used to deliver photosensitizer that presents favorable gene editing efficiency and photo properties [29]. They demonstrated that tumor eradication and gradual normalization of white blood cell levels were achieved in some mice [29]. In addition, lung metastasis was significantly suppressed [29]. Nanoparticles were implemented in an experiment done by Wang et al. where gRNAs specific to legumain (AEP) were designed [30]. AEP knockdown by co-delivered Cas9 mRNA/gRNA by lipid nanoparticles (LNP) in vitro compromised autophagic and lysosomal degradation and impaired cancer cell survival, migration, and invasion [30]. Guo et al. have started to use CRISPR/Cas9 drug delivery methods in TNBC research, where non-cationic, deformable, and TNBC-specific tNLGs were designed to deliver CRISPR plasmids in vivo [31]. They successfully suppressed the Lcn2 gene (breast cancer oncogene) and attenuated TNBC tumor growth (77%). CRISPR/Cas9-mediated therapy seems promising in in vitro settings, but more work still needs to be done [31]. These innovative delivery strategies demonstrate the potential of non-viral CRISPR systems to overcome current barriers in gene editing for cancer therapy. However, despite promising in vitro and in vivo results, further optimization and clinical translation remain necessary for widespread therapeutic use.

Current utility of CRISPR/Cas9 anticancer therapy in non-breast tumors

CRISPR/Cas9 has not only aided in TNBC research, but it has also been applied in gastrointestinal cancers as well. CRISPR/Cas9 has been used in GI cancer research to identify potential target genes that can cause cancer tumors to progress. D'Antonio et al. found that the deletion of Interleukin-30 (IL-30) in colorectal cancer stem cells (CR-CSCs) using CRISPR/Cas9 prevents or delays tumor onset, reduces lung metastases, and prolongs survival [21]. Zhang et al. found that knocking out the PDEF gene using CRISPR/Cas9 lowered the ability of migration, invasiveness, and proliferative capacity of cell lines [22]. This shows that the PDEF gene may play an important role in gastrointestinal cancers [22]. Li et al. successfully knocked out the CD133 gene using CRISPR/Cas9 and found that there was decreased cell migration and invasion in colon cancer cells [23]. Additionally, CRISPR/Cas9 methods of therapy have been applied to blood malignancies. Hu et al. introduced CTA101, a CRISPR-Cas9-engineered, dual-targeted CD19/CD22 CAR-T therapy designed for relapsed or refractory acute lymphoblastic leukemia (r/r ALL) [32]. It overcomes the limitations of autologous CAR-T cells (CAR-T cells belonging to the same individual) by offering an off-the-shelf solution with high gene-editing efficiency,

safety, and potent antileukemia activity [32]. A Phase I trial demonstrated an 83.3% response rate and a manageable safety profile, highlighting its potential as a universal, cost-effective therapy [32]. These findings illustrate the broad applicability of CRISPR/Cas9 across various cancer types, from gastrointestinal tumors to blood malignancies, offering promising avenues for targeted gene therapies and innovative treatment strategies.

DISCUSSION

CRISPR/Cas9 is a groundbreaking gene-editing tool that has transformed our ability to study and modify DNA. Unlike older methods like RNA interference or zinc-finger nucleases, CRISPR is faster, more precise, and easier to use [3]. It works by using a guide RNA to direct the Cas9 enzyme to a specific DNA sequence, where it cuts the strand, allowing scientists to disable, repair, or insert genes [2]. Originally discovered as a bacterial immune system, CRISPR has become essential for studying genetic diseases like Duchenne muscular dystrophy [5]. Cancer research scientists have been utilizing CRISPR/Cas9 to study the roles of specific genes in tumor progression, resistance, and metastasis. For example, Mao et al. and Ahmed et al. showed that EZH2 and CDK4 play a critical role in TNBC tumorigenesis and suggested that these two genes have the potential to be targeted to treat TNBC, a breast cancer subtype that lacks effective targeted therapies [8, 16].

The application of CRISPR/Cas9 technology in treating TNBC holds tremendous promise, but the field is still in its early stages. CRISPR/Cas9-mediated therapies have demonstrated better anticancer efficacy in vitro than conventional treatments such as chemotherapy and radiation [33]. For instance, studies suggest that CRISPR can enhance the genetic engineering of CAR-T cells, boosting their ability to target and destroy cancer cells [34, 35]. Additionally, the potential to edit T-cells using CRISPR opens new avenues for treating TNBC more effectively [34, 35]. While these findings are encouraging, they have primarily been validated in other cancer types or generalized cancer models, leaving a gap in specific TNBC-focused research. Additionally, as of now, no CRISPR-based products for TNBC have entered clinical trials.

This lack of clinical advancement is likely due to several key barriers. Despite promising in vitro results, significant challenges remain in translating these findings into clinical settings. One major limitation is the absence of a universal target gene for TNBC. Each study identifies genes as potential targets, such as CDK7, CXCR4, CXCR7, EGFR, FOSL1, FOXC1, MYC, and SOX9. While these genes are implicated in TNBC progression and metastasis, the heterogeneity of the disease complicates efforts to pinpoint a singular therapeutic target. This complexity underscores the need for more research to identify actionable targets tailored to individual TNBC subtypes.

Going from bench to bedside for CRISPR/Cas9 therapies faces numerous obstacles. One significant concern is the potential for unintended off-target mutations, which could lead to adverse effects, including oncogenesis [36]. Strategies to improve the specificity and precision of CRISPR editing are essential to mitigate these risks. In addition, delivering CRISPR effectively to living cells remains a major challenge [36]. While lab methods like microinjection are precise, they aren't practical for patients [37]. Viral vectors offer a solution but come with risks such as immune responses [37]. Non-viral delivery systems, such as nanoparticles and biosynthetic nanobubbles, are showing promise due to their safety and flexibility [37]. However, immune reactions to foreign delivery materials, such as viral vectors or nanoparticles, could hinder the efficacy and safety of these therapies [37]. The manufacturing process for CRISPR-edited cells is also a significant barrier. Producing

these therapies at a clinical scale is both complex and costly, with projected expenses limiting widespread adoption [38]. Ethical concerns further complicate the landscape, as CRISPR editing could cause chromosomal alterations, such as irreparable double-stranded breaks or acentric chromosome fragments. These risks demand robust regulatory frameworks and ethical guidelines to ensure patient safety.

One of the notable limitations of CRISPR-based cancer therapies is their efficacy in treating solid tumors like TNBC, as opposed to non-solid tumors, where the technology has shown more consistent success. Solid tumors present unique challenges, such as a heterogeneous tumor microenvironment and difficulty in delivering therapeutic agents effectively. Addressing these issues will require innovative delivery mechanisms, such as targeted nanoparticles or enhanced viral vectors, to ensure efficient and precise delivery to tumor sites. Efforts should focus on collaborative research to identify TNBC-specific gene targets and refine delivery systems. Additionally, addressing cost barriers through scalable manufacturing processes and fostering global collaborations can help make these therapies more accessible. Ethical considerations must also be central to the development process, ensuring that the benefits of CRISPR therapies outweigh the risks.

Despite the known challenges, CRISPR/Cas9-based treatments have demonstrated considerable promise for cancer therapy in both preclinical and early clinical studies. For instance, preliminary trials have shown that CRISPR can be used safely to modify immune cells and target tumor cells in humans, bolstering optimism about its therapeutic potential in difficult-to-treat cancers. These findings align with research demonstrating CRISPR's capacity to eliminate genes essential for tumor survival, as well as the technology's flexibility for engineering novel, targeted approaches that overcome resistance mechanisms. Overall, while much work remains—particularly in refining gene-editing precision and ensuring long-term safety—emerging evidence points to CRISPR/Cas9's potential to fundamentally shift the paradigm in cancer treatment.

CRISPR/Cas9-mediated therapies hold the potential to revolutionize the treatment landscape for TNBC, but significant challenges remain. While the technology can be developed into effective anticancer therapies, overcoming barriers such as off-target effects, delivery limitations, manufacturing costs, and ethical concerns will require time and concerted effort. By addressing these challenges, CRISPR-based therapies can pave the way for more effective and personalized treatments for TNBC and other cancers, offering hope to patients who currently have limited options.

Declaration by Author

Ethical Approval: Not applicable

Acknowledgement: None

Source of Funding: None

Conflict of Interest: The author declares no conflict of interest.

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How to cite this article: Yichen Zhang. Review of current applications of CRISPR gene editing techniques in breast cancer therapeutics development. *Int J Health Sci Res.* 2025; 15(7):388-399. DOI: *10.52403/ijhsr.20250745*

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