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The Great Immune Escape: Understanding the Divergent Immune Response in Breast Cancer Subtypes

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Abstract

Breast cancer, the most common type of cancer affecting women, encompasses a collection of histological (mainly ductal and lobular) and molecular subtypes exhibiting diverse clinical presentation, disease trajectories, treatment options, and outcomes. Immunotherapy has revolutionized treatment for some solid tumors but has shown limited promise for breast cancers. In this review, we summarize recent advances in our understanding of the complex interactions between tumor and immune cells in subtypes of breast cancer at the cellular

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and microenvironmental levels. We aim to provide a perspective on opportunities for future immunotherapy agents tailored to specific features of each subtype of breast cancer.

Keywords

estrogen receptor positive breast cancer; HER2+ breast cancer; triple negative breast cancer; immunotherapy; breast cancer

Introduction

Breast cancer is a highly heterogenous disease that affects 1 in 8 women in the United States alone(1). While great progress has been made in the early detection and treatment of breast cancer, it still remains the second largest cause of mortality due to cancer in women after lung cancer(2).

Breast cancers are typically classified according to both molecular and histological subtypes(3, 4). Molecularly, tumors are divided into hormone receptor positive (HR+), HER2+, and triple negative (TNBC). Histologically, invasive lobular carcinoma (ILC) is the most common special subtype, in addition to the no special subtype (NST), also frequently called invasive ductal cancer (IDC). These molecular and histological classifications confer different prognoses and treatment options. While breast cancer can perhaps be distinguished as one of the first malignancies to be approached with precision medicine strategies, through the use of estrogen receptor- (ER) and HER2-targeted therapies, additional strategies are required to improve long-term outcome of patients with this disease. Our current classification of breast cancers is largely naïve to the immune state of each tumor type. Nevertheless, the genomic landscape of HR+, HER2+, and triple negative cancer cells, along with the tumor-immune microenvironment (TIME), often leads to different immune infiltration and functionality. It stands to reason that breast cancer classification will eventually be refined to not only include the current cancer cell-centric molecular and histologic features, but to also incorporate the characteristics of the accompanying immune infiltrate at the single-cell and spatially resolved levels in an effort to better understand the ecosystem of the tumor(5, 6).

Immune-targeting therapies have now become a mainstay of treatment for many patients with cancer; despite early success in melanoma and lung cancer, immune-targeting therapy uptake in breast cancer has been relatively slow, as breast cancer has historically been classified as an “immune cold” or “immune inert” cancer type since the 1980s(7). In breast cancer, PD-1- or PD-L1-targeting drugs such as pembrolizumab and atezolizumab have shown some initial promise; however, rates of overall response remain underwhelming when used as a single agent or in combination with traditional cytotoxic chemotherapies(8, 9). Thus, there is an urgent need to expand immune-targeted agents beyond T cells and gain a better understanding of the diversity in the tumor-immune microenvironment (TIME) in various breast cancer subtypes, which may yield better drugs, trial designs, and ultimately improved outcomes for patients(10).

In this review, we aim to compare and contrast the immune response across the different molecular and histological subtypes of early-stage breast cancer, including HR+ (Luminal A and Luminal B), HER2+, and TNBC, as well as between HR+ IDC and HR+ ILC (given the majority of ILC diagnoses are HR+) (Figure 1). We will discuss cancer-cell intrinsic factors (including immunogenicity, tumor mutation burden (TMB), PD-L1 expression, and secreted factors), cancer-cell extrinsic factors (including immune infiltration landscape and functionality), and the tumor-immune microenvironment in totality (including influence of the spatial architecture and stroma). Finally, we will summarize current challenges, barriers, and next-generation opportunities to harness the immune system for the treatment of breast cancer.

Cancer Cell-Intrinsic Factors Contributing to the TIME

Owing to the advancement of bulk and single-cell sequencing technologies, a wealth of genomic and transcriptomic data is now available that underscores the differences between breast cancer subtypes. These underlying differences in genomic landscape, including tumor mutational burden (TMB), HLA expression, and display of neoantigens, may contribute to divergence in immune infiltration and responses. Further, differences in metabolic reprogramming between the three main subtypes may also impact TIL composition and response to immune-targeting agents. While breast cancers have been historically considered to have a low TMB and immunogenicity compared to other tumor types, emerging evidence suggests resolving the cellular composition and heterogeneity between clonal regions in breast cancer may allow for a more precise understanding. In this section, we aim to shed light on how cancer cell-intrinsic properties may lead to differences in immune interactions.

Classification of Tumors Beyond Traditional Receptor Status

Efforts to further refine molecular classifications based on intrinsic subtypes have dominated over the past decade. In TNBC, four major subtypes have been identified using RNA-sequencing, which suggests there may be differences in immune response even between sub-classifications within TNBC: [i] basal-like immune suppressed (chemotherapy most effective); [ii] immunomodulatory, defined by high tumor-infiltrating lymphocyte (TIL) presence (immune checkpoint therapy may be most effective according to the authors of the study); [iii] mesenchymal subtype, defined by high JAK/STAT activation (suggesting STAT3 inhibitors may be most effective), and [iv] luminal androgen receptor, defined by androgen receptor (AR) expression and *ERBB2* activating mutations (suggesting hormone therapy or ERBB2-targeting tyrosine kinase inhibitors may be effective)(11). Similarly, not all HER2+ disease has the same genomic profile, and only 50% of clinically HER2+ tumors actually fall into the HER2-Enriched (HER2-E) intrinsic subtype category(12, 13). HER2-E, which is typically HR negative, is characterized by the highest levels of *ERBB2* mRNA and therefore the highest activation of the HER2 signaling pathway (including FGFR4 and EGFR activation). This leads to higher proliferation rates compared to HER2+ tumors that may fall into the Luminal B category and ultimately to different levels of immune interaction (more immune infiltration in HER2-E tumors compared to those that are Luminal B)(14).

Further, it is now appreciated that traditional receptor-based classification of breast cancer has limitations, and recent data show that classification based on gene modules can better connect tumors with immune status and is associated with prognosis. For example, a recent single cell sequencing analysis of intrinsic subtype classification showed significant heterogeneity among cancer epithelial cells traditionally belonging to the Luminal, HER2-E, and TNBC subtypes(15). Based on the gene modules, Luminal A cells were enriched in pathways related to estrogen response, but also enriched with respect to TNF signaling through NF-κB, hypoxia, and apoptosis, suggesting a role for secretory products interacting with the immune system in the HR+ subtype (Figure 2). Basal-like TNBCs exhibited a strong intrinsic phenotype towards interferon response(15). Interestingly, interrogation of HER2-E tumors showed more enrichment of proliferation pathways rather than specifically immune-related or cytokine secretory products(15). Significantly, very few tumors are homogenous entities – cellular heterogeneity shows an amalgamation of different intrinsic subtypes within the TIME, which may confer different interactions with non-tumor cells depending on clone location and may create many local environments within a single TIME(15, 16). In an analysis using TCGA data, TNBC and HER2+ tumors that had increased immune metagene expression had lower clonal heterogeneity and neoantigen loads, suggesting a strong immunoediting effect in these tumor types that was not seen in ER+ tumors(17). This may underlie why stronger immune responses are observed in TNBC and HER2+ tumors. Taken together, these data suggest that efforts to better classify tumors beyond receptor status have shed light on subtypes that may be more immune stimulatory or responsive. In the future, clinical trials and studies should provide sub-analyses to determine if agents targeted for each subtype validate these preclinical data.

Tumor Mutational Burden

While intrinsic differences exist between breast cancer subtypes and can contribute to differences in immune interactions, more traditional markers of TMB or HLA expression may also contribute to differing TIMEs. A large analysis of 3969 patients with breast cancer identified subtype differences in TMB, with median TMB in TNBC higher than in HER2+ and HR+, with HR+ being the lowest(18). Across all included samples, the median TMB was 2.63 mut/Mb. When grouping TMB categorically, Luminal A and Luminal B tumors have significantly lower proportions of tumors classified as high TMB (which was defined by the authors as greater than 1.63mut/Mb, the mean TMB rate of the BRCA cohort in TCGA) than HER2+ and TNBC(19). While cutoffs may be helpful for the purposes of research studies, in reality TMB should be considered as a continuous variable with immune responses varying accordingly across the TMB spectrum as well. Across these studies, it becomes apparent that compared to other cancers, breast cancers as a whole harbor a much lower level of hypermutation, with only around 5% of all tumors classified as having TMB > 10 mut/Mb(18). Breast cancers that do have a high TMB were more often enriched with favorable immune infiltrates, including the presence of CD8+ T cells, CD4+ T cells, activated NK cells, and gamma delta T cells(18). Consistent with an antigen-stimulated immune response, patients with a high TMB and high immune infiltrate survived longer than those with weak or poor immune infiltrates. While important, TMB may not tell the whole story: for example, one study demonstrated that 36% of basal-like tumors were classified as having high TMB; yet only 24% of these high TMB tumors had strong immune infiltrates.

This suggests that only a relatively small fraction of these tumors may be particularly immunogenic(19).

HLA Class I Expression

Cancer cell HLA class I expression may also drive differences in antigen presentation and subsequent T cell interactions. In an immunohistochemistry (IHC) analysis of tumors from patients in the GeparTrio trial, HR+/HER2− tumors showed significantly lower HLA class I expression compared to other subtypes. While lower in the HR+ subtype, HLA downregulation is likely involved in immune evasion in other subtypes as well(20). Additional evidence from the AURORA US Network further investigates *HLA-A* dysregulation primarily in the TNBC setting. This study found that in TNBC, genetic (deletion) or epigenetic (hypermethylation) alterations in *HLA-A* led to a general decrease in *HLA-A* gene expression and an associated lower number of MHC-I-associated neoantigens, which were independent of the TMB(21). This was also associated with lower immune cell infiltrates using DNA methylation-based assessments of leukocyte infiltration. In TCGA, primary tumors that had HLA-A methylation had associated decreased expression of multiple adaptive immunity signatures, possibly indicating evidence of immune escape(21). Speculatively, the authors state that immune checkpoint inhibitors (IC) may have little effect in these *HLA-A*-low, TNBC tumors, which may explain modest response rates in clinical trials for TNBC. While it is difficult to say that ER+ tumors are MHC-I “low,” it is likely that TNBCs are just comparatively higher because of more interferon signaling, which is negatively associated with estrogen receptor signaling and *ESR1* expression(22).

Cancer Cell PD-L1 Expression

An additional layer of cancer cell-intrinsic immune differences may be due to underlying expression of stimulatory or inhibitory ligands, the most characterized of which is PD-L1 (encoded by the *CD274* gene). While PD-L1 may be expressed on many different cells in the TIME(23), we focus here on PD-L1 expression on tumor cells given our focus on cancer cell-intrinsic properties in this section. Appreciable differences are observed between the three main molecular breast cancer subtypes(24): TNBCs seem to harbor the highest levels of both PD-L1+ tumor and immune cells, with concomitant decreases in PD-L1+ tumor cells seen in HER2+ and ER+ disease when analyzed by IHC and by gene expression(25, 26). Luminal A tumors, characterized by high ER positivity and low Ki-67 indices, have the lowest proportion of PD-L1+ tumor and immune cells in an analysis of multiplex immunohistochemistry (mIHC)(27, 28). In multivariate analysis, PD-L1 positivity was associated with positive lymph node metastases, higher histological grades, ER negativity, and TNBC(26). As noted above, it could be that PD-L1 expression is highly influenced by strong interferon signaling in TNBCs (a phenomenon that is observed less so in HER2+ and ER+ tumors). No relationship was observed between PD-L1 positivity and HER2 status. At the gene expression level, recent studies have shown that TNBCs harbor higher levels of *CD274* compared to non-TNBCs(29). The prognostic significance of PD-L1 positivity on breast tumor cells remains unresolved.

An additional layer of complexity is that subpopulations of breast cancer cells, such as cancer stem cells, may harbor up to three-fold higher levels of PD-L1 compared to their

more differentiated counterparts when measured by quantitative immunofluorescence(30). This additional layer of immune evasion may help tumors sustain the tumorigenic process. As TNBCs seem to harbor higher amounts of cancer stem cells, this subgroup of cells may help to drive immune evasion, and combinatorial targeting of newly identified pathways, such as Notch3 through mTOR or the beta catenin pathway, in addition to anti-PD-L1 agents may better target this group(31, 32).

Metabolic Programming of the TIME

As breast epithelial cells progress from non-cancer to cancerous, changes in cellular metabolism occurs to meet new demands for energy and nutrients. As such, intrinsic factors, such as genetic and epigenetic alterations, may contribute to the unique metabolic programs of the breast cancer subtypes(33). Recent efforts have focused on identifying metabolic pathway-driven heterogeneity within the subtypes. Metabolic dysregulation has been most pronounced in TNBC, which have shown to be most distinct metabolically from non-TNBC and normal samples in a pathway enrichment analysis using gene expression data from tumors included in TCGA(34). Further scrutiny of TNBCs, again using pathway-based analysis, revealed three distinct metabolic pathway-based subtypes (MPSs), including one with upregulated lipid metabolism, one with upregulated carbohydrate and nucleotide metabolism, and one with mixed pathway activation and dysregulation(34). Interestingly, the MPS group of TNBCs with upregulated glycolytic and nucleotide metabolism had the worst prognosis out of the three identified groups and high rates of homologous recombination deficiency(34). Recent data suggest that highly glycolytic breast tumors, such as TNBCs, create a local lactic acidosis in the TIME, which is a known driver of immune evasion(35). It is then not surprising that these tumors also had higher grades and were most responsive to anti-PD-1 therapy *in vitro* in patient derived organoids, suggesting that therapy targeting lactate dehydrogenase (which would decrease the local lactic acidosis) in combination with anti-PD-1 agents may be an optimal combination for further testing(36). Additionally, ectonucleotidases, specifically CD73 and CD39, have received increased attention. These ectonucleotidases rapidly convert extracellular ATP into the immunsuppressive adenosine(37, 38). Given that TNBCs seem to harbor the most immunosuppressive TIME, inhibiting ectonucleotidases to enhance immunotherapy effect (reprogramming the TIME to be less immunosuppressive) is actively being tested *in vitro* and in clinical trials(39). On the other hand, metabolomic efforts to identify distinct dysregulation in ER+ disease has been more elusive. However, amino acid pathway metabolism may show differences across the subtypes, with the lowest levels of amino acid metabolism in the Luminal A subtype compared to all others(40, 41). Estrogen and ER signaling itself may also alter cellular metabolism, and the potential between pathway crosstalk is an active area of investigation. Further research is needed to determine how intrinsic metabolic reprogramming can lead to promising new combination therapies with currently used immune-targeting agents.

Taken together, the cancer cell-intrinsic factors in HR+, HER2+, and TNBCs are one component in the varying interactions between tumor and non-tumor cells in the TIME (Figure 2A). TNBCs harbor higher TMB rates, HLA downregulation (either through genetic or epigenetic mechanisms) likely indicating immune escape, higher PD-L1 expression,

and higher energy demands. Evidence already shows a role for immunoediting in the TIME for these tumors and finding the right populations of patients for immune-targeted therapy is important given the heterogeneity of the disease. The picture is less clear for HER2+ and ER+ tumors. While ER+ tumors are thought to be the “coldest” out of the three, there is clearly a subset of these tumors that do have strong immune interactions even beyond expression of PD-L1, and though not harboring as high of a value of TMB, PD-L1 expression, or metabolic demand as TNBCs, HER2+ tumors lie somewhere between TNBCs and ER+ tumors. While T cell targeted therapy may not be the most effective immune-targeted therapy for HER2+ or ER+ tumors, assessment of immune cell infiltration and functionality may yield additional insights(42).

Cancer Cell-Extrinsic Factors Contributing to the TIME

Breast cancer was historically thought to be immunologically silent but a growing body of literature over the last decade demonstrates a role for immune cells in the development, progression, and therapy responsiveness of the disease (43–46). Through multiple retrospective analyses using a wide array of methods (including but not limited to IHC, flow cytometry, single-cell RNA-sequencing, and imputation from bulk RNA-sequencing), it is now well established that all subtypes of breast cancer show varying degrees of presence of both innate and adaptive immune cells. These cells have been shown to be involved in patient responses to not just immunotherapy, but also chemotherapy in both the adjuvant and neoadjuvant setting across all subtypes of breast cancer(47). However, the functional impact of these cells, the quality of this immune response, and prognostic value remains a matter of great debate and curiosity. Additionally, during the inflammatory reaction to the tumor, nearly all immune cell types can be recruited into the TIME, and across immune cell types, it generally holds true that infiltration and the number of cells follow a similar pattern: TNBCs > HER2+ tumors > ER+ Luminal B > ER+ Luminal A. Specifically, ER+ Luminal A tumors generally have poor infiltration but much better prognoses due to endocrine therapies. However, any tumor features that oscillate a tumor toward a more aggressive, “basal-like” state will typically illicit a stronger immune reaction.

Further contributions to the TIME include the surrounding stromal cells, including cancer-associated fibroblasts (CAFs), which can be active cytokine- and chemokine-secreters that can influence TIL infiltration and the balance of the local inflammatory reaction (48, 49). CAFs are typically believed to orchestrate tumor-promoting inflammation and modulate the TIME toward immunosuppression, functions that are mediated through intricate reciprocal signaling interactions with cancer cells, matrix components, and infiltrating immune cells. Tumors hijack the inherent repair mechanisms in fibroblasts as their natural state is to sense tissue damage and promote repair through inflammatory responses. CAFs may also recruit myeloid and regulatory T cells and promote M2 macrophage polarization and infiltration (48).

In the following sub-sections, we discuss a key component of the TIME – tumor infiltrating lymphocytes (TILs). While TILs are a broad term, and their importance and association with survival has led to contradictory results in various studies, recent efforts to reproducibly standardize TILs terminology and evaluation has been spearheaded by an international group

of oncologists and pathologists(50). The studies cited in the section below regarding the prognostic or predictive value of TILs were largely performed using these criteria, which includes technical considerations, how to perform calculations, what cell types are to be included, and inclusion/exclusion considerations. In some cases, TILs were assessed in the tumors themselves; in most cases, we report on stromal TILs, which is a distinct metric from those in the tumor. It should also be cautioned that to date, no phase III studies have identified TIL scoring to be associated with benefit for patients taking immunotherapies. While TILs themselves are likely quite important in the TIME, quantitative TIL scoring may have little immunobiological association, and further insight into TIL functional status and cellular composition may be more biologically meaningful.

TIME in TNBC

Of all the breast cancer subtypes, TNBC is the most extensively studied tumor type with respect to the role of immune system. This is partially due to the observation that the majority of TNBCs are highly immunogenic and harbor significantly higher stromal TILs, which are consistently shown to be associated with good prognosis(51–54). Recent analyses indicate that an association between stromal TILs and response to neoadjuvant chemotherapy is strongest in patients with TNBC(55). In this analysis, stromal TILs were quantified using H&E slides both as a continuous measurement as well as by predefined cutoffs (low 0%–10%, intermediate 11%–59%, and high 60%–100%)(55). Patients with TNBC had higher stromal TIL infiltration overall, and 10% increases in TILs were associated with both disease-free survival (DFS) and overall survival (OS)(55).

At the cellular level, TNBCs have significantly higher stromal and intra-tumoral CD8+ and CD4+ T cell infiltration compared to HR+ tumors. CD8+ T cells and tissue-resident memory CD8+ T cells (TRMs) are independently correlated with better prognosis (53, 56–59). Although CD4+ T cell quantities were not prognostic, they have been shown to correlate with CD68+ macrophages as well as B cells within the TIME. The TNBC TIME also shows significant presence of tumor infiltrating B cells (TIL-Bs) by IHC and B cell associated metagene signatures from single-cell RNA-sequencing and from bulk RNA-sequencing(60), which are positively associated with both improved OS and DFS (referring to general prognosis as this was analyzed retrospectively for patients included in the BIG 02–98 trial) (61, 62). B cells together with CD4+ follicular helper T cells have in fact been shown across multiple TNBC genetically engineered mouse models (GEMMs) to mediate response to immune checkpoint blockade therapy (63). The presence of peritumoral tertiary lymphoid structures (TLS), which are ectopic lymph node-like aggregates of CD4+ T cells and B cells that can be identified by multiplexed immunofluorescence, with clonal expansion of B cells and associated chemokine CXCL13 expression, demonstrate the potential for ongoing humoral immune responses at the tumor site, stroma (including those mediated by CAFs), and sentinel lymph nodes in human disease and mouse models of TNBC (61, 64–67). This might be particularly interesting in the context of potentiating the anti-tumor immune response beyond the current T cell-targeting approaches. Recent reports that utilize single-cell and spatial mapping technologies, such as CITE-seq, largely corroborate findings from de-convolution of bulk sequencing, IHC, and immunofluorescence. By single-cell sequencing and spatial mapping, enrichment of TILs and CD8+ T cells in TNBC was

observed, and these T cells exhibited substantially higher dysfunction scores in patients with TNBC(15).

As a countermeasure for this immune activation, patients with TNBC have been shown to harbor increased number of regulatory T cells (Tregs), myeloid derived suppressor cells (MDSCs), and tumor associated macrophages (TAMs) in the TIME as well as in systemic circulation(68–73). Neutrophils, typically assessed by a trained breast pathologist from H&E stained tumor sections, which can play both pro- and anti-tumor roles have been observed to be enriched in TNBC tumors compared to HR+ and HER2+ tumors and are found to be associated with disease progression(74). Mechanistically, this effect is attributed to the support of metastatic niche formation by neutrophils and other myeloid cells and their associated cytokines and chemokines like CCL9 and Prokineticin-2, as observed in murine model (4T1) and in vitro cell line models (MDA-MB-231) of TNBC(75–77). Paradoxically, despite being immunosuppressive and pro-tumorigenic, Tregs when considered as a single variable, are associated with better outcome in TNBC. This surprising correlation may be explained by the finding that elevated Tregs are also associated with a high CD8+ T cell:Treg ratio suggesting that the cytotoxic, anti-tumor CD8+ T cell effect is still predominant over the regulatory effect of the Tregs (69, 78, 79).

Complementing and contributing to the immune milieu and function is the local CAF population. The crosstalk between the immune cells and CAFs has also been most studied in the context of TNBC(80). In addition to their roles in driving pro-tumorigenic inflammation, CAFs may also affect MHC II presentation and immune regulation along with ECM remodeling(48). Four primary CAF subsets have recently been identified in each of the subtypes of breast cancer using multiple methods, including multicolor flow cytometry, immunohistochemical verification, and RNA-sequencing(81). These four CAF populations were distinguishable using six markers: CD29, FAP, αSMA, PDGFRβ, FSP1, and CAV1. Interestingly, in TNBC both the CAF-S1 (characterized by high expression of all markers except for CAV1) and CAF-S4 (characterized by the highest levelsof CD29 expression and αSMA) subsets were identified. If the TNBC TIME was enriched for CAF-S1 fibroblasts, an inflammatory myofibroblast subset, the balance between Tregs and CD8+ T cells was tipped in favor of increased Tregs, leading to a highly immunosuppressive locale. Recruitment of Tregs and differentiation of CD4+ T cells to Tregs was in part mediated by the chemokine CXCL12. On the other hand, some TNBC TIMEs were enriched for the CAF-S4 subset, which was associated with more CD8+ T cells.

Overall, in TNBC, there is a high degree of infiltration of T cells (including CD8+, CD4+, and Tregs) compared to other subtypes. Despite this, only a small proportion of TNBCs respond favorably to current T cell-targeted immunotherapies. A sizable proportion of TNBCs seem to harbor an immunosuppressive TIME characterized dysfunctional CD8+ T cells, Treg cells, and CAF-S1 fibroblasts, suggesting potential synergistic opportunities to boost anti-PD-1/PD-L1 agents(82).

TIME in HER2+ tumors

The HER2+ TIME is more immunogenic than HR+ TIME and similarly, ER−/HER2+ tumors show greater infiltration of TILs compared to ER+/HER2+ when evaluated

immunohistochemically(51, 83, 84). There is a growing body of evidence supporting the idea of intrinsic immunogenicity of HER2+ tumors and a role for adaptive immune responses mediated by T and B lymphocytes in these tumors(24). However, compared to TNBCs, HER2+ tumors seem to be less likely to exhibit high TIL levels(24).

In the HER2+ TIME, the protein HER2 itself serves as a unique target that has been shown to be recognized by T cells and B cells and is amenable to immunotherapeutic intervention(85). Increased CD8+ T cells including CD8+ TRMs and higher immune cell expression of interferon gamma (IFNγ) have been correlated with better prognosis, improved pathological complete responses (pCR) with therapy, and significantly increased OS in HER2+ disease after analysis of sTILs in the GeparQuattro and GeparQuinto trials(51, 86–91). Similarly, Tbet+ (hallmark transcription factor that controls the expression of IFNγ) type 1 CD4+ T cells as well as HER2-specific CD4+ T cells in the peripheral circulation of patients with HER2+ disease were also indicative of better outcome(92). Abundance of tumor infiltrating B cells (TIL-Bs) in HER2+ TIME was demonstrated by IHC as well as using *in silico* B cell-gene signature analysis, both associating with better DFS (60, 62, 93). From the gene signature analysis, more B cell receptor (BCR) chain variations were found in HER2+ tumors indicative of somatic hypermutation and suggesting a role for a productive antibody response in anti-tumor immunity(62). Although not significantly associated with outcome, increased number of TLSs have also been observed in HER2+ TIME via IHC(83). HER2+ tumors respond well to neoadjuvant chemotherapy (NAC) and there is evidence for cooperative anti-tumor immunity in patients treated with NAC. In that setting, patients with HER2+ breast cancer who achieved pCR harbored increased NK cells, B cells, and Th17 CD4+ T cell frequency, assessed by multispectral flow cytometry, as well as polyfunctional CD8+ T cells capable of producing IFNγ upon stimulation *in vitro*(88, 94, 95). Additionally, iNOS+ CD68+ M1-like macrophages and increased CD8+ effectors as a combined factor were significantly associated with improved survival suggesting the importance of a multiparametric approach to understanding the impact of various immune cells (96).

HER2-targeted therapies also rely on interactions with the immune system to enhance efficacy. Interestingly, through an immune gene signature analysis on a set of HER2+ samples treated with the HER2-targeted monoclonal antibody trastuzumab, it was demonstrated that therapeutic benefit was correlated with immune gene enrichment suggesting that HER2-directed antibody benefit is at least in part immune-cell mediated(87, 97–99). One such mechanism of anti-HER2 therapy is now recognized to be antibody dependent cellular cytotoxicity (ADCC). In tumors with HER2 overexpression, the trastuzumab or pertuzumab therapeutic antibodies bind to HER2 receptors expressed on the surface of cancer cells. This binding facilitates NK cells recruitment via FcγR ligation, bringing NK cells into proximity to cancer cells, thereby allowing for a prolonged cytotoxic interaction(98). Beyond ADCC, a novel approach that combines the tumoricidal effects of chemotherapy with the benefit of targeted therapy has resulted in development of HER2-directed antibody-drug conjugates (ADCs) like T-DM1 (ado-trastuzumab emtansine) which contains trastuzumab conjugated with emtansine, a microtubule inhibitor. After binding the HER2 receptor, T-DM1 is internalized, degraded inside the cell, which in turn releases DM1. Preclinical studies have shown that T-DM1 enhances T cell infiltration and turns the

TIME into a T cell-inflamed phenotype(100). Recent investigations have now focused on the role of trastuzumab deruxtecan (T-DXd), which has been recently shown to be superior to T-DM1 in the metastatic setting (although associated with increased risk of interstitial lung disease and pneumonitis)(101).

While CAF characterization is less well-described in HER2+ tumors, CAF-S4 fibroblasts seem to be the most predominant type of CAF in the HER2+ TIME(81). Unlike their CAF-S1 counterparts, CAF-S4 fibroblasts were less able to attract FOXP3+ T cells and enhance their functioning to inhibit proliferation of effector T cells. While deeper subtyping has been studied for the CAF-S1 cells in the context of immunotherapy resistance, further investigation into the role of CAF-S4s is certainly warranted as recent evidence suggests that stromal cells may be a better predictor of HER2-directed therapy efficacy than looking at just the tumor epithelial component(102).

TIME in HR+ tumors

HR+ BC encompasses two distinct molecular subtypes (Luminal A and Luminal B). It is hypothesized that TIL levels and function are quite different in HR+ tumors. For example, in a recent analysis of stromal TILs, increased TILs in HR+ tumors were not linked to DFS and actually associated with decreased OS(55). These data suggest a different immunobiology for HR+ tumors with a different cellular composition(103).

Although limited, the existing data suggest that the immune infiltrate in the primary HR+ TIME is dominated by CD8+ and CD4+ T cells and tumor associated macrophages (TAMs) (104–108). Paradoxically, despite having anti-tumor effector functions, higher TILs have been shown to be associated with poor prognosis and worse breast cancer free survival in the ER+ setting(55, 105, 109, 110). In a single-cell RNA seq analysis of the tumor and immune ecosystem in breast cancers, more than half of ER− tumors but only 12% of ER+ tumors had more than 10% of T cells that express PD-1. When comparing Luminal A and Luminal B tumors, a higher proportion of Luminal B tumors had more than 10% of T cells expressing PD-1(16). Beyond T cells, in HR+ tumors, the immune cell type most associated with poor clinical outcome was increased presence of TAMs. Interestingly, when comparing the most immune-rich HR+ tumors against immune-rich TNBCs, M2-like macrophages were particularly increased in HR+ tumors with a corresponding enrichment of TGF-beta-related genes(111). However, recent evidence indicates that heterogeneity exists with respect to different macrophage populations within luminal breast cancer, and a recent study indicates that an enrichment of FOLR2+ macrophages associate with higher levels of CD8+ T cell infiltration and better overall survival(112). Recent developments indicate ER− tumors are enriched for PD-L1+ TAMs compared to ER+ tumors. Using the TCGA database, a different study found that M2-like, pro-tumor macrophage polarization markers were significantly upregulated in both Luminal A and Luminal B tumors compared to basal-like tumors(113). If validated further, it might offer insight into the cancer cell-intrinsic suppressive ability of luminal tumors and the observed lack of benefit from T cell infiltration as a result of presence of more immunosuppressive M2-like macrophages.

As in TNBC and HER2+ tumors, CAFs are believed to play an important role in tumor-immune-stromal interactions in ER+ tumors as well. Several studies have shown different

proportions and characteristics of the CAFs in ER+ tumors(48, 81, 114). In ER+ tumors, the predominant CAF is the CAF-S2, which is a less activated and less inflammation-promoting myofibroblastic CAF compared to the CAF-S2 and CAF-S4 subsets(81). Spatial pathological evidence also suggests that Luminal A tumors harbor the highest content of fibroblasts compared to other breast cancer subtypes, and using a novel spatial model for better characterizing the interactions between TILs and fibroblasts, the authors identified heterogeneity across an array of ER+/HER2− tumors(115). Interestingly, a poorer survival was observed for patients with high CAFs but low TILs, and a better survival rate was noted for patients with TIMEs characterized by spatial mixing of CAFs and TILs(115).

In addition to ER, other hormone receptors, such as the progesterone receptor (PR) and the androgen receptor (AR), may also have immunomodulatory properties in breast cancer(116). Several studies have suggested that the progesterone (P4)-PR signaling axis has immunosuppressive and immune-evasion properties that is mediated through the dampening of IFN-STAT1 signaling(117). Like E2, P4 also seems to suppress the immune system, which is beneficial during pregnancy and fetal development. However, this effect can be pathological and can contribute to increased tumorigenesis(118). This suggests that high levels of P4 in the TIME may lead to fewer TILs and decreased immune gene expression signatures, which could thus be a potential target to boost immunotherapy efficacy. In addition to PR, a significant number of breast tumors express AR independent of ER, PR, and HER2. However, the role of AR in ER+ breast cancer is currently controversial, and these findings have prompted clinical trials for both AR agonist and AR antagonists. Recent findings shed light on this question, suggesting AR is a tumor suppressor in ER+ breast cancer, including endocrine-resistant tumors, supporting a role for AR agonism(119). Additional work is warranted to investigate AR interactions with the immune system in this setting, but new work in the setting of castration-resistant prostate cancer showed that AR downregulation in intratumoral T cells from patients who responded to anti-PD-L1 therapy(120, 121). In patients who were resistant to anti-PD-L1 therapy, AR mediated suppression of genes, such as TNF, granzyme B, and IFNγ, that were essential to coordinate a potent anti-tumor response. These findings may necessitate a look at the role of ER expressed in TILs.

An additional layer of complexity in breast cancers is the presence of hormones, such as estradiol (E2), and hormone receptor signaling. Studies have also shown that immune cells, especially macrophages and MDSCs, express ERα and are impacted by estradiol in the HR+ TIME(122–124). E2 is historically known to be an anti-inflammatory molecule, which may in part explain poor immune infiltration, poor functioning, and pro-tumor immune cells in the TIME(125). Increased estradiol in the breast TIME has been shown to enhance polarization of monocytes into M2-like macrophages, differentiation of CD4+ T cells into Tregs, and increased infiltration and suppression by Tregs and MDSCs making the TIME highly immunosuppressive for effective anti-tumor immunity(126–129). This also suggests that hormone receptor signaling and the presence of hormones in the TIME shapes not only tumor cell phenotypes but also has strong effects on immune infiltrates. Given that ER+ breast cancer is an age-related disease and the peak incidence of ER+ tumors occurs around the age of 70, further research is also warranted to characterize how chronic age-related inflammation may impact TIL proportion and functioning(130).

Taken together, the TIME varies markedly between the three major molecular breast cancer subtypes (131) (Figure 2B). In TNBC and HER2+ tumors, evidence supports enrichment of TILs, which is prognostic with respect to DFS and OS(45). In HR+ tumors, evidence is controversial for the prognostic role of TILs, but it is clear that these tumors harbor lower levels overall of TILs. Additionally, key features of TNBC and HER2+ tumors compared to HR+ tumors include increased TLSs, increased immunoediting, increased numbers of PD-L1+ TAMs, and a higher proportion of CAF-S1 (immunosuppressive; predominantly in some TNBCs) and CAF-S4 (primarily immunostimulatory; observed in both TNBC and HER2+ tumors) cells. HR+ tumors accumulate higher amounts of M2-like macrophages, which seem to drive a more immunosuppressed TIME, and the spatial mixing of CAFs and TILs seems to be particularly important for these tumors. Despite similarities between TNBC and HER2+ tumors compared to HR+, NK cell engagement and enhancement may be particularly promising for HER2+ tumors given the use of trastuzumab and may be a target to further enhance ADCC action. Additional study of the immunosuppressive nature of macrophages, the role of E2 in the TIME, and the interplay between age-related chronic inflammation and TIL functioning may also facilitate additional therapeutic opportunities. These data should be used to guide new subtype-specific, immune-targeting therapies.

Influence of Histology on the TIME

The two main histological subtypes of breast cancer, IDC and ILC, are also associated with differences in immune state within their respective TIME. Overall, ILCs represent 10%–15% of all breast cancer diagnoses; the distinguishing hallmark of these tumors is the loss of *CDH1*, which leads to cancer cell discohesion and the characteristic single-file infiltration of cancer cells within stroma(132, 133). The vast majority of these tumors are HR+ and fall into the Luminal A PAM50 subtype; distinct from patients with IDC, patients with ILC are more often diagnosed at older ages, typically have larger, multifocal tumors, and may have a unique propensity for late tumor recurrences (Figure 1). It is hypothesized that the spatial architecture of these tumors and influence of the stroma (surrounding non-tumor cells and extracellular matrix) also lead to differences in immune infiltration, functionality, and response. Given that the vast majority of ILCs are considered HR+, we will discuss differences between HR+ ILC and HR+ IDC in this section.

One of the most comprehensive studies to date analyzing immune cell infiltration in HR+ BC, accounting for over 750 samples each for ER+ ILC and IDC that assessed TILs using both IHC and deconvolution by CIBERSORT for tumors included in TCGA and METABRIC, showed that ER+ ILCs have statistically significantly lower total immune cell infiltrates compared to ER+ IDCs(105). Importantly, this study also analyzed the relative distribution of CD3+ T cells, CD8+ T cells, CD68+ macrophages, CD20+ B cells and FOXP3+ Tregs within intra-epithelial, adjacent stromal, and distant stromal compartments and found that ILC had lower levels in all measures of CD3+, CD8+, and CD68+ cells compared to IDC. Thus, as it stands now, ILCs are believed to harbor fewer immune cells overall than IDCs. Along this line, a recent study from our group suggests that while 86% of IDCs harbor higher levels of total immune cells in the TIME compared to normal breast tissue from mammoplasty, only 52% of ILCs harbor this increase (Onkar S et al, in revision).

Despite the low overall infiltration, there are perhaps subsets of ILC that do elicit a strong immune response; it is not known what proportion of ILCs fall into this category. An “immune-related” (IR) subtype within ER+ ILC was discovered by *in-silico* genomic and proteomic approaches on three large cohorts of samples: The Cancer Genome Atlas (TCGA), METABRIC, and Rational Therapy of Breast Cancer (RATHER) consortium(134, 135). This immune-related subtype showed higher reads in markers for CD4+ and CD8+ T cells along with increased expression of T cell inhibitory markers including PD-1, PD-L1, and CTLA-4 compared to the other ILC genomically-identified subtypes. There is limited evidence suggesting that CD8+ T cell infiltration and PD-L1 positivity of tumor and immune cells is correlated in ER+ ILC but not in IDC(136). As a step towards identifying patients who may benefit from immune-targeted agents, a recent large study focusing on 459 ILCs found that stromal TILs assessed by IHC were associated with younger age, larger tumors, lymph node involvement and HER2 amplification(137). A corroborating study also showed similar findings, with younger age, lymph node involvement, and highly proliferative tumors (such as those with pleomorphic features) showing more TIL involvement(105). Recent studies have also shown that there are subsets of Luminal ILCs that harbor enriched immune cell infiltration and high immune checkpoint gene expression(138). For these cases, *CD274*, *PDCD1*, and *CTLA4* in high immune ILCs were comparable to levels seen with basal-like and HER2-E tumors. These enriched immune cases were largely due to increased dendritic and natural killer cell infiltration, identified by immune gene signatures, and less driven by T cell differences between ILC and IDC. In total, these findings suggest that the negative association of TILs with ILC may reflect the fact that TILs are found in the more aggressive ILC subtypes but are largely absent in classical ILCs.

In our recent study that utilized fresh, treatment-naïve tumors to examine differences between HR+ IDC and ILC, M2-like macrophages were found to be a key cell type enriched within the TIME of ILCs (Onkar S et al, in revision). This enrichment was seen both in the tumor and surrounding stromal regions. Functional analysis suggested that ILC tumor cells specifically could be driving macrophages towards the M2 phenotype. Cellular neighborhood analysis using data from mIHC revealed the importance of a tissue architecture-wide approach in understanding differences between ILC and IDC, as specific cell type and neighborhood frequencies, specifically the interaction between T cells and macrophages, showed differential association with outcome in the two histological subtypes.

With the histological differences between ILC and IDC, ILCs typically have a more prominent stromal (non-epithelial) component than IDCs(139), and studies suggest that the loss of *CDH1* drives an entirely different TIME structure with changes to associated infiltrative immune cells(140, 141). This may provide one reason why ILCs exhibit fewer infiltrating immune cells, as some believe the stromal component provides a physical barrier that is more difficult for immune cells to penetrate through(142). ILCs can be additionally categorized into a “reactive” breast cancer subtype, which is defined by a high intratumoral stromal component without many infiltrating immune cells (Figure 3). This stromal component is associated with good prognosis and high breast cancer-specific survival(143). Interestingly, given the unique interactions with the stroma in ILC, treatment targeting stromal contents such as *LOXL1* decreased tumor growth, invasion, and metastasis in preclinical studies(144). Additionally, in a recent analysis of ER+ ILC CAFs, a recent

analysis showed a unique transcriptome for ER+ ILC CAFs compared to ER+ IDC CAFs(145). The gene *PAPPA* was the most enriched gene in the ILC stroma compared to the tumor epithelium, and higher *PAPPA* was associated with increased IGF-1 bioavailability. Targeting this stromal or CAF compartment of ILCs, with recent evidence pointing to *LOXL1* or the IGF pathway, may also be a therapeutic opportunities. Further investigation into the spatial architecture of ILCs and how this leads to fewer immune cells but a better prognosis is certainly warranted.

While important albeit small subsets of these tumors exhibit higher immune infiltration, these results could be explained by the finding that a very small fraction (about 5%) of primary ER+ IDC and ILC tumors display high (> 10mut/Mb) TMB and potential neoantigen load with ER+ IDC > ER+ ILC (146, 147). Interestingly there are new reports suggesting that metastatic HR+ disease and those that harbor ER mutations specifically display higher TMB (ILC > IDC) and are better infiltrated than the primary disease(148, 149).

Overall, ILC have a distinct interaction with the immune system given the enrichment of stromal content (Figure 3). This may impart less immune cell infiltration compared to IDC but confers a better prognosis for HR+ ILC. Immune-targeting agents may be better geared toward the advanced setting for patients with ILC. We continue to advocate for additional and lobular-specific results from immunotherapy clinical trials to drive data for this group of patients(150).

Metastatic TIME

Since primary breast tumors are routinely resected and breast cancer mortality is nearly exclusively a consequence of metastatic disease, an additional point of consideration should be the distinct organs in which metastases occur. First, it is worth noting that intrinsic subtype switching may occur during metastatic spread, and this switching can impact tumor-immune cell interaction in the host organ. Basal-like TNBCs tend to be the most stable and remain basal-like in the metastatic site, while Luminal and HER2-E tumors seem to exhibit higher rates of switching. A recent analysis from the AURORA US Network, which used supervised learning of gene expression data transformed into a set of 740 signatures, showed that signatures of stromal cells, endothelial cells, and many adaptive immune cells are lower in metastatic sites as compared to in the paired primary tumors(21). A subtype-specific approach revealed that while basal-like/TNBCs had far less enrichment of T cells, B cells, NK cells, and antigen presentation in the metastases, ER+ tumors showed little changed with respect to these signatures in the metastases. Further analysis by site of metastasis showed that immune features were much lower in liver and brain metastases, but little was changed between primary tumors and associated lung metastases. This may have a profound impact on those who receive immunotherapy in the metastatic setting, and initial clinical observations from other primary tumor types confirm these findings of immunosuppression in the liver(151).

Promising strategies for new immune-modulating therapies

Given the heterogeneity of the tumor and the associated TIME in ER+, HER2+ and TNBC, it is not surprising that the immune response itself is also quite diverse with some unique areas of interest in each molecular and histologic subtype. Increasing insight from preclinical and clinical research focusing on each subtype has confirmed that breast cancer is in fact a group of different tumor types that require distinctive approaches for optimal treatment. Given the wealth of clinical trial data over the past five years, only modest benefit has been seen from T cell-targeted agents such as pembrolizumab and atezolizumab. Even clearer is the fact that benefit from anti-PD-1 or anti-PD-L1 monotherapy is minimal; thus, approaches that harness other aspects of the immune response and in tumor subtype-specific settings will likely be necessary. In the subsequent section, we describe a primer of some emerging approaches that provide opportunities for future immune-targeted therapies guided by the aforementioned observations.

Combination therapies with immune checkpoint inhibitors (ICI)

While monotherapy with ICI has been limited, many trials are now sprouting that leverage a series of combination therapies to boost ICI efficacy. At the top of the list is conventional cytotoxic agents and radiation therapy. Immunostimulation with chemotherapeutics is thought to release immune-enhancing molecules from dying cancer cells(152). In this case, cytotoxic agents have been shown, mainly in the setting of lung tumors, to convert “cold” TIMEs that are poorly infiltrated into a “hot” TIME with an abundance of dendritic cells and CD8+ TILs(153, 154). Interestingly, in the recent TONIC trial, which tested a number of immune induction strategies, doxorubicin administration rendered the metastatic TIMEs more sensitize to anti-PD-1 therapy. The study further showed that following doxorubicin or cisplatin induction, there was enrichment of genes and gene signatures related to inflammation, JAK-STAT, and TNFα pathways(155). While immune induction or stimulation with cytotoxic agents may be beneficial for its systemic effects, local immunostimulation may be achieved with radiation therapy. Preclinical data from a poorly immunogenic mouse model of TNBC showed that radiation therapy induced new tumor mutations, and these new mutations rendered the tumor more immunogenic and recognizable to CD4+ and CD8+ T cells, allowing for more effective tumor control(156). Critically, stimulation of the immune system may also be mediated by DNA damage and local inflammation, which appears to upregulate the local interferon responses(157). Overall, whether pre-treatment of tumors with cytotoxic agents or radiation therapy or utilized at the same time at ICI, chemoimmunotherapy or radiation-containing ICI regimens hold potential for further exploration.

Adoptive cell therapies

In addition to rescuing dysfunctional T cells using ICI, adoptive T cell therapies are another avenue with some promising results in breast cancer patients having mainly been tested in the advanced setting. In a recent study, neoantigen-specific TILs were cultured and were used for treatment in combination with pembrolizumab (158). Interestingly, there was heterogeneity in tumor response by subtype, with a patient with ER+ cancer having the longest duration of response. Limitations of this approach include its time- and resource-

intensive nature as well as the limited number of patients who actually qualify for the therapy after screening for TILs recognizing immunogenic somatic mutations. For subtypes like HER2+ and TNBC with slightly higher mutational burden, generating a tumor-specific (c-Met or mesothelin) chimeric antigen receptor T (CAR-T) cell has opened the door to a newer area of immunotherapy for patients with advanced disease (159, 160). A case report describing complete and durable (ongoing) regression of chemo-refractory metastatic HR+ BC upon infusion of expanded autologous T cells (specific for somatic mutations in 4 genes) in conjunction with IL-2 and ICI represents an exciting and novel frontier for application of immunotherapeutic treatment for advanced HR+ tumors (161).

Dampening the immunosuppressive response

A real challenge to the efficacy of effector cells is the pro-tumor, immunosuppressive microenvironment and immune evasion within breast cancer. Many breast TIMEs (*ex vivo* and *in vivo* in mouse models) have already been shown to be rich in pro-tumor molecules that modulate immune suppression like IL-4, IL-6, IL-8, IL-10, IL-17, TGFβ, and IDO (162–166). These are generally higher in TNBC compared to HER2+ and HR+ breast cancer in different stages across primary and metastatic disease (167). As such, dissecting these underlying factors of resistance is an area of intense ongoing research. Some examples of potential strategies targeting resistance that are being tested include anti-TGFβRI and TGFβRII for tumor secreted TGFβ, anti-CSFR1 for TAMs, and HDAC inhibitors to decrease MDSC burden which could specifically be geared toward the HR+ TIME(103, 168). Interestingly, in a recent phase I trial testing fresolimumab, a TGFβ blocking antibody, patients receiving higher doses of the drug experienced longer median OS than the lower dose group(169). Both groups experienced favorable systemic immune responses.

Targeting TAMs and myeloid cells may be another strategy to dampen the pro-tumor TIME. As key pro-tumor cells, TAMs may function primarily by promoting extracellular matrix remodeling, angiogenesis, sustaining inflammation, and by acting as a hub for regulating anti-tumor adaptive immune responses(170). Emerging strategies to target TAMs include the use of anti-CD47 therapies and CSF-1R inhibitors. Anti-CD47 checkpoint immunotherapy inhibits the CD47 surface protein and messages macrophages to proceed with phagocytosis. Typically, tumor cells coat themselves with the CD47 molecules, which act as a “don’t eat me” signal to macrophages. While piloted in HER2+ disease(171), additional research is warranted in the ER+ setting given the enrichment of TAMs. CSF-1R inhibitors, such as pexidartinib, target monocyte homing to tumors prior to differentiation into TAMs(172, 173). Early responses in phase Ib clinical trials warrant further testing.

Beyond these strategies, further consideration of various immune checkpoint markers is also warranted. LAG3, for example, has been shown to be enriched in basal and HER2+ tumors and leads to poor survival(174). Given the recent FDA approval of a fixed dose combination of relatlimab (anti-LAG3) and nivolumab (anti-PD1) for non-resectable or metastatic melanoma(175), further study is warranted for its use in breast cancer.

Interestingly, across all subtypes, despite the higher tumor burden in the metastatic setting, the immune infiltration is significantly lower than matched primary tissues with the exception of macrophages which tend to increase in metastatic disease (166, 176). One study

reported that the magnitude of reduction in infiltration across T and B cells from primary to metastatic disease is more pronounced in TNBC compared to HR+ and HER2+ disease, a finding that needs to be validated further through cross-platform analyses (177). These key observations, although limited at this point, might have a significant impact on the design of all immunotherapy trials in advanced setting using only ICI which target just T cells but not pro-tumor macrophages, which might be more abundant in these advanced stages of disease. In order to significantly impact the outcome for patients with advanced disease, it would be prudent to include combination of immunotherapeutic agents that not only support immune activation or T-cell stimulation but also block the pro-tumor suppressor cells which form a bigger barrier to an effective immune response in the metastatic setting.

Targeting CAFs

Given the heterogeneity in the CAF subsets observed in breast cancer subtypes, targeting a specific subset (such as the immunosuppressive CAF-S1 group in TNBC) may be challenging. Prevailing hypotheses suggest targeting specific signaling molecules or pathways may be a more viable option(48). For example, most of the immunosuppressive CAFs in TNBC upregulate IL-6/IL-6R signaling, so that therapeutics, such as tocilizumab, an anti-IL6R antibody, may help to dampen the immunosuppressive TIME. ICIs have also shown limited benefit in tumors that have prominent stromal, which provides a physical barrier for immune cells. Targeting the extracellular matrix may also help to remodel this barrier and allow more immune infiltration(48). In total, targeting CAFs may be a tool in multi-pronged approach to revert an immunosuppressive TIME.

Metabolic vulnerabilities

Recent investigation into the metabolic subpopulations in breast cancer subtypes has yielded some insight into potential targets to boost anti-tumor responses. For example, in TNBC the MPS subtyping showed that particular tumors may be more sensitive to lactate dehydrogenase therapy in combination with anti-PD-1 agents(34). Another interesting avenue for consideration is the anti-CD73 agents, which target the CD73 ectonucleotidase, preventing downstream adenosine production into the TIME(178). Adenosine is thought to be pro-tumorigenic in the TIME as it promotes generation of Tregs and MDSCs and impairs functioning of T cells and NK cells(179). Recent studies in EGFR-mutated non-small cell lung cancer have shown that while neither anti-PD-L1 nor anti-CD73 therapy alone significantly inhibited a xenograft mouse tumor model, combination therapy not only prevented tumor growth but also increased the number of CD8+ TILs while enhancing secreted IFNγ and TNFα from these cells. With a number of ongoing clinical trials in breast cancer, this could be a promising link to inhibit the metabolic TIME(180, 181).

In summary, within the different breast cancer subsets, there is growing evidence that HER2+ and TNBC subsets will benefit most from targeting the effector arm of the immune system while HR+ TIME will require immunotherapeutic interventions that focus on antigen presentation and immune cell activation of existing T cells within the TIME. Additionally, all three TIMEs tend to be immunosuppressive to varying degrees and will benefit substantially from therapies that target Tregs (TNBC > HER2+ > HR+), MDSCs (TNBC > HER2+ > HR+) and TAMs (HR+ > TNBC / HER2+).

Conclusion

It has become clear that novel strategies to better manage breast cancer are essential. Immunotherapy is one such powerful weapon in our arsenal against cancer as demonstrated through its revolutionary impact on outcomes for a number of solid tumors. For the success of immunotherapeutic modulation, it is imperative that future studies are designed with an eye on the unique features for each of the subtype TIMEs. It is becoming increasingly clear that immunotherapy as monotherapy might not be optimal to treat any subtype of breast cancer. Multipronged, innovative approaches for targeting tumor and immune cells together might be the path forward for breast cancer. For further rational design of immunotherapy with maximal benefit, we must thoroughly characterize the TIME of the different molecular and histological breast cancer subtypes to understand and address a series of outstanding questions (Figure 4).

In conclusion, to aid the interrogation of the aspects of TIME stated above, recently developed sophisticated approaches for sequencing, imaging, and in vivo syngeneic animal studies that are currently under-utilized in subtype-specific breast cancer research must be leveraged. These include mIHC, spatial transcriptomics, multiparametric flow cytometry and single cell RNA sequencing. Lessons from such studies when translated to the clinic can yield exciting new opportunities to treat patients with breast cancer. It is conceivable that immunotherapy, when combined with other targeted agents specific for each breast cancer subtype, will develop into an indispensable therapeutic modality for enhancing patient care across all subtypes of breast cancer.

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REFERENCES

1. Harbeck NP-LF, Cortes J, Gnant M, Houssami N, Poortmans P et al. Breast Cancer. Nat Rev Dis Primers. 2019;5: 66. [PubMed: 31548545]
2. DeSantis CE MJ, Gaudet MM, Newman LA, Miller KD, Sauer G et al. Breast cancer statistics 2019. CA- Cancer J Clin. 2019;69:438–451. [PubMed: 31577379]
3. Malhotra GK ZX, Band H, Band V. Histological, molecular and functional subtypes of breast cancers. Cancer Biol ther. 2010;10:955–60. [PubMed: 21057215]
4. Sims AH HA, Howell SJ, Clarke RB. Origins of breast cancer subtypes and therapeutic implications. Nat Clin Pract Oncol. 2007;9:516–25.
5. Jackson HW, Fischer JR, Zanotelli VRT, Ali HR, Mechera R, Soysal SD, et al. The single-cell pathology landscape of breast cancer. Nature. 2020;578(7796):615–20. [PubMed: 31959985]

6. Wellenstein MD, and de Visser KE. Cancer-Cell-Intrinsic Mechanisms Shaping the Tumor Immune Landscape. Immunity. 2018;48(3):399–416. [PubMed: 29562192]
7. Esteva FJ, Hubbard-Lucey VM, Tang J, and Pusztai L. Immunotherapy and targeted therapy combinations in metastatic breast cancer. Lancet Oncol. 2019;20(3):e175–e86. [PubMed: 30842061]
8. Schmid P, Adams S, Rugo HS, Schneeweiss A, Barrios CH, Iwata H, et al. Atezolizumab and Nab-Paclitaxel in Advanced Triple-Negative Breast Cancer. N Engl J Med. 2018;379(22):2108–21. [PubMed: 30345906]
9. Schmid P, Cortes J, Pusztai L, McArthur H, Kümmel S, Bergh J, et al. Pembrolizumab for Early Triple-Negative Breast Cancer. N Engl J Med. 2020;382(9):810–21. [PubMed: 32101663]
10. Franzoi MA, Romano E, and Piccart M. Immunotherapy for early breast cancer: too soon, too superficial, or just right? Ann Oncol. 2021;32(3):323–36. [PubMed: 33307202]
11. Jiang YZ, Ma D, Suo C, Shi J, Xue M, Hu X, et al. Genomic and Transcriptomic Landscape of Triple-Negative Breast Cancers: Subtypes and Treatment Strategies. Cancer Cell. 2019;35(3):428–40.e5. [PubMed: 30853353]
12. Schettini F, and Prat A. Dissecting the biological heterogeneity of HER2-positive breast cancer. Breast. 2021;59:339–50. [PubMed: 34392185]
13. Comprehensive molecular portraits of human breast tumours. Nature. 2012;490(7418):61–70. [PubMed: 23000897]
14. Pernas S, and Tolaney SM. Targeting HER2 heterogeneity in early-stage breast cancer. Curr Opin Oncol. 2020;32(6):545–54. [PubMed: 32925204]
15. Wu SZ, Al-Eryani G, Roden DL, Junankar S, Harvey K, Andersson A, et al. A single-cell and spatially resolved atlas of human breast cancers. Nat Genet. 2021;53(9):1334–47. [PubMed: 34493872]
16. Wagner J, Rapsomaniki MA, Chevrier S, Anzeneder T, Langwieder C, Dykgers A, et al. A Single-Cell Atlas of the Tumor and Immune Ecosystem of Human Breast Cancer. Cell. 2019;177(5):1330–45.e18. [PubMed: 30982598]
17. Safonov A, Jiang T, Bianchini G, Győrffy B, Karn T, Hatzis C, et al. Immune Gene Expression Is Associated with Genomic Aberrations in Breast Cancer. Cancer Res. 2017;77(12):3317–24. [PubMed: 28428277]
18. Barroso-Sousa R, Jain E, Cohen O, Kim D, Buendia-Buendia J, Winer E, et al. Prevalence and mutational determinants of high tumor mutation burden in breast cancer. Ann Oncol. 2020;31(3):387–94. [PubMed: 32067680]
19. Thomas A, Routh ED, Pullikuth A, Jin G, Su J, Chou JW, et al. Tumor mutational burden is a determinant of immune-mediated survival in breast cancer. Oncoimmunology. 2018;7(10):e1490854. [PubMed: 30386679]
20. Sinn BV, Weber KE, Schmitt WD, Fasching PA, Symmans WF, Blohmer JU, et al. Human leucocyte antigen class I in hormone receptor-positive, HER2-negative breast cancer: association with response and survival after neoadjuvant chemotherapy. Breast Cancer Res. 2019;21(1):142. [PubMed: 31829264]
21. Perou C, Garcia-Recio S, Hinoue T, Wheeler G, Kelly B, Garrido-Castro A, et al. Multiplatform Analysis of Primary and Metastatic Breast Tumors from the AURORA US Network identifies microenvironment and epigenetics drivers of metastasis. Research Square; 2022. DOI: 10.21203/rs.3.rs-1221704/v1.
22. Lee HJ, Song IH, Park IA, Heo SH, Kim YA, Ahn JH, et al. Differential expression of major histocompatibility complex class I in subtypes of breast cancer is associated with estrogen receptor and interferon signaling. Oncotarget. 2016;7(21):30119–32. [PubMed: 27121061]
23. Kornepati AVR, Vadlamudi RK, and Curiel TJ. Programmed death ligand 1 signals in cancer cells. Nat Rev Cancer. 2022;22(3):174–89. [PubMed: 35031777]
24. Savas P, Salgado R, and Loi S. Seeing the forest and the tree: TILs and PD-L1 as immune biomarkers. Breast Cancer Res Treat. 2021;189(3):599–606. [PubMed: 34487294]
25. Matikas A, Zerdes I, Lövrot J, Richard F, Sotiriou C, Bergh J, et al. Prognostic Implications of PD-L1 Expression in Breast Cancer: Systematic Review and Meta-analysis of Immunohistochemistry

and Pooled Analysis of Transcriptomic Data. Clin Cancer Res. 2019;25(18):5717–26. [PubMed: 31227501]

26. Zhang M, Sun H, Zhao S, Wang Y, Pu H, Wang Y, et al. Expression of PD-L1 and prognosis in breast cancer: a meta-analysis. Oncotarget. 2017;8(19):31347–54. [PubMed: 28430626]
27. Noske A, Möbus V, Weber K, Schmatloch S, Weichert W, Köhne CH, et al. Relevance of tumour-infiltrating lymphocytes, PD-1 and PD-L1 in patients with high-risk, nodal-metastasised breast cancer of the German Adjuvant Intergroup Node-positive study. Eur J Cancer. 2019;114:76–88. [PubMed: 31075727]
28. Sobral-Leite M, Van de Vijver K, Michaut M, van der Linden R, Hooijer GKJ, Horlings HM, et al. Assessment of PD-L1 expression across breast cancer molecular subtypes, in relation to mutation rate, BRCA1-like status, tumor-infiltrating immune cells and survival. Oncoimmunology. 2018;7(12):e1509820. [PubMed: 30524905]
29. Mittendorf EA, Philips AV, Meric-Bernstam F, Qiao N, Wu Y, Harrington S, et al. PD-L1 expression in triple-negative breast cancer. Cancer Immunol Res. 2014;2(4):361–70. [PubMed: 24764583]
30. Mansour FA, Al-Mazrou A, Al-Mohanna F, Al-Alwan M, and Ghebeh H. PD-L1 is overexpressed on breast cancer stem cells through notch3/mTOR axis. Oncoimmunology. 2020;9(1):1729299. [PubMed: 32313717]
31. Castagnoli L, Cancila V, Cordoba-Romero SL, Faraci S, Talarico G, Belmonte B, et al. WNT signaling modulates PD-L1 expression in the stem cell compartment of triple-negative breast cancer. Oncogene. 2019;38(21):4047–60. [PubMed: 30705400]
32. Hsu JM, Xia W, Hsu YH, Chan LC, Yu WH, Cha JH, et al. STT3-dependent PD-L1 accumulation on cancer stem cells promotes immune evasion. Nat Commun. 2018;9(1):1908. [PubMed: 29765039]
33. Fukano M, Park M, and Deblois G. Metabolic Flexibility Is a Determinant of Breast Cancer Heterogeneity and Progression. Cancers (Basel). 2021;13(18).
34. Gong Y, Ji P, Yang YS, Xie S, Yu TJ, Xiao Y, et al. Metabolic-Pathway-Based Subtyping of Triple-Negative Breast Cancer Reveals Potential Therapeutic Targets. Cell Metab. 2021;33(1):51–64.e9. [PubMed: 33181091]
35. Naik A, and Decock J. Lactate Metabolism and Immune Modulation in Breast Cancer: A Focused Review on Triple Negative Breast Tumors. Front Oncol. 2020;10:598626. [PubMed: 33324565]
36. Xiao Y, Ma D, Yang YS, Yang F, Ding JH, Gong Y, et al. Comprehensive metabolomics expands precision medicine for triple-negative breast cancer. Cell Res. 2022;32(5):477–90. [PubMed: 35105939]
37. Allard B, Longhi MS, Robson SC, and Stagg J. The ectonucleotidases CD39 and CD73: Novel checkpoint inhibitor targets. Immunol Rev. 2017;276(1):121–44. [PubMed: 28258700]
38. Aymeric L, Apetoh L, Ghiringhelli F, Tesniere A, Martins I, Kroemer G, et al. Tumor cell death and ATP release prime dendritic cells and efficient anticancer immunity. Cancer Res. 2010;70(3):855–8. [PubMed: 20086177]
39. Mao C, Yeh S, Fu J, Porosnicu M, Thomas A, Kucera GL, et al. Delivery of an ectonucleotidase inhibitor with ROS-responsive nanoparticles overcomes adenosine-mediated cancer immunosuppression. Sci Transl Med. 2022;14(648):eabh1261. [PubMed: 35675434]
40. Kulkoyluoglu-Cotul E, Arca A, and Madak-Erdogan Z. Crosstalk between Estrogen Signaling and Breast Cancer Metabolism. Trends Endocrinol Metab. 2019;30(1):25–38. [PubMed: 30471920]
41. Yue M, Jiang J, Gao P, Liu H, and Qing G. Oncogenic MYC Activates a Feedforward Regulatory Loop Promoting Essential Amino Acid Metabolism and Tumorigenesis. Cell Rep. 2017;21(13):3819–32. [PubMed: 29281830]
42. Kos Z, Roblin E, Kim RS, Michiels S, Gallas BD, Chen W, et al. Pitfalls in assessing stromal tumor infiltrating lymphocytes (sTILs) in breast cancer. NPJ Breast Cancer. 2020;6:17. [PubMed: 32411819]
43. Gatti-Mays ME, Balko JM, Gameiro SR, Bear HD, Prabhakaran S, Fukui J, et al. If we build it they will come: targeting the immune response to breast cancer. NPJ Breast Cancer. 2019;5:37. [PubMed: 31700993]

44. Emens LA, Adams S, Cimino-Mathews A, Disis ML, Gatti-Mays ME, Ho AY, et al. Society for Immunotherapy of Cancer (SITC) clinical practice guideline on immunotherapy for the treatment of breast cancer. J Immunother Cancer. 2021;9(8).
45. Tarantino P, Corti C, Schmid P, Cortes J, Mittendorf EA, Rugo H, et al. Immunotherapy for early triple negative breast cancer: research agenda for the next decade. NPJ Breast Cancer. 2022;8(1):23. [PubMed: 35181659]
46. Jhunjhunwala S, Hammer C, and Delamarre L. Antigen presentation in cancer: insights into tumour immunogenicity and immune evasion. Nat Rev Cancer. 2021;21(5):298–312. [PubMed: 33750922]
47. DeNardo DG BD, Rexhepaj E, Ruffel B, Shiai SL, Madden SF et al. Leukocyte complexity predicts breast cancer survival and functionally regulates response to chemotherapy. Cancer Discov. 2011;1(1):54–67. [PubMed: 22039576]
48. Lavie D, Ben-Shmuel A, Erez N, and Scherz-Shouval R. Cancer-associated fibroblasts in the single-cell era. Nat Cancer. 2022;3(7):793–807. [PubMed: 35883004]
49. Sahai E, Astsaturov I, Cukierman E, DeNardo DG, Egeblad M, Evans RM, et al. A framework for advancing our understanding of cancer-associated fibroblasts. Nat Rev Cancer. 2020;20(3):174–86. [PubMed: 31980749]
50. Salgado R, Denkert C, Demaria S, Sirtaine N, Klauschen F, Pruneri G, et al. The evaluation of tumor-infiltrating lymphocytes (TILs) in breast cancer: recommendations by an International TILs Working Group 2014. Ann Oncol. 2015;26(2):259–71. [PubMed: 25214542]
51. Loi S SN, Piette F, Salgado R, Viale G, Van Eenoo F, Rouas G, Francis P, Crown JP, Hitre E, de Azambuja E, Quinaux E, Di Leo A, Michiels S, Piccart MJ, Sotiriou C. Prognostic and predictive value of tumor-infiltrating lymphocytes in a phase III randomized adjuvant breast cancer trial in node-positive breast cancer comparing the addition of docetaxel to doxorubicin with doxorubicin-based chemotherapy: BIG 02–98. J Clin Oncol. 2013;31(7);860–7. [PubMed: 23341518]
52. Adams S GR, Demaria S, Goldstein L, Perez EA, Shulman LN, Martino S, Wang M et al. Prognostic value of tumor-infiltrating lymphocytes in triple-negative breast cancers from two phase III randomized adjuvant breast cancer trials: ECOG 2197 and ECOG 1199. J Clin Oncol. 2014;32(27):2959–66. [PubMed: 25071121]
53. Sahar MA Mahmoud ECP, Powe Desmond G., Macmillan R. Douglas, Grainge Matthew J., Lee Andrew H.S., Ellis Ian O., Green Andrew R.. Tumor-Infiltrating CD8+ Lymphocytes Predict Clinical Outcome in Breast Cancer. J Clin Oncol 29:1949–1955. 2011. [PubMed: 21483002]
54. Zhixian Liu ML, Jiang Zehang, Wang Xiaosheng. A Comprehensive Immunologic Portrait of Triple-Negative Breast Cancer. Translational Oncology. 2018;11(2):311–329. [PubMed: 29413765]
55. Denkert C, von Minckwitz G, Darb-Esfahani S, Lederer B, Heppner BI, Weber KE, et al. Tumour-infiltrating lymphocytes and prognosis in different subtypes of breast cancer: a pooled analysis of 3771 patients treated with neoadjuvant therapy. Lancet Oncol. 2018;19(1):40–50. [PubMed: 29233559]
56. SV J Evaluation of CD4+ T-cells and CD8+ T-cells in triple-negative invasive breast cancer. Indian J Pathol Microbiol. 2018;61:477–8. [PubMed: 30303132]
57. Ali HRGS, Blows FM, Provenzano E, Dawson SJ, Liu B, Hiller L, et al. PD-L1 protein expression in breast cancer is rare, enriched in basal-like tumours and associated with infiltrating lymphocytes. Ann Oncol. 2015;(7):1488–93. [PubMed: 25897014]
58. Loi SMS, Salgado R, Sirtaine N, Jose V, Denkert C1, Smyth MJ, Joensuu H, Sotiriou C. Tumor infiltrating lymphocytes are prognostic in triple negative breast cancer and predictive for trastuzumab benefit in early breast cancer: results from the FinHER trial. Ann Oncol 25(8); 1544–50. 2014. [PubMed: 24608200]
59. Byrne ASP, Sant S, Li R. Tissue-resident memory T cells in breast cancer control and immunotherapy responses. Nat Rev Clin Oncol. 2020;17, 341–348. [PubMed: 32112054]
60. Achim Rody TK, Liedtke Cornelia, Pusztai Lajos, Ruckhaeberle Eugen, Hanker Lars, Gaetje Regine et al. A clinically relevant gene signature in triple negative and basal-like breast cancer. Breast Cancer Res. 2011;12(5):R97.

61. Soizic Garaud LB, Solinas Cinzia, Gu-Trantien Chunyan, de Wind Alexandre, Van den Eynden Gert, Naveaux Celine et al. Tumor infiltrating B-cells signal functional humoral immune responses in breast cancer. JCI Insight. 2019;2(11), e91487.
62. Iglesia MD, Vincent BG, Parker JS, Hoadley K, Carey LA, Perou CM, & Serody JS. Prognostic B-Cell Signatures using mRNA-Seq in Patients with Subtype-Specific Breast and Ovarian Cancer. Clinical Cancer Research; 20(14), 3818–3829. 2014. [PubMed: 24916698]
63. Hollern DP XN, Thennavan A, Glodowski C, Garcia-Recio S, Mott KR. B cells and T Follicular Helper cells mediate response to checkpoint inhibitors in high mutation burden mouse models of breast Cancer. Cell. 2019;179(5): 1191–1206. [PubMed: 31730857]
64. Gu-Trantien C, Migliori E, Buisseret L, de Wind A, Brohée S, Garaud S, Willard-Gallo K CXCL13-producing TFH cells link immune suppression and adaptive memory in human breast cancer. JCI Insight, 2(11), e91487. 2017. [PubMed: 28570278]
65. Gu-Trantien C, Loi S, Garaud S, Equeter C, Libin M, de Wind A,Willard-Gallo K. CD4+ follicular helper T cell infiltration predicts breast cancer survival Journal of Clinical investigation 123(7), 2873–2892. 2013. [PubMed: 23778140]
66. Marcus Schmidt VW-E, Hengstler Jan G., Heimes Anne-Sophie, Almstedt Katrin, Gerhold-Ay Aslihan, Lebrecht Antje et al. Prognostic impact of CD4-positive T cell subsets in early breast cancer: a study based on the FinHer trial patient population. Breast Cancer Res. 2018;20(1):15. [PubMed: 29482642]
67. Singh MA-EG, Carswell S, Fergusson JM, Blackburn J, Barton K et al. High-throughput targeted long-read single cell sequencing reveals the clonal and transcriptional landscape of lymphocytes. Nat Commun. 2019;10(1):3120. [PubMed: 31311926]
68. Catharina Medrek FP, Jirström Karin and Leandersson Karin. The presence of tumor associated macrophages in tumor stroma as a prognostic marker for breast cancer patients. BMC Cancer 12:306. 2012. [PubMed: 22824040]
69. Plitas G, Konopacki C, Wu K, Bos PD, Morrow M, Putintseva EV, et al. Regulatory T Cells Exhibit Distinct Features in Human Breast Cancer. Immunity. 2016;45(5):1122–34. [PubMed: 27851913]
70. Halvorsen EC, Mahmoud SM, and Bennewith KL. Emerging roles of regulatory T cells in tumour progression and metastasis. Cancer Metastasis Rev. 2014;33(4):1025–41. [PubMed: 25359584]
71. Mahmoud SM LA, Paish EC, Macmillan RD, Ellis IO, Green AR. Tumour-infiltrating macrophages and clinical outcome in breast cancer. J Clin Pathology; 65(2): 159–63. 2011.
72. Mahmoud SM PE, Powe DG, Macmillan RD, Lee AH, Ellis IO, Green AR. An evaluation of the clinical significance of FOXP3+ infiltrating cells in human breast cancer. Breast Cancer Res Treat 127(1): 99–108. 2011. [PubMed: 20556505]
73. Sushil Kumar DWW, Samuel Nina, Blanco Mario Andres, Nayak Anupma, Alicea-Torres Kevin, Gluck Christian, Sinha Satrajit et al. J Clin Invest. 2018;128(11):5095–5109. [PubMed: 30295647]
74. Soto-Perez-de-Celis EC-GY, Leon-Rodriguez E, Gamboa-Dominguez A. Tumor associated neutrophils in breast cancer subtypes. Asian Pac J Cancer Prev. 2017;18(10): 2689–2694. [PubMed: 29072393]
75. Hagerling C GH, Salari K, Wang CY Immune effector monocyte- neutrophil cooperation induced by the primary tumor prevents metastatic progression of breast cancer. PNAS. 2019;116 (43): 21704–21714. [PubMed: 31591235]
76. Sasaki S BT, Muranaka H, Tanabe Y Involvement of prokineticin 2-expressing neutrophil infiltration in 5-Fluorouracil-induced aggravation of breast cancer metastasis to lung. Mol Cancer Ther. 2018;17 (7): 1515–1525. [PubMed: 29643149]
77. Snoderly HT BB, Bennewitz MF. Neutrophil extracellular traps in breast cancer and beyond: current perspectives on NET stimuli, thrombosis and metastasis, and clinical utility for diagnosis and treatment. Breast Cancer Res. 2019;21 (145).
78. Shuzhen Liu WDF, Samuel Leung, Dongxia Gao, Sherman Lau, Zuzana Kos, Torsten O Nielsen. Prognostic significance of FOXP3+ tumor-infiltrating lymphocytes in breast cancer depends on estrogen receptor and human epidermal growth factor receptor-2 expression status and concurrent cytotoxic T-cell infiltration. Breast Cancer Res. 2014;16, 432. [PubMed: 25193543]

79. Lin Zhang XIW, Ding Jianmin, Sun Qigang, Zhang Songlin. The Predictive and Prognostic Value of Foxp3+/CD25+ Regulatory T Cells and PD-L1 Expression in Triple Negative Breast Cancer. Ann Diagn Pathol. 2019;40:143–151. [PubMed: 31096176]
80. Soongsathitanon J, Jamjuntra P, Sumransub N, Yangngam S, De la Fuente M, Landskron G, et al. Crosstalk between Tumor-Infiltrating Immune Cells and Cancer-Associated Fibroblasts in Tumor Growth and Immunosuppression of Breast Cancer. J Immunol Res. 2021;2021:8840066. [PubMed: 34337083]
81. Costa A, Kieffer Y, Scholer-Dahirel A, Pelon F, Bourachot B, Cardon M, et al. Fibroblast Heterogeneity and Immunosuppressive Environment in Human Breast Cancer. Cancer Cell. 2018;33(3):463–79.e10. [PubMed: 29455927]
82. Monteran L, and Erez N. The Dark Side of Fibroblasts: Cancer-Associated Fibroblasts as Mediators of Immunosuppression in the Tumor Microenvironment. Front Immunol. 2019;10:1835. [PubMed: 31428105]
83. Hee Jin Lee JYK, In Ah Park, In Hye Song, Yu ong Han, Ahn Jin-Hee, Gong Gyungyub. Prognostic Significance of Tumor-Infiltrating Lymphocytes and the Tertiary Lymphoid Structures in HER2-Positive Breast Cancer Treated With Adjuvant Trastuzumab. Am J Clin Pathol. 2015;144:278–288. [PubMed: 26185313]
84. Papaioannou E SM, Melachrinou M, Tzoracoleftherakis E, Kalofonos H, Kourea E. A standardized evaluation method for FOXP3+ Tregs and CD8+ T-cells in breast carcinoma: Association with breast carcinoma subtypes, stage and prognosis. Anticancer Res. 2019;39 (3): 1217–1232. [PubMed: 30842152]
85. Banchereau JPA. Dendritic cells as therapeutic vaccines against cancer. Nat Rev Immunol. 2005;5(4):296–306. [PubMed: 15803149]
86. Ingold Heppner BUM, Denkert C, Pfitzner BM, Lederer B, Schmitt W, Eidtmann H et al. Tumor-Infiltrating Lymphocytes: A Predictive and Prognostic Biomarker in Neoadjuvant-Treated HER2-Positive Breast Cancer. Clin Cancer Res. 2016;(23):5747–5754. [PubMed: 27189162]
87. Perez EABK, Tenner KS, Thompson EA, Badve SS, Bailey H, Baehner FL. Association of Stromal Tumor-Infiltrating Lymphocytes With Recurrence-Free Survival in the N9831 Adjuvant Trial in Patients With Early-Stage HER2-Positive Breast Cancer. JAMA Oncol. 2016;2(1):56–64. [PubMed: 26469139]
88. Salgado RDC, Campbell C, Savas P, Nuciforo P, Aura C, de Azambuja E et al. Tumor-Infiltrating Lymphocytes and Associations With Pathological Complete Response and Event-Free Survival in HER2-Positive Early-Stage Breast Cancer Treated With Lapatinib and Trastuzumab: A Secondary Analysis of the NeoALTTO Trial. JAMA Oncol. 2015;1(4):448–54. [PubMed: 26181252]
89. Johan Staaf MR, Johan Vallon-Christersson, Jönsson Göran, Bendahl Pär-Ola, Arason Karolina HolmAdalgeir, Gunnarsson Haukur. Identification of Subtypes in Human Epidermal Growth Factor Receptor 2–Positive Breast Cancer Reveals a Gene Signature Prognostic of Outcome. Journal of Clinical Oncology. 2010;(28):1813–1820. [PubMed: 20231686]
90. Peter Savas BV, Ye Chengzhong, Salim Agus, Mintoff Christopher P.,, and Franco Caramia RS, Byrne David J., ……., Neeson Paul J. and Loi Sherene. Single-cell profiling of breast cancer T cells reveals a tissue-resident memory subset associated with improved prognosis. Nature Medicine Letters. 2018;24:986–93.
91. Kuznetsova M LJ, Shevchenko J, Silkov A, Maksyutov A, Sennikov S. Cytotoxic activity and memory T Cell subset distribution of in vitro-stimulated CD8+ T cells specific for HER2/neu epitopes. Front Immunol. 2019;10:1017. [PubMed: 31143180]
92. Stanton SE DM. Clinical significance of tumor-infiltrating lymphocytes in breast cancer. J Immunother Cancer. 2016;4:59. [PubMed: 27777769]
93. Mahmoud AHSL SMA, Paish EC, Macmillan RD, Ellis IO, Green AR. The prognostic significance of B lymphocytes in invasive carcinoma of the breast. Breast Cancer Res Treat 132(2): 545–553. 2012. [PubMed: 21671016]
94. Muraro E CE, Talamini R, Turchet E, Miolo G, Scalone S. Improved Natural Killer cell activity and retained anti-tumor CD8(+) T cell responses contribute to the induction of a pathological complete response in HER2-positive breast cancer patients undergoing neoadjuvant chemotherapy. J Transl Med. 2015;13:204. [PubMed: 26116238]

95. Ali HR PE, Dawson S-J, Blows FM, Liu B, Shah M et al. Association between CD8+ T-cell infiltration and breast cancer survival in 12,439 patients. Ann Oncol. 2014;25: 1536–1543. [PubMed: 24915873]
96. Tiia J. Honkanen AT, Karihtala Peeter, Mäkinen Markus, Väyrynen Juha P., Koivunen Jussi P.. Prognostic and predictive role of tumour-associated macrophages in HER2 positive breast cancer. Sci Rep 2019;(9)10961 [PubMed: 31358801]
97. Andre F DM, Dubsky P, Sotiriou C, Curigliano G, Denkert C, et al. Molecular pathways: involvement of immune pathways in the therapeutic response and outcome in breast cancer. Clin Cancer Res. 2013;19:28e33. [PubMed: 23258741]
98. Bianchini G GL. The immune system and response to HER2-targeted treatment in breast cancer. Lancet Oncol. 2014;15:58e68.
99. Pruneri G BG, Vingiani A. Biomarkers for the identification of recurrence in human epidermal growth factor receptor 2 positive breast cancer patients. Curr Opin Oncol. 2016;(6):476e83. [PubMed: 27649027]
100. Griguolo G, Pascual T, Dieci MV, Guarneri V, and Prat A. Interaction of host immunity with HER2-targeted treatment and tumor heterogeneity in HER2-positive breast cancer. J Immunother Cancer. 2019;7(1):90. [PubMed: 30922362]
101. Cortés J, Kim SB, Chung WP, Im SA, Park YH, Hegg R, et al. Trastuzumab Deruxtecan versus Trastuzumab Emtansine for Breast Cancer. N Engl J Med. 2022;386(12):1143–54. [PubMed: 35320644]
102. Janiszewska M, Stein S, Metzger Filho O, Eng J, Kingston NL, Harper NW, et al. The impact of tumor epithelial and microenvironmental heterogeneity on treatment responses in HER2+ breast cancer. JCI Insight. 2021;6(11).
103. Goldberg J, Pastorello RG, Vallius T, Davis J, Cui YX, Agudo J, et al. The Immunology of Hormone Receptor Positive Breast Cancer. Front Immunol. 2021;12:674192. [PubMed: 34135901]
104. Wagner J RM, Chevrier S, Anzeneder T, Langwieder C, Dykgers A et al. A Single-Cell Atlas of the Tumor and Immune Ecosystem of Human Breast Cancer. Cell. 2019;177(5): 1330–1345. [PubMed: 30982598]
105. Christine Desmedt RS, Fornili Marco, Pruneri Giancarlo, Van den Eynden Gert, Zoppoli Gabriele, Green Andrew R, Ellis Ian O, Rakha Emad A, Larsimont Denis, Biganzoli Elia and Sotiriou Christos. Immune Infiltration in Invasive Lobular Breast Cancer. J Natl Cancer Inst. 2018;110(7): 768–776. [PubMed: 29471435]
106. Woosung Chung HHE, Lee Hae-Ock, Lee Kyung-Min, Lee Han-Byoel, Kim Kyu-Tae, Ryu Han Suk et al. Single-cell RNA-seq enables comprehensive tumour and immune cell profiling in primary breast cancer. Nat Commun. 2017;8: 15081. [PubMed: 28474673]
107. Azizi ECA, Plitas G, Cornish AE, Konopacki C, Prabhakaran S, Nainys J, Wu K, Kiseliovas V, Setty M, Choi K, Fromme RM, Dao P, McKenney PT, Wasti RC, Kadaveru K, Mazutis L, Rudensky AY, Pe'er D. Single-Cell Map of Diverse Immune Phenotypes in the Breast Tumor Microenvironment. Cell 2018 Aug 23;. 2018;174(5):1293–1308.e36. [PubMed: 29961579]
108. Bense RD SC, Piccart-Gebhart MJ, Haanen JB, van Vugt MA, de Vries EG, et al. Relevance of tumor-infiltrating immune cell composition and functionality for disease outcome in breast cancer. J Natl Cancer Inst. 2017;109(1).
109. Chung YR KH, Jang MH, Park SY. Prognostic value of tumor infiltrating lymphocyte subsets in breast cancer depends on hormone receptor status. Breast Cancer Res Treat 161(3): 409–420. 2017. [PubMed: 27913931]
110. Sobral- Leite M SI, Opdam M, Kruger DT, Beelen KJ, van der Noort V et al. Cancer-immune interactions in ER-positive breast cancers: PI3K pathway alterations and tumor-infiltrating lymphocytes. Breast Cancer Res. 2019;21, 90. [PubMed: 31391067]
111. O'Meara T, Marczyk M, Qing T, Yaghoobi V, Blenman K, Cole K, et al. Immunological Differences Between Immune-Rich Estrogen Receptor-Positive and Immune-Rich Triple-Negative Breast Cancers. JCO Precis Oncol. 2020;4.

112. Nalio Ramos R, Missolo-Koussou Y, Gerber-Ferder Y, Bromley CP, Bugatti M, Núñez NG, et al. Tissue-resident FOLR2(+) macrophages associate with CD8(+) T cell infiltration in human breast cancer. Cell. 2022;185(7):1189–207.e25. [PubMed: 35325594]
113. Hachim MY TI, Hachim Y. Macrophage M2 polarization markers are downregulated in Basal compared to Luminal A and Luminal B Breast Cancer. The FASEB Journal. 2020;34: 1–1.
114. Ali HR, Jackson HW, Zanotelli VRT, Danenberg E, Fischer JR, Bardwell H, et al. Imaging mass cytometry and multiplatform genomics define the phenogenomic landscape of breast cancer. Nat Cancer. 2020;1(2):163–75. [PubMed: 35122013]
115. Nederlof I, Hajizadeh S, Sobhani F, Raza SEA, AbdulJabbar K, Harkes R, et al. Spatial interplay of lymphocytes and fibroblasts in estrogen receptor-positive HER2-negative breast cancer. NPJ Breast Cancer. 2022;8(1):56. [PubMed: 35484275]
116. Jahan N, Jones C, and Rahman RL. Androgen receptor expression in breast cancer: Implications on prognosis and treatment, a brief review. Mol Cell Endocrinol. 2021;531:111324. [PubMed: 34000352]
117. Goodman ML, Trinca GM, Walter KR, Papachristou EK, D'Santos CS, Li T, et al. Progesterone Receptor Attenuates STAT1-Mediated IFN Signaling in Breast Cancer. J Immunol. 2019;202(10):3076–86. [PubMed: 30936295]
118. Werner LR, Gibson KA, Goodman ML, Helm DE, Walter KR, Holloran SM, et al. Progesterone promotes immunomodulation and tumor development in the murine mammary gland. J Immunother Cancer. 2021;9(5).
119. Hickey TE, Selth LA, Chia KM, Laven-Law G, Milioli HH, Roden D, et al. The androgen receptor is a tumor suppressor in estrogen receptor-positive breast cancer. Nat Med. 2021;27(2):310–20. [PubMed: 33462444]
120. Guan X, Polesso F, Wang C, Sehrawat A, Hawkins RM, Murray SE, et al. Androgen receptor activity in T cells limits checkpoint blockade efficacy. Nature. 2022;606(7915):791–6. [PubMed: 35322234]
121. Pala L, De Pas T, and Conforti F. Boosting anticancer immunotherapy through androgen receptor blockade. Cancer Cell. 2022;40(5):455–7. [PubMed: 35537411]
122. Huang HZJ, Chen H, Li J, Zhang C, Jiang X et al. The immunomodulatory effect of endocrine therapy in breast cancer. J Exp Clin Cancer Res. 2021;40, 19. [PubMed: 33413549]
123. Segovia-Mendoza MM-MJ. Immune tumor microenvironment in breast cancer and the participation of estrogen and its receptors in cancer physiopathology. Front Immunol. 2019;10:348. [PubMed: 30881360]
124. Oner GAS, Canturk Z, Tjalma W, van Berckelaer C, Broeckx G et al. The immunologic aspects in hormone receptor positive breast cancer. Cancer Treat Res Commun. 2020;25:100207. [PubMed: 32896829]
125. Márquez-Garbán DC, Deng G, Comin-Anduix B, Garcia AJ, Xing Y, Chen HW, et al. Antiestrogens in combination with immune checkpoint inhibitors in breast cancer immunotherapy. J Steroid Biochem Mol Biol. 2019;193:105415. [PubMed: 31226312]
126. Tai P WJ, Jin H, Song X, Yan J, Kang Y, Zhao L, An X, Du X, Chen X, Wang S, Xia G, Wang B. Induction of regulatory T cells by physiological level estrogen. J Cell Physiol; 214(2):456–64. 2008. [PubMed: 17654501]
127. Polanczyk MJ1CB, Subramanian S, Afentoulis M, Vandenbark AA, Ziegler SF, Offner H Cutting edge: estrogen drives expansion of the CD4+CD25+ regulatory T cell compartment. J Immunol (2004) 173(4):2227–30 2004. [PubMed: 15294932]
128. Toniolo AFG, Tedesco S, Cappellari R, Vegeto E,Maggi A et al. Alternative activation of human macrophages is rescued by estrogen treatment in vitro and impaired by menopausal status. J Clin Endocrinol Metab. 2015;100(1):E50–8. [PubMed: 25303489]
129. Svoronos NP-PA, Allegrezza MJ, Rutkowski MR, Payne KK, Tesone AJ et al. Tumor cell–independent estrogen signaling drives disease progression through mobilization of myeloid-derived suppressor cells. Cancer Discov. 2017;7(1): 72–85. [PubMed: 27694385]
130. Carleton N, Nasrazadani A, Gade K, Beriwal S, Barry PN, Brufsky AM, et al. Personalising therapy for early-stage oestrogen receptor-positive breast cancer in older women. Lancet Healthy Longev. 2022;3(1):e54–e66. [PubMed: 35047868]

131. El Bairi K, Haynes HR, Blackley E, Fineberg S, Shear J, Turner S, et al. The tale of TILs in breast cancer: A report from The International Immuno-Oncology Biomarker Working Group. NPJ Breast Cancer. 2021;7(1):150. [PubMed: 34853355]

132. Sflomos G, Schipper K, Koorman T, Fitzpatrick A, Oesterreich S, Lee AV, et al. Atlas of Lobular Breast Cancer Models: Challenges and Strategic Directions. Cancers (Basel). 2021;13(21).

133. Mouabbi JA, Hassan A, Lim B, Hortobagyi GN, Tripathy D, and Layman RM. Invasive lobular carcinoma: an understudied emergent subtype of breast cancer. Breast Cancer Res Treat. 2022 Jun;193(2):253–264. doi: 10.1007/s10549-022-06572-w. Epub 2022 Mar 26. [PubMed: 35347549]

134. Michaut M, Chin S-F, Majewski I, Severson TM, Bismeijer T, de Koning L, Bernards R. Integration of genomic, transcriptomic and proteomic data identifies two biologically distinct subtypes of invasive lobular breast cancer Scientific Reports, 6, 18517. 2016. [PubMed: 26729235]

135. Ciriello G, Gatza ML, Beck AH, Wilkerson MD, Rhie SK, Pastore A Perou CM. Comprehensive molecular portraits of invasive lobular breast cancer. Cell, 163(2), 506–519. 2015. [PubMed: 26451490]

136. Thompson ED TJ, Asch-Kendrick RJ, Ogurtsova A, Xu H, Sharma R, Meeker A, Argani P, Emens LA, Cimino-Mathews A. PD-L1 expression and the immune microenvironment in primary invasive lobular carcinomas of the breast. Mod Pathology. 2017;30(11):1551–1560.

137. Tille J-CVA, Saint-Martin C, Djerroudi L, Furhmann L, Bidard FC et al. Tumor-infiltrating lymphocytes are associated with poor prognosis in invasive lobular breast carcinoma. Mod Pathol. 2020; 33(11):2198–2207. [PubMed: 32404955]

138. Du T, Zhu L, Levine KM, Tasdemir N, Lee AV, Vignali DAA, et al. Invasive lobular and ductal breast carcinoma differ in immune response, protein translation efficiency and metabolism. Sci Rep. 2018;8(1):7205. [PubMed: 29739984]

139. Dennison JB, Shahmoradgoli M, Liu W, Ju Z, Meric-Bernstam F, Perou CM, et al. High Intratumoral Stromal Content Defines Reactive Breast Cancer as a Low-risk Breast Cancer Subtype. Clin Cancer Res. 2016;22(20):5068–78. [PubMed: 27172895]

140. Chen F, Ding K, Priedigkeit N, Elangovan A, Levine KM, Carleton N, et al. Single-Cell Transcriptomic Heterogeneity in Invasive Ductal and Lobular Breast Cancer Cells. Cancer Res. 2021;81(2):268–81. [PubMed: 33148662]

141. Tasdemir N, Bossart EA, Li Z, Zhu L, Sikora MJ, Levine KM, et al. Comprehensive Phenotypic Characterization of Human Invasive Lobular Carcinoma Cell Lines in 2D and 3D Cultures. Cancer Res. 2018;78(21):6209–22. [PubMed: 30228172]

142. Danenberg E, Bardwell H, Zanotelli VRT, Provenzano E, Chin SF, Rueda OM, et al. Breast tumor microenvironment structures are associated with genomic features and clinical outcome. Nat Genet. 2022; 54:660–669. [PubMed: 35437329]

143. Forsare C, Vistrand S, Ehinger A, Lövgren K, Rydén L, Fernö M, et al. The Prognostic Role of Intratumoral Stromal Content in Lobular Breast Cancer. Cancers (Basel). 2022;14(4).

144. Sflomos G, Battista L, Aouad P, De Martino F, Scabia V, Stravodimou A, et al. Intraductal xenografts show lobular carcinoma cells rely on their own extracellular matrix and LOXL1. EMBO Mol Med. 2021;13(3):e13180. [PubMed: 33616307]

145. Gómez-Cuadrado L, Bullock E, Mabruk Z, Zhao H, Souleimanova M, Noer PR, et al. Characterisation of the Stromal Microenvironment in Lobular Breast Cancer. Cancers (Basel). 2022;14(4).

146. Alexandra Thomas EDR, Pullikuth Ashok, Jin Guangxu, Su Jing, Chou Jeff W.,Hoadley Katherine A. et al. . Tumor mutational burden is a determinant of immune-mediated survival in breast cancer. Oncoimmunology. 2018;7(10): e1490854. [PubMed: 30386679]

147. Barroso-Sousa RJE, Kim D, Partridge AH, Cohen O, Wagle N. Determinants of high tumor mutational burden (TMB) and mutational signatures in breast cancer. J Clin Oncol. 2018;36:15–6.

148. Sokol ESFY, Jin DX, Basudan A, Lee AV, Atkinson JM et al. Loss of function of NF1 is a mechanism of acquired resistance to endocrine therapy in lobular breast cancer. Ann Oncol. 2019;30(1):115–123. [PubMed: 30423024]

149. Williams MMSN, Arnesen S, O'Neill KI, Christenson JL, Reese J, Torkko KC et al. Steroid hormone receptor and infiltrating immune cell status reveals therapeutic vulnerabilities of ESR1-mutant breast cancer. Cancer Res. 2021;81(3): 732–746. [PubMed: 33184106]

150. Pate L, Desmedt C, Metzger O, Burgess Hutcheson L, Turner C, Freeney S, et al. How Researchers, Clinicians and Patient Advocates Can Accelerate Lobular Breast Cancer Research. Cancers (Basel). 2021;13(13).

151. Lee JC, Green MD, Huppert LA, Chow C, Pierce RH, and Daud AI. The Liver-Immunity Nexus and Cancer Immunotherapy. Clin Cancer Res. 2022;28(1):5–12. [PubMed: 34285059]

152. Galluzzi L, Humeau J, Buqué A, Zitvogel L, and Kroemer G. Immunostimulation with chemotherapy in the era of immune checkpoint inhibitors. Nat Rev Clin Oncol. 2020;17(12):725–41. [PubMed: 32760014]

153. Liu P, Zhao L, Pol J, Levesque S, Petrazzuolo A, Pfirschke C, et al. Crizotinib-induced immunogenic cell death in non-small cell lung cancer. Nat Commun. 2019;10(1):1486. [PubMed: 30940805]

154. Pfirschke C, Engblom C, Rickelt S, Cortez-Retamozo V, Garris C, Pucci F, et al. Immunogenic Chemotherapy Sensitizes Tumors to Checkpoint Blockade Therapy. Immunity. 2016;44(2):343–54. [PubMed: 26872698]

155. Voorwerk L, Slagter M, Horlings HM, Sikorska K, van de Vijver KK, de Maaker M, et al. Immune induction strategies in metastatic triple-negative breast cancer to enhance the sensitivity to PD-1 blockade: the TONIC trial. Nat Med. 2019;25(6):920–8. [PubMed: 31086347]

156. Lhuillier C, Rudqvist NP, Yamazaki T, Zhang T, Charpentier M, Galluzzi L, et al. Radiotherapy-exposed CD8+ and CD4+ neoantigens enhance tumor control. J Clin Invest. 2021;131(5).

157. McLaughlin M, Patin EC, Pedersen M, Wilkins A, Dillon MT, Melcher AA, et al. Inflammatory microenvironment remodelling by tumour cells after radiotherapy. Nat Rev Cancer. 2020;20(4):203–17. [PubMed: 32161398]

158. Zacharakis N, Huq LM, Seitter SJ, Kim SP, Gartner JJ, Sindiri S, et al. Breast Cancers Are Immunogenic: Immunologic Analyses and a Phase II Pilot Clinical Trial Using Mutation-Reactive Autologous Lymphocytes. J Clin Oncol. 2022:Jco2102170.

159. Gatti-Mays MEBJ, Gameiro SR, Bear HD, Prabhakaran S, Fukui J et al. If we build it they will come: targeting the immune response to breast cancer. NPJ Breast Cancer. 2019;5:37. [PubMed: 31700993]

160. Tchou JZY, Levine BL, Zhang PJ, Davis MM, Melenhorst JJ et al. Safety and efficacy of intratumoral injections of chimeric antigen receptor (CAR) T cells in metastatic breast cancer. Cancer Immunol Res. 2017;5(12):1152–1161. [PubMed: 29109077]

161. Zacharakis NCH, Black M, Xu H, Lu Y-C, Zheng Z et al. Immune recognition of somatic mutations leading to complete durable regression in metastatic breast cancer. Nat Med. 2018;24(6):724–730. [PubMed: 29867227]

162. Aspord CP-GA, Gallegos M, Tindle S, Burton EC, Su D et al. Breast cancer instructs dendritic cells to prime interleukin 13–secreting CD4+ T cells that facilitate tumor development. J Exp Med. 2007;204: 1037–1047. [PubMed: 17438063]

163. Carvajal-Hausdorf DEMN, Velcheti V, Schalper KA, Rimm DL. Objective measurement and clinical significance of IDO1 protein in hormone receptor-positive breast cancer. J Immunother Cancer. 2017;5:21. [PubMed: 28331613]

164. Coffelt SBKK, Doornebal CW, Weiden J, Vrijland K,Hau CS et al. IL-17-producing γδ T cells and neutrophils conspire to promote breast cancer metastasis. Nature. 2015;522, pages345–348.

165. Kawaguchi KSM, Yamamoto Y, Suzuki E, Tsuda M, Kataoka TR et al. Alteration of specific cytokine expression patterns in patients with breast cancer. Sci Rep. 2019;9: 2924. [PubMed: 30814616]

166. Zhu LNJ, Onkar S, Joy M, Broadwater G, Luedke C et al. Metastatic breast cancers have reduced immune cell recruitment but harbor increased macrophages relative to their matched primary tumors. J Immunotherapy Cancer. 2019;7: 265.

167. Liu ZLM, Jiang Z, Wang X. A Comprehensive Immunologic Portrait of Triple-Negative Breast Cancer. Translational Oncology. 2018;11(2):211–329.

168. Mehta AK, Kadel S, Townsend MG, Oliwa M, and Guerriero JL. Macrophage Biology and Mechanisms of Immune Suppression in Breast Cancer. Front Immunol. 2021;12:643771. [PubMed: 33968034]

169. Formenti SC, Lee P, Adams S, Goldberg JD, Li X, Xie MW, et al. Focal Irradiation and Systemic TGFβ Blockade in Metastatic Breast Cancer. Clin Cancer Res. 2018;24(11):2493–504. [PubMed: 29476019]

170. Pucci F, and Coussens LM. Redirecting tumor macrophage activity to fight cancer: Make room for the next era of anti-cancer drugs. Cancer Cell. 2021;39(10):1300–2. [PubMed: 34637745]

171. Upton R, Banuelos A, Feng D, Biswas T, Kao K, McKenna K, et al. Combining CD47 blockade with trastuzumab eliminates HER2-positive breast cancer cells and overcomes trastuzumab tolerance. Proc Natl Acad Sci U S A. 2021;118(29).

172. Wesolowski R, Sharma N, Reebel L, Rodal MB, Peck A, West BL, et al. Phase Ib study of the combination of pexidartinib (PLX3397), a CSF-1R inhibitor, and paclitaxel in patients with advanced solid tumors. Ther Adv Med Oncol. 2019;11:1758835919854238. [PubMed: 31258629]

173. Saung MT, Muth S, Ding D, Thomas DL 2nd, Blair AB, Tsujikawa T, et al. Targeting myeloid-inflamed tumor with anti-CSF-1R antibody expands CD137+ effector T-cells in the murine model of pancreatic cancer. J Immunother Cancer. 2018;6(1):118. [PubMed: 30424804]

174. Liu Q, Qi Y, Zhai J, Kong X, Wang X, Wang Z, et al. Molecular and Clinical Characterization of LAG3 in Breast Cancer Through 2994 Samples. Front Immunol. 2021;12:599207. [PubMed: 34267742]

175. Tawbi HA, Schadendorf D, Lipson EJ, Ascierto PA, Matamala L, Castillo Gutiérrez E, et al. Relatlimab and Nivolumab versus Nivolumab in Untreated Advanced Melanoma. N Engl J Med. 2022;386(1):24–34. [PubMed: 34986285]

176. Szekely B BV, Li X, Wali VB, Patwardhan GA, Frederick C et al. Immunological differences between primary and metastatic breast cancer. Ann Oncol. 2018;29(11): 2232–2239. [PubMed: 30203045]

177. Cimino-Mathews A YX, Meeker A, Argani P, Emens LA. Metastatic triple-negative breast cancers at first relapse have fewer tumor-infiltrating lymphocytes than their matched primary breast tumors: a pilot study. Hum Pathol. 2013;44(10):2055–2063. [PubMed: 23701942]

178. Chen S, Wainwright DA, Wu JD, Wan Y, Matei DE, Zhang Y, et al. CD73: an emerging checkpoint for cancer immunotherapy. Immunotherapy. 2019;11(11):983–97. [PubMed: 31223045]

179. Vigano S, Alatzoglou D, Irving M, Ménétrier-Caux C, Caux C, Romero P, et al. Targeting Adenosine in Cancer Immunotherapy to Enhance T-Cell Function. Front Immunol. 2019;10:925. [PubMed: 31244820]

180. Buisseret L, Pommey S, Allard B, Garaud S, Bergeron M, Cousineau I, et al. Clinical significance of CD73 in triple-negative breast cancer: multiplex analysis of a phase III clinical trial. Ann Oncol. 2018;29(4):1056–62. [PubMed: 29145561]

181. De Caluwé A, Buisseret L, Poortmans P, Van Gestel D, Salgado R, Sotiriou C, et al. Neo-CheckRay: radiation therapy and adenosine pathway blockade to increase benefit of immuno-chemotherapy in early stage luminal B breast cancer, a randomized phase II trial. BMC Cancer. 2021;21(1):899. [PubMed: 34362344]

SIGNIFICANCE

While there are currently over 200 ongoing clinical trials testing immunotherapeutics, such as immune checkpoint blockade agents, these are largely restricted to the triple negative and HER2+ subtypes and primarily focus on T cells. With the rapid expansion of new in vitro, in vivo, and clinical data, it is critical to identify and highlight the challenges and opportunities unique for each breast cancer subtype to drive the next generation of treatments that harness the immune system.

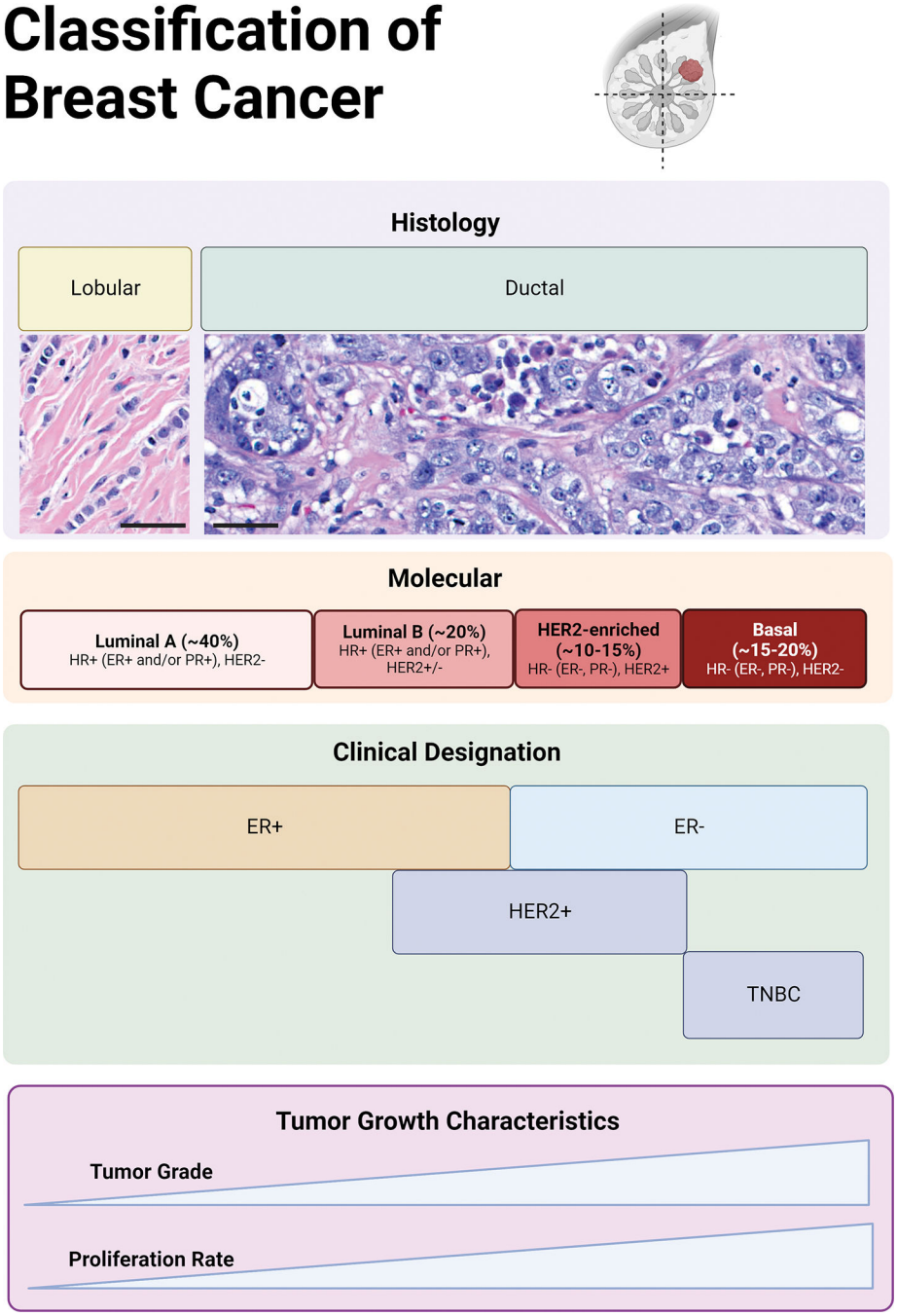

FIGURE 1:
Summary of histological, molecular, and clinical subtypes of breast cancer. Scale bars for the images of ILC and IDC indicate 50μm lengths.

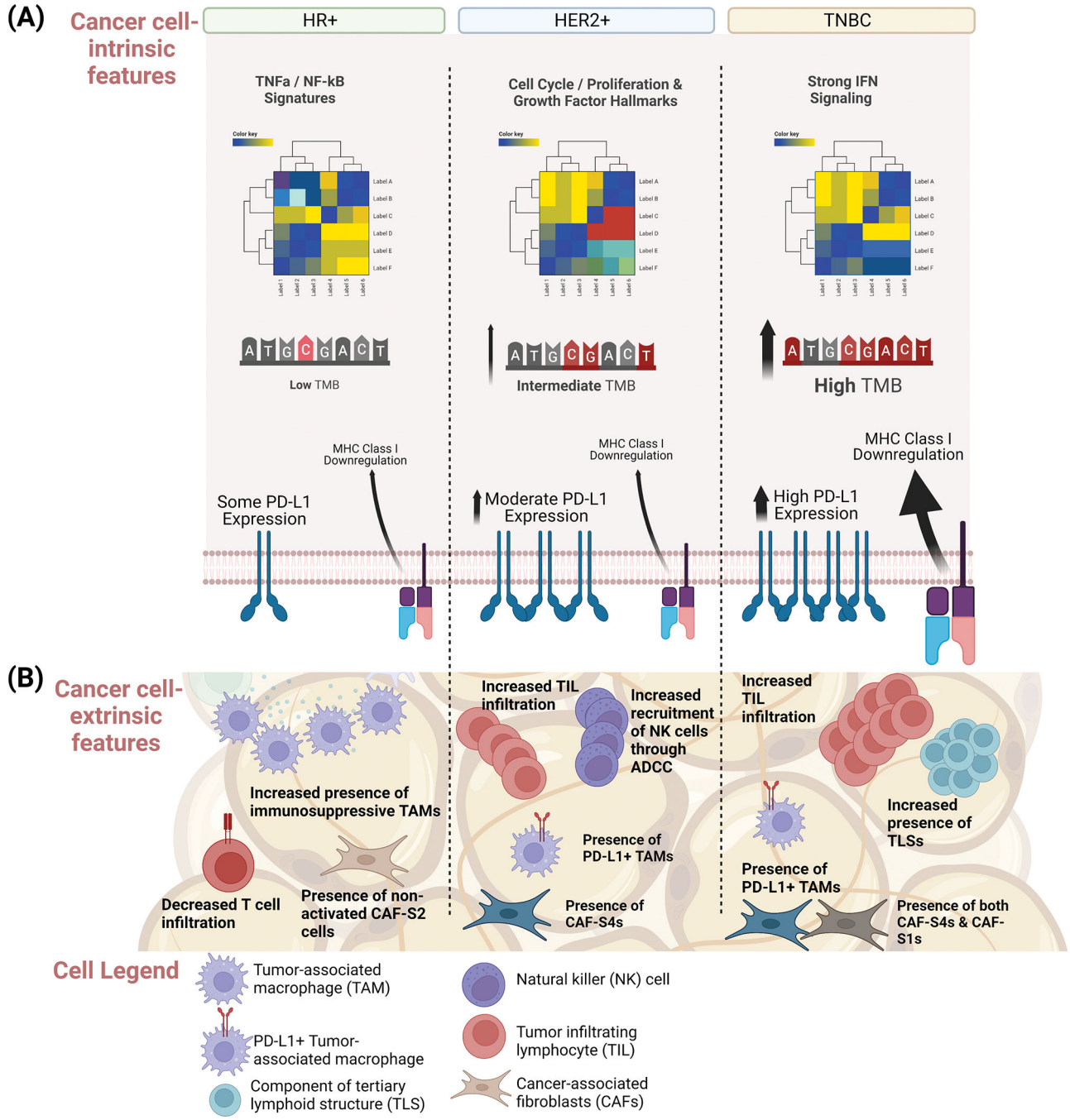

FIGURE 2:

(A) Comparison of unique tumor-intrinsic features of the breast cancer subtypes. Key differences in intrinsic genomically-upregulated pathways, tumor mutational burden (TMB), tumor PD-L1 expression, and MHC Class I downregulation can all contribute to different TIMEs for each breast cancer subtype. **(B) Comparison of unique cancer cell-extrinsic, immune cell-related features across the main breast cancer subtypes.** In HR+ breast cancer, there are generally increased proportions of tumor-associated macrophages (TAMs) that tend to be immunosuppressive along with decreased T cell infiltration and the presence

of non-activated CAF-S2 cells. In HER2+ breast cancer, there is increased TIL proportions compared to HR+ breast cancer as well as increased recruitment of NK cells and PD-L1+ TAMs. Lastly, in TNBC, data suggests the highest level of TILs compared to both HR+ and HER2+ breast cancer along with TLS presence and PD-L1+ TAMs. In TNBC, there are both CAF-S1 and CAF-S4 cells, which may help in steering the Treg:CD8+ T cell balance in the TIME.

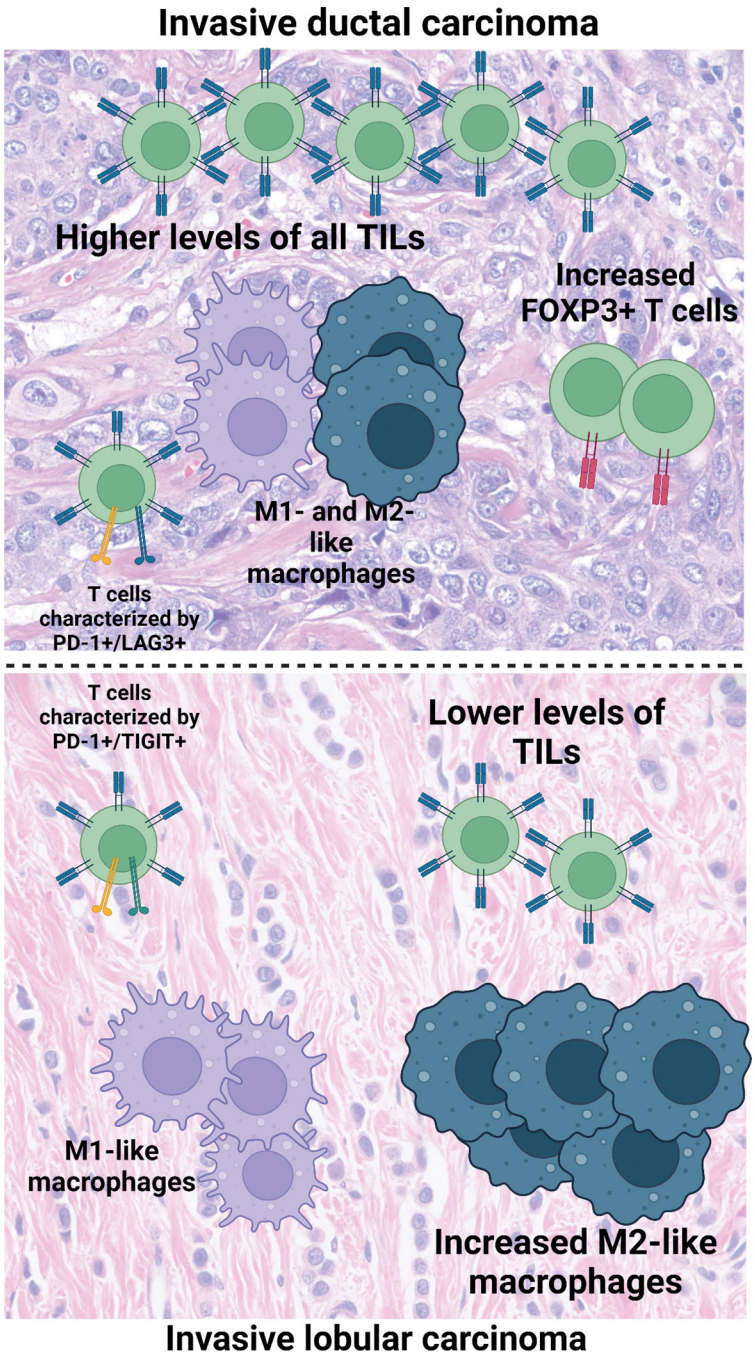

FIGURE 3: Key differences in the TIME between ER+ ILC and ER+ IDC.

In IDC, data suggests higher levels of all TILs, including FOXP3+ T cells. T cells in the TIME for IDCs are typically characterized by PD-1+ and LAG3+. Additionally, there are both M1-like and M2-like macrophages present. However, in ILCs, there tends to be increased levels of M2-like macrophages, lower levels of TILs, and T cells characterized by PD-1+ and TIGIT+ compared to IDCs.

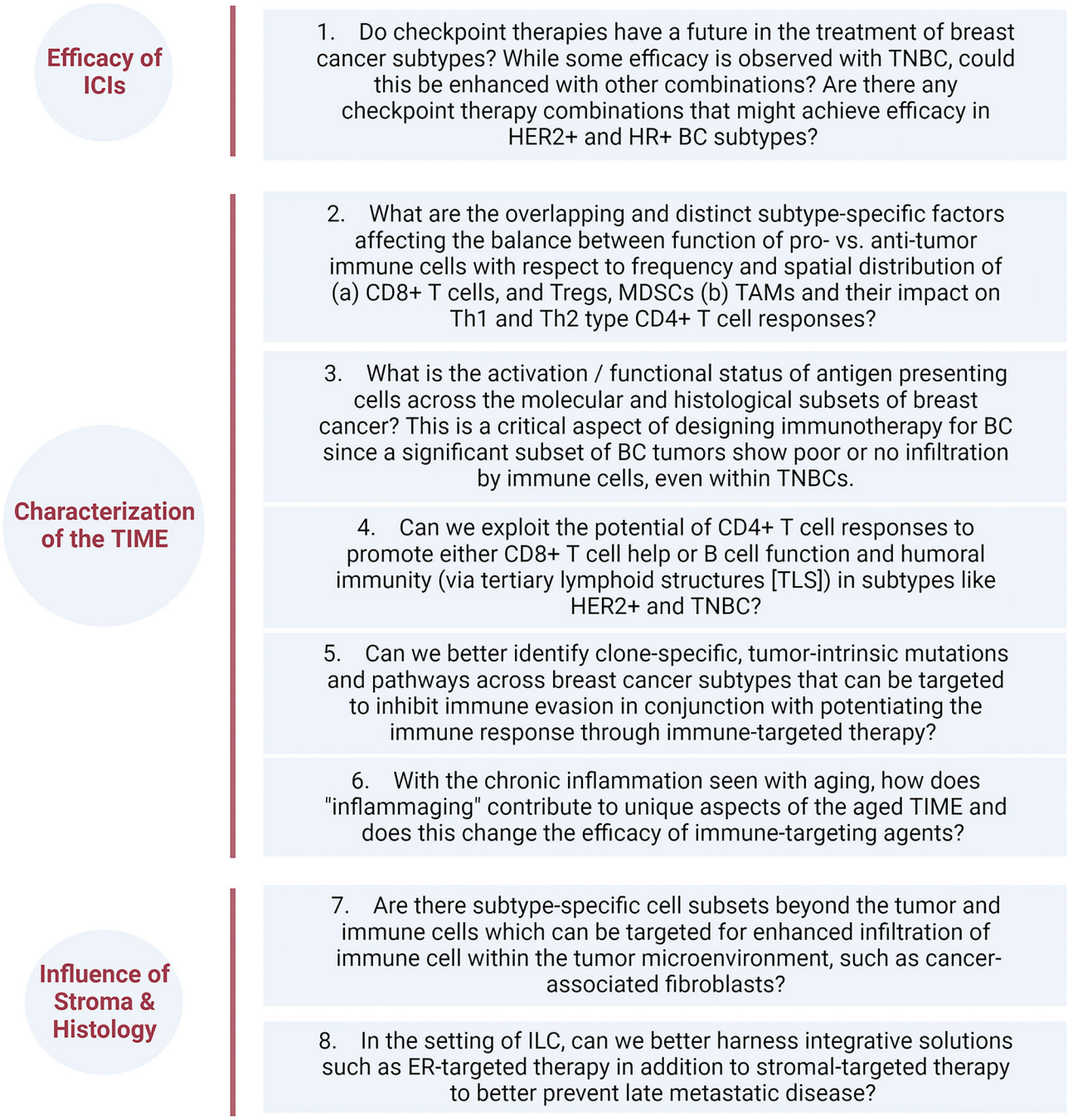

FIGURE 4:
Summary of key questions to guide the future of immune system investigation across breast cancer molecular and histological subtypes.