Narrative Review Article

A Dual Approach: Combining CRISPR-Cas9 with Immunotherapy and PARP Inhibition to Enhance Efficacy in BRCA-Mutated Hereditary Breast and Ovarian Cancers

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ABSTRACT

Approximately 1 in 200 individuals is affected by mutations in either BRCA1 or BRCA2, which increase the lifetime risk of developing breast and ovarian cancer by around 5 times. However, current treatment options for BRCA-mutated cancers present major limitations due to the risk of both resistance and recurrence in certain cancer therapies. Approximately 40% of BRCA-mutated cancers acquire secondary resistance mutations that restore homologous recombination function. Similarly, 40-50% of BRCA-mutated cancers develop resistance to PARP inhibitors, another option for cancer treatment. This is further exemplified by the fact that platinum-based chemotherapy drugs like cisplatin are often highly toxic to the body. To address these limitations, alternative systems such as CRISPR-Cas9 offer a promising solution, as they could lower the risk of resistance and increase treatment efficacy. Overall, combining therapies such as PARP inhibitors or immunotherapy with CRISPR in BRCA-mutated cancers has yielded promising results not only in increasing the efficiency of treatments—the time it takes for them to show significant results—but also improving their efficacy. Due to the promising results of these combinations, research is being conducted with larger studies to investigate and prove the safety of these treatments. to investigate larger studies that will prove the safety of these treatments. Therefore, this paper examines the benefits of combining CRISPR-Cas9 with existing therapies to address the current limitations of BRCA-mutated cancer treatments.

Keywords: BRCA1; BRCA2; CRISPR-Cas9; PARP inhibitors; immunotherapy; combination therapy

INTRODUCTION

Clustered Regularly Interspaced Short Palindromic Repeats (CRISPR) is a gene editing mechanism that utilizes a guide RNA to identify targeted DNA sequences and make precise cuts using the enzyme Cas9 (1). Using this tool is beneficial for gene addition, or knock-in; gene removal, or knockout; and alteration of gene expression, or knockdown. CRISPR-Cas9 mechanisms are often used to treat genetic disorders, such as sickle cell disease (SCD), or to alter gene expression, thereby improving clinical outcomes in combination therapy. PARP (poly ADP-ribose polymerase) inhibitors are a method of cancer therapy that inhibit PARP enzymes, which are crucial for repairing single-strand breaks (SSBs) in DNA (2). The purpose of inhibiting PARP enzymes is to prevent DNA repair in cancerous cells, causing an accumulation of DNA damage (Figure 1B). Immunotherapy is a method of cancer treatment that uses the immune system to target and attack cancer cells

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(3). Checkpoint inhibitors, a type of immunotherapy, remove the blindfold placed by cancer cells on the body's T-cells, which are a type of immune cell. This allows them to recognize and kill cancer cells. In addition to immune checkpoint inhibitors, chimeric antigen receptor (CAR) T-cell therapy, another type of immunotherapy, utilizes modified T-cells collected from a patient's body to attach to antigens on the cancer cells and kill them. Figure 1 provides a visual overview of these therapeutic mechanisms to illustrate how they work at the cellular level. Therefore, this review examines the benefits of combining CRISPR-Cas9 with existing therapies to address current limitations of BRCA-mutated cancer treatments.

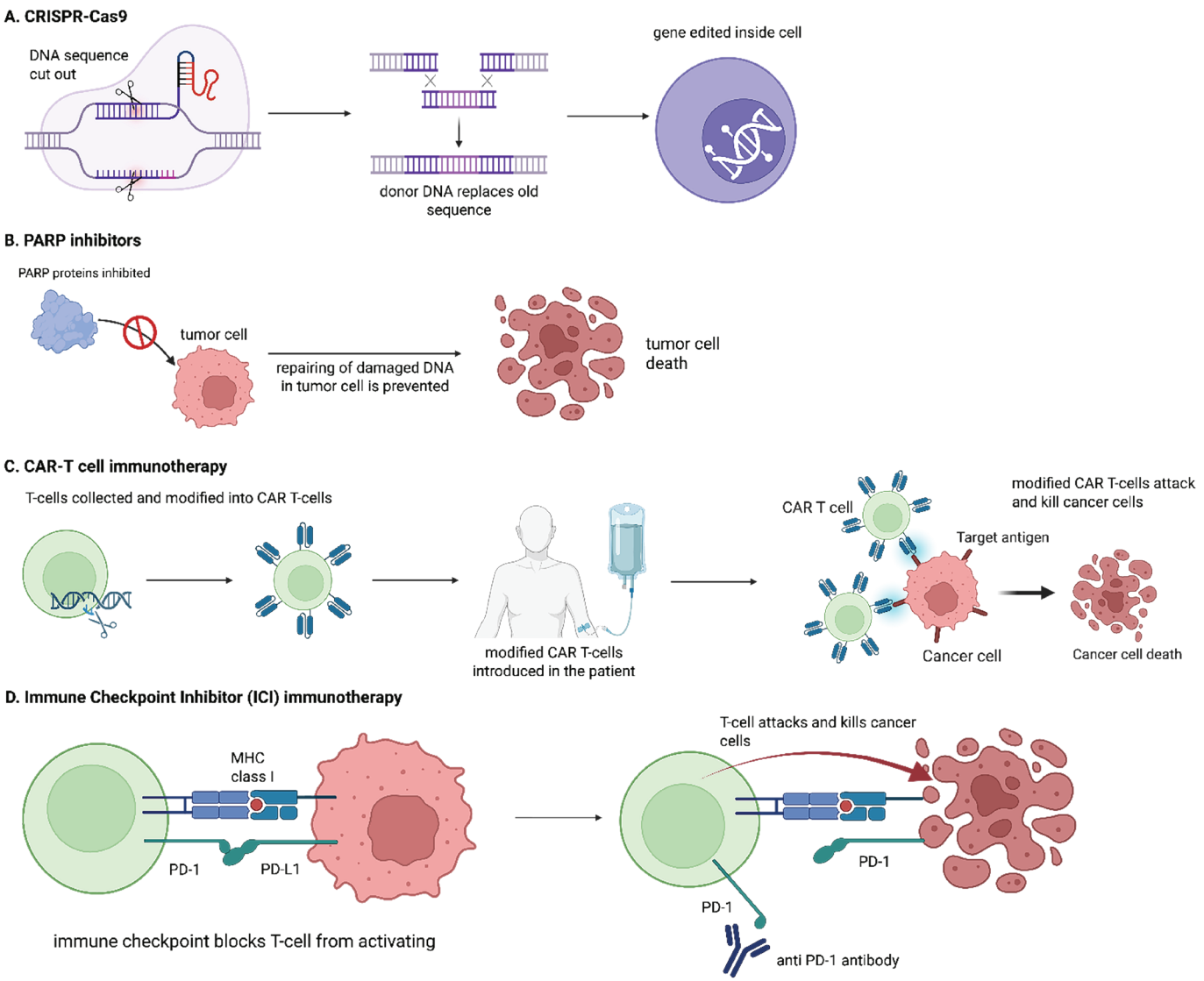

Figure 1. *Overview of emerging and current treatment strategies for cancers involving BRCA mutations. Diagram created with BioRender™. (A) CRISPR-Cas9 gene editing enables precise replacements of gene sequences inside the nucleus (B) PARP-inhibiting proteins block tumor cells from repairing DNA damage by blocking PARP protein production, causing tumor cell death (C) T-cells collected from the bloodstream are modified to CAR T-cells, which are then inserted into the blood and kill cancer cells by attaching to their antigens (D) Programmed Cell Death Ligand 1 (PD-L1) binds to Programmed Cell Death Protein 1 (PD-1) in the T-cell, blocking it from activating; immune checkpoint inhibitor immunotherapy releases an anti PD-1 antibody which allows the T-cells to kill the cancer cell.*

BRCA GENES IN THE CELL CYCLE

Genetic Susceptibility and Cancer Risk

BRCA1 and BRCA2 are tumor suppressor genes that, when mutated, increase the chances that a person develops cancer (4). BRCA1 was first located in 1994 at the 17q21.3 chromosomal locus, the long arm of chromosome 17 at band 21.3 (5). The BRCA1 and BRCA2 genes prevent cells from becoming cancerous. Later, in 1995, BRCA2 was discovered at loci 13q12-13 (long arm of the 13th chromosome at bands 12 to 13) (6). When wild-type, which means non-mutated, BRCA1 encodes around a 220 kDa nuclear protein (a relatively large protein) that is present in multiple tissues, such as the breast and ovaries (7). As a result, BRCA1 mutations increase the risk of breast and ovarian cancer. In individuals without BRCA1 and BRCA2 mutations, the average risk of developing breast cancer in women is 12.5% and 0.1% for men (NIH). However, women with BRCA1 mutations have approximately a 65% chance of developing breast cancer and a 39% chance of developing ovarian cancer by age 70 (8). Similarly to BRCA1, BRCA2 encodes a 384 kDa nuclear protein that is present in the gallbladder, bile ducts, stomach, and skin (9). This increases the risk of breast, ovarian, stomach, pancreatic, and prostate cancer (10). Female carriers of the BRCA2 mutation have an approximately 45% risk of developing breast cancer and an 11% risk of ovarian cancer by age 70 (8). Furthermore, men with BRCA2 mutations have approximately a 27% risk of developing prostate cancer and a 2.5% chance of developing pancreatic cancer by age 80 (11). Despite the known increased risk of stomach cancer in BRCA2 carriers, no precise number has been calculated. In the general population, approximately 1 in 200 individuals (0.5%) carry a BRCA mutation (12). However, the appearance of BRCA mutations is higher in groups like Ashkenazi Jews, at about 1 in 40 (13).

Molecular Mechanisms in DNA Repair

Both BRCA genes play a crucial role in the S and G2 phase checkpoints of the cell cycle, which ensure sufficient DNA replication and repair before the cell divides. The BRCA1 and BRCA2 genes are essential in DNA double-strand break (DSB) repair through the regulation of the homologous recombination (HR) pathway, a DNA repair method that utilizes sister chromatids as templates to repair damage (15). In DSB repair, the BRCA1 protein detects DNA damage and signals the cell to stop replicating (16). In addition, BRCA1 plays a crucial role in monitoring the translation of genes to proteins during protein synthesis, a process involved in the creation of tumors (tumorigenesis) and apoptosis (17). A BRCA1 protein deficiency prevents cell cycle arrest at the G2 checkpoint, allowing the cell to divide uncontrollably and become cancerous. Likewise, BRCA2 regulates the RAD51 protein, which is critical in DSB repair because it facilitates HR; therefore, BRCA2 mutations impair this process (15). Overall, both the BRCA1 and BRCA2 proteins aid in preventing cell DNA damage and therefore decrease the likelihood of tumorigenesis (18).

The BRCA1 gene acts as a regulator of gene expression in chromatin remodeling (the process of changing chromatin structures to monitor gene expression), ensuring appropriate structures for DNA repair and other crucial cellular processes (19). Therefore, inadequate chromatin remodeling may cause DNA damage, worsening the risks of developing cancer. As a result, when BRCA genes are mutated, cells are more susceptible to DNA damage, as their DNA repair mechanisms are compromised. DNA damage in cells disrupts the functions of proteins and can lead to uncontrollable cell division, or cancer (18).

Limitations of Current Treatment Strategies

Some of the current treatment options for BRCA-mutated cancers include surgery, chemotherapy, and PARP inhibitors. However, these treatments are limited. For example, in metastatic cancers, surgery often does not improve survival because the cancer has already spread to other body parts (20). Chemotherapy, like cisplatin, can be toxic to the body, meaning it could also damage healthy cells. Typical symptoms include hair loss, vomiting, anemia, fatigue, and a weakened immune system (21). Despite this, chemotherapy is typically effective at killing cancerous cells. Furthermore, BRCA-mutated cancers can regain function because of other mutations through secondary mutations (called reversion mutations, which restore original BRCA functionality), causing resistance to cisplatin and PARP inhibitors (22). 40-50% of BRCA-mutated breast and ovarian cancers develop resistance to PARP inhibitors, a treatment that stops cancer cells from repairing their DNA (23). PARP inhibitors are effective because they kill BRCA-mutated cancer cells that are unable to repair DSBs, but keep healthy cells alive (2). Response rates in these mutated cancers were about 26% in breast and 41% in ovarian (24). Despite being effective, these treatments leave room for improvement because of the many side effects they may carry, such as toxicity and limited results.

The Justification for Gene Editing Approaches

Due to the limitations of current treatments, researchers have begun to explore gene editing for BRCA-mutated cancers. Gene editing tools, such as CRISPR-Cas9, can replace bases, meaning they can cut out and insert new DNA sequences, and restore genes to wild-type function at targeted DNA sequences. The use of gene editing has the potential to correct BRCA mutations in patients, and doing so would be extremely beneficial in mutation carriers because of BRCA's role in DNA repair. By editing the genes directly, we can restore wild-type function to BRCA. Restoring the BRCA1 and BRCA2 genes in mutation carriers has the potential to decrease the risk of tumorigenesis, though further in vivo validation is required. For example, restoring regular BRCA function in combination with PARP inhibitors could protect non-cancerous cells from toxicity (14). However, while CRISPR has a lot of promise, it is not currently 100% effective because it doesn't target every cell in a population.

STRATEGY I: ENHANCING PARP INHIBITION

PARP inhibitors and Synthetic Lethality: What Are They?

PARP (poly ADP-ribose polymerase) inhibitors are a method of cancer therapy that inhibit PARP enzymes, which are severely important for the repair of single-strand breaks (SSB) in DNA (2). The purpose of inhibiting PARP enzymes is to prevent DNA repair in cancerous cells, causing an accumulation of DNA damage (Figure 1B). As a result of the accumulated DNA damage, the cell experiences death through synthetic lethality (a DNA damage combination that leads to cell death). PARP inhibitors are primarily effective in cancer cells with BRCA mutations because of the homologous recombination (HR) deficiencies, preventing further DNA repair. An example of an FDA-approved PARP inhibitor is olaparib. This treatment was initially created for BRCA-mutated breast and ovarian cancers (25). However, PARP inhibitors demonstrated efficacy in pancreatic and prostate cancer as well (26). In BRCA mutations, PARP inhibitors greatly extend progression-free survival (time without progression of the cancer), the SOLO1 trial showing 56 months versus 13.8 months in the group that took placebo tablets (27). Similarly, in wild-type BRCA cancers, PARP inhibitors reduce the risk of progression by 20-30% (28). In all, PARP inhibitors can be a very effective method of cancer treatment and hold great possibilities in the future of medicine with advancements in biotechnology.

Limitations of PARP inhibitors: Acquired Resistance

Mechanisms of Acquired Resistance

One of the primary limitations of using PARP inhibitors on their own to treat cancer is the risk of acquired resistance to the treatment (29). This happens so often that it affects roughly 40-70% of patients. This resistance is typically caused by reversion mutations (secondary mutations in BRCA1 and BRCA2) that restore typical HR function. The restoration of HR reduces the overall efficacy of PARP inhibitors because less DNA damage can be inflicted on the cancer cells. Nonetheless, PARP inhibitors are highly effective in BRCA-mutated tumors because of their HR deficiency due to the nature of the mutation, meaning that they accumulate more DNA damage (2).

Toxicity and Bioavailability

Other problems include the potential toxicity to the body of non-cancerous cells, which can cause anemia, fatigue, and nausea (21). These effects can often lead to discontinuation of the treatment because of their severity (29). Another major drawback of PARP inhibition on its own is its restricted delivery in complex environments. This is due to their poor bioavailability, or ability to be absorbed into the bloodstream and delivered to the target site, because they are orally delivered drugs (30). The lack of penetration from this allows tumors to survive and continue to progress (29).

Maximizing DNA Damage

Due to the limitations of PARP inhibitors alone in BRCA-mutated cancers, combining CRISPR with PARP inhibition holds promise. A 2025 *in vitro* study revealed that using CRISPR-Cas9 to create SSBs combined with PARP inhibitors increased synthetic lethality in BRCA2-mutated cancer cells by 95% *in vitro*, which means taking place outside of the organism (31). The induced SSBs, combined with PARP inhibitors that block SSB repair, resulted in a greater accumulation of DNA damage, thereby increasing synthetic lethality. This strategy limited off-target effects (editing of incorrect sequences) by using four adjacent guide RNAs: RNA sequences that guide the Cas9 mechanism to the desired DNA sequences. In cell lines (cultured cells), this method resulted in reduced toxicity from the PARP inhibitors to the non-cancerous cells. This study highlights one

potential strategy for using CRISPR in combination with PARP inhibitors, which can enhance treatment efficacy. However, they have yet to be replicated in human clinical trials.

Another 2025 study by Lazardides *et al*. utilized a CRISPR mechanism to recognize DNA polymerase beta (POLB) as an enhancer in PARP inhibition in BRCA-mutated cancers (32). This is possible because POLB aids in DNA replication, and when mutated, it can aid in acquiring DNA damage. This study used a method of knocking out (using CRISPR to remove) POLB in combination with certain PARP inhibitors like niraparib. This combination substantially increased both DSBs and SSBs, thereby enhancing PARP inhibitor efficacy by inducing apoptosis in BRCA-mutated cancer cells. As a result, within 6 weeks, POLB knockout in combination with PARP inhibition greatly decreased tumor progression. Overall, POLB knockout with PARP inhibitors demonstrated a significant increase in efficacy compared to typical PARP inhibitors in BRCA-mutated models *in vitro*.

Preventing Tumor Escape Mechanisms

Disrupting PARP1 and Restoring Sensitivity

In 2020, an *in vitro* study used CRISPR to disrupt the PARP1 (which encodes PARP enzymes) gene in BRCA1-mutated and regular BRCA1 triple-negative breast cancer (TNBC) cell lines (33). This use of CRISPR prevented regular PARP enzymes from being made within these cancer cells, providing a similar functionality to PARP inhibitors. Furthermore, editing the PARP gene could avoid mutations of reversion through HR. The efficacy of this CRISPR-Cas9 system proved approximately an 85% disruption of the PARP1 gene in the BRCA1-mutated group and a 70% disruption in the wild-type BRCA1 group. As a result, PARP protein levels in both cell groups decreased by around 80%, allowing for increased synthetic lethality.

Synergy with Chemotherapy

This study also tested three chemotherapy drugs (doxorubicin (DOX), gemcitabine (GEM), and docetaxel (DTX)) on the PARP-disrupted BRCA1-mutated cancer cell lines. The combination of the drugs and the mutations reduced the concentration needed to kill half of the cancer cells (IC50) by around 45% for DOX, 60% for GEM, and 40% for DTX. It works because it allows more DNA damage to accumulate, aiding in the three drugs' functions. DOX disrupts DNA replication and DNA repair, GEM prevents DNA replication, and DTX impedes cancer cell division.

Discrepancies in Tumor Models

This study also tested their method on a tumor recreation device (which is more accurate than in cell lines because it mimics natural body processes like blood flow). However, the results of this scenario were far different from those of the cell lines. There was no major IC50 decrease in either of the tumors when tested in the tumor recreation device because the cell lines didn't account for the body's natural processes, which may affect tumor penetration. The distance between success in a lab and clinical use of a medicine is illustrated by this drastic difference in IC50. Their results highlight that researchers still face limitations when using CRISPR in tumor microenvironments. These three studies prove that CRISPR-Cas9 can be useful in treating BRCA-mutated tumors when combined with PARP inhibitors. However, these methods still need refining and *in vivo* testing before we can ensure their increased effectiveness.

Expanding the Scope of These Treatments: BRCA Knock-In

Because BRCA-mutated cancers demonstrate improved responses to PARP inhibitors, there have also been studies where a BRCA mutation is knocked in (edited in) to BRCA wild-type cancer cells (34). As mentioned previously, this occurs because BRCA mutations lead to greater DNA damage accumulation in response to PARP inhibitors (2). In a 2023 *in vitro* study, researchers used CRISPR-Cas9 to knock in two pathogenic BRCA mutations into both pancreatic and breast cancer cell lines. As a result, these cell lines stopped producing functional BRCA proteins and impaired their DNA repair mechanisms. The study used an accurate gRNA system, which resulted in approximately 90% efficiency in the breast cancer group and one of the pancreatic cancer cell lines. However, only around 20% in the other pancreatic cancer group. The BRCA-mutated cell lines revealed a significantly lower olaparib IC50 compared to the wild-type BRCA cell lines, demonstrating PARP inhibitor effectiveness. One of the cell groups was 3 times more responsive to olaparib with a mutated BRCA gene than wild-type. Therefore, this treatment could prove effective for cancer patients born without BRCA1 and BRCA2 mutations because the mutations are edited specifically in the cancer cells. However, *in vivo* application of this system still faces challenges with the potential of off-target effects and ineffective CRISPR delivery (34).

STRATEGY II: COMMANDING IMMUNE RESPONSES

The Immunogenic Profile of BRCA Tumors: Potential and Risks

Neoantigens and TILs

BRCA-mutated tumors are a good candidate for immunotherapy because they increase immune system activation and tumor recognition in breast cancers (36). This is because BRCA mutations increase the production of neoantigens (proteins that are only produced by tumors), which are recognized as foreign by the immune system, increasing immune response rates.

Challenges of Inflammation

However, an overreaction to antigens can cause local inflammation and other immune-related adverse events (irAEs) like cytokine release syndrome (37). Cytokine release syndrome is an immune response in which systemic inflammation occurs; it is often caused by immunotherapy. Additionally, BRCA-mutated tumors have higher levels of tumor-infiltrating lymphocytes (TILs: immune cells that enter cancers to attack them), which can be more effectively targeted by immunotherapies (38). Higher TIL levels are commonly associated with better patient outcomes because they make targeting cancers more efficient for the immune system. Despite this, it is important to note that there exist limitations to immunotherapy. For starters, immunotherapy provides inconsistent responses between BRCA1 and BRCA2-mutated tumors, providing better results in BRCA2 tumors (39). BRCA2 tumors provide better results because they typically have higher tumor mutational burdens (TMB; accumulation of DNA damage). More importantly, there is a likelihood of irAEs (40). Common irAEs include diarrhea (low-grade), blood conditions like anemia, and skin conditions (37).

Heterogeneity in BRCA1 vs. BRCA2

A 2020 study by Samstein *et al*. looked at how BRCA mutations can affect immunotherapy responses in breast and ovarian cancer models with human and mouse cancer cells (39). Because BRCA2-mutated tumors demonstrate a higher accumulation of DNA damage compared to BRCA1-mutated tumors, BRCA2 tumors have increased neoantigen production (because of their increased TMB), making them more immunogenic. Furthermore, the Brca2null tumors (the BRCA2 mouse tumors) have far larger deletions than the parental (regular mouse tumors), which further increased the TMB and neoantigen production. This study analyzed immune checkpoint inhibitor (ICI) responses, revealing that using CRISPR to edit immunoregulatory (regulate immune responses) genes can reduce immune suppression and increase T-cell activation. In fact, Brca2null demonstrated approximately a 75% reduction in tumor volume compared to roughly 25% in the parental group. CRISPR can also target genes that can enhance signals to the immune system, which increases ICI efficacy by increasing immune responses. Therefore, these methods may eventually increase overall survival rates and provide more consistent immune responses if translation barriers are overcome through extensive *in vivo* validation. Overall, the research conducted in this study highlights the potential of targeting BRCA2-mutated tumors in combination with ICIs and CRISPR-Cas9 systems to enhance immune response rates.

Mechanisms of Checkpoint Blockade and CAR-T Cell Therapy

Although CRISPR and PARP inhibitors are demonstrating hope in the treatment of BRCA1 and BRCA2 cancers, immunotherapy is emerging as another strategy, showing great potential. Immunotherapy is a method of cancer treatment that uses the immune system to target and attack cancer cells (3). Immunotherapy is like retraining a guard dog; it is already on watch, but sometimes it cannot see the intruder (cancer cells). Immunotherapy trains the immune system to follow the correct scent trail. There are five classes of cancer immunotherapy: checkpoint inhibitors, cytokines, CAR T-cell therapy, vaccines, and agonistic antibodies. However, this paper will primarily focus on checkpoint inhibitors and CAR T-cell therapy.

Immune Checkpoint Inhibition

Checkpoint inhibitors work by blocking proteins used by tumors to evade immune detection. These proteins are also known as cell cycle checkpoints, which control cell division. In cancer cells, these checkpoints are disrupted, causing the cells to grow uncontrollably. Checkpoint inhibitors thereby allow T-cells to locate and attack the cells that have antigens identified as “foreign”, such as tumor cells. The most common checkpoint inhibitors block proteins like PD-L1, which bind to a protein on the T-cells called PD-1 (Figure 1D). Cancer cells use this to render T-cells inactive. Inhibiting these proteins allows the immune system to attack tumors more efficiently. Preventing the interaction between cancer cells and

PD-1/PD-L1 enables the T-cells to inflict cell death upon the tumor cells. Another common form of checkpoint inhibition is blocking CTLA4, a molecule that reduces T-cell activity and therefore allows for tumor progression.

CAR-T Cell Therapy

Chimeric antigen receptor (CAR) T-cell therapy is an immunotherapy method in which a patient's T-cells are removed from the body and genetically modified to express CARs (35; Figure 1C). CARs specifically attack tumor antigens (immune response triggering substances), allowing the immune system to recognize and eliminate them. CAR-T cell therapy has demonstrated high response rates in hematologic malignancies (blood cancer). However, CAR-T cell therapy for solid tumors is still in development due to its unique tumor targets and ability to suppress immune responses. In summary, checkpoint inhibitors and CAR T-cell therapy use the immune system's ability to attack tumors and enhance them, presenting promise for BRCA-mutated cancer treatment despite ongoing challenges in targeting solid tumors.

CRISPR-Enhanced Tumor Infiltration: A Novel Innovation

A systematic review by Feng *et al.* outlined the role of CRISPR-Cas9 in increasing immunotherapy responses in tumors (43). BRCA1-mutated breast and ovarian cancers typically have suppressive tumor microenvironments, noted by their lower T-cell activation levels. By using CRISPR-Cas9 to knock out genes like PD-1 in CAR-T cells, T-cell infiltration (the ability of T-cells to penetrate the tumor's microenvironment) is greatly enhanced. Knocking out PD-1 prevents T-cell exhaustion (where T-cells get tired from being active for too long), increasing cytokine (immune messenger proteins) production. It is important to decrease T-cell exhaustion and increase cytokine production because it allows for T-cells to more actively and effectively target tumor cells. In BRCA-mutated breast cancers, editing out PD-1 in CAR-T cells can greatly increase penetration into the tumor's microenvironment, enhancing clinical results. In fact, in a 2019 study by Hu *et al*., hCD3+ T-cell infiltration (a type of T-cell) to the TNBC tumor microenvironment was reported as nearly 2.5 times higher through knockout of PD-1 in CAR-T cells (44). Moving back, the review also mentions clinical trial NCT02793856, where CRISPR was used to knock out PD-1 in lung cancer cells (43). The results of the trial proved that using CRISPR to edit PD-1 was generally safe. However, the trial never mentioned the application in BRCA1 and BRCA2 patients. These studies illustrate the potential use of CRISPR-Cas9 for enhancing patient outcomes in CAR-T cell immunotherapies. However, more clinical studies will be needed to prove to the utmost extent that these methods are safe cancer treatment options.

Risks of Toxicity

In the KEYNOTE-522 trial, a breast cancer treatment combining chemotherapy with pembrolizumab, a PD1-blocking immunotherapy drug, serious treatment-related adverse events occurred in 32.5% of patients receiving pembrolizumab (41). One patient in this group died from an irAE. Even if these toxicities are low-grade, they still create limitations in dosage (which can alter overall survival if lowered). The higher the dose of a treatment, the more irAEs the patient experiences. These detrimental side effects can potentially put an end to a patient's immunotherapy treatment, lowering their risk of survival. The evidence presented suggests that BRCA-mutated tumors are effective immunotherapy candidates because they increase neoantigen and TIL production. However, the irAE risk emphasizes the need for optimized and monitored patient treatments.

Furthermore, a 2020 clinical trial called NCT02771088 (MEDIOLA) analyzed the effectiveness of olaparib and duvalumab (an anti-PD-L1 immunotherapy drug) in BRCA-mutated breast cancer patients (45). The findings of the study show that 32% of the patients enrolled in the study experienced grade 3 (severe) or worse irAEs, highlighting possible safety concerns. However, this combination demonstrated a promising antitumor activity, where 24 of the 30 patients experienced complete cancer control at 12 weeks. Furthermore, throughout the study, there were no deaths related to the treatment. Despite this, it's important to note that 3 patients discontinued the treatment due to irAEs. Combining this method with CRISPR could be used to achieve similar results. Overall, combining CRISPR-Cas9 with immunotherapy drugs in BRCA-mutated cancer patients greatly increases the efficacy of the treatment and may better long-term outcomes. While antitumour activity is promising in both of these trials, the risk of toxicity from immunotherapy limits survival.

Resensitizing Tumors with Immunogenic Editing

Due to the limitations of immunotherapy in BRCA-mutated cancers, combining CRISPR with immunotherapy has the potential to increase response rates. A 2024 study by Eskandari *et al.* focused on the

use of CRISPR-Cas9 in breast cancer treatment (42). The study describes that editing PARP1 in BRCA1-mutated cancer cells increased tumor cell death. Due to the nature of BRCA mutations, lowering resistance to PARP inhibitors in BRCA1 cancer cells made the tumor's microenvironment more immunogenic (able to induce immune responses) because it increased neoantigen production. Therefore, the immune system was more effectively able to target the tumor. Knocking down (editing out) PARP1 is important because many cancers develop resistance to PARP inhibitors, limiting their effectiveness. As a result, the efficacy of immune checkpoint inhibitor immunotherapy improved because it increased T-cell activity (by blocking PD-1/PD-L1, an inhibitor on T-cell activation gets removed, causing more immune responses), increased cancer cell clearance, and increased neoantigen production. This combination is mainly effective in BRCA2-mutated tumors, as they typically have more DNA damage accumulation. Despite these benefits, CRISPR delivery to BRCA-mutated tumors is complicated *in vivo* because they're buried deeply inside normal tissue. Therefore, CRISPR efficacy can be reduced.

BARRIERS TO BEDSIDE IMPLEMENTATION

Delivery Bottlenecking

Current delivery options, like AAV vectors (Adeno-associated virus vectors; using viruses to deliver modified genes to cells), are often delivered by electroporation (using electricity to allow easier delivery of substances to the cell) or microinjection (direct insertion into the cell). However, microinjection is only plausible for *ex vivo* delivery. However, *ex vivo* delivery isn't an option for many diseases, including breast and ovarian cancer, as they are embedded into solid masses (51). Furthermore, AAV delivery can often lead to many off-target effects, greatly lowering treatment outcomes. To address these concerns, research should focus on alternative delivery methods that improve CRISPR efficiency and reduce off-target effects.

Immune Responses to Cas9

Other limitations to CRISPR include immunotoxicity, DNA damage toxicity, and safe delivery (48). When using CRISPR-Cas9 models to edit genes, oftentimes, the immune system can recognize Cas9 as a threat. In a clinical study done by Charlesworth *et al*., more than half of the human patients in the study already had antibodies against SaCas9 and SpCas9, different forms of Cas9 used in CRISPR gene editing (49). This is a threat to a CRISPR patient because the body's immune system can destroy the edited cells, thereby neutralizing the effects of the treatment. Another concern of CRISPR delivery is the risk of DNA damage toxicity (48). DSBs induced by CRISPR-Cas9 systems can sometimes cause the cell to trigger apoptosis (cell death). Leading to adverse effects like inflammation (50). These unfavorable effects can be a major concern, as they can create long-term health problems or interfere with treatments. Also, safe delivery is often a concern for CRISPR patients, which builds upon off-target effects, immunotoxicity, and DNA damage toxicity (48).

CONCLUSION

Recently, BRCA-mutated cancer treatment has significantly evolved. However, resistance and toxicity limit the long-term efficacy of therapies like PARP inhibitors and chemotherapy. This review paper showcases the potential of combining CRISPR-Cas9 with these existing treatment models. For example, by enhancing synthetic lethality in PARP inhibitors and improving infiltration in immunotherapy, using gene editing can be a transformative approach to improving patient outcomes. However, translating research to treatments is difficult because of off-target effects, resistance to Cas9, and limits to tumor delivery systems *in vivo*. Down the line, research must prioritize long-term safety studies to improve and assess delivery for these treatments.

Improving the Safety of These Treatments

<u>Optimizing Clinical Trial Design</u>

To improve the safety and efficacy of BRCA-mutated cancer treatment options with CRISPR, early-phase safety and dose-escalation trials need to be prioritized. This is necessary because it would validate the safety of CRISPR when combined with PARP inhibitors or immunotherapies. For example, because PARP inhibitors work so well with BRCA-mutated cancers, trials with this combination are highly beneficial for cancer research. However, a significant translational gap remains: although preclinical models have demonstrated synergy with treatment mechanisms, they don't fully explain the immunotoxicity observed in human trials like MEDIOLA (45). Longitudinal studies should focus on the long-term impacts of combining CRISPR, PARP inhibitors, and immunotherapies in determining optimal

dosages to increase the efficiency of these treatments with minimal side effects.

Technological Innovation and Personalization

Additionally, personalized CRISPR systems (specifically, guide RNA to match specific patients) could be used to reduce off-target effects and inconsistencies in patient outcomes. This data would be extremely helpful in better understanding BRCA2-mutated tumors and their response to treatments, because of their higher TMB. Recent developments in machine learning (AI) could also be utilized to accurately find optimal targets for CRISPR guides, further increasing the efficiency and efficacy of these treatments. However, before testing this on patients, it would need to be verified *in vitro* due to limitations with many AI software. Lastly, studies are needed to test the long-term impact of CRISPR in combination with cancer therapies for BRCA-mutated cancers. These studies should primarily focus on testing cancer reemergence rates and tracking adverse effects, giving us a better understanding of the safety concerns regarding these treatments. Furthermore, research should specifically follow high-risk BRCA-mutated groups in these studies, such as Ashkenazi Jews. Therefore, the efficacy of these treatments can be attributed to diverse genetic factors. Advancements in CRISPR, PARP inhibition, and immunotherapy have a significant amount of promise, and further research makes overcoming drug resistance and extending progression-free survival for BRCA1 and BRCA2-mutated cancer much more tangible.

Ethics of CRISPR Treatments

Bioethics and Germline Editing

While many of these treatment combinations could work in a clinical setting, there are still limitations to combining CRISPR with BRCA treatment. One major concern for the use of CRISPR-Cas9 lies in the field of medical ethics. For example, there is the possibility that editing genes could lead to genetic manipulation for desirable traits (rather than targeting disease-causing genes). This would likely be regarded as unethical because it does not protect patient well-being and causes harm. Additionally, a major concern is affordable access to these gene editing technologies for public use (46).

Genetic diversity is critical for the survival of a species, especially in a rapidly evolving environment. Having a variety of different traits may allow some individuals to better adapt to the environment, allowing them to survive and reproduce, passing those traits to their offspring. Given this, if too many people were to edit their genes for either cultural "desirable" traits or even some genetic diseases, it could lower genetic diversity, which could have negative effects for the survival of the human species. While there are many ethical concerns about using CRISPR, it's evident that it's highly promising for application in BRCA, but safety should be of utmost importance.

Socioeconomic Barriers

Finally, affordable access to CRISPR-Cas9 treatments is critical to ensure that the benefits of new technology can be spread to all individuals in society. This is already a concern; in 2024, the first FDA-approved CRISPR treatment for sickle cell disease (SCD) was released. This treatment is intended for those who are homozygous for the pathogenic allele of SCD. However, the estimated cost of treatment is around $ 2.2 million per individual (47). This presents an ethical problem because it brings up the question: What is the purpose of developing these treatments if very few can access them? Whether this is unethical is up for debate, and regulating bodies for the affordability and access to CRISPR treatments do not yet exist. Despite the tremendous cost of CRISPR treatments, many still need access to these life-saving treatments, which is concerning for patients who cannot afford the treatment.

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CONFLICT OF INTEREST

The author declares that there is no conflict of interest related to this work.

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