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REVIEW

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PD-1/PD-L1 immune checkpoint blockade in breast cancer: research insights and sensitization strategies

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Abstract

Immunotherapy targeting programmed cell death-1 (PD-1) and PD-L1 immune checkpoints has reshaped treatment paradigms across several cancers, including breast cancer. Combining PD-1/PD-L1 immune checkpoint blockade (ICB) with chemotherapy has shown promising efficacy in both early and metastatic triple-negative breast cancer, although only a subset of patients experiences durable responses. Identifying responders and optimizing immune drug selection are therefore critical. The effectiveness of PD-1/PD-L1 immunotherapy depends on both tumor-intrinsic factors and the extrinsic cell-cell interactions within the tumor microenvironment (TME). This review systematically summarizes the key findings from clinical trials of ICBs in breast cancer and examines the mechanisms underlying PD-L1 expression regulation. We also highlight recent advances in identifying potential biomarkers for PD-1/PD-L1 therapy and emerging evidence of TME alterations following treatment. Among these, the quantity, immunophenotype, and spatial distribution of tumor-infiltrating lymphocytes stand out as promising biomarkers. Additionally, we explore strategies to enhance the effectiveness of ICBs in breast cancer, aiming to support the development of personalized treatment approaches tailored to the unique characteristics of each patient's tumor.

Keywords Breast cancer, PD-1/PD-L1, Immune checkpoint blockade, Clinical trials, Biomarkers, Sensitization strategies

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Introduction

Over the past decade, cancer immunotherapy has revolutionized treatment, leading to significant improvements in patient survival [1]. Despite more than two decades of research on immunotherapy for breast cancer, the field experienced significant advancement primarily with the introduction of immune checkpoint blockades (ICBs) [2]. Even though breast cancer has traditionally been considered poorly immunogenic, programmed cell death-1 (PD-1)/PD-L1 ICBs have shown promise, particularly in triple-negative breast cancer (TNBC) [3]. In 2020, the Food and Drug Administration (FDA) approved the use of anti-PD-1 pembrolizumab combined with nab-paclitaxel as a first-line treatment for metastatic TNBC, following the successful outcomes of the KEYNOTE-355 study [4]. Additionally, the KEYNOTE-522 trial in 2021 marked a significant advance with the FDA's approval of pembrolizumab in combination with chemotherapy for early-stage TNBC, demonstrating improved pathologic complete remission rates in patients [5].

However, the clinical benefits of ICBs in TNBC are inconsistent, with response rates ranging from 15 to 60%, whether used as monotherapy or in combination [6]. This variability is partly due to the complex and dynamic nature of the tumor microenvironment (TME), which influences breast cancer progression and treatment outcomes. The efficacy of ICBs is closely linked to both the intrinsic properties of the tumor and the interactions within the TME, yet reliable biomarkers to predict therapeutic success remain limited, making it challenging to optimize their use in breast cancer [7]. Although PD-1/PD-L1 ICBs have revolutionized cancer therapy, monotherapy response rates in solid tumors remain around 20%, with many patients ultimately developing primary or acquired resistance. To enhance the effectiveness of ICBs, strategies are needed to identify biomarkers that can predict response and to explore combination therapies that increase sensitivity.

This review provides an overview of clinical trials involving PD-1/PD-L1 ICBs in breast cancer, clarifies key factors within the breast TME that regulate PD-L1 expression, and summarizes current research on prognostic and predictive biomarkers for immunotherapy. Additionally, we explore strategies to enhance the responsiveness of tumors to immune checkpoint blockades (ICBs), with the aim of advancing the development of personalized medicine in breast cancer treatment.

Clinical efficacy and updates on PD-1/PD-L1 Immune checkpoint blockades in breast cancer

PD-1/PD-L1 are among the most extensively researched targets for ICBs. The PD-1 receptor, part of the CD28 superfamily, suppresses T cell activation and immune responses when it binds to its ligand, PD-L1. This selective interaction between PD-L1, expressed by tumor cells, and PD-1, on T cells, enables tumors to evade immune detection. Numerous clinical trials have assessed PD-1/PD-L1 as therapeutic targets in breast cancer, with both anti-PD-1 therapies, like pembrolizumab, and anti-PD-L1 therapies, such as atezolizumab, being extensively investigated. A search on clinicaltrials.gov identified 304 clinical trials investigating anti-PD-1/PD-L1 therapies specifically for breast cancer. Our analysis reveals that anti-PD-1 therapies have been studied more extensively than anti-PD-L1 therapies (Fig. 1a). While research into neoadjuvant immunotherapy has increased in recent years, the majority of clinical trials still focus on advanced metastatic breast cancer (Fig. 1b), with most of these trials targeting subtypes of triple-negative breast cancer (TNBC) (Fig. 1c). Additionally, clinical studies targeting PD-1 and PD-L1 therapies have consistently focused more on advanced breast cancer than on early-stage disease (Fig. 1d and e); (Table 1) .

Clinical evidence of PD-1/PD-L1 immune checkpoint blockades in metastatic breast cancer

The main clinical trials involving ICBs in breast cancer initially focused on their efficacy in metastatic disease. The KEYNOTE-012 trial was an early study that explored single-agent PD-1/PD-L1 ICBs therapy in patients with advanced metastatic breast cancer, particularly targeting those with PD-L1-positive triple-negative breast cancer (TNBC). The study reported an overall response rate (ORR) of 18.5%, highlighting the potential of ICBs in treating breast cancer [8]. However, subsequent studies indicated that only a minority of patients achieved long-term survival benefits [9]. In the phase III KEYNOTE-119 trial, pembrolizumab monotherapy did not significantly improve overall survival (OS) compared to chemotherapy in patients with advanced TNBC who had failed previous systemic therapy, thus laying a foundation for exploring combination therapy options [10]. The IMpassion-130 trial investigated atezolizumab plus nab-paclitaxel, finding no statistically significant OS benefit in the overall population. However, in patients with ≥1% PD-L1-expressing immune cells, the combination showed a 7.5-month improvement in OS and a 33% reduction in the risk of death [11, 12]. Despite these findings, the Food and Drug Administration (FDA) withdrew atezolizumab's approval due to the lack of statistically significant improvements in progression-free survival (PFS) and OS in the intent-to-treat (ITT) population [11]. The IMpassion-131 study, which compared atezolizumab with paclitaxel, was terminated early due to a lack of clinical benefit and safety concerns [13]. In the KEYNOTE-355 study, pembrolizumab plus chemotherapy was compared to placebo plus chemotherapy as a first-line treatment for patients with metastatic TNBC [14]. Due to significant

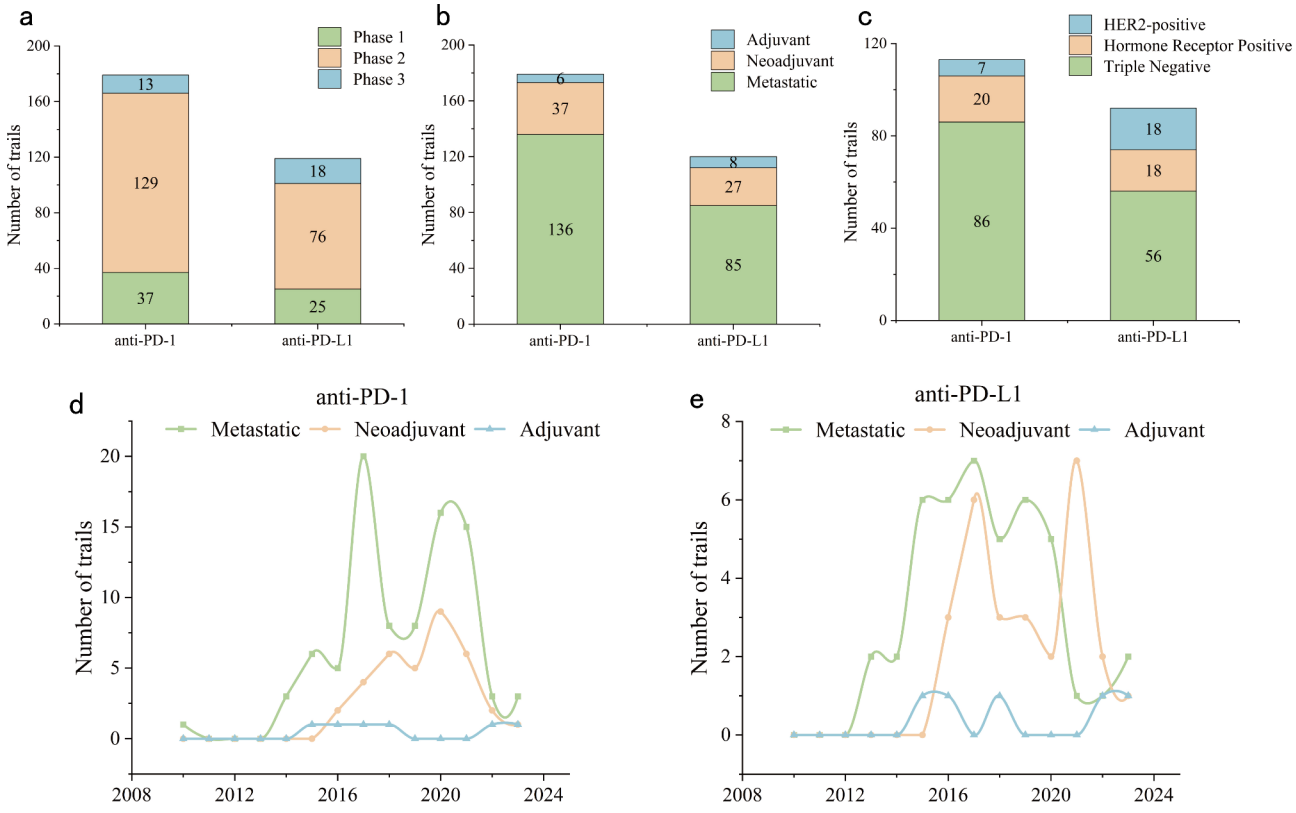

Fig. 1 Search results clinical trials in breast cancer for anti-PD-1/PD-L1. a–c show the histogram of clinical trials number in breast cancer since 2010, by trial phase (**a**), by trial setting (**b**), and by subtype (**c**). **d**-**e** show the number of anti-PD-1/PD-L1 clinical trials in breast cancer per year by starting date

and clinically relevant improvements in PFS, the FDA approved pembrolizumab in combination with chemotherapy in November 2020 for PD-L1-positive tumors. The SAFIR02-Breast Immuno study investigated the use of PD-L1 antibody monotherapy as maintenance treatment for metastatic breast cancer [15]. Overall, durvalumab did not significantly improve progression-free or overall survival in the general study population. However, exploratory analysis showed a hazard ratio (HR) of death of 0.37 in PD-L1-positive triple-negative breast cancer (TNBC) patients and 0.49 in PD-L1-negative TNBC patients, suggesting some potential benefit in these subgroups.

In the MEDIOLA trial, the combination of the PARP inhibitor olaparib with durvalumab was tested in patients with germline BRCA-mutated metastatic breast cancer [16]. Results showed a disease control rate (DCR) of 80% at 12 weeks, with an objective response rate (ORR) of 63.3%, and a DCR of 50% at 28 weeks. These findings suggest that olaparib combined with durvalumab offers promising efficacy, with a durable therapeutic effect and favorable survival outcomes.

However, the combination of ICBs with target therapy has not consistently met expectations in metastatic breast cancer with other subtypes. A phase 1b trial investigating the combination of abemaciclib with pembrolizumab, with or without endocrine therapy, in estrogen receptor (ER) positive metastatic breast cancer, revealed increased hepatotoxicity and interstitial lung disease [17]. Additionally, the response rate for trastuzumab plus pembrolizumab in trastuzumab-resistant, PD-L1-positive metastatic HER2-positive breast cancer was only 15% [18]. Atezolizumab, when combined with trastuzumab-emtansine (T-DM1), did not improve PFS and was associated with an increase in adverse effects [19].

Clinical evidence of PD-1/PD-L1 immune checkpoint blockades in early breast cancer

Recent research has increasingly focused on early-stage breast cancer. Untreated or early-stage breast cancer is generally more biologically homogeneous and less immunosuppressed, making it a promising target for immunotherapy. Additionally, the role of neoadjuvant therapy in localized breast cancer has evolved significantly. The KEYNOTE-522 trial marked a pivotal moment in neoadjuvant studies, demonstrating that stage III patients with newly diagnosed, non-metastatic TNBC who received pembrolizumab combined with neoadjuvant chemotherapy had a higher pathologic complete remission (pCR) rate (64.8% vs. 51.2%) compared to those receiving placebo [3]. The continued use of pembrolizumab as adjuvant therapy post-surgery also improved event-free

Table 1 The main trials of immune checkpoint blockade in metastatic and early breast cancer.

Trial	Phase	Drug	Patients	Main results	Reference
KEYNOTE-012	Ib	Pembrolizumab	Metastatic TNBC	ORR: 18.5%	[8]
KEYNOTE-119	III	Pembrolizumab vs chemotherapy	Metastatic TNBC	CPS≥1: OS: 10.7 vs 10.2 months (HR=0.86, P≥0.073) CPS≥10: OS: 12.7 vs 11.6months (HR=0.78, P=0.057)	[10]
IMpassion -130	III	Atezolizumab+Nab-Paclitaxelvs placebo+Nab-Paclitaxel	Metastatic TNBC	OS: 21.0 vs 18.7 months (HR=0.87, P=0.077) PD-L1+: OS 25.4 vs 17.9 months (HR=0.67)	[11]
KEYNOTE-355	III	Pembrolizumab+Nab-Paclitaxel/Paclitaxel/Gemcitabine+Carboplatin vs placebo+Nab-Paclitaxel/Paclitaxel/Gemcitabine+Carboplatin	Metastatic TNBC	CPS≥10: PFS: 9.7 vs 5.6 months (HR=0.67, P=0.0012) CPS≥1: PFS: 7.6 vs 5.6 months (HR=0.74, P=0.0014)	[14]
SAFIR02-BREAST IMMUNO	II	Durvalumab vs chemotherapy	Metastatic HER2-	mPFS: 2.7 vs 4.6months (HR=1.4, P=0.047) mOS: 21.7 vs 17.9months (HR=0.84, P=0.423) TNBC PD-L1+ : mOS=27.3 vs 12.1months (HR=0.37, P=0.0678)	[15]
MEDIOLA	I/II	Olaparib+Durvalumab	Metastatic HER2-	Disease control at week 12: 80%;	[16]
KEYNOTE-522	III	Pembrolizumab+Carboplatin+Paclitaxel+4xAC+adjuvant pembrolizumab vs placebo+Carboplatin+Paclitaxel+4xAC+adjuvant placebo	Neoadjuvant Adjuvant TNBC	pCR: 64.8 vs 51.2% (p=0.00055) PD-L1+: pCR 68.9 vs 54.9%	[3]
GeparNUEVO	II	Durvalumab+nab-paclitaxel+EC vs Placebo+nab-paclitaxel+EC	Neoadjuvant TNBC	pCR: 53.4 vs 44.2% (p=0.224) 3-year iDFS 84.9 vs 76.9% (HR=0.54, P=0.0559) 3-year OS: 95.1 vs 83.1% (HR=0.26, P=0.0076)	[22]
IMpassion031	III	Atezolizumab+Nab-paclitaxel+4xAC vs placebo+Nab-paclitaxel+4xAC	Neoadjuvant TNBC	pCR: 57.6 vs 41.1% (p=0.0044) PD-L1+: pCR 68.8 vs 49.3% (p=0.021)	[19]

Table 1 (continued)

Trial	Phase	Drug	Patients	Main results	Reference
GIADA	II	EC+Nivolumab+triptorelin+exemestane	Neoadjuvant Luminal B	pCR: 16.3%	[24]
I-SPY2	II	Weekly paclitaxel followed by AC+pembrolizumab vs weekly paclitaxel followed by AC	Neoadjuvant HER2-	pCR: HER2-: 44 vs 17% HR+/HER2-: 30 vs 13% TNBC: 60 vs 22%	[20]
CheckMate 7FL	III	NACT+Nivolumab vs NACT+placebo	Neoadjuvant ER+/HER2-	CPS≥1: pCR: 40.4% vs 23.8%; CPS≥10: pCR: 65.7% vs 33.3; CPS≥20: pCR: 78.9% vs 26.7%	[25]
KEYNOTE-756	III	Pembrolizumab+paclitaxel+4xAC+adjuvant pembrolizumab+adjuvant endocrine therapy vs placebo+paclitaxel+4xAC+adjuvant pembrolizumab+adjuvant endocrine therapy	Neoadjuvant Adjuvant ER+/HER2-	pCR: 24.3% vs 15.6% (p=0.00005)	[26]

TNBC, triple-negative breast cancer; ORR, overall response rate; CPS, combined positive score; OS, overall survival; AC, anthracycline-cyclophosphamide; pCR, pathological complete response; PFS, progression-free survival; mPFS, Median PFS; mOS, Median OS; EC, epirubicin-cyclophosphamide; HR+, hormone-receptor-positive; ER+, estrogen receptor positive; NACT, neoadjuvant chemotherapy

survival (EFS) [5]. As a result, in July 2021, the FDA approved pembrolizumab in combination with chemotherapy as a treatment option for early TNBC. Following this, the NeoTRIPaPDL1 trial explored the combination of nab-paclitaxel and carboplatin as neoadjuvant therapy [20]. The IMpassion031 trial combined nab-paclitaxel and atezolizumab with sequential anthracycline-based chemotherapy, resulting in an increased pCR rate (41% vs. 58%) [21]. The GeparNUEVO trial combined nab-paclitaxel with durvalumab but did not show a significant increase in the pathologic complete response (pCR) rate [22]. Despite this, adding durvalumab to neoadjuvant chemotherapy (NACT) significantly improved 3-year overall survival (OS), indicating that pCR may not fully capture long-term survival benefits in neoadjuvant immunotherapy trials. This suggests that the use of pCR as a surrogate endpoint in these trials should be further evaluated. Despite these advances, most clinical trials have focused on TNBC, with limited data available for luminal and HER2-positive subtypes.

In luminal breast cancer, the combination of chemotherapy and ICBs tends to result in lower pCR rates, likely due to the "colder" immunophenotype of this subtype. However, in the I-SPY2 trial for early-stage breast cancer, combining pembrolizumab with chemotherapy (weekly paclitaxel followed by doxorubicin-cyclophosphamide) showed a high pCR rate in patients with HER2-negative BC [23]. The neoadjuvant phase II GIADA trial evaluated epirubicin/cyclophosphamide followed by nivolumab in patients with Luminal B-like breast cancer, alongside concurrent triptorelin with chemotherapy and exemestane with nivolumab [24]. Pathologic complete response (pCR) was achieved in 16.3% of patients, indicating that Luminal B-like breast cancers with certain molecular subtypes or immune activation may respond to sequential anthracycline and anti-PD-1 therapy.

The CheckMate7FL trial found that nivolumab was increasingly effective in breast cancer patients with higher PD-L1 expression levels [25]. This suggests that higher PD-L1 expression is associated with greater pCR rates, highlighting that adding nivolumab to neoadjuvant chemotherapy may be particularly effective in PD-L1+, ER+/HER2- breast cancer. Additionally, the KEYNOTE-756 study recently demonstrated that adding pembrolizumab to neoadjuvant chemotherapy improved pCR in early-stage hormone receptor (HR)-positive breast cancer (24.3% vs. 15.6%) [26]. The next step may involve identifying which HR-positive breast cancer patients are most likely to benefit from these treatment strategies, potentially leading to changes in clinical practice.

Mechanisms regulating PD-1/PD-L1 expression

The immune profile of tumors is influenced by a complex interplay of diverse factors (Fig. 2). Genomic alterations play a crucial role in cancer immune escape, and epigenetic mechanisms are also involved in the regulation of PD-1/PD-L1 expression. In addition, mechanisms exist to regulate PD-L1 expression at the level of transcriptional, post-transcriptional, and post-translational modifications. Cytokines and the presence of sufficient inflammation between different cell types are crucial for eliciting effective anti-tumor immune responses following immunotherapy. However, not all patients with PD-L1-expressing tumors respond well to immune checkpoint inhibitors, suggesting that varying tumor-associated immune states may influence the response to PD-1/PD-L1 ICBs.

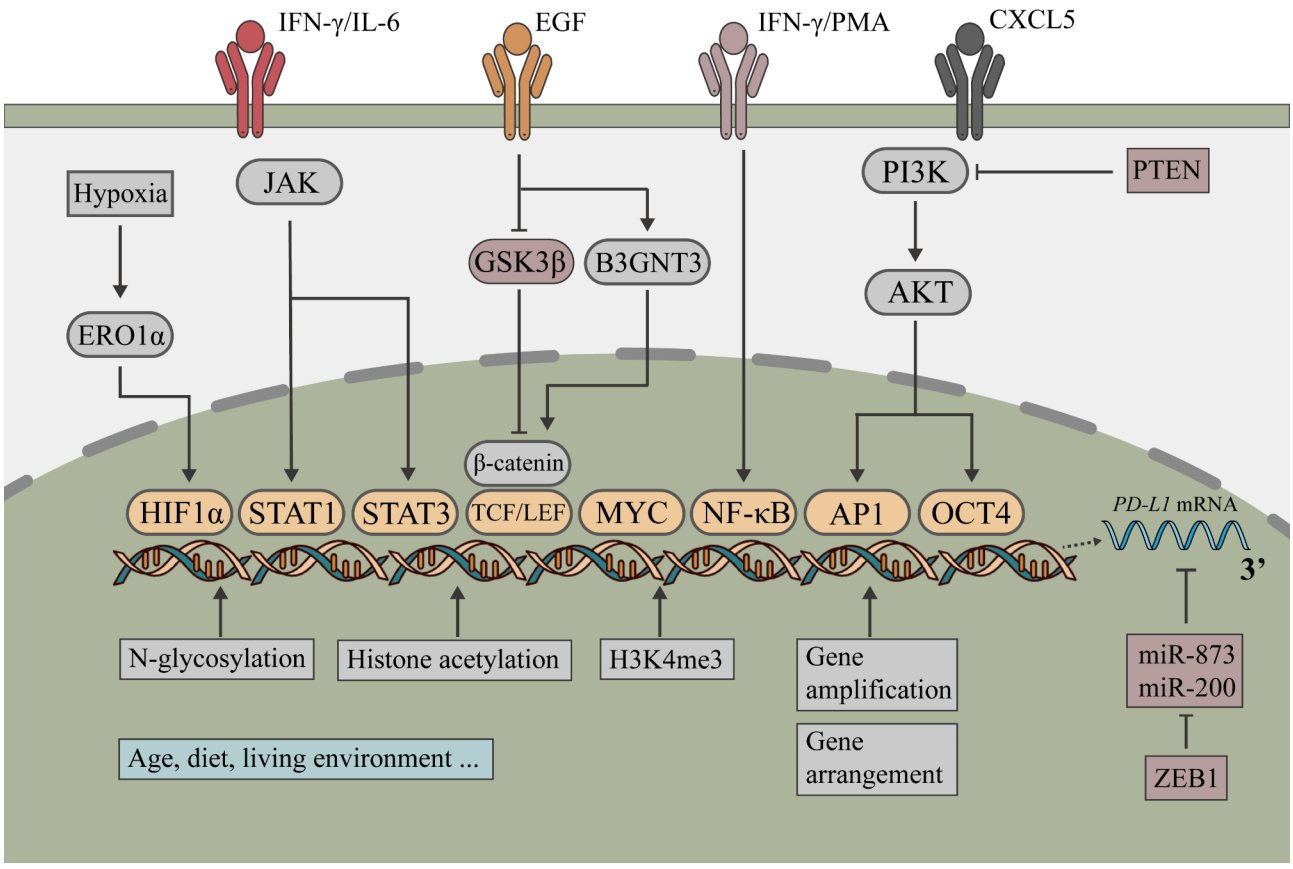

Fig. 2 The Regulatory Mechanism of PD-L1 Expression. The complicated regulation of PD-L1 expression includes various transcription factors, epigenetic and genetic alterations. ERO1α, endoplasmic reticulum oxidoreductase; IFN-γ, Interferon-γ; IL-6, Interleukin-6; JAK, Janus Kinase; EGF, Epidermal growth factor; GSK3β, Glycogen synthase kinase 3β; B3GNT3, β-1,3-N-acetylglucosaminyltransferase 3; PMA, phorbol 12-myristate 13-acetate; PI3K, Phosphatidylinositide 3-kinases; HIF1α, hypoxia-inducible factor 1α; STAT, signal transducer and activator of transcription; TCF, T cell-specific transcription factor; LEF, lymphoid enhancer-binding factor; NF-κB, nuclear factor κB; AP1, activator protein 1; H3K4me3, histone H3 lysine 4 trimethylation; miRNA, microRNAs

Genetic mutations and epigenetic modifications

The mutational load of a tumor plays a significant role in shaping its immune profile. Tumors with a higher mutational burden are more likely to present immunogenic mutations, which are recognized as foreign by the immune system and can serve as targets for immune cells [27]. Genomic rearrangements in the chromosomal region 9p24.1, where the CD274 gene (encoding PD-L1) is located, have been shown to upregulate PD-L1 expression, thereby enhancing immune escape [28]. Mutations in the oncogene p53, found in more than 50% of malignant tumors, are associated with elevated levels of PD-L1 mRNA and protein [29]. This effect is mediated through the IFN-gamma-Janus kinase (JAK) signaling pathway and transcriptional activator (STAT) pathway, with JAK2, located on chromosome 9p, playing a key role in regulating PD-L1 production. Mutations or amplifications within the JAK family can further increase PD-L1 RNA expression, promoting immune evasion. Additionally, PTEN deletion, common in HR-negative breast cancer, has been shown to induce PD-L1 expression via activation of the PI3K signaling pathway [30]. The activation and nuclear translocation of STAT1 and STAT3 further support the steady-state expression of PD-L1 by forming a heterodimer that interacts with the PD-L1 promoter [31].

Epigenetic modifications also play a crucial role in regulating PD-L1 expression. Histone acetylation, for instance, recruits bromodomain and extra-terminal (BET) proteins to the CD274 locus, enhancing PD-L1 mRNA synthesis during transcription [32]. Mixed Lineage Leukemia 1 (MLL1) shows a strong affinity for the CD274 promoter, leading to the upregulation of proteins associated with H3K4 trimethylation (H3K4me3), a marker for histone methylation. This indicates that H3K4me3 trimethylation at histone H3 lysine 4 enhances PD-L1 expression in tumor cells [33]. MicroRNAs (miRNAs) also play a crucial role in regulating PD-L1 expression by targeting transcripts through the 3′ UTR and coding sequence [34]. The absence of specific miRNAs can regulate the expression level of PD-L1 in certain tumor cells. For instance, miR-200 interacts with the

3′ UTR of CD274, downregulating PD-L1 and thereby impairing tumor metastasis. Conversely, increased expression of zinc finger E-box binding homeobox 1 (ZEB1) suppresses miR-200 in lung cancer, leading to elevated PD-L1 levels and reduced cytotoxic T cell activity [35]. Moreover, miR-873, which regulates stemness and drug resistance by modulating PD-L1/PD-1 signaling, acts as a tumor suppressor in various cancers [36]. In TNBC, miR-195/miR-497 regulates CD274 expression by binding to its 3′ UTR [37].

Post-translational regulation

Furthermore, N-glycosylation is essential for PD-L1 activation and stability, facilitating its interaction with the PD-1 receptor. Partial glycosylation of the N-linked glycan on PD-L1 regulates its binding with PD-1 and stabilizes PD-L1 expression [38]. This stable expression is promoted by EGF/EGFR signaling, which upregulates B3GNT3 glycosyltransferase and inhibits GSK3β activity [39]. In contrast, GSK3β prevents PD-L1 glycosylation, leading to its proteasomal degradation, underscoring the intricate molecular balance that regulates PD-L1 expression in cancer. Therefore, targeting protein glycosylation may offer a potential strategy to enhance immune checkpoint therapy.

Aberrant inflammatory and carcinogenic signaling pathways

The expression of immune checkpoint molecules like PD-1/PD-L1 is intricately regulated by aberrant inflammatory and carcinogenic signaling pathways, enabling malignancies to evade immune detection [40]. Recent studies have shown that the oncogenic transcription factor MYC binds to the PD-L1 promoter, increasing its expression across various cancer types [41, 42]. Notably, inhibiting MYC leads to a decrease in PD-L1 mRNA levels, thereby enhancing anti-tumor immunity. Tumor cells in the microenvironment are subject to surveillance and attack by both innate and adaptive immune systems, yet they can upregulate PD-L1 expression through inflammatory pathways to suppress these anti-tumor responses. For example, the proinflammatory cytokine IFN-γ, secreted by T cells and natural killer cells, typically enhances immune surveillance by increasing major histocompatibility complex (MHC) expression. However, tumor cells exploit the IFN-γ/JAK/STAT1 pathway to boost PD-L1 expression, which inactivates cytotoxic T cells and dampens the immune response [43]. In triple-negative breast cancer (TNBC), PD-L1 transcription is regulated by the lipid kinase PIPK1-γ. The activation of NF-κB by IFN-γ and PMA induces PIPK1-γ expression, thereby elevating PD-L1 levels in TNBC cells [39]. In addition to IFN-γ, other inflammatory stimuli such as IL-6 can also induce PD-L1 expression. The IL-6-JAK-STAT3 pathway promotes PD-L1 expression and leads to resistance to immune killing [44]. Additionally, myeloid-derived suppressor cells (MDSCs) within the tumor microenvironment exert strong immunosuppressive effects by activating the PI3K, NF-κB, and AKT signaling pathways in PD-1-/PD-L1+regulatory T cells (Tregs), further hindering the anti-tumor immune response [45]. CXCL5, a subfamily of CXC chemokines, is one of the cytokines secreted by cancer-associated fibroblasts. Studies have shown that CXCL5 secreted by cancer-associated fibroblasts promotes PD-L1 expression by activating PI3K and AKT signal transduction, thereby inhibiting anti-tumor immunity [46].

Epithelial-mesenchymal transition (EMT), a process by which epithelial cells acquire mesenchymal characteristics, promoting tumor progression and metastasis, also influences PD-L1 expression. Alterations in the PI3K/AKT pathway—such as the loss of PTEN—lead to increased PD-L1 protein levels in tumor cells by affecting downstream proteins like eIF4E and S6K1 [47]. Overexpression of PD-1/PD-L1 in tumor cells activates the PI3K/AKT pathway, which also upregulates Nanog and OCT4, transcription factors associated with embryonic stem cells and cancer stem cell traits [48]. Consequently, identifying EMT characteristics in breast cancer patients may help identify relevant inhibitors, potentially enhancing the efficacy of anti-PD-1/PD-L1 therapies.

Furthermore, the overexpression of endoplasmic reticulum oxidoreductase (ERO1α) during oxidative protein folding increases PD-L1 expression in breast cancer. ERO1α induces hypoxia-inducible factor 1-alpha (HIF1α) to respond to the hypoxic tumor microenvironment by elevating reactive oxygen species (ROS) levels, which in turn upregulate PD-L1 mRNA and protein levels [49]. Other extrinsic factors, such as the gut microbiome, have been shown to influence cancer progression, particularly in gastrointestinal tumors [50]. However, research into the microbiome's role in breast cancer is also exploring [51]. Moreover, factors such as age [52], diet [53], and living environment [54] may affect systemic immune responses, potentially influencing cancer progression and treatment outcomes.

Changes of the tumor microenvironment (TME) after therapy

The tumor microenvironment (TME), a complex and dynamic milieu influenced by tumor formation, plays a crucial role in cancer development. The interactions within the TME are critical in shaping tumor progression and the immune response. The importance of the immune system in the breast cancer microenvironment has become increasingly recognized, with the composition and interaction of immune cells within the TME being vital to successful tumor elimination [55].

Tumor-infiltrating lymphocytes (TILs), which include both T and B lymphocytes, have emerged as a potential biomarker due to their simplicity and effectiveness [56]. Breast cancer, however, typically exhibits fewer TILs compared to other cancers. The extent of lymphocyte infiltration varies across breast cancer subtypes, with higher infiltration observed in hormone receptor (HR)-negative or HER2-positive breast cancer [57]. Different T cell subtypes exert distinct effects on the TME. For instance, CD4+T lymphocytes, which include diverse subpopulations such as Th1, Th2, Th17, and FoxP3+Tregs, have a less straightforward prognostic value compared to CD8+T lymphocytes [58]. C. Gu-Trantien and colleagues discovered that CD4+T follicular helper (Tfh) cells producing CXCL13 are associated with extensive immune infiltration and are primarily located in the germinal centers of tertiary lymphoid structures, potentially enhancing immune cell recruitment to the TME [59].

Interestingly, even PD-L1-negative tumors at baseline can respond to therapy in early settings [19, 60]. This variation in the predictive value of PD-L1 may partly result from treatment-induced PD-L1 expression, reflecting changes in the TME during therapy (Fig. 3). Analysis of residual lesions post-neoadjuvant therapy revealed an increase in total stromal TILs (sTILs), though this was not statistically significant [61]. Pre-treatment tumor biopsies often reveal lymphocyte infiltrates predominantly composed of CD4+T cells. However, following anti-PD-1 therapy, there is a marked increase in CD8+T cells and a notable decrease in CD4+FoxP3+regulatory T cells (Tregs). Blomberg et al. [62] found that patients and mice responding to ICBs therapy exhibited increased eosinophils both intratumorally and systemically, with ICBs amplifying systemic eosinophilia by stimulating eosinophil production in the bone marrow and enhancing IL-5 production by CD4+T cells (Fig. 3).

Additionally, in patients responding to a combination of paclitaxel and atezolizumab, elevated levels of conventional dendritic cells 1 (cDC1), myeloid dendritic cells (mDC), and plasmacytoid dendritic cells (pDC) were observed compared to paclitaxel alone (Fig. 3). This suggests that atezolizumab contributes to the effectiveness of anti-PD-L1 therapeutic strategies [63]. Transcriptome

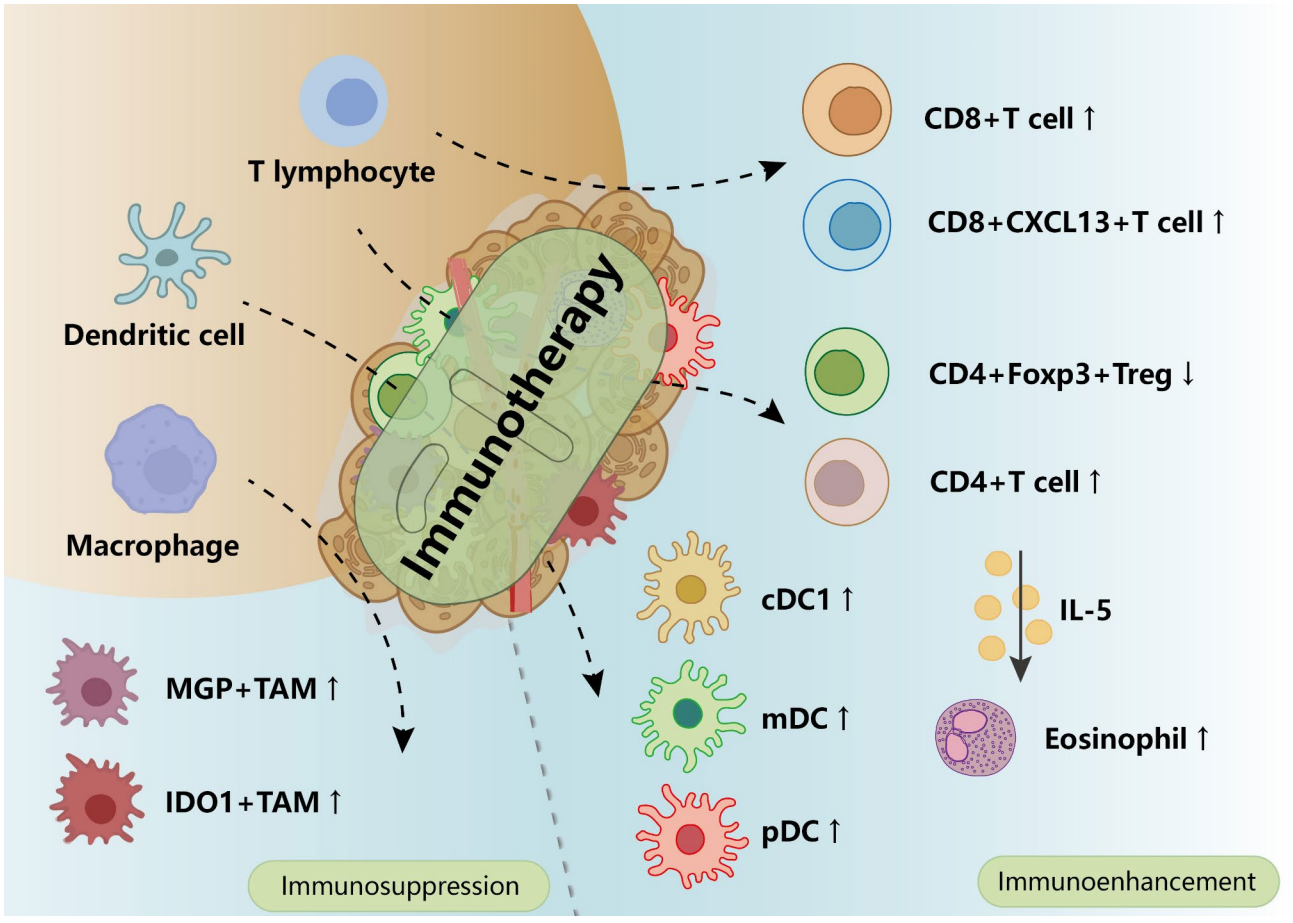

Fig. 3 Tumor microenvironment and variation of associated immune cells after immunotherapy in breast cancer. DC, dendritic cell; TAM, tumor-associated macrophage; IL, Interleukin

analysis revealed that genes associated with effector and memory functions, such as IFNG, GZMK, GZMA, and CD44, were upregulated in CD8-CXCL13+cells after treatment (Fig. 3). Transcription factors like TBX21, BHLHE40, and BCL6 were also upregulated, while genes associated with T cell exhaustion were downregulated. This phenotypic shift in CD8-CXCL13+cells showed a decrease in exhaustion markers and an increase in effector-memory characteristics, indicating enhanced cytotoxic functions following anti-PD-L1 therapy. Furthermore, these cells showed increased expression of genes involved in T-cell-mediated cytotoxicity, antigen processing and presentation, and IFN-γ-mediated signaling pathways, further indicating their enhanced effector functions post-therapy.

However, tumor-promoting MGP+tumor-associated macrophages (TAMs) and IDO1+TAMs were also found to increase in the TME (Fig. 3), with IDO1 and MGP being secreted via the NF-κB signaling pathway [64]. These TAMs may alter the TME by secreting tumor-promoting growth factors, suppressing the immune response, and contributing to the development of immunotherapy resistance in breast cancer. Given these complex interactions, more sophisticated follow-up studies are needed to determine the exact prognostic significance of residual disease with different TILs immune characteristics after immunotherapy.

Potential efficacy biomarkers of PD-1/PD-L1 immune checkpoint blockades

Tumor-infiltrating lymphocytes

Tumor-infiltrating lymphocytes (TILs) have emerged as a readily available and effective marker for assessing tumor immune profiles (Fig. 4). The pathological evaluation of TILs offers a convenient approach to identifying patients who are likely to respond favorably to immunotherapy. For instance, a randomized trial found that a TIL level of ≥5% could predict a positive response to pembrolizumab. Additionally, a retrospective analysis from another clinical trial demonstrated that patients with moderate to high TILs who were treated with nab-paclitaxel, with or without atezolizumab, experienced improved

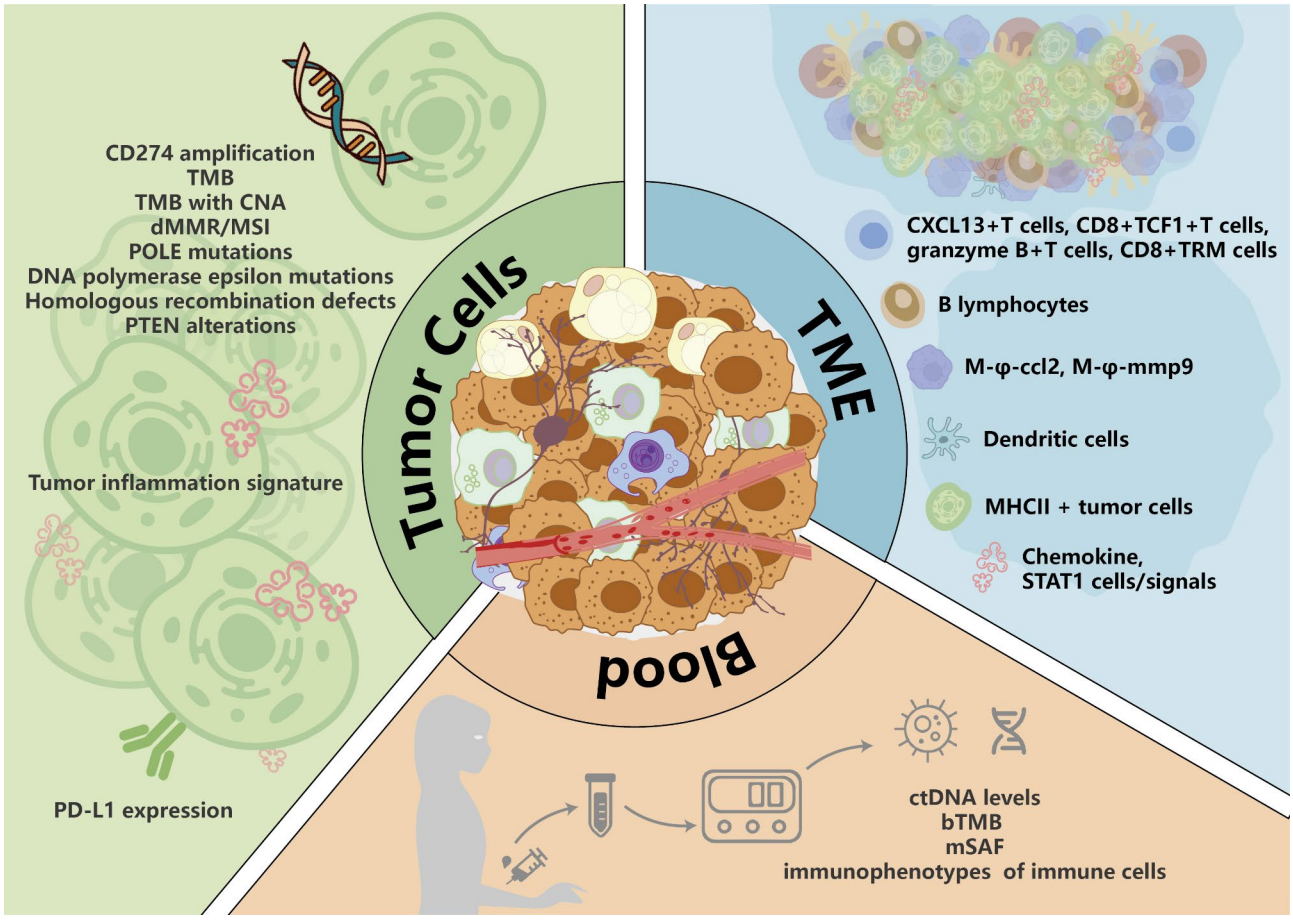

Fig. 4 An overview of biomarkers for immunotherapy response in breast cancer. TMB, tumor mutational burden; TME, tumor microenvironment; CNA, copy number alterations; dMMR, defects in mismatch repair; MSI, microsatellite instability; POLE, polymerase epsilon; mSAF, maximum somatic allele frequency

progression-free survival (PFS) and overall survival (OS) in PD-L1-positive tumors [65]. Furthermore, combining atezolizumab with T-DM1 has shown greater benefits in patients with higher TIL percentages compared to those with lower TIL levels [19]. Early-stage triple-negative breast cancer (TNBC) patients with high TIL levels tend to have a better prognosis [66].

TILs have also been associated with an increased frequency of pathological complete remission (pCR) following neoadjuvant chemotherapy across all early-stage breast cancer subtypes, providing both predictive and prognostic value in anti-HER2 and chemotherapy treatments for HER2-positive breast cancer [19, 67, 68]. Recent studies have suggested that the subpopulations and immunophenotypes of TILs may be optimal indicators of ICB response. For example, CD8+intratumoral TILs (iTILs) have been identified as superior predictors of tumor immunogenicity compared to total sTILs. In a randomized study, Loi et al. demonstrated that PD-L1 co-positive scores, sTIL levels, CD8+iTIL counts, and tumor mutational burden (TMB) were all correlated with favorable responses to pembrolizumab in previously treated or untreated metastatic breast cancer patients. Additionally, in patients responding to the combination of atezolizumab and paclitaxel, an increase in CD8-CXCL13 and CD4-CXCL13 T cells was observed, indicating that CXCL13+T cells play a crucial role in the clinical outcome of anti-PD-L1 therapy [58].

Furthermore, mϕ-CCL2 and mϕ-MMP9 macrophage subpopulations were found to be highly correlated with CXCL13+T cells, with higher pre-treatment levels suggesting a better response to combination therapy. CD8+tissue-resident memory (TRM) cells have also been investigated as potential biomarkers for immune checkpoint suppression responses. For instance, in metastatic TNBC patients treated with pembrolizumab alone, enriched CD8+TRM cell profiles predicted a favorable treatment response [69]. Additionally, CD39 expression has been proposed as a marker for identifying tumor-specific CD8+TRM cells, with studies showing that CD39 is expressed on TRM cells derived from the tumor beds of breast cancer patients [70, 71]. Moreover, abundant B-cell tumor infiltration, particularly within tertiary lymphoid structures (TLS), has been positively associated with better clinical prognosis [72].

High levels of dendritic cells, chemokines, and STAT1 signaling were most predictive of TNBC response, while high B-cell levels combined with low mast cell signaling were most predictive of achieving pCR in HR+HER2- breast cancer [73]. Recent advances in single-cell and region-based complex immunophenotyping have enabled new methods for analyzing spatial relationships within tumor tissues. By comparing multicellular spatial organization before and after treatment, researchers have identified tissue features predictive of ICB efficacy. For example, Wang et al. found that the expansion of CD8+TCF1+T cells and MHCII+cancer cells were primary indicators of therapeutic response, while interactions between cancer cells and immune cells, particularly B cells and granzyme B+T cells, served as secondary indicators [74]. During therapy, responsive tumors were enriched in granzyme B+T cells, whereas resistant tumors exhibited characteristics of CD15+cancer cells.

Programmed death ligand-1

PD-L1 expression is frequently used as the primary biomarker for immune checkpoint blockades (ICB) therapy (Fig. 4). Studies have shown that higher percentages of PD-L1 expression are often linked to favorable responses. However, in breast cancer, PD-L1 expression levels are typically lower than in other solid tumors, with an expression rate of approximately 10–20% [75, 76]. The IMpassion031 trial demonstrated that the combination of atezolizumab with neoadjuvant chemotherapy (NACT) improved the rate of pathological complete remission (pCR) in patients with early-stage TNBC, irrespective of their PD-L1 expression status [21]. Similarly, in a randomized neoadjuvant trial involving early-stage TNBC, patients treated with chemotherapy and pembrolizumab experienced an increased pCR rate, independent of PD-L1 status. This may be due to the dynamic changes in PD-L1 expression induced by NACT, which can lead to differences in PD-L1 levels between early and late stages of treatment [77]. These findings suggest that even early-stage PD-L1-negative tumors can respond to ICB therapy due to fluctuations in PD-L1 levels during treatment.

The gene encoding PD-L1, CD274, is located in the chromosome 9p24.1 region, and recent studies have shown that amplification of this region is associated with frequent and durable responses to ICBs. In a cohort of patients with metastatic breast cancer, 9p24.1 amplification was observed in approximately 1.2% of tumors, with significantly higher levels in TNBC compared to other subtypes [78]. This underscores the need for further research on PD-L1 gene amplification to validate its representativeness as a predictive biomarker.

Tumor mutation load

Tumor mutation burden (TMB) serves as a valuable biomarker for predicting responses to immune checkpoint blockades (ICBs) due to its association with neoantigen generation and T cell activation (Fig. 4). TMB quantifies the number of somatic mutations per megabase pair (mut/Mb) of DNA [79]. These mutations can lead to the formation of neoantigens, which are then presented on major histocompatibility complex (MHC) molecules. If these neoantigens are immunogenic—particularly when TMB reaches or exceeds 10 mut/Mb—they can stimulate

T cells and enhance the response to immunotherapy [80]. Research consistently shows that high TMB is associated with better responses to immunotherapy across various cancer types [81, 82]. For example, a prospective study highlighted that high TMB was linked to positive outcomes with pembrolizumab in patients across ten different cancer types [83].

In breast cancer, TMB is generally low, with hypermutation occurring in fewer than 5% of cases [84]. TMB levels are highest in TNBC and lowest in hormone receptor-positive (HR-positive) breast cancers [85]. Although preliminary evidence suggests that high TMB may indicate a favorable response to immune checkpoint blockades (ICBs) in breast cancer, this is based on a limited number of patients [86]. Recent studies are examining the potential of high TMB as a biomarker for immunotherapy response in metastatic breast cancer, where TMB tends to be higher in metastatic lesions compared to primary tumors. However, statistical significance remains elusive due to small sample sizes. A randomized trial investigating the effects of durvalumab combined with anthracycline/paclitaxel chemotherapy in early-stage TNBC found that patients achieving a pathological complete response (pCR) had significantly elevated TMB, regardless of the treatment group [87]. Despite TMB-high being approved by the FDA in 2020 as a biomarker for ICB therapy, its role in breast cancer remains complex, and TMB-high alone may not fully predict response [88].

Combining TMB with other biomarkers can enhance predictive accuracy. For instance, copy number alterations (CNA) have been linked to immune escape mechanisms and poor responses to CTLA-4 blockade [89]. Liu et al. demonstrated that patients with high TMB and low CNA (TMBhighCNAlow) responded more effectively to ICB treatment than those with high TMB or low CNA alone [90]. This dual classification of blood tumor mutation burden (bTMB) and maximum somatic allele frequency (mSAF) has emerged as a promising approach for predicting responses to immunotherapy, particularly in advanced TNBC [91]. A bTMB cutoff of 6.7 mutations per megabase indicated that patients with a lower mSAF achieved better responses to the combination of anlotinib and TQB2450. Moreover, an mSAF threshold of 10% correlated with improved objective responses and extended median survival times. Notably, patients with both low mSAF and low bTMB showed significantly enhanced responses to this regimen.

Mismatch repair deficiency/microsatellite instability

Tumor microsatellite instability (MSI) [92] is a significant biomarker for predicting responses to cancer immunotherapy (Fig. 4). Microsatellites are short, repeated sequences of 1 to 10 nucleotides found in tandem throughout the genome. The mismatch repair (MMR) system is crucial for correcting errors that occur during DNA replication, ensuring high fidelity of DNA synthesis [93]. Deficiencies in MMR (dMMR) lead to increased mutational loads due to errors that are not corrected, which can disrupt DNA replication accuracy. Tumors with dMMR often exhibit high MSI due to these replication errors. Since dMMR/MSI status can influence how tumors respond to PD-1/PD-L1 therapies, pembrolizumab is approved for use in any tumor type with dMMR/MSI [94].

While the predictive value of MSI in breast cancer is less well established, there is evidence suggesting that patients with dMMR/MSI metastatic breast cancer can achieve sustained responses to pembrolizumab [95]. Additionally, the effectiveness of pembrolizumab is enhanced when combined with trastuzumab in patients with dMMR HER2+metastatic breast cancer [96]. Thus, identifying dMMR/MSI status in breast cancer patients could be pivotal for determining their suitability for such combination therapies and improving treatment outcomes.

Gene expression profile-based biomarkers

Studies have identified several potential predictors of response to ICBs based on gene expression profiles (Fig. 4). One of the most extensively studied biomarkers is the tumor inflammatory signature (TIS), which involves an 18-gene panel associated with cytotoxic cells, antigen presentation, and interferon gamma (IFN-γ) activity [97]. IFN-γ plays a key role in stimulating T cell proliferation, enhancing T cell differentiation, and initially increasing the expression of MHC class I and II molecules [98]. However, sustained IFN-γ signaling can lead to the upregulation of PD-1 ligands and other immunosuppressive molecules, resulting in feedback inhibition and suppression of the immune response [99].

Ayers et al. identified a genetic marker linked to elevated IFN-γ signaling and T-lymphocyte activation in baseline tumor tissues from melanoma patients treated with pembrolizumab. This marker, known as the T-cell inflammatory gene expression profile (GEP), was subsequently found to predict response to PD-1 therapy across various cancers [97]. In patients with advanced metastatic solid tumors, a high inflammatory GEP was associated with increased response rates and prolonged progression-free survival (PFS) [100]. Data from TCGA revealed higher TIS scores in basal-like and HER2-positive breast cancer subtypes compared to Luminal subtypes, correlating with better responses to pembrolizumab due to greater TIL infiltration [101]. Additionally, combining TIS and tumor mutational burden (TMB) biomarkers showed improved predictive efficacy for identifying responders to pembrolizumab in over 300 patients

across 22 tumor types from four KEYNOTE clinical trials [102]. Thus, T-cell inflammatory GEP represents a promising genetic biomarker for predicting responses to anti-PD-1 treatments, especially in certain breast cancer subtypes.

BRCA mutations have been linked to increased responses to PD-1 blockade in melanoma and lung cancer [80, 103]. In breast cancer, patients with PD-L1 positive tumors have benefited from atezolizumab combined with nab-paclitaxel, regardless of BRCA1/2 mutation status [62]. A study of 89 patients with BRCA1/2 mutations showed balanced PD-L1 immune cell statuses. Microsatellite-stable tumors with DNA polymerase epsilon (POLE) mutations have exhibited elevated TMB and are considered good candidates for ICB treatment due to high neoantigen expression and significant immune cell infiltration. The safety and efficacy of pembrolizumab have been validated in POLE-hypermutated endometrial cancers with PD-L1 positive advanced tumors. However, because POLE mutations occur in less than 3% of breast cancers, there is a need for further research to evaluate ICB responses in POLE-mutant breast cancer cases.

Additional genomic anomalies

The activation of B cells by ICB is dependent on the role of Tfh cells, which is similar to the activation of B cells in germinal center (Fig. 4) [104]. ICB activated B cells were associated with increased class-switching antibody (IgG) production and activation of T cell subsets, as shown by a decrease in the number of memory T cells following B-cell suppression. Zhao et al. established a B cell marker gene score (BCMG score) based on 9 B cell marker genes and revealed that the marker genes were mainly related to immune-related pathways [105]. Homologous recombination defects (HRDs) are general molecular features of genomic instability and have been shown to be biomarkers for targeted therapies (Fig. 4). In the upper 20% of HRD scores, genes with differential expression displayed significant up-regulation within immune-related signaling pathways [106]. These pathways include immune response, chemokine signaling, and cytokine-cytokine receptor interactions. Notably, six genes appeared multiple times in these immune-related pathways. Among them, CXCL10 exhibited the strongest prognostic value for survival (Fig. 4). Its expression correlated positively with neoantigen load, dendritic cells, and antitumor lymphocyte subsets. Both experimental in mice data and clinical trial findings have identified CXCL10 expression as a possible biomarker for anti-PD-1/PD-L1 therapy [58].

Importantly, studies have evaluated the association of elevated TMB or PTEN alterations (defined as non-synonymous mutations or deletions of one or two copies) with clinical outcomes in metastatic TNBC treated with ICBs (Fig. 4) [85]. In these patients, higher TMB was associated with longer survival, while the opposite was true for changes in PTEN. In detail, high TMB (18%) was associated with a longer clinical outcome of longer survival, while changes in PTEN (29%) were associated with a lower response rate ORR, a shorter PFS, and a shorter OS.

Circulating tumor DNA

Circulating tumor DNA (ctDNA) represents a promising non-invasive tool for precision oncology, offering real-time insights into the genomic landscape of tumors (Fig. 4) [107]. As a liquid biopsy, ctDNA analysis provides crucial information on tumor burden and acts as a prognostic marker, especially in metastatic cancers undergoing treatment [108]. Its non-invasive nature also facilitates the detection of biomarkers relevant to immunotherapy, such as microsatellite instability (MSI) and tumor mutational burden (TMB). Studies have demonstrated that pre-treatment plasma analysis revealing MSI and TMB-high status correlates with progression-free survival (PFS) and may indicate potential benefits from immune checkpoint blockades (ICBs) [109]. Moreover, monitoring ctDNA levels during treatment can serve as a surrogate marker for assessing response to ICB therapy in breast cancer [107]. Recent research has highlighted that ctDNA clearance is associated with improved survival outcomes in patients receiving pembrolizumab [110].

Peripheral blood cell subpopulations

Systemic immunity plays a crucial role in the effectiveness of cancer immunotherapy, as the activation of immune checkpoint blockades (ICBs) often leads to an increase in effector T cells both within tumor tissues and in peripheral blood (Fig. 4). Analyzing peripheral blood immune cell subsets has become a valuable approach for monitoring ICB responses. For example, a pan-cancer study found that an increased frequency of naive B cells in peripheral blood is linked to improved survival following ICB treatment [111]. Additionally, monocytes in blood can provide valuable insights into the immune characteristics of the tumor microenvironment [58].

Multiparametric flow cytometry has been used to assess immune cell heterogeneity in blood samples from both healthy donors and patients with advanced cancer. Dyikanov et al. identified five distinct immune subtypes, each associated with different cell types and gene expression profiles, which may reflect systemic immunity and influence patient responses to cancer treatments, including immunotherapy [112].

Assessments of immune cell populations both at baseline and after treatment have provided useful information on treatment effects. For example, in

an exploratory analysis of the GeparNuevo trial, durvalumab treatment led to a near complete loss of detectable PD-L1+CD4+and CD8+T cells in peripheral blood during and after neoadjuvant therapy, compared to no significant change in the placebo group [113]. Additionally, higher baseline levels of CD4+T cells and post-treatment expansion of γδT cells were associated with a better response to durvalumab and chemotherapy [113].

Eosinophilia has also been linked to positive responses to ICB treatment in breast cancer [114]. In a longitudinal study of patients with metastatic triple-negative breast cancer (mTNBC), those who responded to nivolumab showed a significant increase in circulating eosinophils, with increased expression of eosinophil-related genes correlating with higher levels of CD8+T cells and IFN-g gene signatures [62]. Blomberg et al. further found that eosinophilia, both intratumorally and systemically, was associated with responses to ICB therapy in patients and mice [57]. These findings suggest that eosinophils may play a supportive role in the ICB response.

Additionally, functional CD4+T cells are necessary to restore CD8+T cell cytotoxicity following anti-PD-(L)1 treatment. In non-small cell lung cancer patients, a high proportion of highly differentiated CD4+T cells (greater than 40%) in peripheral blood at baseline was predictive of a positive treatment response [115]. A low percentage of CD25+FOXP3+CD4+regulatory T cells (Tregs) was also associated with higher response rates, as well as longer progression-free survival (PFS) and overall survival (OS). Blomberg's study similarly found a significant reduction in CD4+FoxP3+Tregs after anti-PD-1 therapy in breast cancer [57].

However, it is important to note that changes in peripheral blood immune cell populations do not always correspond with changes within the tumor microenvironment [116]. Despite this, the T cell immunophenotype and count in peripheral blood remain promising predictive biomarkers for immunotherapy responses in breast cancer.

Combination therapy strategy for sensitization

The integration of combination therapy strategies, including clinically approved and investigational approaches, is proving to be a critical advancement in enhancing the efficacy of immunotherapy for breast cancer (Fig. 5). Chemotherapy, radiotherapy, and targeted therapies are

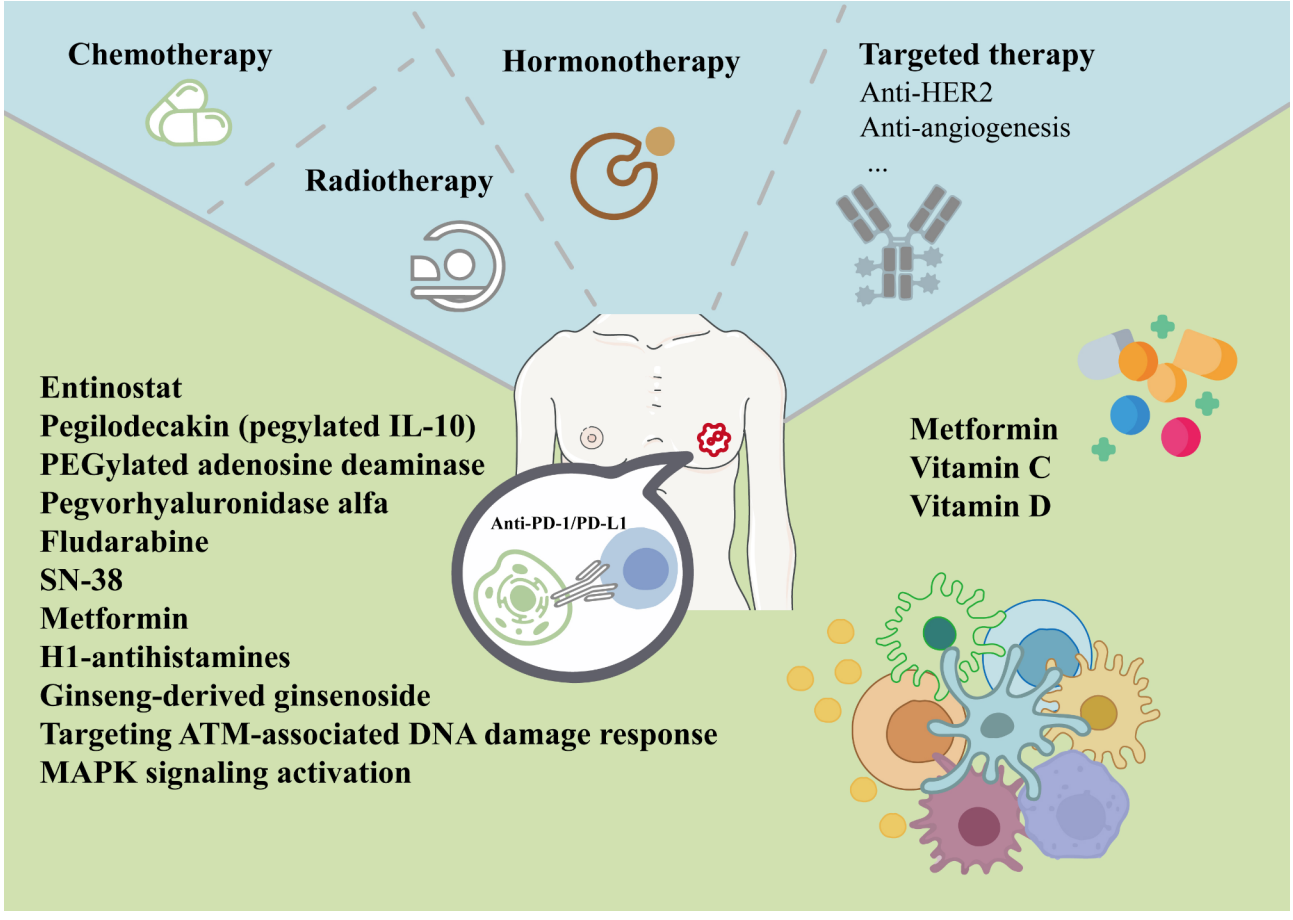

Fig. 5 Summary of combination therapy strategies for sensitization with PD-1/PD-L1 immune checkpoint blockade for breast cancer therapy

not only effective as stand-alone treatments but also play crucial roles in modulating the tumor microenvironment, enhancing antigen presentation, and stimulating immune responses that complement immunotherapy. The promising potential of combining cytokines, anti-angiogenic agents, and metabolic pathway inhibitors with immune checkpoint blockades (ICBs) highlights the dynamic interplay between various treatment modalities in overcoming resistance and improving patient outcomes (Table 2).

Clinically approved approaches of combination therapy

Chemotherapy drugs have been primarily developed for their direct cytotoxic effects on cancer cells. However, recent studies have shown that these agents can also promote anti-cancer immunity by inducing immunogenic cell death (ICD), which enhances the effectiveness of immunotherapy [117]. Cytotoxic chemotherapy can damage cancer cells, leading to the release and relocalization of damage-associated molecular patterns (DAMPs). Various cytotoxic drugs, such as anthracyclines, cyclophosphamides, and platinum-based compounds, stimulate cell cycle arrest and apoptosis in proliferating cells. The dying cancer cells are then engulfed by antigen-presenting cells (APCs), which subsequently present new tumor antigens to the immune system. Moreover, chemotherapeutic agents can influence antigen presentation processes; for instance, gemcitabine has been shown to increase β2-microglobulin levels, significantly enhancing the expression of human

Table 2 Investigational approaches of combination therapy

Therapeutic categories	Agents	Immunotherapy combined	Immune cells	Mechanisms	References
Histone deacetylase inhibitor	Entinostat	Anti-PD-1, anti-CTLA-4	CD8+T cells, neutrophils, MDSC, TAM	Promote the accumulation of immune cytokine NHS-rmIL12, reprogramming the TME into an inflamed landscape	[144, 145]
Immunostimulant cytokines	Pegilodecakin (pegylated IL-10)	Anti-PD-1	CD8+T cells	Reinvigoration, proliferation, and expansion of antigen experienced PD-1+Lag-3+CD8+cytotoxic T cells and expansion of novel CD8+T cell clones	[146]
Targeting ATM-associated DNA damage response and/or MAPK signaling activation	NA	Anti-PD-1	CD4+T cells	Prevent senescence of T cells mediated by tumor cells and Treg cells	[156]
STAT1 inhibitor	Fludarabine	Anti-PD-1	CD4+T cells, CD8+T cells, DC	Inhibit STAT1 regulon to facilitate local immune tolerance through up-regulating immunosuppressants due to CXCL-16^{high} cells	[157]
Hyaluronan (HA) degrading enzyme	Pegvorhyaluronidase alfa (PEGPH20; PVHA)	Anti-PD-L1	CD8+T cells, NK	Reduce tumor interstitial pressure and decompressing tumor blood vessels	[159]
Adenosine deaminase	PEGylated adenosine deaminase (PEG-ADA)	Anti-PD-1	M-MDSCs, CD8+T cells	Depletion of adenosine reduces the inhibitory effect of anti-tumor CD8+T cells	[160]
Active metabolite of irinotecan	SN-38	Anti-PD-1	NK, CD8+T cells	Inhibit c-Myc and STAT3 through FOXO3 activation, and enhance the anti-tumor immune function of TME by infiltrating NK or CD8+T cells and secreting interferon-γ and granzyme B	[161]
Biguanide	Metformin				
H1-antihistamines	Fexofenadine, loratadine, desloratadine, cetirizine, levocetirizine, and azelastine	Anti-PD-1	TAM, CD8+T cells	Revert macrophage immunosuppression and revitalize T cell cytotoxic function via the histamine-HRH1 axis,	[162]
Ginseng-derived ginsenoside	Rh2	Anti-PD-L1	CD8+T cells	Increase the expression of CXCL10 through activating TBK1-IRF3 signaling pathway to increase infiltration of T cells	[163]
Vitamin	Vitamin C	Anti-CTLA-4, anti–PD-1	CD8+T cells, CD4+T cells	Induce activation of CD4 and CD8+T cells for the activation marker CD69 and the effector/memory CD44 marker	[164]
Vitamin	Vitamin D	Anti–PD-1, anti-CTLA-4	CD8+T cells, CD4+T cells	Act through intestinal epithelial cells and is beneficial for increasing the gut microbiome for immune-mediated cancer control.	[165, 166, 168]

PD-1, programmed cell death-1; CTLA-4, cytotoxic T Lymphocyte associated antigen-4; MDSC, myeloid-derived suppressor cell; TAM, tumor-associated macrophage; IL, interleukin; ATM, ataxia telangiectasia mutated; NA, not available; DC, dendritic cells; PD-L1, programmed death ligand-1; NK, natural killer

leukocyte antigens (HLA) A, B, and C [118]. Chemotherapy can also modify the tumor microenvironment (TME) to favor immune cell differentiation, such as cyclophosphamide, which promotes the differentiation of M1 tumor-associated macrophages, contributing to anti-cancer effects [119]. Cytotoxic chemotherapeutic agents like platinum and cyclophosphamide significantly reduce myeloid-derived suppressor cells (MDSCs) in mice [120]. Trabectedin selectively depletes macrophages by inducing caspase-8-dependent apoptosis [121]. Human regulatory T (Treg) cells are particularly sensitive to cyclophosphamide due to their lack of the efflux transporter protein ABCB1, which other immune cells express [122]. A key adverse effect of chemotherapy is lymphocyte depletion, given its immunosuppressive properties. It remains debated whether chemotherapy-induced lymph node clearance suppresses anticancer immunity.

Radiation therapy, another widely used cancer treatment, can enhance antigenicity and adjuvant properties, thereby promoting anti-cancer immunity. Radiotherapy increases tumor antigen expression by inducing MHC-I expression [123], triggers ICD [124], down-regulates CD47 expression on tumor cell surfaces [125], and generates reactive oxygen species [126]. One of the key contributions of radiotherapy to anti-cancer immunity is its ability to strengthen the adjuvant effect. Radiation-induced DNA damage and cytoplasmic DNA release from micronuclei activate the cGAS/STING pathway, leading to increased type I interferon pathway expression and an enhanced immune response [127]. Radiation therapy enhances CD8+T cell infiltration and induces expression of MHC-1, death receptors (Fas/CD95), and NKG2D ligands on CD8+T cells and natural killer (NK) cells [128]. These changes allow tumor cells to be more readily recognized and destroyed by immune cells. However, radiation also triggers the secretion of immunosuppressive cytokines, such as transforming growth factor-β (TGF-β), which suppresses CD8+T cells and promotes regulatory T cell transformation, leading to a more immunosuppressive tumor microenvironment (TME). Additionally, radiation-induced DNA damage upregulates PD-L1 expression on tumor cells through the ATM/ATR/Chk1 pathway, further contributing to immune evasion [129].

Radiation can also lead to an immunosuppressive TME by killing normal and immune cells, particularly with extensive exposure. Studies indicate that radiation increases infiltration and accumulation of myeloid-derived suppressor cells (MDSCs) [130], tumor-associated macrophages [131], and cancer-associated fibroblasts [132], all of which support an immunosuppressive TME. These complex radiation-induced effects raise important questions regarding the optimal timing and dosage for combining immunotherapy with radiation therapy to maximize therapeutic benefits.

Targeted therapies, designed to exploit specific genomic alterations in tumors, can also enhance anti-tumor immunity. These therapies can stimulate and improve the function of APCs, initiate and activate immune responses, and strengthen overall immunity against cancer, as seen with ICD [133]. For example, PI3K inhibitors have been studied for breast cancer treatment, where they reverse interferon gamma inhibition of antigen presentation mechanisms [134]. Additionally, targeted therapies may focus on immune cells themselves; for instance, they can inhibit T cell receptor (TCR) dependent activation [135] and T cell cytotoxic activity [136], thereby enhancing the immune response against tumors. Many FDA-approved or investigational targeted drugs have direct effects on immune cells. For example, ibrutinib [137], approved for B-cell chronic lymphocytic leukemia, modulates T cells by inhibiting Bruton's tyrosine kinase (BTK) and IL-2-inducible T-cell kinase (ITK), promoting Th1 responses while suppressing Th2 responses in T lymphocytes. This leads to significant increases in CD4+and CD8+T cell numbers, a reduced Treg/CD4+T cell ratio, and decreased production of the immunosuppressive cytokine IL-10.

Hormone therapy, which targets estrogen production and/or estrogen receptor (ER) signaling, is a cornerstone in treating patients with localized or metastatic ER-positive breast cancer [138]. Studies suggest that inhibiting ER signaling in immune cells can improve immunotherapy efficacy in disease models of various cancers. Analysis of a breast tumor cohort revealed that increased eosinophil and monocyte counts were significantly associated with improved prognosis [139]. Additionally, increased tumor eosinophilia has been associated with a better immunotherapy response in patients with triple-negative breast cancer (TNBC) [62]. Mouse models of breast cancer and melanoma have shown that estrogen administration reduces peripheral eosinophils, supporting tumor growth [140]. Estrogen signaling in healthy female mice similarly inhibits peripheral eosinophil increases by reducing the proliferation and survival of mature eosinophils. Inhibition of ER signaling has been found to reduce tumor growth in an eosinophil-dependent manner, and combining immune checkpoint blockades (ICBs) with anti-estrogen therapy enhances efficacy. In summary, hormone therapy affects remodeling of the tumor immune microenvironment, a benefit that may extend to all breast cancer subtypes and other cancers typically defined as ER-negative, potentially increasing the effectiveness of immunotherapy.

Investigational approaches of combination therapy

Cytokines, including tumor necrosis factors, interleukins, chemokines, and interferons, are small glycoproteins or proteins that play crucial roles in modulating immune responses by interacting with cell surface receptors. They influence the growth, progression, and activity of immune cells. Chemokines primarily regulate immune cell trafficking, while interleukins, interferons, and transforming growth factor-β (TGF-β) modulate various aspects of the immune response. CD8+effector T (Teff) cells, IFN-γ-expressing T helper 1 (TH1) cells, and natural killer (NK) cells can be recruited to the tumor microenvironment by CXC-chemokine ligands 9 (CXCL9), 10 (CXCL10), and 11 (CXCL11), where they exert potent antitumor effects [141]. Regulatory T (Treg) cells express high levels of IL-2 receptor α (IL-2Rα), a component of the high-affinity IL-2 receptor, allowing IL-2 to promote the expansion of T lymphocytes toward Treg cells [142]. Additionally, TGF-β regulates various immune cell subtypes and is a key factor in the immunosuppressive environment of the tumor microenvironment (TME). In Treg cells, TGF-β induces the expression of FOXP3, a critical transcriptional regulator for Treg cell differentiation [143]. In tumors with a poorly inflamed microenvironment, immunotherapy may not be effective. For example, the histone deacetylase inhibitor Entinostat has been shown to promote the accumulation of the necrosis-targeted recombinant mouse immune cytokine NHS-rmIL12 in experimental mouse models of colon cancer and low immunogenic breast tumors, reprogramming the tumor's innate and adaptive immune environment into an inflamed state [144]. Combination therapies can reprogram the tumor's innate and adaptive immune environment into an inflammatory landscape, where the synergistic actions of highly active CD8+T cells and activated neutrophils promote the polarization of macrophages toward an M1-like phenotype [145].

For immunostimulatory cytokines like IL-10 and IL-2, natural and genetically engineered forms that bind to ICBs are being developed. Combinations of IL-10 with pembrolizumab or nivolumab have demonstrated controlled toxicity profiles and preliminary anti-tumor activity [146]. A key strategy for combining cytokines with immunotherapy has been to limit their activity to the tumor site, reducing systemic pro-inflammatory side effects. Techniques include direct injection of cytokines into tumors or gene therapy to localize their expression.

Aberrant signaling pathways in immune cells also play critical roles in modulating the tumor immune environment and can be targeted for combination therapy. Anti-angiogenic drugs, for instance, exhibit immunomodulatory effects across various immune cell subsets [147]. Many targeted therapies can influence immune cell function, leading to numerous clinical trials exploring the effectiveness of combining these therapies with immune checkpoint blockades (ICBs) in cancer treatment [148]. For example, combining camrelizumab with apatinib has shown a favorable response rate (ORR) and progression-free survival (PFS) in advanced triple-negative breast cancer (TNBC), regardless of PD-L1 status [149]. In a multicenter retrospective study, patients receiving apatinib alongside chemotherapy or immunotherapy achieved the highest PFS in the immunotherapy group.

Research indicates a dose-dependent synergy between anti-angiogenic therapy and ICBs [150]. Low-dose VEGFR2 blockade promotes immune cell infiltration and activation, stimulating CD8+T cells to secrete osteoblast protein (OPN), which induces tumor cells to produce TGF-β, subsequently upregulating PD-1 on immune cells. Anti-angiogenic therapy also enhances the effectiveness of anti-PD-1/PD-L1 therapy by increasing PD-L1 expression and CD8+T cell infiltration in tumors [154]. VEGFR is found on activated and memory T cells [151], where VEGF suppresses T cell receptor activation and cytotoxicity. In regulatory T cells (Tregs), VEGFR2 is selectively expressed in FOXP3-high Tregs, and VEGF also promotes the accumulation of myeloid-derived suppressor cells (MDSCs) through JAK2 and STAT3 activation [152]. In dendritic cells, tumor-produced VEGF disrupts maturation via the NF-κB pathway [153], while elevated plasma VEGF levels are associated with immature dendritic cells in circulation—effects that may partially reverse with tumor resection [154].

PI3K inhibitors, used in treating lymphoma and breast cancer, not only exhibit direct anti-tumor activity but also alter immune cell metabolism, inhibit antigen presentation, and influence the local tumor environment [155]. In a mouse homozygous tumor model, PI3K inhibitors showed strong antitumor activity, which was associated with improved CD8+T cell activation and memory in the tumor microenvironment. Moreover, Treg cells can induce T cell senescence through DNA damage associated with ataxia telangiectasia mutated (ATM) protein. Inhibiting ATM-related DNA damage and MAPK signaling in T cells can effectively block tumor and Treg cell-induced senescence, enhancing anti-tumor immunity when combined with anti-PD-L1 checkpoint inhibitors [156].

Recent research in triple-negative breast cancer (TNBC) has shown that neoadjuvant low-dose metronomic chemotherapy increases the presence of CXCL16+myeloid cells, which elevate STAT1 regulatory activity and result in immature myeloid cells expressing PD-L1 [157]. Inhibiting STAT1 signaling induced by chemotherapy, such as Fludarabine, can sensitize TNBC to ICB therapy, suggesting a promising combination strategy for patients with this aggressive breast cancer subtype.

Additionally, targeting metabolic pathways such as acetyl-CoA synthetase 2 (ACSS2), an enzyme involved in converting acetate to acetyl-CoA, can shift tumor cells from consumers to producers of acetate, thereby supporting the effector function and proliferation of tumor-infiltrating lymphocytes [158]. This metabolic reprogramming may enhance the anti-tumor immune response. The degradation of hyaluronic acid (HA) by polyethylene glycol hyaluronidase α (PEGPH20) reduces tumor interstitial pressure and decompresses tumor vasculature, improving the uptake and efficacy of PD-L1 antibodies in HA-accumulating breast cancer models [159]. Furthermore, combining PEG-ADA, which degrades adenosine in the tumor microenvironment, with ICB therapy has been shown to increase CD8+T cell activity and improve responsiveness to immunotherapy [160].

Several approved drugs also exhibit synergy with immunotherapy. For example, the anti-tumor agents SN-38 and metformin inhibit c-Myc and STAT3 through FOXO3 activation, enhancing TME immune function by promoting the infiltration of NK or CD8+T cells and increasing the secretion of interferon-γ and granzyme B [161]. Moreover, cancer patients who use antihistamines during immunotherapy have shown significantly improved survival outcomes. Histamine receptor H1 (HRH1) on macrophages promotes an immunosuppressive M2-like phenotype and upregulates immune checkpoint VISTA, leading to T cell dysfunction. HRH1 gene knockout or antihistamine therapy restores macrophage immunosuppression, reactivates cytotoxic T cell function, and improves immunotherapy response [162].

Natural compounds such as Rh2 ginsenoside, derived from traditional Chinese medicine, have been shown to enhance the anti-cancer effects of anti-PD-L1 antibodies in preclinical models. This combination therapy promotes the activation and infiltration of CD8+T cells by stimulating the TBK1-IRF3 signaling pathway and increasing the M1/M2 macrophage ratio within the TME [163]. Additionally, high doses of vitamin C have been found to modify immune cell infiltration within the TME, enhancing the cytotoxic activity of CD8+T cells and synergizing with ICBs in breast cancer models with high mutational loads [164]. Vitamin D, which has immunomodulatory and anti-cancer properties, has also been linked to reduced cancer incidence and improved responses to ICBs in various cancer types [165–168]. Mice fed a vitamin D-rich diet demonstrated better immune resistance to cancer and an improved response to ICB therapy [168].

Conclusion

The integration of immune checkpoint blockades (ICBs) into breast cancer treatment marks a promising development in therapeutic strategies. While encouraging results have been achieved, particularly in triple-negative breast cancer (TNBC), extending these benefits to other subtypes presents notable challenges. The complex and evolving immunological landscape of the tumor microenvironment (TME) appears to significantly impact ICB efficacy. Enhancing patient outcomes may require more comprehensive, biomarker-driven approaches that go beyond PD-L1 expression to include the broader immunological profile of individual tumors.

The current focus on investigating biomarkers such as tumor-infiltrating lymphocytes (TILs), genetic alterations, and immune cell subpopulations is crucial for identifying patients most likely to benefit from ICBs. However, further clinical evidence is needed to validate these biomarkers. Additionally, the development of novel combination therapies and more sophisticated analytical methods, such as single-cell and spatial immunophenotyping, holds promise for enhancing the precision and effectiveness of treatment. Ultimately, advancing personalized cancer care will depend on a concerted effort to refine biomarker strategies, improve our understanding of the TME, and tailor therapies to individual patient profiles. Collaborative research and continued innovation in immunotherapy will be key to achieving sustained improvements in breast cancer treatment outcomes and ensuring that the benefits of ICBs extend across all breast cancer subtypes.

In addition, the strategic combination of therapies shows great potential for enhancing the effectiveness of immunotherapy in breast cancer, especially in aggressive subtypes like triple-negative breast cancer. Ongoing research and clinical trials are essential to refine these combinations, optimize dosing regimens, and identify biomarkers that predict response, ultimately leading to more personalized and effective cancer treatment strategies.

Abbreviations

PD-1	Programmed Cell Death-1
ICBs	Immune Checkpoint Blockades
TNBC	Triple-Negative Breast Cancer
TME	Tumor Microenvironment
FDA	Food and Drug Administration
ORR	Overall Response Rate
OS	Overall Survival
PFS	Progression-Free Survival
ITT	Intent-To-Treat
ER	Estrogen Receptor
T-DM1	Trastuzumab-Emtansine
pCR	Pathologic Complete Remission
EFS	Event-Free Survival
HR	Hormone Receptor
JAK	Janus Kinase
STAT	Signal Transducer and Activator of Transcription

BET	Bromodomain and Extra-Terminal
MLL1	Mixed Lineage Leukemia 1
H3K4me3	H3K4 Trimethylation
miRNAs	MicroRNAs
ZEB1	Zinc finger E-box Binding Homeobox 1
MHC	Major Histocompatibility Complex
MDXCs	Myeloid-Derived Suppressor Cells
Tregs	Regulatory T Cells
EMT	Epithelial-Mesenchymal Transition
ERO1α	Endoplasmic Reticulum Oxidoreductase
HIF1α	Hypoxia-Inducible Factor 1-alpha
ROS	Reactive Oxygen Species
TILs	Tumor-Infiltrating Lymphocytes
Tfh	CD4 + T follicular helper
STILs	Stromal Tumor-Infiltrating Lymphocytes
cDC1	Conventional Dendritic Cells 1
mDC	myeloid Dendritic Cells
pDC	plasmacytoid Dendritic Cells
TAMs	Tumor-Associated Macrophages
iTILs	intratumoral Tumor-Infiltrating Lymphocytes
TMB	Tumor Mutational Burden
TRM	Tissue-Resident Memory
TLS	Tertiary Lymphoid Structures
NACT	Neoadjuvant Chemotherapy
CNA	Copy Number Alterations
bTMB	blood Tumor Mutation Burden
mSAF	Maximum Somatic Allele Frequency
MSI	Microsatellite Instability
MMR	Mismatch Repair
dMMR	deficiencies in MMR
TIS	Tumor Inflammatory Signature
IFN-γ	Interferon Gamma
GEP	Gene Expression Profile
POLE	Polymerase Epsilon
HRDs	Homologous Recombination Defects
ctDNA	circulating tumor DNA
ICD	Immunogenic Cell Death
DAMPs	Damage-Associated Molecular Patterns
APCs	Antigen-Presenting Cells
HLA	Human Leukocyte Antigens
TCR	T Cell Receptor
TGF-β	Transforming Growth Factor-β
ATM	Ataxia Telangiectasia Mutated
ACSS2	Acetyl-CoA Synthetase 2
HA	Hyaluronic Acid
PEGPH20	Polyethylene Glycol Hyaluronidaseα
HRH1	Histamine Receptor H1

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Author contributions
ZG.C. conceived and designed this project. ML.J., J.F., and JW.P. wrote the draft of the manuscript. ML.J., KP.J., and JM.H. did the literature search and review. ZG.C., P.X., XT.W, DT.W., and YX.D. revised the manuscript. WL.L., JW.P. and XY.W. prepared and edited the tables and figures. ZG.C. and WL.L. supervised the project. All authors have read and approved the article.

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Data availability
No datasets were generated or analysed during the current study.

Declarations

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