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Breast Cancer Immune Microenvironment Pathology: Cellular Composition, Spatial Organization, and Clinical Implications

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Abstract.
Breast cancer develops within a complex tumor microenvironment that includes not only neoplastic epithelial cells but also a diverse repertoire of immune and stromal cells, soluble mediators, and extracellular matrix. The immune compartment—comprising tumor-infiltrating lymphocytes, tumor-associated macrophages, myeloid-derived suppressor cells, regulatory T cells, and other subsets—plays a decisive role in tumor initiation, progression, metastatic spread, and response to systemic therapy. Distinct breast cancer subtypes, particularly triple-negative and HER2-positive tumors, display characteristic immune phenotypes that can be captured by histopathology, immunohistochemistry, and emerging spatial and single-cell technologies. These immune patterns have important prognostic and predictive value, informing patient stratification for chemotherapy and immunotherapy, especially immune checkpoint blockade. This paper reviews the cellular and molecular components of the breast cancer immune microenvironment, emphasizes their spatial organization and functional crosstalk, summarizes pathological methods for their assessment, and discusses how these features are being leveraged to develop microenvironment-targeted therapies and to refine personalized treatment strategies.

Introduction

The tumor microenvironment (TME) of breast cancer is a dynamic ecosystem composed of malignant cells, immune infiltrates, fibroblasts, endothelial cells, extracellular matrix, and soluble mediators [1–3]. Within this ecosystem, the immune microenvironment—tumor-infiltrating lymphocytes (TILs), macrophages, myeloid-derived suppressor cells (MDSCs), regulatory T cells (Tregs), and other populations—exerts both anti-tumor and pro-tumor influences, shaping clinical behavior and therapeutic response [1,2,4]. Breast cancer has historically been viewed as less immunogenic than cancers such as melanoma or non-small-cell lung cancer; however, accumulating evidence shows that subsets, particularly triple-negative breast cancer (TNBC) and some HER2-positive tumors, can be highly immune-infiltrated and responsive to immunotherapy [4–6]. These observations have driven intensive investigation into the pathology of the immune microenvironment, including the density, phenotype, and spatial organization of immune cells and their relationships with prognosis and treatment outcomes [3,7,8].

Cellular Composition of the Breast Cancer Immune Microenvironment

2.1 Tumor-infiltrating lymphocytes (TILs)

Tumor-infiltrating lymphocytes (TILs) are among the most clinically important immune components in breast cancer and include $CD8^+$ cytotoxic T cells, $CD4^+$ helper T cells, and $FOXP3^+$ regulatory T cells (Tregs) [4,9,10].

Multiple studies and international working groups have shown that higher levels of stromal TILs are generally associated with improved prognosis and better responses to therapy, particularly in TNBC and HER2-positive disease [4,10,11]. Guidelines from the International TILs Working Group recommend standardized visual assessment of stromal TILs on hematoxylin–eosin sections and recognize TILs as robust prognostic and predictive biomarkers in early-stage TNBC and HER2-positive breast cancer [10,11]. These recommendations emphasize scoring the percentage of stromal area occupied by mononuclear immune cells within the borders of the invasive tumor, excluding areas of in situ disease and necrosis, to enable reproducible reporting in both research and clinical practice [11]. High TIL levels—sometimes operationalized using thresholds such as approximately 30–50% stromal lymphocytic infiltration—define "lymphocyte-predominant" breast cancers, which

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tend to have better outcomes and enhanced responses to chemotherapy [9,10]. The prognostic impact is particularly marked in TNBC, where TIL-high tumors show lower recurrence risk and improved survival compared with TIL-low counterparts [4,9,10].

2.2 Regulatory T cells (Tregs)

Within the TIL compartment, Tregs (commonly characterized immunohistochemically as $CD4^+FOXP3^+$) play an important immunosuppressive role [1,2,4]. They inhibit effector $CD8^+$ T-cell function, dampen antigen presentation, and contribute to an immunosuppressive milieu that allows tumor cells to escape immune attack [2,8,12]. Enrichment of Tregs in the breast cancer microenvironment is frequently associated with worse prognosis and attenuated responses to immunotherapies, reflecting their central role in immune evasion [4,8,13].

2.3 Tumor-associated macrophages (TAMs)

Tumor-associated macrophages (TAMs) are often the most abundant immune infiltrate in breast cancer and exert profound effects on tumor biology [2,13,14]. They originate from circulating monocytes and tissue-resident macrophages and polarize along a spectrum between pro-inflammatory, anti-tumor "M1-like" and anti-inflammatory, pro-tumor "M2-like" phenotypes [13–15]. In many breast cancers, especially hormone receptor–positive tumors, macrophages exhibit predominantly M2-like characteristics, supporting tumor growth, angiogenesis, invasion, and metastasis through secretion of cytokines (e.g., IL-10, TGF-β), pro-angiogenic factors such as VEGF, and matrix-remodeling enzymes [2,13,14]. These M2-like TAMs also recruit and activate Tregs and MDSCs, contributing to an immunosuppressive TME that hampers effective anti-tumor T-cell responses [13–15].

High TAM density and a skewed M2:M1 ratio have been associated with aggressive disease and poor survival in breast cancer cohorts, highlighting TAMs as both prognostic markers and therapeutic targets [12–14]. Across gynecologic and breast malignancies, dysregulated TAMs are implicated in carcinogenesis, progression, and therapeutic resistance, leading to growing interest in strategies that inhibit TAM recruitment, deplete TAMs, or reprogram them toward an M1-like phenotype [13–15]. A transcriptionally distinct subset of TAMs expressing secreted phosphoprotein 1 (SPP1, also known as osteopontin) has been identified across multiple cancers, including breast cancer [15,16]. $SPP1^+$ macrophages are enriched in hypoxic regions, interact with cancer-associated fibroblasts and other stromal cells, and form physical and functional barriers that impede $CD8^+$ T-cell infiltration while promoting T-cell exhaustion and immune escape [15,16]. Targeting $SPP1^+$ TAMs is emerging as a promising approach to remodel the immunosuppressive microenvironment and enhance responses to immunotherapy [15,16].

2.4 Myeloid-derived suppressor cells (MDSCs)

Myeloid-derived suppressor cells (MDSCs) are a heterogeneous population of immature myeloid cells that expand in cancer and exert potent immunosuppressive effects, including depletion of amino acids such as L-arginine, production of reactive oxygen species and nitric oxide, and promotion of Treg development [2,5,8]. Their accumulation in breast cancer is associated with immune evasion, tumor progression, and resistance to systemic therapies, including chemotherapy and immune checkpoint inhibitors [1,5,8]. MDSCs cooperate with TAMs and Tregs to create a suppressive microenvironment that inhibits cytotoxic T-cell and NK-cell function [2,8,12]. Understanding the signaling and metabolic pathways that sustain MDSCs—such as those involving PI3K–AKT–mTOR, hypoxia-inducible factors, and lipid metabolism—is informing development of therapeutic strategies aimed at depleting MDSCs or neutralizing their suppressive activity [1,2,8].

2.5 Other immune and stromal populations

Beyond T cells, B cells, and macrophages, breast tumors contain dendritic cells, NK cells, and various innate lymphoid cells, whose contributions to tumor immunity are being increasingly recognized [1,2,8]. B cells may form part of tertiary lymphoid structures (TLSs) in some breast cancers, where they contribute to local antigen presentation and support anti-tumor T-cell responses, although the prognostic significance of TLSs remains an active area of investigation [1,8,17].

Cancer-associated fibroblasts (CAFs) are key non-immune stromal cells that modulate immune

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infiltration by secreting chemokines, remodeling the extracellular matrix, and interacting directly with immune cells [16–18].
Distinct CAF subpopulations can either facilitate or restrict immune cell entry and function within the tumor bed, influencing responses to immunotherapy and making CAFs themselves promising therapeutic targets [16–18].

2.6 Soluble Mediators, Immune Checkpoints, and Spatial Immunology

Immune and stromal cells in the breast cancer microenvironment communicate via cytokines, chemokines, growth factors, and immune checkpoint pathways that collectively determine whether anti-tumor or pro-tumor immunity predominates [1,2,8]. Pro-inflammatory cytokines can promote anti-tumor responses, whereas immunosuppressive cytokines (e.g., IL-10, TGF-β) and chemokines that recruit Tregs, TAMs, and MDSCs favor immune escape [2,8,12]. TAMs and MDSCs also secrete VEGF and matrix-modifying enzymes, promoting angiogenesis and facilitating tumor invasion [2,13,14]. Immune checkpoint molecules, including PD-1, PD-L1, CTLA-4, and others such as LAG-3, TIM-3, and TIGIT, are expressed by tumor and immune cells in the breast cancer TME and play a central role in T-cell exhaustion and functional impairment [4,8,19]. PD-L1 expression on tumor and immune cells, together with TIL levels, has become a critical biomarker for selecting patients—particularly with TNBC—for immune checkpoint blockade in both early-stage and metastatic settings [4,6,10]. The spatial distribution of immune cells and their checkpoints within the tumor has strong prognostic and predictive implications [1,7,8]. "Hot" tumors with dense intratumoral $CD8^+$ T-cell infiltration generally have better outcomes and are more likely to respond to immunotherapy, whereas "immune-desert" or "immune-excluded" tumors, where T cells are sparse or confined to the periphery, often show primary resistance [1,7,19].
Advanced spatial analysis techniques—including raster and vector-based metrics, neighborhood analysis, and spatial clustering—are increasingly applied to quantify how immune cells are arranged relative to tumor cells, stroma, and vasculature [7,17]. These methods complement established spatial biomarkers such as the breast cancer stromal TIL score and the colon cancer Immunoscore and provide refined prognostic information [7].

4. Pathological Assessment of the Immune Microenvironment

4.1 Conventional histopathology and TIL scoring

Routine hematoxylin–eosin (H&E) histology remains the foundation for assessing immune infiltration in breast cancer [3,11].
The International TILs Working Group provides detailed recommendations for evaluating stromal TILs on standard sections, including defining the invasive tumor borders, excluding areas of necrosis or in situ carcinoma, and expressing TILs as a percentage of stromal area [11]. These standardized criteria have improved reproducibility across laboratories and supported the incorporation of TILs into clinical and translational research, particularly in TNBC and HER2-positive disease [10,11].
In addition to global TIL density, pathologists may qualitatively describe patterns such as band-like infiltrates at the invasive margin, diffuse stromal infiltrates, or focal aggregates, reflecting underlying immune dynamics [3,10].

4.2 Immunohistochemistry and PD-L1 testing

Immunohistochemistry (IHC) is widely used to phenotype immune cells (e.g., CD3, CD8, CD4, FOXP3, CD68, CD163) and to assess PD-L1 expression on tumor and immune cells [3,4,6].
Pathology-based assessment of PD-L1 has become essential for selecting patients with TNBC for PD-1/PD-L1 inhibitors, with different assays and scoring algorithms used in clinical trials and regulatory approvals [4,6,10].
IHC also aids in the identification of TAM subsets and other myeloid cells, although finer distinctions among macrophage and MDSC populations often require advanced techniques [2,13,14].

4.3 Multiplex imaging and spatial profiling

Multiplex immunofluorescence and related technologies permit simultaneous visualization of multiple markers in a single tissue section, enabling detailed characterization of immune cell phenotypes and their spatial relationships with tumor cells, vasculature, and stroma [1,7,17].
Quantitative spatial metrics derived from these images—such as distances between $CD8^+$ T cells and tumor cells or the co-localization of Tregs and

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TAMs—are increasingly linked to prognosis and response to immunotherapy [1,7,19].
Digital image analysis enhances reproducibility by providing automated, objective measures of immune cell densities and spatial patterns, which can be integrated with molecular data to generate comprehensive immune profiles [3,7,8].

4.4 Emerging multi-omics and single-cell approaches

Single-cell and spatially resolved transcriptomic approaches, although not yet routine in diagnostics, are rapidly expanding our understanding of the immune landscape in breast cancer [1,8,17].
These technologies can resolve the diversity of T-cell states (activated, exhausted, regulatory), TAM and MDSC subpopulations, and CAF subsets, mapping their spatial relationships and interactions within the tissue [1,2,15].
By revealing previously unappreciated heterogeneity—such as $SPP1^+$ macrophage niches associated with T-cell exclusion—these methods are helping identify novel prognostic signatures and therapeutic targets within the TME [15,16].

5. Subtype-Specific Features of the Immune Microenvironment

5.1 Triple-negative breast cancer (TNBC)

TNBC, lacking estrogen receptor, progesterone receptor, and HER2 expression, is characterized by aggressive behavior and limited targeted therapies [4,5,6].
Among breast cancer subtypes, TNBC frequently exhibits high TIL levels, immune gene signatures, and PD-L1 expression, making it a primary candidate for immunotherapy [4,5,10].
The TNBC microenvironment often includes abundant $CD8^+$ T cells, but also significant populations of TAMs, MDSCs, and Tregs, collectively driving immune suppression [1,2,5].
Spatial organization is critical: TNBCs with T cells deeply infiltrating tumor nests have better outcomes, whereas T cells confined to stroma or excluded from tumor nests correlate with poor responses to chemotherapy and immunotherapy [5,7,19].
Disparities in TNBC outcomes across racial and socioeconomic groups may partly reflect differences in TME composition and access to therapies, underscoring the need for immune-targeted strategies [5].

5.2 HER2-positive breast cancer

HER2-positive tumors often harbor substantial immune infiltrates, and higher TIL levels are associated with improved responses to HER2-targeted therapies and better survival [1,4,10].
Combinations of HER2-directed agents with immune checkpoint inhibitors are under investigation, aiming to enhance antibody-dependent cellular cytotoxicity and anti-tumor T-cell responses [1,6,8].
TAMs and Tregs contribute to immune suppression in HER2-positive disease, and $SPP1^+$ macrophage–rich niches may drive resistance, supporting macrophage-targeted strategies [13,15,16].

5.3 Hormone receptor–positive breast cancer

Hormone receptor–positive, HER2-negative tumors generally have lower TIL densities and more immunosuppressive microenvironments than TNBC, which may contribute to modest responsiveness to checkpoint blockade [1,4,8].
Subsets with elevated immune infiltration and immune gene signatures—"immunoreactive" or "lymphocyte-rich" ER-positive tumors—may have distinct prognostic profiles and could benefit from immunotherapy in the future [1,2,8].
TAMs (including M2-like) and Tregs often predominate, reinforcing immunosuppression and potentially influencing endocrine therapy resistance [2,12,13].

6. Prognostic and Predictive Significance of the Immune Microenvironment

6.1 Prognostic value

Across studies, the density and composition of immune infiltrates correlate with survival outcomes [1,3,4].
High stromal TIL levels are associated with lower recurrence and mortality in early-stage TNBC and HER2-positive disease [4,10,11].
Conversely, enrichment of M2-like TAMs, MDSCs, and Tregs is linked to worse prognosis [2,13,14].
In ER-positive disease, heavy TAM infiltration and high M2:M1 ratio correlate with shorter survival [12–14].
Spatial distribution adds prognostic information: tumors with effector T cells intermingled with tumor cells have better outcomes than tumors with T cells segregated in stromal regions [1,7,8].

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6.2 Predictive value for chemotherapy and targeted therapy

The immune microenvironment influences responses to conventional therapies [1,6,8].

Higher baseline TILs predict better responses to neoadjuvant chemotherapy, including higher pathological complete response rates [4,10,11].

Chemotherapy can modulate the TME by inducing immunogenic cell death, increasing antigen release, and altering effector–suppressor cell balance [1,6,8].

TAMs and MDSCs contribute to chemotherapy resistance through drug tolerance, tumor survival support, and suppression of therapy-induced anti-tumor immunity [13–15].

These insights have encouraged combining cytotoxic agents with TAM- or MDSC-targeted therapies [1,14,15].

6.3 Predictive value for immunotherapy

Immune checkpoint blockade has expanded options for metastatic and high-risk early-stage TNBC [6,8,19]. Clinical responses to PD-1/PD-L1 inhibitors correlate with high TIL levels, PD-L1 expression, and inflamed gene signatures [4,6,8]. Resistance often involves low T-cell infiltration, dominance of suppressive myeloid cells, dense stromal barriers, or alternative immunosuppressive pathways [8,15,19]. Understanding TME resistance mechanisms is critical for designing effective combination regimens and patient selection [6,8,19].

7. Therapeutic Targeting of the Immune Microenvironment

7.1 Modulating tumor-associated macrophages

TAM-directed therapies aim to reduce pro-tumor activity or reprogram TAMs toward anti-tumor functions [13–15]. Approaches include blocking monocyte recruitment (CCL2–CCR2, CSF1–CSF1R), depleting macrophages, and repolarizing M2-like TAMs into M1-like phenotypes [13–15]. Targeting $SPP1^{+}$ macrophages may dismantle immunosuppressive niches and enhance checkpoint inhibitor efficacy [15,16].

7.2 Targeting MDSCs and immunosuppressive myeloid networks

MDSC-directed strategies inhibit expansion, block recruitment, or neutralize suppressive functions via metabolic or signaling modulation (e.g., PI3K–AKT–mTOR) [1,2,8]. Combination strategies disrupting TAMs, MDSCs, and Tregs may be necessary for effective immune reactivation [2,8,12].

7.3 Regulatory T-cell–directed approaches

Treg depletion or functional inhibition is challenging due to systemic immune tolerance [8,12,13]. Strategies include targeting intratumoral chemokine receptors, checkpoint differences, or selective agents (e.g., low-dose cyclophosphamide) combined with checkpoint inhibitors [8,12,13].

7.4 Enhancing TILs and tertiary lymphoid structures

Therapies that increase TIL infiltration or TLS formation may enhance local anti-tumor immunity [1,8,17]. Radiotherapy, chemotherapy, oncolytic viruses, and intratumoral adjuvants can modulate TIL density and distribution [1,6,8].

Adoptive T-cell therapies (TIL therapy, CAR-T) are in early development, particularly promising in immunologically “hot” TNBC [6,8].

7.5 Targeting CAFs and stromal barriers

CAFs and extracellular matrix can exclude immune cells and sustain immunosuppression [16–18]. Interventions target CAF-derived cytokines, matrix-modifying enzymes, and pathways promoting fibrosis, facilitating T-cell trafficking and improving immunotherapy efficacy [16–19].

7.6 Combination immunotherapy strategies

Combination regimens integrating checkpoint blockade with TME-modifying agents (TAM/MDSC modulators, anti-angiogenics, CAF-targeted therapies) are under investigation [1,6,8,15]. These aim to convert “cold” or myeloid-dominated tumors into “hot” tumors more susceptible to T-cell–mediated killing [1,8,15].

8. Future Directions and Conclusions

The pathology of the breast cancer immune microenvironment has evolved from descriptive lymphocyte assessments to multi-dimensional analyses incorporating TIL scoring, immune phenotyping, spatial analytics, and single-cell/multi-omics technologies [3,7,11].

Key insights include:

- TILs are robust prognostic and predictive markers, particularly in TNBC and HER2-positive disease, and standardized scoring enables clinical and trial use [4,10,11].
- Immunosuppressive populations—M2-like TAMs, SPP1^{+} macrophages, MDSCs, and Tregs—drive tumor progression, therapeutic resistance, and immune escape, representing high-value intervention targets [2,13–16].
- Spatial organization of immune cells provides prognostic and predictive information beyond cell counts [1,7,8].
- Immune landscapes differ across subtypes: TNBC is inflamed yet heterogeneous, hormone receptor–positive tumors are often myeloid-dominated and immunosuppressive [1,4,5,8].
- Future work will integrate immune profiling into diagnostics, refine immune-based classifications, and deploy rational combination therapies tailored to specific TME states [1,8,17].

A deeper understanding of cellular composition, spatial architecture, and functional circuitry is essential for improving prognostication, overcoming therapy resistance, and delivering personalized immuno-oncology [1,2,8].

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