Commentary

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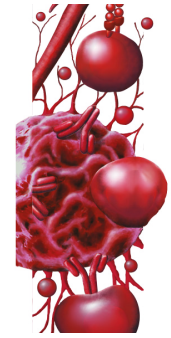
Immunotherapy



Potential role of immunotherapy for advanced breast cancer

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"The severity of advanced BC and the still incurable nature of this increasingly incident disease demand that doctors and researchers think outside of the box for the development of new therapies. Immunotherapy is a vast field with multiple possibilities of action mechanisms for new drugs."

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Breast cancer (BC) is the most common malignancy among women – in 2018 alone, 2.1 million new cases were diagnosed worldwide. Advanced BC includes both locally advanced/inoperable tumors and metastatic/stage IV tumors. The most common metastasis sites for breast malignancies are the bones (67% of advanced BCs show bone metastasis), the liver (40.8%) and the axillary lymph node chains (30–50%). The international medical literature is notoriously lacking in research on advanced BC epidemiology. However, one of the most recent studies estimated an incidence of 160,000 new cases of the metastatic disease in the USA in 2017. Experts believe that the incidence of advanced BC is much higher in middle and low-income countries, due to late tumor diagnosis owing to deficient health systems and lack of well-established screening programs [1].

Treatment options for advanced BC are limited, even in developed countries, and this is considered an incurable disease. The 5-year survival rates are estimated at 25% and are especially dismal in the subgroup of recurrent metastatic BC. The European Society of Medical Oncology's 2020 guidelines for advanced BC treatment includes recommendations such as: removal of the primary tumor in stage IV BC is not associated with prolonged survival, but can improve quality of life in specific cases; first-line therapy for advanced BC tumors that test positive for hormone receptors is endocrine therapy, consisting of drugs such as tamoxifen, an antagonist of estrogen receptors; first-line therapy for HER-2-positive advanced tumors consists of HER-2 blockers, such as pertuzumab and trastuzumab; first line chemotherapy for HER-2 negative advanced tumors is based on single-agent cytotoxic regimens, consisting of drugs such as anthracyclines and taxanes [2].

Since the aforementioned traditional forms of treatment have not been successful in further extending the survival rates for advanced BC, the development of new mechanisms of fighting this malignancy is of utmost importance. One of the most promising areas for development in this field lies in immunotherapy. Immune system evasion by tumor cells involves three main pillars: the establishment of a tumor microenvironment characterized by chronic inflammation, the triggering of lymphocyte apoptosis by the tumor cells, and the low recognition of tumor cell antigens. Regarding the first pillar, the chronic inflammation of the tumor microenvironment stimulates the production of anti-inflammatory cytokines, such as IL-10, IL-4 and TGF-β, leading to the activation of cells involved in suppressing the immune response, such as Th2 lymphocytes and regulatory T cells. The mechanism of

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lymphocyte apoptosis is based on the activation of the PD-1 present on the surface of T cells, by the expression of its ligand, PD-L1, on the surface of tumor cells. Cancer cells can also inhibit the activation of lymphocytes and lead them to apoptosis by expressing molecules that bind to cytotoxic T-lymphocyte antigen 4, an inhibitory receptor present on the surface of lymphocytes. Finally, the low immunogenicity of the tumor cells is a way of escaping recognition of tumor antigens by T cells' MHC receptors [3,4].

Each of these pillars of immune system evasion presents possibilities for emerging BC treatment modalities. On the front of decreasing T-cell apoptosis triggered by BC cells, anti-PD-1 antibodies, such as pembrolizumab, and anti-PD-L1 antibodies, such as avelumab and atezolizumab, have had their potential confirmed by several clinical trials. This potential is especially relevant in triple negative BC (TNBC), BC negative for estrogen, progesterone and HER-2 receptors, because of the more limited treatment options for this molecular subtype of BC. The KEYNOTE-086 trial obtained an overall response rate (ORR) of 23% for the antitumor activity of pembrolizumab against metastatic TNBC. The same research group found an ORR of 12% for the same drug against estrogen receptor positive BC, suggesting that the molecular characterization of the BC tumor is correlated with PD-1 activation [5]. The JAVELIN trial obtained a response rate of 5.2% for avelumab against TNBC, and Schmid *et al.* showed a 10% ORR and a 23% disease control rate for atezolizumab for TNBC [6].

The somewhat modest benefits of these antibodies can be increased by their association with traditional methods of chemotherapy. The IMpassion130 trial associated atezolizumab with the cytotoxic chemotherapy agent paclitaxel in treatment of TNBC and obtained a median overall survival of 25 months for the treated patients, against an overall survival of 15.5 for the patients who received paclitaxel alone [7]. The recently published KEYNOTE-355 trial associated pembrolizumab with standard chemotherapy in TNBC. The findings showed that patients who received chemotherapy alone had a median progression-free survival (PFS) of 5.6 months, while patients who received the associated antibody had different PFS according to the level of expression of PD-1 in the tumor cells. Patients with high PD-1 expression had a higher clinical benefit, obtaining PFS of 9.7 months, while patients with lower PD-1 expression had a PFS of 7.6 months [8]. The hope is that thoroughly researched trials with a high number of participants such as KEYNOTE-355 could bring forth more evidence to establish PD-1 and PD-L1 antibodies as first-line options for TNBC patients with high expression of these molecules.

While PD-1 and PD-L1 inhibition is relatively well documented in the literature, the role of CTLA-4 antagonists (such as tremelimumab and ipilimumab) in increasing immune response to BC cells demands more attention. Tremelimumab was associated with endocrine therapy in patients with tumors positive for estrogen receptors, and 42% of patients obtained a response of stable disease for over 12 weeks. However, five out of the 26 patients showed dose-limiting toxicities [4]. Ipilimumab is under investigation in the ICON trial, which is testing its efficacy in hormone receptor positive BC tumors in association with chemotherapy. The estimated primary completion date is in 2022 [9].

One emerging possibility for BC immunotherapy is adoptive T-cell therapy, which consists of collecting tumor-specific T cells from the patient's bloodstream, followed by an *in vitro* expansion of these cells and a reinfusion of the expanded cell population into the patient. This therapy ideally allows for a higher count of T cells that specifically attack tumor antigens, counteracting the increased apoptosis rate of T cells in the tumor environment [5].

A similar emerging therapy consists in collecting T cells from healthy subjects and exposing them to a viral vector that inserts a gene for a chimeric tumor antigen receptor (CAR) into the cell's DNA, which is known as CAR-T therapy. This treatment is well established for hematological diseases such as chronic and acute lymphocytic leukemia but is very new in treating malignancies such as BC. A 2018 study reported the synthesis of a chimeric antigen receptor with a specificity for the mucin1 protein expressed in BC cells, which was then inserted in donated T cells via a retroviral vector. These cells were also induced to express an inverted cytokine receptor, with an exodomain of the IL-4 receptor and an endodomain of the IL-7 receptor, with the purpose of increasing T-cell activation in the tumor microenvironment. The modified T cells were inserted into *in vivo* mice models, and they successfully identified the tumor antigens and underwent T-cell expansion. This caused tumor reduction and eventually tumor clearance in all of the mice for 4 weeks [10].

In another clinical trial, the antigen receptor added to the T cells was synthesized to attack c-Met (hepatocyte growth factor receptor), a growth factor receptor that is frequently amplified in BC cells and is associated with more aggressive tumor progression. In an *in vitro* model, when the ratio between these CAR-T cells and a commercial BC cell line was 1:1, the modified T cells caused the death of 40% of the tumor cells, and when the ratio was increased to 5:1, 100% of the tumor cells were eliminated. The same group conducted a Phase 0 clinical trial where they injected the c-Met CAR-T cells into patients' BC tumor tissue. The patients then underwent biopsy,

and the histopathological analysis of the tissues showed that the CAR-T cells caused tumor necrosis and increased inflammatory cell infiltration in the tumor environment [11]. The results of these studies are promising, which demands further investigation into CAR-T therapy for BC from the international research community, since there are limited experiments on this front, and most of them are still focused on animal models.

Additional efforts to increase immune response to BC tumors lie in the field of vaccine therapies. The main antitumor vaccine categories are peptide-based, dendritic cell and phage display vaccines. The peptide-based vaccines consist of BC-specific antigens, that is, molecules that are overexpressed in BC cells and not in normal body cells. These antigens are already present in the tumor microenvironment, but the vaccine greatly increases their concentration, therefore increasing the presentation of these antigens to T cells. This augments T-cell activation and immune response to the tumor and generates memory T cells that are specific to tumor antigens. One of the most promising studies regarding peptide-based vaccines was a Phase III clinical trial in which patients with stage II BC received a vaccine containing mucin-1, after receiving traditional forms of treatment. The 15-year disease recurrence rate for the vaccinated patients was 12.5%, versus a recurrence rate of 60% in patients who received traditional treatment plus a placebo vaccine ($p = 0.002$). Another peptide-based vaccine under investigation is the E75 vaccine, which contains an extracellular epitope of the HER-2 protein. The 5-year disease-free survival of patients with node-positive BC who received the vaccine was 14 percentage points higher than the disease-free survival of patients who received placebo ($p = 0.05$). These results demanded more investigation into the E75 vaccine, however a Phase III trial exploring the correlation between levels of HER-2 expression and vaccine response was unfortunately deemed 'futile' by research monitoring boards [12].

The dendritic cell vaccine group, instead of injecting free peptides into the patient which will then be identified and presented by *in vivo* dendritic cells, consists of *ex vivo* generated dendritic cells that are already presenting the tumor-specific antigens on their surface. This production of the dendritic cells can be achieved by isolating mononuclear cells from peripheral blood and culturing them in a cytokine-rich environment containing extracted BC cells. The mononuclear cells will mature into dendritic cells and incorporate the tumor cells' antigens in order to present them on their surface. Another technique for the dendritic cell vaccine is to combine the peptide tumor antigen with specific antibodies that bind to surface molecules of dendritic cells. Therefore, the vaccine does not contain the dendritic cells *per se*, but the antigen will be directly driven to these cells by the antibody [13,14]. One important study regarding dendritic cell vaccines in BC created dendritic cells presenting the Runt-associated transcription factor 2 (Runx2) antigen, which is a gene associated with tumor progression/invasion and is frequently overexpressed in TNBC. The study concluded that these dendritic cells were able to generate a satisfactory level of T-cell activation *in vitro* [15]. *In vivo* studies with dendritic vaccines are rare, and most of them are still animal experiments, but this area is certainly one to watch in the coming years.

Finally, it is worth noting recent efforts in developing phage display vaccines, which contain bacteriophage viruses that can display an array of tumor peptides for recognition by the body's immune system. Therefore, a phage vaccine is multivalent and can cause the body to react to many tumor antigens at once. Another advantage of this vaccine is that the bacteriophages have higher penetration in the tumor tissue due to the miniscule dimensions of the virus. One study has already shown the efficacy of a phage display vaccine in mice with HER-2+ BC, which correctly stimulated T-cell activation and humoral production in the animal models [16]. Viral vector vaccines have been tested in humans for clinical trials focused on other types of cancer – the TG4010 vaccine presents mainly murine antigen specific to non-small-cell lung cancer, and in a Phase II study it has been confirmed to display satisfactory safety and tolerance by the patients and has improved overall survival at the 6-month end point [17]. Current trials on viral phage vaccines focusing specifically on BC were not found through the National Health Institute's Clinical Trials registry, showcasing the prospects of BC immunotherapy that remain relatively unexplored.

The severity of advanced BC and the still incurable nature of this increasingly incident disease demand that doctors and researchers think outside of the box for the development of new therapies. Immunotherapy is a vast field with multiple possibilities of action mechanisms for new drugs. While research on drugs that act in the PD-1/PD-L1 pathway are the most advanced branch of immunotherapy in BC, other therapies such as CTLA-4 blockers, adoptive T-cell therapy, CAR-T therapy and cancer vaccines have shown promise in the fight against this malignancy.

Author contributions

All authors contributed the same to the manuscript.

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