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Review Article

Immunotherapy and immunobiomarker in breast cancer: current practice and future perspectives

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Abstract: Among the new cancer cases and resulting deaths among women worldwide, breast cancer is the most significant threat to women's health. In recent years, immunotherapy was initially used to treat patients with metastatic breast cancer, where it demonstrated its unique value by providing a novel way to improve therapeutic effects and prolong survival time. With the development of clinical trials related to immunotherapy for breast cancer, tumour vaccines, such as DNA vaccines, have been observed to improve the disease-free survival (DFS) and overall survival (OS) of patients. Monoclonal antibodies have also shown good efficacy, and adoptive cell therapies, such as CAR-T, exhibit strong tumour killing ability and good safety, and thus, these therapies may comprise a new strategy for the treatment of breast cancer. These breakthrough successes have promoted the achievement of "individualized" breast cancer treatment. Moreover, a recent study showed that patients with various cancer types with a higher tumour mutational burden (TMB) are more likely to benefit from immunotherapy. As research progresses, TMB may also demonstrate a certain clinical significance in the treatment of breast cancer. This paper reviews the latest research progress on breast cancer immunotherapy and the predictive value and application status of TMB in immunotherapy regimens for breast cancer patients to provide a reference for further in-depth studies of breast cancer immunotherapy.

Keywords: Breast cancer, immunotherapy, tumour vaccine, adoptive cell therapy, immunobiomarker, tumour mutational burden

Introduction

Breast cancer is a global disease and is the most common malignant cancer in females worldwide [1]. Although substantial progress has been made in the diagnosis and treatment of breast cancer, the effects of current conventional treatment are not ideal for patients with advanced breast cancer, especially for those with triple-negative breast cancer (TNBC). The median survival time of patients with advanced TNBC who received conventional chemotherapy was reported to be only 13 months [2]. In recent years, immunotherapy has achieved remarkable results in tumour treatment and has become the fourth bell mode in addition to surgery, chemotherapy, and radiotherapy. Immunotherapy plays an antitumour role by regulating the immune system of patients. Historically, breast cancer has not been regarded as a highly immunogenic tumour because of the low mutation rate of breast cancer genes and a limited ability to form tumour-causing neoantigens [3]. However, a growing body of research has confirmed a strong link between breast cancer and the immune system. Studies have found that immunotherapy also has a good therapeutic effect in some breast cancer patients, among which studies are also called anti-programmed cell death-1/programmed death ligand-1 (PD-1/PD-L1) at most [4]. Moreover, a recent study showed that among various cancer types, patients with higher tumour mutational burden (TMB) are more like-

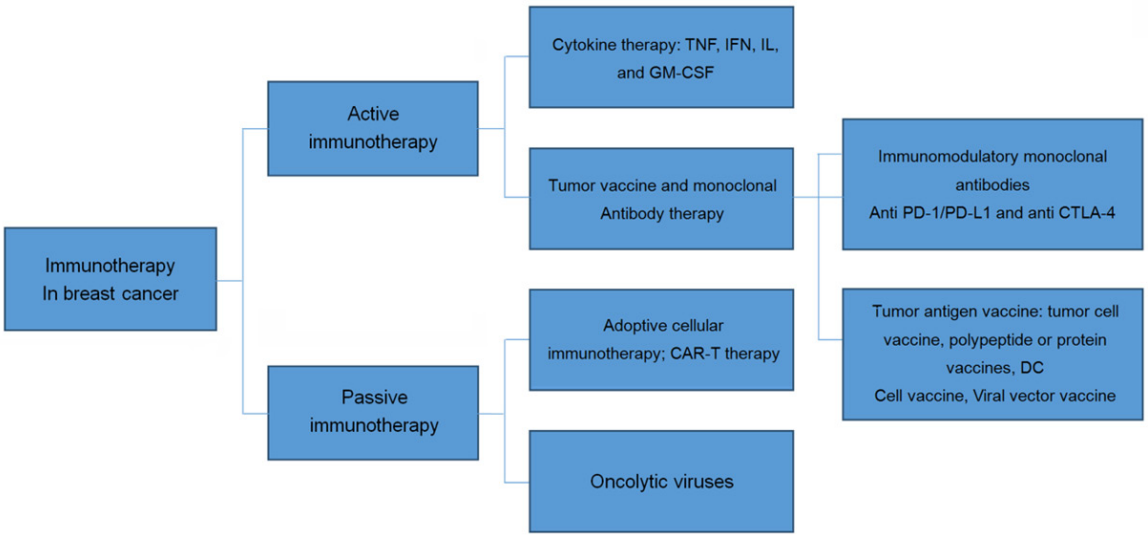

Figure 1. Classification of cancer immunotherapy [42]. Progress of Immunotherapy and Its Application in Triple Negative Breast Cancer. Note: AR-T, chimeric antigen receptor T cell; TNF, tumor necrosis factor; IFN, interferon; IL, interleukin; GM-CSF, granulocyte-macrophagecolony stimulating factor; PD-1, programmed cell death-1; PD-L1, programmed cell death-ligand 1; CTLA-4, cytotoxic T lymphocyte-associated antigen 4.

ly to benefit from immunotherapy. This article reviews the latest research and current immunobiomarkers of breast cancer immunotherapy to provide a reference for further research on immune-precision therapy for breast cancer.

Immunotherapy strategies for breast cancer

Immunotherapy for breast cancer can be divided into three categories according to its mechanism of action: active immunotherapy (including various tumour vaccines), passive immunotherapy (including monoclonal antibodies, adoptive cell therapy, etc.), and nonspecific immunomodulator therapy (mainly anti-PD-1/PD-L1 antibodies, anti-CTLA-4 antibodies, etc.) [5]. Current immunotherapy that targets breast cancer has progressed in regard to tumour vaccines, monoclonal antibodies, and immunomodulators (**Figure 1**).

Tumour vaccines

Tumour vaccines play an antitumour role by stimulating specific immune responses to tumours through immunization. Tumour vaccines can maintain long-term immune memory and exert long-lasting effects.

Tumour cell vaccine

A tumour whole cell vaccine is rich in tumour antigens and can theoretically generate non-specific costimulatory signals through antigen presenting cells (APCs) to promote an immune response. Whole cell vaccines were administered to patients with seven types of advanced tumours, including breast cancer, to prolong OS [currently in a phase I/II clinical trial (NCT00722228)]. Due to the absence of costimulatory molecules, an adjuvant is often added to enhance the immune response to tumour whole cell vaccines. However, adjuvants can also induce autoimmunity, which limits their usefulness [6].

Cancer polypeptide (protein) vaccine

DNA vaccine: DNA vaccines can induce the synergistic effect of humoral and cellular immunity by taking up the DNA sequence encoding the target tumour-associated antigen (TAA), translating it, and expressing it into protein, which is then treated and presented as an antigen. DNA vaccines can be produced on a large scale and can easily induce antitumour immune responses, but low long-term levels of tumour antigens can cause immune tolerance. Guardino et al. [7] found that the MVA-BN®-HER2 vaccine has biological activity against metastatic breast cancer with HER2 overexpression and can break HER2 tolerance. At present, although it is still a challenge to find suitable vectors for a DNA vaccine, the electroporation method has

achieved preliminary results, which may be a breakthrough [8].

DC vaccine: Dendritic cells (DCs) are the only specialized APCs that can activate primary T cells, and thus, they play an important role in the immune response. A DC vaccine is not restricted by HLA and can induce class I and II immune responses. Studies [9] found that a DC vaccine based on HER2/Neu could induce a decrease and even cause loss of HER2/Neu expression. In addition, the Indoximod + AD.p53DC vaccine may be sensitized to chemotherapy, but its safety and efficacy in combination with chemotherapy drugs require further study [10]. Abdellateif et al. [11] induced mature DCs using viable MCF-7 breast cancer cells *in vitro* and reported that $CD83^+$, $CD86^+$, and MHC-II^+ DCs were significantly increased after induction and that $CD4^+$ and $CD8^+$ T cells were also increased. Numbers of $CD4^+$, $CD25^+$, and Foxp3+ Tregs were significantly decreased, and Foxp3 gene expression levels were significantly decreased. In addition, the secretion of IL-12 and IFN-γ was increased, while the release of lactate dehydrogenase was significantly increased, which indicates the enhanced cytotoxicity of CTLs to the tumour. Therefore, a live breast cancer cell-DC vaccine is a novel approach for breast cancer immunotherapy.

HER2/neu: The HER2/Neu protein is immunogenic and can induce antigen-specific immune responses by $CD4^+$ and $CD8^+$ T lymphocytes. High expression of HER2/Neu can therefore be an effective target for immunotherapy in breast cancer. At present, advanced research is being performed on three types of polypeptide vaccines, namely, E75, GP2 and AE37.

(I) The HER2 antigenic peptide E75 is an HLA-A2/A3-restricted polypeptide derived from the extracellular domain of the HER2/Neu protein that plays an antitumour role by effectively stimulating the expansion of E75-specific cytotoxic lymphocytes (CTLs). Preocular phase I/II clinical trials [12] have confirmed that the E75 vaccine can increase disease-free survival (DFS) in patients with early breast adenocarcinoma, and related phase III clinical trials have also been completed [13]. Benavides et al. [14] found that breast cancer patients with HER2/Neu overexpression may have immune tolerance to HER2/Neu, as patients with low expression of HER2/Neu experience a greater benefit.

(II) GP2 is derived from the HER2/Neu transmembrane domain and is also an HLA-A2 restricted polypeptide, which means it is more immunogenic than E75. Early results of a phase II clinical trial of GP2 for the treatment of breast cancer (NCT00524277) [15] showed that DFS was higher in the experimental group than in the control group (94% vs. 85%).

(III) AE37 is a HER2/Neu-derived MHC class II epitope peptide vaccine hybridized with the Ii-Key peptide, which significantly enhances the activity of $CD4^+$ T cells and has a longer-lasting immune effect. A phase II clinical trial showed that the relative risk of patients with non-HER2 overexpression was reduced after treatment with the AE37 vaccine and that AE37 could benefit patients with non-HER2 overexpression, especially those with TNBC.

Monoclonal antibody therapy

Monoclonal antibody against HER2/Neu

Previous studies [16, 17] showed that dual-targeted therapy can improve the pathologic complete response (pCR) of patients, but the difference was not statistically significant. Recently, a phase III randomized controlled trial (CALGB40601) of a dual target (lapatinib + trastuzumab) combined with paclitaxel in HER2-positive breast cancer [18] suggested that the prolongation of invasive disease-free survival (iDFS) was correlated with pCR. Powles et al. [19] found that patients with high levels of the T-cell receptor β-chain variable (TRBV) 11-3 gene or TRBV-MG2 benefited more from dual-targeted therapy. Thus, the TRBV gene pattern can predict the efficacy of dual-targeted therapy. In addition, primary or secondary drug resistance is a key factor that affects the clinical efficacy of trastuzumab. In the Panacea study [20] trastuzumab-resistant HER2-positive metastatic breast cancer was treated with a combination of pembrolizumab and trastuzumab, the results of which are forthcoming. Pertuzumab is a novel HER2 recombinant monoclonal antibody that binds to the extracellular domain II of HER2 and remains effective against breast cancers with low HER2 underexpressed breast expression and poor trastuzumab response. A phase III randomized clinical trial (NCT00567190) found that the median overall survival (OS) and median progression-free survival (PFS) in patients with nonearly HER2-positive breast cancer treated with

trastuzumab plus docetaxel were significantly longer after the addition of pertuzumab. In another phase III clinical trial (APHINITY; LBA500) 4,805 patients with early HER2-positive breast cancer were treated with standard chemotherapy, and the results showed that iDFS in the pertuzumab group was 94.1% vs. 93.2% (P = 0.045) in the other group [21]. The benefit in the lymph node-positive group was greater than that in the trastuzumab group (92% vs. 90.2%). Therefore, dual-targeted therapy combined with chemotherapy is recommended for the first time as an adjunctive therapy for patients with lymph node-positive HER2-positive breast cancer [22]. Keith et al. [23] further confirmed that T-DM1 can effectively delay the growth of brain metastasis (BMS) in patients with HER2-positive BMS and results in a survival benefit. In addition, Borges et al. [24] showed that tucatinib + T-DM1 exhibited preliminary antitumour activity and acceptable toxicity in refractory HER2-positive breast cancer patients with or without brain metastasis.

Recently, the results of the open-label phase III trial of margetuximab were presented [25]. SOPHIA trial (NCT02492711) enrolled 538 patients with advanced HER2 positive metastatic BC and randomly assigned in a 1:1 fashion to margetuximab (15 mg/kg intravenously every 3 weeks) plus chemotherapy or trastuzumab (6 mg/kg [8-mg/kg loading dose]) plus chemotherapy given every 3 weeks. All patients had received trastuzumab and pertuzumab, and more than 90% had also received T-DM1. Most patients were treated with taxane, more than 40% were treated with anthracycline, and almost half had received an endocrine drugs. Compared with trastuzumab, magtuximab improved primary PFS with a 24% relative risk reduction (P = 0.03), with a median PFS of 5.8 months versus 4.9 months with trastuzumab. Margetuximab improved primary PFS over trastuzumab with 24% relative risk reduction (p = 0.03) with a median PFS of 5.8 vs. 4.9 months. The ORR was higher in the magtuximab group: 25.2% vs. 13.7%, increasing the clinical benefit rate from 35.6% with trastuzumab to 48.1% with margetuximab (p = 0.0025). The median duration of response was similar in the two arms. In the planned exploratory analysis of the FcRIII genotype, the benefit was enhanced in patients with low-affinity FcγIIIa genotypes containing a 158F allele, in which disease progression was reduced by 32% [25, 26]. In the second interim analysis, for 85% of patients with the FcIIIa 158F allele, median OS was extended by 4.3 months (23.7 months) in the Margetuximab arm compared with 19.4 months (P = 0.087) in the trastuzumab arm. Safety profiles and treatment discontinuation rates were comparable in the margetuximab and trastuzumab arms [27].

Bispecific monoclonal antibody (mAb)

Bispecific mAbs can bind two different TAAs, such as HER3/EGFR, VEGF/HER2, and HER2/HER3, and this double blockade can improve the efficacy of immunotherapy. MM 111 is a bispecific single chain antibody that simultaneously targets HER2/HER3 in HER2-overexpressing breast cancer. Preclinical studies [28] found that MM-111 showed antitumour activity when combined with trastuzumab and lapatinib. T cells lack FcyR, and thus, typical antibodies cannot directly recruit T cells. The Fv region of the triple functional antibody can bind to tumour cells and T cells, while the Fc region can bind to FCR-expressing immune cells, such as NK cells, and kill tumours through antibody-dependent cellular cytotoxicity. *In vitro* studies [29] found that ertumaxomab, a trifunctional antibody against HER2 and CD3, can be used to treat tumours with low HER2 expression that do not respond to trastuzumab. In addition, many other types of bispecific antibodies, such as BiTEs, are also under investigation [30]. To overcome the problems of immunogenicity and short half-life, we focused on a T-cell-dependent bispecific antibody (HER2-TDB) that can target HER2 and induce an antitumour immune response of polyclonal T cells, which reduces the occurrence of tumour immunity. Other studies [31] found that HER2-TDB has a strong effect at low concentrations in HER2-overexpressing cell lines and showed certain efficacy in cell lines resistant to trastuzumab, lapatinib, TDM-1, and other drugs. HER2-TDB was also effective in hormone receptor-positive breast cancer (HPBC). Although PD-L1 expression limits HER2-TDB activity, the inhibitory effect can be reversed with anti-PD-L1 antibodies, which suggests that a combination with immune checkpoint inhibitors can increase immunotherapy efficacy. However, since HER2 is also expressed at low levels in

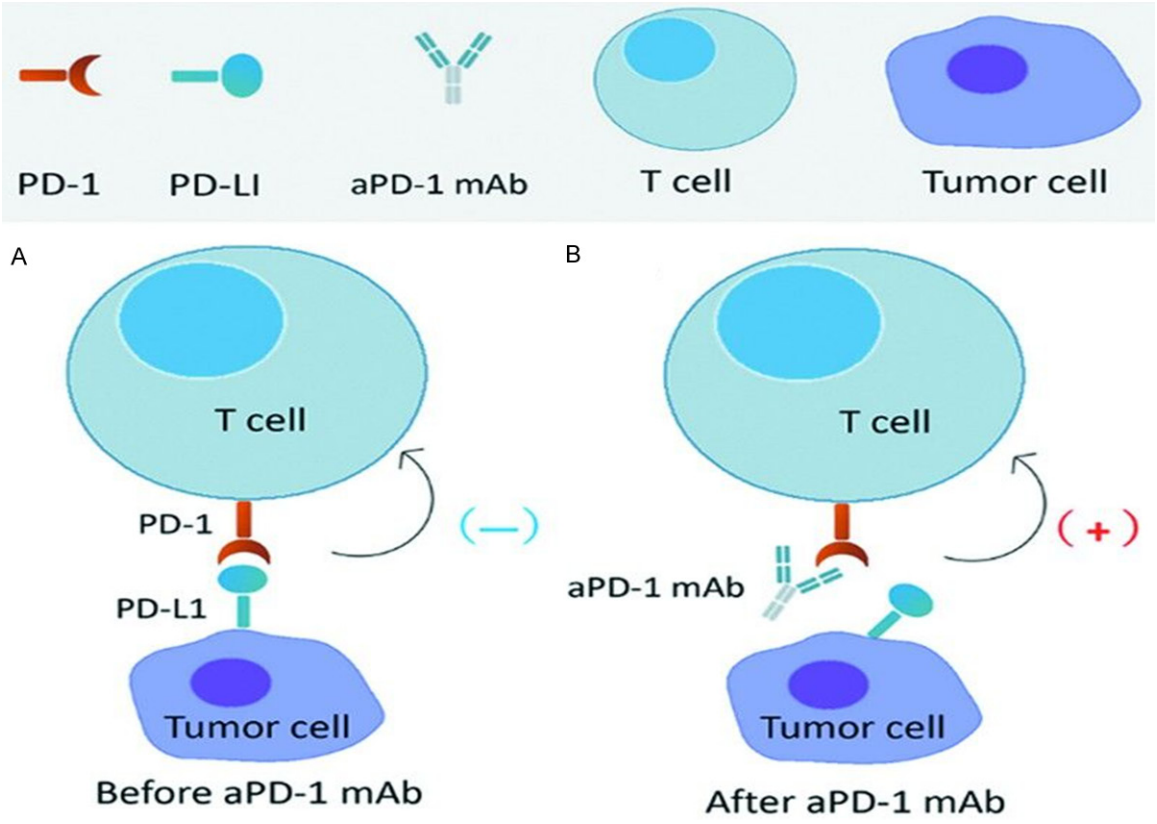

Figure 2. Schematic diagram of aPD-1mAb in treatment of triple negative breast cancer [42]. Progress of Immunotherapy and Its Application in Triple Negative Breast Cancer. A: PD-L1 expressed on tumor cells combing with PD-1 expressed on TILs play an immunosuppressive role by inhibiting the anti-tumor activity of T cells and tumor cells survive through immune escape before anti-PD1 or anti-PD-L1 monoclonal antibody (aPD-1/PD-L1 mAb); B: PD-1/PD-L1 axis becomes immunoreactive after adding aPD-1/PD-L1 mAb owing to the activation of CD8+ T cells. aPD-1 mAb, anti-PD-1 monoclonal antibody; TILs, tumor infiltrating lymphocytes.

many normal somatic cells, such as cardiomyocytes, the safety of HER2-TDB should be further evaluated.

Non-specific immunomodulator therapy

PD-1/PD-1/PD-L1 inhibitors

A meta-analysis showed that PD-L1 expression was significantly correlated with lymph node metastasis and histological grade, which suggests that PD-L1 expression may be a prognostic biomarker for breast cancer (**Figure 2**).

Although patients with metastatic TNBC who received immunotherapy benefited more in terms of objective response rate (ORR) compared with second-line and above chemotherapy, no significant difference was observed in overall survival time (OS) between the two groups [32]. Advanced breast cancer patients with rapid tumour progression or visceral crisis often require rapid control of tumour growth in the short term to alleviate symptoms. Most breast cancer patients are resistant to monotherapy with checkpoint inhibitors, and radiation therapy has many immune-stimulating effects, including activation of the immune system, recruitment of immune cells to the tumour environment and immunosuppressive effects that alter the tumour microenvironment. Radiotherapy combined with checkpoint inhibitors can not only synergistically enhance antitumour efficacy but can also induce long-lasting field responses to radiotherapy [33, 34]. A phase II study [35] reported that in patients with advanced TNBC who were treated with pembrolizumab combined with radiotherapy, 3 of 9 evaluable patients (33%) responded outside the irradiation zone and survived up to 49 weeks. Several additional clinical trials evaluating the efficacy of radiotherapy combined with new immunotherapy approaches are ongoing

[36]. PD-1/PD-L1 inhibitors are also emerging in TNBC neoadjuvant therapy [37]. One study [38] evaluated the efficacy of adding durvalumab to standard neoadjuvant chemotherapy in primary TNBC, and the results showed that the addition of durvulumab to anthracycline/taxane standard neoadjuvant chemotherapy increased pCR rates (53% vs. 44%) compared with durvulumab alone prior to the initiation of chemotherapy. Although immunotherapy is not used as a neoadjuvant or advanced treatment, its combination with chemotherapy significantly increased the benefit in patients compared with conventional chemotherapy or immunotherapy alone, and no unacceptable immune-related adverse reactions were observed. In addition to TNBC, in which efficacy has been established, clinical trials of PD-1/PD-L1 have been conducted in HER2-positive and hormone-receptor-positive (HR+) breast cancer patients. Tumour-infiltrating lymphocytes (TILs) and PD-L1 expression are lower in HR+/HER2- breast cancer than in HER-2+ breast cancer and TNBC, and thus, patients with HR+/HER2- cancer may not respond significantly to checkpoint inhibitor therapy. The Keynote-028 study treated patients with PD-L1-positive HR+/HER2- advanced breast cancer with pembrolizumab monotherapy, and the ORR was approximately 12%, whereas the ORR was only 3% in patients with metastatic HR+/HER2- breast cancer treated with avelumab. In early HR+ breast cancer, the opposite result is likely to be observed; with the addition of pembrolizumab to standard neoadjuvant chemotherapy, the estimated pCR rate increased from 13% to 34%, which suggests that the combination in neoadjuvant therapy may be effective for this subtype [39, 40]. The 229 studies performed on trastuzumab and durvalumab reported disappointing results, with no significant clinical activity observed in patients with HER-2-positive PD-L1-negative metastatic breast cancer [41]. These are similar to the results of PD-L1-negative patients in the Keynote-14 study [42, 43], where the ORR of PD-L1-positive patients was 15%. In addition, overall survival data from the Pherexa trial [44] suggest that trastuzumab and pertuzumab combined with the clinical activity of double HER-2 inhibition may induce immune activation in patients with advanced HER-2-positive progressive breast cancer who previously received trastuzumab. HER2-positive-resistant advanced breast cancer was resensitized to trastuzumab therapy, although the prolongation in PFS was not significant.

CTLA-4 inhibitors

Similar to PD-1, CTLA-4 is a negative costimulatory molecule expressed on the surface of the T-cell membrane, and its ligand is the B7 molecule present on the surface of APCs and target cells (tumour cells). The combination of the two can inhibit T-cell activation, proliferation, and cytokine secretion and can negatively regulate the body's immune response to tumour cells [45]. In addition, CTLA-4 can also negatively regulate the immune response by inhibiting the maturation and antigen presenting ability of APCs, promoting the amplification of Tregs, and inducing the production of indoleamine 2,3-dioxygenase in APCs [46]. Thus, blocking the CTLA-4 signalling pathway restores the immune system's ability to recognize tumour cells. Currently, two humanized CTLA-4 monoclonal antibodies are clinically available: tremelimumab and ipilimumab. Vonderheide RH [47] combined tremelimumab and exemestane for the treatment of patients with ER-positive, HER2-negative advanced breast cancer, and of these, approximately 42% (11 cases) achieved disease stability over 12 weeks, and the main adverse reactions were mild to moderate diarrhoea, itching, constipation, and fatigue. Ipilimumab is mainly used as a single drug or in combination with PD-1/PD-L1 inhibitors for melanoma and other tumours [48]. Preclinical studies have demonstrated significant antitumor efficacy of combination therapy targeting both PD-1 and CTLA-4 immune checkpoints. The combination of blocking and PD-1 and CTLA-4-based vaccines can promote the rejection of B16 melanoma tumour cells in mice and can synergistically increase the proportion of $CD4^+$ TILs and MDSCs on the tumour surface of these mice [49]. The phase III Check Mate 067 study [50] confirmed that nivolumab combined with ipilimumab significantly improved PFS and 3-year survival in patients with melanoma. However, the exact mechanism and efficacy of this immunocombination therapy in breast cancer are still unclear and require further exploration in relevant animal models and clinical trials.

Adoptive cell therapy

Adoptive cell therapy can improve the state of low cellular immune function in the body by

activating immune effects in autologous or allogeneic cells *in vitro* and then transferring them back into the body where they exert antitumour effects. This therapy can also directly play an antitumour role and repair immune damage to a certain extent. However, limited cell sources, low cytotoxicity, and a low number of amplifications limit its application. Cytokine-induced killer (CIK) cells are mainly $CD3^+$ $CD56^+$ T cells, with the non-MHC restriction of natural killer cells and the antitumour activity of T lymphocytes, that can directly kill tumour cells. Activated CIK cells can inhibit and kill tumour cells by expressing high levels of cytokines. Their tumour killing spectrum is broad, their proliferation rate is fast, their killing activity is high, and their associated adverse reactions are infrequent. Therefore, these cells have certain advantages as an immunotherapy treatment for breast cancer.

Pan et al. [51] found that CIKs are an effective treatment for TNBC patients with positive lymph nodes, high TNM stage, and poor pathological grade.

TILs

TILs are cells with MHC restriction and tumour specificity that exert a novel antitumor effect [52]. Zhang et al. [53] purified and expanded TILs from breast cancer and lymph nodes to obtain $>1\times10^9$ cells after which they were transfused back into patients. The results showed that TILs had the highest killing rate at 78.0%, which suggests that TILs can enhance cellular immune function in breast cancer patients. Recently, Zacharakis et al. [54] reported a case of hormone receptor-positive and HER2-negative metastatic breast cancer that failed to respond to multiple chemotherapy regimens. TILs were cultured *in vitro*, and TILs that responded to only four somatic mutant proteins were further cultured to a certain number and transfused back into the body. At the same time, the combination of TILs with IL-2 and an immune checkpoint inhibitor (ICI) showed that the disease foci completely disappeared for more than 22 months, which represents a new way for immunotherapy to treat refractory cancer.

Genetically modified T cells

CAR T therapy (CAR-T) is an adoptive cell immunotherapy based on the use of genetically engineered lymphocytes that express chimeric antigen receptors that kill tumour cells in a nonmajor histocompatibility complex-restrictive manner [55]. CAR-T therapy has been successful in haematological malignancies, but challenges remain in solid tumours. Clinical trials of CAR-T cells targeting HER2 in breast cancer are ongoing. A phase I/II clinical trial (NCT01935843) [56] investigated the clinical efficacy of CAR-T cells in the treatment of HER2-positive advanced solid tumours. Another phase II trial (NCT01022138) [57] evaluated the efficacy of activated T cells modified with an anti-CD3X, anti-HER2/Neu bispecific monoclonal antibody in HER2/Neu-negative metastatic breast cancer. Byrd et al. [58] proposed that specific CAR-T cells targeting TEM8/ANTXR1 could be used as an immunotherapy for TNBC. In addition, clinical trials of CAR-T cells targeting other breast cancer antigens, such as cMet and tumour-associated antigen-mesothelin, are also ongoing. In conclusion, in solid tumours such as breast cancer, some key issues related to CAR-T therapy persist, such as scarcity of target antigens and limited CAR-T-cell duration, which require further research (**Figure 3**).

Immunobiomarkers

Tumour mutation load

Tumour mutational burden (TMB) refers to the total number of somatic mutations detected per million bases or the number of mutations detected per tumour. Neoantigens generated by somatic mutations in tumours can induce the body's antitumour immune response. Therefore, patients with higher TMB levels respond better to immunotherapy drugs. Compared with the TMB of lung cancer, the TMB of breast cancer is lower and related to molecular typing. TNBC has the highest TMB, followed by $HER2^+$ breast cancer [59]. Among patients with TNBC and $HER2^+$ refractory metastatic breast cancer, those with high TMB have significantly better OS than patients with low TMB [60, 61]. TMB is closely related to the prognosis of breast cancer, and studies on the ability of TMB to predict the efficacy of PD-1/PD-L1 antibody therapy for breast cancer need to be performed.

The predictive role of TMB in triple-negative breast cancer (TNBC) immunotherapy

The IMpassion130 phase III clinical trial demonstrated that treatment with atlizumab plus

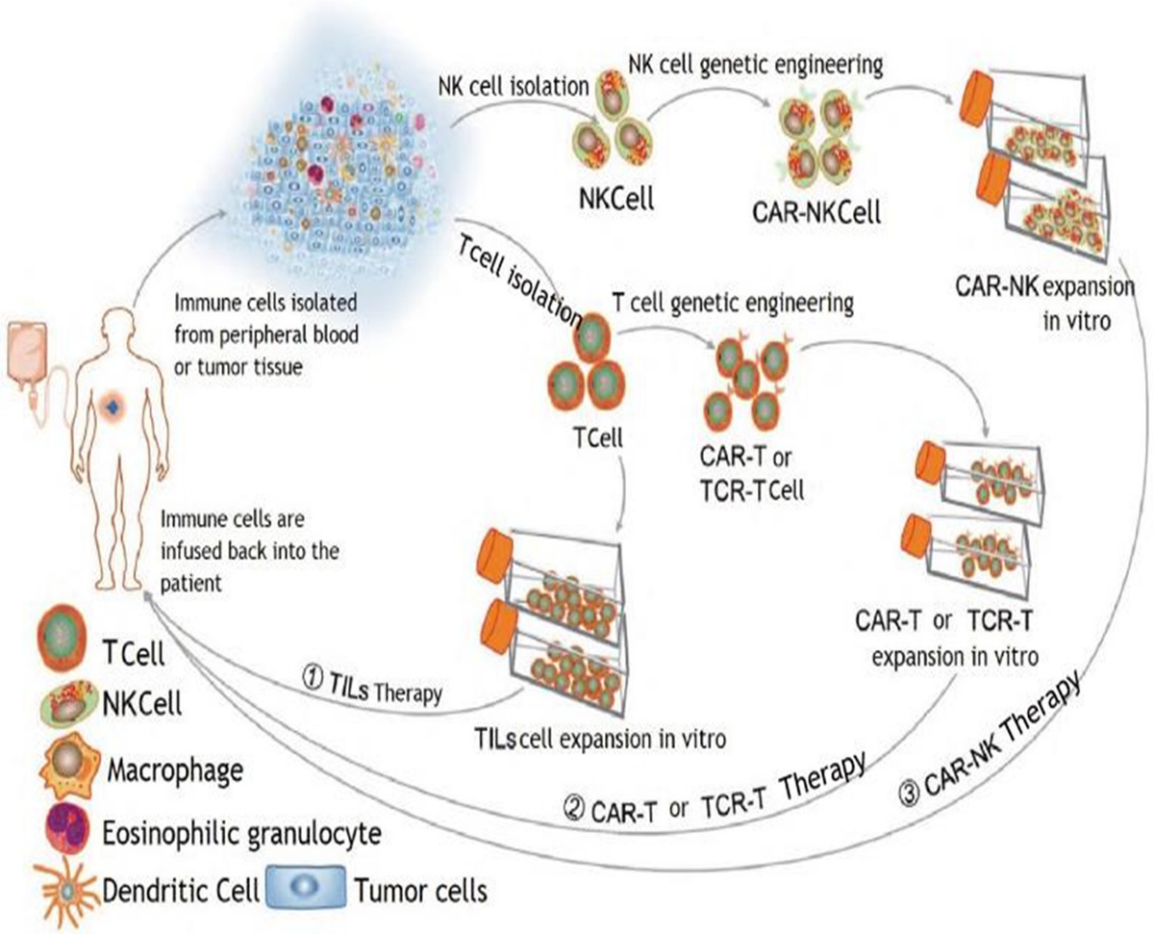

Figure 3. Chimeric antigen receptor T cell immune therapy [56]. Advances in research on tumor immunotherapy. CAR-T, Chimeric antigen receptor T cell immune therapy; TILs, Tumor-infiltrating lymphocytes.

albumin-paclitaxel significantly extended PFS (7.5 months vs. 5 months) and overall survival (25 months vs. 18 months) in patients with advanced TNBC with PD-L1-positive tumours compared with placebo plus albumin-paclitaxel [62]. Emens et al. [63] performed a retrospective study based on this finding and confirmed that higher TMB was associated with an overall survival benefit in the atlizumab combined with albumin-paclitaxel group; however, a clinical benefit was only observed in patients with PD-L1-positive tumours, and TMB was not related to PD-L1 expression. TMB also plays a predictive role in immune neoadjuvant therapy for breast cancer. In a study of needle neoadjuvant therapy for early TNBC, the predictive value of TMB and TMB combined with gene expression profiling (GEP) for pathologic complete response (pCR) was shown. The results showed that both TMB and immune GEP were independent predictors of pCR, and that when patients were stratified according to the upper third and median GEP of TMB, the pCR rate was 82% in patients with higher TMB and GEP (95% CI: 60-95%), while the pCR rate was only 28% (95% CI: 16-43%) in patients with low TMB and GEP [64, 65].

Predictive effect of TMB on immunotherapy efficacy in other molecular types of breast cancer

Yin et al. [66] analysed whole-exon sequencing data of 366 cases of breast cancer in the Cancer Genome Atlas (TCGA) database and next-generation sequencing data of 335 cases of patients with breast adenocarcinoma. We confirmed that human epithelial growth factor receptor 2 (HER2) amplification is associated with higher TMB in breast cancer, which may be

helpful in screening breast cancer patients to determine who is more suitable for immunotherapy. TMB can not only predict the efficacy of immunotherapy for HER2-positive breast cancer but can also serve as a prognostic factor for patients treated with HER2-targeted drugs combined with chemotherapy. In a clinical study of patients with metastatic breast cancer, 31 of 46 patients (67.4%) with HER2-positive metastatic breast cancer elected to receive docetaxel plus HER2-targeting trastuzumab, while 7 patients (15.2%) received docetaxel and trastuzumab plus pertuzumab; 16 patients (34.6%) were predefined as part of the high TMB group, and 30 patients (65.4%) were in the low TMB group. The results showed that the difference in median overall survival of those in the low and high TMB groups (44.9 months vs. 85.8 months) was statistically significant [61]. TMB and antitumour immunogenicity were found to be higher in HR-negative than in HR-positive breast cancer patients. HR-negative breast cancer patients may show higher immunogenicity. Patients with HER2-positive breast cancer may exhibit higher immunogenic activity than those with HER2-negative breast cancer [67]. HR-negative or HER2-positive breast cancers exhibit higher TMB and immunogenic activity, which suggests that patients with these tumours may benefit from immunotherapy. Generally, the effect of TMB on the ICI treatment of breast cancer varies according to the molecular type of breast cancer and antitumour immune response. TMB in breast cancer was reported to be the highest in clinically aggressive TNBC, followed by HER2-positive, luminal B, and luminal A cancers [68, 69]. It is important to note that the primary source of TMB detection is primary or metastatic tumour tissue, which may lead to systematic bias because metastatic tumours tend to have more monoclonal structures. Schnidrig et al. [70] retrospectively analysed 1,662 patients with advanced cancer who were treated with ICI therapy and whose TMB was measured using the MSK-IMPACT technology platform; they then explored whether primary or metastatic TMB predicted overall survival after ICI treatment. The results showed revealed a positive correlation between primary TMB and metastatic TMB. However, primary TMB and metastatic TMB were equally effective in predicting overall survival during ICI treatment (primary TMB: HR = 0.61, 95% CI: 45-82%; metastatic TMB: HR = 0.59, 95% CI: 45-76%).

TMB combined with other biomarkers to predict the efficacy of immunotherapy for breast cancer

TMB has been demonstrated to be a biomarker that can predict the efficacy of immunotherapy in breast cancer. However, as additional research has been performed, methods to optimize the integration of TMB and other biomarkers have been proposed to improve the accuracy of prediction. A meta-analysis published in JAMA Oncology showed that the combination of multiple biomarkers had the best predictive value for response to ICI therapy compared with individual biomarkers (e.g., PD-L1, GEP, TMB) [71]. Thus, while higher TMB is associated with better treatment outcomes for ICI therapy, the complexity of the immune response means that TMB should be considered in conjunction with other factors to optimize the prediction of ICI outcomes. As for other potential biomarkers in breast cancer immunotherapy, such as new antigen load, mismatch repair defects, BRCA1/2 mutation, immunogenicity of cell death, MHC II expression, tumour-infiltrating lymphocytes, oestrogen receptor expression, and some markers of serum lactate dehydrogenase, their integration as a composite biomarker may lead to better prediction of tumour immunotherapy efficacy.

Tumour-infiltrating lymphocytes

Tumour-infiltrating lymphocytes (TILs) are a heterogeneous population of lymphocytes that are primarily present in tumour nests and in the interstitium [72]. The predictive effect of TILs on immunotherapy for breast cancer is related to the type of TIL and the molecular classification and development stage of breast cancer, but the relationship between the two is still controversial. Studies have shown that TNBC has the highest degree of TIL invasion, followed by $HER2^+$ breast cancer [73]. The higher the level of TILs in TNBC patients, the better the effect of immunotherapy, and the number of TILs is positively correlated with prognosis, such as disease-free survival [74]. The Keynote-086 study found that in metastatic TNBC, a higher abundance of interstitial TILs was closely associated with better therapeutic efficacy of pembrolizumab [75]. However, the pre-

dictive effect of TILs on immunotherapy for breast cancer is still unclear, and further studies are needed.

PD-L1

With FDA approval of PD-1/PD-L1 antibodies for the treatment of malignant tumours, PD-L1 expression has also been included in all levels of guidelines and has thus become an important immunobiomarker for screening patients for appropriate antibody therapy. In addition to tumour cells that express PD-L1, immune cells such as lymphocytes and macrophages also express PD-L1. Therefore, the following three methods can be used to evaluate the expression level of PD-L1: (1) the tumour proportion score (TPS), which refers to the proportion of PD-L1$^+$ tumour cells; (2) the combined positive score (CPS), which refers to the ratio of the total number of PD-L1$^+$ tumour cells, lymphocytes, and macrophages to the total number of tumour cells; and (3) the expression of PD-L1 in immune cells only [76]. PD-L1 expression is detected by immunohistochemistry. Currently, FDA-approved antibodies for immunohistochemical detection include 28-8, 22C3, SP-142, and SP263 [77]. The Blueprint project compared these four antibodies and showed that 28-8, 22C3, and SP263 predominantly stained tumour cells with similar results, while SP142 showed strong staining of immune cells. All four antibodies stained immune cells and showed both greater heterogeneity compared with tumour cell staining and different judgement criteria due to different critical values [78]. The IMpassion130 study used SP142 to detect PD-L1 expression in tumour and immune cells. In TNBC, PD-L1 expression is primarily found on tumour-infiltrating immune cells, and the PD-L expression level predicts whether patients will benefit from alemtuzumab combined with albumin-bound paclitaxel. Patients in the PD-L1$^+$ group had longer median PFS and OS [79]. CD8$^+$ T cells were abundant in the population of PD-L1$^+$ immune cells. Only when the PD-L1 expression of immune cells was positive could CD8$^+$ T cells predict any benefit. More PD-L1$^+$ cells were also observed in the TIL population. Similarly, patients with high TIL content could benefit only when immune cells were positive for PD-L1 expression. PD-L1 expression on tumour cells and immune cells was evaluated by SP-142 and SP263. The CPS score was used for 22C3. Over 97% of patients with positive PD-L1 by SP142 staining were also positive by 22C3 and SP263 staining, and when both SP142 and 22C3 were positive, the PFS and OS benefits of alemtuzumab combined with albumin-bound paclitaxel could be predicted. In conclusion, in different studies, the predictive effect of PD-L1 expression on the efficacy of PD-1/PD-L1 antibodies is highly variable. Due to the heterogeneity of PD-L1 expression, nonunified detection methods and interpretation standards, as well as for other reasons, more studies are needed on the molecular typing of breast cancer, detection methods, and PD-L1 expression to accurately predict the efficacy of PD-1/PD-L1 antibodies.

Conclusion and prospects

Immunotherapy has been shown to prolong the survival time of patients with a variety of solid tumours and is a promising treatment option for metastatic breast cancer. Immune checkpoint inhibitors and secondary cellular immunotherapy, which enable immune-mediated tumour clearance, are two major achievements in cancer immunotherapy that play a leading role in overcoming tumour-induced immunosuppression.

However, some patients will still experience drug resistance to existing immunotherapies, which will lead to immune escape and a poor overall therapeutic effect. In triple-negative breast cancer, the combination of chemotherapy, radiotherapy, and new targeted therapies with existing immunotherapy methods, especially immune checkpoint inhibitors, has greatly improved the efficacy of immunotherapy. This suggests that an effective combination of immune checkpoint inhibitors with chemotherapy or other immune-targeted therapies may be an effective means to improve long-term survival of patients with triple-negative breast cancer who do not have options for specific effective endocrine therapy or targeted therapy.

As breast cancer has entered a new era, immunotherapy has shown an ability to treat metastatic breast cancer. Among patients with breast cancer, those with PD-L1-positive and triple-negative metastatic breast cancer are most likely to benefit from immunotherapy, and

multiple retrospective studies have preliminarily confirmed that TMB is an effective biomarker for predicting immunotherapy efficacy in breast cancer. Additional studies have analysed TMB in different molecular types of breast cancer. Due to the complexity of the immune system, the integration of TMB and several other immune-related biomarkers into a composite biomarker can more accurately predict the outcome of ICI treatment. Since the definition of high TMB is not unified with the detection method of TMB, and because clinical trial data support is lacking, TMB is not currently able to better guide clinical treatment.

It is believed that with the increasing momentum of TMB as a biomarker of immunotherapy response and the emergence of standardized methods for clinical application, TMB detection as a way to guide treatment will become a new trend in precision medicine in the foreseeable future.

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Disclosure of conflict of interest

None.

Abbreviations

TNBC, Negative breast cancer; DFS, Disease-free survival; OS, Overall survival; PD-L1, Programmed cell death 1; TMB, Tumor mutational burden; AR-T, chimeric antigen receptor T cell; TNF, tumor necrosis factor; IFN, interferon; IL, interleukin; GM-CSF, granulocyte-macrophagecolony stimulating factor; CTLA-4, cytotoxic T lymphocyte-associated antigen 4; CTL, cytotoxic lymphocyte; MUC1-C, t MUC1 transmembrane C-terminal; ADCC, antibo-dependent cell-mediated cytotoxicity; ADCP, antibose-dependent cell-mediated phagosis; CDC, Complement dependent cytotoxicity; TRBV, cell β-chain variable; HPBC, hormone receptor positive breast cancer; PFS, disease-free progression; LAK, flymphokine-activated killer cell; ICI, immune checkpoint inhibitor; CAR T, Chimeric antigen receptor T cell immune therapy; TMB, Tumor mutation burden; HER2, Human epithelial growth factor receptor 2; TIL, Tumor-infiltrating lymphocyte; TPS, Tumor positive score; CPS, Combined positive score.

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