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Review

Immune System Effects on Breast Cancer

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Abstract—Breast cancer is one of the most common cancers in women, with the ability to metastasize to secondary organs, which is the main cause of cancer-related deaths. Understanding how breast tumors progress is essential for developing better treatment strategies against breast cancer. Until recently, it has been considered that breast cancer elicits a small immune response. However, it is now clear that breast tumor progression is either prevented by the action of antitumor immunity or exacerbated by proinflammatory cytokines released mainly by the immune cells. In this comprehensive review we first explain antitumor immunity, then continue with how the tumor suppresses and evades the immune response, and next, outline the role of inflammation in breast tumor initiation and progression. We finally review the current immunotherapeutic and immunoengineering strategies against breast cancer as a promising emerging approach for the discovery and design of immune system-based strategies for breast cancer treatment.

Keywords—Antitumor immunity, Immune suppression, Immune evasion, Immunotherapy, Immune cells, immune engineering, Proinflammatory cytokines, Breast cancer progression.

INTRODUCTION

One in eight women will develop breast cancer in their lifetime. It is estimated that there will be 279,100 new cases of breast cancer and 42,690 deaths in 2020 worldwide.[102] Breast tumors have the potential to metastasize to other organs such as bone, lung, and brain,[112] which is the main cause of cancer-related deaths.[15] Now it is well established that to progress and metastasize successfully, the breast tumor remodels its surrounding tissue and creates its own microenvironment.[5] This new microenvironment, known as the tumor microenvironment (TME), includes changes in the extracellular matrix (ECM), the vasculature, and the supporting cells such as stromal cells (fibroblasts and adipocytes), immune cells, and endothelial cells,[5,6,13,117] all contributing to tumor progression.

Breast cancer has traditionally been considered one of the cancers with the least immune responses.[37,103] Recently, in the last 20 years, there has been an increase in the literature for studies on the immune effects on breast cancer. This has caused a more thorough understanding of the immune system and how it interacts with breast cancer. There are many cells and cytokines involved in the breast cancer immune environment, some with antitumorigenic roles, others with protumorigenic or immunosuppressive functions. These cells and cytokines can also promote the progression of breast cancer through chronic inflammation. Investigating how the immune system functions and affects breast cancer is useful for designing treatments to utilize the immune system and microenvironment to treat breast cancer. It can also enable engineering more predictive models for a better understanding of the breast cancer biology, as well as a more accurate prognosis and better treatment options for breast cancer patients. Scientists have already begun to use the immune response to breast cancer to their advantage and are creating immunotherapies to perform various tasks such as vaccinating against breast cancer, helping enrich the immune system to fight breast cancer, or working in conjunction with chemotherapy to reduce the breast cancer mortality.

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This comprehensive review seeks to provide a glance at the whole tumor immune microenvironment in the case of breast cancer by looking at the ways the immune system attacks the tumor, the methods through which the tumor evades the immune response, the role chronic inflammation plays, and strategies used to combat breast cancer. To this end, first, we give a brief introduction on the antitumor immunity and the immune cells involved in antitumor response, then we continue with how the tumors suppress and evade the immune system and give an overview of the cells involved. Next, we explain how inflammation supports cancer progression. Finally, we give brief information on immunotherapy and immune engineering, where state-of-art treatment strategies and engineered models of the immune system are discussed.

ANTITUMOR IMMUNITY

Antitumor immunity is one of the body's first line of defenses against tumors. In this section, we start by reviewing the immune cells that generate an antitumor response against breast cancer. Innate immune system cells, such as natural killer (NK) cells, attack the primary tumor. These cells have also been thought to prevent neoplasms from developing into fully formed tumors.[25] Cytotoxic T-cells are adaptive immune cells that also attack the primary tumor. However, some adaptive immune cells, such as B-cells may have dual roles in the breast tumor immune microenvironment, where they either promote tumor progression or prevent it. A summary of the cell types discussed, and their actions are shown in Fig. 1. The cells referenced in this section are meant to highlight the antitumor immunity function of the specific cells.

Cytotoxic T Lymphocytes (CTLs)

Cytotoxic T lymphocytes are $CD8^+$ cells that play a role in the antitumor immune response. First, an antigen presenting cell (APC), in this case a tumor cell, presents an antigen on its surface that activates the CTLs. The CTLs respond first through clonal expansion, then through lysing the target cell. CTLs act through Fas/FasL binding at the surface of the target cell, which drives the target cell to apoptosis.[44]

It has been shown previously that elevated levels of CTLs correspond to a better prognosis and better overall survival in triple negative breast cancer[23,104] as well as other types of invasive breast ductal carcinomas.[84] CTLs, T-cells, and B-cells that have infiltrated into the tumor, tumor infiltrating lymphocytes (TILs), are found more often in higher grade ductal carcinoma *in situ* and in more invasive carcinomas.[113] Overall, an increase in TILs have been proven to have a better prognosis in breast neoplasms.[23] Many researchers are currently developing immunotherapeutic strategies based on the use of CTLs as they have more specific targets and are good at killing breast cancer cells and cancer stem cells.[23]

Natural Killer (NK) Cells

The NK cells interact with a variety of surface receptors on target cells to distinguish between the self and non-self. This interaction allows the NK cells to find and destroy enemies, while avoiding tissue damage. Upon activation, NK cells produce a number of inflammatory cytokines such as CCL5 and XCL1 to crosstalk with T-cells and dendritic cells and initiate an immune response. The presence of these cytokines has been linked to increased cancer patient survival.[11] The NK cells, typically, take action through releasing cytotoxic granules to kill the target cells.

The NK cells work to eliminate early tumors and early metastases,[76] but the role of NK cells within breast cancer has not been fully elucidated. Various studies[87,91] have shown that the NK cell number does not increase in breast cancer as compared to normal breast tissue, but the number of lymphokine-activated killer cells, a population of cells consisting of NK, natural killer T cells, and T cells, activated by lymphokines to kill tumor cells, is increased. On the other hand, in advanced breast cancer, studies showed that the NK cell activity is decreased,[8] suggesting that NK cells are active during initial breast tumor development.[27] NK cell dysfunction has also been connected to breast cancer progression, indicating that as the disease progresses, the breast cancer cells decrease the activation of NK cells in order to promote immune evasion.[74]

B-Cells

B-cells are another cell type involved in adaptive immunity. They act by secreting antibodies or immunoglobulins as well as various cytokines. Additionally, the receptors on the surface of the B-cells, surface immunoglobulins, allow the cell to attach to specific antigens and neutralize them. B-cells normally reside in the secondary lymphoid organs, and upon activation they travel to the specific target site.

B-cell's role in breast cancer is bilateral, as they secrete cytokines that inhibit the antitumorigenic CTLs and NK cells, but have also been found to infiltrate the tumor and promote antitumor immunity.[96] The immunoglobulins they secrete may reduce early neoplasms.[25] An increase in B-cell count has also been linked to an improved breast cancer prognosis.[2,36,116]

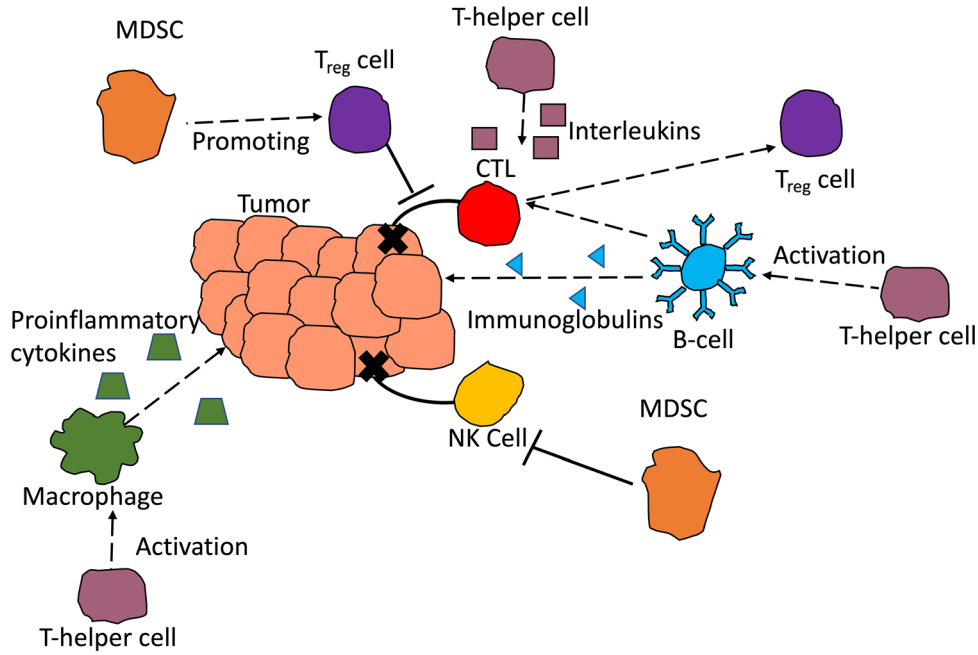

FIGURE 1. A graphical representation of the cells discussed and their actions on breast tumors. Cytotoxic T-Lymphocytes (CTLs) and Natural Killer (NK) cells directly act on the breast cancer cells and promote antitumor immunity through destroying the cells. B-cells promote antitumor immunity by secreting immunoglobulins that reduce early neoplasms. They promote tumor progression through acting on CTLs and transforming them into T_{reg} cells. T_{reg} cells act by inhibiting CTLs to promote tumor progression. T-helper cells secrete interleukins that promote a proinflammatory environment, activate B-cells and macrophages, and promote CTL anergy. Macrophages secrete proinflammatory cytokines that promote tumor progression. Myeloid Derived Suppressor Cells (MDSCs) promote T_{reg} cell population and suppress NK cells.

Despite their role in reducing early neoplasms, B-cells may also lead to breast cancer progression and metastasis. Olkhanud *et al.* showed that B-cells convert $CD4^+$ or T-helper cells to regulatory T-cells (T_{regs}), which mediate breast cancer metastasis.[81] Inactivation of B-cells has been proven to inhibit metastasis of breast adenocarcinoma cells in a mouse model.[60] These findings suggest that B-cells work towards anti-tumor immunity in the beginning stages of the breast tumor, but once the tumor progresses, B-cells begin to be pro-tumorigenic and promote metastasis.

IMMUNE SUPPRESSION AND EVASION

There are many factors that contribute to breast tumor's capacity to evade the immune system and eventually suppress the antitumor response. One factor is the immune cells' inability to identify the tumor as "non-self" or "foreign", which allows the tumor to escape antitumor activity. As discussed previously, there are multiple different cell types with bilateral roles in breast tumor progression. In this section, we briefly explain the immune cells involved in immune suppression and evasion, and thus breast tumor progression. The cells referenced in this section are meant to highlight the immune suppression and evasion functions of the specific cells.

Regulatory T-Cells (T_{regs})

Regulatory T-cells (T_{regs}), that regulate the immune response by maintaining "self-tolerance", are one of the most prevalent cell types when it comes to immune suppression and immune evasion, although they account for a low percentage of the overall T-lymphocytes.[25] "Self-tolerance" ensures that the immune system is not attacking the body itself. T_{regs} can inhibit the function of $CD8^+$ CTLs as well as the response of T-helper cells, DCs, NK cells, and B-cells[3,16,65,90,108] in order to limit the attack on self. T_{regs} also promote "self-tolerance" by inhibiting effector cytokines like interleukin (IL)-2 and by producing immunosuppressive cytokines like IL-10.[20,24,109] T_{regs} are $CD4^+$, $CD25^+$ and can be identified by the expression of FOXP3. T_{reg} populations have also been shown to diminish in the case of autoimmune disorders to reduce the immune response against the self.[32]

It has been proven in breast adenocarcinomas and in spontaneous mouse models of breast cancer that there is an increase of T_{regs} in the TME.[54,71] This corresponds to a poor prognosis with shorter recurrence-free survival and a decrease in overall patient

survival.[7,77] The ratio of T_{regs} to CTLs has also been shown to be a predictor of adverse outcomes in patients, with worse outcomes and shorter disease-free survival when T_{reg} to CTL ratio is higher.[23] The increase in this ratio is associated with the molecular subtype of breast cancer, with the largest T_{reg} to CTL ratio coming from triple negative breast cancer. These T_{regs} are members of the T-cell population that infiltrates the breast tumor.[38,70] Once they have infiltrated into the breast tumor, they start to expand, which then suppress the function of other effector immune cells so as to help the breast tumor evade the antitumor immune response. The percentage of T_{regs} was even shown to increase with increasing disease stage in breast cancers from normal tissue to DCIS to invasive carcinoma,[7] proving that as the breast cancer becomes more invasive, the TME expands the T_{reg} population. It is thought that an increase in the production of prostaglandin E2 from tumor cells and production of CCL22 from tumor-associated macrophages (TAMs) leads to increased T_{reg} population.[21,22,28,40] As the breast tumor proliferates and becomes more invasive, the production of these cytokines increases, which again leads to immune evasion.

T-Helper Cells

T-helper cells are members of the adaptive immune system as they cannot mount an immune response until they have been activated through an APC. T-helper cells activate B-cells, macrophages, cytotoxic T-cells, and other cells involved in the adaptive immune response. T-cells have the potential to differentiate into T-helper 1 (Th1), T-helper 2 (Th2), T-helper 17 (Th17), or T follicular helper (Tfh) cells upon stimulation. The different types of T-helper cells secrete different cytokines in response to an insult. Th1 cells secrete IFN-γ and TNF-α which activate macrophages or CTLs, but can also favor immune evasion.[14] Th2 cells secrete interleukins that activate antibody production in B-cells. Th17 cells primarily produce IL-17, while Tfh cells mainly mediate the B-cell response.

Because the T-helper cells can activate various other cells in the adaptive immune system, they are an important mediator in breast cancer antitumor immunity and in immune evasion. Namjoshi *et al.* showed that the cytokines produced by Th1 cells promoted apoptosis as well as the loss of the HER-2 oncodriver in breast cancer, displaying the role Th1 cells could play in antitumor immunity.[79] Conversely, the presence of T-helper cells within the TME correlates with breast tumor progression, metastasis, and an increase in tumor diameter.[18,55] The same study[14] also proved that the ratio of T-helper cells to CTLs was important, with a greater ratio of T-helper cells to CTLs corresponding to an increase in breast tumor progression, metastasis, and tumor diameter. The interleukins secreted by the Th2 cells decrease T-cell cytotoxicity and mediate T-cell anergy, leading to immune suppression.[83] Fu *et al.* also reported that in postoperative breast cancer patients, the circulating T-helper cells showed immunosuppressive properties.[33] These T-helper cells were linked to poor prognosis in these postoperative patients. Gruber *et al.* showed a correlation between the number of T-helper cells and circulating tumor cells, which both were associated with higher grade breast tumors.[42] T-helper cells may also promote both antitumor immunity and immune evasion.

The role of Th17 and Tfh within the immune response to cancer has not been fully elucidated. Th17 cells have been shown to have both a protumorigenic effect and antitumor immunity.[45] Il-17, secreted by Th17 cells, decreases with increasing tumor progression, while Th17 cell content was consistent for healthy women, pre-, and post-operative breast cancer patients.[4,34] While the Th17 content did not change between patients with or without breast cancer, the ratio of Th17 cells to T_{regs} in TILs was increased in early breast cancer and decreased in advanced breast cancer, proving that as breast cancer progresses, less Th17 cells and more T_{regs} infiltrate the breast tumor.[110] Th17 cells promote tumorigenesis through secreting angiogenic and anti-apoptotic factors.[45] Th17 cells also show antitumor immunity through cytotoxic activity and expressing MHC antigens.[45] Tfh cells have been studied even less in the case of breast cancer. Tfh cells were found to activate B-cells in immune therapies.[47] In one study, when Tfh cells were detectable in breast tumors, there was an increase in patient survival,[43] however, another study has shown no differences in the levels of Tfh cells in breast cancer patients as compared to healthy women.[121] The full extent of Th17 and Tfh cells' response to breast cancer is still under investigation, as conflicting reports of their function and presence within breast cancer leaves their function yet to be discovered.

Macrophages

Macrophages are effector cells within the immune system that use phagocytosis to digest various targets such as cellular debris, cancer cells, and other foreign substances. There are two different subclasses of macrophages entitled M1 and M2 macrophages. M1 macrophages are more proinflammatory while M2 macrophages are more anti-inflammatory. Macrophages change their phenotype based on the immune response needed and the microenvironment. They may

promote antitumor immune response, as well as tumor progression.

The first response of macrophages to a neoplasm is from the tissue-resident macrophages that secrete various factors in order to draw other immune cells like NK cells, CTLs, and DCs to the site of action to destroy the neoplasm.[25] If the defense is prolonged and becomes chronic, the macrophage response can also become protumorigenic. With chronic infiltration of macrophages, the macrophages secrete cytokines and factors that contribute to tumor cell survival, TME remodeling, and immune suppression.[25]

TAMs, macrophages that reside in the TME, are altered by the tumor in such a way that they help with tumor development. In a mouse model of breast cancer, increased tumor progression was reported when macrophages infiltrated into the mammary tissue,[73] while decreased tumor development as well as decreased metastasis was reported when macrophages did not infiltrate into the tissue.[68] Macrophages also regulate vascular endothelial growth factor levels, which controls angiogenesis.[67] In an early neoplasm, typically the M1 macrophages invade into the breast tumor and secrete proinflammatory cytokines, which draw other cells to the tumor site. If the macrophages cannot kill the tumor, the proinflammatory cytokines will convert the M1 macrophages into TAMs to promote tumor progression. In the late stages of breast cancer, the TAMs transition to M2 macrophages and secrete other cytokines to drive T-cell differentiation to Th2 cells, as well as immune suppression by $T_{regs.}$[10] Those breast tumors with high number of M2-TAMs have a higher tumor grade and a lower survival.[53]

Specifically, within the breast tissue, adipocytes only further aggravate the macrophage response to promote tumorigenesis. The macrophages within the breast tissue form a crown-like structure (CLS) around dead or dying adipocytes. The macrophages phagocytose the dying or dead adipocytes, and thus become lipid-loaded ''foam'' cells that release a number of proinflammatory cytokines.[101] Increased inflammation within the adipose tissue of the breast has been linked with a shorter recurrence-free survival post-mastectomy,[49] and with an increase in CLSs caused by macrophages, corresponding to a reduced survival in breast cancer.[58] Macrophages have also been shown to help disseminate early breast cancer cells and promote early metastasis.[69]

Myeloid-Derived Suppressor Cells (MDSCs)

Myeloid-derived suppressor cells (MDSCs) are another immune cell population that is involved in regulating the immune response in a healthy individual as well as in response to various diseases and infections. This population of cells mainly consists of myeloid progenitor cells, and immature dendritic cells, granulocytes, and macrophages. The MDSC population expands in response to various factors, most of which are present at high levels in the TME. MDSCs promote T_{regs} which suppress the antitumor immune response, while reducing the activity of CTLs. They interact with and affect other cells through cell-to-cell contact, reactive oxygen species production, and multiple other mechanisms. MDSCs have also been shown to suppress DCs and NK cells which have antitumor response.[75] As a result, MDSCs also contribute to immune suppression and evasion specifically within the breast tumor environment. An increase in MDSCs in patients with metastatic breast cancer showed worse outcomes and lower overall survival,[19] while inhibiting MDSCs improves outcomes and increases survival.[75] MDSCs have also been correlated with breast cancer progression and metastasis.[9]

INFLAMMATION

Inflammation can cause irreparable damage within the body and is typically protumorigenic. Inflammation is caused by various immune and non-immune cells secreting inflammatory cytokines. It also helps with the recruitment of more immune cells to the inflamed tissue. There is both acute and chronic inflammation. Acute inflammation is usually seen in the case of an environmental insult such as injury and infection and lasts a shorter period of time. Chronic inflammation is more persistent and typically does not show symptoms of inflammation. Chronic inflammation can cause a lot of damage through various lymphocytes actually attacking the healthy tissue. This occurs frequently in the breast tissue. In this section, we review the immune cells that secrete proinflammatory cytokines and the resulting consequences these cells have on breast cancer.

Immune Cells Secreting Inflammatory Cytokines

Neutrophils, macrophages, NK cells and T-helper cells all secrete proinflammatory cytokines that contribute to the inflamed breast environment. Macrophages are one of the main mediators of inflammation. They secrete a whole suite of inflammatory cytokines such as tumor necrosis factor (TNF), IL-1, IL-6, IL-8, IL-23, and IL-27.[114] IL-6 is an important mediator of breast tumorigenesis through increasing inflammation. T-helper cells recruit the macrophages to the target tissue by producing IFN-γ and TNF-α, as mentioned earlier, which promote an inflammatory environment. Neutrophils also release IFN-γ as well as other

proinflammatory interleukins.[106] NK cells also secrete proinflammatory interleukins as well as granulocyte-macrophage colony-stimulating factor (GM-CSF), IFN-γ, and TNF-α, which, again, lead to recruitment of macrophages to the target site. The inflammatory environment can promote proliferation and expansion of the breast tumor cells and cancer stem cells, as well as angiogenesis.

Proinflammatory Cytokines

There are many different cytokines, mainly produced by macrophages, that contribute to a proinflammatory environment within the breast tissue. Proinflammatory cytokines typically are protumorigenic and can even induce chemoresistance.[17] Interleukins are a primary mediator of the inflammatory environment. IL-6 is one of the strongest players involved in chronic inflammation and tumorigenesis. It has been shown that IL-6 creates a feed-forward loop with inflammation that expands the cancer stem cell population.[57] IL-6 can also inhibit apoptosis, which benefits breast cancer cell survival.[12,62] In addition, IL-6 promotes inflammation, which allows the breast tumor to proliferate and have increased angiogenesis. The advancement of inflammation leads to increases in IL-6 in this feed-forward loop, which is further supports tumor progression. Some interleukins such as IL-12 cause tumor regression through decreasing angiogenesis and remodeling. These cytokines have been used for cytokine therapy to help activate the immune system to promote tumor regression.

TNF-α is another cytokine that contributes to inflammation and, therefore, increases tumorigenesis. TNF-α does this through multiple mechanisms such as interacting with the apoptosis and NF-kB pathways to activate inflammation,[118] driving remodeling of blood vessels, and inducing angiogenesis,[98] all which contribute to tumor progression. TNF-α has also been shown to increase matrix metalloproteinase (MMP) expression and correlate with disease progression in breast cancer.[59] An increase in MMP expression leads to increased breast tumor invasion and progression.[80] TNF-α creates an inflammatory phenotype within the cancer associated fibroblasts and mesenchymal stem cells that promote tumor progression through releasing other proinflammatory cytokines.[52] In summary, TNF-α promotes breast cancer progression through inducing a proinflammatory environment with other cells and cytokines.

Other interleukins such as IL-1β, IL-23, and IL-27 are also important proinflammatory cytokines. IL-1β promotes breast tumor growth and metastasis through a proinflammatory and proangiogenic environment.[46] IL-23 also correlates with a negative prognosis for breast cancer but does this through expanding and maintaining a subset of the T_{reg} cell population.[35] IL-27 is similar to IL-1β in that it supports tumor growth and metastasis through a proinflammatory and proangiogenic environment when it is upregulated in the case of breast cancer.[72] Important cytokines and their effects on breast cancer are summarized in Table 1. It should be noted that, these are only a summary of a few of the many proinflammatory interleukins and proinflammatory cytokines that play a role in breast cancer proliferation, invasion and metastasis. A thorough review of the role of proinflammatory cytokines in breast cancer can be found elsewhere.[39]

TABLE 1. Proinflammatory cytokines and their effect on breast cancer.

Cytokine	Effects	Sources
TNF-α	Activates inflammation, induces angiogenesis, increases MMP production, creates inflammatory environment within stromal cells	52,59,80,98,118
IFN-γ	Promotes inflammatory environment, recruits macrophages to the tumor site, can inhibit the macrophage from processing the tumor antigen	61
GM-CSF	Proinflammatory cytokine, activates macrophages to TAMs, associated with EMT and poor prognosis	105
IL-1β	Creates proinflammatory, proangiogenic environment	46
IL-6	Creates a proinflammatory environment, expands cancer stem cell population, inhibits cancer cell apoptosis	12,57,62
IL-8	Promotes angiogenesis, promotes cancer cell growth and survival, promotes breast cancer cell invasion	107
IL-12	Anti-angiogenic, decreases VEGF and MMP levels, causes breast cancer regression	26
IL-18	Increases immunosuppression, promotes migration and invasion	64,82
IL-23	Expands and maintains subset of T_{reg} cell population	35
IL-27	Creates proinflammatory, proangiogenic environment	72

IMMUNOTHERAPY

Current treatments for breast cancer include surgical intervention, radiotherapy, chemotherapy, and hormone replacement therapy. These are usually applied to non-invasive or non-metastatic breast cancers. A method that would address invasive and metastatic breast cancers is still missing. Immunotherapy is one promising approach to tackle this need. There are multiple different techniques for immunotherapy. One of the first methods of immunotherapy created was cytokine therapy which uses cytokines to activate certain immune cells to antitumor immunity. Some of these have shown promising results on invasive or metastatic breast cancers, while others have shown an enhanced effect when used in conjunction with chemotherapy.[120] Many of these immunotherapies are experimental and are in the process of clinical trials for efficacy and safety but show positive outcomes for the treatment of breast cancer. These methods of immunotherapy are displayed in Fig. 2. This section will cover four of the main treatments being studied for immunotherapy of breast cancer. These immunotherapies have been used as building blocks, with each method building on the base of immunotherapy knowledge and the studies within each method building on each other to better enhance the immunotherapy.

Antibody-Based Immunotherapy

Antibodies can bind to the antigens present on the surface of the tumor cells and mark them, which allows the immune system to recognize and destroy the tumor through a variety of immune responses such as phagocytosis, tumor lysis, inhibiting the invasion and spread of the tumor, or apoptosis that occurs as a result of the immune response. Antibody-based immunotherapy typically focuses on developing antibodies that target antigens specific to a particular cell type, in this case, breast cancer cells. Antibodies have recently been created to target certain cytokines or cytokine receptors to inhibit immune functions or target various points in the immune checkpoint to help inhibit immune evasion by the tumor.[120]

The main antibodies that have been tested for breast cancer have been monoclonal antibodies for HER-2$^+$ breast cancers. The monoclonal antibody tratsuzumab increased the survival in patients with metastatic breast cancer and had a response rate of 23.1% in patients with HER-2 overexpressing breast cancer.[50,63,93,100] This response was only increased when combined with

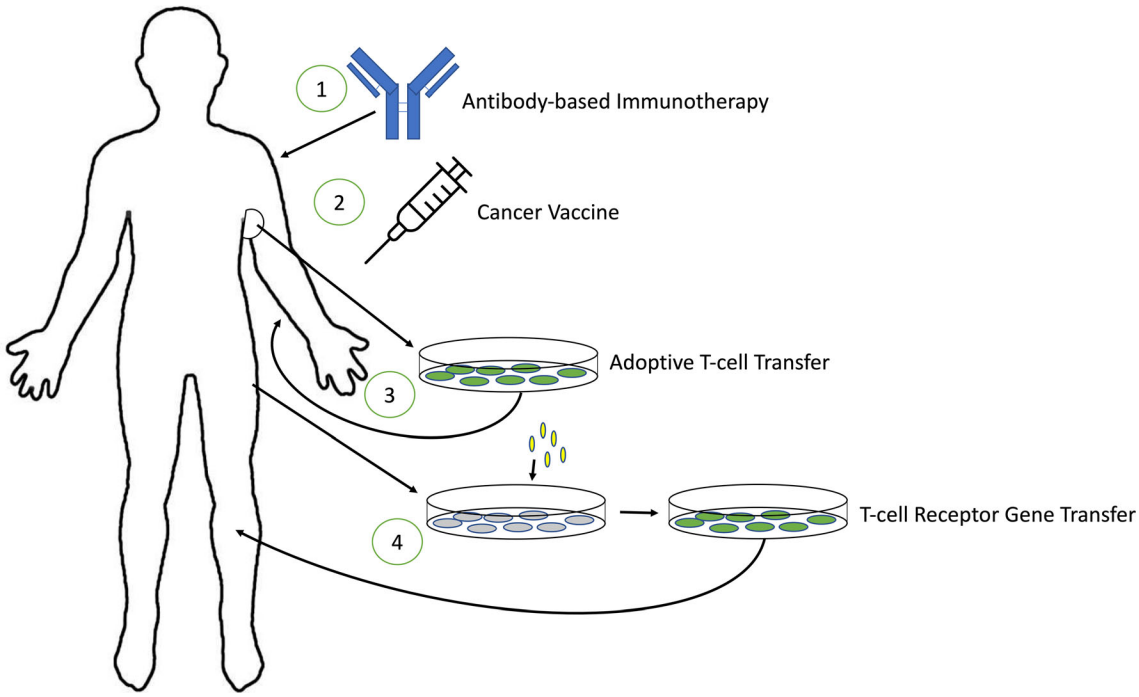

FIGURE 2. Graphical representation of four immunotherapy methods discussed in this review. (1) Antibody-based immunotherapy: antibodies are created to target the specific tumor and can be used to target drugs to the site or target specific cytokines. (2) Cancer vaccine: peptides, DNA, or other proteins are injected into the body to activate CTLs to lyse the tumor cells. (3) Adoptive T-cell transfer: a portion of the tumor is resected, and the TILs are cultured from this resection and implanted back into the body to lyse the tumor cells. (4) T-cell receptor gene transfer: T-cells are removed from the body and the TCR gene is transferred to one that can recognize a specific breast cancer antigen. These cells are then implanted back into the body to lyse the tumor cells.

chemotherapy. Another antibody being tested is a monoclonal antibody against the VEGF receptor which showed a 19.1% response rate by itself and a 30.2% response rate in combination with chemotherapy.[50] Antibodies are an attractive treatment option as they are more specific than general chemotherapy and can, therefore, mitigate some of the off-target effects. Antibodies could even be used as a targeted drug delivery system to make a drug more specific. There has been some success with targeted antibodies atezolizumab, which targets a transmembrane protein PD-L1 found in breast cancers, and pembrolizumab, which targets another cell surface protein PD-1 found in breast cancers, against triple negative breast cancers.[30] The use of these antibodies against triple negative breast cancer shows promise that antibody-based immunotherapies can be developed for more than just HER-2$^+$ tumors.

Cancer Vaccine

Cancer vaccines use CTLs to help kill the tumor cells. This method uses a variety of different tumor specific antigens, such as peptides, DNA, and proteins, to activate the CTLs to lyse the tumor cells expressing that specific antigen. Currently, there are only a few breast cancer specific antigens: HER-2/neu, MUC-1, or NY-ESO-1[1,85,111] The limited number of available antigens has made it challenging to develop a breast cancer vaccine. However, many more antigens are currently being investigated for the development of a vaccine.

A study showed that 89% of the patients with HER-2 overexpressing breast cancer developed a T-cell immunity in response to the breast cancer vaccine and 82% developed immunoglobulin G antibody immunity, but no clinical response such as tumor lysis or improved survival was observed.[89] There have been no promising results from the trials of the NY-ESO-1 breast cancer vaccine. The only breast cancer vaccine that has shown an effect is MUC-1, although not very promising. In a trial containing patients with metastatic breast cancer, only two out of 28 had a partial regression of the tumor in response to the MUC-1 breast cancer vaccine.[95] Again, many of these patients had an increase in antigen-specific immune response, but this did not translate to a clinical response.[56] There has also been a vaccine developed to stimulate T-cell activation, but the T-cell response has been low with little to no clinical response.[29] After more thorough testing and identifying more breast cancer antigens, cancer vaccines could be a viable immunotherapy option in the future.

Adoptive T-Cell Transfer

Adoptive T-cell transfer has been shown to be an effective method for immunotherapy, especially in the case of metastatic cancer. Adoptive T-cell transfer works by resecting a portion of the primary tumor and generating the anti-tumor T lymphocytes. These anti-tumor T lymphocytes are then expanded *ex vivo* and activated. After expansion and activation, the anti-tumor T lymphocytes are infused back into the patient's body to elicit an anti-tumor immune response. This immunotherapy has been achieved with great success in the case of melanoma patients who showed a reduction in the metastatic tumors as well as some patients that achieved regression of their cancer.[120]

It has been shown previously that TILs are present in breast tumors, meaning that adoptive T-cell transfer could be a viable immunotherapy option. TILs were able to be cultured from primary breast tumors in 15 out of 19 patients.[97] These cultures were primarily CD4$^+$ T-helper cells with only 21% being CTLs, meaning the cultures had poor lysis capabilities. The TILs lysed the autologous tumor in one out of 12 patients with low lysis and low specificity. Other labs have shown that anti-tumor TILs with tumor reactivity can be generated from primary tumor tissues.[29] A clinical trial found that 12 out of 81 patients treated with adoptive T-cell transfer survived over 5 years.[51] More research into adoptive T-cell transfer needs to be done before it can be considered as an effective immunotherapy for breast cancer.

T-Cell Receptor Gene Transfer

There are two problems related to the adoptive T-cell transfer: (i) not all patients will have the correct size and accessible tumors, and (ii) only 50% of TIL cultures can generate the specific anti-tumor TILs necessary for this treatment. In order to work around these issues, T-cell receptor (TCR) gene transfer is also being studied. The T-cell is activated through the TCR, which is necessary for specific antigen recognition. Hence, researchers could, theoretically, transfer the specific gene for a TCR to recognize a specific antigen, such as one pertaining to breast cancer. There has been some success with generating specific T-cells that target and kill the melanocytes containing the antigen recognized due to TCR gene transfer[94] in the case of melanomas.

In the case of breast cancer, researchers have constructed a chimeric gene of the HER-2/neu monoclonal antibody with the TCR in order to express the TCR with HER-2/neu antigen specificity. This was successful with the cells releasing cytokines upon recognition of HER-2$^+$ breast cancer cells and primary tumors.

Cells with the TCR gene transfer also lysed the target cells *in vitro* with specificity to the HER-2/neu antigen.[41] Currently, this is the only work that has been done with HER-2^+ cancers, so it will need to be researched if this treatment could be applied to other types of breast cancer. Early results are promising for TCR gene transfer as an immunotherapy, but there are many more clinical trials needed before this will be considered for immunotherapy.

IMMUNE ENGINEERING

Immune engineering, as it relates to breast cancer, is a large field that ranges from machine learning for better breast cancer diagnoses to engineering immune cells for targeting breast cancer. There have been many recent developments in immune engineering. For example, Sahan *et al*. used machine learning to create a "fuzzy" artificial immune system to better model breast cancer diagnoses.[92] They engineered a method to quantitatively capture immune system cells such as B-cells, T-cells, and macrophages through interactions with antigens, cell size, cell shape, and other factors. Polat *et al*. made the Feature Select Artificial Immune Recognition System to better diagnose breast cancers.[86] This is an algorithm that uses an artificial immune system to help make diagnostic decisions based on previous data. Shafiee *et al*. invented a tissue engineered bone model to investigate the effects of the immune system on breast cancer metastasis.[99] Mice models have also become more sophisticated through implanting human immune cells and human cancer cells within the mice in order to study tumor-immune effects.[48]

There have been recent advances in the field of engineering micro and nanoparticles for immune suppression or stimulation for a therapy to be more effective.[78] Recently, researchers have also been using particle-laden cells to target for anti-cancer drugs. Zhao *et al*. treated lung metastases with erythrocytes attached to chemokine encapsulating nanoparticles.[119] This allowed more effector immune cells to infiltrate into the lung metastasis and showed greater animal survival. This could provide a much-needed therapy in the future for breast cancer metastases to the lungs. PEGylated bilirubin nanoparticles were even created to increase the effectiveness of immune-chemotherapy in breast cancer.[115]

Escobar *et al*. used genetic engineering to introduce IFN-α to hematopoietic stem cells that will differentiate into macrophages.[66] This approach was able to limit tumor progression and metastasis in mouse models of breast cancer. T-cells have long been an attractive cell for immune engineering purposes. Another method for immune engineering has been the development of chimeric antigen receptor (CAR) T-cells. Researchers create chimeric antigen receptors for specific breast tumor antigens and transfer these receptors to the T-cells. CAR-T cells directly interact with these external antigens. Once activated within the body, the CAR-T cells will lyse the target tumor cells presenting these antigens. These CAR-T cells are engineered to target specific breast cancer cells and there have been many new developments with using these cells for breast cancer therapy.[31] CAR-NK cells have also been investigated as another potential therapeutic for breast cancer, although most of the trials have been preclinical.[88]

As the immune system is one of the most complex systems in the body, and some cells may have both antitumor and pro-tumor effects, care must be taken to avoid unwanted outcomes that could lead to tumor progression instead of treating the tumor. For example, B-cells have anti-tumor effects against new neoplasms, they may contribute to tumor progression in the later stages of cancer. Therefore, therapeutics should be applied at the right time, stage, and circumstances.

CONCLUDING REMARKS

The immune system plays a dynamic role in the prevention and progression of breast cancer. Cells such as CTLs and NK cells promote antitumor immunity through targeting breast cancer cells. T_{regs}, macrophages, MDSCs, and T-helper cells aid in the progression of breast cancer through various mechanisms such as inhibiting the function of cytotoxic T-cells, secreting proinflammatory cytokines, promoting metastasis, and others. B-cells may have an antitumorigenic role through releasing tumor-neutralizing antibodies, and a protumorigenic role through suppressing antitumor immunity. Inflammation can cause serious damage to the breast tissue and make it easier for the progression of breast cancer through the increase of proinflammatory cytokines such as interleukins and TNF-α. Immunotherapy and immune engineering are relatively new fields with new discoveries being made constantly. These fields seek to engineer the immune microenvironment or the immune cells to either attack the cancer cells themselves or to release chemotherapeutic drugs to be able to destroy the breast tumor. There has also been work in the immune engineering field to be able to diagnose breast tumors better and give a more accurate prognosis. There is still a long way to go before many of these treatments become valid therapy possibilities, but the studies show promise to be able to engineer the im-

mune system to destroy the breast tumor and promote antitumor immunity.

This paper reviewed the complex tasks that immune cells have in relation to breast cancer. Some cells promote antitumor immunity, while others contribute to tumor progression. Other cells have bilateral roles, as they may either promote cancer or prevent it. Inflammation also plays a key part with proinflammatory cytokines also promoting tumor development. Understanding the immune effects on breast cancer can lead to better therapeutic strategies by improving the abilities of the antitumor cells or inhibiting the cells that promote tumor progression. This knowledge can also be used for creating better models of the immune system. Immune cells should be included in 3D engineered tumors to better study cancer biology and the reaction of immune system against the tumor before they are tested in animal models. Immune cells would allow researchers to create more precise and physiologically relevant breast cancer models. These models would serve as more realistic drug testing platforms, as well as tools for studying the biology of breast cancer. Incorporating immune cells into the model systems will allow for high-throughput evaluation of breast cancer drug candidates. The immune system is very complex, and it may be explored in more detail through engineered models for a better understanding of the immune system's reactions.

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CONFLICT OF INTEREST

Jensen N. Amens, Gökhan Bahçecioglu, and Pinar Zorlutuna declare that they have no conflicts of interest.

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