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# Biomarker Predictors for Immunotherapy Benefit in Breast: Beyond PD-L1

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## Abstract

**Purpose of Review** Immune checkpoint blockade (ICB) has changed the clinical course of multiple cancer types and durable responses have now been observed in breast cancer (BC) patients. Most data suggest that, compared to other subtypes, triple-negative BC (TNBC) patients are more responsive to ICB, and anti-PD-L1 therapy is now approved in PD-L1+ metastatic TNBC, in combination with chemotherapy.

**Recent Findings** Nearly 40% of PD-L1+ TNBC patients did not respond to this combination. Thus, additional biomarkers appear to be necessary to more precisely identify potential responders. A comprehensive analysis of the breast tumor microenvironment (TME) and peripheral blood may identify potential biomarkers for a more accurate selection of patients likely to respond to ICB.

**Summary** Herein, we summarize key features of the breast TME, and beyond, that may hold predictive power in determining immunotherapy benefit. Incorporation of these features in controlled clinical trials may help further guide personalized care for BC immunotherapy.

**Keywords** Breast cancer · Biomarkers · Programmed death-ligand 1

## Introduction

The PD-1/PD-L1 axis is a critical component of peripheral tolerance. This pathway is meant to serve as an 'off switch' for T cells following a successful adaptive immune response and suppress prolonged or chronic inflammation, limiting potential autoimmunity. However, tumors can evade T cell-mediated responses by expressing PD-L1 which, when engaged with PD-1 on T cells, disrupts effector T cell activity and promotes exhaustion [1]. Importantly, PD-L1 can be expressed on many cell types in the TME, including tumor cells, stromal cells, and immune cells (macrophages, dendritic cells, and rare lymphocytes) [2].

To date, PD-L1 expression on tumor-associated stromal cells is the only biomarker shown to be predictive of ICB benefit in phase III randomized controlled BC trials. However, lack of harmony in evaluating PD-L1 status could impact the utility of this biomarker. The Food and Drug Administration (FDA) has approved four unique PD-L1 antibody clones, including Dako 28-8, Dako 22C3, Ventana SP142, and Ventana SP263. In addition to the different PD-L1 epitopes recognized by these clones, various staining protocols and scoring systems have been used to evaluate PD-L1 positivity, which negatively impacts study-to-study comparisons. Since the results of the IMpassion130 trial were reported, multiple groups have sought to understand the differences in performance between these assays. Two groups recently found that the SP142 assay could erroneously identify PD-L1-positive tumors as PD-L1-negative, regardless of the percent cut-offs set for benefit assessment [3]. Oyan et al. found that compared to the SP263 and 22C3 assays, the SP142 assay underperformed at detecting PD-L1 expression on tumor cells

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[3]. More importantly, for ICB prediction in BC, another group that produced a similar result using a single pathologist also reported that the SP142 assay detects significantly fewer PD-L1-positive immune cells compared to all other assays [4•].

Despite the perception that the studies comparing anti-PD-L1 clones might clearly identify the superior PD-L1 detection assay, other experimental details must be carefully examined when interpreting the data. First, it must be considered that although the two studies mentioned above compared assays among the same tissues, intratumor heterogeneity can skew this comparison. In addition, formalin-fixed, paraffin-embedded tissues may have reduced sensitivity versus frozen tissues when using the same PD-L1 staining protocol [5, 6]. Thus, it is crucial that standards be set for evaluating PD-L1 positivity, as PD-L1 expression currently stands as the most widely accepted biomarker.

Much of the work addressing anti-PD-L1 safety and efficacy in BC has focused on PD-L1-positive patients. Early studies, such as the phase 1 trial conducted by Schmid et al. in 115 metastatic TNBC patients, revealed that PD-L1-positive (≥ 5%) patients had higher responses to atezolizumab than PD-L1-negative patients (ORR, 17% vs. 8%) [7••]. In contrast, the phase 1b JAVELIN study, which included locally advanced or metastatic BC of all subtypes, three different criteria were used to stratify patients based on PD-L1 expression on either tumor cells or immune infiltrates [8••]. The study showed that PD-L1 expression, whether on tumor cells or immune infiltrates, did not predict response to avelumab [8••]. Instead, it was found that TNBC patients with dense aggregates of tumor-associated immune cells ($n$ = 12) had an ORR of 16.7% compared to a 1.6% ORR in the entire cohort ($n$ = 24) [8••]. These studies suggest that PD-L1-positive immune cells are associated with anti-PD-L1 benefit, but only in a subset of patients. The small percentage of responders with PD-L1-negative tumors also indicates a need to refine biomarker strategies. Finally, one should consider that (1) since the SP142 assay appears to be the least sensitive assay and (2) since the number of infiltrating immune cells likely demonstrates some linearity with prevalence and intensity of PD-L1 expression, that PD-L1 staining of immune cells may simply be a proxy for those with the most robust tumor-immune infiltrate.

Indeed, in the IMpassion130 trial, which led to the approval of atezolizumab and nab-paclitaxel in metastatic TNBC, it was PD-L1 expression on infiltrating leukocytes, not tumor cells, that was associated with benefit to anti-PD-L1 [9••]. Among patients with PD-L1-expressing tumors (≥ 1%), those receiving atezolizumab and nab-paclitaxel had an ORR of 58.9% (95% CI, 51.5–66.1) compared to 42.6% (95% CI, 35.4–50.1) of patients who received placebo and nab-paclitaxel, although these responses were more durable in the atezolizumab arm [9••]. Since the results from the IMpassion130 study and others mentioned above suggest that PD-L1 expression alone does not provide enough information to fully predict ICB response, several other potential biomarkers are being evaluated in the clinic. Table 1 displays several selected ongoing clinical trials in which some of these potential biomarkers are included in the enrollment criteria. This review focuses on recent clinical findings on the predictive capacity of potential biomarkers for ICB response and survival. We discuss the mechanisms that contribute to the behavior of these biomarkers in the context of tumor-immune interactions, which are largely governed by host and tumor genetics.

## Breast Cancer Immunogenicity

BC typically harbors a lower intratumoral immune presence than most other malignancies. However, some BC subtypes tend to be more immunogenic with increased inflammation compared to others [10, 11]. Spatially, the composition of various immunologic compartments can add to this complexity, enhancing the potential sources of heterogeneity. The tumor and immune system interact on different levels, whether in the tumor itself, the tumor-adjacent stroma, or any adjacent or regional lymph nodes or tertiary lymphoid structures [12]. The immune composition in these compartments may have vastly different physiological consequences and can change as disease progresses. In addition, treatment options used as standard-of-care (e.g., radiotherapy or chemotherapy) can have immunologic consequences [13].

Nearly two-thirds of diagnosed breast tumors are estrogen receptor (ER) and/or progesterone receptor (PR) positive, collectively referred to as hormone-receptor (HR)-positive [14]. Immune cells are scarce in most HR-positive tumors, which has been attributed to factors including general immune suppression and the lack of sufficient numbers of tumor-expressed T cell antigens to trigger anti-tumor immunity [15]. Interestingly, while patients with HR-positive tumors respond well to hormone therapy initially, many acquire resistance or progress to metastatic disease and demonstrate higher numbers of mutations, possibly via enhanced activity of APOBEC nucleotide-modifying enzymes [16].

Human epidermal growth factor-receptor 2 (HER2)-positive tumors, caused by the *HER2/ERBB2* gene amplification, are also often HR-positive [17]. However, these tumors are often more immunogenic than luminal, HER2-negative tumors [18]. Some evidence shows that HER2-positive tumors exhibit greater numbers of antigens than ER+/PR+ HER2-negative counterparts [19]. Importantly, HER2 overexpression sustains tumor growth, but can also trigger an HLA-A2-restricted cytotoxic lymphocyte (CTL) response to HER-2/neu extracellular domain-derived peptide p369-377 [20]. Thus, T cells directed toward the p369-377 peptide in tumors

**Table 1** Current clinical trials testing immune checkpoint blockade

| Clinical trial no. | Phase | *n* | Therapeutic agents | BC criteria for enrollment |
|---|---|---|---|---|
| NCT03989089 | II | 44 | Pembrolizumab | HER2− with *APOBEC3B* mutation |
| NCT03025035 | II | 20 | Pembrolizumab | *BRCA*-mutated BC |
| NCT03820141 | II | 39 | Trastuzumab and pertuzumab + durvalumab | HER2-enriched and HER2-amplified BC |
| NCT03801369 | II | 28 | Olaparib + durvalumab | *BRCA*-wild-type metastatic BC |
| NCT03789110 | II | 14-30 | Nivolumab + ipilimumab | hypermutated HER2− metastatic BC |
| NCT02693535 | II | 28 | Pembrolizumab | TMB-high mBC |
| NCT03044730 | II | 30 | Capecitabine ± pembrolizumab | endocrine-refractory, HR+/HER− metastatic BC |
| NCT03608865 | II | 30 | Durvalumab + tremelimumab | hypermutated HR+ metastatic BC |

overexpressing HER2 could be potential targets in an ICB setting.

Finally, TNBCs (i.e., those lacking ER/PR expression or *HER2* gene amplification) account for 15–20% of disease and are usually the most aggressive and invasive of the BC subtypes [21, 22]. Given the inherent resistance to hormone therapy and the high volume of TILs often found in TNBC tumors, it is no surprise that the first FDA approval for ICB in BC is in the TNBC population.

## Genetic Biomarkers

### Tumor Mutational Burden

The accumulation of genomic alterations is a hallmark of cancer. Regardless of origin, most cancers increase in genetic instability during disease progression [23]. Loss of DNA repair and subsequent genetic damage creates many avenues for tumor cells to be recognized by the immune system as 'non-self' [24]. Tumor mutational burden (TMB) is a measure of the number of missense mutations encoded in the genome per megabase [25]. Cells with deficient DNA repair can acquire mutations which are carried on through daughter cell generations. While "driver" mutations (e.g., those inducing oncogene activation or loss of negative suppression) permit a gain of function, most alterations to the genome have no direct effect on cell fitness [24–26]. Thus, as a function of inherent genetic instability, "passenger" mutations accumulate through subsequent tumor cell generations [24, 26]. TMB can be calculated using targeted next-generation sequencing (NGS) panels, although the measurement is more precise when a higher percentage of the genome is assayed [25]. For improved utility, TMB scoring might also involve bioinformatic algorithms that include a minimal cut-off for total genomic content and methods to exclude genetic variants that are not likely immunogenic [25]. Consistent with their reduced inflammatory nature, HR-positive, HER2-negative luminal-like tumors generally have a lower TMB than the more clinically aggressive HER2-enriched and basal-like/TNBC tumors [27•]. Barroso-Sousa et al. also found that BC tumors with a high TMB (> 10 mutations per megabase) were more commonly metastatic versus primary tumors [28•]. Thus, more aggressive BC subtypes and more advanced BC tumors generally have higher TMBs, which may be important for determining potential ICB benefit.

The accumulation of TMB increases the "foreignness" of cancer cells, which increases the probability of an anti-tumor immune response. A higher TMB can stochastically lead to more encoded neoantigens, and if recognized by cognate T cell receptors (TCRs), stronger T cell effector responses. After the stratification of breast tumors based on TMB, patients with high TMB tumors survived longer, particularly when they had robust immune infiltrates, while the degree of immune infiltrate did not prognosticate low-TMB tumors [27•]. This suggests that the immune system, even if capable of homing to and interfacing with the tumor, cannot mount an efficient anti-tumor response without a high TMB. Although the utility of TMB as a biomarker of ICB outcome in BC remains underexplored, there have now been preliminary reports testing this hypothesis. For instance, the TAPUR phase II basket study enrolled heavily pre-treated metastatic BC patients with high TMB, regardless of HR expression ($n = 28$) for treatment with pembrolizumab (anti-PD-1) [29]. The study preliminarily reported a 21% ORR (95% CI, 8–41), which is promising for ICB given as a monotherapy. Although this study did not include a group with low TMB, it is important to note that the KEYNOTE-086 phase II study tested pembrolizumab in pre-treated metastatic BC patients regardless of TMB status and observed only a 5.3% ORR (95% CI, 2.7–9.9) [30].

### Neoantigen Load

While TMB is intimately linked to the number of potential neoantigens encoded in the tumor genome, it does not provide information on those mutations most likely to be actively processed and loaded on major histocompatibility complex molecules (MHCs) [31, 32•]. Neoantigen load is a refinement of TMB and consists of altered peptide sequences derived from the mutant gene that are predicted to bind to a particular

MHC family member [31]. In contrast, TMB could include many mutations that cannot be loaded onto MHC molecules, and thus inconsequential in guiding an adaptive response [31]. Using structural and experimental binding-affinity data, neoantigen load provides information about newly formed tumor antigens that are most likely to be presented to T cells as non-self [31, 32•]. As with TMB, HER2-enriched and TNBC tumors are usually associated with a higher neoantigen load, which aligns with the presence of tumor-infiltrating lymphocytes (TILs) [33]. However, it is important to note that calculating true neoantigen load requires acquisition of several parameters, including whole exome sequencing, RNA sequencing (to limit the analysis to alterations that are expressed), and a reliable method for HLA-typing the individual patient. Thus, in addition to being computationally intensive, neoantigen load as a biomarker could be difficult to employ routinely in the clinic.

## T Cell Receptor Repertoire Analysis

As assessing genomic features like TMB and neoantigen load are informative methods for determining tumor antigenicity, these tools are indirect. For this reason, several groups have explored T cell receptor sequencing (TCR-seq) data using NGS platforms to evaluate TCR clonality, which provides a measurement of the proliferation or expansion of distinct populations of antigen-specific T cells [34]. In contrast, measurements of TCR diversity inform on the robustness or fitness of the general adaptive immune system, representing the size of the pool of potential TCR clonotypes that could be leveraged for anti-tumor immunity [34]. The CDR3 region of TCRβ is the most variable and can be sequenced to determine the diversity of clonotypes, as well as the abundance of specific clonotypes within the tumor [34]. Low TCRβ diversity was associated with poor overall survival in metastatic BC patients [35]. More recently, in a small cohort of 18 patients with metastatic ER-positive or TNBC tumors treated with durvalumab (anti-PD-1) and tremelimumab (anti-CTLA-4), expansion of multiple TCRβ clonotypes was observed in TNBC tumors compared to ER-positive tumors. The authors speculated that this increase in TCRβ clonotypes may reflect expansion and proliferation of T cell clones with anti-tumor activity in response to ICB and could be an early on-treatment biomarker for benefit to therapy [32•]. Thus, the current data indicate that a diverse TCR repertoire is meaningful for general survival and control of disease, and the presence of oligoclonal T cells may be targets and/or biomarkers for immunotherapy outcome. Whether sequencing tumor genes to determine neoantigen loads or analyzing the TCR repertoire, these potential biomarkers have financial and computational limitations. Furthermore, a gold standard method for prediction of therapeutic outcomes must be determined in large cohorts where all information is captured, and predictive capabilities are assessed in an unbiased manner.

## Mismatch Repair Deficiency

Mismatch repair genes correct mismatched nucleotide bases (e.g., A/C, G/T) within the genome during DNA replication. These errors often occur in non-coding short-tandem repeats in the genome known as microsatellites. Mismatch repair deficiency (dMMR), a form of genetic instability, occurs when MMR genes become mutated or epigenetically silenced, which ultimately leads to high microsatellite instability (MSI-H) [36]. Although defective DNA repair can lead to general increases in mutations, Vaderwalde et al. showed that a higher MSI does not necessarily translate to a higher TMB [37]. Thus, high MSI has been studied as a potentially independent predictive biomarker in other cancers like colorectal because of the high (15–20%) rate of occurrence [38]. After promising results from the phase II CheckMate142 study for metastatic colorectal cancer patients, the FDA-approved nivolumab (anti-PD-1) plus ipilimumab (anti-CTLA-4) for patients with MSI-H or dMMR tumors that progressed after chemotherapy [39]. MSI-H and dMMR tumors are much less common in BC patients (1–2%), but MSI-H is associated with lower survival rates and poor disease prognosis [40, 41]. Because of the success of MSI-H as a biomarker biomarker for pembrolizumab response, the FDA's approval included all solid tumor types, a first for tumor-intrinsic genetic features [42]. The predictive capacity of this biomarker was further demonstrated in BC when one group observed a significant response in a metastatic dMMR TNBC patient after nivolumab treatment [43••]. In another case testing pembrolizumab monotherapy, a rapid response was seen in a metastatic luminal BC patient with dMMR [44••]. Thus, dMMR deficiency may be a universally predictive biomarker, extending across subtypes. However, this is anecdotal evidence and will require larger numbers of BC patients to conclusively determine if this association holds true.

## BRCA1/2 Mutation Status

BRCA1 and BRCA2 are tumor suppressors involved in the repair of DNA double-strand breaks. The mutation of one or both of these genes and the resulting DNA repair deficiency increases the risk of mutations in other genes and the development of BC and ovarian cancer [45]. Germline *BRCA1/2* mutations occur in about 5% of BC patients [46]. TNBC tumors with germline *BRCA1* mutations have higher stromal TILs and higher *PD-L1* and *CTLA-4* gene expression than tumors with wild-type *BRCA1*, suggesting an increased probability of a positive ICB response [47, 48]. Anti-PD-1 plus anti-CTLA-4 therapy (but not monotherapy) significantly increased the response to cisplatin in these patients [47]. The

FDA has approved PARP inhibitors to treat patients with metastatic *BRCA1*-mutated BCs [49]. Importantly, PARP inhibitors display immunoregulatory effects in murine BC models, leading to speculation over whether these inhibitors might work in combination with ICB [50, 51]. Similarly, in human cells, long-term BRCA2 inhibition was shown to induce cell-intrinsic immune signaling through the STING pathway [52]. Interestingly, in the phase III IMpassion130 trial, patients with BRCA1/2 mutant tumors had a significantly increased PFS from nab-paclitaxel/atezolizumab compared to BRCA1/2 wildtype tumors, but only when immune infiltrates expressed PD-L1, suggesting that the BRCA1/2 mutation does not independently predict response [53•]. As PD-L1 positivity on immune cells was found to be the most prognostic biomarker in this case, considering BRCA1/2 mutations provides opportunity for a more personalized approach to ICB. This hypothesis is being tested in multiple malignancies, including BC, ovarian cancer, and bladder cancer [54–56].

## Non-genetic TME Biomarkers

### Immunogenic Cell Death

For neoantigens to effectively prime T cells, they must be released from dying cells and engulfed by phagocytic professional antigen-presenting cells (e.g., dendritic cells). Thus, one way to leverage potential neoantigens existing in the tumor genome for therapy is to prime adaptive immunity by potentiating their release through tumor cell death. Immunogenic cell death (ICD), or any cell death mechanism that by nature triggers an adaptive immune response, appears crucial for clinical response to ICB. ICD is characterized by hallmark increases in surface calreticulin, ATP secretion, and HMGB1 release [57]. Calreticulin expression at the cell surface sends phagocytosis signals to antigen presenting cells [58]. Extracellular ATP recruits dendritic cells and macrophages by binding to the P2Y2 receptor on target cells and further triggers the maturation of myeloid-derived dendritic cells and the expansion of macrophages [59]. The ectonucleotidases CD39 and CD73, expressed on immune and endothelial cells, also interact with ATP by hydrolyzing it into adenosine, giving ATP added immunosuppressive effects, including shunting T cells away from effector activity and memory formation toward immunosuppressive activity and apoptosis [60, 61]. After its release from necrotic cells and immune cells that have recognized antigen, HMGB1 targets TLR4 to activate dendritic cells and optimize the presentation of tumor-associated antigens [62]. ICD inducers, such as some chemotherapy agents, have commonly been used to drive immune responsiveness as an 'adjuvant' [62, 63•]. These have been theorized as part of the primary mechanism behind the efficacy of chemotherapy, which indirectly releases tumor-associated antigens to induce a clinically meaningful anti-tumor response. Thus, ICD markers could be effective predictive biomarkers in patients who have previously undergone chemotherapy as part of a priming regimen for ICB. Importantly, this line of thinking also in part forms the basis for the nab-paclitaxel plus atezolizumab treatment schedule for metastatic TNBC, although in most experimental studies, taxanes are generally considered poor ICD-inducers, as opposed to other agents, such as topoisomerase inhibitors [57].

### MHC-II Expression

Tumor cells can present cancer antigens to T cells, and most, if not all, of these antigens are intracellular self-antigens that trigger cytotoxic CD8+ T cell-directed immunity. CTL responses require antigens to be presented by MHC-I. CD4+ T helper cells, on the other hand, also have significant roles in anti-tumor immunity that have become more realized in recent years. In addition to classical T helper activity, CD4+ T cells are crucial for an optimal anti-tumor CD8+ T cell response [64]. Antigen presentation by MHC-II is required for CD4+ T cell-dependent effector activity. Unlike MHC-I, MHC-II expression is usually restricted to antigen presenting cells. However, some tumors can express MHC-II and potentially present CD4+ T cell antigens through MHC-II through mechanisms that are not completely understood. Although the factors driving tumor-specific MHC-II are unknown, Ras/MAPK activation has been shown to suppress MHC-II expression in BC [65]. Tumor-specific MHC-II expression appears to drive enhanced CD4+ and CD8+ T cell recruitment and/or expansion, interferon signaling, and high expression of immune checkpoint molecules [66, 67]. Tumor-specific MHC-II expression has now been reported as a biomarker for anti-PD-1 therapy outcomes in a variety of studies and cancer types [66, 68, 69]. Most recently, reverse protein array analysis was conducted on tumor epithelium from 156 HR+/HER2-negative BC and TNBC patients treated with pembrolizumab in the I-SPY2 trial. This analysis, performed on over 30 individual potential biomarkers, revealed tumor-specific MHC-II expression as one of the only predictive markers of anti-PD-1 benefit and was not associated with benefit to chemotherapy alone [70••].

### TILs

The abundance of TILs, a crude but easily measurable marker of immunologic presence in the TME, has been a proposed biomarker for response to ICB. In BC, however, TILs have a strong prognostic value and predictive capacity when used as a biomarker for chemotherapy response, complicating their use as a biomarker specifically for ICB. Importantly, in BC, the prognostic and predictive power of TILs likely depends on regional location in association to the intratumoral space. The

recommended method (International TILs working group) is to evaluate stromal TILs [71]. Intratumoral TILs include immune cells that have penetrated past the stroma and are directly interacting with tumor cells. Stromal TILs have no physical contact with tumor cells and are found within the stromal areas that run between tumor cell nests. Intratumoral TILs were found to be difficult to evaluate on H&E-stained slides, more heterogeneous than stromal TILs, and less reproducible [71]. A robust TIL presence was associated with disease-free and overall survival in TNBC [72], and higher TILs predicted response to neoadjuvant chemotherapy (NAC) in HER2-positive BC and TNBC, but not ER-positive BC [73].

Although total TILs carry some prognostic power, individual TIL populations tell a more complex story. While high CD8+ T cell infiltration indicates a positive prognosis, CD4+ Tregs and gamma-delta T cells were found to be negative prognostic indicators [74, 75]. In addition, high CD8+ T cell/Treg ratio predicted DFS and OS after NAC [76]. Molecular profiling of tumor-associated cells additionally revealed that a plasma cell signature was associated with a good prognosis, while a neutrophil signature associated with a less-favorable prognosis [77]. These findings suggest that not only a strong immune presence contributes to survival, but also a relative lack of immunosuppressive features may also contribute. In the phase Ia trial that tested atezolizumab as a monotherapy in metastatic TNBC patients, trends toward higher ORR and longer OS were found in patients who had > 10% TILs or > 1.35% intratumoral CD8 T cells prior to treatment [7••]. These findings further suggest a need for a panel of immune markers to more appropriately evaluate immune presence and the dominance of anti-tumor immune cells versus immunosuppressive cells within the TIL population. Despite its utility in prognosticating and predicting benefit to chemotherapy, the composition of stromal TILs, and the association with immunotherapy outcomes remain to be more thoroughly tested clinically.

## ER Expression

Surface markers and genomic qualities of tumor cells can shed light on the likelihood of a positive response to ICB. These findings reinforce the idea that the less hormone-dependent tumors are, the more genetically unstable and immunogenic they are likely to be. The more immune-permissive ER/PR-negative tumors are more likely to develop neoantigens, trigger anti-tumor immunity, and foster a durable response to ICB [15]. ER-positive tumors, particularly the luminal A subtype, are usually accompanied by low immunogenicity, few TILs, and a low mutational burden compared to HER2-enriched tumors and TNBC, both of which are generally more responsive to ICB [19, 71, 78, 79]. In fact, high TILs correlated with negative prognosis in ER-positive tumors, whereas positive associations were found in ER-negative tumors [80]. This discrepancy might be best attributed to immunosuppressive features of the ER that potentially enhance tumor progression. ER can be putatively expressed on virtually all cell types that can be found in the TME, including tumor cells, stromal cells, endothelial cells, and immune cells [81–84]. Endogenous estrogen, 17β-estradiol (E2), was shown to enhance immunosuppressive functionality in multiple cell types, contributing to tumor progression. In humans and mice, E2 can induce the recruitment of M2 tumor-associated macrophages and the expansion of myeloid-derived suppressor cells within the tumor [82, 84]. In addition, E2 treatment reduced the sensitivity of human liver carcinoma cells to natural killer cell- and CD8+ T cell-directed killing by inducing the expression of proteinase inhibitor-9, a granzyme B inhibitor [81, 83]. Finally, E2-treated mice showed higher numbers of Foxp3+ Tregs in one study, while another study further demonstrated higher PD-1 expression and immunosuppressive functionality in Tregs isolated from tumors of E2-treated mice [85, 86]. The propensity of estrogen to mediate immunologic suppression suggests that ER expression may be a biologically functional predictor of ICB benefit and suggest new strategies to modulate anti-tumor immunity in ER-positive tumors.

## Serum Lactate Dehydrogenase

The minimally invasive nature of collecting peripheral blood makes circulating biomarkers attractive to study as predictive indicators for immunotherapy. Serum lactate dehydrogenase (LDH) has shown promise in the clinic and has a high level of evidence as a reasonable biomarker in multiple malignancies including melanoma and non-small cell lung cancer [87–89]. Serum LDH serves as a surrogate for cell turnover, which increases as tumors gain proliferative momentum and overall aggression [90]. Under physiologically normal anaerobic conditions like muscle fatigue, LDH converts pyruvate, the final product of glycolysis, into lactate. LDH is released by cells upon lysis or death, and increased serum LDH levels indicates active disease [91]. Healthy humans maintain low LDH levels that only reflect normal systemic cell turnover, which makes LDH a potentially suitable biomarker for diseases that involve excessive tissue breakdown, which includes not only cancer, but also heart failure, anemia, lung, and liver disease [92].

In the TME, high LDH suppresses T cell cytokine production and cytolytic activity. Tregs further gain metabolic advantage over effector T cells through a Foxp3-mediated shift away from glycolysis in the glucose-poor, lactate-rich TME [93]. LDH levels usually associate with worse outcomes but seem to provide some predictive benefit for immunotherapy. In the phase Ib KEYNOTE-012 study that tested pembrolizumab in patients with metastatic BC tumors that expressed ≥ 1% PD-L1 by IHC, all five patients with high LDH levels showed rapid tumor progression [94, 95•]. However, in a cohort of

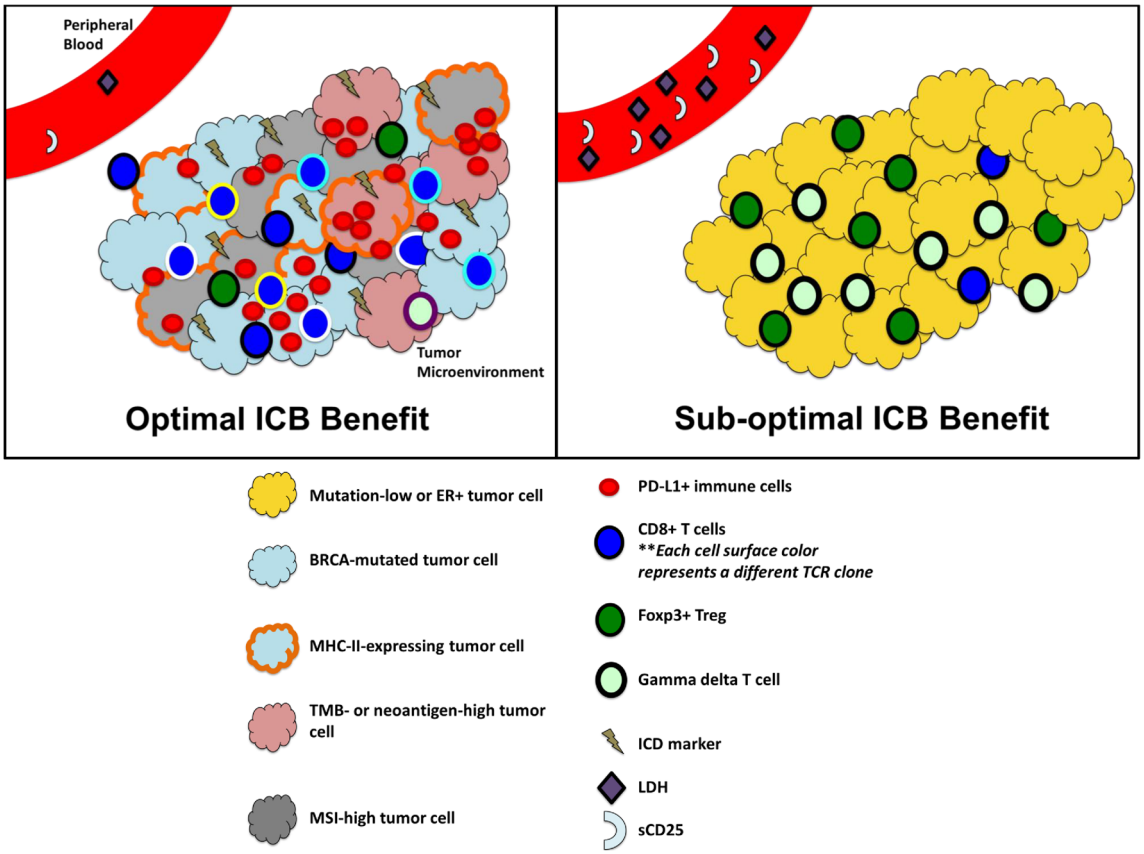


**Fig. 1** Proposed biomarkers for anti-PD-L1 benefit

170 metastatic TNBC patients from the KEYNOTE-086 study, a 2% ORR (95% CI 0.1–9) was observed in patients with high LDH levels compared to 7% ORR (95% CI 3–15) in patients with normal or low LDH levels, but the difference was not significant [96•]. Of note, the KEYNOTE-012 excluded PD-L1-negative tumors (41.4% of all tumors) and included patients who had abnormally high LDH levels [95•]. Nonetheless, the predictive benefit of serum LDH seems to vary between studies. Further investigation should help determine a standard for LDH cut-off levels and refinement of exclusion criteria.

## Conclusions

The identification of more accurate biomarkers of ICB benefit remains an ongoing area of study. Figure 1 represents the potential ICB biomarkers that have shown the most promise for BC patients. Understanding the immune landscape of the TME across BC subtypes can help guide ICB therapies toward patients who are most likely to respond. Since many 'classical' biomarkers are either rare in BC, or do not adequately predict response in patients, future studies integrating multiple biomarkers could be useful in optimally predicting ICB response.

## Compliance with Ethics Standards

**Conflict of Interest** Justin Balko reports research support from Genentech/Roche, Bristol Myers Squibb, and Incyte Corporation; has received consulting/expert witness fees from Novartis; is an inventor on provisional patents regarding immunotherapy targets and biomarkers in cancer; and has a patent 15/376,276 pending on the use of MHC-I/II to predict response to immunotherapy. Jamaal James declares no conflicts of interest relevant to this manuscript.

**Human and Animal Rights and Informed Consent** All reported studies/experiments with human or animal subjects performed by the authors have been previously published and complied with all applicable ethical standards (including the Helsinki declaration and its amendments, institutional/national research committee standards, and international/national/institutional guidelines).

## References

Papers of particular interest, published recently, have been highlighted as:
- • Of importance
- •• Of major importance

1. Jiang Y, Li Y, Zhu B. T-cell exhaustion in the tumor microenvironment. Cell Death Dis. 2015;6:e1792. https://doi.org/10.1038/cddis.2015.162.

2. Sun C, Mezzadra R, Schumacher TN. Regulation and Function of the PD-L1 Checkpoint. Immunity. 2018;48(3):434–52. https://doi.org/10.1016/j.immuni.2018.03.014.
3. Oyan B, Sonmez O, Yazar A, Teomet M. Comparison of SP142 (Ventana) and SP263 (Ventana) assays to test PD-L1 expression in metastatic cancer patients to be treated with immune checkpoint inhibitors. J Clin Oncol. 2018;36(15). https://doi.org/10.1200/JCO.2018.36.15_suppl.e24306.
4.• Scott M, Scorer P, Barker C, Al-Masri H. Comparison of patient populations identified by different PD-L1 assays in in triple-negative breast cancer (TNBC). Ann Oncol. 2019;30. **Comparison FDA-approved PD-L1 IHC assays testing predictive accuracy in TNBC.**
5. Gadiot J, Hooijkaas AI, Kaiser AD, van Tinteren H, van Boven H, Blank C. Overall survival and PD-L1 expression in metastasized malignant melanoma. Cancer. 2011;117(10):2192–201. https://doi.org/10.1002/cncr.25747.
6. Hunter KA, Socinski MA, Villaruz LC. PD-L1 Testing in Guiding Patient Selection for PD-1/PD-L1 Inhibitor Therapy in Lung Cancer. Mol Diagn Ther. 2018;22(1):1–10. https://doi.org/10.1007/s40291-017-0308-6.
7.•• Schmid P, Cruz C, Braiteh FS, Eder JP, Tolaney S, Kuter I et al. Atezolizumab in metastatic TNBC (mTNBC): Long-term clinical outcomes and biomarker analyses. Cancer Res. 2017;77. https://doi.org/10.1158/1538-7445.Am2017-2986. **Dose-escalation study evaluating safety, tolerability and pharmokinetics of atezolizumab in metastatic TNBC regardless of PD-L1 expression.**
8.•• Dirix LY, Takacs I, Jerusalem G, Nikolinakos P, Arkenau HT, Forero-Torres A, et al. Avelumab, an anti-PD-L1 antibody, in patients with locally advanced or metastatic breast cancer: a phase 1b JAVELIN Solid Tumor study. Breast Cancer Res Treat. 2018;167(3):671–86. https://doi.org/10.1007/s10549-017-4537-5. **Dose-escalation study testing avelumab activity and the predictive capacity of PD-L1 in metastatic BC patients who resisted standard-of-care treatment.**
9.•• 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. https://doi.org/10.1056/NEJMoa1809615. **Randomized trial testing standard-of-care nab-paclitaxel in combination with atezolizumab in patients with untreated metastatic TNBC.**
10. Althobiti M, Aleskandarany MA, Joseph C, Toss M, Mongan N, Diez-Rodriguez M, et al. Heterogeneity of tumour-infiltrating lymphocytes in breast cancer and its prognostic significance. Histopathology. 2018;73(6):887–96. https://doi.org/10.1111/his.13695.
11. Dannenfelser R, Nome M, Tahiri A, Ursini-Siegel J, Vollan HKM, Haakensen VD, et al. Data-driven analysis of immune infiltrate in a large cohort of breast cancer and its association with disease progression, ER activity, and genomic complexity. Oncotarget. 2017;8(34):57121–33. https://doi.org/10.18632/oncotarget.19078.
12. Varn FS, Mullins DW, Arias-Pulido H, Fiering S, Cheng C. Adaptive immunity programmes in breast cancer. Immunology. 2017;150(1):25–34. https://doi.org/10.1111/imm.12664.
13. Wargo JA, Reuben A, Cooper ZA, Oh KS, Sullivan RJ. Immune Effects of Chemotherapy, Radiation, and Targeted Therapy and Opportunities for Combination With Immunotherapy. Semin Oncol. 2015;42(4):601–16. https://doi.org/10.1053/j.seminoncol.2015.05.007.
14. Cortet M, Bertaut A, Molinie F, Bara S, Beltjens F, Coutant C, et al. Trends in molecular subtypes of breast cancer: description of incidence rates between 2007 and 2012 from three French registries. BMC Cancer. 2018;18(1):161. https://doi.org/10.1186/s12885-018-4080-8.
15. Luen S, Virassamy B, Savas P, Salgado R, Loi S. The genomic landscape of breast cancer and its interaction with host immunity. Breast. 2016;29:241–50. https://doi.org/10.1016/j.breast.2016.07.015.
16. Venkatesan S, Rosenthal R, Kanu N, McGranahan N, Bartek J, Quezada SA, et al. Perspective: APOBEC mutagenesis in drug resistance and immune escape in HIV and cancer evolution. Ann Oncol. 2018;29(3):563–72. https://doi.org/10.1093/annonc/mdy003.
17. Iqbal N, Iqbal N. Human Epidermal Growth Factor Receptor 2 (HER2) in Cancers: Overexpression and Therapeutic Implications. Mol Biol Int. 2014;2014:852748. https://doi.org/10.1155/2014/852748.
18. Salgado R, Loi S. Tumour infiltrating lymphocytes in breast cancer: increasing clinical relevance. Lancet Oncol. 2018;19(1):3–5. https://doi.org/10.1016/S1470-2045(17)30905-1.
19. Shah SP, Roth A, Goya R, Oloumi A, Ha G, Zhao Y, et al. The clonal and mutational evolution spectrum of primary triple-negative breast cancers. Nature. 2012;486(7403):395–9. https://doi.org/10.1038/nature10933.
20. Henle AM, Erskine CL, Benson LM, Clynes R, Knutson KL. Enzymatic discovery of a HER-2/neu epitope that generates cross-reactive T cells. J Immunol. 2013;190(1):479–88. https://doi.org/10.4049/jimmunol.1201264.
21. Cheang MC, Chia SK, Voduc D, Gao D, Leung S, Snider J, et al. Ki67 index, HER2 status, and prognosis of patients with luminal B breast cancer. J Natl Cancer Inst. 2009;101(10):736–50. https://doi.org/10.1093/jnci/djp082.
22. Dai X, Li T, Bai Z, Yang Y, Liu X, Zhan J, et al. Breast cancer intrinsic subtype classification, clinical use and future trends. Am J Cancer Res. 2015;5(10):2929–43.
23. Yao Y, Dai W. Genomic Instability and Cancer. J Carcinog Mutagen. 2014;5. https://doi.org/10.4172/2157-2518.1000165.
24. Chan TA, Yarchoan M, Jaffee E, Swanton C, Quezada SA, Stenzinger A, et al. Development of tumor mutation burden as an immunotherapy biomarker: utility for the oncology clinic. Ann Oncol. 2019;30(1):44–56. https://doi.org/10.1093/annonc/mdy495.
25. Buttner R, Longshore JW, Lopez-Rios F, Merkelbach-Bruse S, Normanno N, Rouleau E, et al. Implementing TMB measurement in clinical practice: considerations on assay requirements. ESMO Open. 2019;4(1):e000442. https://doi.org/10.1136/esmoopen-2018-000442.
26. Bozic I, Antal T, Ohtsuki H, Carter H, Kim D, Chen S, et al. Accumulation of driver and passenger mutations during tumor progression. Proc Natl Acad Sci U S A. 2010;107(43):18545–50. https://doi.org/10.1073/pnas.1010978107.
27.• 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. https://doi.org/10.1080/2162402X.2018.1490854. **A study using publicly available datasets to determine the prognostic value of TMB and its relationship with immune cell infiltration and BC subtype.**
28.• Barroso-Sousa R, Jain E, 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). https://doi.org/10.1200/JCO.2018.36.15_suppl.1010. **A study using publicly available datasets to understand relationships between TMB and BC subtype, tumor stage, metastatic site, and mutational patterns.**
29. Alva AS, Mangat PK, Garrett-Mayer E, Halabi S, Alvarez RH, Calfa CJ, et al. Pembrolizumab (P) in patients (pts) with metastatic breast cancer (MBC) with high tumor mutational burden (HTMB): Results from the Targeted Agent and Profiling Utilization Registry (TAPUR) Study. J Clin Oncol. 2019;37(suppl):1014.

30. Adams S, Schmid P, Rugo HS, Winer EP, Loirat D, Awada A, et al. Pembrolizumab monotherapy for previously treated metastatic triple-negative breast cancer: cohort A of the phase II KEYNOTE-086 study. Ann Oncol. 2019;30(3):397–404. https://doi.org/10.1093/annonc/mdy517.
31. Miller A, Asmann Y, Cattaneo L, Braggio E, Keats J, Auclair D, et al. High somatic mutation and neoantigen burden are correlated with decreased progression-free survival in multiple myeloma. Blood Cancer J. 2017;7(9):e612. https://doi.org/10.1038/bcj.2017.94.
32.• Santa-Maria CA, Kato T, Park JH, Kiyotani K, Rademaker A, Shah AN, et al. A pilot study of durvalumab and tremelimumab and immunogenomic dynamics in metastatic breast cancer. Oncotarget. 2018;9(27):18985–96. https://doi.org/10.18632/oncotarget.24867. **A single arm pilot study aimed at evaluating ORR in metastatic ER-positive or TNBC patients treated with durvalumab and tremelimumab.**
33. Hammerl D, Smid M, Timmermans AM, Sleijfer S, Martens JWM, Debets R. Breast cancer genomics and immuno-oncological markers to guide immune therapies. Semin Cancer Biol. 2018;52(Pt 2):178–88. https://doi.org/10.1016/j.semcancer.2017.11.003.
34. Rosati E, Dowds CM, Liaskou E, Henriksen EKK, Karlsen TH, Franke A. Overview of methodologies for T-cell receptor repertoire analysis. BMC Biotechnol. 2017;17(1):61. https://doi.org/10.1186/s12896-017-0379-9.
35. Manuel M, Tredan O, Bachelot T, Clapisson G, Courtier A, Parmentier G, et al. Lymphopenia combined with low TCR diversity (divpenia) predicts poor overall survival in metastatic breast cancer patients. Oncoimmunology. 2012;1(4):432–40. https://doi.org/10.4161/onci.19545.
36. Poulogiannis G, Frayling IM, Arends MJ. DNA mismatch repair deficiency in sporadic colorectal cancer and Lynch syndrome. Histopathology. 2010;56(2):167–79. https://doi.org/10.1111/j.1365-2559.2009.03392.x.
37. Vanderwalde A, Spetzler D, Xiao N, Gatalica Z, Marshall J. Microsatellite instability status determined by next-generation sequencing and compared with PD-L1 and tumor mutational burden in 11,348 patients. Cancer Med. 2018;7(3):746–56. https://doi.org/10.1002/cam4.1372.
38. Nojadeh JN, Behrouz Sharif S, Sakhinia E. Microsatellite instability in colorectal cancer. EXCLI J. 2018;17:159–68. https://doi.org/10.17179/excli2017-948.
39. Lenz HJJ, Van Cutsem E, Limon ML, Wong KY, Hendlisz A, Aglietta M, et al. Durable clinical benefit with nivolumab (NIVO) plus low-dose ipilimumab (IPI) as first-line therapy in microsatellite instability-high/mismatch repair deficient (MSI-H/dMMR) metastatic colorectal cancer (mCRC). Ann Oncol. 2018;29:714.
40. Paulson TG, Wright FA, Parker BA, Russack V, Wahl GM. Microsatellite instability correlates with reduced survival and poor disease prognosis in breast cancer. Cancer Res. 1996;56(17):4021–6.
41. Davies H, Morganella S, Purdie CA, Jang SJ, Borgen E, Russnes H, et al. Whole-Genome Sequencing Reveals Breast Cancers with Mismatch Repair Deficiency. Cancer Res. 2017;77(18):4755–62. https://doi.org/10.1158/0008-5472.CAN-17-1083.
42. Le DT, Uram JN, Wang H, Bartlett BR, Kemberling H, Eyring AD, et al. PD-1 Blockade in Tumors with Mismatch-Repair Deficiency. N Engl J Med. 2015;372(26):2509–20. https://doi.org/10.1056/NEJMoa1500596.
43.•• Kok M, Horlings HM, Snaebjornsson P, Chalabi M, Schumacher TN, Blank CU, et al. Profound Immunotherapy Response in Mismatch Repair-Deficient Breast Cancer. Jco Precis Oncol. 2017;1. https://doi.org/10.1200/Po.17.00052. **An ongoing report of a single patient with pre-treated, MSI-H metastatic TNBC who benefitted from nivolumab treatment.**
44.•• Fremd C, Hlevnjak M, Zapatka M, Zoernig I, Halama N, Fejzibegovic N, et al. Mismatch Repair Deficiency Drives Durable Complete Remission by Targeting Programmed Death Receptor 1 in a Metastatic Luminal Breast Cancer Patient. Breast Care (Basel). 2019;14(1):53–9. https://doi.org/10.1159/000492580. **A report of rapid response and durable complete remission in a single patient with MSI-H metastatic luminal breast cancer treated with pembrolizumab monotherapy.**
45. Godet I, Gilkes DM. BRCA1 and BRCA2 mutations and treatment strategies for breast cancer. Integr Cancer Sci Ther. 2017;4(1). https://doi.org/10.15761/ICST.1000228.
46. van Beers EH, van Welsem T, Wessels LF, Li Y, Oldenburg RA, Devilee P, et al. Comparative genomic hybridization profiles in human BRCA1 and BRCA2 breast tumors highlight differential sets of genomic aberrations. Cancer Res. 2005;65(3):822–7.
47. Nolan E, Savas P, Policheni AN, Darcy PK, Vaillant F, Mintoff CP, et al. Combined immune checkpoint blockade as a therapeutic strategy for BRCA1-mutated breast cancer. Sci Transl Med. 2017;9(393). https://doi.org/10.1126/scitranslmed.aal4922.
48. Lakhani SR, Jacquemier J, Sloane JP, Gusterson BA, Anderson TJ, van de Vijver MJ, et al. Multifactorial analysis of differences between sporadic breast cancers and cancers involving BRCA1 and BRCA2 mutations. J Natl Cancer Inst. 1998;90(15):1138–45. https://doi.org/10.1093/jnci/90.15.1138.
49. Robson M, Im SA, Senkus E, Xu B, Domchek SM, Masuda N, et al. Olaparib for Metastatic Breast Cancer in Patients with a Germline BRCA Mutation. N Engl J Med. 2017;377(6):523–33. https://doi.org/10.1056/NEJMoa1706450.
50. Huang J, Wang L, Cong Z, Amoozgar Z, Kiner E, Xing D, et al. The PARP1 inhibitor BMN 673 exhibits immunoregulatory effects in a Brca1(-/-) murine model of ovarian cancer. Biochem Biophys Res Commun. 2015;463(4):551–6. https://doi.org/10.1016/j.bbrc.2015.05.083.
51. Higuchi T, Flies DB, Marjon NA, Mantia-Smaldone G, Ronner L, Gimotty PA, et al. CTLA-4 Blockade Synergizes Therapeutically with PARP Inhibition in BRCA1-Deficient Ovarian Cancer. Cancer Immunol Res. 2015;3(11):1257–68. https://doi.org/10.1158/2326-6066.CIR-15-0044.
52. Reislander T, Lombardi EP, Groelly FJ, Miar A, Porru M, Di Vito S, et al. BRCA2 abrogation triggers innate immune responses potentiated by treatment with PARP inhibitors. Nat Commun. 2019;10(1):3143. https://doi.org/10.1038/s41467-019-11048-5.
53.• Emens LA, Loi S, Rugo HS, Schneeweiss A, Dieras V, Iwata H, et al., editors. IMpassion130: Efficacy in immune biomarker subgroups from the global, randomized, double-blind, placebo-controlled, phase III study of atezolizumab + nab-paclitaxel in patients with treatment-naïve, locally advanced or metastatic triple-negative breast cancer. San Antonio Breast Cancer Symposium; 2018 December 4-8, San Antonio, Texas; 2018. **Further analysis of data gathered in the IMpassion130 trial exploring nab-paclitaxel/atezolizumab efficacy in biomarker-defined subgroups.**
54. Zhang G, Liu C, Bai H, Cao G, Cui R, Zhang Z. Combinatorial therapy of immune checkpoint and cancer pathways provides a novel perspective on ovarian cancer treatment. Oncol Lett. 2019;17(3):2583–91. https://doi.org/10.3892/ol.2019.9902.
55. Criscuolo D, Morra F, Giannella R, Visconti R, Cerrato A, Celetti A. New combinatorial strategies to improve the PARP inhibitors efficacy in the urothelial bladder Cancer treatment. J Exp Clin Cancer Res. 2019;38(1):91. https://doi.org/10.1186/s13046-019-1089-z.
56. Sulai NH, Tan AR. Development of poly(ADP-ribose) polymerase inhibitors in the treatment of BRCA-mutated breast cancer. Clin Adv Hematol Oncol. 2018;16(7):491–501.
57. Kroemer G, Galluzzi L, Kepp O, Zitvogel L. Immunogenic cell death in cancer therapy. Annu Rev Immunol. 2013;31:51–72. https://doi.org/10.1146/annurev-immunol-032712-100008.

58. Chao MP, Jaiswal S, Weissman-Tsukamoto R, Alizadeh AA, Gentles AJ, Volkmer J, et al. Calreticulin is the dominant pro-phagocytic signal on multiple human cancers and is counterbalanced by CD47. Sci Transl Med. 2010;2(63):63ra94. https://doi.org/10.1126/scitranslmed.3001375.
59. Kronlage M, Song J, Sorokin L, Isfort K, Schwerdtle T, Leipziger J, et al. Autocrine purinergic receptor signaling is essential for macrophage chemotaxis. Sci Signal. 2010;3(132):ra55. https://doi.org/10.1126/scisignal.2000588.
60. Antonioli L, Pacher P, Vizi ES, Hasko G. CD39 and CD73 in immunity and inflammation. Trends Mol Med. 2013;19(6):355–67. https://doi.org/10.1016/j.molmed.2013.03.005.
61. Beavis PA, Stagg J, Darcy PK, Smyth MJ. CD73: a potent suppressor of antitumor immune responses. Trends Immunol. 2012;33(5):231–7. https://doi.org/10.1016/j.it.2012.02.009.
62. Wang YJ, Fletcher R, Yu J, Zhang L. Immunogenic effects of chemotherapy-induced tumor cell death. Genes Dis. 2018;5(3):194–203. https://doi.org/10.1016/j.gendis.2018.05.003.
63.• Lu J, Liu X, Liao YP, Wang X, Ahmed A, Jiang W, et al. Breast Cancer Chemo-immunotherapy through Liposomal Delivery of an Immunogenic Cell Death Stimulus Plus Interference in the IDO-1 Pathway. ACS Nano. 2018;12(11):11041–61. https://doi.org/10.1021/acsnano.8b05189. **A preclinical study testing the effects of stimulating ICD with doxorubicin before treating with IDO-1-inhibitor, indoximod in mice with BC tumors.**
64. Zanetti M. Tapping CD4 T cells for cancer immunotherapy: the choice of personalized genomics. J Immunol. 2015;194(5):2049–56. https://doi.org/10.4049/jimmunol.1402669.
65. Loi S, Dushyanthen S, Beavis PA, Salgado R, Denkert C, Savas P, et al. RAS/MAPK Activation Is Associated with Reduced Tumor-Infiltrating Lymphocytes in Triple-Negative Breast Cancer: Therapeutic Cooperation Between MEK and PD-1/PD-L1 Immune Checkpoint Inhibitors. Clin Cancer Res. 2016;22(6):1499–509. https://doi.org/10.1158/1078-0432.CCR-15-1125.
66. Johnson DB, Nixon MJ, Wang Y, Wang DY, Castellanos E, Estrada MV, et al. Tumor-specific MHC-II expression drives a unique pattern of resistance to immunotherapy via LAG-3/FCRL6 engagement. JCI Insight. 2018;3(24). https://doi.org/10.1172/jci.insight.120360.
67. Johnson DB, Estrada MV, Salgado R, Sanchez V, Doxie DB, Opalenik SR, et al. Melanoma-specific MHC-II expression represents a tumour-autonomous phenotype and predicts response to anti-PD-1/PD-L1 therapy. Nat Commun. 2016;7:10582. https://doi.org/10.1038/ncomms10582.
68. Roemer MGM, Redd RA, Cader FZ, Pak CJ, Abdelrahman S, Ouyang J, et al. Major Histocompatibility Complex Class II and Programmed Death Ligand 1 Expression Predict Outcome After Programmed Death 1 Blockade in Classic Hodgkin Lymphoma. J Clin Oncol. 2018;36(10):942–50. https://doi.org/10.1200/JCO.2017.77.3994.
69. Rodig SJ, Gusenleitner D, Jackson DG, Gjini E, Giobbie-Hurder A, Jin C, et al. MHC proteins confer differential sensitivity to CTLA-4 and PD-1 blockade in untreated metastatic melanoma. Sci Transl Med. 2018;10(450). https://doi.org/10.1126/scitranslmed.aar3342.
70.•• Wulfkuhle JD, Yau C, Wolf DM, Gallagher RI, Swigart LB, Hirst GL, et al. Quantitative MHC II protein expression levels in tumor epithelium to predict response to the PD1 inhibitor pembrolizumab in the I-SPY 2 Trial. J Clin Oncol. 2019;37(15_suppl):2631. https://doi.org/10.1200/JCO.2019.37.15_suppl.2631. **A phase 2 trial testing MHC-II as a predictive biomarker in TNBC patients treated with pembrolizumab.**
71. 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. https://doi.org/10.1093/annonc/mdu450.
72. Adams S, Goldstein LJ, Sparano JA, Demaria S, Badve SS. Tumor infiltrating lymphocytes (TILs) improve prognosis in patients with triple negative breast cancer (TNBC). Oncoimmunology. 2015;4(9):e985930. https://doi.org/10.4161/2162402X.2014.985930.
73. Mao Y, Qu Q, Zhang Y, Liu J, Chen X, Shen K. The value of tumor infiltrating lymphocytes (TILs) for predicting response to neoadjuvant chemotherapy in breast cancer: a systematic review and meta-analysis. PLoS One 2014;9(12):e115103. https://doi.org/10.1371/journal.pone.0115103.
74. Siegers GM, Dutta I, Lai R, Postovit LM. Functional Plasticity of Gamma Delta T Cells and Breast Tumor Targets in Hypoxia. Front Immunol. 2018;9:1367. https://doi.org/10.3389/fimmu.2018.01367.
75. Ziai J, Gilbert HN, Foreman O, Eastham-Anderson J, Chu F, Huseni M, et al. CD8+ T cell infiltration in breast and colon cancer: A histologic and statistical analysis. PLoS One. 2018;13(1):e0190158. https://doi.org/10.1371/journal.pone.0190158.
76. Takada K, Kashiwagi S, Goto W, Asano Y, Takahashi K, Takashima T, et al. Use of the tumor-infiltrating CD8 to FOXP3 lymphocyte ratio in predicting treatment responses to combination therapy with pertuzumab, trastuzumab, and docetaxel for advanced HER2-positive breast cancer. J Transl Med. 2018;16(1):86. https://doi.org/10.1186/s12967-018-1460-4.
77. Sugie T. Immunotherapy for metastatic breast cancer. Chin Clin Oncol. 2018;7(3):28. https://doi.org/10.21037/cco.2018.05.05.
78. Adams S, Gray RJ, Demaria S, Goldstein L, Perez EA, Shulman LN, 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. https://doi.org/10.1200/JCO.2013.55.0491.
79. Santa-Maria CA, Kato T, Park JH, Flaum LE, Jain S, Tellez C, et al. Durvalumab and tremelimumab in metastatic breast cancer (MBC): Immunotherapy and immunopharmacogenomic dynamics. J Clin Oncol. 2017;35. https://doi.org/10.1200/JCO.2017.35.15_suppl.3052.
80. Kurozumi S, Yamaguchi Y, Matsumoto H, Kurosumi M, Hayashi SI, Fujii T, et al. Utility of Ki67 labeling index, cyclin D1 expression, and ER-activity level in postmenopausal ER-positive and HER2-negative breast cancer with neoadjuvant chemo-endocrine therapy. PLoS One. 2019;14(5):e0217279. https://doi.org/10.1371/journal.pone.0217279.
81. Jiang X, Orr BA, Kranz DM, Shapiro DJ. Estrogen induction of the granzyme B inhibitor, proteinase inhibitor 9, protects cells against apoptosis mediated by cytotoxic T lymphocytes and natural killer cells. Endocrinology. 2006;147(3):1419–26. https://doi.org/10.1210/en.2005-0996.
82. Svoronos N, Perales-Puchalt A, 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. https://doi.org/10.1158/2159-8290.CD-16-0502.
83. Rothenberger NJ, Somasundaram A, Stabile LP. The Role of the Estrogen Pathway in the Tumor Microenvironment. Int J Mol Sci. 2018;19(2). https://doi.org/10.3390/ijms19020611.
84. Kozasa K, Mabuchi S, Matsumoto Y, Kuroda H, Yokoi E, Komura N, et al. Estrogen stimulates female cancer progression by inducing myeloid-derived suppressive cells: investigations on pregnant and non-pregnant experimental models. Oncotarget. 2019;10(20):1887–902. https://doi.org/10.18632/oncotarget.26711.
85. Polanczyk MJ, Hopke C, Vandenbark AA, Offner H. Treg suppressive activity involves estrogen-dependent expression of programmed death-1 (PD-1). Int Immunol. 2007;19(3):337–43. https://doi.org/10.1093/intimm/dxl151.

86. Polanczyk MJ, Carson BD, Subramanian S, Afentoulis M, Vandenbark AA, Ziegler SF, et al. Cutting edge: estrogen drives expansion of the CD4+CD25+ regulatory T cell compartment. J Immunol. 2004;173(4):2227–30. https://doi.org/10.4049/jimmunol.173.4.2227.
87. Mezquita L, Auclin E, Ferrara R, Charrier M, Remon J, Planchard D, et al. Association of the Lung Immune Prognostic Index With Immune Checkpoint Inhibitor Outcomes in Patients With Advanced Non-Small Cell Lung Cancer. JAMA Oncol. 2018;4(3):351–7. https://doi.org/10.1001/jamaoncol.2017.4771.
88. Weide B, Martens A, Hassel JC, Berking C, Postow MA, Bisschop K, et al. Baseline Biomarkers for Outcome of Melanoma Patients Treated with Pembrolizumab. Clin Cancer Res. 2016;22(22):5487–96. https://doi.org/10.1158/1078-0432.CCR-16-0127.
89. Ribas A, Shin DS, Zaretsky J, Frederiksen J, Cornish A, Avramis E, et al. PD-1 Blockade Expands Intratumoral Memory T Cells. Cancer Immunol Res. 2016;4(3):194–203. https://doi.org/10.1158/2326-6066.CIR-15-0210.
90. Jurisic V, Radenkovic S, Konjevic G. The Actual Role of LDH as Tumor Marker, Biochemical and Clinical Aspects. Adv Exp Med Biol. 2015;867:115–24. https://doi.org/10.1007/978-94-017-7215-0_8.
91. Alegre E, Sammamed M, Fernandez-Landazuri S, Zubiri L, Gonzalez A. Circulating biomarkers in malignant melanoma. Adv Clin Chem. 2015;69:47–89. https://doi.org/10.1016/bs.acc.2014.12.002.
92. Petrelli F, Cabiddu M, Coinu A, Borgonovo K, Ghilardi M, Lonati V, et al. Prognostic role of lactate dehydrogenase in solid tumors: a systematic review and meta-analysis of 76 studies. Acta Oncol. 2015;54(7):961–70. https://doi.org/10.3109/0284186X.2015.1043026.
93. Angelin A, Gil-de-Gomez L, Dahiya S, Jiao J, Guo L, Levine MH, et al. Foxp3 Reprograms T Cell Metabolism to Function in Low-Glucose, High-Lactate Environments. Cell Metab. 2017;25(6):1282–93 e7. https://doi.org/10.1016/j.cmet.2016.12.018.
94. Voorwerk L, Kat M, Kok M. Towards predictive biomarkers for immunotherapy response in breast cancer patients. Breast Cancer Manag. 2018;7(1). https://doi.org/10.2217/bmt-2017-0014.
95.• Nanda R, Chow LQ, Dees EC, Berger R, Gupta S, Geva R, et al. Pembrolizumab in Patients With Advanced Triple-Negative Breast Cancer: Phase Ib KEYNOTE-012 Study. J Clin Oncol. 2016;34(21):2460–7. https://doi.org/10.1200/JCO.2015.64.8931. **A non-randomized phase Ib study of testing safety and clinical activity of pembrolizumab monotherapy in patients with heavily pretreated, advanced TNBC.**
96.• Adams S, Schmid P, Rugo HS, Winer EP, Loirat D, Awada A, et al. Phase 2 study of pembrolizumab (pembro) monotherapy for previously treated metastatic triple-negative breast cancer (mTNBC): KEYNOTE-086 cohort A. J Clin Oncol. 2017;35. https://doi.org/10.1200/JCO.2017.35.15_suppl.1008. **A phase 2 trial testing pembrolizumab safety and responses in metastatic TNBC patients who had previously received chemotherapy.**