

Breast cancer immunology and immunotherapy: targeting the programmed cell death protein-1/programmed cell death protein ligand-1

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Abstract

Historically, breast cancer has been regarded as an immunogenic “cold” tumor. However, the discovery of immune checkpoint inhibitors has made immunotherapy becoming an emerging new treatment modality for breast cancer. This review discusses the immune system, immune features of breast cancer, and the programmed cell death protein-1/programmed cell death protein ligand-1 (PD-1/PD-L1) inhibitors used in the treatment of breast cancer. High T lymphocyte infiltration and mutation burden were observed in triple-negative breast cancer and human epidermal growth factor receptor 2 positive breast cancer. Increasing breast cancer immunogenicity and modulating the tumor microenvironment has been reported to improve the therapeutic efficacy of immunotherapy. Recent clinical trials involving PD-1/PD-L1 inhibitors monotherapy in breast cancer has revealed little efficacy, which highlights the need to develop combinations of PD-1/PD-L1 inhibitors with chemotherapy, molecularly targeted therapies, and other immunotherapies to maximize the clinical efficacy. Collectively, the immunotherapy might be a promising therapeutic strategy for breast cancer and several clinical trials are still on-going.

Keywords: Breast cancer; Immune microenvironment; Immunotherapy; Programmed cell death protein ligand-1 inhibitors; Programmed cell death protein-1 inhibitors

Introduction

Breast cancer is the most common cancer in women and globally, 2.1 million breast cancer cases were diagnosed in 2018.^[1] In China, breast cancer ranks sixth as the leading cause of cancer-related deaths and it is estimated that about 268,600 new cancer cases were reported in 2015.^[2] Over the past two decades, breast cancer-related deaths have declined due to recent advances in screening, detection, and treatment. However, metastatic breast cancer is considered to be largely incurable. The heterogeneous nature of the disease and resistance to treatment are some of the challenges influencing the success of the therapeutic process. In recent years, immunotherapy has emerged as a new treatment approach, particularly the immune checkpoint proteins cytotoxic-T-lymphocyte-associated antigen 4 (CTLA-4) inhibitor and programmed cell death protein-1 (PD-1) inhibitor which has resulted in enhanced patient survival including those with advanced cancer such as melanoma, renal cancer, and lung cancer. Although breast cancer has

traditionally been thought to be poorly immunogenic “cold tumor,” recent clinical trials involving checkpoint inhibitors either as monotherapy or in combination with local or systemic strategies have yielded promising results. However, increasing breast cancer immunogenicity and modulating the tumor microenvironment are some of the strategies needed to improve therapeutic efficacy. In this review, we discuss the immune system, immune features of breast cancer and the current immunotherapy strategies under investigation specifically focusing on PD-1/PD-L1 inhibitors.

Overview of the Immune System and Breast Cancer

The immune system plays a dual role in cancer in that it suppresses tumor growth and promotes tumor progression. In recent years, intensive research to understand the complex interaction of the immune system with cancer has increased. However, uncovering the complex relationship between the immune system and breast cancer has been quite challenging.

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The concept of the immune system protecting the host against cancer was first postulated in 1909 by Ehrlich who proposed that the host immune system suppressed tumor cells. Further, in 1970, Burnet refined the hypothesis of immune surveillance which described that genetic changes common in somatic cells led to malignancy and the host immune system identified and eliminated the changes to protect the host from neoplastic disease.^[3] However, Schreiber proposed the theory of “immunoediting”^[4] which illustrates that the host immune system not only protects the host against tumor formation but also shapes tumor fate by activating both the innate and adaptive immune systems. Cancer immunoediting comprises of three distinct phases: elimination, equilibrium, and escape.^[5] During the escape phase, the tumor has developed the ability to evade immune surveillance and there is a progressively growing tumor. This can occur due to a number of mechanisms which include the absence of antigens, the loss of major histocompatibility complex (MHC), the expression of immune inhibitory co-stimulatory receptors (PD-1, CTLA-4, and lymphocyte activation gene [LAG]-3). In addition, tumors have the ability to create a tolerant microenvironment by producing immunosuppressive cytokines (vascular endothelial growth factor, transforming growth factor- β , galectin, or indoleamine 2,3-dioxygenase [IDO]) and recruiting regulatory immune cells (regulatory T cells [Treg cells] and myeloid-derived suppressor cells [MDSCs]).

In breast cancer, different sub-types evade the immune system via different mechanisms. In hormone receptor (HR) positive breast cancer, the absence of tumor antigens and low expression of MHC-I allow the tumor not to be recognized by the immune system.^[6] In addition, estrogen plays an immunosuppressive role in the tumor microenvironment by polarizing the immune response to T helper 2 (Th2) rather than T helper 1 (Th1) effector immune response.^[7] In HER2-positive cancer cells, there exists an inverse correlation between MHC-I expression and HER2 expression.^[8] This shows that overexpression of HER2 leads to the downregulation of MHC-I. In triple-negative breast cancers (TNBC), which is reported to be the most immunogenic sub-type, the immune escape is related to the development of the immunosuppressive tumor microenvironment.^[9]

Immunogenicity of Breast Cancer

Identification of tumor antigen by the immune system triggers anti-tumor immunity and it is important in immunotherapy. Neo-antigens are newly formed antigens that arise from gene mutations, viral oncogenes or are derived from overexpressed proteins or aberrant expression.^[10] The mutational load which is the average number of somatic mutations per cancer cell is associated with antigenicity.^[9] Advancements in sequencing technology have yielded a catalog of somatic mutation.^[11]

In a study by Barroso-Sousa *et al*,^[12] 3869 breast cancer samples were evaluated and the Median tumor mutation burden (TMB) was 1.55 mutations per megabase (mut/Mb). In addition, Chinese scholars Zhuang *et al*^[13] proposed a new 381-cancer-gene panel and demonstrated a higher

median TMB of 4.03 mut/Mb after the evaluation of 196 breast cancer samples. However, the median TMB of breast cancer is lower compared to other “hot tumors” such as lung cancer and melanoma^[14] which are more responsive to checkpoint immunotherapies.^[15,16]

Breast cancer is a highly heterogeneous tumor, therefore, TMB varies significantly according to tumor sub-types. HER2-positive breast cancer shows a higher TMB and increased immune gene expression compared with HER2-negative sub-type.^[17] Estrogen receptor (ER)-negative tumors have a significantly higher tumor somatic mutation load (SML) than ER-positive tumors. Therefore, ER-positive, but not ER-negative tumors with high SML is associated with poor overall survival (OS).^[18] Other studies have shown no significant differences in TMB in ER-positive and ER-negative groups.^[19] The different outcomes may contribute to the different gene test panel and method of calculation. Barroso-Sousa *et al*'s^[12] study revealed that the TMB decreased based on the sequence of HR-/HER2+, TNBC, HR+/HER2+, and HR+/HER2- with significant differences reported. For all the sub-types, patients with low TMB had a better disease-free survival than those with high TMB.^[19] In addition, the metastatic tumor showed higher TMB than the primary tumor,^[12] and this was accompanied by accumulated genomic alterations during tumor evolution.^[20]

Some specific mutations in breast cancer are associated with high mutational loads. Defects in BRCA1 and BRCA2 result in homologous repair (HR) deficiency which is associated with genomic instability and high mutational loads.^[21] Mismatch repair (MMR) deficiency is another DNA repair mechanism which also associated with increased immunogenicity.^[22] Tumors with MMR deficiency have a promising response to immune checkpoint inhibitors regardless of their primary site. This leads to the approval of the anti-PD-1 monoclonal antibody pembrolizumab used in treating advanced or recurrent solid tumors. However, the MMR rate in breast cancer is extremely low at <1%.^[22,23] High TMB represents high rates of neo-antigens which are associated with better OS due to the use of checkpoint inhibitors in non-small cell lung cancer,^[16,24-26] However, evidence of predictive value in breast cancer remains insufficient.

Role of the Tumor-infiltrating Lymphocyte in Breast Cancer

Tumor-infiltrating lymphocyte (TILs) is linked to pre-existing anti-tumor immunity and clinical responses in breast cancer.^[27] Research demonstrates that TILs are measurable prognostic and predictive biomarkers of the response to treatment.^[28] Lymphocyte-predominant breast cancer is defined as having more than 50% to 60% lymphocyte infiltrating in the stroma, which is more common in TNBC (20%), HER2-positive tumors (16%), and rare in ER-positive luminal sub-type (6%).^[29]

High TILs level in the TNBC^[30-32] and HER2-positive^[32,33] predicts better prognosis and it has been suggested as a potential biomarker for the identification of patients who may respond well to immunotherapy.^[34] However, the prognostic value of TILs in breast cancer remains relatively

conflicting.^[35-38] For neo-adjuvant treatment, TILs are considered to be a reliable biomarker for predicting the pathological complete response (pCR) for all molecular subtypes,^[39] especially for TNBC and HER2-positive subtype.^[40,41] In addition, different subset of TILs represents different prognosis information. Increased number of CD8 positive T cells^[38] has been associated with a favorable prognosis, while increased CD4 positive T cells are debatable. This can be attributed to the CD4+ T-cell composition which is complicated as Th1, Th2, Th17, and Tregs with distinct functional characteristics.^[42] CD4-positive Th1 cells are predicted to show favorable prognosis and Th1-mediated immunity has been considered to be antitumoral.^[43] However, Th2 cells reveal an opposite function.^[44] Treg cells play a major role in the development of an immunosuppressive tumor microenvironment.^[45] However, the prognostic role of tumor infiltrated Tregs is varied in different breast cancer sub-types. Liu *et al*^[37] demonstrated that Tregs TILs were associated with a poor prognosis in ER+ breast cancer but not in HER2+/ER- subtype. However, another study showed that patients with high Tregs TILs had a significantly shorter OS and progression free survival (PFS) and high CD8+/FOXP3 ratio which was associated with improved survival.^[46]

PD-1/PD-L1 Pathway in Breast Cancer

PD-1/PD-L1 pathway is a major checkpoint inhibitor in immune responses. PD-1 is expressed on the surface of T-cells, B-cells, natural killer T-cells, monocytes, and

dendritic cells (DCs). It is activated by its ligands PD-L1, which expressed on antigen-presenting cells such as DCs, macrophages, or B cells and it is also highly expressed on tumor cells.^[47] The binding of PD-L1 to PD-1 attenuates lymphocyte activation and inhibits the immune response which is one of the tumor evasion mechanisms^[48] [Figure 1].

In breast cancer, upregulated PD-L1 on tumor cells ranges from 20% to 34% and it is heterogeneous across different sub-types. It is positively associated with young age, high grade, presence of TILs and aggressive molecular sub-types (triple negative, basal, HER2-enriched).^[49-52] The prognostic value of PD-L1 in breast cancer has been reported but with divergent results. Ali *et al*'s study^[53] revealed that PD-L1 was not associated with the outcome in either ER-positive or ER-negative cancer. A study by Muenst *et al*^[54] revealed that the expression of PD-L1 is associated with poor prognosis in breast cancer. In contrast, Baptista *et al*^[55] studied 192 breast cancer patients and demonstrated that PD-L1 expression was significantly associated with better OS. These conflicting results can be attributed to different populations, molecular sub-types and different antibodies used for IHC and the use of different scoring systems. Studies focusing on triple-negative or basal-like sub-type revealed that PD-L1 expression was associated with longer survival.^[49,56-58] This can be explained by PD-L1 being a marker of the strong cytotoxic local immune response. The gene expression profile of breast cancer shows that PD-L1 overexpression is associated with the

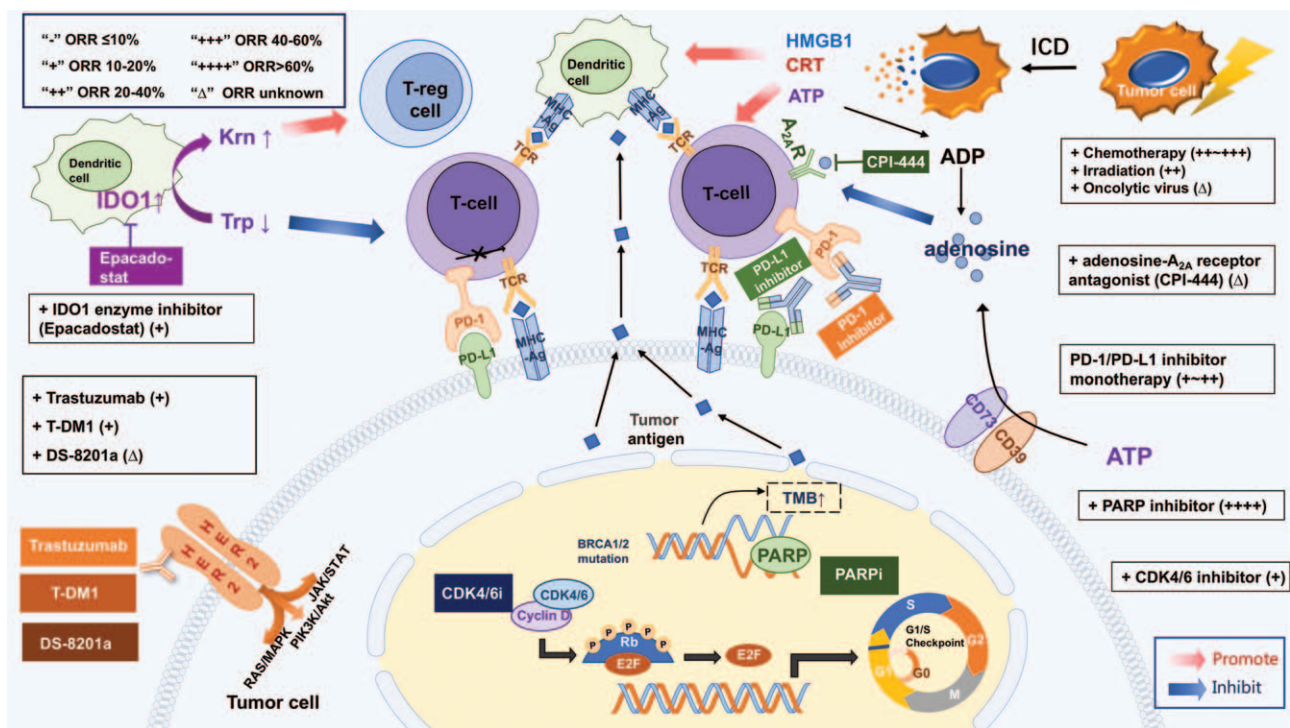


Figure 1: PD-1/PD-L1 pathway and anti-PD-1/PD-L1 agents combination strategies in breast cancer. The binding of PD-L1 to PD-1 attenuates lymphocyte activation and inhibits the immune response. A_{2A}R: Adenosine A_{2A} receptor; ATP: Adenosine triphosphate; CDK4/6: Cyclin-dependent kinase 4/6; CRT: Calreticulin; E2F: Transcription factor E2F; HER2: Human epidermal growth factor receptor 2; HMGB1: Mobility group box protein B1; ICD: Immune cell death; IDO1: Indoleamine 2,3-dioxygenase 1; JAK/STAT: Janus kinase/signal transducer and activator of transcription; Krn: Kynurenine; MHC-Ag: Major histocompatibility complex-antigen; ORR: Overall response rate; PARP: Poly (ADP-ribose) polymerase; PD-1: Programmed cell death-1; PD-L1: Programmed cell death ligand-1; PI3K3K/Akt: Phosphatidylinositol 3-kinase/protein kinase-B; Rb: Retinoblastoma; RAS/MAPK: RAS/mitogen-activated protein kinase; T-DM1: Ado-trastuzumab emtansine; Trp: Tryptophan; TCR: T cell receptor; TMB: Tumor mutation burden.

activation of immune-related pathways such as interferon (IFN)- α , IFN- γ , signal transducer and activator of transcription 3, and tumor necrosis factor (TNF)- α .^[49] All these provide a basis for the therapeutic targeted PD-1/PD-L1 pathway.

Anti-PD-1/PD-L1 Agents in Breast Cancer

Harnessing the body's immune system to fight the tumor has worked in some hematological and solid malignancies. However, as an immunogenic "cold" tumor, immunotherapy progress in breast cancer is slow. Increased research on the interaction between breast cancer and the immune system, for example, recent developments in immunotherapy especially the combination strategy encompassed PD-1/PD-L1 inhibitor gives hope that it can also be effective in breast cancer. A number of clinical trials have focused on evaluating PD-1/PD-L1 inhibitors in Breast cancer.

Anti-PD-1/PD-L1 agents in breast cancer: monotherapy

TNBC is known as a highly immunogenic sub-type breast cancer and possesses higher levels of TILs infiltration.^[39] In most of the clinical trials, metastatic TNBC patients have been considered as the study population. KEYNOTE-012 was the first phase Ib trial to evaluate the role of anti-PD-1 inhibitor pembrolizumab monotherapy in TNBC. The findings revealed in PD-L1 expression $\geq 1\%$ patients, overall response rate (ORR) was 18.5% and the median time to response as 17.9 weeks.^[59] Phase II KEYNOTE-086 trial revealed ORR of 21.4% for PD-L1 $\geq 1\%$ in an untreated cohort B and 5.3% for treated patients in cohort A. The PFS and OS was 2.1 months, 18 months in cohort B and 2 months, 9 months in cohort A, respectively. Besides, stromal TILs in both cohorts were positively correlated with ORR.^[60,61] These results suggest that pembrolizumab therapy may possess durable antitumor activity in a subset of metastatic TNBC patients with PD-L1 positive expression. Phase III randomized KEYNOTE-119 study was designed to further verify the efficacy of pembrolizumab monotherapy compared to chemotherapy in metastatic TNBC pretreated (NCT02555657) patients. Unfortunately, these results were unpublished. Besides, in phase 3 SWOG S1418/NRG-BR006 study, which is currently at its recruitment status explored the application of pembrolizumab as adjuvant therapy.^[62]

Anti-PD-L1 inhibitor activity was also investigated. In phase 1 study PCD4989g, the ORR of atezolizumab monotherapy was 10% for the entire population and 26% for the first-line treatment subset.^[63] High-levels of TILs ($>10\%$) were observed and associated with better survival. In the JAVELIN phase Ib study, another anti-PD-L1 inhibitor avelumab was investigated and showed ORR of 8.3% for TNBC sub-group.^[64] Besides the TNBC sub-type, other studies enrolled HR+/HER2- patients; however, regardless of the anti-PD-1 or anti-PD-L1 inhibitor, the tumor did not respond well.^[65]

Anti-PD-1/PD-L1 agents monotherapy does not show high efficacy in breast cancer. Although positivity for PD-L1 and TIL infiltration seems to give a better response in TNBC sub-types, whether it can be considered as a

potential biomarker in future clinical practice is controversial.

Anti-PD-1/PD-L1 agents in breast cancer: combination therapy

Since breast cancer is moderate immunogenic, the use of single-agent Anti-PD-1/PD-L1 does not show promising activity. Lack of immunogenicity and the immunosuppressive tumor microenvironment is considered to be the potential mechanism. Numerous studies have explored combination therapy where anti-PD-1/PD-L1 inhibitors are combined with other therapeutics to enhance efficacy [Figure 1].

Combined with chemotherapy

Traditionally, chemotherapy has been considered to be immune suppressive. However, pre-clinical studies have demonstrated that chemotherapy augments anti-tumor immunity and shows synergism with anti-PD-1/PD-L1 agents.^[66,67] These mechanisms include: (1) induction of immunogenic cell death (ICD) by certain chemotherapeutic drugs during tumor cell death. This involves the release of tumor antigens, pre-apoptotic exposure of calreticulin or another endoplasmic reticulum proteins on the plasma membranes, production of adenosine triphosphate and emission of high mobility group box protein B1 from dead tumor cells. All of the above promote DCs maturation and support anti-tumor T-cell cytotoxicity.^[68] Increased tumor infiltrated lymphocytes have been reported in breast cancer after neo-adjuvant chemotherapy^[69,70]; (2) chemotherapy as a cytotoxic drug which decreases the number of immunosuppressive cells such as Tregs and MDSCs in the tumor microenvironment^[71,72]; (3) chemotherapy modifies several cytokines levels and promotes tumor immunity (up-regulation of TNF- α , interleukin-2, and IFN- γ).^[73] This shows that the combination of cytotoxic chemotherapy with anti-PD-1/PD-L1 agents in breast cancer patients both at advanced and early stages needs to be investigated.

Some of the cytotoxic agents in pembrolizumab combined regimens include capecitabine, paclitaxel, and eribulin. In a phase Ib study, the ORR of capecitabine-treated and paclitaxel-treated metastatic TNBC patients was 43% and 25%, respectively.^[74] However, the same regimen reported only 14% ORR when used in another single-arm phase II study which may be attributed to the different dosage of capecitabine and the small sample size.^[75] Phase 1b/2 ENHANCE-1 trial assessed the activity of pembrolizumab combined with eribulin in 104 TNBC patients and reported an ORR of 26%.^[76] This regimen was further investigated in a randomized phase II study in 88 HR+/HER2- metastatic breast cancer patients. The ORR and median PFS with or without pembrolizumab was similar and there was no benefit from pembrolizumab in PD-L1 positive sub-group was observed.^[77] This result suggested TNBC but not luminal sub-types might be the benefit populations of immunotherapy.

The TONIC trial is a phase II study for metastatic TNBC which was conducted to evaluate different cytotoxic agents induction treatments such as doxorubicin, cyclophospha-

mide, cisplatin and no induction treatment before the administration of nivolumab which is another PD-1 inhibitor. The findings revealed that doxorubicin followed by nivolumab was the most efficient induction strategy with an ORR of 35%.^[78]

Atezolizumab combined with nanoparticle albumin-bound paclitaxel (nab-paclitaxel) regimen indicated some role of immunotherapy in breast cancer, by showing an ORR of 54% as a first-line treatment in the phase I trial^[79] while phase 3 trial IMpassion130 confirmed the promising efficacy.^[80] A total of 902 metastatic TNBC patients were randomly assigned to atezolizumab plus nab-paclitaxel or placebo plus nab-paclitaxel as first-line therapy. For patients with PD-L1-positive tumors, the median PFS and OS were significantly improved in atezolizumab treatment arm as 7.5, 25 months *vs.* 5.0 and 18 months in the placebo group, respectively. Based on these results, atezolizumab has been approved by the Food and Drug Administration in adult patients with unresectable locally advanced or metastatic TNBC whose tumors express PD-L1. This is the first indication of a checkpoint inhibitor in breast cancer. Currently, weekly paclitaxel combined with atezolizumab regimen in Impassion 131 (NCT03125902), one of the three different chemotherapies (paclitaxel, nab-paclitaxel, or gemcitabine with carboplatin) combined with pembrolizumab regimen KEYNOTE-355 (NCT02819518) trial is under investigation in metastatic TNBC.

The chemotherapy combined strategies have also been investigated in neo-adjuvant treatment. In the I-SPY 2 trial, pembrolizumab has been reported to increase the pCR rates from 19.3% to 71.4% in TNBC patients and 14.8% to 28% in HR+/HER2- patients.^[81] In the KEYNOTE-173 phase Ib study involving TNBC patients, the overall pCR rate was 60%.^[82] In the GeparNuevo randomized phase II trial, the pCR rate was higher in durvalumab and weekly nab-paclitaxel combination-treated patients (53.4% *vs.* 44.2% in placebo-treated patients).^[83] In addition, biomarker analysis showed that the presence of stromal TILs was associated with higher pCR rate in both treatment groups. Overall, chemotherapy combined strategies have bright prospects in the neoadjuvant setting.

Combined with molecularly targeted therapies

Combined with HER2-targeting therapies

HER2 positive breast cancer is a relatively immunogenic sub-type that is associated with higher levels of tumor infiltrated lymphocytes. In current practice, Trastuzumab, a humanized monoclonal antibody, combined with chemotherapy is the standard treatment for HER2-positive breast cancer. Besides inhibiting the HER2 signal transmission, it has been reported to recruit immune cells by Fc fragment and antibody-dependent cellular cytotoxicity.^[84] Preclinical studies using animal models have also shown that dual blockade of HER2 and PD-1/PD-L1 may be synergistic against HER2-positive breast cancer.^[84] These findings support the exploration of anti-PD-1/PD-L1 immunotherapy in combination with anti-HER2 treatments in HER2-positive breast cancer patients.

In the PANACEA phase 1b/2 trial, trastuzumab combined with pembrolizumab was administered in HER2-positive breast cancer patients who had shown progression in previous trastuzumab-based therapy.^[85] However, the response rate in the PD-L1-positive cohort and PD-L1-negative cohort was 15% and 0%, respectively. Further correlation analyses showed that the lymphocytic infiltration counts were associated with the tumor response. These findings showed that a subset of patients who were positive for PD-L1 and TILs can benefit from checkpoint inhibition and trastuzumab-based therapies. The CCTGIND.229 (NCT02649686) phase 1 trial investigated the combination of durvalumab (anti-PD-L1) and trastuzumab in patients with metastatic HER2-positive breast cancer.^[86] The study reported that there were no responses observed and only 29% of the patients had stable disease at week 6 of the treatment.

Ado-trastuzumab emtansine (T-DM1) is a kind of antibody-drug-conjugate (ADC) designed to target HER2 and release the cytotoxic drug maytansine. In an animal model, T-DM1 shows immunomodulatory effects and synergy with checkpoint inhibitors.^[87] The randomized KATE2 trial evaluated T-DM1 in combination with atezolizumab or not in patients with HER2-positive metastatic breast cancer. The benefit of time to progression in atezolizumab combined group was only observed in PD-L1-positive tumors.^[88] Therefore, there was a need for further investigation to explore this novel combination pattern as ADC with a checkpoint inhibitor. Currently, the phase 1 trial evaluating the combination of T-DM1 with pembrolizumab (NCT03032107) and Trastuzumab Deruxtecan (DS-8201a) with nivolumab in HER2-expressing breast is recruiting participants (NCT03523572).

Combined with inhibitors of cyclin-dependent kinase (CDK) 4 and CDK6

Inhibitors that target CDK4 and CDK6 cell cycle kinase palbociclib, ribociclib, and abemaciclib have been shown to suppress retinoblastoma phosphorylation in cancer cells, arrest the cell cycle, and inhibit cell proliferation.^[89] Several clinical trials have confirmed that it significantly improves PFS in patients with ER-positive advanced breast cancer.^[90,91] However, recent studies show that it can also promote anti-tumor immunity and increase tumor immunogenicity through the following mechanism: First, CDK4/6 inhibitors enhance tumor antigen presentation by increasing the expression of endogenous retroviral sequence fragments, which in turn stimulate the production of type III IFNs; second, CDK4/6 inhibitors suppress the proliferation of regulatory T-cells.^[92] These effects lead to immune cell activation which may act synergistically with immune checkpoint blockade. In the murine cancer model, abemaciclib combined with anti-PD-L1 therapy led to complete tumor regression and increased the tumor T-cell inflammatory signature.^[93] Abemaciclib in combination with pembrolizumab was estimated in a phase Ib trial with a total of 28 hormone-resistant advanced breast cancer patients.^[94] The ORR at 24 weeks was 14% which was higher than that reported from abemaciclib monotherapy data (11% from MONARCH 1 study). Other combinations such as avelumab with palbociclib (NCT03147287),

pembrolizumab with palbociclib (NCT02778685) pembrolizumab with dinaciclib (NCT01676753) are under evaluation in clinical trials.

Combined with poly(ADP-ribose) polymerase (PARP) inhibitors

Mutations in *BRCA1/2* genes account for 2% to 3% of breast cancer and more than 10% of TNBC.^[95] Cells harboring *BRCA1/2* mutation lack the ability to repair the DNA double-strand breaks through the homologous recombination pathway.^[96] Poly (ADP-ribose) PARP plays an important role in the single-strand DNA repair pathway.^[97] The damage of two different DNA repair pathways leads to synthetic lethality which is the rationale for antitumor activity of PARP inhibitor (PARPi) in *BRCA* mutated patients. PARPi olaparib and talazoparib have been reported to significantly improve PFS in single-agent chemotherapy and in *HER2*-negative metastatic breast cancer patients with *BRCA1/2* mutation.^[98,99] In addition, tumors with *BRCA1/2* mutations are associated with a higher mutation burden because of the abbreviated protein accumulation in the cell.^[21] Preclinical studies have revealed an increase in stromal TILs and a higher TMB in *BRCA1* mutation-associated breast cancer.^[100] On the other hand, PARPi up-regulates PD-L1 expression in breast cancer cells while a combination of PARPi and anti-PD-L1 therapy significantly increases the therapeutic efficacy compared with single agents in animal breast cancer model.^[101] These studies support the combination of PARPi and PD-1/PD-L1 inhibitors as potential therapeutic strategies in breast cancer.

MEDIOLA study is a phase II basket study aiming to investigate the combination of olaparib and durvalumab in germline *BRCA*-mutated *HER2*-negative metastatic breast cancer. The preliminary results reported that 25 of the enrolled patients' disease control rate (DCR) was 80% at 12 weeks and 50% at 28 weeks.^[102] The KEYNOTE-162/TOPACIO trial investigated the effects of a combination of niraparib and pembrolizumab in patients with metastatic TNBC regardless of the *BRCA* status.^[103] The ORR and DCR for all the patients was 29% and 49%, respectively. In a total of 12 patients harboring *BRCA* mutation, ORR and DCR were higher and reported as 67% and 75%, respectively. These studies indicate that the antitumor activity of PARPi combined with an immune checkpoint inhibitor for the *BRCA* mutation carrier promising.

Combined with radiotherapy

Radiotherapy is a local control approach for malignant tumors. Sometimes radiotherapy causes tumor shrinkage at a distant site out of the radiation field which results from a systemic immune response known as abscopal effect.^[104] This effect is associated with ICD-releasing dangerous signals induced by DNA damage from the irradiated tumor cells.^[97] Radiotherapy induced ICD also increases cytokine release, promotes antigen presentation, and stimulates T-cell response and this makes a combination of radiotherapy with PD-1/PD-L1 inhibitor a promising strategy. Animal model studies have demonstrated a

synergistic relationship between radiation and checkpoint inhibitors.^[105,106] A phase II single-arm study was designed to investigate whether radiation therapy enhances the efficacy of the PD-1 inhibitor pembrolizumab in metastatic TNBC.^[107] Pembrolizumab was administered intravenously at 200 mg within 3 days of the first radiation therapy fraction. A total of 3 of 9 (33%) patients showed a partial response, one patient had stable disease and 5 (56%) patients had disease progression. Although the sample size was small, the results are promising and a similar strategy is being evaluated in patients with HR +/*HER2*- breast cancer.^[108] However, there is a need to further evaluate the radiotherapy dose, schedule, and treatment sequence.

Combined with other immunotherapies

CTLA-4 is another immune checkpoint pathway that binds CD80/CD86 to provide negative feedback at the initial priming phase of the T cell activation process. This differs from PD-1 which is expressed on activated T cells, B cells or myeloid cells and is inhibitory at a later effector phase.^[109] The strategy simultaneously targeting both pathways may result in a synergistic effect. A combination of anti-CTLA-4 and anti-PD-1 inhibitor in the treatment of metastatic melanoma and NSCLC have reported better responses compared to each of them alone.^[26,110] A phase I/2 study evaluated the combination of durvalumab (anti-PD-L1) and tremelimumab (anti-CTLA-4) in patients with metastatic TNBC and HR-positive cancer. A total of three out of 28 (17%) of the enrolled patients achieved an overall response.^[111] Besides CTLA-4 inhibitor, a combination of PD-1/PD-L1 inhibitor with antibodies targeting other co-inhibitory molecules, such as anti-LAG3, anti-TIM3, or anti-TIGIT (NCT02913313 and NCT03099109) and with an antibody targeting co-stimulatory molecules like OX40 (NCT03971409) or 4-1BB (NCT03364348, NCT03414658) have triggered research interest in breast cancer.

Cancer vaccines suppress malignancy by stimulating immune responses specific for tumor antigens and these can be delivered as a variety of vaccine platforms. These platforms include those targeting antigens through peptide, protein or engineered plasmid DNA, those targeting cells such as DCs, autologous tumor cells and those using tumor cell lysates derived from patients.^[112] In breast cancer, vaccine monotherapy has shown evidence of modest immune response but limited anti-tumor activity. This may be associated with immunosuppression caused by the activation of checkpoint signaling pathways. The combination of PD-1/PD-L1 inhibitor with DC vaccine showed anti-tumor activity and survival benefits in an animal model.^[14] Several clinical trials are underway to evaluate these strategies.

The oncolytic virus is designed to lyse tumor cells; however, it causes a strong change in the tumor immune microenvironment including improving the transfer of anti-tumor immunity associated cytokines IFN- α and granulocyte-macrophage colony-stimulating factor and increases the release of damage-associated molecular patterns.^[113,114] These suggest that oncolytic virotherapy can be used to improve the efficacy of anti-PD-1 therapy.

Immunosuppressive agents can be used to transfer the tumor microenvironment to facilitate anti-tumor immunity. IDO is an enzyme that converts tryptophan to kynurenine which causes apoptosis of effector T cells and activation of immunosuppressive cells.^[115] Epacadostat is a highly selective oral inhibitor of the IDO1 enzyme. A phase I clinical trial demonstrated that a combination of pembrolizumab and epacadostat showed antitumor activity in multiple advanced solid tumors.^[116] Adenosine is another mediator of immunosuppression that is generated from the hydrolysis of nucleotides by CD39 and CD73.^[117] When adenosine binds to its receptors (particularly the adenosine A2a receptor) expressed on natural killer and T cells, it exhibits the brake effects on anti-tumor immunity. An oral antagonist of the adenosine-A2A receptor (CPI-444) can reverse the immune suppression.^[118] In a study of a range of advanced treatment-refractory cancers, including TNBC, adenosine has been tested with atezolizumab.^[119]

Conclusions and Perspectives

Traditionally, breast cancer has been viewed as a cold tumor with relative immunogenicity. Several breast cancer sub-types (HER2 positive BC and TNBC) have shown higher TMB and TIL infiltration indicating the role of anti-PD-1/PD-L1 agents. Therefore, the development of anti-PD-1/PD-L1 agents combination strategies in breast cancer is promising. This is because single-agent anti-PD-1/PD-L1 inhibitors have shown limited efficacy in breast cancer. How to inflame the immunologically “cold tumor” to “hot tumor,” to clarify the complex positive and negative feedback loops and regulatory mechanisms of the immune system is a key issue. Therefore, in combination with other agents, the synergistic anti-tumor activity can be achieved. Some of the agents involved in current clinical trials include those target HER2 pathway (trastuzumab, T-DM1, DS-8201a, etc), increase the cancer antigen presentation (chemotherapy, a CDK4/6 inhibitor, PARPi, radiotherapy, oncolytic virus, etc), augment the activity of effector T cells (checkpoint inhibitor, cancer vaccine) and improve the immunosuppressive microenvironment (IDO inhibitor, adenosine-A2A receptor antagonist, etc). There is a need to investigate predictive biomarkers required to define the population that would benefit the most from the treatment. However, with respect to PD-L1 expression, clinical trials have demonstrated mixed results. In addition, the different antibodies used in immunohistochemical tests have reported different cut-off values, different expression in primary and metastatic biopsies^[120] and the staining of tumor cells *vs.* immune cells is one of the unresolved issues when it comes to PD-L1 expression analysis. TILs are potential biomarkers but need further validation. Tumor mutational burden and oncogenic mutations are all markers under current investigations. Collectively, the immunotherapy might be a promising therapeutic strategy for breast cancer and several clinical trials are still ongoing.

Conflicts of interest

None.

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