State-of-the-Art Combination Therapies and Nanomedicines for Triple Negative Breast Cancer

By
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Triple-negative breast cancer (TNBC) is a particularly aggressive molecular subtype of malignant cells that tends to proliferate quickly and is commonly resistant to traditional chemotherapy leading to relapses in prognosis more frequently than other forms of breast cancer (BCa) [1][2][3]. Current treatment options include surgical resection of cancerous tissue and chemotherapy. The purpose of this review is to explore recent publications and clinical trials to highlight the ongoing advances in treatment methods for mid-to-late-stage TNBC. As an assessment of the safety and effectiveness of novel neoadjuvant chemotherapy, immunotherapy treatments, and optimal drug delivery systems, my goal here is to identify future directions for the treatment of TNBC. Recently, new treatment strategies using PARP inhibitors and immunotherapy have emerged. In particular, the use of PARP inhibitors, such as olaparib and talazoparib, in the treatment of patients with BRCA1 and BRCA2 mutations has emerged as a promising treatment option [2][4]. Furthermore, the use of PARP inhibition in combination with immunotherapy and/or DNA damaging agents may be a practical therapeutic strategy for the treatment of both BRCA-mutated tumors and other tumors that exhibit BRCA-like characteristics or dysfunctional DNA damage response [5]. The current literature also provides mounting evidence that a variety of promising nanoparticle-based technologies combined with individual drugs, or therapeutic combinations, may improve the efficiency of TNBC treatment. Advantages, such as reducing the side effects through
targeting specific cancer cell types, increasing the solubility and half-life of drugs (thus increasing the bioavailability of many chemotherapeutic drugs), and improve drug accumulation in tumor tissues, all provide strong justification for the ongoing development of these novel treatment approaches [6].

Key Words: Breast Cancer, Triple-Negative, Immunology, Olaparib, BRCA, Nanoparticles
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Purpose

Breast cancer (BCa) is a type of cancer that begins when healthy breast tissue cells undergo genetic mutations and begin to divide uncontrollably [7]. Approximately 15% of diagnosed BCa cases are triple-negative breast cancers (TNBC) [8], referring to malignant cells characterized by a lack of expression of estrogen and progesterone receptors [7]. Unfortunately, this means that TNBC cells will not be responsive to otherwise effective hormone therapy [9]. Triple-negative breast cancer cells also do not have extra copies of the HER2 gene, which leads to extra production of HER2 receptor proteins capable of binding growth factor causing uncontrolled growth, and therefore are also not able to be treated using targeted therapy, such HER2 antibodies [7][10]. Figure 1 shows the classification of different cancer subtypes based on immunohistochemistry and microarrays [11] as well as a visualization of receptor-positive BCa and TNBC.
Figure 1: Left, examples of receptor positive and TNBC cells and the subtypes of tumor each cell type leads to. The majority of cancerous breast cells have at least one of the mentioned receptors, potentially all three types [7][10]. Right, 5 main intrinsic subtypes of cancer ranked in terms of prognosis.

Triple-negative breast cancers are often basal-like BCa [7], which refers to cancers of a particularly aggressive molecular subtype that tend to proliferate quickly and lead to relapses in prognosis more frequently than non-basal subtypes [1]. Because of the high proportion of basal-like TNBCs, they are characterized by an aggressive biological behavior with a distant recurrence peak observed early at only 3 years following diagnosis. Furthermore, metastatic TNBC bears a dismal prognosis with an average survival of 12 months [3][12]. Tumor growth rates in percent per day increase for estrogen receptor-positive tumors, HER2 positive/estrogen receptor-negative tumors, and triple-negative tumors are .208, .859, and 1.003, respectively [13], demonstrating the aggressive nature of TNBC cells. Considering the severity of the condition as well as the lack of effective treatment options available, there is a clear need for further research into viable treatments for reducing the mortality rate of TNBC.

As cancer progresses beyond the primary tumor, it is known as metastatic BCa, also known as stage IV or advanced BCa. For advanced BCa other organs involved typically include the lymph nodes in the armpit, lung (16%-34%), liver (19%-20%), bone (47%-60%), or the brain (10%-16%) [2]. This means that treatment of the primary tumor, or even secondary tumors, is not enough, as microscopic metastasis may remain throughout the body, allowing lesions to return after treatment and potentially spread further. Diagnosis of metastatic BCa may occur with initial diagnoses or any time after the initial diagnosis, sometimes up to years later. The risk of returning metastasis is generally highly unpredictable and varies by patient, depending largely on individual tumor microenvironment and biology. However, approximately 30% of the women diagnosed with early-stage BCa will develop a metastatic form of the disease, demonstrating a need for patient-specific late-stage interventions [14]. Additionally, mutations found in metastasis typically continue to acquire mutations that are not seen in the primary tumor, making
treatment through certain pathways more or less effective for different metastasis. This means that as cancer progresses, single-strategy treatments become less effective. Thus, detection and treatment of cancer metastasis at the earliest stage possible is important for the management of BCa progression [2].

Another concern with TNBC is that it is more likely to be diagnosed in women younger than 50, with other types of BCa generally being diagnosed in women older than 60. This means that there is a greater average years of life lost in deaths associated with TNBC. Moreover, younger women are more likely to be diagnosed at later stages, further increasing mortality [7]. There is a significantly higher prevalence of TNBCs and mortality rates among African-American women compared to other cohorts as well [7], with the age-adjusted mortality rate for black women younger than 40 being twice that of white women [15]. Less than 1% of all types of BCa occur in men, including TNBC [9]. Although transgender women have an increased risk of BCa compared to cisgender men, most of these cases are hormone-receptor-related [16]. Although recent advances in the prevention, screening, and treatment of BCa have resulted in a drastic reduction of incidence and mortality rates across all subtypes, TNBC rates have not been impacted by these advances on the same scale, specifically in vulnerable populations, providing additional evidence that there is a greater need for research in the treatment of TNBC [16][15]. This review focuses specifically on mid-to-late-stage interventions in cisgender adult women, the population identified as being the most vulnerable.

Background

In healthy tissue, damaged cells are destroyed and replaced through highly regulated cell division. Breast cancer occurs when cell growth in the breast is not appropriately regulated by a variety of means, allowing cells to grow out of control. Through analysis of cancerous breast tissues, many parallels have been drawn between the normal development of breast tissue and BCa progression at the molecular level, suggesting that BCa may often derive from mammary
cancer stem cells. Figure 2a depicts the mammary cell hierarchy including BCa stem cells. Figure 2b maps the locations of different cell types within a breast ductal-alveolar unit.

**Figure 2:** a) differentiation of mammary stem cells and potential paths leading to cancerous tissue formation. A multipotent stem cell present during development gives rise to luminal epithelial and basal stem cells, which further divide into luminal and basal progenitors during puberty. These cells then
Breast cancer stem cells are a small population of BCa cells that play a critical role in metastasis to other organs in the body. Breast cancer stem cells have both self-renewal and differentiation properties, both of which contribute to the aggressiveness of metastasis [18]. Mammary stem cells have an important role in developing and replacing mammary glands and have a role in the cellular origin of BCa stem cells, which has been traced extensively using various methods and is visualized in figure 2 [14]. These cells exhibit a slow cell turnover rate and the potential to divide asymmetrically to seed tumors in vivo. It has also been demonstrated that BCa stem cells have antioxidative, tumorsphere formation, tumorigenicity, and chemoresistance properties, further complicating treatment [18]. Emerging evidence also suggests that noncoding RNAs and their role in epigenetic regulations may be involved in TNBC development and may also contribute to the heterogeneity and metastatic aspects of the disease [14].

**Diagnosis and Treatment Options**

Most individuals with BCa notice common symptoms, such as a mass in the breast, swelling, and skin or nipple changes, leading them to seek a diagnosis. The diagnosis of BCa is usually achieved through diagnostic testing, most commonly including a combination of physical breast exams, mammograms, breast ultrasounds, MRI, and biopsies [19]. The most used radiological exam is the mammogram, which uses an x-ray. However, the lack of abnormal features in triple-negative tumors can sometimes lead to inaccurate diagnosis and higher sensitivity tests such as MRI may be required [20]. After initial diagnosis, further testing such as blood tests and scans of other regions of the body may be required in order to determine the stage of cancer [19].

Although TNBCs have fewer treatment options than other BCa subtypes, there are currently several treatment options including surgery and neoadjuvant chemotherapy using poly(ADP-ribose) polymerase (PARP) inhibitors and immunotherapy. Specifically, various molecular targeted therapies including agents that target the Notch signaling pathway, the Wnt/β-catenin
pathway, the Hedgehog pathway, the PI3K/Akt/mTOR pathway, as well as poly(ADP-ribose) polymerase 1 (PARP-1), androgen receptors, angiogenesis, and epidermal growth factor receptors have been found to be active against TNBC [12].

Surgical options
Invasive BCa is categorized as a malignancy that the cells of which have spread outside of the basement membrane of the lobules and ducts into adjacent healthy tissues. Different types of breast cancer characterized by growth pattern and location include ductal carcinoma, lobular carcinoma, and inflammatory mammary cancer [21]. The Tumor, Node, Metastasis (TNM) is the system used to classify various stages of breast carcinoma. The early stages of invasive cancer, which are traditionally considered operable, are the TNM stages I, II, and IIIA, before significant invasion into surrounding tissues occurs [22]. However, with the aggressive nature of TNBC, tumors progress quickly and full surgical removal of the cancerous tissue without additional intervention becomes highly unlikely in many cases [1].

Radiation and Chemotherapy
Radiation therapy, such as external beam radiation therapy (EBRT) or brachytherapy, is typically used post-breast-conserving surgery, if the tumor was large, or if the cancer was widespread to many lymph nodes or in tissues, such as skin or muscle, in order to lower the chance of relapse in nearby breast tissue or lymph nodes. More widespread radiation is also used if the cancer cells have spread to distant locations, such as the bones or the brain [8].

Traditional chemotherapy involves anti-cancer drugs given intravenously or orally, whereupon they provide systemic treatment to the entire body. Chemotherapy can be given before or after surgery. Post-surgery, chemotherapy is administered to kill any residual cancer cells or microtumors remaining at the surgical site or beyond. Prior to surgery, chemotherapy can be used to shrink the tumor so it can be removed less invasively, especially if the tumors are initially locally advanced complicating their safe removal. Neoadjuvant chemotherapy may also be used to test how resistant to cancer drugs a cancer is and determine if additional treatments are required. Treatment length depends on how well the drug is working and how well treatment is tolerated by the patient. Currently, there are complimentary diagnostics that can help determine which individuals will most likely benefit from chemotherapy after breast surgery.
such as Oncotype DX [8]. Recently, the FDA approved another diagnostic test called the VENTANA PD-L1 Assay, which can be used to identify patients with TNBC who may benefit from an immunotherapy–chemotherapy combination treatment [23].

Emerging Treatment Options

Immunotherapy

Immunotherapy, or immuno-oncology, is emerging as an effective treatment for several subtypes of BCa, including TNBC. An improved understanding of cancer cells’ ability to evade the immune system, as well as the discovery of selective immune checkpoint inhibitors, has allowed for the development of treatment methods employing the patient’s immune system to target and destroy cancer cells. Although TNBC cells are commonly resistant to traditional chemotherapy, they are highly sensitive to the inhibition of the polyamine synthesis pathway, which allows for the sensitization of cancerous cells to chemotherapy [3]. However, the associated adverse effects of chemotherapy to normal tissue may still limit its therapeutic benefit [24]. Chemotherapy is almost always employed at the maximum tolerated dose of the patient, maximizing the potential for tumor destruction but also potentially depleting the immune-related cells that are stimulated in an immunotherapeutic approach. However, tumor cell death results in the release of neoantigens into the tumor microenvironment as well as the release of danger signals that stimulate immunological memory [25]. This means that finding the right drug combinations and doses in combination with chemotherapy can provide a synergistic effect in stimulating immune cells and destroying cancer cells, improving treatment outcomes.

The immune system has several important functions in the development and control of a tumor; typically, the immune system is capable of destroying cancer by drawing immune cells into the area, causing an enhancement of tumor cell death and leading to an increase in tumor cell antigen release, which facilitates tumor-antigen recognition by dendritic cells [19]. However, cancer cells can suppress the immune system, taking advantage of immune checkpoints that typically protect tissues, allowing them to evade immune system detection that would regularly prevent abnormal tissue growth [19]. Cancer immunosurveillance has three distinct phases: elimination,
equilibrium, and escape. In this process, type II interferons and lymphocytes prevent the development of a primary tumor. During the elimination phase of tumor growth, the immune response prompts an effective extrinsic tumor-suppressor system. However, the partial elimination of a primary tumor may lead to the selection of tumor cells that are better able to survive in the host, leading to the escape phase. Between these phases lies an equilibrium phase, in which tumor growth remains under pressure by the immune system. This process is shown in Figure 3. Both the innate and adaptive immune systems are involved in controlling the tumor [20].

![Figure 3](image)

**Figure 3:** The three phases of cancer immunosurveillance: elimination, equilibrium, and escape. As the tumor progresses through the phases shown in the figure, genetic instability and tumor heterogeneity increase [21]. NK = natural killer cell, NKT = natural killer T-cell, CTL = cytotoxic T-cell, CD4 = cytotoxic T-cells with CD4 protein. Tumor cells that are capable of evading immune surveillance are shown in darker red.

One of the most significant molecules identified in the immune evasion of cancer cells is the programmed cell death protein 1 (PD-1). Generally, PD-1 downregulates T cell activation to control immunological homeostasis. The upregulation of programmed death-ligand 1 (PDL-1) by tumor cells gives them resistance to the immune system and allows them to escape the body’s immunosurveillance mechanisms. To allay this immune evasion, anti-PD-1/PDL-1 treatments
have been developed and approved for many cancer types and have resulted in an objective response in approximately a quarter of treated patients [20]. This low efficacy is most likely primarily due to the low number of tumor-infiltrating immune cells [19]. However, with TNBC having the highest immune cell infiltration of all other BCa subtypes, this treatment method is more promising, especially given the low efficacy of other treatments methods for this cancer type [9]. The PDL-1/PD-1 interaction for T-cell activity modulation is shown in Figure 4.

**Figure 4**: The PDL-1/PD-1 interaction for T-cell activity modulation with PD-1 and PD-L1 inhibitors. Activation of the immune checkpoint results in the deactivation of T-cells, allowing tumor cells to survive [21]. Created with BioRender.com

Tumor-infiltrating lymphocytes have important functions in tumor control and may be able to be used to predict the tumor response to immunotherapies. In particular, CD8+ T lymphocytes are integral to the antitumor immune response and studies have shown that their tumor-infiltrating capacity directly correlates with patient survival. The infiltration of specific activated CD8+ lymphocytes against tumor antigens induces a response against the tumor, making tumor-
infiltrating lymphocytes key to controlling tumor proliferation. Tumor-associated antigens that are recognized by T lymphocytes induce this specific immune response [20].

Because the value of immunotherapy is amplified with the expression of tumor antigens, coupling PARP inhibitors and immunotherapy may be a promising treatment method. PARP inhibitors are also combined with radiation therapy or chemotherapy, inhibiting DNA repair functions and enhancing the effects of radiation and chemotherapy by increasing the rate of cell death. Additionally, this association may also interact with the antitumor immune response; PARP inhibitors and ionizing radiation can enhance the infiltration of cytotoxic T lymphocytes into the tumor bed as well as enhance PD-1/PDL-1 expression. Therefore, the addition of immune checkpoint inhibitors with PARP inhibitors in combination with radiation treatment could counterbalance immunosuppressive effects of tumors [20].

An example of an immune checkpoint inhibitor that has been approved for use when used in combination with the chemotherapy drug nab-paclitaxel is Atezolizumab. The combination is approved for women with locally advanced or metastatic TNBC that cannot be treated surgically and whose tumors are positive for the PD-L1 protein. By inhibiting immune checkpoint proteins, these drugs in combination allow the immune system to find and attack cancer cells more aggressively [22]. By continuing to explore combinations of immunotherapy and molecularly targeted therapies in combination with DNA-damaging chemotherapy, more effective treatments for late-stage TNBC can be developed.

**PARP inhibitors**

As knowledge of BCa’s molecular mechanisms increases, several targeted PARP inhibiting agents have recently been identified and studied for the treatment of TNBC. These PARP inhibitors work by exploiting the synthetic lethality concept to prevent the repair of DNA damage by blocking PARP enzymes, which aid in DNA damage repair, leading to cancer cell death [23][26]. The concept of synthetic lethality refers to two genes where the mutation of either gene alone does not harm cell viability, but the mutation of both genes leads to cell death.
Thus, damaging genes that are synthetically lethal to cancer-causing mutations should, by
definition, kill cells that harbor such mutations, while having no effect on normal, healthy tissues
[24]. Figure 5 shows how PARP inhibitors control DNA damage repair and the relationship
between cell DNA repair and cell survival.

![Figure 5: PARP inhibitor mechanism, specifically in BRCA-deficient cells compared to normal cells. BRCA-proficient cells can repair double-strand breaks, resulting in cell survival and making treatment more difficult. Adapted from [25].]

Currently, several members of the PARP protein family have been identified. Among them,
PARP-1 accounts for approximately 90% of total activity and is one of the main components of
DNA base-excision repair and repair of DNA single-strand breaks. PARP-1 consists of three
conserved, major domains. These domains are an NH2-terminal DNA-damage sensing and
binding domain consisting of three zinc fingers, the auto-modification domain, and a C-terminal
catalytic domain [12]. The basic structure of PARP-1 is pictured in Figure 6.
Figure 6: A diagram of simplified PARP-1 protein domains. PARP-1 has a carboxyl-terminal domain with an active site where enzymatic activity occurs. PARP inhibitors work by binding to this domain, leading to reversible inhibition of PARP enzyme. PARP-1’s amino-terminal consists of three zinc finger motifs, a nuclear localization signal, and an auto-modification domain that functions as the target of covalent auto-poly(ADP-ribosyl)ation. The phosphorylation of PARP-1 at Ser 372 and Thr 373 residues is required for the maximal activation of the enzyme in response to DNA damage [23].

DNA damage occurs either due to cellular exposure to damaging agents such as chemotherapy, or because of a failure to repair endogenous DNA damage in cells. DNA damage itself can take many different forms depending on the mechanism of action of the agent used, with DNA double-strand breaks considered to be the most cytotoxic to cancer cells, especially cancer cells with variants of the breast cancer susceptibility gene (BRCA mutation) [27]. Everyone has the BRCA 1 and BRCA 2 genes. The normal role of BRCA is to scan cellular DNA for damage and trigger DNA repair processes when mutations are found, however, individuals with mutated versions of the BRCA gene may not be able to detect and repair damage or mutations to DNA, leading to cancer [10]. The three major epigenetic modifications occurring in BCa proliferation are DNA methylation, post-translational modifications of histones, and miRNA dysregulation [28]. Point mutations in the first BRCA mutation type interferes with the formation of the BRCA1-CBP/P300 complex, resulting in dysfunction of BRCA1 genes. Carriers bearing BRCA1, but not BRCA2, mutations present a great amount of rearrangement signature 3 small tandem duplications. Cancers with BRCA1 or BRCA2 mutations exhibit substantial numbers of rearrangement signature 5 deletions (18). The position 859 mutation of BRCA 2 is likely pathogenic and the cause of tumor development, as it generates an early stop codon at position 881 of the BRCA 2 protein and truncates proteins that cause cellular damage. This evidence
supports BRCA-1/2 mutation as a biomarker for PARP inhibitor sensitivity in primary and metastatic BCa [29].

As previously discussed, tumor-associated antigens that are recognized by T lymphocytes induce a fundamental response against cancer cells [20]. Tumor-associated antigens most likely result from tumor mutations, meaning that tumors with a high mutational burden might respond better to immunotherapies [20]. However, with TNBCs not displaying specific tumor-associated antigens, cytotoxic molecules that target DNA repair functions, such as PARP inhibitors, could enhance the mutational load in tumors with a pre-existing deficiency in DNA repair function. This concept has been demonstrated in mismatch repair-deficient colorectal cancers, but there is still much opportunity for applications in other tumor types [20]. Thus, PARP inhibitors and ionizing radiation might improve the efficacy of such immunotherapies as those using immune checkpoint inhibitors [20].

**PI3K/AKT/mTOR pathway**

Triple-negative breast cancers display aberrant activation of the PI3K pathway due to a variety of mechanisms. Direct inhibition of the PI3K/AKT/mTOR pathway is, therefore, a relevant therapeutic strategy [12].

**PARP inhibitors combined with histone deacetylase inhibitors**

Studies have shown that treatment with histone deacetylase inhibitors sensitizes cancer cells to PARP inhibition. Due to the BRCAness effect, which is a set of characteristics of DNA repair defects caused by BRCA1 dysfunction, histone deacetylase inhibitors have been found to enhance the responsiveness of TNBC cells to PARP-1 inhibitors [30]. First, histone deacetylase inhibitors block the deacetylation of heat shock protein 90 (HSP90), leading to hyperacetylation and inhibition of the protein. Consequently, several proteins, including BRCA1, cannot interact with HSP90, resulting in cell death [12]. At present, a variety of histone deacetylase inhibitors
have either been obtained from natural sources or have been developed in the laboratory for testing in clinical research [31].

**PARP inhibitors combined with EGFR inhibitors**

Epidermal growth factor receptor (EGFR) regulates various cellular processes relevant to the growth of cancer cells including proliferation, differentiation, and survival. Overexpression of EGFR leads to undesirable outcomes and carcinogenic effects, including cell growth and invasion, angiogenesis, and metastasis [31]. It has been shown in preclinical models that EGFR inhibition alters the ability of treated cells to repair DNA double-strand breaks. The mechanism of this increased sensitivity involves the reduction of nuclear BRCA1 and EGFR induced by chemotherapy, which impairs the repair of the HR pathway, produces sustained DNA damage, and subsequently makes sporadic triple-negative breast cancer susceptible to PARP inhibition [12]. PARP inhibition prevents nuclear retention of PKM2, thereby inhibiting cell proliferation and tumor growth [32].

**PARP inhibition and ATM downregulation**

Ataxia-telangiectasia mutated (ATM) kinase is a key DNA damage response protein with heterozygous germline mutations that represent a moderate risk factor for breast cancer development. The data suggest that PARP inhibition has a potential role in triple-negative breast cancer treatment with low ATM protein expression/activity. In addition, it has been reported that transforming growth factor β (TGF-β) down-regulates ATM in BCa cells by inducing the miR-181 family that targets the 3' untranslated region of ATM transcripts. Through this pathway, TGF-β could sensitize TNBC cells to PARP inhibition, as has been previously demonstrated in preclinical *in vitro* and *in vivo* models [12].

**PARP inhibitors and androgen receptors**

In a recent study reported that enzalutamide, a nonsteroidal antiandrogen drug that is in clinical use for the treatment of prostate cancer, followed by olaparib, promoted DNA damage-induced cell death, inhibited proliferation of prostate cancer cells in culture, and suppressed the growth of
prostate cancer in mice. Given that one of the TNBC subtypes (LAR) expresses the androgen receptor, the combination of anti-androgens and PARP inhibitors may represent an effective treatment for patients with this subtype [12].

**PARP inhibitors and the Wnt/b-catenin pathway**

The Wnt/b-catenin pathway has been shown to be highly activated in basal breast tumors, and its nuclear localization is associated with a poor prognosis [2]. With a large proportion of Wnt ligands promoting the progression of breast tumors, tumor growth has been shown to be attenuated by the restoration of many Wnt inhibitors that have been silenced by mechanisms such as DNA methylation and microRNAs in tumors. Furthermore, Wnt pathway activation has been shown to increase radiation resistance of progenitor cells in both mouse mammary gland and human BCa cell lines, indicating that Wnt signaling is involved in resistance to current anticancer drugs and may be a good potential target in reducing resistance [2].

**PARP inhibitors and CTLA-4**

The first immune checkpoint receptor to be clinically targeted was CTLA-4 (also CD152), an immune checkpoint protein [20]. T cell activation requires the interaction between a T cell receptor and antigen-bound major histocompatibility complex as well as costimulatory signals, such as those provided by the interaction between CD28, a protein required for T cell activation and survival, on the T cells and B7, a membrane protein on the antigen-presenting cell. CTLA-4 interacts with B7 and initiates regulatory signals, leading to T cell inhibition. In melanoma, the CTLA-4 antibody ipilimumab was the first therapy to improve patient survival and several additional anti-CTLA-4 immunotherapies have been tested in many tumor models both in clinical and trial settings, such as tremelimumab [20].

According to additional reports, PARP inhibitors can activate interferon I through replication stress in the cGAS-STING pathway. The activation of interferon I triggers the immune system to target cancerous cells as well as elevating the levels of PD-L1 in the cells [29]. As immune checkpoint inhibitors rely on the phosphorylation of the cGAS-STING pathway using PARP
inhibition, the combination of PARP inhibitors and PD-L1 antibodies has been again demonstrated to be a promising method for cancer treatment. The synergistic effects of combination therapy have been identified both in breast cancer cell lines and patient-derived tissue samples. As measured by tumor regression and survival, results from several phase II clinical trials show that duel treatment using antibodies against PD-L1 such as durvalumab or pembrolizumab as well as a PARP inhibitor, such as olaparib or niraparib, are effective in treating germline BRCA-1/2-mutated breast cancer [29]. Several similar clinical trials are still ongoing.

*PARP inhibitors and Luteinizing Hormone-Releasing Hormone*

Existing cytotoxic chemotherapy drugs have been proven to effectively target, eliminate or shrink tumors at early, mid and late stages without any apparent cytotoxicity when conjugated to Luteinizing Hormone-Releasing Hormone (LHRH) for the specific targeting and treatment of TNBC [33]. In patient-derived tissue samples of TNBC, expression of the LHRH-receptor was present in 49% of samples [34]. The LHRH-conjugated drugs inhibit the growth of breast cells in both *in vitro* and *in vivo* experiments [33].

Figure 7 gives a visual overview of several of the described pathways.
**Figure 7**: An overview of main pathways targeted using PARP inhibitors in triple-negative breast cancer.  
A) PI3K/Akt/mTOR pathway as a target for treating triple-negative breast cancer [31]. B) When DNA damage occurs, ATM actuates TGF-β signaling by stabilizing the receptor TβRII. In addition, ATM phosphorylates and stabilizes c-Cbl, enhancing TβRII neddylation. DNA damage enhances this interaction [35]. C) Histone deacetylases inhibition as a therapeutic strategy against triple-negative breast cancer. H = Histone; A = Acetylgroup; HAT = Histone acetyltransferase; HDAC = Histone deacetylase; HDACI = Histone deacetylase inhibitor [31]. D) PARP inhibition suppresses cell proliferation by preventing the nuclear retention of PKM2, thus inhibiting tumor growth [32]. E) Blockade of B7 ligands via CTLA4-Ig promoting T-cell anergy and/or apoptosis. Co-stimulation signals to follicular T cells via B7:CD28 and from stimulatory signals from CD40L:CD40 interactions are also important for B-cell activation and differentiation [36]. Only relevant interactions are pictured.

**> Recent and Ongoing Trials**

**PARP Inhibitors**

Trial 6, described in table 1, began May 30, 2017, and was most recently updated Aug 13, 2021. The study enrolled adults with histologically documented triple-negative, inoperable, locally advanced, metastatic, not amenable to resection adenocarcinoma of the breast, and who have received a minimum of 4 cycles of platinum-based chemotherapy in the 1st or 2nd line setting and derived clinical benefit with platinum-based therapy. Eligible subjects were randomized to either treatment group with the two groups being olaparib or olaparib in combination with durvalumab. Study treatment will continue until disease progression, intolerable toxicity, elective withdrawal from the study, or study completion or termination. Upon individual discontinuation of treatment, survival is noted every two months [37]. Table 1 reviews similar studies with similar enrollment requirements and study designs, several of which will be discussed in this section; Studies include adult participants over 18 years of age diagnosed with TNBC.

Another specific area of focus is combining olaparib with an immunotherapy agent. Promising options include atezolizumab (Tecentriq), durvalumab (Imfinzi), and tremelimumab [25]. Trial 2, a phase I/II study, evaluated the combination of durvalumab, an anti-PD-L1 antibody, and
olaparib as a first or second-line treatment. The study included 32 individuals with germline BRCA mutations. Overall, the combination of durvalumab and olaparib had an objective response rate of 53% and a 12-week disease control rate of 47% with minimal adverse events [38] [39]. An early-stage trial, trial 2, evaluates durvalumab in combination with olaparib in 12 patients, two of which had TNBC and 11 of which had the wild-type BRCA gene, two women achieved PR and eight women had disease stability, achieving an 83% disease control rate overall [40][39]. Currently, there are several studies that are investigating the use of olaparib in patients with somatic BRCA mutations compared to germline BRCA mutations, in combination with radiation therapy, and in an adjuvant setting post traditional first-line treatment. There are also several studies evaluating the use of olaparib in combination with cytotoxic chemotherapy, specifically carboplatin [25].

Trial 3 is another promising study (phase I/II ) of the Anti-Programmed Death Ligand-1 Antibody MEDI4736 in combination with olaparib and/or cediranib. The study began June 29, 2015 with a total of nine patients being treated initially, one of whom had TNBC. The study hypothesized that cediranib, a VEGFR1-3 inhibitor, would reduce VEGF signaling and complement the anti-tumor activity of durvalumab. The study also aimed to assess if the three-drug combination would be tolerable to patients. Patients were dosed with 20 mg of cediranib five days a week in succession with full doses of durvalumab and olaparib. One out of nine patients experienced anemia, one hypertension, and three experienced lymphopenias. However, no patients experienced dose-limiting toxicity. Four patients exhibited an incomplete response and three patients exhibited a stable disease state with no progression for longer than six months, yielding an overall 67% clinical benefit rate. PD-L1 expression of the tumor cells was correlated with clinical benefit. Owing to these promising results, the study was expanded in phase II to 384 participants and was last updated June 11, 2021 [41]. In a similar vein, results from other studies support the investigation of pamiparib, a PARP 1/2 inhibitor, combined with tislelizumab, an anti-PD-1 monoclonal antibody, in patients with advanced solid tumors, including those with homologous recombination deficiency mutations [42]. Trial 8 began October 19, 2017 and was last updated January 20, 2021) involves treatment with avelumab and talazoparib. Patients with
advanced solid tumors who had received at least one prior chemotherapy regimen were treated with avelumab by IV every 2 weeks in combination with talazoparib at full dose, with a 25%-50% de-escalation permitted if toxicity occurred [43]. Preliminary results from this trial showed that the combination of avelumab, a human IgG1 anti–PD-L1 monoclonal antibody and talazoparib in patients with advanced solid tumors showed anti-tumor activity and controllable safety which was equivalent to the safety of single agents [43].

Analyzing the data and results from several trials described or listed in table 1 shows clear evidence that the combination of a PARP inhibitor and immunotherapy can yield positive results in controlling cancerous growth in both BRCA-1/2 mutated and BRCA wild-type patients. Furthermore, it was demonstrated that the PARP capture potential of talazoparib is 100 times that of other PARP inhibitors examined in the investigation. Median progression-free survival was longer in the talazoparib group compared to other drugs (8.6 months vs. 5.6 months). This study also showed a great benefit in patients with a history of central nervous system metastases, a subtype with particularly adverse outcomes [25]. Additionally, anti-tumor activity and controllable safety of combining the various types of cancer drugs described was shown to be equivalent to the safety of single agents [43].

**Table 1: List of currently ongoing trials with interventions used and study phase.**

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<th>Title</th>
<th>Interventions</th>
<th>Study Type/Phase</th>
<th>Source</th>
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<tr>
<td>1. A Study Of Ipatasertib in Combination With Atezolizumab and Paclitaxel as a Treatment for Participants With Locally Advanced or Metastatic TripleNegative Breast Cancer.</td>
<td>• Drug: Atezolizumab • Drug: Ipatasertib • Drug: Paclitaxel • Drug: Placebo for Atezolizumab • Drug: Placebo for Ipatasertib</td>
<td>interventional/phase 3</td>
<td>[44]</td>
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<td>2. An open-label, phase II basket study of Olaparib and durvalumab (MEDIOLA): Results in germline BRCA -mutated (gBRCA m) platinum-sensitive relapsed (PSR) ovarian cancer (OC)</td>
<td>• Drug: Olaparib • Durvalumab (PD-L1) +/bevacizumab</td>
<td>Phase 1/2</td>
<td>[45][38]</td>
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<tr>
<td>Study ID</td>
<td>Description</td>
<td>Drugs</td>
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| 3       | Phase I/II Study of the Anti-Programmed Death Ligand-1 Antibody MEDI4736 in Combination With Olaparib and/or Cediranib for Advanced Solid Tumors and Advanced or Recurrent Ovarian, Triple Negative Breast, Lung, Prostate and Colorectal Cancers | • Drug: Olaparib  
• Drug: Cediranib  
• Drug: MEDI4736 | Phase 1/2   | [41]      |
| 4       | Pamiparib in combination with tislelizumab in patients with advanced solid tumours | • Drug: Pamiparib  
• Drug: tislelizumab | Phase 1a/b | [42]      |
| 5       | Ipatasertib Plus Non-Taxane Chemotherapy for Advanced or Metastatic Triple-Negative Breast Cancer | • Drug: Ipatasertib  
• Drug: Capecitabine  
• Drug: Eribulin  
• Drug: Carboplatin  
• Drug: Gemcitabine | Interventional/phase 2 | [44]      |
| 6.9     | Phase II Multicenter Study of Durvalumab and Olaparib in Platinum tReated Advanced Triple Negative Breast Cancer (DORA) | • Drug: Olaparib Oral Product  
• Drug: Olaparib Oral Product in combination with Durvalumab | Interventional/phase 2 | [44]      |
| 7       | Neoadjuvant Study of Two Platinum Regimens in Triple Negative Breast Cancer | • Drug: Paclitaxel  
• Drug: Paclitaxel  
• Drug: Carboplatin  
• Drug: Doxorubicin  
• Drug: Cyclophosphamide | Interventional/phase 2 | [44]      |
| 8       | Avelumab Plus Talazoparib In Locally Advanced Or Metastatic Solid Tumors | • Talazoparib) II  
• Avelumab (PD-1) | Phase 2     | [44][43]  |
| 9       | A Trial of Camrelizumab in Combination With Nab-paclitaxel and Faminitinib as a First Line Treatment in Patients With Unresectable Locally Advanced or Metastatic Immunomodulatory Triple Negative Breast Cancer(FUTURE-C-PLUS) | • Drug: camrelizumab in combination with nab-paclitaxel and faminitinib | Interventional/phase 2 | [44] |
| Study of Pembrolizumab (MK-3475) Plus Chemotherapy vs. Placebo Plus Chemotherapy for Previously Untreated Locally Recurrent Inoperable or Metastatic Triple Negative Breast Cancer | • Biological: Pembrolizumab  
• Drug: Nab-paclitaxel  
• Drug: Paclitaxel  
• Drug: Gemcitabine  
• Drug: Carboplatin  
• Drug: Normale Saline Solution | Phase 3 | [44][46] |
| --- | --- | --- | --- |
| Radiation, Immunotherapy and PARP Inhibitor in Triple Negative Breast Cancer (NADiR) | • Drug: Niraparib  
• Drug: Dostarlimab  
• Radiation: Radiation therapy | Phase 2 | [44] |

**Sensitizing Triple-Negative Breast Cancer to PARP Inhibitors**

In order to expand new PARP inhibitor treatments to patients with non-BRCA mutated cancers, it is important to test whether there is room for the application of PARP inhibitors outside of BRCA-1/2 mutated cancers. Olaparib, when used to treat BRCA-1 and BRCA-2 mutated carriers has been shown to have cell-autonomous immunomodulatory properties in [47]. However, based on several distinct studies, there is strong evidence from preclinical studies that support the use of PARP inhibition in BRCA wild-type BCa as well. Several groups have reported sensitivity to olaparib, independent of BRCA-mutation status in a panel of BCa cell lines [48][49]. BRCA wild-type tumors were also found to be sensitive to olaparib *in-vivo*, in two distinct cohorts of BCa patient-derived tissue samples [50].

One potential solution to improving the sensitivity of wild-type BRCA TNBC to PARP inhibition involves Fra-1, a member of an activator protein that regulates gene expression in response to stimuli such as cytokines and growth factors, therefore playing a critical role in tumor progression and treatment resistance [4]. The AP-1 member Fra-1 is overexpressed in TNBC regardless of BRCA status, identifying it as a potential target. In preliminary screening, one study identified PARP-1 to interact with endogenous chromatin-bound Fra-1 in TNBC cells. As discussed, the PARP-1 inhibitor olaparib has recently been approved for clinical use for the treatment of some cancer types. However, the relevant study showed that the Fra-1-PARP-1
interaction directly correlates with the efficacy of olaparib treatment. Additionally, because a large fraction of PARP1-regulated genes are dependent on Fra-1, by inhibiting Fra-1, non-BRCA-mutated TNBC cells can become sensitized to olaparib treatment [4].

Another targeted approach that has yielded similar results in TNBC cells is the targeting of the PI3K pathway. As previously discussed, the inhibition of PI3K leads to the downregulation of BRCA-1/2, an increase of poly-ADP-ribosylation, and subsequent sensitization of cancer cells to PARP inhibition. In a recent study, TNBC tumor samples, dual inhibition with buparlisib (a PI3K inhibitor) and olaparib reduced the growth of tumors displaying BRCA-1/2 downregulation following PI3K inhibition. The combination of a PI3K inhibitor with a PARP inhibitor was shown to provide improved synergy in an endogenous mouse model for BRCA-1 related breast cancers [12]. A recent (2020) study was able to further legitimize this concept using several lines of basal-like breast cancer cells examined the effect of 13 different PARP inhibitors in 12 BCa cell lines with and without BRCA-mutations through the use of cell viability assays. The results showed that five of the eight TNBC cell lines were significantly susceptible to PARP inhibition regardless of BRCA status. Figure 8, adapted from the study, illustrates these results [5].
Figure 8: Inhibition curves in the cells under the indicated PARP treatments. Points: mean of three and bars represent standard errors. Metastatic and non-metastatic triple negative breast cancer cells without BRCA1 mutation treated from 0.001 to 200 µM for 7 days. Only results of the most promising PARP inhibitors for this treatment method were included (talazoparib and olaparib) [5].

Drug Delivery Systems

Cancer therapeutics are typically administered via intravenous or oral routes. However, with concerns about toxicity to healthy tissues remaining, the use of nanoparticles in cancer treatment is actively being investigated. Nanoparticle-based carriers are a class of drug delivery system that is defined as ultra-dispersed solid supramolecular structures within a size range of 10-1000 nm [51]. The use of nanoparticles in drug delivery increases the solubility and half-life of some hydrophobic chemotherapeutics thus increasing the bioavailability and improving drug
accumulation in the cancer tissues due to the enhanced permeability and retention (EPR) effect [6]. Nanocarriers have been found to release more drug in acidic environments, which mimics tumor microenvironments, than in typical physiological conditions, protecting healthy tissues from cellular damage while focusing damage in cancerous tissue [52]. Nanoparticles can also protect drugs from being destroyed by the body once administered and alleviate the problems encountered by free drug combination therapy [51][53]. In addition to serving as novel drug delivery vehicles, nanoparticles have shown a great deal of promise as inhibiting agents for key signaling pathways in BCa stem cells. As previously discussed, several signal transduction pathways are deregulated in BCa stem cells including Wnt/β-catenin, hedgehog, Notch, BMPs, and PI3K/Akt/NFkB. These signaling pathways stimulate BCa stem cell proliferation, migration, invasion, EMT, chemotherapy and radiotherapy resistance, and interact in complex ways. An example of this is the use of short non-coding RNAs to regulate the stemness characteristics and tumorigenesis of BCa stem cells through these pathways [18].

Currently, there is active investigation on improving BCa treatment using various novel nano-formulations, such as liposomes, hydrogels, exosomes, dendrimers, microspheres, microbubbles, phytosomes, micelles, and more [51]. These nanoparticle subtypes are defined and visualized in table 2. The drugs can either be encapsulated, entrapped, dissolved, or attached to a nanoparticle matrix, acting as a reservoir for the drug [51].

<table>
<thead>
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<th>Table 2: Background on existing types of nanoparticles studied in cancer therapeutic applications</th>
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<td><strong>Delivery System</strong></td>
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| **Nanosphere** | Nanospheres are made up of a porous polymer or polystyrene that acts as a mini reservoir, allowing the nanosphere to absorb and deliver the therapeutic. The absence of oil in nanospheres leads the polymeric chains to form a matrix structure capable of loading with cancer therapeutics [54]. |
| **Nanocapsule** | A nanocapsule is a shell made from a nontoxic polymer. The presence of oil in the nanocapsules leads to a vesicular structure [54]. Drug-filled nanocapsules can be covered with antibodies or cell surface receptors that are capable of binding to cancer or various cells and releasing their biological compound on contact with the specified tissue [55]. |
| **Liposomes** | Liposomes are biodegradable and biocompatible lipid bilayer vesicles that can be used to carry both hydrophobic and hydrophilic bioactives [56]. They are typically around 400 nm in diameter [57]. |
| **Hydrogels** | Hydrogels for the delivery of therapeutics are typically prepared by introducing non-reversible covalent bonds via self-assembly either through chemical reactions or by UV/photo polymerization [52]. Hydrogels have a broad range of applications, including the detection of TNBC and the delivery of therapeutics. [51] |
| **Exosomes** | Artificial exosome-like carriers are biocompatible and within the optimal size range for cancer cell targeting, typically between 30–150 nm. Exosomes have also been found to play important roles in cell communication, acting as natural carriers of biological cargoes throughout the body [58]. | [51] |
| **Denrimer** | Dendrimers are nano-sized, radially symmetric molecules with well-defined, homogeneous, and monodisperse structure with a symmetric core, an inner shell, and an outer shell. Dendrimers consist of three classes that are broadly recognized to generate rather polydisperse products of different molecular weights [59]. | Created with BioRender.com |
| **Microbubble** | Biomedical microbubbles are liquid microscopic bubbles, typically .5-10 um in size [60], stabilized by a coating of magnetic or drug-containing nanoparticles. Nanoparticle-coated microbubbles can be made to be stable and echogenic and may be used to release the cargo of drug-containing | [60] |
nanoparticles with an ultrasound trigger, providing a clear and targeting treatment strategy [61].

Phytosomes

Phytosomes are vesicular systems formed by the interaction between hydrophilic parts and the phyto-active components of phospholipids. The structural difference between liposomes and phytosomes is that liposomes have their active ingredient inside the hydrophilic cavity or within the layers of membranes while in phytosomes, the components are a part of the membrane itself, allowing for easy transfer into tissues [62].

Micelles

Polymeric micelles are core/shell structures formed by amphiphilic block copolymers. Both the inherent and modifiable properties of polymeric micelles make them particularly well suited for drug delivery purposes [63] Micelles are typically within 5-100 nm [57].
Optimal size of nanoparticles:
The similarity between the size of nanoparticles and the size of cells, bacteria, and other biological entities leads to close and effective interactions. This interaction can be used to achieve the controlled transport of drugs and their bonding to a patient's cells and tissues, plus the determination of disease-agent concentrations in complex environments such as blood, saliva, and urine [55]. Typically, drug-loaded nanoparticles need to be injected into the bloodstream to target the cancer site, and therefore must be of the right size to pass through the endothelial barrier. The size, shape, charge, and density of nanoparticles are each important parameters that may determine the trajectory, dynamics, stability, and distribution of nanoparticles in the bloodstream, impacting the subsequent infiltration of tumor tissues and cells [57].

Although the range of what is considered a nanoparticle is broad on the nanometer scale, the most efficient nanocarriers seem to be on the lower end of that size range. One study compared 14 nm, 30 nm, 50 nm, 74 nm, and 100 nm gold nanoparticles and reported that the highest brain cancer cell uptake was detected for the 50 nm nanoparticles, similarly to what has been reported in other studies, while another group reported that 20 nm nanoparticles gave the best results [64]. In a separate study that found the use of various nanoparticles to be highly effective, the magnetic nanoparticles were measured to be between 38.7 ± 4.3 nm and 114 ± 7.6 nm depending on drug loading, environment, and surface coatings [65]. Another study saw success in the delivery of cancer therapeutics using uncoated magnetic nanocarriers proven through dynamic light scattering analysis to be an average of 34.2 ± 8.4 nm [66]. On the larger end of nanoparticles reported effective for cancer drug delivery were doxorubicin-loaded micelles that were spherical in shape that began closer to the typical size range reported in other studies but have average particle size around 300-320 nm post-drug-loading. The drug entrapment efficiency of untargeted and targeted doxorubicin-micelles was between 71-93% [66]. However, differences in size, shape, and material of the nanoparticles and the variability of divergent cell types make it difficult to make definite conclusions about ideal size across these studies [64].
Stability of the nanoparticles \textit{in vivo}: 
In addition to physiologic size constraints, the stability of nanocarriers is significant to ensure proper delivery to diseased tissues and reduced toxicity to the patient. \textit{In vivo} stability is a significant issue for many PARP inhibitors, limiting the doses that patients can handle. An important hurdle to overcome when working with DNA damage response inhibitors is their relative insolubility and high toxicity. As such, various drug carriers have been employed to solubilize these compounds and co-deliver them with traditional chemotherapeutics, other inhibitors, and/or radiation therapy [67]. In order to reach cancerous and infiltrate a tumor in order to successfully deliver the therapeutic drug, the vascular barrier needs to be crossed. Nanoparticles target a cancer site through passive action. In this model, nanoparticles passively cross into leaky vessels of tumor tissues, accumulating in cancer cells, with more vascularized tumors accumulating drug more quickly. Once in circulation, it is crucial that nanocarriers can avoid immune detection as to not be destroyed. This is typically achieved using biocompatible coatings such as PEG, leading to longer half-life of circulation in blood [57].

In a recent study (2021), black pomegranate peel extract was tested as a potential treatment for BCa. The study compared the stability and efficacy of the drug when delivered as a drug-loaded nanoparticle, free drug, and unloaded nanoparticle (no drug). The chitosan-coated magnetic nanoparticles (BPPE-CCMNPs) were loaded with novel black pomegranate peel extract (BPPE) as the drug-loaded nanoparticles and blank magnetic nanoparticles were used as the unloaded nanoparticles. Two different lines of BCa cells and a normal fibroblastic cell line were used to assess the nanoparticle-drug delivery system [65]. The stability of the nanoparticles, loaded with drug and blank, was tested in both serum-free and serum-containing cell media and was incubated for 48 hours at 37 °C, with updates being logged at 24 hours and 48 hours. In serum-free media, magnetic nanoparticles and BPPE-CCMNPs formed large aggregates, changing their surface charge and pointing to instability of the nanoparticles. However, BPPE-CCMNPs were shown to maintain their stability in serum-containing media and no significant size changes were observed. On the contrary, magnetic nanoparticles displayed far more instability in serum-containing media, showing that the drug-loaded nanoparticles were far more stable in
physiological-like conditions [65]. In addition, it has been shown that both short- and long-term administration of magnetic nanoparticles did not lead to significant toxicity, as evaluated through blood chemistry, histology, and weight change [67]. Figure 9, adapted from the study in question, displays the stability of the magnetic nanoparticles and BPPE-CCMNP as measured in the study.

**Figure 9**: Describes the stability of the magnetic nanoparticles and BPPE-CCMNPs in cell media with and without serum. Figure 9(a, c) shows the results of dynamic light scattering and figure 9(b, d) presents the zeta potential of the nanoparticles in serum-free and serum-containing cell media, respectively. Adapted from [65]. Two different cell media types (RPMI and DMEM, both with and without serum) were used in the stability evaluation, with similar results. The dynamic light scattering and zeta potential results of the MNPs and BPPE-CCMNPs in deionized water (DI water) are also shown in order to compare the size and stability changes [65]. RPMI = Roswell Park Memorial Institute, DMEM = Dulbecco's Modified Eagle Medium

Hydrogels, also known as nanogels, have also shown a lot of promise in drug delivery to cancer cells in that they are highly biocompatible and show strong, sustained pH-dependent release
behavior. When compared to other nanocarriers, nanogels have several therapeutic advantages, including high drug-loading capacities, greater storage stability than liposomes and micelles, relative ease of production, and reactivity to external stimuli [52]. Using the normal cell line COS7 and cancer cell lines HepG2 and A549 in a 2018 study, these concept were effectively demonstrated [52]. Drug release is facilitated under acidic conditions as the polymer becomes protonated, disrupting the electrostatic interaction between the drug and the hydrogel at pH < 5.5. With pH 5 representing the tumor microenvironment, drug-loaded hydrogels can safely travel through the body, accumulate in cancerous areas, and release drug. This concept was shown in A549 and HepG2 cells using doxorubicin-loaded hydrogels [52]. Figure 10 shows cell uptake of drug in the two different cell lines for drug-loaded hydrogels compared to free drug (traditional delivery) exhibiting the efficacy of drug delivery of the drug-loaded hydrogels.
Correspondingly, another study tested the pH stability of magnetic nanoparticles with the drugs talazoparib and buparlisib. The study investigated several different scenarios including release in circulation, in the tumor microenvironment, and in the endosome. Under pH conditions mimicking in vivo circulation at 25 °C, talazoparib was fully released after eight hours, while buparlisib was fully released after 48 hours. pH conditions similar to the tumor microenvironment (pH 6.8) had little influence on talazoparib release. However, under the same conditions, the release time of buparlisib within 24 hours is half of the release time exhibited at neutral pH. Under pH conditions meant to mimic the endosomal environment (pH 5.5), the release for both drugs occurred rapidly due to the denaturation and swelling of the nanoparticles. Overall, the release of talazoparib showed little dependence on pH, indicating that this delivery method may not be beneficial for all cancer drug types. However, the pH-dependent release demonstrated by buparlisib supports the idea that nanoparticles may maintain stability as drug carriers in the bloodstream until they reach cancerous environments where the nanocarriers quickly become denatured and release large doses of drugs directly to the affected tissues [67].

Efficacy and toxicity compared to traditional drug delivery

Although many studies have demonstrated cancer drugs delivered with nanocarriers are able to reach cancer tissues and promote cell death while preserving healthy tissues, further evidence must be collected to make conclusions about nanoparticle drug delivery systems’ true impact on patient outcomes. As demonstrated in a previously discussed study employing magnetic nanoparticles, developed nanoparticles effectively inhibited the growth of tumors for a longer period by invading the human BCa cell line and dramatically inhibiting the expression of the cell cycle protein cyclin D1 [65]. Figure 11 shows LDH release studies of MDA-MB-231 (human breast adenocarcinoma) cells exposed to magnetic nanoparticles, free black pomegranate peel extract (BPPE), and black pomegranate peel extract loaded chitosan-coated magnetic nanoparticles (BPPE-CCMNPs). Higher LDH release correlates to higher rates of cell death. At the same time, the researchers also tested drug-loaded nanoparticles and free drug treatments on
healthy cell lines. Neither the drug-loaded nanoparticles or blank nanoparticles showed any
cytotoxicity, meaning that the treatment can effectively protect healthy tissues while destroying
cancerous tissues [65].

Figure 11: LDH release studies of MDA-MB-231 (human breast adenocarcinoma) cells expose
to magnetic nanoparticles, free black pomegranate peel extract (BPPE), and black pomegranate
peel extract loaded chitosan-coated magnetic nanoparticles (BPPE-CCMNP) with 1000 to 7.81
µg/ml concentration at 48 hours [65].

Although many nanoparticle drug delivery systems have shown a lot of promise, liposomes are
noted as one of the most versatile nanocarriers for cancer treatment due to the feasibility of
encapsulating anti-cancer drugs in both the lipid membrane and aqueous core. One study
targeted the overexpressed CD49c protein in TNBC models by using ligand-attached liposomes
carrying a dual drug treatment and reported that the effectiveness of the treatment compared to
traditional drug delivery was significantly improved [57]. Comparably, liposomes loaded with
doxorubicin and sorafenib, a type of chemotherapy, enhanced antitumor activity in a TNBC in
vivo mouse model. A liposomal drug delivery system for the co-delivery of a microRNA and
paclitaxel (chemotherapy) has also been developed specifically to prevent lung metastasis in
BCa patients, with different variations of the technology and drug loading seeing 82%-87%
inhibition of tumor growth in lung metastasis and cancerous breast tissue, respectively [57].
In a similar vein, another study demonstrated co-loaded polymer-lipid hybrid nanoparticles can pass through various efflux transporter pumps that often aid multidrug resistance, resulting in enhanced efficacy in breast tumor models. The superior efficacy of co-loaded polymer-lipid hybrid nanoparticles was attributed to the synergistic pharmacokinetics of doxorubicin and Mitomycin C as well as the increased bioavailability of the drugs in tumor cells enabled by the nanocarrier polymer-lipid hybrid nanoparticles [53]. Another group developed two types of doxorubicin-loaded micelles with different targeting peptides, QND or HSQ. Doxorubicin-loaded nanoparticles had an average particle size around 300-320 nm when filled with drug to maximum capacity. The drug encapsulation efficiency of non-targeted and targeted DOX micelles was very high. Targeted micelles exhibited significantly higher cytotoxicity when compared to free doxorubicin and untargeted doxorubicin micelles. In addition, significantly greater binding and uptake were observed for the targeted micelles on BT549-Luc and T47D (mammary) cells [66].

Although the PD-1/L1 approach has been promising in studies as well as in clinical use, as demonstrated through various studies, approximately 80% of TNBC patients still fail to respond fully to current therapies [68]. However, recent studies have identified a correlation between higher intratumoral T lymphocyte infiltration with higher patient response towards the PD-1/L1 antibody therapy. One solution that is currently under investigation involves the use of nanoparticles to efficiently deliver antigens and/or adjuvants to peripheral immune organs and cells, improving response in drug-resistant cancers. This potential has recently been demonstrated in a phase III trial in which paclitaxel and atezolizumab were co-loaded into nanoparticles for the effective treatment of patients with advanced TNBC. "Cancer vaccines" also continue to be developed, with one study using mRNAs encoding patient-specific antigens developed using ionizable lipids. By adjusting the properties of the nanoparticles such as size, surface charge, and stability, the researchers were able to develop a drug delivery system that accumulates in and transfects the spleen antigen-presenting cells, stimulating T cells to respond to cancer antigens in both mouse and human models. This method addressed another extremely significant but often overlooked issue: high treatment costs for patients. In the same study,
peripheral T cells were successfully able to be engineered to express the intended antigen receptor as a part of an alternative approach for CAR-T therapy, which has the potential to reduce the high cost of adoptive T cell therapy [69].

Another notable study demonstrates the ability of a novel puerarin nanoemulsion (NanoPue) to reduce the immunosuppressive nature of the tumor microenvironment and significantly reduce tumor growth. In the study, nanoPue was combined with α-PD-L1 when examining the combination of nanoPue and a paclitaxel polymer nanoformulation (nanoPTX) for the treatment of TNBC, specifically. The study aimed to deactivate tumor-associated fibroblasts T rather than directly damaging fibroblasts due to damage caused to tumor-associated fibroblasts by chemotherapy potentially enhancing drug resistance in tumor cells. The study concluded that their nanoparticle-drug formulation successfully deactivated the stromal microenvironment (p < 0.0001) and facilitated the chemotherapy effect of nano-paclitaxel in the triple-negative breast cancer model. Moreover, the removal of the physical barrier increased intratumoral effectively doubled the infiltration of cytotoxic T cells. An activated immune microenvironment allowed nanoPue to cooperate with PD-L1 blocking therapy in a TNBC model [68]. In a similar study, researchers developed a nanoparticle delivery system that was shown to sequentially release CCL25 proteins and CD47 small interfering RNA in a mouse TNBC model. Since CCL25 is not expressed in TNBC tumors, delivery of CCL25 inside the tumor microenvironment offers an effective strategy for increasing CCR9+ T cell tumor infiltration and enhancing immunotherapy through PD-1/PD-L1 signaling pathways [70].

Manufactured exosomes created by genetic engineering of the exosome-producing tumor cells are also a promising tool to attain a better antitumor immune response. The expression induction of artificial neoantigens or neoepitopes on the surface of exosomes can bring out anti-tumor recognition by immune cells. It has been previously demonstrated that early secretory antigen target-6 (ESAT-6) could be a powerful antigen able to initiate an immune response. ESAT-6 carrier exosomes from genetically modified tumor cells and showed significant tumor growth reduction in mice treated with these exosomes [71]. Another
successful method was to use biotin to mark donor cell membranes before exposing them to a potent anti-neoplastic drug. Exosomes expressing both biotin and avidin on the membrane surface were obtained and encapsulated the drug following cell functionalization with avidin to improve targeting efficiency. These synthetic exosomes showed high target ability to tumor cells and receptor-mediated cellular uptake, resulting in significantly reduced tumor growth [72][58].

> Conclusions and Future Outlook

Overall, there have been numerous recent successes involving the use of combinations of PARP inhibitors, specific checkpoint inhibitors, and immunotherapy in both wild-type and mutated BRCA TNBC. In malignant tumors with BRCA1 or BRCA2 mutations, the therapeutic combination of PARP inhibitors and immunotherapeutic drugs works by inducing a more favorable anti-tumor immune response in the context of activating the tumor microenvironment [45]. More research on other PARP targets is needed to better understand the underlying mechanisms of PARP inhibition in BRCA-positive and BRCA-negative tumors. After reviewing numerous studies, the conclusion that PARP inhibition using drugs such as olaparib and talazoparib in combination with immunotherapy may be a useful therapeutic strategy for the treatment of both BRCA-1/2 mutated tumors, as well as other tumors that exhibit BRCA-like characteristics or dysfunctional DNA damage response, may be drawn [5]. Immunotherapies for PD-L1–negative TNBC patients and patients that have not responded to previous PD-1/L1 treatments, as well as methods to reduce immune-related toxicity, are critical areas that require additional research [73].

Ultimately, a variety of promising nanoparticles combined with drug combinations or individual drugs can improve the efficiency of the therapy by reducing the side effects through targeting specific cancer sites, can increase the solubility and half-life of drugs thus increasing the
bioavailability of many chemotherapeutic drugs, and can improve drug accumulation in the
cancer tissues due to the enhanced permeability and retention of drug in cancerous tissue [6]. In
addition, it has been demonstrated that nanocarriers release more drugs in an acidic environment
that mimics the tumor microenvironment than under typical physiological conditions, thereby
protecting healthy tissues from cell damage [52].

However, many challenges must be overcome before nanomedicines can be used in clinical
settings. Because of interactions with biological components such as the corona protein, which
has been found to affect the size of nanocarriers in several studies, nanomedicines can have
dramatically changing physicochemical properties after administration, making it difficult to predict in vivo functions [67]. To fully realize the benefits of integrating nanomedicines with an
immunotherapeutic approach, a number of challenges must be overcome. Because of the greater
complexity of nanomedicine formulations, large-scale production is more difficult than that of
traditional drugs, also impacting the reproducibility of preclinical studies involving
nanomedicines. One potential solution to this is to introduce more standardized protocols for
nanomedicine production and results reporting. Furthermore, scaling up the production of
nanomedicine, particularly those with multiple components, is difficult. Complex nanomedicines
are difficult to produce in large quantities for late-stage preclinical and clinical trials. As a result,
in order to promote clinical translation of immunomodulatory nanomedicines, nanomedicines
should be built with as few components as possible and using scalable manufacturing
procedures. By addressing these concerns, nano-immunotherapy will become more widely used,
resulting in better treatments available for patients [69].

Another potential research area is whether omics baseline and surgical biopsy analysis can be
used to determine patients who can receive neoadjuvant chemotherapy alone, patients with
immune control inhibitors, and patients with possible drug resistance mechanisms, although
several studies have identified immune response to genomic instability itself as an independent
biomarker in identifying candidates for immune targeting treatments [74]. Nanoparticles may
also have potential applications as cancer detection systems (bioimaging agents) because they
tend to accumulate in malignant tissues. Analysis of patients' immune systems is also important because the immune system needs to have a minimal level of function in order to be recruited as an anti-cancer agent. [75].

> References


A. Jamburidze, A. Huerre, D. Baresh, V. Poulichet, M. De Corato, and V. Garbin, “Nanoparticle-


