Am J Cancer Res 2026;16(5):1823-1846
www.ajcr.us /ISSN:2156-6976/ajcr0172176

Review Article

Regulatory T cells in the breast cancer tumor microenvironment: new mechanisms, potential therapeutic strategies and future perspectives

Yunxia Wang[1,2*], Yu Yu[1,2*], Yongling Pei[1], Jiayao Wang[1], Linye Hua[1,2], Minjie Li[1], Yamei Li[1]

[1]Key Laboratory for Quality Evaluation of Bulk Herbs of Hunan Province, School of Pharmacy, Hunan University of Chinese Medicine, Changsha 410208, Hunan, China; [2]Medical College, Hunan University of Chinese Medicine, Changsha 410208, Hunan, China. [*]Equal contributors.

Received February 19, 2026; Accepted May 7, 2026; Epub May 15, 2026; Published May 30, 2026

Abstract: Regulatory T cells (Tregs) are key immunosuppressive components of the tumor microenvironment (TME). Their marked enrichment in breast cancer tissues, compared with normal or benign tissues, promotes tumor immune evasion and represents a major barrier to effective immunotherapy. This review systematically elucidates the multifaceted roles of Tregs in breast cancer progression. We first outline the fundamental characteristics and origins of Tregs within the breast cancer TME. We then examine the molecular mechanisms underlying Treg recruitment, highlighting how tumor cells and stromal components cooperatively establish an immunosuppressive niche. In addition, we analyze the specific pathways through which Tregs promote tumor angiogenesis, facilitate immune escape, and drive metastasis. Finally, we summarize emerging therapeutic strategies targeting Tregs, including biological agents, natural and synthetic compounds, combination therapies, and traditional Chinese medicine formulations, and discuss current challenges and future directions. This review aims to provide a comprehensive framework and novel insights for the development of more effective Treg-targeted immunotherapies for breast cancer.

Keywords: Breast cancer, tumor cell immune escape, tumor metastasis, immunotherapy

Introduction

Breast cancer is the most commonly diagnosed malignancy among women worldwide and imposes a substantial burden on both physical and mental health. According to the International Agency for Research on Cancer (IARC), a specialized agency of the World Health Organization (WHO), approximately 20 million new cancer cases were reported globally in 2022, of which 2.3 million were breast cancer cases in women. This ranks breast cancer as the second most frequently diagnosed cancer, accounting for 11.6% of all new cancer cases [1]. In China, the National Cancer Center reports more than 420,000 new breast cancer cases annually, with a steadily increasing incidence and a declining mean age at onset [2]. Projections based on GLOBOCAN 2022 data suggest that by 2050, global breast cancer incidence will increase by 38% and mortality by 68%. Notably, in approximately half of the 185 countries analyzed, annual incidence rates are already rising by 1-5%. Encouragingly, advances in early detection and treatment have led to a sustained decline in breast cancer mortality. In the United States, the 5-year relative survival rate has reached 91%, representing a substantial improvement over historical levels [3, 4].

Breast cancer is a highly heterogeneous disease. Based on the expression of estrogen receptor (ER), progesterone receptor (PR), human epidermal growth factor receptor 2 (HER2), and the proliferation marker Ki-67, breast cancer is classified into four intrinsic molecular subtypes: Luminal A, Luminal B, HER2-enriched, and triple-negative breast cancer (TNBC). These subtypes differ significantly in clinical outcomes, therapeutic responses, and underlying molecular drivers. For example, TNBC lacks targetable hormone receptors and HER2 expression, rendering it the most aggressive subtype with limited treatment options [5].

https://doi.org/10.62347/FZMV2668

The management of breast cancer has evolved from a uniform treatment approach to subtype-specific precision medicine. For hormone receptor-positive tumors, endocrine therapy combined with CDK4/6 inhibitors has become the standard of care. HER2-positive breast cancer is effectively treated with anti-HER2 therapies, including trastuzumab, pertuzumab, and antibody-drug conjugates. In contrast, chemotherapy remains the cornerstone of treatment for TNBC, although immune checkpoint inhibitors and PARP inhibitors have recently expanded therapeutic options [6]. Despite these advances, current treatments—including chemotherapy, targeted therapy, and immunotherapy—are associated with substantial adverse effects, such as bone marrow suppression [7], cardiotoxicity [8], hepatic and renal dysfunction [9], febrile neutropenia [10], osteoporosis [11], and gastrointestinal and metabolic disturbances, as well as severe alopecia [12]. These toxicities impose significant physical and psychological burdens on patients and adversely affect their quality of life. Therefore, there is an urgent need to develop more effective and less toxic therapeutic strategies to improve treatment adherence, survival outcomes, and overall quality of life in patients with breast cancer.

Among emerging immunotherapeutic approaches, programmed cell death protein 1 (PD-1) and programmed death-ligand 1 (PD-L1) checkpoint inhibitors have demonstrated promising efficacy in a subset of breast cancer patients, particularly those with TNBC [13]. However, resistance to anti-PD-1/PD-L1 therapy remains common. This resistance is closely associated with the accumulation of a specialized $CD4^+$ T-cell subset—Tregs—within the TME. Tregs play critical roles in immune regulation, modulation of antitumor T-cell responses, and tumor immune evasion [14]. Recent studies have shown that increased Treg infiltration in the TME is significantly correlated with higher histological grade, enhanced invasiveness, and poorer prognosis in breast cancer patients [15]. Moreover, the abundance of Tregs is markedly higher in breast cancer tissues than in normal breast tissues [16].

Treg Profile

As early as 1983, a subset of $CD4^+$ T cells with immunosuppressive properties was identified [17]. In physiological conditions, these cells protect host tissues from immune-mediated damage and maintain immune tolerance, functions now well recognized as characteristic of Tregs [18]. Subsequent studies demonstrated that Tregs highly express forkhead box P3 (FOXP3), a lineage-specific transcription factor that serves as a master regulator of Treg development and function and is widely accepted as a definitive marker of Tregs [19, 20]. Moreover, ectopic expression of FOXP3 can confer suppressive properties on conventional T cells [21].

Tregs are widely distributed throughout the body, and the balance between Tregs and effector T cells across tissues is tightly regulated to ensure effective immune surveillance and pathogen control while preventing chronic inflammation and autoimmune diseases [22]. Disruption of Treg distribution can therefore lead to tissue-specific inflammatory disorders [23]. Accumulating evidence indicates that Tregs are enriched in malignant tumors, where they promote tumor progression by suppressing antitumor immune responses [24]. Their immunosuppressive role in attenuating antitumor immunity is now well established [25].

In breast cancer, the TME is characterized by substantial Treg infiltration, which positively correlates with tumor stage as defined by the tumor-node-metastasis (TNM) classification [26]. The classification and clinical management of breast cancer, particularly aggressive subtypes such as TNBC, are closely associated with the abundance of activated Tregs within the TME. Compared with normal tissues, $CD4^+$ T cells derived from tumor tissues exhibit significantly elevated mRNA expression of *Foxp3, IL-10,* and *TGF-β*. Clonal expansion of Tregs has been observed in both peripheral tissues and the TME of breast cancer patients [27]. Although a minority of Tregs arise from local proliferation of pre-existing Tregs [28], the majority are recruited from draining lymph nodes or peripheral blood and subsequently undergo phenotypic adaptation within the local microenvironment. Notably, patients with lymph node or distant metastasis exhibit lower absolute counts of peripheral blood Tregs than those without metastasis, and reduced peripheral Treg levels are associated with poorer clinical outcomes. These findings

suggest that Tregs in the TME are predominantly recruited from peripheral sources and that quantification of peripheral blood Tregs may serve as a potential biomarker for identifying high-risk patients and predicting metastatic recurrence [29].

Mechanism of action of Treg recruitment to the TME in breast cancer

Tregs play a critical role in breast cancer progression, making it essential to elucidate the mechanisms governing their recruitment in order to develop targeted therapeutic strategies. Multiple pathways contribute to Treg accumulation within the breast cancer TME, particularly those driven by tumor cells.

Induction of breast cancer cells

Cytokines and chemokines: A variety of cytokines and chemokines present in the TME of breast cancer play a role in the recruitment and accumulation of Tregs. Breast cancer cells secrete interferon-inducible protein-10 (IP-10), which selectively attracts Tregs to the TME. Additionally, the expression of ER or HER2 in breast cancer does not influence the chemotactic activity of IP-10 on Tregs. Notably, IP-10 is the only functional factor capable of inducing migration of γδ T regulatory cells (γδ Tregs), one subset of Tregs, into the breast cancer TME, and the extent of γδ Treg infiltration is independent of IP-10 expression levels [30].

Similarly, the tumor-secreted chemokine ligand 22 (C-C motif chemokine ligand 22, CCL22) and CCL17 are also positively correlated with the infiltration of Tregs in tumors. CCL22 and CCL17 act via their common receptor CCR4, which is expressed at significantly higher levels on tumor-infiltrating Tregs than on peripheral Tregs [31, 32]. Mechanistically, the FOXP3/HAT1 complex induces acetylation of the CCR4 promoter in tumor Tregs, thereby promoting Treg infiltration into the TME [33]. The chemokine-CCR4 axis is further reinforced by elevated prolactin (PRL), which enhances CCL17-mediated Treg recruitment via the long-form prolactin receptor [34]. Beyond its chemotactic function, CCL22 also exhibits a synergistic effect with TGF-β1. Specifically, CCL22 enhances the capacity of TGF-β1 to promote Treg differentiation, thereby further facilitating Treg accumulation in the TME [35].

Beyond chemotaxis, specific cytokines also promote Treg expansion and suppressive function. Upregulation of IL-33 in the TME correlates with tumor stage and enhances the expansion of immunosuppressive cells, including Tregs, thereby accelerating tumor progression and metastasis [36, 37]. The role of TGF-β in Treg differentiation is modulated by various factors. For example, ionizing radiation induces the expression of activin A, a member of the TGF-β family, whose levels positively correlate with Treg abundance in the TME [38]. Furthermore, compared with non-stem cancer cells, breast cancer stem cells (BCSCs) secrete higher levels of TGF-β, thereby promoting the differentiation of $CD4^+$ T cells into $CD4^+CD25^+FOXP3^+$ Tregs [39].

Collectively, these chemotactic and inductive signals cooperatively shape the Treg landscape in breast cancer.

Regulation of cell membrane receptors: Multiple cell surface receptors in the breast cancer TME regulate Treg accumulation through distinct yet potentially convergent mechanisms.

Leptin and its receptor are overexpressed in breast cancer compared with normal breast tissue, indicating a close association with the development of breast cancer [40]. Overexpression of leptin increases the number and proportion of Tregs, as well as the levels of TGF-β1, in the peripheral blood and spleen. The formation of a substantial number of Tregs within an organism indirectly facilitates their accumulation in the TME [41]. Similarly, histamine H4 receptor (H4R) is also highly expressed in cancer cells, and its elevated expression induces the accumulation of immunosuppressive molecules such as IL-10 in the microenvironment, which results in the recruitment of more Tregs [42].

Inducible costimulator-ligand (ICOSL) has immunomodulatory functions in various malignant tumors. Research has demonstrated that ICOSL is positively expressed in the cytoplasm and nuclear membrane of specific breast cancer subtypes, including TNBC, and the abundance of Tregs within the TME of breast cancer models is positively correlated with ICOSL expression. Moreover, ICOSL overexpression promotes tumor progression [43]. ICOSL overexpression leads to the differentiation of co-

cultured $CD4^+$ T cells into $FOXP3^+$ Tregs and increased the secretion of the tumor-promoting factors IL-10 and IL-4. ICOSL also suppressed p38 phosphorylation and facilitated the proliferation, invasion, and metastasis of the corresponding cancer cells [44].

Although direct interactions among leptin receptor, H4R, and ICOSL have not been fully elucidated, these pathways may converge on shared immunosuppressive mechanisms, particularly through the upregulation of IL-10. Collectively, they contribute to the establishment of an immunosuppressive network that facilitates immune evasion in breast cancer.

Activation of protease expression: Several enzymes and metabolic regulators in the breast cancer TME contribute to Treg recruitment and expansion. Cyclooxygenase-2 (COX-2) is overexpressed in aggressive breast cancer cells, leading to increased prostaglandin E2 (PGE2) production, which promotes Treg infiltration via EP2/EP4 receptors [45, 46]. Indoleamine 2,3-dioxygenase (IDO) is also upregulated and promotes FOXP3 expression and Treg expansion through tryptophan metabolism [47-49]. Thymidine kinase 1 (TK1), a proliferation marker, positively correlates with $FOXP3^+$ Treg infiltration in TNBC, although its precise mechanism remains unclear [50-53]. These factors act through largely independent pathways but collectively contribute to the immunosuppressive TME.

Induction of signaling pathway dysregulation: Beyond these extracellular regulators, intracellular signaling pathways also play critical roles in modulating Treg function and differentiation within the breast cancer TME. The Hedgehog (Hh) signaling pathway is closely associated with malignant tumor progression, particularly the invasiveness and metastatic potential of breast cancer [54]. Within the breast cancer TME, Hh signaling fosters an immunosuppressive milieu by modulating Treg differentiation and activity, as well as the balance between Tregs and Th17 cells. Mechanistically, Hh signaling facilitates O-linked N-acetylglucosamine (O-GlcNAc) modification of the transcription factors FOXP3 and transcription activator 3 (STAT3) in Tregs and Th17 cells. These modifications enhance Treg differentiation and immunosuppressive capabilities. Disruption of the Hh signaling pathway induces metabolic reconfiguration in Tregs, suppresses their immunosuppressive functions, and promotes their conversion into inflammatory Th17 cells, thereby augmenting cytotoxic $CD8^+$ T cell infiltration into the TME [55]. The Hippo pathway, via its key downstream effectors YAP and TAZ, plays a role in multiple cancers, including breast cancer [56]. In the breast cancer TME, disruption of Hippo signaling leads to increased TAZ expression, which then promotes Treg recruitment. Specifically, TAZ increases the levels of cytokines such as CCN4 and IL-23, thereby facilitating Treg accumulation [57].

In summary, breast cancer cells recruit and activate Tregs through chemokines, receptors, enzymes, and signaling pathways (**Figure 1**). These interconnected mechanisms collectively establish an immunosuppressive niche that promotes tumor progression.

Induction of tumor mesenchymal cells

Tregs: Following infiltration into the TME, Tregs undergo multiple adaptations that enhance their survival, expansion, and suppressive function. One key mechanism involves remodeling of the T-cell receptor (TCR) repertoire. Intratumoral Tregs exhibit a distinct yet partially overlapping TCR repertoire compared with circulating Tregs, enabling tumor antigen-specific activation and clonal expansion [58]. This process further promotes Treg recruitment, forming a positive feedback loop.

At the genetic level, overexpression of miR-126 in Tregs promotes the induction and activation of Tregs cultured in vitro and significantly increases the expression levels of FOXP3, Cytotoxic T-lymphocyte-associated protein 4 (CTLA-4), IL-10 and TGF-β, thereby strengthening their immunosuppressive function. The underlying mechanism involves miR-126 promoting the aggregation of Tregs in the TME by inhibiting the expression of its target phosphatidylinositol-3-kinase regulatory subunit 2 (P85β), which in turn activates the phosphatidylinositol 3-kinase (PI3K)-serine/threonine kinase (AKT) signaling pathway and upregulates the expression of FOXP3 [59].

At the protein level, increased expression of stemness-related genes such as SOX2 activates the NF-κB-CCL1 signaling axis, promoting Treg recruitment through epigenetic regula-

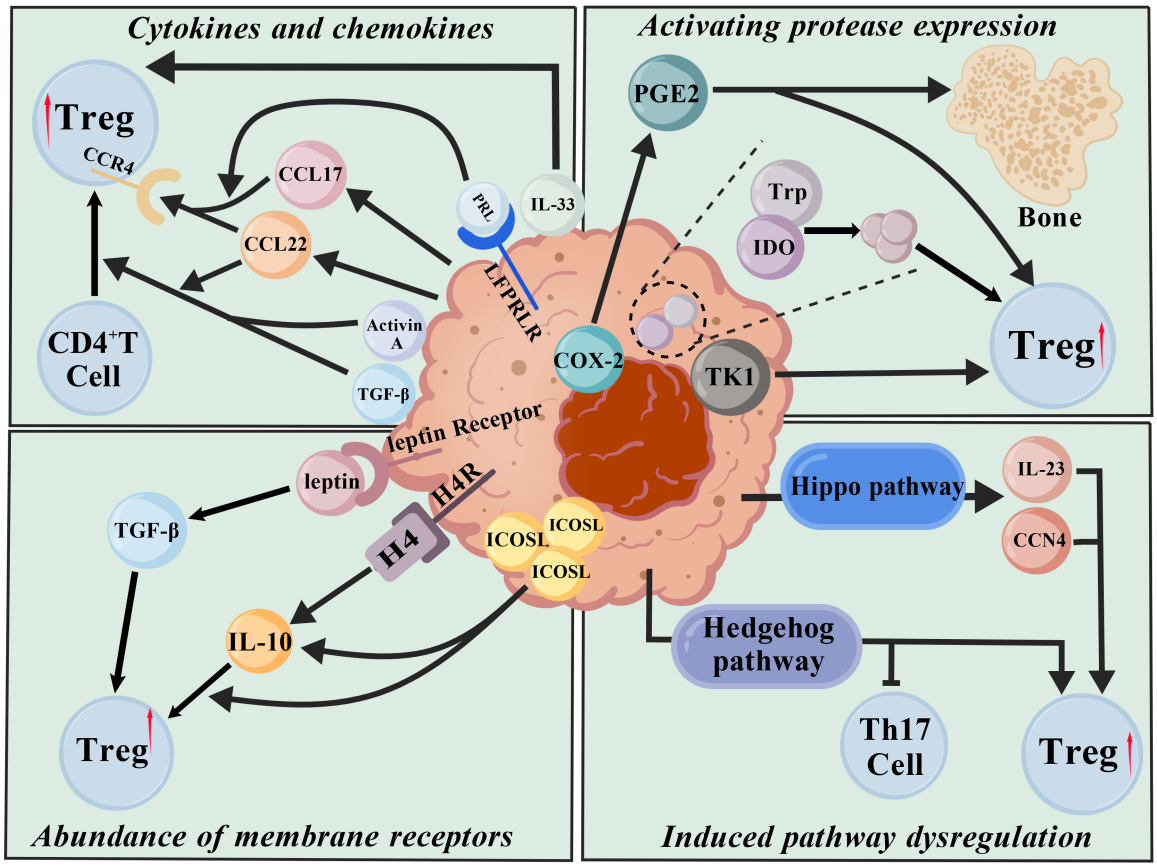

Figure 1. Induction of breast cancer cells (by BioGDP.com). Breast cancer cells promote Treg accumulation via four mechanisms. They secrete chemokines/cytokines (IP-10, CCL17/CCL22, TGF-β, IL-33), upregulate membrane receptors (leptin receptor, H4R, ICOSL), activate metabolic enzymes (COX-2/PGE2, IDO, TK1), and dysregulate signaling pathways (Hedgehog, Hippo). These cooperative events shape an immunosuppressive TME that drives breast cancer progression.

tion of target gene promoters [60]. In addition, CD28 activation enhances Treg proliferation and chemotaxis while inducing metabolic reprogramming, including increased glucokinase expression, allowing Tregs to adapt to low-glucose, high-lactate conditions within the TME [61].

These genetic, molecular, and metabolic adaptations synergistically enhance Treg accumulation and immunosuppressive function within the breast cancer TME.

Myeloid-derived suppressor cells: Myeloid-derived suppressor cells (MDSCs) are a subset of immature bone marrow cells that exhibit immunosuppressive properties in vivo under pathological conditions such as cancer [62]. In the breast cancer TME, MDSCs exert their immunosuppressive function by inhibiting T cell differentiation and proliferation or by promoting Treg expansion, thereby suppressing the host immune response. MDSCs and Tregs reciprocally induce and recruit each other through intercellular communication. MDSCs can directly enhance the proliferation of Tregs and indirectly promote the differentiation of $CD4^+$ T cells into Tregs via cytokine secretion [63].

Cancer-associated fibroblasts: Studies have shown that tumors manipulate ECM remodeling to create a tumorigenic and metastatic niche [64]. Cancer-associated fibroblasts (CAFs) in the TME restructure the ECM by paracrine release of matrix metalloproteinases (MMPs) and other factors, leading to increased collagen deposition [65]. CAFs promote Treg recruitment via ECM collagen accumulation [66]. The underlying mechanism involves accumulated

collagen activating discoid domain receptor 1 (DDR1), which upregulates interferon-induced chemokines 5 (CXCL5) expression, thereby promoting neutrophil extracellular trap (NET) formation and Treg infiltration in the TME [67].

In addition to ECM-mediated indirect recruitment, CAFs can also directly recruit Tregs through surface and secreted proteins. A distinct subset of CAF-S1 exhibits markedly elevated CD73 protein levels in the TME [68]. Analysis of the TME in 215 breast cancer patients revealed a significant correlation between $FOXP3^+$ Treg infiltration and stromal CD73 expression. Moreover, CD73 expression in CAF-S1 cells, but not in cancer cells, enhances CD73-mediated immunosuppression. Co-culture of purified CAF-S1 cells with $CD4^+CD25^+$ T cells significantly upregulated FOXP3 expression, confirming that CAF-S1 promotes Treg proliferation in the breast cancer TME. Silencing CD276/B7H3 and dipeptidyl peptidase 4 (DPP4) substantially reduced the total $CD25^+FOXP3^+$ T cell population induced by CAF-S1. Thus, CAF-S1 not only enhances Treg infiltration in the TME but also potentiates their capacity to suppress the proliferation of effector T cells, thereby promoting breast cancer metastasis [69]. Another recent study further demonstrated that CAFs can induce T-cell differentiation into Tregs by downregulating the antigen-presenting function of co-stimulatory molecules [70].

Collectively, CAFs employ multiple mechanisms to establish an immunosuppressive niche that promotes tumor progression and metastasis.

Tumor-associated macrophages: The recruitment of naïve $CD4^+$ T cells into the TME correlates with the abundance of tumor-associated macrophages (TAMs) that produce CCL18, and the infiltration of naive $CD4^+$ T cells into human breast cancer tissues is contingent upon CCL18. CCL18 promotes the infiltration of naïve $CD4^+$ T cells into tumors through interaction with phosphatidylinositol transfer protein 3 (PITPNM3), thereby significantly increasing the intratumoral Treg count, which in turn indirectly affects Treg proliferation [71].

Similarly, CXCL1 is one of the most common chemokines secreted by TAMs [72]. Inhibiting TAM/CXCL1 activity in the TME significantly suppresses immune evasion and metastasis of breast cancer [73], which is associated with CXCL1 recruiting peripheral naïve $CD4^+$ T cells and promoting their differentiation into Tregs via activation of the NF-κB/FOXP3 pathway [74]. In vitro experiments further demonstrated that 4T1 cells stimulate mouse TAMs to produce monocyte chemotactic protein 1 (MCP-1/CCL2) through the release of granulocyte-macrophage colony-stimulating factor [75]. Subsequently, CCL2 attracts peripheral macrophages to the breast cancer TME, where they differentiate into TAMs. These TAMs then affect the breast cancer TME and enhance Treg recruitment through the CCL17/CCL22-CCR4 axis [76].

Others: Regulatory B cells (Bregs) also contribute to Treg recruitment. Bregs express high levels of CD40, CD80, CD86, and TGF-β, facilitating the conversion of $CD4^+$ T cells into $FOXP3^+$ Tregs through direct cell-cell interactions. Persistent tumor activity sustains Breg production, thereby maintaining an immunosuppressive environment that promotes metastasis, particularly to the lungs [77].

In summary, Tregs within the breast cancer TME are supported by a network of mesenchymal cells, including MDSCs, CAFs, TAMs, and Bregs. These cells cooperate to enhance Treg recruitment, expansion, and immunosuppressive function (**Figure 2**).

Mechanisms of Treg-promoted breast cancer development

Tregs are highly enriched within the breast cancer TME, where they establish an immunosuppressive milieu that attenuates antitumor immune responses and facilitates tumor progression. The marked accumulation of Tregs within the TME provides a permissive environment for tumor metastasis, rendering invasive breast cancer more immunologically quiescent than in situ disease. Accumulating evidence indicates that Tregs primarily contribute to breast cancer progression by promoting tumor metastasis and mediating immunosuppression [27].

Tregs promote intratumor angiogenesis

Angiogenesis, a complex process involving multiple cell types, is a fundamental pathological basis for tumor growth and metastasis. Tregs within the TME contribute to neovascularization through both direct and indirect mecha-

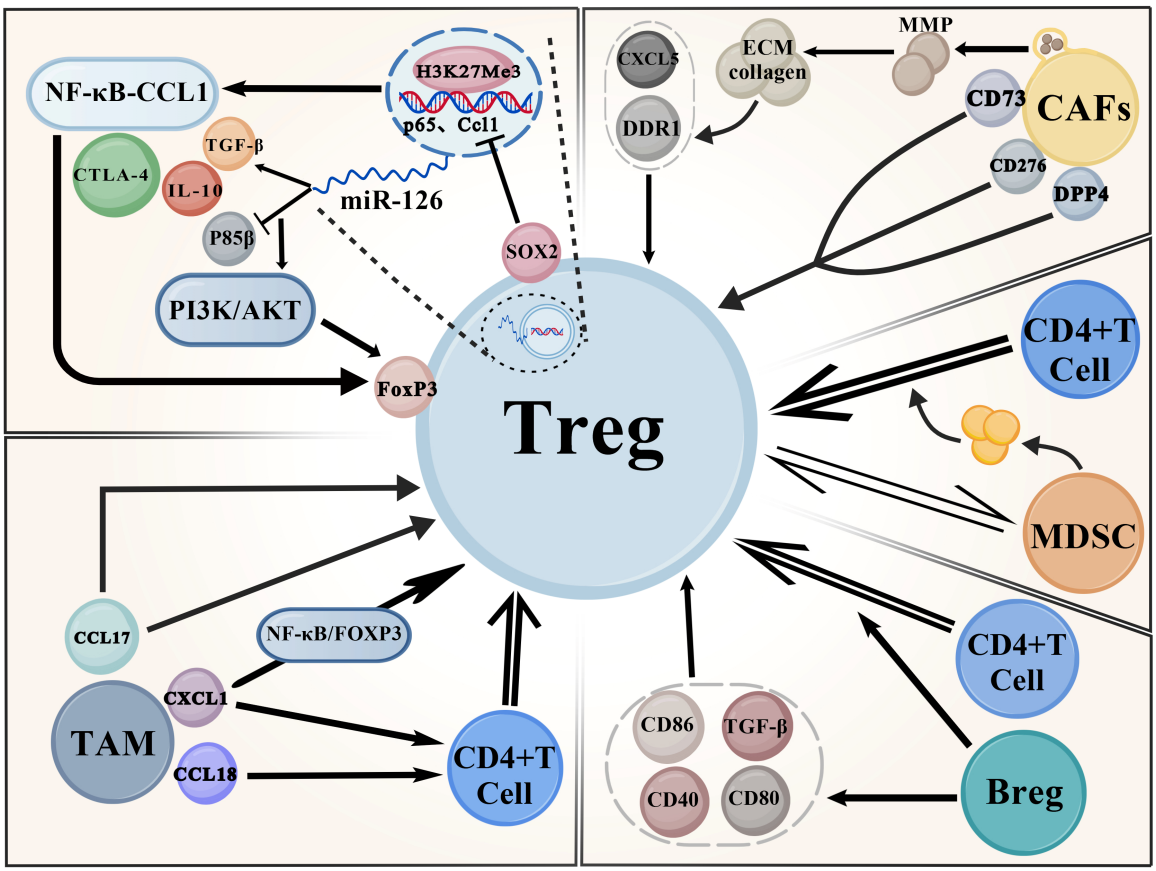

Figure 2. Mechanism of Tregs recruitment (by BioGDP.com). Tregs remodeling by mesenchymal cells and intrinsic pathways in breast cancer TME. Tregs are modified at genetic (miR-126/PI3K-AKT/FOXP3), protein (SOX2/NF-κB-CCL1), and metabolic (CD28) levels. Mesenchymal cells - CAFs, Bregs, MDSCs - further boost Treg accumulation. CAFs remodel ECM via MMP/DDR1/CXCL5; CAF-S1 expresses CD73/CD276/DPP4. Bregs use CD40/CD80/CD86/TGF-β to convert $CD4^+$ T cells into $FOXP3^+$ Tregs. MDSCs enhance Treg expansion via cytokines. Together, these pathways create a positive feedback loop driving immunosuppression and metastasis.

nisms, thereby remodeling the tumor vascular microenvironment. This results in increased microvessel density and the establishment of vascular networks that supply nutrients and oxygen, supporting tumor expansion and dissemination. Consequently, angiogenesis is a key determinant of disease progression and poor prognosis [78-80].

(1) Direct contribution: Tregs secrete vascular endothelial growth factor (VEGF), thereby directly promoting tumor angiogenesis [81]. Mechanistically, FOXP3 interacts with hyperactivated STAT3 to form a complex that binds to the VEGF promoter and enhances its transcription [82].

(2) Indirect promotion: Effector T cells produce antiangiogenic cytokines, including tumor necrosis factor-α (TNF-α) and interferon-γ (IFN-γ), the latter of which has potent antiangiogenic activity. $CD4^+$ T-cell-mediated antitumor responses rely heavily on IFN-γ production [83]. Additionally, chemokines such as CXCL9, CXCL10, and CXCL11 inhibit integrin expression, angiogenesis, and tumor proliferation. Tregs suppress these antiangiogenic effects by inhibiting effector T-cell activation and proliferation and by reducing the production of TNF-α and IFN-γ, thereby indirectly promoting angiogenesis [84] (**Figure 3**).

Mediate immune evasion

Tregs suppress antitumor immunity mainly by releasing immunosuppressive cytokines such as TGF-β, IL-10, and IL-35, by engaging in direct cell-cell interactions, and by competing for key immune mediators. Together, these mechanisms inhibit immune cell proliferation and

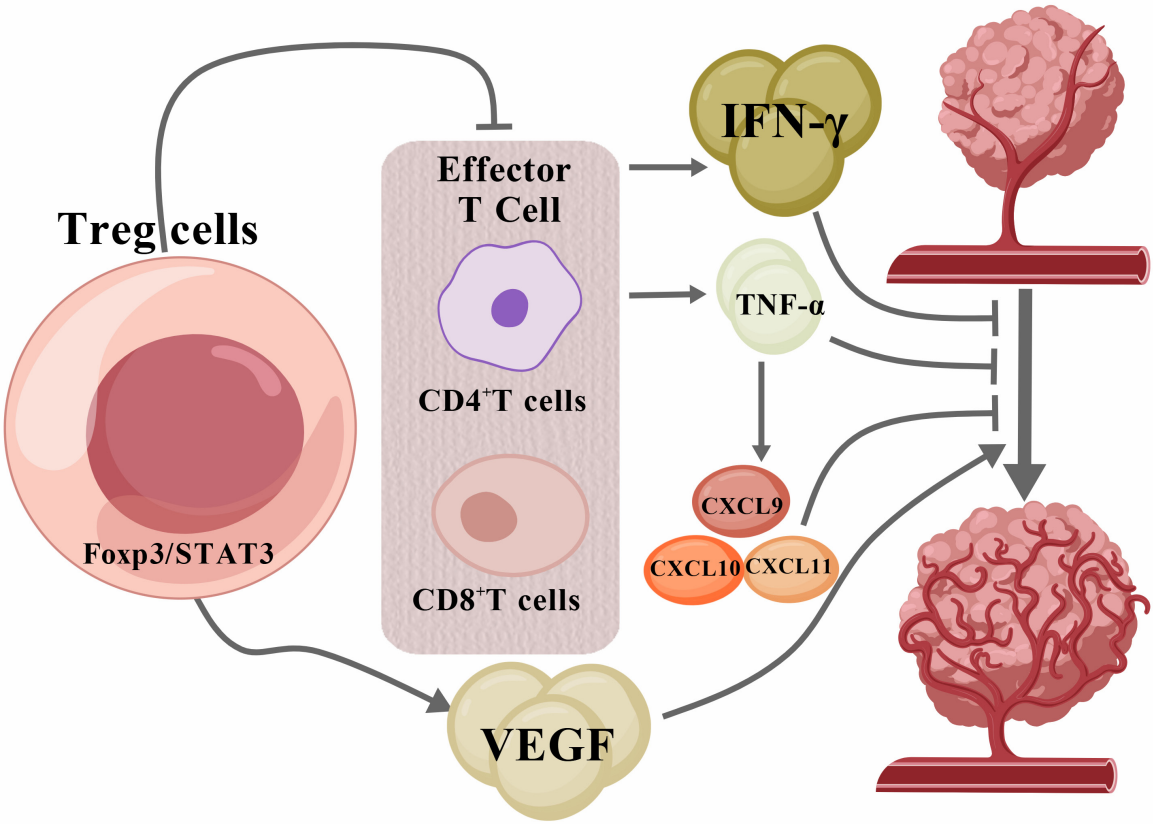

Figure 3. Tregs promote intratumor angiogenesis (by BioGDP.com). Tregs directly promote tumor angiogenesis by expressing VEGF. Additionally, they suppress the expression of TNF-α, IFN-γ, CXCL9, CXCL10, and CXCL11 in effector T cells; these factors inhibit tumor angiogenesis, thereby playing an indirect role in promoting it.

differentiation, cause immune cell depletion, and ultimately weaken the body's ability to fight tumors (**Figure 4**).

Secretion of immunosuppressive mediators: (1) TGF-β: Tregs are a major source of TGF-β in the breast cancer TME [85]. TGF-β suppresses the expression of cytotoxic molecules, including granzyme A (GzmA), granzyme B (GzmB), perforin, Fas ligand (FASL), and IFN-γ, thereby impairing immune-mediated tumor cell killing [86, 87]. It inhibits $CD8^+$ T-cell cytotoxicity via activation of SMAD proteins and activating transcription factor 1 (ATF1), leading to reduced GzmB and IFN-γ expression [88]. Additionally, TGF-β modulates antigen-presenting cell (APC) function, for example by inducing indoleamine 2,3-dioxygenase (IDO) in plasmacytoid dendritic cells and upregulating CCL22 in myeloid dendritic cells, thereby promoting tumor immune escape [89, 90].

(2) IL-10: IL-10 exerts potent immunosuppressive effects, including direct inhibition of APC activation via Toll-like receptor (TLR) signaling [91]. It suppresses IFN-γ-dependent APC activation and promotes the expression of the E3 ubiquitin ligase MARCH1 in monocytes and macrophages, leading to degradation of MHC-II and CD86 through lysosomal pathways. This impairs antigen presentation and inhibits activation of tumor-specific $CD4^+$ T cells [92, 93]. IL-10 also inhibits MyD88-dependent TLR signaling by suppressing NF-κB activation and promotes AMP-activated protein kinase (AMPK) signaling, further dampening immune responses [94-96].

(3) IL-35: Tregs produce IL-35, which cooperates with IL-10 to promote T-cell exhaustion via pathways involving B lymphocyte-induced maturation protein 1 (Blimp-1), thereby contributing to immune dysfunction [97].

Induction of apoptosis and down-regulation of immune protein expression in immune cells: (1) Tregs eliminate effector immune cells through

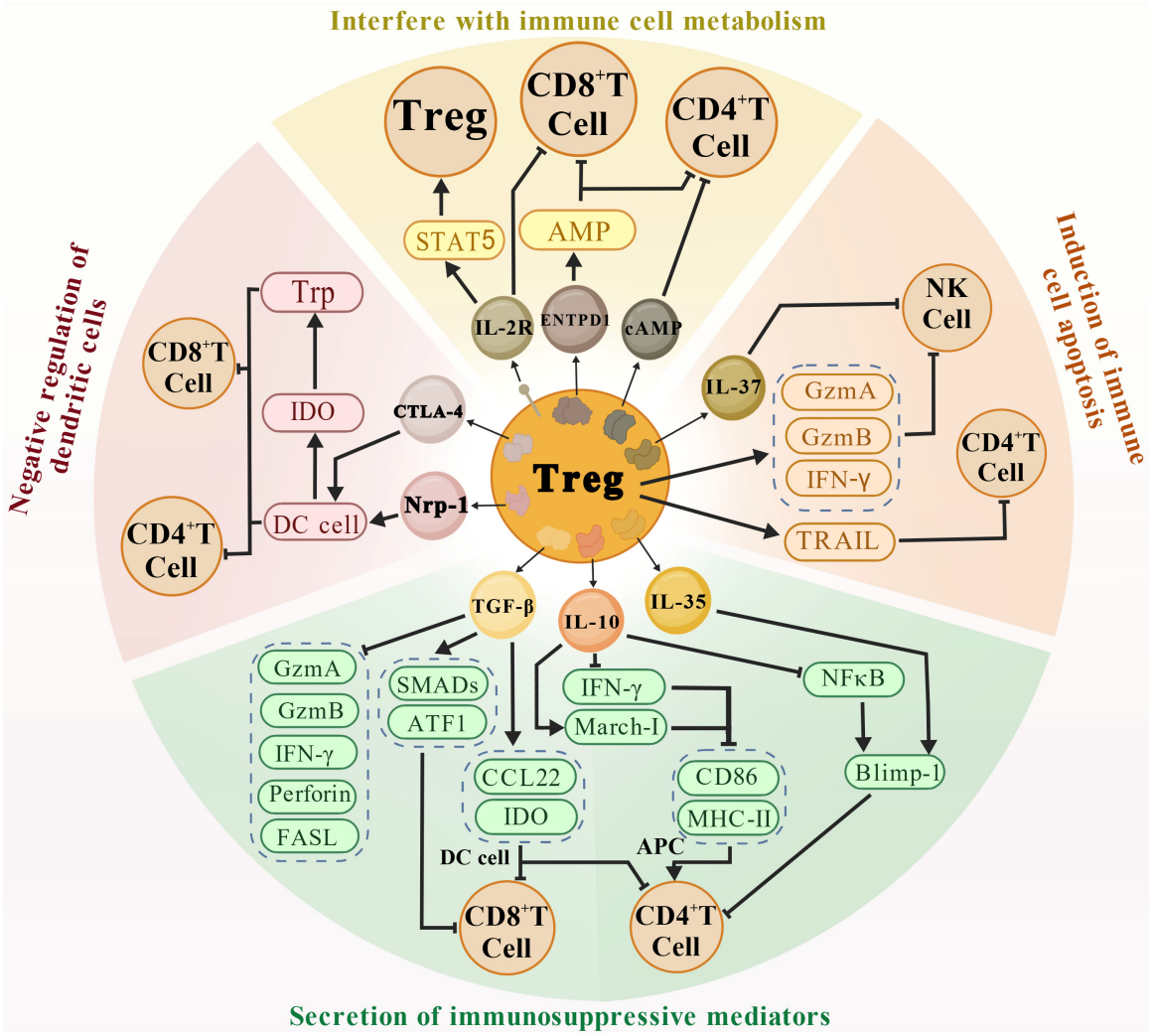

Figure 4. Mechanisms of Tregs-mediated immune escape (by BioGDP.com). Tregs primarily evade the immune system by downregulating the expression of $CD8^+$ T cells, $CD4^+$ T cells, and NK cells, and by increasing their own numbers. It can produce immunosuppressive mediators such as TGF-β, IL-10, and IL-35, induce apoptosis in immune cells and downregulates the expression of immune proteins via IL-37, GzmA, GzmB, IFN-γ and TRAIL. The remaining factors, such as IL-2R, ENTPD1, and cAMP, can interfere with the metabolism of immune cells, CTLA-4, Nrp-1 can reduce DC cell expression.

granzyme- and perforin-dependent cytotoxicity. Activated natural Tregs (nTregs) primarily produce GzmA and perforin, whereas induced Tregs (iTregs) predominantly express GzmB. Both subsets exhibit cytotoxic activity against target cells [98, 99]. Within the TME, Tregs can eliminate B cells via GzmB-dependent and partially perforin-dependent mechanisms [100].

(2) The tumor necrosis factor-related apoptosis-inducing ligand (TRAIL)/death receptor 5 (DR5) pathway contributes to Treg-mediated immunosuppression. Upregulation of TRAIL in activated Tregs induces apoptosis of $CD4^+$ T cells. Inhibition of this pathway reduces Treg suppressive capacity and cytotoxicity, highlighting its functional importance [101].

(3) IL-37 is produced by a range of immune cells, including Tregs, B cells, plasma cells, and NK cells [102]. When researchers isolated NK cells from healthy individuals and cultured them with Tregs at varying ratios, they observed downregulation of TIM-3 in NK cells and a reduction in NK cell cytotoxic function. Further investigation revealed that IL-37 within the microenvironment mediated this effect [103].

Interference with immune cell metabolism: Tregs disrupt immune cell metabolism through the expression of key receptors and enzymes, including IL-2 receptor (IL-2R), ENTPD1 (CD39), and cyclic adenosine monophosphate (cAMP).

(1) IL-2R: Tregs express high levels of IL-2R but do not produce IL-2, enabling them to competitively sequester IL-2 and deprive effector T cells of this critical growth factor. This suppresses $CD8^+$ T-cell activation and function. Meanwhile, IL-2/IL-2R signaling activates JAK/STAT5, PI3K/AKT, and MAPK pathways, promoting Treg proliferation and suppressive function [104, 105].

(2) Ectonucleoside triphosphate diphosphohydrolase-1 (ENTPD1), also known as CD39, is the main ectonucleotidase expressed on Tregs and is essential for their ability to suppress antitumor immunity. It converts immunogenic ATP and ADP into AMP [106, 107]. Unlike ATP, which promotes immune activation, AMP suppresses immune responses. Therefore, overexpression of ENTPD1 leads to AMP accumulation in the tumor microenvironment, weakening overall immune function and promoting immune evasion. In addition, Tregs that produce IL-27 can drive CD39-mediated ATP hydrolysis through the IL-27/STAT1 axis, thereby inhibiting cytokine production by $CD8^+$ T cells in vitro [108].

(3) cAMP effectively suppresses T cell growth, differentiation, and proliferation. Naturally occurring Tregs contain high intracellular levels of cAMP and can transfer this molecule to reactive $CD4^+$ T cells through gap junctions within the tumor microenvironment. Such transfer subsequently inhibits the proliferation and function of $CD4^+$ T cells, thereby dampening their antitumor immune activity [109].

Negative regulation of dendritic cells: (1) CTLA-4: CTLA-4 is a receptor highly expressed on Tregs. On conventional T cells, CD28 binds to CD80/CD86 on dendritic cells (DCs), promoting effector T cell proliferation, differentiation, and cytokine production, thereby enhancing antitumor immunity. CTLA-4 competitively binds the same CD80/CD86 ligands [110], blocking this costimulatory pathway and delivering negative signals to APCs, which inhibits effector T cell function. In addition, CTLA-4 removes CD80/CD86 from the DC surface through trans-endocytosis [111]. This reduction in CD80/CD86 on APCs exerts dual suppressive effects: it limits costimulation for naïve T cells and increases the amount of free PD-L1, which in turn inhibits PD-1-expressing effector T cells [112]. Furthermore, CTLA-4 signaling induces IDO activity, leading to tryptophan catabolism and accumulation of immunosuppressive tryptophan metabolites that further suppress effector T cell function [113].

(2) Neuropilin 1 (Nrp-1): Nrp-1 is a transmembrane glycoprotein that binds class III/IV semaphorins, VEGF, and TGF-β. It is expressed on the surface of Tregs and is involved in tumor cell migration, angiogenesis, cancer progression, and suppression of antitumor immunity [114]. In the tumor microenvironment, Tregs upregulate Nrp-1 expression, which enhances their interaction with dendritic cells (DCs). This interaction blocks effector T cells from entering APCs and downregulates costimulatory molecules on DCs [115], thereby inhibiting the cytotoxic activity of antigen-specific T cells and reducing the body's antitumor capacity [116].

Facilitating tumor invasion and escape

The main metastatic organs of patients with breast cancer are bone, brain, lung, liver, and lymphatic tissues [117], and the expression of chemokine receptors, vascular endothelial growth factor, and chemokines, as well as the pathways associated with their expression by Tregs, is the key to the ability of Tregs to facilitate the spread of breast tumors, providing a "hotbed" for breast cancer metastasis (**Figure 5**).

The core general mechanism of Tregs mediating metastasis: (1) SDF-1-CXCR4: Tregs express CXCR4, and its corresponding chemokine is SDF-1/CXCL12 [118]. Many target organs promote the metastasis of breast cancer cells by highly expressing SDF-1, which attracts CXCR4. For example, Leptin is an important promoter of bone metastasis in cancer, and it induces breast cancer cell metastasis through activation of the SDF-1/CXCR4 axis, resulting in increased bone metastasis and poor prognosis [119]. (2) CCR4: CCR4 is highly expressed in breast cancer cells under the influence of FOXP3, while its ligands, CCL22 and CCL17, are released by tumor cells and tumor-associated macrophages, attracting CCR4-expressing

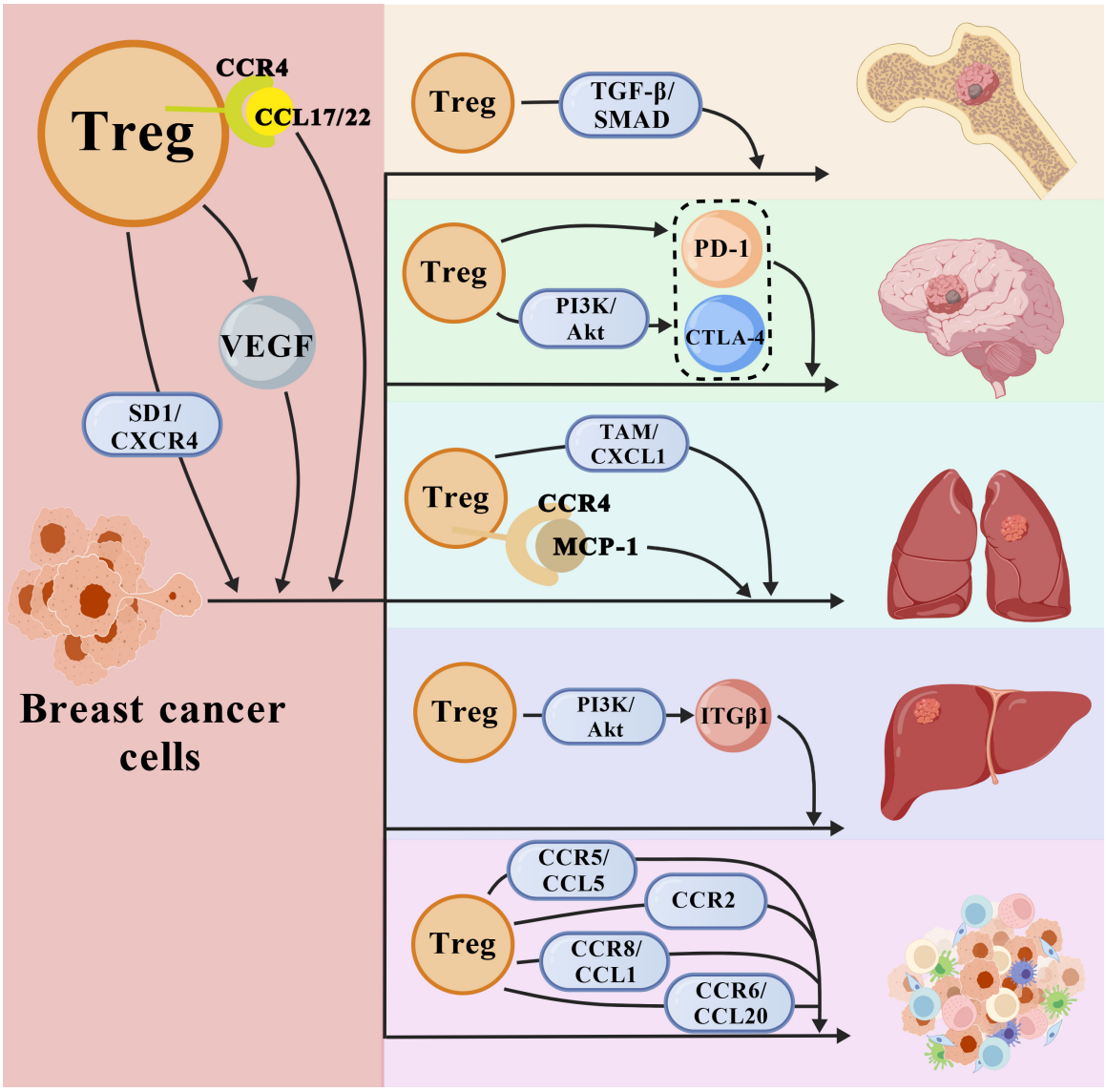

Figure 5. Mechanisms by which Tregs promote tumor invasion and escape (by BioGDP.com). During the metastasis of breast cancer cells to various target organs, Tregs play a central role in metastasis through SDF-1-CXCR4, CCR4, and VEGF. TGF-β-SMAD primarily targets bone metastases. PI3K-AKT, PD-1, and CTLA-4 in brain metastases. TAM/CXCL1 and MCP-1 act on lung metastases. PI3K-AKT promotes liver metastasis via ITGβ1. Receptors CCR2, CCR5/CCL5, CCR8/CCL1 and CCR6/CCL20 facilitate the invasion of normal tissues by tumor cells.

Tregs to the tumor site and thereby facilitating the metastasis of breast cancer cells [120]. In pulmonary metastasis, Tregs expressing CCR4 are attracted to CCL17 and CCL22 secreted by lung tissues, directing breast cancer tumor cells to metastasize to lung tissues and invade them [121]. (3) VEGF: As mentioned earlier, VEGF is secreted by Tregs and induces angiogenesis within tumors [81], thereby promoting the proliferation and metastasis of breast cancer cells in various target organs. In particular, brain metastasis is based on breaching the blood-brain barrier [122], and VEGF can be involved in promoting tumor endothelial adhesion and extravasation, causing vascular remodeling and increased permeability of tumor cells as they pass through the blood-brain barrier and facilitating tumor cell entry into the brain [123].

(1) Bone metastasis: TGF-β secreted by Tregs promotes epithelial-mesenchymal transition (EMT) through the TGF-β/SMAD pathway, which drives cell cycle progression and contributes

to bone metastasis in triple-negative breast cancer (TNBC) [124].

(2) Brain metastasis: The PI3K-AKT signaling pathway, which is associated with Treg activation, is highly active in brain-metastatic breast cancer [125]. Downstream immunosuppressive modulators, including PD-1 and CTLA-4, are also significantly elevated in brain metastatic tissues from breast cancer patients, suggesting their involvement in brain metastasis [126].

(3) Pulmonary metastasis: RANK, a receptor produced by Tregs, together with its ligand RANKL, facilitates lung metastasis of breast cancer cells [127]. Additionally, MCP-1 promotes lung metastasis by binding to CCR2 on Tregs, thereby fueling tumor spread [128]. The TAM/CXCL1/Treg axis remodels the immunosuppressive TME and inhibits immune evasion and lung metastasis in breast cancer [129].

(4) Liver metastasis: Overexpression of AKT2 in the PI3K-AKT pathway promotes integrin β1 synthesis, enhancing the binding of breast cancer cells to type IV collagen (an integrin β1 ligand) and facilitating their adhesion to and invasion of normal liver tissue [130].

(5) Other: CCR2 also promotes metastasis to other organs; $CCR2^+$ Tregs accumulate in draining lymph nodes and attract tumor cells to these nodes [131]. CCR5 is highly expressed on Tregs in breast cancer tissues and binds to CCL5 secreted by tumor cells or tumor-recruited mesenchymal stromal cells, enabling bidirectional communication that supports breast cancer growth and progression [132]. Activated by CCL20, CCR6 promotes breast cancer proliferation and metastasis by recruiting protumorigenic macrophages to the tumor site, playing an important role in tumor initiation and early development [133]. In addition, CCR8, activated by CCL1, is highly expressed on Tregs in the TME. CCR8-activated Tregs produce GzmB and IL-10 - markers of potent immunosuppression - which are associated with breast cancer survival and prognosis [134].

Tregs-based strategies for the treatment of breast cancer

Currently, a range of targeted inhibitors has been developed to interfere with cytokines secreted by Tregs or receptors highly expressed on their cell surface. In addition, several drugs originally developed for other indications, as well as traditional Chinese herbal formulations, have been shown to suppress Treg function. Some of these agents, including β-D-mannuronic acid and FLX475, have entered phase II randomized controlled clinical trials. However, most remain at the preclinical stage, limited to in vitro or animal studies. Nonetheless, these investigations provide valuable insights for the development of future Treg-targeted therapies.

Targeting agents for Tregs in the TME

Cytotoxic T-lymphocyte-associated protein 4 (CTLA-4) is highly expressed on Tregs within the breast cancer TME. Targeting CTLA-4 with specific inhibitors, such as ipilimumab, can partially deplete Tregs, although complete elimination is not achieved [135]. However, prolonged use of ipilimumab has been associated with autoimmune adverse events. To address this limitation, a fully human heavy-chain antibody (HCAb 4003-2) targeting CTLA-4 has been developed. This antibody effectively suppresses Tregs while exhibiting lower systemic exposure and reduced immune-related toxicity, suggesting an improved safety and efficacy profile compared with ipilimumab [136].

The chemokine CCL22 recruits peripheral Tregs into the TME via interaction with the CCR4 receptor [28]. FLX475, an oral CCR4 antagonist, has demonstrated good tolerability in healthy volunteers and effectively reduces Treg recruitment. Clinical trials evaluating FLX475 as monotherapy or in combination with pembrolizumab are currently ongoing in phase I/II settings [137].

Tregs are characterized by high expression of interleukin-2 receptor (IL-2R), particularly its α-chain CD25, despite minimal IL-2 production [42]. Targeting CD25 with monoclonal antibodies such as baliximab selectively inhibits $CD25^+$ Tregs, thereby enhancing antitumor immunity [138]. The novel CD25 blocker RG6292 overcomes some limitations of earlier agents by reducing immunotoxicity while maintaining efficacy [139]. Additionally, PF-08046032, an antibody-drug conjugate targeting CD25, selectively depletes intratumoral Tregs while sparing

peripheral Tregs. Preclinical studies have demonstrated favorable tolerability and efficacy, suggesting a promising therapeutic strategy with an improved safety profile [140].

Natural compound

Artemisinin (ART), a sesquiterpene lactone isolated from Artemisia annua by Tu Youyou in 1971, is widely known for its antimalarial activity but also exhibits anti-inflammatory and antitumor properties [141, 142]. In breast cancer models, ART has been shown to inhibit proliferation and induce apoptosis of 4T1 cells in a dose-dependent manner in vitro, while delaying tumor progression and prolonging survival in vivo. These effects are associated with reduced TGF-β levels and decreased Treg proportions within the TME, suggesting a role in reversing immunosuppression [143].

MHO7, a marine-derived small molecule, induces immunogenic cell apoptosis via the endoplasmic reticulum stress (ERS)-CHOP pathway. It enhances the activation of $CD86^+$ dendritic cells and $CD4^+/CD8^+$ T cells, increases MHC-II expression, and significantly suppresses Tregs in vivo, highlighting its therapeutic potential in TNBC [144].

Quercetin (QUE), a naturally occurring flavonoid, possesses anti-inflammatory, antioxidant, and antitumor properties. It inhibits proliferation, migration, and invasion of breast cancer cells and suppresses the IL-6/JAK/STAT3 signaling pathway. Additionally, QUE reduces Treg infiltration in the TME and enhances antitumor immune responses, supporting its potential as an adjunct to immunotherapy [145-147].

Synthetic compound

Zoledronic acid (ZA), a bisphosphonate widely used for bone-related diseases, inhibits bone resorption and prevents metastasis [148]. In breast cancer models, ZA suppresses Treg proliferation, differentiation, and function in a dose-dependent manner. These effects are associated with reduced expression of cytokines such as CCL2, CCL5, and IDO, thereby limiting Treg recruitment and tumor progression [149].

β-D-mannuronic acid (M2000), a novel nonsteroidal anti-inflammatory drug, has demonstrated promising antitumor activity. In experimental models, M2000 reduces the expression of MMP-2, MMP-9, CCL22, and TGF-β1, decreases Treg frequency, and inhibits angiogenesis and metastasis without significant adverse effects [150, 151].

DC101, a VEGFR2 inhibitor, suppresses tumor angiogenesis, growth, and metastasis. It also reduces Treg infiltration and partially reverses immunosuppression mediated by myeloid-derived suppressor cells, thereby improving the tumor immune microenvironment [152-154].

Pentoxifylline (PF), a methylxanthine analog, has been shown to inhibit proliferation and migration and induce apoptosis in breast cancer cells. It reduces Treg proportions in a dose-dependent manner and modulates cytokine levels, increasing IFN-γ and decreasing TGF-β, thereby enhancing antitumor immunity [155, 156].

Atovaquone, a hydroxynaphthoquinone compound, inhibits mitochondrial electron transport and is commonly used as an antimalarial agent [157]. In breast cancer models, it significantly reduces tumor growth and decreases TGF-β and IL-10 levels, accompanied by a reduction in tumor-associated Tregs [158].

Drug combination strategies

Although anthracyclines and paclitaxel remain standard treatments for TNBC, their use is limited by toxicity and the development of resistance [159]. Novel chemoimmunotherapy strategies aim to overcome these limitations. For example, integrin-targeting micelles delivering gemcitabine and paclitaxel (ATN-mG/P), combined with polymeric CpG (NanoCpG), enhance immunogenic cell death, promote dendritic cell maturation, and reduce Treg and MDSC populations. This combination significantly enhances $CD8^+$ T-cell responses and effectively suppresses lung metastasis [160].

Combination therapy with AMD3465 (a CXCR4 antagonist) and D1MT (an IDO1 inhibitor) has shown efficacy in inhibiting breast cancer bone metastasis. AMD3465 reduces Treg and MDSC infiltration, while D1MT enhances $CD8^+$ T-cell activity, collectively improving antitumor immunity [161].

Targeting tumor necrosis factor receptor 2 (TNFR2), which is highly expressed on Tregs,

represents another promising approach. Anti-TNFR2 antibodies inhibit Treg proliferation and FOXP3 expression and reduce their suppressive function. In preclinical models, these antibodies suppress tumor growth and induce tumor-specific immunity, with enhanced efficacy when combined with anti-PD-L1 therapy [162, 163].

Although anti-PD-1/PD-L1 therapies have demonstrated clinical benefit in TNBC, their efficacy is often limited by Treg-mediated immunosuppression. Notably, combining ursodeoxycholic acid (UDCA) with anti-PD-1 therapy enhances $CD8^+$ T-cell responses and reduces Treg infiltration, significantly improving therapeutic outcomes [164].

Chinese herbal soup

Traditional Chinese medicine (TCM) has gained increasing attention due to its multi-target, multi-component characteristics and favorable safety profile.

The Aiduqing (ADQ) formula, developed based on TCM principles, has demonstrated significant antitumor efficacy in both in vitro and in vivo models. In murine breast cancer models, ADQ inhibits tumor growth and lung metastasis without significant toxicity. Mechanistically, ADQ enhances antitumor immune cell infiltration, reduces Treg recruitment, and remodels the immunosuppressive TME. It suppresses CXCL1 expression in tumor-associated macrophages, thereby inhibiting Treg differentiation via the NF-κB/FOXP3 pathway and enhancing $CD8^+$ T-cell cytotoxicity [165, 166].

Jiawei Yanghe Decoction (JWYHD), another widely used TCM formulation, exhibits antitumor activity in breast cancer models. It inhibits tumor growth, reduces Treg and M2 macrophage populations and increases M1 macrophages. These effects are associated with decreased levels of proinflammatory cytokines and suppression of the JAK2/STAT3 signaling pathway [167, 168].

Conclusion and perspectives

Metastasis is a complex, multistep process that includes immune evasion by cancer cells at the primary tumor site, survival within the circulatory system, dissemination to distant organs, and eventual colonization. Escape from immune surveillance represents a critical early step in successful metastasis [169]. Tumor cells reside within the TME, a dynamic and heterogeneous milieu composed of blood and lymphatic vessels, extracellular matrix, stromal cells, immune and inflammatory cells, as well as secreted proteins, RNA, and extracellular vesicles. Through continuous interactions with tumor cells, stromal components participate in all stages of tumorigenesis, progression, metastasis, recurrence, and therapeutic response, ultimately influencing clinical outcomes [170].

Emerging evidence indicates that early-stage breast cancer can recruit Tregs into the TME through multiple mechanisms [171], followed by their local expansion. Uncontrolled accumulation of Tregs contributes to the establishment of a profoundly immunosuppressive microenvironment, which facilitates tumor progression and may also be implicated in autoimmune and metabolic disorders. In this review, we comprehensively summarized the roles of Tregs in breast cancer progression and outlined current therapeutic strategies targeting their accumulation and function within the TME. Numerous studies have elucidated the mechanisms by which Tregs suppress antitumor immunity and accumulate within the breast cancer microenvironment.

The immunosuppressive function of Tregs in the TME is well established, and a variety of therapeutic agents targeting Tregs have been developed in recent years. Early attempts to transiently deplete Tregs using anti-CD25 antibodies resulted in enhanced effector T-cell activation, delayed tumor growth, and prolonged survival in breast cancer models [172]. However, these strategies were limited by insufficient specificity, as they also impaired normal effector T cells, thereby reducing overall antitumor immunity. In addition, Treg depletion was transient, with rapid reconstitution driven by cytokines and chemokines within the TME, highlighting the complexity of targeting Tregs in vivo [173]. Consequently, such approaches are gradually being replaced by more refined strategies. Recent advances have led to the development of more selective and potentially safer inhibitors capable of targeting intratumoral Tregs, although most remain at the preclinical stage.

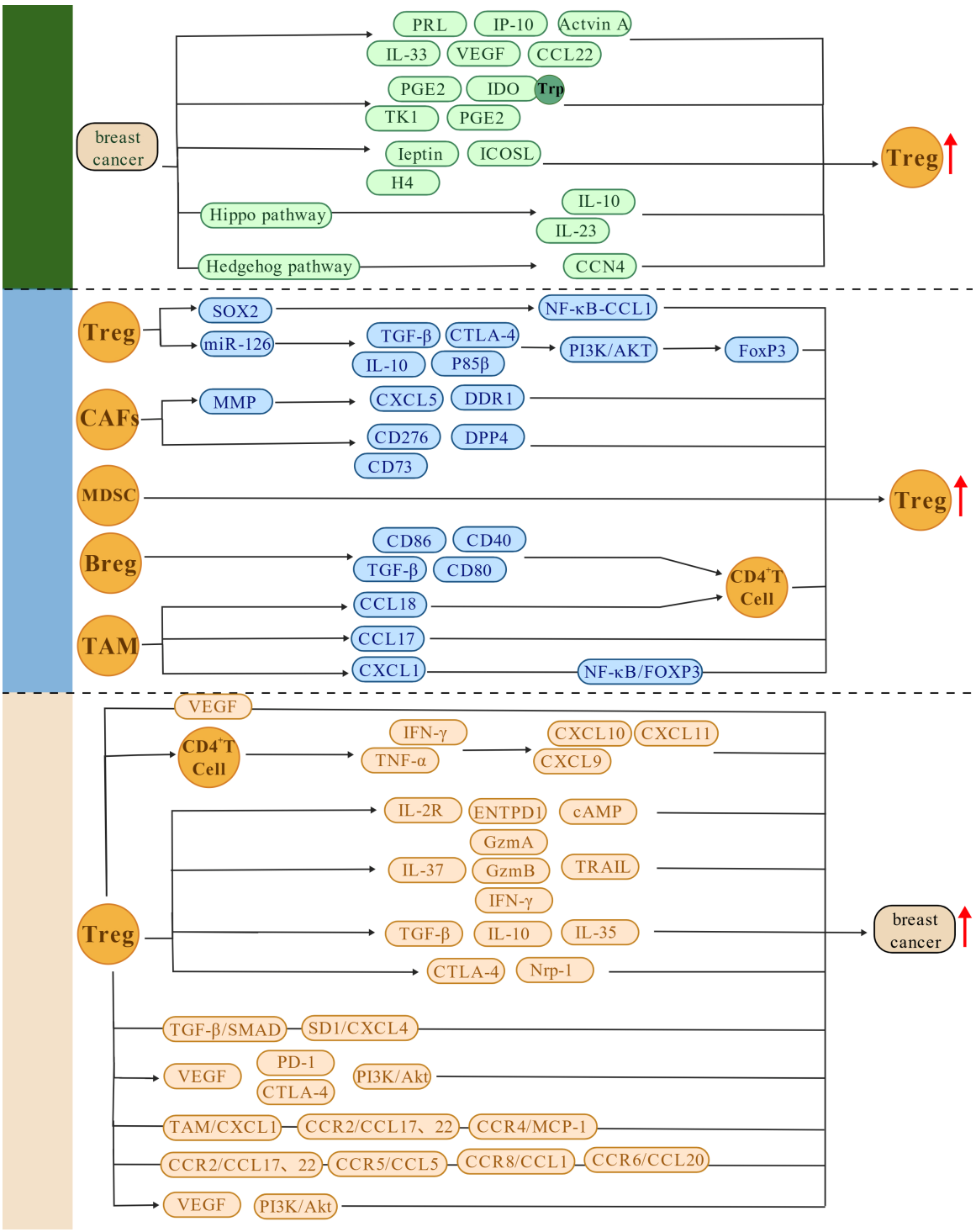

Figure 6. Role and recruitment mechanism of Tregs in breast cancer (by BioGDP.com).

Tregs within tumors often express high levels of PD-1 and persist in patients with TNBC who are resistant to PD-1 blockade. Targeted depletion or functional modulation of Tregs may therefore enhance the efficacy of anti-PD-1 therapies [174]. Accordingly, future therapeutic strategies should focus on targeting key functional pathways of Tregs that regulate their suppressive phenotype and effector interactions [175]. The rational design of combination thera-

pies will require a deeper understanding of the dynamic interactions within the TME.

In summary, comprehensive investigation of the biological characteristics, functional roles, and mechanisms underlying Treg recruitment and accumulation in the breast cancer TME will provide new opportunities for immunotherapy. Although most current Treg-targeted therapies remain in preclinical development, they offer important insights for future drug design. Continued research is expected to further elucidate Treg biology in breast cancer and to facilitate the development of more precise, less toxic, and more effective therapeutic strategies for patients (**Figure 6**).

Acknowledgements

This work was supported by National College Students' Innovation and Entrepreneurship Training Program (No. S202410541094), Hunan Provincial Natural Science Foundation (Nos. 2025JJ80089, 2025JJ80092), project of the Health Commission of Hunan Province (Grant No.: 20254184), Excellent Youth Fund Support Project of Research Project of Education Bureau of Hunan Province (No. 23B0383), The 2024 Undergraduate Research Innovation Fund Project of Hunan University of Chinese Medicine (Nos. 2024BKS066, 2024BKS065).

Disclosure of conflict of interest

None.

Address correspondence to: Yamei Li, Key Laboratory for Quality Evaluation of Bulk Herbs of Hunan Province, School of Pharmacy, Hunan University of Chinese Medicine, Changsha 410208, Hunan, China. E-mail: yameili@hnucm.edu.cn

References

[1] Wilkinson L and Gathani T. Understanding breast cancer as a global health concern. Br J Radiol 2022; 95: 20211033.

[2] Shang Q, Wang HP and Wang J. Age-period-cohort model analysis and prediction of breast cancer incidence and mortality among Chinese women from 1992 to 2021. Med J Chine PLA 2025; 50: 436-443.

[3] Kim J, Harper A, McCormack V, Sung H, Houssami N, Morgan E, Mutebi M, Garvey G, Soerjomataram I and Fidler-Benaoudia MM. Global patterns and trends in breast cancer incidence and mortality across 185 countries. Nat Med 2025; 31: 1154-1162.

[4] Siegel RL, Kratzer TB, Giaquinto AN, Sung H and Jemal A. Cancer statistics, 2025. CA Cancer J Clin 2025; 75: 10-45.

[5] Gradishar WJ, Moran MS, Abraham J, Abramson V, Aft R, Agnese D, Allison KH, Anderson B, Bailey J, Burstein HJ, Chen N, Chew H, Dang C, Elias AD, Giordano SH, Goetz MP, Jankowitz RC, Javid SH, Krishnamurthy J, Leitch AM, Lyons J, McCloskey S, McShane M, Mortimer J, Patel SA, Rosenberger LH, Rugo HS, Santa-Maria C, Schneider BP, Smith ML, Soliman H, Stringer-Reasor EM, Telli ML, Wei M, Wisinski KB, Yeung KT, Young JS, Schonfeld R and Kumar R. Breast Cancer, Version 3.2024, NCCN Clinical Practice Guidelines in Oncology. J Natl Compr Canc Netw 2024; 22: 331-357.

[6] Carvalho E, Canberk S, Schmitt F and Vale N. Molecular subtypes and mechanisms of breast cancer: precision medicine approaches for targeted therapies. Cancers (Basel) 2025; 17: 1102.

[7] Feng W, He Y, Xu J, Zhang H, Si Y, Xu J and Li S. A meta-analysis of the effect and safety of platinum-based neoadjuvant chemotherapy in treatment of resectable triple-negative breast cancer. Anticancer Drugs 2022; 33: e52-e60.

[8] Jafari L and Akhter N. Heart failure prevention and monitoring strategies in HER2-positive breast cancer: a narrative review. Breast Cancer Res Treat 2021; 186: 295-303.

[9] Ulker D, Ozyurt R, Erkasap N and Butun V. Magnetic targeting of 5-fluorouracil-loaded liposome-nanogels for in vivo breast cancer therapy and the cytotoxic effects on liver and kidney. AAPS PharmSciTech 2022; 23: 289.

[10] Mason SR, Willson ML, Egger SJ, Beith J, Dear RF and Goodwin A. Platinum-based chemotherapy for early triple-negative breast cancer. Cochrane Database Syst Rev 2023; 9: CD014805.

[11] Goel B, Virmani T, Jain V, Kumar G, Sharma A and Al Noman A. Unveiling the link between breast cancer treatment and osteoporosis: implications for anticancer therapy and bone health. Biomed Res Int 2024; 14: 5594542.

[12] Palmer BV, Walsh GA, McKinna JA and Greening WP. Adjuvant chemotherapy for breast cancer: side effects and quality of life. Br Med J 1980; 281: 1594-7.

[13] Fu T, Jin X, He M, Chen YY, Yang YS, Chen L, Zhang HY, Fan L, Wu J, Wang ZH, Chu YW, Liu RH, Jiang YZ and Shao ZM. Interferon-induced senescent CD8+ T cells reduce anti-PD1 immunotherapy efficacy in early triple-negative breast cancer. Sci Transl Med 2025; 17: eadj7808.

[14] Ge J, Yin X and Chen L. Regulatory T cells: masterminds of immune equilibrium and future therapeutic innovations. Front Immunol 2024; 15: 1457189.
[15] Fusco C, Di Rella F, Liotti A, Colamatteo A, Ferrara AL, Gigantino V, Collina F, Esposito E, Donzelli I, Porcellini A, Feola A, Micillo T, Perna F, Garziano F, Maniscalco GT, Varricchi G, Mottola M, Zuccarelli B, De Simone B, di Bonito M, Matarese G, Accurso A, Pontillo M, Russo D, Insabato L, Spaziano A, Cantone I, Pezone A and De Rosa V. CD4+ FOXP3 Exon2+ regulatory T cell frequency predicts breast cancer prognosis and survival. Sci Adv 2025; 11: eadr7934.
[16] Bates GJ, Fox SB, Han C, Leek RD, Garcia JF, Harris AL and Banham AH. Quantification of regulatory T cells enables the identification of high-risk breast cancer patients and those at risk of late relapse. J Clin Oncol 2006; 24: 5373-80.
[17] Hu SK, Eardley DD, Cantor H and Gershon RK. Definition of two pathways for generation of suppressor T-cell activity. Proc Natl Acad Sci U S A 1983; 80: 3779-81.
[18] Voarino M, Consonni F and Gambineri E. Expanding the spectrum of IPEX: from new clinical findings to novel treatments. Curr Opin Allergy Clin Immunol 2024; 24: 457-463.
[19] Smith EL, Finney HM, Nesbitt AM, Ramsdell F and Robinson MK. Splice variants of human FOXP3 are functional inhibitors of human CD4+ T-cell activation. Immunology 2006; 119: 203-11.
[20] Hori S, Nomura T and Sakaguchi S. Control of regulatory T cell development by the transcription factor Foxp3. Science 2003; 299: 1057-61.
[21] Ramsdell F. Foxp3 and natural regulatory T cells: key to a cell lineage? Immunity 2003; 19: 165-8.
[22] Sakaguchi S, Mikami N, Wing JB, Tanaka A, Ichiyama K and Ohkura N. Regulatory T cells and human disease. Annu Rev Immunol 2020; 38: 541-566.
[23] Sather BD, Treuting P, Perdue N, Miazgowicz M, Fontenot JD, Rudensky AY and Campbell DJ. Altering the distribution of Foxp3(+) regulatory T cells results in tissue-specific inflammatory disease. J Exp Med 2007; 204: 1335-47.
[24] Ge J, Yin X and Chen L. Regulatory T cells: masterminds of immune equilibrium and future therapeutic innovations. Front Immunol 2024; 15: 1457189.
[25] Ichiyama K, Long J, Kobayashi Y, Horita Y, Kinoshita T, Nakamura Y, Kominami C, Georgopoulos K and Sakaguchi S. Transcription factor Ikzf1 associates with Foxp3 to repress gene expression in Treg and limit autoimmunity and anti-tumor immunity. Immunity 2024; 57: 2043-2060, e10.
[26] Gupta S, Joshi K, Wig JD and Arora SK. Intratumoral FoxP3 expression in infiltrating breast carcinoma: its association with clinicopathologic parameters and angiogenesis. Acta Oncol 2007; 46: 792-7.
[27] Tay C, Tanaka A and Sakaguchi S. Tumor-infiltrating regulatory T cells as targets of cancer immunotherapy. Cancer Cell 2023; 41: 450-465.
[28] Zhang H, Felthaus O, Eigenberger A, Klein S and Prantl L. Treg cell therapeutic strategies for breast cancer: holistic to local aspects. Cells 2024; 13: 1526.
[29] Mao XD, Chen SZ, Shen SQ and Liu KS. Clinical significance of regulatory T cells and T lymphocyte subsets in peripheral blood in neoadjuvant chemotherapy for breast cancer. Am J Cancer Res 2023; 13: 3091-3099.
[30] Yan W, Dunmall LSC, Lemoine NR, Wang Y, Wang Y and Wang P. The capability of heterogeneous γδ T cells in cancer treatment. Front Immunol 2023; 14: 1285801.
[31] Imai T, Chantry D, Raport CJ, Wood CL, Nishimura M, Godiska R, Yoshie O and Gray PW. Macrophage-derived chemokine is a functional ligand for the CC chemokine receptor 4. J Biol Chem 1998; 273: 1764-8.
[32] Sirven P, Faucheux L, Grandclaudon M, Michea P, Vincent-Salomon A, Mechta-Grigoriou F, Scholer-Dahirel A, Guillot-Delost M and Soumelis V. Definition of a novel breast tumor-specific classifier based on secretome analysis. Breast Cancer Res 2022; 24: 94.
[33] Sarkar T, Dhar S, Chakraborty D, Pati S, Bose S, Panda AK, Basak U, Chakraborty S, Mukherjee S, Guin A, Jana K, Sarkar DK and Sa G. FOXP3/HAT1 axis controls treg infiltration in the tumor microenvironment by inducing CCR4 expression in breast cancer. Front Immunol 2022; 13: 740588.
[34] Chen KE, Ghosh M, Rivera L, Lin S, Kumar A, Swaminathan S, Lorenson MY and Walker AM. Prolactin enhances T regulatory cell promotion of breast cancer through the long form prolactin receptor. Transl Oncol 2021; 14: 101195.
[35] Marshall LA, Marubayashi S, Jorapur A, Jacobson S, Zibinsky M, Robles O, Hu DX, Jackson JJ, Pookot D, Sanchez J, Brovarney M, Wadsworth A, Chian D, Wustrow D, Kassner PD, Cutler G, Wong B, Brockstedt DG and Talay O. Tumors establish resistance to immunotherapy by regulating Treg recruitment via CCR4. J Immunother Cancer 2020; 8: e000764.
[36] Acharya S, Hegde U and Acharya AB. The clinicopathological implications of serum IL-33 and sST2 as cancer biomarkers: a narrative review. J Oral Biol Craniofac Res 2025; 15: 645-658.

[37] Stojanovic B, Gajovic N, Jurisevic M, Stojanovic MD, Jovanovic M, Jovanovic I, Stojanovic BS and Milosevic B. Decoding the IL-33/ST2 Axis: its impact on the immune landscape of breast cancer. Int J Mol Sci 2023; 24: 14026.

[38] De Martino M, Daviaud C, Diamond JM, Kraynak J, Alard A, Formenti SC, Miller LD, Demaria S and Vanpouille-Box C. Activin a promotes regulatory T-cell-mediated immunosuppression in irradiated breast cancer. Cancer Immunol Res 2021; 9: 89-102.

[39] Mukherjee S, Chakraborty S, Basak U, Pati S, Dutta A, Dutta S, Roy D, Banerjee S, Ray A, Sa G and Das T. Breast cancer stem cells generate immune-suppressive T regulatory cells by secreting TGFβ to evade immune-elimination. Discov Oncol 2023; 14: 220.

[40] Artac M and Altundag K. Leptin and breast cancer: an overview. Med Oncol 2012; 29: 1510-1514.

[41] Modak S, Aktar T, Majumder D, Singha AK and Maiti D. A systematic review on leptin's role in defining cancer: special emphasis on immunomodulation, inflammation, and therapeutic interventions. Genes Immun 2025; 26: 266-286.

[42] Sterle HA, Nicoud MB, Massari NA, Táquez Delgado MA, Herrero Ducloux MV, Cremaschi GA and Medina VA. Immunomodulatory role of histamine H4 receptor in breast cancer. Br J Cancer 2019; 120: 128-138.

[43] Wang B, Jiang H, Zhou T, Ma N, Liu W, Wang Y and Zuo L. Expression of ICOSL is associated with decreased survival in invasive breast cancer. PeerJ 2019; 7: e6903.

[44] Ma N, Chen T, Zhang Y, Chen L, Li J, Peng X, Wang Y, Zhou D and Wang B. ICOSL expressed in triple-negative breast cancer can induce FoxP3+ Treg cell differentiation and reverse p38 pathway activation. Am J Cancer Res 2022; 12: 4177-4195.

[45] Tsuji S, Tsujii M, Kawano S and Hori M. Cyclooxygenase-2 upregulation as a perigenetic change in carcinogenesis. J Exp Clin Cancer Res 2001; 20: 117-29.

[46] Jin K, Qian C, Lin J and Liu B. Cyclooxygenase-2-prostaglandin E2 pathway: a key player in tumor-associated immune cells. Front Oncol 2023; 13: 1099811.

[47] Mansfield AS, Heikkila PS, Vaara AT, von Smitten KA, Vakkila JM and Leidenius MH. Simultaneous Foxp3 and IDO expression is associated with sentinel lymph node metastases in breast cancer. BMC Cancer 2009; 9: 23.

[48] Sarangi P. Role of indoleamine 2, 3-dioxygenase 1 in immunosuppression of breast cancer. Cancer Pathog Ther 2023; 2: 246-255.

[49] Fallarino F, Grohmann U, You S, McGrath BC, Cavener DR, Vacca C, Orabona C, Bianchi R, Belladonna ML, Volpi C, Santamaria P, Fioretti MC and Puccetti P. The combined effects of tryptophan starvation and tryptophan catabolites down-regulate T cell receptor zeta-chain and induce a regulatory phenotype in naive T cells. J Immunol 2006; 176: 6752-61.

[50] McCartney A and Malorni L. Thymidine kinase-1 as a biomarker in breast cancer: estimating prognosis and early recognition of treatment resistance. Biomark Med 2020; 14: 495-498.

[51] Matikas A, Wang K, Lagoudaki E, Acs B, Zerdes I, Hartman J, Azavedo E, Björhle J, Carlsson L, Einbeigi Z, Hedenfalk I, Hellström M, Lekberg T, Loman N, Saracco A, von Wachenfeldt A, Rotstein S, Bergqvist M, Bergh J, Hatschek T and Foukakis T. Prognostic role of serum thymidine kinase 1 kinetics during neoadjuvant chemotherapy for early breast cancer. ESMO Open 2021; 6: 100076.

[52] Weagel EG, Burrup W, Kovtun R, Velazquez EJ, Felsted AM, Townsend MH, Ence ZE, Suh E, Piccolo SR, Weber KS, Robison RA and O'Neill KL. Membrane expression of thymidine kinase 1 and potential clinical relevance in lung, breast, and colorectal malignancies. Cancer Cell Int 2018; 18: 135.

[53] Huang P, Zhou X, Zheng M, Yu Y, Jin G and Zhang S. Regulatory T cells are associated with the tumor immune microenvironment and immunotherapy response in triple-negative breast cancer. Front Immunol 2023; 14: 1263537.

[54] Cao ZJ, You J, Fan YM, Yang JY, Sun J, Ma X, Zhang J, Li Z, Wang X and Feng YX. Noncanonical UPR factor CREB3L2 drives immune evasion of triple-negative breast cancer through Hedgehog pathway modulation in T cells. Sci Adv 2025; 11: eads5434.

[55] Hinshaw DC, Benavides GA, Metge BJ, Swain CA, Kammerud SC, Alsheikh HA, Elhamamsy A, Chen D, Darley-Usmar V, Rathmell JC, Welner RS, Samant RS and Shevde LA. Hedgehog signaling regulates Treg to Th17 conversion through metabolic rewiring in breast cancer. Cancer Immunol Res 2023; 11: 687-702.

[56] Cordenonsi M, Zanconato F, Azzolin L, Forcato M, Rosato A, Frasson C, Inui M, Montagner M, Parenti AR, Poletti A, Daidone MG, Dupont S, Basso G, Bicciato S and Piccolo S. The Hippo transducer TAZ confers cancer stem cell-related traits on breast cancer cells. Cell 2011; 147: 759-72.

[57] Gershoni A, Hassin O, Nataraj NB, Baruch S, Avioz-Seligman A, Pirona AC, Fellus-Alyagor L, Meir Salame T, Mukherjee S, Mallel G, Yarden Y, Aylon Y and Oren M. TAZ facilitates breast tumor growth by promoting an immune-sup-

pressive tumor microenvironment. Mol Oncol 2023; 17: 2675-2693.
[58] Ahmadzadeh M, Pasetto A, Jia L, Deniger DC, Stevanović S, Robbins PF and Rosenberg SA. Tumor-infiltrating human CD4+ regulatory T cells display a distinct TCR repertoire and exhibit tumor and neoantigen reactivity. Sci Immunol 2019; 4: eaao4310.
[59] Guo B, Gu J, Zhuang T, Zhang J, Fan C, Li Y, Zhao M, Chen R, Wang R, Kong Y, Xu S, Gao W, Liang L, Yu H and Han T. MicroRNA-126: from biology to therapeutics. Biomed Pharmacother 2025; 185: 117953.
[60] Li J, Xu Y, Han Y, Yang A, Qian M and Wang B. Role of the SOX family in cancer immune evasion: emerging player and promising therapeutic opportunities. Medicine (Baltimore) 2025; 104: e41393.
[61] Saravia J and Chi H. Immunometabolism of regulatory T cells in cancer. Oncogene 2025; 44: 2011-2024.
[62] Lasser SA, Ozbay Kurt FG, Arkhypov I, Utikal J and Umansky V. Myeloid-derived suppressor cells in cancer and cancer therapy. Nat Rev Clin Oncol 2024; 21: 147-164.
[63] Alshetaiwi H, Pervolarakis N, McIntyre LL, Ma D, Nguyen Q, Rath JA, Nee K, Hernandez G, Evans K, Torosian L, Silva A, Walsh C and Kessenbrock K. Defining the emergence of myeloid-derived suppressor cells in breast cancer using single-cell transcriptomics. Sci Immunol 2020; 5: eaay6017.
[64] Winkler J, Abisoye-Ogunniyan A, Metcalf KJ and Werb Z. Concepts of extracellular matrix remodelling in tumour progression and metastasis. Nat Commun 2020; 11: 5120.
[65] Li Y, Wang C, Huang T, Yu X and Tian B. The role of cancer-associated fibroblasts in breast cancer metastasis. Front Oncol 2023; 13: 1194835.
[66] Gao H, Tian Q, Zhou Y, Zhu L, Lu Y, Ma Y, Feng J, Jiang Y and Wang B. 3D collagen fiber concentration regulates Treg cell infiltration in triple negative breast cancer. Front Immunol 2022; 13: 904418.
[67] Li H, Li J, Bai Z, Yan S and Li J. Collagen-induced DDR1 upregulates CXCL5 to promote neutrophil extracellular traps formation and Treg infiltration in breast cancer. Int Immunopharmacol 2023; 120: 110235.
[68] Magagna I, Gourdin N, Kieffer Y, Licaj M, Mhaidly R, Andre P, Morel A, Vincent-Salomon A, Paturel C and Mechta-Grigoriou F. CD73-mediated immunosuppression is linked to a specific fibroblast population that paves the way for new therapy in breast cancer. Cancers (Basel) 2021; 13: 5878.
[69] Costa A, Kieffer Y, Scholer-Dahirel A, Pelon F, Bourachot B, Cardon M, Sirven P, Magagna I, Fuhrmann L, Bernard C, Bonneau C, Kondratova M, Kuperstein I, Zinovyev A, Givel AM, Parrini MC, Soumelis V, Vincent-Salomon A and Mechta-Grigoriou F. Fibroblast Heterogeneity and immunosuppressive environment in human breast cancer. Cancer Cell 2018; 33: 463-479, e10.
[70] Liu Y, Sinjab A, Min J, Han G, Paradiso F, Zhang Y, Wang R, Pei G, Dai Y, Liu Y, Cho KS, Dai E, Basi A, Burks JK, Rajapakshe KI, Chu Y, Jiang J, Zhang D, Yan X, Guerrero PA, Serrano A, Li M, Hwang TH, Futreal A, Ajani JA, Solis Soto LM, Jazaeri AA, Kadara H, Maitra A and Wang L. Conserved spatial subtypes and cellular neighborhoods of cancer-associated fibroblasts revealed by single-cell spatial multi-omics. Cancer Cell 2025; 43: 905-924, e6.
[71] Xu A, Ayoub S, Zhang H, Wu Y, Rau M and Ma X. Regulatory T cells in invasive breast cancer: prognosis, mechanisms and therapy. Cancers (Basel) 2025; 17: 3172.
[72] Wang N, Liu W, Zheng Y, Wang S, Yang B, Li M, Song J, Zhang F, Zhang X, Wang Q and Wang Z. CXCL1 derived from tumor-associated macrophages promotes breast cancer metastasis via activating NF-κB/SOX4 signaling. Cell Death Dis 2018; 9: 880.
[73] Wang S, Wang N, Huang X, Yang B, Zheng Y, Zhang J, Wang X, Lin Y and Wang Z. Baohuoside i suppresses breast cancer metastasis by downregulating the tumor-associated macrophages/C-X-C motif chemokine ligand 1 pathway. Phytomedicine 2020; 78: 153331.
[74] Li J, Wang S, Wang N, Zheng Y, Yang B, Wang X, Zhang J, Pan B and Wang Z. Aiduqing formula inhibits breast cancer metastasis by suppressing TAM/CXCL1-induced Treg differentiation and infiltratio. Cell Commun Signal 2021; 19: 89.
[75] Yoshimura T, Imamichi T, Weiss JM, Sato M, Li L, Matsukawa A and Wang JM. Induction of monocyte chemoattractant proteins in macrophages via the production of Granulocyte/Macrophage colony-stimulating factor by breast cancer cells. Front Immunol 2016; 7: 2.
[76] Korbecki J, Kojder K, Simińska D, Bohatyrewicz R, Gutowska I, Chlubek D and Baranowska-Bosiacka I. CC chemokines in a tumor: a review of pro-cancer and anti-cancer properties of the ligands of receptors CCR1, CCR2, CCR3, and CCR4. Int J Mol Sci 2020; 21: 8412.
[77] Guo R and Wang P. The complex role of regulatory cells in breast cancer: implication for immunopathogenesis and immunotherapy. Breast Cancer 2025; 32: 227-241.
[78] Li Y, Singh N, Dong C, Sharma S, Zhou Z, Du J, Haouili M, Jiao Y, Hopewell E, Liu Y, Opyrchal M, Zhang X, Zhou B and Lu X. Reprogramming intratumoral Treg cells by morpholino-mediated

splicing of FOXP3 for cancer immunotherapy. Sci Immunol 2025; 10: eadr9933.
[79] Zhang H, Felthaus O, Eigenberger A, Klein S and Prantl L. Treg cell therapeutic strategies for breast cancer: holistic to local aspects. Cells 2024; 13: 1526.
[80] Sumida TS, Cheru NT and Hafler DA. The regulation and differentiation of regulatory T cells and their dysfunction in autoimmune diseases. Nat Rev Immunol 2024; 24: 503-517.
[81] Mergia Terefe E, Catalan Opulencia MJ, Rakhshani A, Ansari MJ, Sergeevna SE, Awadh SA, Polatova DS, Abdulkadhim AH, Mustafa YF, Kzar HH, Al-Gazally ME, Kadhim MM and Taherian G. Roles of CCR10/CCL27-CCL28 axis in tumour development: mechanisms, diagnostic and therapeutic approaches, and perspectives. Expert Rev Mol Med 2022; 24: e37.
[82] Qin JJ, Yan L, Zhang J and Zhang WD. STAT3 as a potential therapeutic target in triple negative breast cancer: a systematic review. Exp Clin Cancer Res 2019; 38: 195.
[83] Basurto-Olvera P, Serrano H and Maldonado-Bernal C. Regulatory T cells in cancer: from immunosuppression to therapeutic targeting. Front Immunol 2025; 16: 1703211.
[84] Müller-Hermelink N, Braumüller H, Pichler B, Wieder T, Mailhammer R, Schaak K, Ghoreschi K, Yazdi A, Haubner R, Sander CA, Mocikat R, Schwaiger M, Förster I, Huss R, Weber WA, Kneilling M and Röcken M. TNFR1 signaling and IFN-gamma signaling determine whether T cells induce tumor dormancy or promote multistage carcinogenesis. Cancer Cell 2008; 13: 507-518.
[85] Yang L, Pang Y and Moses HL. TGF-beta and immune cells: an important regulatory axis in the tumor microenvironment and progression. Trends Immunol 2010; 31: 220-227.
[86] Dou A and Fang J. Heterogeneous myeloid cells in tumors. Cancers (Basel) 2021; 13: 3772.
[87] Peña-Romero AC and Orenes-Piñero E. Dual effect of immune cells within tumour microenvironment: Pro- and anti-tumour effects and their triggers. Cancers (Basel) 2022; 14: 1681.
[88] Batlle E and Massagué J. Transforming growth factor-β signaling in immunity and cancer. Immunity 2019; 50: 924-940.
[89] Arteaga CL, Hurd SD, Winnier AR, Johnson MD, Fendly BM and Forbes JT. Anti-transforming growth factor (TGF)-beta antibodies inhibit breast cancer cell tumorigenicity and increase mouse spleen natural killer cell activity. Implications for a possible role of tumor cell/host TGF-beta interactions in human breast cancer progression. Clin Invest 1993; 92: 2569-2576.
[90] Hanks BA, Holtzhausen A, Evans KS, Jamieson R, Gimpel P, Campbell OM, Hector-Greene M, Sun L, Tewari A, George A, Starr M, Nixon AB, Augustine C, Beasley G, Tyler DS, Osada T, Morse MA, Ling L, Lyerly HK and Blobe GC. Type III TGF-β receptor downregulation generates an immunotolerant tumor microenvironment. Clin Invest 2013; 123: 3925-40.
[91] Lou J, Gong C, Gao X, Zhou J, Wu Q, Zheng X and Cheng L. TIR domain proteins: regulatory mechanisms in the tumor immune microenvironment, clinical translation strategies, and prospects for precision therapy applications. Front Immunol 2025; 16: 1695754.
[92] de Waal Malefyt R, Haanen J, Spits H, Roncarolo MG, te Velde A, Figdor C, Johnson K, Kastelein R, Yssel H and de Vries JE. Interleukin 10 (IL-10) and viral IL-10 strongly reduce antigen-specific human T cell proliferation by diminishing the antigen-presenting capacity of monocytes via downregulation of class II major histocompatibility complex expression. Exp Med 1991; 174: 915-24.
[93] Kaul S, Cole NB, Bandola-Simon J, Lu Y, Tondini E, Walseng E and Roche PA. CD83 suppresses endogenous March-I-dependent MHC class II ubiquitination, endocytosis, and degradation. Proc Natl Acad Sci U S A 2025; 122: e2504077122.
[94] Bhattacharyya S, Sen P, Wallet M, Long B, Baldwin AS Jr and Tisch R. Immunoregulation of dendritic cells by IL-10 is mediated through suppression of the PI3K/Akt pathway and of IkappaB kinase activity. Blood 2004; 104: 1100-9.
[95] Guo Z, He Q, Zhang Y, Li Y and Zhang Z. Mechanisms of interleukin-10-mediated immunosuppression in viral infections. Pathogens 2025; 14: 989.
[96] Sag D, Carling D, Stout RD and Suttles J. Adenosine 5'-monophosphate-activated protein kinase promotes macrophage polarization to an anti-inflammatory functional phenotype. J Immunol 2008; 181: 8633-41.
[97] Sawant DV, Yano H, Chikina M, Zhang Q, Liao M, Liu C, Callahan DJ, Sun Z, Sun T, Tabib T, Pennathur A, Corry DB, Luketich JD, Lafyatis R, Chen W, Poholek AC, Bruno TC, Workman CJ and Vignali DAA. Adaptive plasticity of IL-10$^+$ and IL-35$^+$ Treg cells cooperatively promotes tumor T cell exhaustion. Nat Immunol 2019; 20: 724-735.
[98] Thompson R and Cao X. Reassessing granzyme B: unveiling perforin-independent versatility in immune responses and therapeutic potentials. Front Immunol 2024; 15: 1392535.
[99] Li C, Jiang P, Wei S, Xu X and Wang J. Regulatory T cells in tumor microenvironment: new mechanisms, potential therapeutic strategies and future prospects. Mol Cancer 2020; 19: 116.

[100] Zhao DM, Thornton AM, DiPaolo RJ and Shevach EM. Activated CD4+CD25+ T cells selectively kill B lymphocytes. Blood 2006; 107: 3925-32.
[101] Luo S, Larson JH, Blazar BR, Abdi R and Bromberg JS. Foxp3+CD8+ regulatory T cells: bona fide Tregs with cytotoxic function. Trends Immunol 2025; 46: 324-337.
[102] Gu M, Jin Y, Gao X, Xia W, Xu T and Pan S. Novel insights into IL-37: an anti-inflammatory cytokine with emerging roles in anti-cancer process. Front Immunol 2023; 14: 1278521.
[103] Li F, Gao C, Huang Y, Qiao Y, Xu H, Liu S and Wu H. Unraveling the breast cancer tumor microenvironment: crucial factors influencing natural killer cell function and therapeutic strategies. Int J Biol Sci 2025; 21: 2606-2628.
[104] Yodoi J and Maeda M. Discovery of ATL: an odyssey in restrospect. Int J Hematol 2011; 94: 423-8.
[105] Zong Y, Deng K and Chong WP. Regulation of Treg cells by cytokine signaling and co-stimulatory molecules. Front Immunol 2024; 15: 1387975.
[106] Kaplinsky N, Williams K, Watkins D, Adams M, Stanbery L and Nemunaitis J. Regulatory role of CD39 and CD73 in tumor immunity. Future Oncol 2024; 20: 1367-1380.
[107] Yang Y, Zhu L, Xu Y, Liang L, Liu L, Chen X, Li H and Liu H. The progress and prospects of targeting the adenosine pathway in cancer immunotherapy. Biomark Res 2025; 13: 75.
[108] Yin X, Yang Y and Liu S. Cancer immunotherapy by adenosinergic CD39 and CD73 as emerging immune checkpoints. Discov Oncol 2025; 16: 2153.
[109] Park YJ, Ryu H, Choi G, Kim BS, Hwang ES, Kim HS and Chung Y. IL-27 confers a protumorigenic activity of regulatory T cells via CD39. Proc Natl Acad Sci U S A 2019; 116: 3106-3111.
[110] Bopp T, Becker C, Klein M, Klein-Hessling S, Palmetshofer A, Serfling E, Heib V, Becker M, Kubach J, Schmitt S, Stoll S, Schild H, Staege MS, Stassen M, Jonuleit H and Schmitt E. Cyclic adenosine monophosphate is a key component of regulatory T cell-mediated suppression. Exp Med 2007; 204: 1303-1310.
[111] Linsley PS, Brady W, Grosmaire L, Aruffo A, Damle NK and Ledbetter JA. Binding of the B cell activation antigen B7 to CD28 costimulates T cell proliferation and interleukin 2 mRNA accumulation. Exp Med 1991; 173: 721-730.
[112] Tekguc M, Wing JB, Osaki M, Long J and Sakaguchi S. Treg-expressed CTLA-4 depletes CD80/CD86 by trogocytosis, releasing free PD-L1 on antigen-presenting cells. Proc Natl Acad Sci U S A 2021; 118: e2023739118.
[113] Robinson MA, Kennedy A, Orozco CT, Chen HC, Waters E, Giovacchini D, Yeung K, Filer L, Hinze C, Lloyd C, Dovedi SJ and Sansom DM. Rigid, bivalent CTLA-4 binding to CD80 is required to disrupt the cis CD80/PD-L1 interaction. Cell Rep 2024; 43: 114768.
[114] Fallarino F, Grohmann U, Hwang KW, Orabona C, Vacca C, Bianchi R, Belladonna ML, Fioretti MC, Alegre ML and Puccetti P. Modulation of tryptophan catabolism by regulatory T cells. Nat Immunol 2003; 4: 1206-1212.
[115] Varanasi SM, Gulani Y, Rachamala HK, Mukhopadhyay D and Angom RS. Neuropilin-1: a multifaceted Target for cancer therapy. Curr Oncol 2025; 32: 203.
[116] Chuckran CA, Liu C, Bruno TC, Workman CJ and Vignali DA. Neuropilin-1: a checkpoint target with unique implications for cancer immunology and immunotherapy. J Immunother Cancer 2020; 8: e000967.
[117] Shevach EM. Mechanisms of foxp3$^+$ T regulatory cell-mediated suppression. Immunity 2009; 30: 636-645.
[118] Park M, Kim D, Ko S, Kim A, Mo K and Yoon H. Breast cancer metastasis: mechanisms and therapeutic implications. Int J Mol Sci 2022; 23: 6806.
[119] Abraham M, Mishalian I, Vainstein A, Shemesh-Darvish L, Sorani E, Eizenberg O and Peled A. The high affinity CXCR4 inhibitor, BL-8040, impairs the infiltration, migration, viability, and differentiation of regulatory T cells. Blood 2021; 138: 2804.
[120] Sarkar T, Dhar S, Chakraborty D, Pati S, Bose S, Panda AK, Basak U, Chakraborty S, Mukherjee S, Guin A, Jana K, Sarkar DK and Sa G. FOXP3/HAT1 Axis controls Treg infiltration in the tumor microenvironment by inducing CCR4 expression in breast cancer. Front Immunol 2022; 13: 740588.
[121] Giannoudis A, Varešlija D, Sharma V, Zakaria R, Platt-Higgins A, Rudland PS, Jenkinson MD, Young LS and Palmieri C. Characterisation of the immune microenvironment of primary breast cancer and brain metastasis reveals depleted T-cell response associated to ARG2 expression. ESMO Open 2022; 7: 100636.
[122] Chen M, Wu C, Fu Z and Liu S. ICAM1 promotes bone metastasis via integrin-mediated TGF-β/EMT signaling in triple-negative breast cancer. Cancer Sci 2022; 113: 3751-3765.
[123] Abbott NJ, Patabendige AA, Dolman DE, Yusof SR and Begley DJ. Structure and function of the blood-brain barrier. Neurobiol Dis 2010; 37: 13-25.
[124] Duan L, Lu Y, Xie W, Nong L, Jia Y, Tan A and Liu Y. Leptin promotes bone metastasis of breast cancer by activating the SDF-1/CXCR4

axis. Aging (Albany NY) 2020; 12: 16172-16182.
[125] Ivanova M, Porta FM, Giugliano F, Frascarelli C, Sajjadi E, Venetis K, Cursano G, Mazzarol G, Guerini-Rocco E, Curigliano G, Criscitiello C and Fusco N. Breast cancer with brain metastasis: molecular insights and clinical management. Genes (Basel) 2023; 14: 1160.
[126] Monteiro M, Santos M and Santos A. Could immune checkpoints PD-1/PD-L1, PD-L2 and CTLA-4 be valuable in the spontaneous canine model of breast cancer? Crit Rev Oncol Hematol 2025; 216: 104973.
[127] Núñez NG, Tosello Boari J, Ramos RN, Richer W, Cagnard N, Anderfuhren CD, Niborski LL, Bigot J, Meseure D, De La Rochere P, Milder M, Viel S, Loirat D, Pérol L, Vincent-Salomon A, Sastre-Garau X, Burkhard B, Sedlik C, Lantz O, Amigorena S and Piaggio E. Tumor invasion in draining lymph nodes is associated with Treg accumulation in breast cancer patients. Nat Commun 2020; 11: 3272.
[128] Tan W, Zhang W, Strasner A, Grivennikov S, Cheng JQ, Hoffman RM and Karin M. Tumour-infiltrating regulatory T cells stimulate mammary cancer metastasis through RANKL-RANK signalling. Nature 2011; 470: 548-53.
[129] Yoshimura T, Li C, Wang Y and Matsukawa A. The chemokine monocyte chemoattractant protein-1/CCL2 is a promoter of breast cancer metastasis. Cell Mol Immunol 2023; 20: 714-738.
[130] Chien MH, Lee LM, Hsiao M, Wei LH, Chen CH, Lai TC, Hua KT, Chen MW, Sun CM and Kuo ML. Inhibition of metastatic potential in breast carcinoma in vivo and in vitro through targeting VEGFRs and FGFRs. Evid Based Complement Alternat Med 2013; 2013: 718380.
[131] Liu H, Li X, Li H, Feng L, Sun G, Sun G, Wu L, Hu Y, Liu L and Wang H. Potential molecular mechanisms and clinical progress in liver metastasis of breast cancer. Biomed Pharmacother 2022; 149: 112824.
[132] Qiu J, Xu L, Zeng X, Wu H, Liang F, Lv Q and Du Z. CCL5 mediates breast cancer metastasis and prognosis through CCR5/Tregs. Front Oncol 2022; 12: 972383.
[133] Jiao X, Nawab O, Patel T, Kossenkov AV, Halama N, Jaeger D and Pestell RG. Recent advances targeting CCR5 for cancer and its role in immuno-oncology. Cancer Res 2019; 79: 4801-4807.
[134] Pant A, Jain A, Chen Y, Patel K, Saleh L, Tzeng S, Nitta RT, Zhao L, Wu CY, Bederson M, Wang WL, Bergsneider BH, Choi J, Medikonda R, Verma R, Cho KB, Kim LH, Kim JE, Yazigi E, Lee SY, Rajendran S, Rajappa P, Mackall CL, Li G, Tyler B, Brem H, Pardoll DM, Lim M and Jackson CM. The CCR6-CCL20 Axis promotes regulatory T-cell glycolysis and immunosuppression in tumors. Cancer Immunol Res 2024; 12: 1542-1558.
[135] Knorr DA, Blanchard L, Leidner RS, Jensen SM, Meng R, Jones A, Ballesteros-Merino C, Bell RB, Baez M, Marino A, Sprott D, Bifulco CB, Piening B, Dahan R, Osorio JC, Fox BA and Ravetch JV. FcγRIIB is an immune checkpoint limiting the activity of treg-targeting antibodies in the tumor microenvironment. Cancer Immunol Res 2024; 12: 322-333.
[136] Gan X, Shan Q, Li H, Janssens R, Shen Y, He Y, Chen F, van Haperen R, Drabek D, Li J, Zhang Y, Zhao J, Qin B, Jheng MJ, Chen V, Wang J, Rong Y and Grosveld F. An anti-CTLA-4 heavy chain-only antibody with enhanced Treg depletion shows excellent preclinical efficacy and safety profile. Proc Natl Acad Sci U S A 2022; 119: e2200879119.
[137] Powderly JD, Chmielowski B, Brahmer JR, Piha-Paul SA, Bowyer SE, LoRusso P, Catenacci DVT, Wu C, Barve MA, Chisamore MJ, Nasrah N, Johnson D and Ho W. Phase I/II dose-escalation and expansion study of FLX475 alone and in combination with pembrolizumab in advanced cancer. J Clin Oncol 2020; 38: TPS3163.
[138] Onizuka S, Tawara I, Shimizu J, Sakaguchi S, Fujita T and Nakayama E. Tumor rejection by in vivo administration of anti-CD25 (interleukin-2 receptor alpha) monoclonal antibody. Cancer Res 1999; 59: 3128-33.
[139] Solomon I, Amann M, Goubier A, Arce Vargas F, Zervas D, Qing C, Henry JY, Ghorani E, Akarca AU, Marafioti T, Śledzińska A, Werner Sunderland M, Franz Demane D, Clancy JR, Georgiou A, Salimu J, Merchiers P, Brown MA, Flury R, Eckmann J, Murgia C, Sam J, Jacobsen B, Marrer-Berger E, Boetsch C, Belli S, Leibrock L, Benz J, Koll H, Sutmuller R, Peggs KS and Quezada SA. CD25-Treg-depleting antibodies preserving IL-2 signaling on effector T cells enhance effector activation and antitumor immunity. Nat Cancer 2020; 1: 1153-1166.
[140] Abdelhamed S, Zhang X, Zeng W, Grogan B, Schilperoort L, James R, Burley KC, Sarrett SM, Bou L, Raha P, Olson DJ, Sigurjonsson HL, Dominguez MMC, Nicholas T, Neff-LaFord HD, Fishburn J, Lim AR, Diolaiti D, Gupta P, Abrahams CL, Carosino CM, Heiser RA, Gardai SJ and Levengood MR. PF-08046032: a novel, investigational CD25-directed antibody-drug conjugate optimized for selective depletion of regulatory T cells in advanced malignant tumors. Mol Cancer Ther 2025; 24: 963-975.
[141] Wang JG, Xu CC, Wong YK, Li YJ, Liao FL, Jiang TL and Tu YY. Artemisinin, the magic drug discovered from traditional Chinese Medicine. Engineering 2019; 5: 72-88.

[142] Zeng W, Yang Y, Xiong J, Li C, He Y, Liang Z and He Y. Artemisinin derivatives maintain fibroblast normalization by acting on tumor-stroma interactions in oral tongue squamous cell carcinoma. Am J Cancer Res 2025; 15: 2657-2681.
[143] Cao Y, Feng YH, Gao LW, Li XY, Jin QX, Wang YY, Xu YY, Jin F, Lu SL and Wei MJ. Artemisinin enhances the anti-tumor immune response in 4T1 breast cancer cells in vitro and in vivo. Int Immunopharmacol 2019; 70: 110-116.
[144] Wen H, Zhong Y, Yin Y, Qin K, Yang L, Li D, Yu W, Yang C, Deng Z and Hong K. A marine-derived small molecule induces immunogenic cell death against triple-negative breast cancer through ER stress-CHOP pathway. Int J Biol Sci 2022; 18: 2898-2913.
[145] Zhu Q, Han Y, He Y, Meng P, Fu Y, Yang H, He G, Long M and Shi Y. Quercetin inhibits neuronal Ferroptosis and promotes immune response by targeting lipid metabolism-related gene PTGS2 to alleviate breast cancer-related depression. Phytomedicine 2024; 130: 155560.
[146] Sun J, Li M, Lin K, Liu Z, Wang Z, Wang W, Zhao Y, Zhen Y and Zhang S. Delivery of quercetin for breast cancer and targeting potentiation via hyaluronic nano-micelles. Int J Biol Macromol 2023; 242: 124736.
[147] Liao Y, Xie X, Zhang C, Zhong H, Shan L, Yu P and Xu L. Quercetin exerts anti-tumor immune mechanism by regulating IL-6/JAK2/STAT3 signaling pathway to deplete Tregs. Toxicon 2024; 243: 107747.
[148] Stopeck AT, Lipton A, Body JJ, Steger GG, Tonkin K, de Boer RH, Lichinitser M, Fujiwara Y, Yardley DA, Viniegra M, Fan M, Jiang Q, Dansey R, Jun S and Braun A. Denosumab compared with zoledronic acid for the treatment of bone metastases in patients with advanced breast cancer: a randomized, double-blind study. J Clin Oncol 2010; 28: 5132-9.
[149] Petruk N, Siddiqui A, Tadayon S, Määttä J, Mattila PK, Jukkola A, Sandholm J and Selander KS. CD73 regulates zoledronate-induced lymphocyte infiltration in triple-negative breast cancer tumors and lung metastases. Front Immunol 2023; 14: 1179022.
[150] Mirshafiey A, Rehm B, Abhari RS, Borzooy Z, Sotoude M and Razavi A. Production of M2000 (β-d-mannuronic acid) and its therapeutic effect on experimental nephritis. Environ Toxicol Pharmacol 2007; 24: 60-66.
[151] Zhang H, Felthaus O, Eigenberger A, Klein S and Prantl L. Treg cell therapeutic strategies for breast cancer: holistic to local aspects. Cells 2024; 13: 1526.
[152] Witte L, Hicklin DJ, Zhu Z, Pytowski B, Kotanides H, Rockwell P and Böhlen P. Monoclonal antibodies targeting the VEGF receptor-2 (Flk1/KDR) as an anti-angiogenic therapeutic strategy. Cancer Metastasis Rev 1998; 17: 155-61.
[153] Wang Z, Shi X, Zhao Y, Zhou J, Zhang S, Wang J, Yu W, Zhang X, Ren X and Zhao H. DC101, an anti-VEGFR2 agent, promotes high-endothelial venule formation and immune infiltration versus SAR131675 and fruquintinib. Biochem Biophys Res Commun 2023; 661: 10-20.
[154] Secondini C, Coquoz O, Spagnuolo L, Spinetti T, Peyvandi S, Ciarloni L, Botta F, Bourquin C and Rüegg C. Arginase inhibition suppresses lung metastasis in the 4T1 breast cancer model independently of the immunomodulatory and anti-metastatic effects of VEGFR-2 blockade. Oncoimmunology 2017; 6: e1316437.
[155] Gupta N and Srivastava SK. Atovaquone suppresses the growth of metastatic triple-negative breast tumors in lungs and brain by inhibiting integrin/FAK signaling axis. Pharmaceuticals (Basel) 2021; 14: 521.
[156] Kazemi MH, Shokrollahi Barough M, Ghanavatinejad A, Momeni-Varposhti Z, Khorrami S, Sadeghi B and Falak R. Decrease of tumor-infiltrating regulatory T cells using pentoxifylline: an Ex Vivo analysis in triple-negative breast cancer mouse model. Iran J Allergy Asthma Immunol 2022; 21: 167-177.
[157] He W and Yang GY. Progress on pharmacological properties of atovaquone. Advances in Veterinary Medicine 2023; 44: 102-106.
[158] Gupta N, Gaikwad S, Kaushik I, Wright SE, Markiewski MM and Srivastava SK. Atovaquone suppresses triple-negative breast tumor growth by reducing immune-suppressive cells. Int J Mol Sci 2021; 22: 5150.
[159] Li F and Qiao N. Application effect of taxanes combined with anthracycline neoadjuvant chemotherapy in breast cancer patients. Clinical Research and Practice 2023; 8: 78-81, +102.
[160] Guo B, Qu Y, Sun Y, Zhao S, Yuan J, Zhang P, Zhong Z and Meng F. Co-delivery of gemcitabine and paclitaxel plus NanoCpG empowers chemoimmunotherapy of postoperative "cold" triple-negative breast cancer. Bioact Mater 2023; 25: 61-72.
[161] Zhang J, Pang Y, Xie T and Zhu L. CXCR4 antagonism in combination with IDO1 inhibition weakens immune suppression and inhibits tumor growth in mouse breast cancer bone metastases. Onco Targets Ther 2019; 12: 4985-4992.
[162] Chen X, Subleski JJ, Kopf H, Howard OM, Männel DN and Oppenheim JJ. Cutting edge: expression of TNFR2 defines a maximally suppressive subset of mouse $CD4^+CD25^+FoxP3^+$ T regulatory cells: applicability to tumor-infil-

trating T regulatory cells. J Immunol 2018; 180: 6467-71.
[163] Fu Q, Shen Q, Tong J, Huang L, Cheng Y and Zhong W. Anti-tumor necrosis factor receptor 2 antibody combined with anti-PD-L1 therapy exerts robust antitumor effects in breast cancer. Front Cell Dev Biol 2021; 9: 720472.
[164] Shen Y, Lu C, Song Z, Qiao C, Wang J, Chen J, Zhang C, Zeng X, Ma Z, Chen T, Li X, Lin A, Guo J, Wang J and Cai Z. Ursodeoxycholic acid reduces antitumor immunosuppression by inducing CHIP-mediated TGF-β degradation. Nat Commun 2022; 13: 3419.
[165] Wang N, Yang B, Zhang X, Wang S, Zheng Y, Li X, Liu S, Pan H, Li Y, Huang Z, Zhang F and Wang Z. Network pharmacology-based validation of caveolin-1 as a key mediator of Ai Du Qing inhibition of drug resistance in breast cancer. Front Pharmacol 2018; 9: 1106.
[166] Li J, Wang S, Wang N, Zheng Y, Yang B, Wang X, Zhang J, Pan B and Wang Z. Aiduqing formula inhibits breast cancer metastasis by suppressing TAM/CXCL1-induced Treg differentiation and infiltration. Cell Commun Signal 2019; 19: 89.
[167] Wang Y, Cui J, Jiang Y, Zhang S, Chen L, Ma Z, Yang D, Zhang Z, Huang X, Yang Y, Guo J, Lu Z and Li C. Jiawei Yanghe decoction attenuate allergic airway inflammation by suppressing group 2 innate lymphoid cells responses. J Ethnopharmacol 2024; 326: 117927.
[168] You Y, Chen X, Chen X, Li H, Zhou R, Zhou J, Chen M, Peng B, Ji S, Kwan HY, Zou L, Yu J, Liu Y, Wu Y and Zhao X. Jiawei Yanghe decoction suppresses breast cancer by regulating immune responses via JAK2/STAT3 signaling pathway. J Ethnopharmacol 2023; 316: 116358.
[169] Han X, Li X, Bai L and Zhang G. Single-cell transcriptomics in metastatic breast cancer: mapping tumor evolution and therapeutic resistance. Front Genet 2025; 16: 1669741.
[170] Rech AJ, Mick R, Martin S, Recio A, Aqui NA, Powell DJ Jr, Colligon TA, Trosko JA, Leinbach LI, Pletcher CH, Tweed CK, DeMichele A, Fox KR, Domchek SM, Riley JL and Vonderheide RH. CD25 blockade depletes and selectively reprograms regulatory T cells in concert with immunotherapy in cancer patients. Sci Transl Med 2012; 4: 134ra62.
[171] Li C, Jiang P, Wei S, Xu X and Wang J. Regulatory T cells in tumor microenvironment: new mechanisms, potential therapeutic strategies and future prospects. Mol Cancer 2020; 19: 116.
[172] Yang S, Guo Y, Jia J, Cui W, Zhang X, Wang Y, Xie Z, Fang Y, Ding X, Chang L and Liu Y. Emerging role of cancer-associated fibroblasts in the premetastatic niche. Mol Cancer 2025; 24: 250.
[173] Solis-Castillo LA, Garcia-Romo GS, Diaz-Rodriguez A, Reyes-Hernandez D, Tellez-Rivera E, Rosales-Garcia VH, Mendez-Cruz AR, Jimenez-Flores JR, Villafana-Vazquez VH and Pedroza-Gonzalez A. Tumor-infiltrating regulatory T cells, CD8/Treg ratio, and cancer stem cells are correlated with lymph node metastasis in patients with early breast cancer. Breast Cancer 2020; 27: 837-849.
[174] Fattori S, Le Roy A, Houacine J, Robert L, Abes R, Gorvel L, Granjeaud S, Rouvière MS, Ben Amara A, Boucherit N, Tarpin C, Pakradouni J, Charafe-Jauffret E, Houvenaeghel G, Lambaudie E, Bertucci F, Rochigneux P, Gonçalves A, Foussat A, Chrétien AS and Olive D. CD25high effector regulatory T cells hamper responses to PD-1 blockade in triple-negative breast cancer. Cancer Res 2023; 83: 3026-3044.
[175] Zheng L, Wu D, Xie H and Zhao H. Revisiting Tregs in cancer and beyond: immunological control and therapeutic potential. Front Immunol 2025; 16: 1581093.