

The Use of Immunotherapies in Triple-Negative Breast Cancer

O'Donnell, A., Pearson AN.

Introduction

Characterized by the abnormal, uncontrolled division of cells, cancer is an increasingly common disease with thousands of subtypes. Due to gene mutations that disrupt the cell cycle, cancer cells are able to resist cell death, evade growth suppressors, and sustain proliferative life signaling, allowing them to grow rapidly without regulation. The immune system, a complex network of organs, cells, and tissues, works to fight infections and diseases like cancer while simultaneously protecting healthy cells. Normally, the immune system should be able to successfully recognize cancerous cells as foreign and induce immune-mediated apoptosis, or cell death. However, cancer cells avoid this immune response by inhibiting immune pathways, restricting antigen recognition, and impeding T cell activity – all of which are essential to the proper functioning of the immune system. Immunotherapy treatments work to counteract cancer immune evasion by stimulating and enhancing the activity of patient immune systems using monoclonal antibodies, immune checkpoint inhibitors, and adoptive cell therapies. Immunotherapies are an attractive treatment option for many cancer patients, especially those struggling to achieve results from traditional treatments such as chemotherapy and radiation. This is because, in comparison to traditional treatments alone, patients exhibit significantly greater results when administered immunotherapy alongside other treatments. In this review, we will discuss triple-negative breast cancer (TNBC) and the effects of immunotherapy treatments on its patient population. Furthermore, we will review ongoing clinical trials and discuss the future possibilities of immunotherapy use in the TNBC patient population.

Overview of TNBC

Although cancer knows no age or gender, breast cancer comprises about 30% of diagnosed cancer cases among women in the United States. TNBC, specifically, makes up approximately 15-20% of those cases [15]. TNBC is defined as a subtype of breast cancer with the negative expression of hormonal receptors for progesterone (HR), estrogen (ER), and human growth factor receptor 2 (HER2). The significant prevalence of TNBC in women can be attributed to the inheritability of the *BRCA1* and *BRCA2* genes, and the high risk of cancer their mutations impose on carriers. Acting as tumor suppressor genes, *BRCA* genes help to repair damaged DNA and prevent cancer from occurring in the body. When these genes become mutated, a result of uncontrolled cell growth produces cancerous tumors, most commonly diagnosed as breast or ovarian cancer. In fact, 55-65% of women with a *BRCA1* mutation will develop breast cancer, and 45% with mutations in *BRCA2* [9]. TNBC itself consists of 6 different subtypes, each with its own gene expression profiles and ontologies. Subtypes include basal-like 1, basal-like 2, immunomodulatory, mesenchymal, mesenchymal stem-like, and luminal androgen receptor. The heterogeneity of these subtypes makes the treatment of TNBC more complex than most breast cancers, as there is no single therapy that is completely effective across all subtypes. Additionally, TNBC is one of the most aggressive breast cancers, as well as one of the fastest metastasizing. These conditions leave advanced TNBC patients with an average life expectancy of about 12-18 months, making TNBC's expectancy one of the lowest in comparison to other advanced breast cancer subtypes. Alongside this low life expectancy, TNBC patients are extremely likely to experience relapses within the first 3 years of their diagnosis. Although people facing these expectancies are usually women under 40,

statistics demonstrate that of these women, African Americans and Hispanics are significantly more susceptible to TNBC than women of European descent [15]. Many speculations of these statistics suggest that this is due to differences in socioeconomic status, population genetics, and lack of healthcare access [16]. Still, no matter the race or age of the patient, TNBC has been proven difficult to treat due to its absence in ER, HR, and HER2 receptors. Chemotherapy, radiotherapy, surgery, and immunotherapy are treatments commonly used to eliminate the TNBC depending on its subtype in patients. Among these treatments, systemic chemotherapy is the most common, yet it poses numerous negative side effects due to frequent patient over- or under-treatment. It has also become less effective, as TNBC is commonly chemoresistant due to poor prognosis, metastasis, and recurrence [15]. These downsides present a need for alternative options like immunotherapy in the TNBC patient population.

Immune Checkpoint Inhibitors in TNBC

In the case of TNBC, immunotherapy treatments, specifically immune checkpoint inhibitors (ICIs), have proven to be significantly effective when used in combination with traditional treatments such as radiation and chemotherapy [12]. Using a patient's own immune system, immunotherapies aim to enhance or change one's immune responses to better attack cancerous cells. Immunotherapies most commonly focus on increasing T cell cytotoxic activity. In order to protect healthy cells from immune-mediated apoptosis, the immune system is highly regulated by immune checkpoints. TNBC evades the immune system by upregulating immune checkpoints such as the programmed cell death protein 1 (PD-1) pathway and cytotoxic T-lymphocyte associated protein 4 (CTLA-4) pathway [18]. Upregulation of these pathways results in the decrease of T cell proliferation and survival, and ultimately leads to poor immune response. In the PD-1 pathway, elevated levels of programmed death ligand 1 (PD-L1) suppress the function of T cells, allowing for the continuation of tumor development. TCGA analyses suggest that TNBC patients express higher levels of PD-L1 in comparison to patients with other breast cancer subtypes, with approximately 20% of TNBC samples expressing significant levels of PD-L1 [13]. Anti-PD-1 ICI treatments work to inhibit the PD-1 pathway, in turn promoting antitumor immune responses. However, only 10% of TNBC patients respond to ICI monotherapy [17]. In a preclinical trial using hematopoietic humanized mouse models (hu-CB-BRGS) containing TNBC cell lines, the efficacy of both anti-PD-1 monotherapy and combination treatment was examined. In the study, mouse models were separated into two groups, control and treated, with treated models receiving anti-PD-1 drug Nivolumab. In the monotherapy setting, treated mice expressed decreased levels of PD-L1 on humanized cells, as well as inhibited tumor growth by 61% in comparison to the control. When the administration of nivolumab was combined with OKI-179, a histone deacetylase (HDAC) inhibitor, tumor growth was inhibited approximately 77% more than untreated tumors [4]. These results demonstrate that the use of PD-1 combination therapy is more effective than monotherapy alone. Similar results also were demonstrated in the Phase Ib KEYNOTE-012 trial, using anti-PD-1 drug Pembrolizumab in both monotherapy and combination therapy settings. In this trial, monotherapy patients displayed an overall response rate of 18.5% [14]. Patients who underwent a combination therapy of Pembrolizumab and chemotherapy, however, demonstrated more

significant responses, as their median survival increased from 5.6 months to 9.7 [6]. Using radiation therapy (RT) in combination with anti-PD-1 treatments has also proven to improve patient immune responses. RT treatments induce interferon signaling [7], which aids in the regulation of cell growth and modulation of immune responses. As a result of these effects, RT can promote improved antitumor responses from T cells [2]. A single-arm Phase II clinical trial studied the combination of radiotherapy and Pembrolizumab in TNBC patients, finding that 17.6% of patients responded well with minor adverse effects [10]. Chemotherapy can also be used alongside anti-PD-1 treatments; however, patients can develop chemoresistance over the course of treatment, limiting its overall effectiveness. Another ICI used in TNBC patients is anti-CTLA-4 therapy. When bound to the B7 protein of an antigen-presenting cell (APC), the CTLA-4 protein prevents full activation of the T cell, in turn resulting in a decreased immune response. Anti-CTLA-4 drugs such as Ipilimumab and Tremelimumab block CTLA-4 from binding to the B7 protein, allowing for the activation of T cells. Similar to anti-PD-1 treatments, combination therapy using anti-CTLA-4 drugs improves immune responses significantly more than when used in monotherapy alone. A preclinical trial demonstrated the benefits of anti-CTLA-4 combination therapy in murine models, using anti-CTLA-4 and DZ-2384, a microtubule-targeting agent. In this study, DZ-2384 was administered once and followed by a bi-weekly dose of an anti-CTLA-4 drug. In comparison to the use of both treatments alone, the combination of DZ-2384 and anti-CTLA-4 significantly enhanced antitumor effects in the models. In fact, 6 of 7 mice demonstrated a complete tumor regression [3]. By eliciting enhanced T cell activity, anti-CTLA-4 and anti-PD-1 therapies can provide an overall improved immune response in patients [5], especially when used in a combination setting.

Future Possibilities of Immunotherapy in TNBC

Although anti-PD-1 and anti-CTLA-4 therapies have proven to improve outcomes in TNBC patients, clinical trials are underway to further improve the efficacy of ICI therapies, as well as pioneer alternative immunotherapy options for the TNBC patient population. Exploring the possibilities of ICI therapies, an open-label phase II trial has combined the use of RT, ICIs, and poly-ADP ribose polymerase (PARP) inhibitors in TNBC patients. For up to 2 years, or until their TNBC worsens, patients receive treatments in 3-week study cycles on a regular schedule. In cycle 1 only, patients receive RT on days 1, 2, and 3 [11]. Using high amounts of radiation in the form of beams, RT damages cancerous cells by degrading their genetic information, or DNA, in turn preventing the cells from growing and dividing. As RT is administered to the patient, Niraparib, a PARP inhibitor, is given once daily during each study cycle [11]. PARP inhibitors work against cancerous cells by inhibiting the function of enzyme poly-ADP polymerase, which is responsible for repairing damaged DNA. By combining RT and Niraparib, researchers can dramatically lessen cancer cell growth and division, in turn possibly decreasing TNBC cell amounts in treated patients. Alongside RT and Niraparib, PD-1 inhibitor Dostarlimab is administered once every cycle for the duration of 4 cycles, and lessened to once every 2 cycles beginning on cycle 5 [11]. As previously explained, PD-1 inhibitors work to elicit an

improved immune response in treated patients by blocking the upregulation of the PD-1 pathway. The combination of Dostarlimab with RT and Niraparib projects to engage patient immune systems while suppressing the growth rate of TNBC cells. However, the FDA has not yet approved the use of Niraparib in TNBC cases, and has not approved Dostarlimab for use in any diseases [11]. TNBC patients looking to enter this trial must meet a series of requirements, including, but not limited to: histologically or cytologically-confirmed TNBC, prior progression on immune checkpoint inhibitor and/or PD-L1-negative, no more than 2 prior lines of systemic therapy for an inoperable, recurrent, or metastatic disease; at least one tumor for which RT is considered clinically appropriate, at least one radiographically-confirmed metastases index lesions that will not undergo RT, available archived tissue of a metastatic tumor collected up to 28 days prior to registration, and an Eastern Cooperative Oncology Group (ECOG) status less than or equal to 1 [11]. The ECOG Performance Status Scale is a measurement used to evaluate a patient's level of functioning based on their daily activity and physical abilities. Statuses less than or equal to 1 describe a fully or significantly active individual with little to no physical restrictions [8]. Additionally, patients must not meet a number of exclusion criteria, including, but not limited to: germline or somatic *BRCA* mutation-positive status, active brain metastases, additional malignancy that progressed or required treatment in the last 2 years, prior treatment of both a PARP inhibitor and ICI, and receipt of a live vaccine within 14 days of trial start date [11]. Overall, this trial stands to push the boundaries of ICI combination treatments and their efficacy in TNBC patients by using innovative drugs alongside long-standing therapies. The trial's efforts suggest a new wave of ICI combination treatments and options within the TNBC patient population. Another phase II open-label trial has taken a different approach on expanding the use of immunotherapy in TNBC patients, using PD-1 positive tumor-infiltrating lymphocytes (TILs) rather than ICIs. TILs are T cells that have been extracted from a patient's blood, altered and enhanced to promote cytotoxic activity, and intravenously reintroduced into the patient after modification. More specifically, the TIL treatment process begins by isolating a patient's tumor-infiltrating T cells from peripheral blood cells. These T cells are then expanded *ex vivo*, treated with interleukin-2 (IL-2) to proliferate, and modified to express a receptor that will bind to the presented tumor antigen [19]. With TIL therapy, treated patients can experience improved immune-regulated responses to cancer cells. In the case of the PD-1+ TILs trial, modified T cells are referred to as NUMARZU-001. NUMARZU-001 is administered once over the course of treatment alongside concomitant medications including a saline solution, paracetamol, and dexchlorfeniramina. The use of NUMARZU-001 has not been FDA approved for use in any clinical setting. Prior to trial admission, potential candidates must undergo a series of molecular pre-screenings for select tumors with a higher enrichment probability by PD-1+ TILs. Further eligibility criteria also includes; an estimated life expectancy of at least 6 months, histologically confirmed diagnosis of advanced TNBC, and ECOG status of 0 or 1, and at least 1 resectable target lesion. Exclusion criteria for the trial includes: patients with a history or evidence of symptomatic autoimmune diseases, a history of HIV or Hepatitis B, those with clinically active cerebral metastases, and



any patient using systemic steroid therapy [1]. This trial projects to expand the use of immunotherapies in TNBC greatly, as ICIs are currently the primary option due to a lack of effective and safe alternatives. By pushing the boundaries of immunotherapies in TNBC beyond current ICI treatments, more TNBC patient demographics could qualify and benefit from immunotherapy treatments.

Conclusion

One of the most aggressive breast cancers, TNBC commonly evades the immune system by upregulating checkpoint pathways. Although immunotherapies such as ICIs can aid in the enhancement of a patient's immune system, they are not sufficient enough to be used in monotherapy settings. However, when used in combination with radiation or chemotherapy, ICIs can have a greater impact on inhibiting tumor growth than when used alone. Efforts to expand the limited possibilities of immunotherapy in TNBC continue to be made, as clinical trials examine different combinations alongside ICI, as well as the use of TILs. As the possibilities of immunotherapies in TNBC expand, the survival rate of the TNBC patient population could increase as well, as more varieties of combination therapies could allow immunotherapy to benefit a larger demographic of TNBC patients.

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