PD-L1/PD-1 blockade in breast cancer: The immunotherapy era (Review)

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Abstract. Breast cancer is a common malignant tumor in women. Triple-negative breast cancer (TNBC) is highly invasive with a high rate of metastasis and poor prognosis. Programmed death ligand 1 (PD-L1) plays an important role in mediating the escape of tumor cells from immune surveillance. There have been significant advances in understanding the biology of TNBC. This review presents a detailed discourse on the available data on the expression of PD-L1 in breast cancer and preliminary clinical outcome of PD-L1/PD-1 inhibitors in breast cancer patients. Early clinical trials involving PD-L1/PD-1 inhibitors have exhibited efficacy in tumor response and/or disease control in patients with refractory metastatic breast cancer, particularly TNBC. Furthermore, the mechanisms and factors that influence the immunoediting process are summarized and their functions in detail are analyzed.

Contents

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1. Introduction

Breast cancer has been identified as one of the leading causes of cancer-related deaths worldwide. Abnormalities in proteins result in malignant transformation of cells that results in 25-40% of recurrence and metastasis of cancer (1). Breast cancer is a common malignant tumor and its rate of incidence was the highest among tumors detected in women in 2019 (2).

Approximately 627,000 people are expected to succumb to breast cancer in 2019. Due to the lack of specific therapeutic targets available for triple-negative breast cancer (TNBC), the prognosis of TNBC remains unsatisfactory. The emergence of anti-PD-L1/PD-1 therapeutics has shown promise for the treatment of TNBC (3). In the present study, the advancements in the efficacy of PD-L1 against TNBC are described. Programmed death ligand 1 (PD-L1) is a ligand of programmed cell death-1 (PD-1) and is expressed on various tumors and immune cells. Once PD-L1 binds to PD-1, it inhibits T-cell migration and proliferation and the secretion of cytotoxic mediators, thereby limiting its killing effect on tumor cells (3). PD-L1 has been revealed to be highly expressed in a variety of tumors and enhance anti-tumor immunity by inhibiting PD-L1 (4). The development and clinical application of targeted drugs against signaling pathways associated with the occurrence and development of breast cancer have become a hot spot in research on breast cancer treatment (5). Targeted therapy has better clinical efficacy and safety than cytotoxic drugs (6). The present study reviewed the current molecular targeted therapeutics in treating breast cancer.

2. Overview of tumor immunotherapy

Tumor immunotherapy is a method of treatment that has been developed in recent years. It stimulates and regulates immune function, enhances antitumor ability, and controls and kills tumor cells (7). Immunotherapy has attracted attention owing to the importance of antitumor immunity, diverse mechanisms in escape of tumors from immune surveillance, continuous discovery of novel therapeutic targets and immunotherapies (8,9). Tumor immunotherapy can be divided into active and passive immunotherapy. Active immunotherapy employs tumor vaccines to mimic tumor antigens to activate immune effects on tumors, thereby directly or indirectly promoting specific antitumor immune responses in humans (10). Passive immunotherapy includes the use of monoclonal antibodies, adoptive immune cells, and cytokines. Monoclonal antibody therapy involves the administration of specific antibodies to stimulate host immune response against tumor antigens (11,12). Adoptive immune cell therapy separates and expands immune cells to induce the production of cytokines before introducing the cells into patients to increase the abundance of immune cells and enhance their anticancer function (13). Cytokine therapy involves the direct injection of cytokines into the human body, which enhances antitumor function in immune cells (14,15). Compared to traditional methods of treatment, tumor immunotherapy has specific advantages: Tumor immune-related monoclonal antibodies and tumor vaccines exhibit fewer adverse reactions, strong specificity, and promising clinical application (13).

An increase in research on immune checkpoints has been observed in recent years. Immune checkpoints are inhibitory signaling pathways present in the immune system that regulate the persistence and intensity of immune response, maintain autoimmune tolerance, and avoid tissue damage (16,17). Dysfunction of key negative regulatory molecules during T-cell activation is important for the tolerance and escape of tumor immunity. Inhibition of the immune checkpoint can reverse the immunosuppressive state of the tumor microenvironment and enhance the clearance of tumor cells (18). Immunological checkpoints include cytotoxic T lymphocyte-associated antigen-4 (CTLA-4), PD-1, B and T lymphocyte attenuator (BTLA), and lymphocyte activation gene 3 (LAG3). The FDA-approved immunological checkpoint inhibitor, ipilimumab, that targets CTLA-4 is used to treat melanoma (19,20). Researchers are further identifying novel therapeutic targets to regulate immune checkpoints. The most important monoclonal antibody currently in use targets the immune checkpoint PD-1 and its ligand PD-L1 (21). PD-L1/PD-1-related immunotherapy has become a hotspot for research on tumor immunotherapy.

3. Biological characteristics of PD-1/PD-L1

PD-1 is a type I transmembrane protein expressed on the surface of activated T cells, B cells, monocytes and dendritic cells and is composed of extracellular, hydrophobic transmembrane, and cytoplasmic regions (22). Its extracellular domain consists of a single IgV-like domain with an immunoreceptor tyrosine-based inhibitory motif (ITIM) and immunoreceptor tyrosine-based motif (23). ITIM can be commonly found in numerous immunosuppressive receptors (24). The immunosuppressive function of PD-1 is primarily exerted via ITIM. PD-L1 and PD-L2 are the ligands of PD-1 (25). PD-L1 is the primary ligand that is upregulated in various solid tumors. This reduces the infiltration of $CD4^+$ and $CD8^+$ T cells into tumors and concomitant cytokine production. PD-L1 is a member of the B7 superfamily and a type I transmembrane glycoprotein (26,27). PD-L1 is expressed in various tumors, such as urothelial, ovarian, breast, cervical, colorectal, pancreatic, gastric, melanoma, malignant glioma, and non-small cell lung cancers among others. This suggests the involvement of PD-1 signaling in tumors for immune evasion (7,28-30). PD-1/PD-Ls exerts a negative immunomodulatory effect. The surface of tumor cells in the tumor microenvironment exhibit increased expression of PD-L1 that binds to PD-1 on activated T cells, which leads to apoptosis or immunological inactivation of tumor antigen-specific T cells, thereby inhibiting immune response and promoting the evasion of tumor cells (31).

PD-L1 is expressed on the surface of various tumor and immune cells, such as T, B, and dendritic cells (13). Tumor cells bind to PD-1 on the surface of tumor infiltrating lymphocytes (TILs) via PD-L1. Activation of TILs enables immunosuppressive signaling, inhibits T-cell migration and proliferation, secretion of cytotoxic mediators, induces T-cell depletion, and limits its antitumor effects, thereby resulting in immune evasion (32,33). Decreased binding of PD-L1 to PD-1 reverses immune escape, enhances antitumor immunity, and inhibits tumor progression (34). However, PD-L1/PD-1-targeted immunotherapy has been revealed to be effective in clinical trials of various tumors, suggesting that the PD-L1/PD-1 pathway plays an important role in tumor progression (13). This targeted immunotherapy has the potential to improve the prognosis of cancer patients by inhibiting the PD-L1/PD-1 pathway (35-37). Thus, it is imperative to further identify and develop anti-PD-L1/PD-1 therapeutics for TNBC.

Detection of PD-L1. Detection of PD-L1 levels in tumors predicts patient response to anti-PD-L1/PD-1 monoclonal antibody therapy. This allows the screening of selective patients to undergo immunotherapy to reduce unnecessary waste of resources and over-treatment (38). Using the PD-1 monoclonal antibody, pembrolizumab, in patients with non-small cell lung cancer has revealed a >50% correlation between the expression of PD-L1 in tumor cells and increased efficacy (39). Immunohistochemistry (IHC) is the most commonly used method for detecting the expression of PD-L1 in tumors. IHC is a simple method that is associated with reduced time for sample processing, low cost, and enhanced visualization. However, in recent years the reliability and reproducibility of this technique have come into question. A disadvantage is with tissue organization (40). Studies have revealed that surgically resected preoperative specimens for tissue biopsy differentially express PD-L1, and biopsy specimens underestimate the expression of PD-L1 as compared to the expression in intraoperative resected specimens (41). PD-L1 levels are affected by focal expression, genetic heterogeneity, and a variety of complex factors within the tumor (42). A routine diagnostic biopsy for measuring PD-L1 expression is highly likely to be a false negative result that biases the sensitivity of PD-L1 targeted therapy (43). Moreover, there is no standardized antibody currently available for the detection of PD-L1. Different investigators use different detection

antibodies whose combined antigenic epitopes do not match. Thus, the same sample may show opposing results. Owing to these differences, an antibody from an effective clinical trial may not be reliable for other patient populations (44). Finally, setting a threshold for a positive readout is tricky and there is no standard for determining the expression of PD-L1 (45).

Researchers have compared the efficacy of determining PD-L1 expression using IHC and quantitative immunofluorescence (QIF). QIF has been revealed to be more consistent and reproducible than IHC, and the measurement of PD-L1 levels by QIF has been demonstrated to be more objective (44). Detecting the mRNA levels of PD-L1 in tumors is also important. A novel RNA detection technology, RNAScope, has been used to determine the mRNA levels of PD-L1 in 636 patients with stage I-III breast cancer. Surface expression of PD-L1 on circulating tumor cells was successfully detected in patients with hormone receptor-positive, HER-2-negative metastatic breast cancer (46,47). Further developing this technology may prove useful as a non-invasive technique for measuring PD-L1 expression in a liquid biopsy format to screen and treat patients with PD-L1/PD-1 immunotherapy by means of clinical trials in the future.

Regulation of PD-L1. The expression of PD-L1 on the surface of immune cells is relatively constant, whereas PD-L1 expression on the surface of tumor cells is dynamic (48). In addition to the effects of interferon-γ, PD-L1 expression is also affected by signaling pathways, chemotherapy, radiation therapy, as well as other factors (49). In anaplastic lymphoma kinase (ALK)-positive T-cell lymphoma, the oncogene NPM/ALK was revealed to activate transcription transducer and activator of transcription 3 (STAT3) (50). It has been revealed to bind to the promoter of PD-L1 to upregulate PD-L1 and promote immunosuppression (50,51).

In breast cancer, the inactivation of PTEN or mutation of PI3K leads to PI3K activation, which in turn activates the downstream pathways Akt and mTOR and promotes PD-L1 transcription and protein expression in a ribosomal protein S6 Kinase 1 (S6K1)-dependent manner (52,53). Tumor cells escape immune surveillance and produce immune resistance (54). In melanoma, tumor cells that are tolerant to BRAF gene inhibitors were revealed to promote PD-L1 expression by activating MAPK signaling via the c-Jun and STAT3 pathways (55). Moreover, tumor intervention also affects PD-L1 expression on tumor cells. Using chemotherapeutics, such as doxorubicin, has been revealed to downregulate PD-L1 on the surface of breast cancer cells. The expression of PD-L1 in the nucleus was increased; this may lead to chemoresistance and inhibition of apoptosis of tumor cells. The inhibition of PD-L1, doxorubicin-induced apoptosis was revealed to be increased (55,56). However, chemotherapeutics, such as paclitaxel, were reveealed to upregulate PD-L1 on the surface of ovarian cancer cells via NF-κB signaling and inhibition of T-cell function, thereby leading to immune evasion. Combining paclitaxel and PD-L1/PD-1 inhibitors was revealed to prolong patient survival (Fig. 1) (57). In addition, radiation therapy also affects the expression of PD-L1. There is increased surface expression of PD-L1 on metastatic cancer cells that are resistant to radiation therapy, resulting in the depletion of TILs and therapeutic resistance (58). Inhibiting PD-L1/PD-1 signaling during radiation therapy reverses this phenotype of T-cell depletion and promotes T-cell proliferation (59,60). This indicates that it is necessary to concurrently inhibit PD-L1/PD-1 signaling during chemotherapy and radiation therapy to reduce the immune resistance of tumor cells, enhance the therapeutic effect, and improve the prognosis of patients.

4. Prognostic significance of PD-1/PD-L1

PD-L1 is overexpressed in most breast cancers, especially TNBC tissues, as compared to its expression in normal breast tissue (59,61-64). Previous research revealed that PD-L1 was expressed in 45% of breast cancers and 59% of TNBCs among 116 breast cancer tissues. Overexpression of PD-L1 has been revealed to be associated with poor prognosis (63), larger tumors, higher tumor grade, estrogen receptor (ER)-negative, progesterone receptor-negative, and HER-2-positive status, cell proliferation, and an increased abundance of TILs in patients with breast cancer (59,64,65). Using high-throughput analysis of 650 breast cancer tissue microarrays, it was revealed that patients with breast cancer and overexpression of PD-L1 had significantly shorter overall survival (OS) (66). Previous research has demonstrated that PD-L1 expression is associated with prolonged recurrence-free survival (62). In patients with TNBC, overexpression of PD-L1 was also associated with metastasis-free survival and specific OS. The higher the expression of PD-L1, the higher the reactivity to chemotherapy (65). The potential of PD-L1 as an independent prognostic factor is unclear. This could be attributed to a variety of factors, such as detection methods and sample heterogeneity; however, it is widely recognized that PD-L1 is associated with factors related to the poor prognosis of breast cancer (42). Tumors overexpressing PD-L1 are often accompanied by the infiltration of PD-1-positive TILs that are associated with shortened OS, indicating a poor prognosis of breast cancer (67,68). This further highlights the need for utilizing PD-L1/PD-1 signaling in developing efficacious therapeutics for the treatment of TNBC.

5. Significance of targeting PD-1/PD-L1 monoclonal antibody

Binding of PD-1 to PD-L1 or PD-L2 activates PD-1 signaling (69). PD-L1 is key for the proliferation of tumor cells during antitumor immunity. Notably, tumor immunogenicity is weakened significantly and this promotes the escape of the tumor cells from immune surveillance and response. PD-L1/PD-1 plays a crucial positive stimulating role in tumor invasion and metastasis (70). Blocking the PD-L1/PD-1 interaction reduces the inhibition of innate immunity and promotes tumor-specific T-cell activation (28).

Application of PD-L1/PD-1 inhibitor. Drugs targeted to block PD-1 signaling have exhibited sustained clinical activity in numerous advanced solid tumors (Fig. 2) (71). The use of the monoclonal antibody BMS-936559 to block PD-L1 in 160 patients with advanced solid tumors during phase I clinical trials revealed an objective response rate of 6-17% (72). The objective response rates of using the PD-1-targeting antibody, nivolumab, in the treatment of advanced non-small cell lung

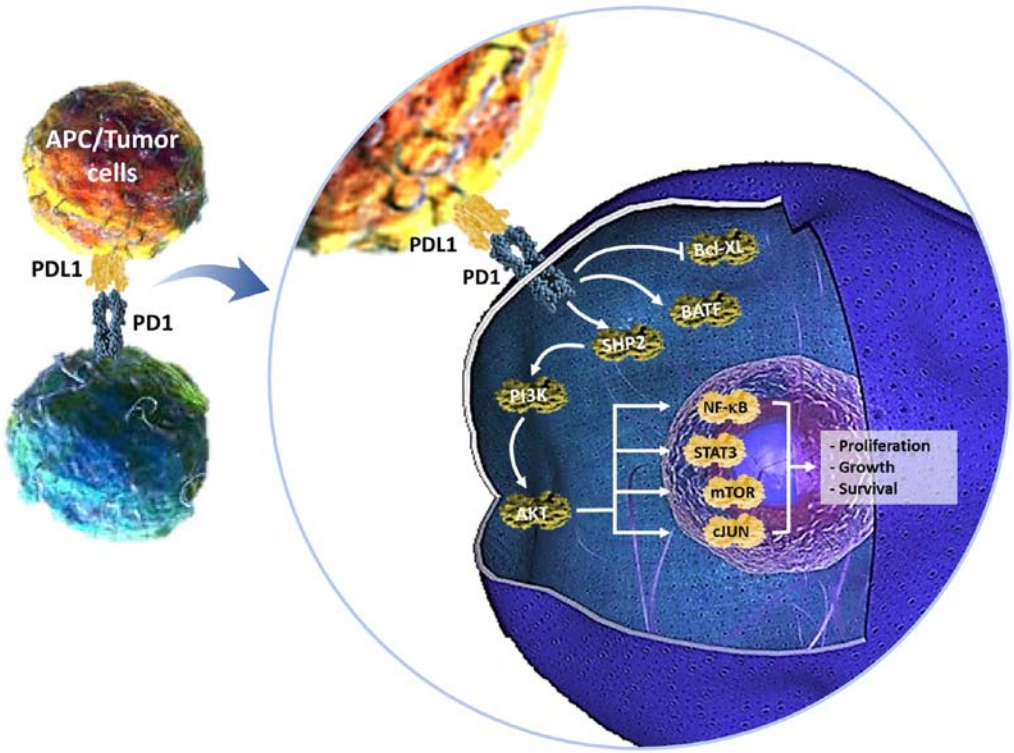

Figure 1. Mechanism of how PD-1 inhibits T-cell receptor signaling is under focus of investigation. APC, antigen-presenting cell; mTOR, mechanistic target of rapamycin; NF-κB, nuclear factor-κB; PD-1, programmed cell death protein 1; PD-L1, programmed death-ligand 1; PTEN, phosphatase and tensin homolog; SHP, Src homology region 2 domain-containing phosphatise.

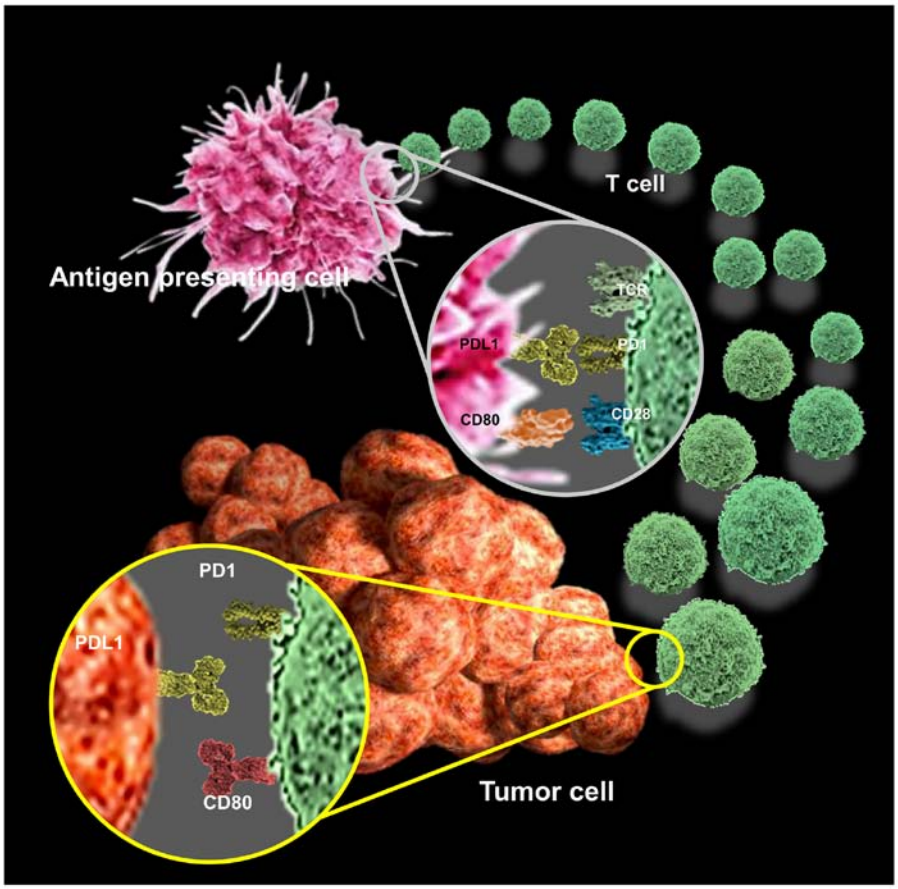

Figure 2. Mechanism of PD-1 receptor and PD-L1/L2 inhibitors-mediated cancer immunotherapy. The interaction between tumor-intrinsic PD-1 and PD-L1 inhibits tumor progression, but the treatment by anti-PD-1 disrupts this inhibitory signaling and promotes tumor progression. This process represents an adverse effect of anti-PD-1 therapy for activating antitumor immunity. PD-1, programmed cell death protein 1; PD-L1, programmed death-ligand 1.

cancer, bladder cancer, chondrosarcoma, melanoma, and renal cell carcinoma were revealed to be 18, 28 and 27%, respectively (35,73-76). The KEYNOTE-028 trial evaluated the safety and efficacy of pembrolizumab in metastatic ER^+ breast cancer. PD-L1 was expressed in 19% of the samples and the efficacy of treatment in 25 evaluable patients was analyzed. The total effective rate was 12% (77). Nanda *et al* evaluated the safety and efficacy of pembrolizumab in 32 patients with advanced TNBC who were positive for PD-L1. Most patients received 1-3 cycles of chemotherapy before being administered pembrolizumab. A minority of patients (21.9%) were administered five or more cycles of chemotherapy. Efficacy analysis of 27 evaluable patients revealed a total effective rate of 18.5%, including one patient with complete response and two patients exhibiting partial remission. Most of the adverse reactions were grade 1-2, including joint pain, fatigue, muscle pain, and nausea (78). The ORR for 21 PD-L1+ patients with metastatic triple-negative breast cancer evaluable for efficacy was 19%, including 2 CRs and 2 PRs; 3 of 4 of these responses were ongoing at the time of data cutoff. The JAVELIN study tested the efficacy of the anti-PD-L1 antibody, avelumab, on patients with a variety of all breast cancer types, regardless of the extent of PD-L1 expression. $ER^+/HER2^-$ patients in the TNBC group (58 cases) had a response rate of 8.6%. Among the 72 patients and 26 $HER2^+$ patients, the response rates were 2.8 and 3.8%, respectively. Preliminary results have revealed that PD-L1-positive tumors exhibit a higher response rate (77).

PD-L1/PD-1 inhibitor combined with anticancer drugs. TILs are an independent prognostic factor for improved OS, reduced distant recurrence, and increased metastasis-free survival in newly diagnosed patients with TNBC (79). Retrospective analysis of several large clinical trials and randomized neoadjuvant studies have demonstrated that high abundance of TILs in tumors have predictive effects on neoadjuvant chemotherapy for pCR or increased disease-free survival and OS (80). In addition, a recent retrospective analysis confirmed that the presence of TILs in patients with residual lesions after neoadjuvant chemotherapy can predict patient outcomes (81). Previous research has confirmed a significant association between activated Ras-MAPK signaling and low abundance of TILs in TNBC patients. Activated MEK inhibits IFN-γ-induced antigen presentation. This is because the Ras/MAPK pathway initiates immune evasion, and inhibition of MEK signaling upregulates PD-L1 expression on TNBC cells (82). Combining MEK and PD-L1/PD-1 inhibitors was revealed to enhance the antitumor immune response in a mouse model of breast cancer (82). Sagiv-Barfi *et al* used ibrutinib in combination with an anti-PD-L1 antibody, ibrutinib, to treat mice with TNBC that were not intrinsically sensitive to ibrutinib. The combination was determined to significantly delay tumor growth and improve survival relative to drug administration alone (83).

Concurrent vaccination with PD-L1/PD-1 inhibitor. For patients with metastatic breast cancer, monotherapy, including vaccination or monoclonal antibodies against immune checkpoints, such as anti-PD-1 or PD-L1, may not be sufficient to eradicate the lesion (84). Vaccines stimulate antigen-specific T cells after combination therapy. Monoclonal antibodies targeting the immune checkpoint allow antigen-specific T cells to proliferate. This approach may enhance antitumor immune responses, thereby increasing tumor cell death and improving patient outcomes (85). IHC of biopsy specimens from 42 patients evaluated the expression of PD-L1 on the surface of tumor cells. Notably, none of the 17 PD-L1-negative patients had an objective response to PD-1 therapy. Despite the small patient cohort, this data suggests that PD-L1 expression on the surface of tumor cells may be a biomarker for the efficacy of anti-PD-1 therapy (35). Melanoma patients and PD-L1-positive tumor patients treated with nivolumab demonstrated higher objective response rates, longer progression-free survival, and improved OS. Ongoing and future studies on the efficacy of nivolumab and other anti-PD-L1/PD-1 drugs will help confirm whether PD-L1 is a potent biomarker for predicting responsiveness to anti-PD-L1/PD-1 therapy (85). Chatterjee *et al* have revealed that the non-invasive detection of PD-L1 expression in the tumor microenvironment will enable the appropriate administration of monoclonal antibodies targeting PD-L1/PD-1 (86).

6. Anti-PD-L1 therapy for breast cancer

Overexpression of PD-L1 and PD-1 and abundance of TILs suggest that PD-L1/PD-1 are reliable candidates for PD-L1/PD-1 immunotherapy (35,38,72,87,88). The current treatment methods for breast cancer primarily include surgery, radiation therapy, chemotherapy, endocrine therapy, and targeted therapy. There are no specific and effective treatment options for TNBC patients owing to the lack specific targets, resulting in unsatisfactory disease prognosis. To that extent, the emergence of PD-L1 as a novel target is an exciting avenue in TNBC treatment (58,70).

A phase 1 clinical trial comprising patients with metastatic TNBC was reported in 2015; the monoclonal antibody, atezolizumab, targeting PD-L1 was demonstrated to be safe, tolerable, and consistently exhibited antitumor effects (89). In addition to the PD-L1/PD-1 antibody, clinical trials have used other immunological targets to enhance its antitumor effects, such as the CTLA-4 monoclonal antibody ipilimumab (90). CTLA-4 monoclonal antibodies block negative co-stimulatory signaling, promote the activation and proliferation of tumor-specific T cells, and prevent T-cell disability. PD-1 and PD-L1-targeting monoclonal antibodies reverse the immunosuppressive state of the tumor microenvironment by inhibiting the PD-L1/PD-1 axis. Compared with the CTL-4 monoclonal antibody, the PD-1 and PD-L1 monoclonal antibodies are associated with reduced adverse reactions, improved tolerance, and safety. Thus, PD-L1 antibodies are also used in combination with chemotherapeutics, such as atezolizumab in combination with white protein and abraxane or MEDI4736 (durvalumab) in combination with ibrutinib, in patients with TNBC (91). Several clinical trials are underway to evaluate the efficacy of PD-L1/PD-1 monoclonal antibodies in treating TNBC (Table I).

7. Future perspectives

The past few decades have seen significant progress in the treatment of breast cancer. This has been associated with decreased rates of mortality rate in patients with breast

Table I. Immunotherapeutic agents (anti-PD-L1) in clinical trials.

No.	Drugs	Target	Phase
NCT02622074	Pembrolizumab	PD-1	I
NCT02795429	INC280	PD-1	I
NCT02530125	Pidilizumab	PD-1	II
NCT02644369	Pembrolizumab	PD-1	II
NCT02447003	Pembrolizumab	PD-1	II
NCT02555657	Pembrolizumab	PD-1	III
NCT02967692	Dabrafenib, Trametinib, LCL161	PD-1	III
NCT02403271	MEDI4736	PD-L1	I, II
NCT02484404	MEDI4736	PD-L1	I, II
NCT02530489	Atezolizumab	PD-L1	II
NCT02724878	Bevacizumab	PD-L1	II
NCT02620280	Atezolizumab	PD-L1	III
NCT02425891	Atezolizumab	PD-L1	III
NCT02008227	Docetaxel	PD-L1	III
NCT02302807	Docetaxel, Paclitaxel, Vinflunine	PD-L1	III

PD-L1, programmed death-ligand 1; PD-1, programmed cell death protein 1.

cancer and improved quality of life. However, the prognosis for most patients with TNBC remains poor (92). Exploring TNBC-specific therapeutic targets is key to prolonging patient survival and further improving quality of life. PD-L1/PD-1 signaling is currently a research hotspot for tumor immunotherapy (84). Although PD-L1/PD-1 immunotargeting drugs have not been approved by the FDA for the treatment of TNBC, clinical trials have revealed encouraging results. Thus, PD-L1/PD-1 immunotargeting drugs are expected to be used in the treatment of patients with TNBC in the near future (92).

Despite this, several issues remain worthy of further discussion: i) PD-L1 detection methods, and outcome judgment criteria need to be further improved to accurately determine the expression of PD-L1 and select patients to avoid over- or ineffective treatment. ii) Recently, new members of the B7 family other than PD-L1/PD-1 have been discovered and may form novel targets for tumor immunotherapy. For example, PD-L2 is also expressed in TNBC; thus, anti-PD-1 antibody may be ineffective for PD-L1-negative patients. The reason why the expressed patient is effective; B7-H3 and B7-H4 have immunosuppressive roles and are expressed in tumor cells. Thus, they are excellent novel candidates to be used in tumor immunotherapy; however, their physiological functions remain to be studied (93). iii) The accuracy and adverse reactions of PD-L1/PD-1 immunotargeting therapy need to monitored. It is important to target PD-L1/PD-1-specific drugs to tumor cells during treatment and reduce the impact on autoimmune function. The growing research on these candidates will soon establish immunotherapy as an indispensable method for the comprehensive treatment of cancers.

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Availability of data and materials

Data sharing is not applicable to this article, as no datasets were generated or analyzed during the current study.

Authors' contributions

CJL, LTL, MFH and PYC conceived the study. CJL and LTL wrote the study. CJL and PYC reviewed and edited the study. PYC supervised the study. All authors reviewed the final version of the manuscript.

Ethics approval and consent to participate

Not applicable.

Patient consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

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