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Review

Determining PD-L1 Status in Patients With Triple-Negative Breast Cancer: Lessons Learned From IMpassion130

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REVIEW

Abstract

Triple-negative breast cancer (TNBC) accounts for approximately 12% to 17% of all breast cancers and has an aggressive clinical behavior. Increased tumor-infiltrating lymphocyte counts are prognostic for survival in TNBC, making this disease a potential target for cancer immunotherapy. Research on immunophenotyping of tumor-infiltrating lymphocytes is revealing molecular and structural organization in the tumor microenvironment that may predict patient prognosis. The anti–programmed death-ligand 1 (PD-L1) antibody atezolizumab plus *nab*-paclitaxel was the first cancer immunotherapy combination to demonstrate progression-free survival benefit and clinically meaningful overall survival benefit in the first-line treatment of metastatic TNBC (mTNBC) in patients with PD-L1–expressing tumor-infiltrating immune cells in 1% or more of the tumor area. This led to its United States and European Union approval for mTNBC and US approval of the VENTANA PD-L1 (SP142) assay as a companion diagnostic immunohistochemistry assay. Subsequently, the anti–programmed death-1 (PD-1) antibody pembrolizumab plus chemotherapy was approved by the US Food and Drug Administration for mTNBC based on progression-free survival benefit in patients with a combined positive score of at least 10 by its concurrently approved 22C3 companion diagnostic assay. Treatment guidelines now recommend PD-L1 testing for patients with mTNBC, and the testing landscape will likely become increasingly complex as new anti–PD-L1 and anti–PD-1 agents and diagnostics are approved for TNBC. Integrating PD-L1 testing into current diagnostic workflows for mTNBC may provide more treatment options for these patients. Therefore, it is critical for medical oncologists and pathologists to understand the available assays and their relevance to therapeutic options to develop an appropriate workflow for immunohistochemistry testing.

Triple-negative breast cancer (TNBC) is a biologically aggressive breast cancer subtype accounting for approximately 12% to 17% of all breast cancers (1). TNBC is clinically and molecularly heterogeneous, and recent years have witnessed the search for specific treatments targeted toward molecular subtypes (1-3). Compared with other breast cancer types, TNBC disproportionately affects women younger than 40 years and of Indian, African American, Hispanic, and sub-Saharan African descent (1,4,5). Chemotherapy is the standard treatment in the metastatic setting, which generally results in a median survival of less than 18 months (6,7). Patients relapse 2 to 3 times faster (8-10) than patients with estrogen receptor (ER)–, progesterone receptor (PR)–, or HER2-positive cancers and are twice as likely to experience distant recurrence (11), especially in the first 5 years (1). Adding the programmed death-ligand 1 (PD-L1) or programmed death-1 (PD-1) –targeting agents atezolizumab or pembrolizumab to chemotherapy was recently shown to improve outcomes in both early-stage and metastatic TNBC (mTNBC) (12-15). In mTNBC, the combination of atezolizumab with *nab*-paclitaxel showed a statistically significant progression-free survival (PFS) benefit (stratified hazard ratio [HR] = 0.62, 95% confidence interval [CI] = 0.49 to 0.78; $P < .001$)

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and a clinically meaningful overall survival (OS) benefit vs *nab*-paclitaxel in PD-L1–positive patients—defined as patients whose tumors contain PD-L1–expressing tumor-infiltrating immune cells (IC) covering at least 1% of the tumor area (15). These results have led to global changes in standards of care (16). In this same setting, pembrolizumab combined with either a taxane or gemcitabine plus carboplatin showed a PFS benefit vs placebo combined with chemotherapy in PD-L1–positive patients—defined as patients whose tumors had a combined positive score (CPS) of at least 10. CPS is defined as the sum of PD-L1–expressing tumor cells (TC), lymphocytes, and macrophages divided by the total number of viable TC multiplied by 100, to result in a score ranging from 0 to 100 (13). In contrast, determination of PD-L1 status may be less important in early-stage TNBC, where a pathological complete response (pCR) was shown to be independent of PD-L1 status when either atezolizumab or pembrolizumab was combined with neoadjuvant chemotherapy regimens (12,14). This review describes how various PD-L1 assays might identify different populations eligible for treatment and the implications for patient outcomes. We will discuss the tumor microenvironment (TME) in early-stage TNBC and mTNBC and highlight differences that may explain why PD-L1 status may matter more for mTNBC. We will also highlight recent developments that inform our understanding of the TME of TNBC and may shed light on the importance of assessing PD-L1 on IC in tumor-associated stroma. We provide an overview of the currently available PD-L1 assays and discuss how PD-L1 testing might be integrated into the current pathology workflow and which samples are suitable for PD-L1 scoring. Finally, we will discuss emerging biomarkers for efficacy in immunotherapy.

Histological and Genetic Subtypes of TNBC

The American Society of Clinical Oncology–College of American Pathologists guidelines define TNBC as invasive breast cancers with less than 1% of cells expressing ER and PR—or less than 1% of cells expressing ER with an unknown PR status—and HER2-negative status (17,18). HER2-negative status is defined as a negative HER2 immunohistochemistry (IHC) result (0 or 1+) or an indeterminate HER2 IHC result (2+) followed by a negative in situ hybridization (ISH) result. Alternatively, HER2 negativity can be defined by a negative ISH result even in the absence of a prior IHC test (17,19). Categories of ER-low positivity were recently added for cases with 1% to 10% of TC staining for ER. In these cases, the pathology report should mention that such tumors are heterogeneous in both behavior and biology and often have gene-expression profiles more similar to ER-negative or PR-negative cancers (18). As a consequence, patients with HER2-negative, ER-low, and PR-low status may have disease with characteristics similar to those of TNBC. Oncologists should consider including these patients when designing clinical trials in advanced TNBC (20); these patients have not been included in phase 3 TNBC studies to date (13,15,21). Furthermore, TNBC itself is heterogeneous and includes diverse histological subtypes (Figure 1, A) (22). Originally, 6 molecular TNBC subtypes were defined based on gene-expression profiles, including a mesenchymal, luminal androgen receptor, immunomodulatory, mesenchymal stem-like, and 2 basal-like subtypes (23). However, this classification has been refined to 4 distinct subtypes (2,24): basal-like immune-activated, basal-like immunosuppressed, luminal androgen receptor, and mesenchymal (24). Additionally, there is a low-grade TNBC category that is molecularly distinct from all other TNBCs, has a more benign clinical behavior, and includes special-type tumors such as adenoid cystic carcinoma, secretory carcinoma, and acinic cell carcinoma. These low-grade TNBCs are not currently within the scope of immunotherapy. Overall, 50% to 70% of TNBC has basal-like features characterized by high proliferation, high tumor grade, pushing borders, and lymphocytic infiltrate (Figure 1, B). Primary basal-like TNBC tumors frequently have *TP53* mutations (84%), mutations or loss of *RB1* (20%), loss of function of *BRCA1/2* (20%), and/or amplification (but not mutation) in *PIK3CA* (49%) or *MYC* (31%); specific percentages can vary between subtypes (25). Although the mutation profile in metastatic tumors appears to be different from that of primary tumors, a statistically significant increase ($q < .05$) in the frequency of a specific gene alteration was only identified for *TP53* (26).

TNBC Pathophysiology

Cancer immunoediting is a process by which mutations in cancer cells are able to modify immune pathways in the TME and help the tumor evade anticancer immunity. The composition of the TME is variable, depending on the prevalence of different immune mediators at any given timepoint, but ultimately, the TME provides a scaffold-like structure for the TC and a source of cytokines and growth factors (27). This microenvironment surrounds the TCs and comprises an extracellular matrix and a heterogeneous population of stromal cells, including immune and inflammatory cells, endothelial cells, fibroblasts, and other mesenchymal cells and bone marrow–derived stem cells. ICs involved in anticancer immunity include dendritic cells, macrophages, natural killer cells, mast cells, neutrophils, myeloid-derived suppressor cells, B lymphocytes, and T lymphocytes (28,29).

Regulatory T cells inhibit the immune response in the TME, and their behavior is mediated by proteins such as forkhead box P (FOXP) and indoleamine-2,3-dioxygenase (30,31). The immune response of CD8+ effector T lymphocytes is regulated by costimulatory receptors such as OXO40, CD40, and 4-1BB and by co-inhibitory receptors such as lymphocyte-activation gene 3, cytotoxic T-lymphocyte–associated protein, and PD-1 (32). Disrupting these co-inhibitory pathways—for example, by inhibiting the interaction between PD-1 and PD-L1 to restore T-cell immune response—has been a successful strategy for treating various cancers. Therefore, there is great interest in the immune response and behavior of T cells within the TME. The so-called tumor-infiltrating lymphocytes (TILs) are detected in varying amounts in the different histological, clinical, and molecular subtypes of breast cancer and can be seen within the tumoral stroma (stromal TILs) or within the TC clusters. Elevated TIL counts are a prognostic factor for survival in metastatic HER2-positive breast cancer and TNBC. Stromal TILs specifically have been linked to better prognosis in TNBC (33-35). The prognosis also depends on the localization of lymphocytes, which in turn can be correlated with certain immunophenotypes (36). The best prognosis was seen in patients whose tumors had CD8+ TILs infiltrated in both the tumor epithelium and stroma, also called *inflamed*. TILs that were restricted to the stroma were associated with an intermediate prognosis, and tumors with TILs restricted to the tumor margins or absent altogether (called the *immune desert*) resulted in the worst prognosis. Furthermore, PD-L1 and other markers of inflammation were enriched in the epithelial compartment of fully inflamed tumors and in the stromal compartment of stroma-restricted tumors.

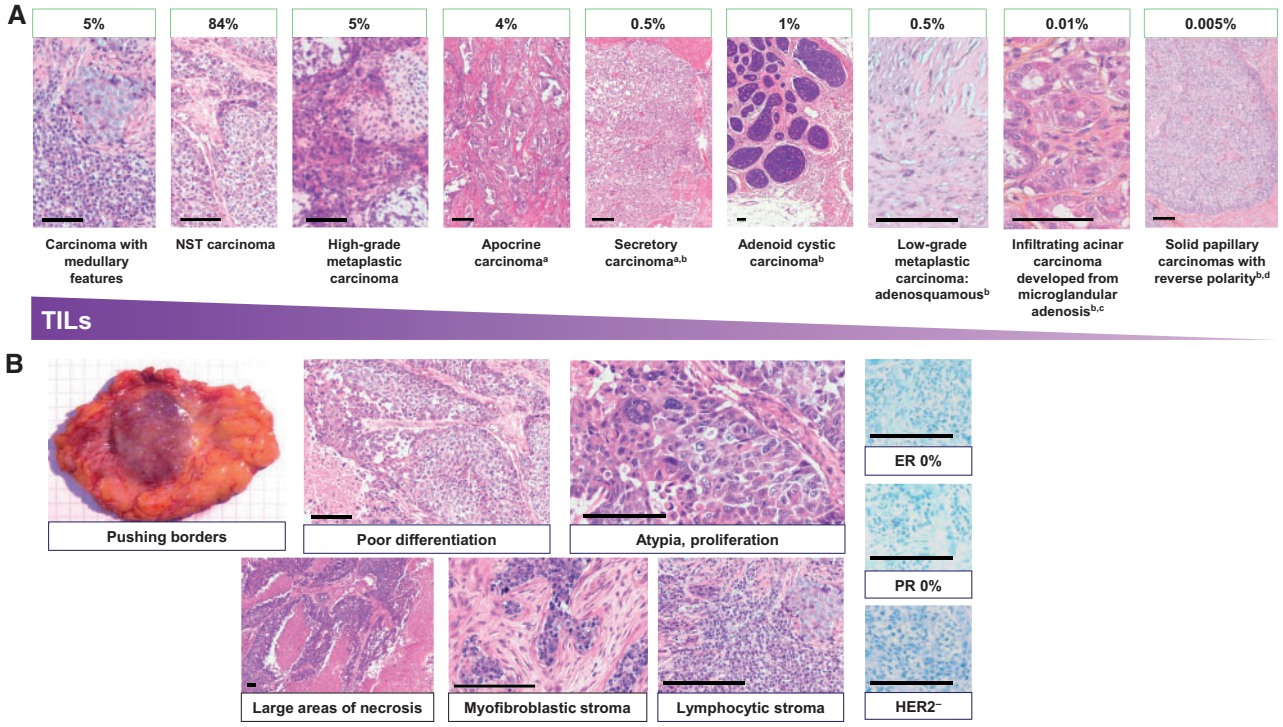

Figure 1. Diversity of histological subtypes in triple-negative breast cancer. **A)** The histological subtypes of triple-negative breast cancer are ordered here by decreasing levels of tumor-infiltrating lymphocytes (TILs) from left to right. Percentages indicate the prevalence of each subtype. **B)** 90% of triple-negative breast tumors are invasive, no special type. Immunohistochemistry may show poorly differentiated necrotic areas and high grade with lymphocytic infiltrate and myofibroblastic proliferation in the stroma. Staining for estrogen receptor (ER), progesterone receptor (PR), and HER2 expression is negative. Scale bars indicate 100 μm. [a]May also be ER+ and/or HER2+. [b]Good prognosis. [c]Image provided courtesy of Dr Magali Lacroix-Triki, Gustave Roussy, Villejuif, France. [d]Image provided courtesy of Gaëtan Mac Grogan, Institut Bergonié, Bordeaux, France. NST = no special type; TIL = tumor-infiltrating lymphocytes .

REVIEW

Immunotherapies for mTNBC

The anti–PD-L1 antibodies atezolizumab, durvalumab, and avelumab and the anti–PD-1 antibodies pembrolizumab and nivolumab have been approved for treatment of numerous cancer types (Table 1) (37,42-44). All approved biomarker assays for these antibodies involve testing for PD-L1. In the phase 3 IMpassion130 trial, atezolizumab plus *nab*-paclitaxel was the first cancer immunotherapy combination to demonstrate efficacy in the first-line treatment of patients with mTNBC expressing PD-L1 on at least 1% of tumor-infiltrating IC in the tumor area, determined using the VENTANA PD-L1 (SP142) assay (Ventana, Tucson, AZ). The co-primary endpoints were PFS and OS in the intention-to-treat (ITT) and PD-L1–positive populations. In PD-L1–positive patients, the addition of atezolizumab to *nab*-paclitaxel statistically significantly improved PFS with a median of 7.5 vs 5.5 months and a hazard ratio of 0.62 (95% confidence interval [CI] = 0.49 to 0.78; $P < .001$). These findings led to accelerated approval by the US Food and Drug Administration (FDA) and approval by the European Medicines Agency and the National Institute for Health and Care Excellence of atezolizumab plus *nab*-paclitaxel. PD-L1–positive patients also showed a clinically meaningful improvement in OS with atezolizumab plus *nab*-paclitaxel, with a median of 25.0 vs 15.5 months (HR = 0.62, 95% CI = 0.45 to 0.86). Importantly, the SP142 assay is the only companion diagnostic IHC assay for this therapy (37,49). Notably, in the final analysis of IMpassion130, the PD-L1–positive subgroup showed an unprecedented 7.5-month improvement in OS in the atezolizumab plus *nab*-paclitaxel arm (HR = 0.67, 95% CI = 0.53 to 0.86, vs placebo plus *nab*-paclitaxel), with practice-changing implications (56).

In the IMpassion130 study, PD-L1 status was determined using the SP142 assay, with positive cases defined as at least 1% PD-L1 IC positivity. IHC was performed on biopsy or resection samples from the primary breast cancer (62% of enrolled patients), biopsy samples from local recurrence, or biopsy samples from metastatic lesions in any organ except bone (Table 2). Tumor samples acceptable for PD-L1 testing included formalin-fixed, paraffin-embedded core biopsies for deep tumor tissue or excision, incision, punch, or forceps biopsies for cutaneous, subcutaneous, or mucosal lesions. PD-L1 status predicted benefit of atezolizumab plus *nab*-paclitaxel regardless of the tissue source. PD-L1–positive status was more common in primary breast tumor tissue samples than in samples from distant metastatic sites. In the metastatic setting, PD-L1–positive status was most common in lymph node metastases and least common in liver metastases (57).

The phase 3 KEYNOTE-355 study showed that anti–PD-1 therapy for mTNBC had antitumor activity in patients with PD-L1–positive tumors, defined as a CPS of at least 10. The anti–PD-1 antibody pembrolizumab demonstrated a PFS benefit vs placebo in combination with investigator's choice of chemotherapy (paclitaxel, *nab*-paclitaxel, or gemcitabine plus carboplatin) for the first-line treatment of patients with previously untreated mTNBC (13). PD-L1 expression was determined using the PD-L1 IHC 22C3 pharmDx assay (Dako, Carpinteria, CA). The dual primary endpoints were PFS and OS by tumor PD-L1 expression (CPS ≥10 and ≥1) and in the ITT population. In the population with a CPS of at least 10, pembrolizumab plus chemotherapy statistically significantly improved PFS (median = 9.7 vs 5.6 months; HR = 0.65, 95% CI = 0.49 to 0.86; $P = .0012$). The hazard ratio for the population with a CPS of at least 1 (HR

Table 1. PD-L1 IHC assays approved for cancer immunotherapy (37-48)

Drug and clone	Developer	PD-L1 IHC-positive cutoff[a]	Indication	Status with the FDA	CE IVD[b]
Nivolumab					
SP263	Ventana	TC ≥1%	Second-line nonsquamous NSCLC	No FDA guidance given	CE mark
28-8	Dako	TPS ≥1%	First-line metastatic melanoma	Complementary diagnostic	CE mark
28.8	Dako	TPS ≥1%	Second-line nonsquamous NSCLC	Complementary diagnostic	CE mark
28-8	Dako	TPS ≥1%	HNSCC	Complementary diagnostic	CE mark
28.8	Dako	TPS ≥1%	Second-line metastatic urothelial cancer	Complementary diagnostic	CE mark
Pembrolizumab					
SP263	Ventana	TC ≥1% second-line; TC ≥50% first-line	First-line, second-line NSCLC monotherapy	No FDA guidance given	CE mark
22C3	Dako	TPS ≥1%	First-line, second-line NSCLC monotherapy	Companion diagnostic	CE mark
22C3	Dako	CPS ≥1	≥ Second-line cervical cancer	Companion diagnostic	Drug indication not approved in the European Union
22C3	Dako	CPS ≥1	Third-line gastric cancer	Companion diagnostic	Drug indication not approved in the European Union
22C3	Dako	CPS ≥1	First-line HNSCC	Companion diagnostic	CE mark
22C3	Dako	CPS ≥10	First-line cis-ineligible metastatic urothelial cancer	Companion diagnostic	CE mark
22C3	Dako	CPS ≥10	Second-line esophageal	Companion diagnostic	Not CE marked
22C3	Dako	CPS ≥10	First-line TNBC	Companion diagnostic	Not CE marked
Atezolizumab					
SP142	Ventana	TC ≥50% or IC ≥10%	First-line NSCLC	Complementary diagnostic	CE mark
SP142	Ventana	IC ≥5%	First-line cis-ineligible metastatic urothelial cancer	Companion diagnostic	CE mark
SP142	Ventana	IC ≥5%	Second-line metastatic urothelial cancer	Drug indication no longer FDA approved	CE mark
SP142	Ventana	IC ≥1%	First-line TNBC	Companion diagnostic	CE mark
Durvalumab					
SP263	Ventana	TC ≥25% or IC ≥1%	Second-line metastatic urothelial cancer	Complementary diagnostic	Drug indication not approved in the European Union
SP263	Ventana	TC ≥1%	Post-CRT stage III NSCLC	No FDA guidance given	CE mark

[a]All assays score cells at any intensity. CE-IVD = CE in vitro diagnostic; CPS = combined positive score; CRT = chemoradiation therapy; FDA = Food and Drug Administration; HNSCC = head and neck squamous cell carcinoma; IC = immune cells; IHC = immunohistochemistry; NSCLC = non-small cell lung cancer; PD-L1 = programmed death-ligand 1; TC = tumor cells; TPS = tumor proportion score.
[b]The CE-IVD mark may be applied to assays that demonstrate conformity with certain requirements and allows the assay to be used to guide treatment choices according to the label for each assay.

= 0.74, 95% CI = 0.61 to 0.90; $P = .0014$) did not cross the boundary for statistical significance, so the ITT population was not formally tested (HR = 0.82, 95% CI = 0.69 to 0.97). Based on the PFS results, pembrolizumab in combination with chemotherapy was given accelerated approval for the treatment of patients with mTNBC whose tumors express PD-L1 (CPS ≥ 10) according to an FDA-approved test. The 22C3 assay was also approved as a companion diagnostic for selecting patients with mTNBC for pembrolizumab treatment.

As a result of IMpassion130 and KEYNOTE-355, guidelines recommend that medical oncologists and pathologists consider testing patients with metastatic breast cancer for PD-L1, in addition to HER2 and hormone receptors (16,20,58,59). Treatment decisions for patients with TNBC will inevitably require the accurate IHC assessment of PD-L1 expression, perhaps with 2 different assays (SP142 and 22C3) and 2 different scoring algorithms (IC and CPS). Eventually, after 2 or more immune checkpoint inhibitors become approved for mTNBC along with their corresponding companion diagnostic assays, it will be challenging to decide whether the choice of the drug should drive the selection of the assays, or conversely, the result of the assays should inform the choice of the drug.

The Importance of Scoring PD-L1 on IC in Metastatic Breast Cancer

It is worth emphasizing that substantial differences exist between the SP142 and 22C3 assays and the corresponding scoring algorithms. First, there is an intrinsic difference in the amounts of positive TC. Second, the algorithms differ in the criteria for cells included. The SP142 IC category includes acute cells and plasma cells, which are excluded from the 22C3 algorithm. Although IMpassion130 and KEYNOTE-355 highlight the importance of PD-L1 testing, the IMpassion130 study also demonstrated that a scoring algorithm that uses PD-L1 positivity on IC only can enrich for clinical benefit of atezolizumab plus *nab*-paclitaxel in mTNBC. The CPS also includes IC and predicts for clinical benefit of pembrolizumab plus chemotherapy but only at the cutoff of at least 10. In IMpassion130, 41% of patients had PD-L1 IC+ tumors and 9% had PD-L1 TC+ tumors (Figure 2, A and B), indicating that the SP142 assay identified more PD-L1 expression on IC vs TC in TNBC tumors (60). Of the PD-L1 TC+ tumors, the 9% includes 7% that are both TC+ and IC+ and 2% that are TC+ only, demonstrating that scoring PD-L1 IC captures most PD-L1 TC+ tumors (Figure 2, C) (61). These findings are noteworthy for TNBC, because these tumors have relatively high TIL counts compared with other breast cancer subtypes. IMpassion130 exploratory analyses demonstrated a PFS and OS benefit in several immune-related biomarker subgroups, including other PD-L1–positive subgroups (≥1% to <5% IC+ and ≥5% IC+) and subgroups with tumors with CD8+ T cells or high stromal TIL counts, but only in conjunction with PD-L1 IC positivity. Response to atezolizumab was seen regardless of *BRCA* status in PD-L1–positive patients (60). Therefore, tumor-infiltrating IC may be key to informing treatment decisions in mTNBC, in contrast with non-small cell lung cancer (NSCLC) in which PD-L1 expression on TC is more pronounced and both TC and IC are predictive of benefit (62,63). Interestingly, despite high TIL levels overall in TNBC, mTNBC tumors may have fewer TILs than early TNBC primary tumors, suggesting that inhibition of T-cell anticancer immunity is facilitating tumor growth and metastasis in these cases (64-66). Emerging results from phase 3 trials in early-stage TNBC may be able to confirm this hypothesis.

Table 2. Preferred standards for tissue samples

Characteristic	Recommendation
New biopsy	Deep tumor tissue or lymph nodes: core-needle biopsies ≥3 cores Cutaneous, subcutaneous, or mucosal lesions Excisional, incisional, punch, or forceps biopsies Bone metastases not acceptable
Biopsy from diagnosis/resection	Representative block from primary tumor or if not available, lymph node metastasis block with ≥50 cells
No. of viable cells	≥50

Immunotherapy in Early-Stage TNBC

Results from phase 3 trials of immunotherapy in early-stage TNBC are emerging, and the evidence to date does not suggest a role for PD-L1 testing in guiding treatment choices in this setting. Potential reasons include that early-stage TNBC tumors might contain a more active immune environment with a higher activity of CD8+ T cells and a greater diversity of T-cell receptor clones that overall are less susceptible to PD-L1–

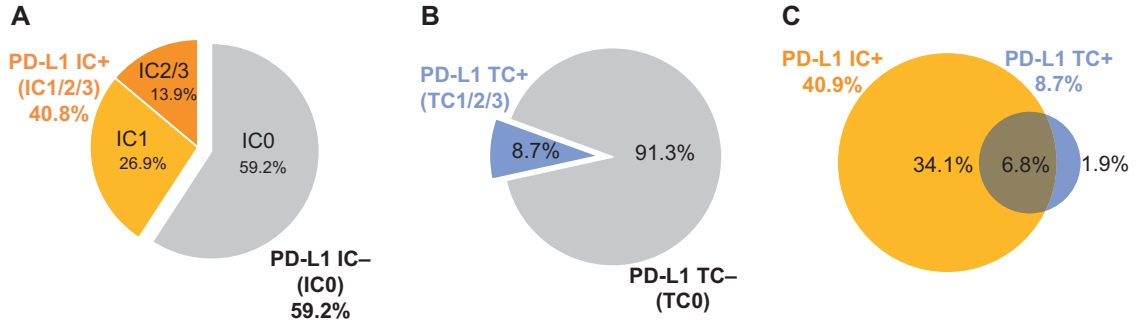

Figure 2. In IMpassion130, programmed death-ligand 1 (PD-L1) in triple-negative breast cancer was expressed mainly on tumor-infiltrating immune cells (IC). **A**) Prevalence of PD-L1 IC subgroups. **B**) Prevalence of PD-L1 tumor cell (TC) subgroups. **C**) The overlap between PD-L1 IC and TC subgroups. Most of the patients with PD-L1 expression on TC are included within the PD-L1 IC+ population. Emens LA. Atezolizumab and nab-paclitaxel in advanced triple-negative breast cancer: Biomarker evaluation of the IMpassion130 study. J Natl Cancer Inst. 2021;113(8):1005-1016, by permission of Oxford University Press.

mediated immune suppression (67). The IMpassion031 study demonstrated that atezolizumab in combination with *nab*-paclitaxel and anthracycline-based chemotherapy had a pCR benefit regardless of PD-L1 status (14). Patients with stages II or III TNBC were treated with neoadjuvant atezolizumab 840 mg or placebo every 2 weeks in combination with *nab*-paclitaxel for 12 weeks followed by doxorubicin and cyclophosphamide every 2 weeks for 8 weeks and then received surgery. Patients with nodal stage N0-N3 were included in IMpassion031. After surgery, treatment allocation was unblinded, and patients in the experimental arm were treated with atezolizumab 1200 mg every 3 weeks for 33 weeks, and patients in the control arm were observed. The coprimary endpoints were pCR rate in the ITT and PD-L1–positive (PD-L1 IC $\geq$1%) populations. The pCR rate in the ITT population for the atezolizumab vs placebo arms was 58% vs 41%, with a rate difference of 17% ($P = .0044$; statistical significance boundary, 0.0184). The pCR rates in PD-L1–positive patients did not cross the statistical significance boundary, although there was a trend toward greater pCR improvement for the PD-L1–positive compared with the ITT population (14). Taken together, current evidence indicates that patients benefit from anti–PD-L1/PD-1 therapy regardless of PD-L1 status. However, PD-L1 IC expression in early-stage TNBC might be induced by atezolizumab treatment (68).

Furthermore, KEYNOTE-522 showed that pembrolizumab in combination with carboplatin-containing chemotherapy had a pCR benefit in stages II and III TNBC, including in patients with nodal stage N0-N2. KEYNOTE-522 included neoadjuvant and adjuvant phases. Patients in the neoadjuvant phase were treated with pembrolizumab 200 mg once every 3 weeks or placebo in combination with 4 cycles of carboplatin plus paclitaxel followed by doxorubicin or epirubicin plus cyclophosphamide. After surgery, the experimental arm was treated with adjuvant pembrolizumab 200 mg once every 3 weeks vs placebo for up to 27 weeks. The primary endpoint pCR rate in the ITT population for the pembrolizumab vs placebo arms was 64.8% vs 51.2% ($P = .00055$). Higher pCR rates were seen in the PD-L1–positive (CPS $\geq$1) patients in both arms, with a pCR rate for pembrolizumab vs placebo arms of 68.9% vs 54.9%. Trial results suggest that PD-L1 status determined before neoadjuvant treatment is favorably prognostic but not predictive of the benefit of immune checkpoint inhibitors. Subsequently, longer-term outcomes for endpoints such as event-free survival were shared with the FDA Oncology Drugs Advisory Committee, and the benefit of pembrolizumab for early TNBC is currently inconclusive (69). PD-L1/PD-1 inhibitors provided additional benefit in both the PD-L1–positive and –negative groups. In contrast, PD-L1–positive status in the metastatic setting strongly predicted for benefit of checkpoint inhibition vs PD-L1–negative status. However, the prognostic value of PD-L1 expression in mTNBC is less clear. A trend toward longer PFS in PD-L1–negative vs –positive subgroups in the chemotherapy control arms was observed in IMpassion130 and KEYNOTE-355, but further study is needed to explore this hypothesis.

The PD-L1 IHC Testing Landscape in mTNBC

In addition to the SP142 and 22C3 assays discussed above, the VENTANA PD-L1 (SP263) assay (Ventana) and 28-8 assay (Dako) are FDA approved to evaluate PD-L1 expression either as complementary (ie, informative for treatment decisions) or companion (required for treatment decisions) diagnostics. These assays can detect expression on both TC and IC, albeit with different staining intensities. However, whether 1 or both are used to determine PD-L1 positivity depends on the approved scoring algorithm for the specific indication and drug (Table 1) (37-48,71).

Each of the 4 assays uses a different primary antibody, with different detection systems, staining platforms, scoring criteria, and definitions of PD-L1 positivity. Depending on the cancer type, PD-L1 status may be determined either by frequency of PD-L1–positive TC and/or an assessment of PD-L1 positivity on IC. Assessing PD-L1 IC positivity is less intuitive than that of TC because IC are more sparsely distributed and may appear in TC clusters, the intratumoral stroma, or the peritumoral stroma. Thus, assays have developed different methods to quantify IC staining, as detailed below. The scoring algorithm and cutoffs for positivity were developed in conjunction with each corresponding anti–PD-L1/PD-1 clinical program. The determination of assay cutoffs is correlated with the clinical activity of those agents, which is the basis for the specified companion diagnostic for each indication (37-44).

SP142 Assay

In the SP142 scoring algorithm, TC are categorized as TC0, TC1, TC2, or TC3, corresponding to the percentage of PD-L1–stained TC at thresholds of less than 1%, less than 5%, less than 50%, and 50% or higher, respectively. IC are categorized as IC0, IC1, IC2, or IC3 based on the percentage of tumor area containing PD-L1–expressing IC, with thresholds of less than 1%, less than 5%, less than 10%, and 10% or higher, respectively. The definition of PD-L1 positivity for atezolizumab treatment using SP142 as a companion diagnostic in mTNBC is at least 1% IC (IC1, IC2, or IC3). IC are defined as the totality of IC (eg, lymphocytes, macrophages, dendritic cells, plasma cells, and granulocytes), and they can be located either among the TC cluster or in the intratumoral or contiguous peritumoral stroma. Breast cancer tissue with PD-L1 staining higher and lower than the positive threshold (IC1, IC2, or IC3) of at least 1% is shown in Figure 3. Guidance for scoring TNBC tissue is presented in Table 3. Further details can be found in the *VENTANA PD-L1 (SP142) Assay: Interpretation Guide for Triple-Negative Breast Carcinoma (TNBC)* (71).

For first-line cisplatin-ineligible metastatic urothelial cancer (mUC), the PD-L1–positive threshold is at least 5% IC (IC2/3). Reproducibility of IC2/3 measurements in mUC samples had 95.8% inter-reader overall agreement and 93.6% intrareader agreement (72). For use of SP142 as a complementary diagnostic in NSCLC, the definition of PD-L1 positivity is at least 50% TC or at least 10% IC.

22C3 Assay

The use of 22C3 has been approved in NSCLC, UC, TNBC, and esophageal, gastric, head and neck, and cervical cancers. The algorithm that the 22C3 assay uses to determine PD-L1 positivity for pembrolizumab treatment of NSCLC calculates a tumor proportion score based only on TC staining, specifically excluding IC, and is the percentage of viable cells staining positive out of 100 TC. UC, mTNBC, esophageal squamous cell carcinoma, gastric and gastroesophageal junction adenocarcinoma, head and neck, and cervical cancers use CPS based on both TC and IC staining

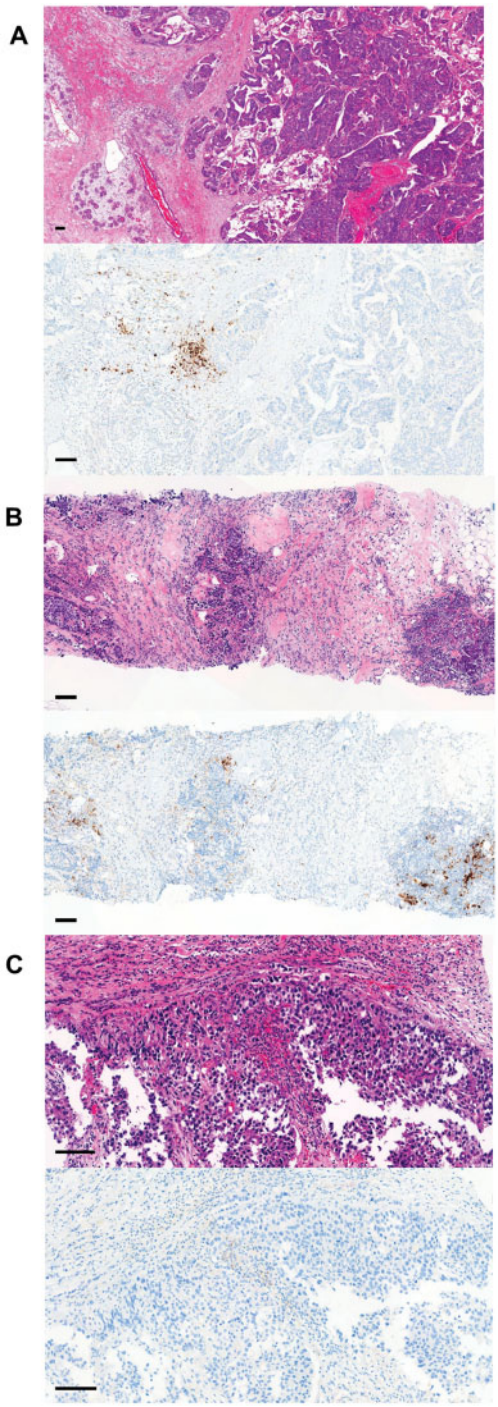

Figure 3. Images of hematoxylin and eosin-stained and the corresponding SP142-stained slides. **A**, **B**) ≥1% programmed death-ligand 1 (PD-L1)–positive tumor-infiltrating immune cell staining. **C**) <1% PD-L1–positive tumor-infiltrating immune cell staining. Scale bars indicate 100 μm. Images were provided courtesy of CellCarta.

(73). The established CPS cutoff for PD-L1 positivity in UC, mTNBC, or esophageal squamous cell carcinoma is at least 10 (73). A CPS of at least 1 is used for gastric and gastroesophageal junction adenocarcinoma, head and neck cancer, and cervical cancer (74-76). A CPS of at least 1 has also been investigated as criteria for PD-L1 positivity in mTNBC (52,77).

Table 3. SP142 scoring guidance[a]

Assessment	Criteria
Tissue evaluation	Is tissue integrity and quality sufficient?
H&E slide evaluation	Is there enough tumor content?
Control slide evaluation	Is the tonsil slide (eg, positive control) acceptable?
Sample evaluation	Is the negative control slide acceptable? Is morphology of PD-L1 (SP142)–stained slide acceptable? Is background of PD-L1 (SP142)–stained slide acceptable?
IC PD-L1 staining assessment	PD-L1 diagnostic assessment based on the percentage of the tumor area covered with discernible PD-L1 staining of any intensity in IC: PD-L1 positive ≥1% (IC1/2/3) PD-L1 negative <1% (IC0)
PD-L1–expressing IC as % of tumor area	Percentage based on visual area size estimation

[a]H&E = hematoxylin and eosin; IC = tumor-infiltrating immune cells; PD-L1 = programmed death-ligand 1.

28-8 Assay

The 28-8 assay is a complementary diagnostic for nivolumab in NSCLC, melanoma, mUC, and head and neck cancer that uses only TC in its tumor proportion scoring algorithm (38,44).

SP263 Assay

The SP263 assay uses a percentage TC scoring algorithm and has a CE in vitro diagnostic mark for pembrolizumab, nivolumab, and atezolizumab in NSCLC but is not FDA approved as a companion diagnostic (46,78).

Consistency Across Assay Platforms

Because of the inconsistent availability and high costs of instrumentation worldwide, studies have investigated the performance of the various assays across platforms in an attempt to harmonize the procedures and scoring algorithms (70,79-86). Many of these harmonization studies have been conducted on NSCLC tissue in which TC is predominantly scored instead of IC. For example, the Blueprint study compared SP142, 22C3, 28-8, and SP263 staining in NSCLC tissue. The 22C3 and 28-8 assays are optimized for detection systems developed for the Dako Autostainer Link 48 staining platform (38,39). The SP142- and SP263-based assays were developed using a different detection technology on the Ventana BenchMark platform. Furthermore, the SP142 assay uses an additional amplification step to enhance visual contrast for IC scoring. In the Blueprint study, SP142 stained fewer TC than the other 3 antibodies tested. Note, however, that in NSCLC, the SP142 scoring algorithm assigns a positive result if either the TC or the IC threshold is met. Thus, despite fewer cases meeting the TC threshold, SP142 identified the greatest number of PD-L1–positive cases because the IC assessment compensated for the lower TC–positive rate. Therefore, the scoring criteria for positivity have a large contribution to the overall PD-L1 status of a case regardless of the amount of TC staining. Furthermore, high variability in IC staining was found across assays, and consistent scoring of IC

between assays and between observers was hampered by lack of training and alignment on scoring criteria. IC scoring is not routine clinical practice and can be approached in different ways. Therefore, the inconsistent results for IC across the 4 antibodies may also depend on pathologist training rather than solely on the performance of the antibodies (79). Furthermore, although these studies may demonstrate analytical concordance between assays, or between different antibodies using a particular scoring algorithm, data correlating the assay results to clinical outcomes are limited—a comparison that would be essential to determine whether these assays identify similar populations of patients and to enable these assays to be used effectively for treatment selection (57,70).

A small exploratory study (n = 30) evaluated the analytical concordance of the SP142, SP263, 22C3, and 28-8 antibodies to score breast cancer tissue using the PD-L1 IC of at least 1% algorithm (87). There was good reproducibility and agreement among the SP142, 22C3, and 28-8 antibodies; however, the SP263 assay showed a much higher rate of positivity. These results showed that some different assays may be analytically comparable using the same algorithm and cutoff. However, correlation analysis with clinical outcomes was not performed; therefore, the results are not able to inform treatment selection.

One of the few correlations of assays with clinical outcomes has been conducted in an exploratory post hoc analysis of IMpassion130. Data from 614 biomarker-evaluable patients were used to assess the analytical concordance of the SP142, 22C3, and SP263 assays and to compare their ability to predict clinical benefit of atezolizumab plus *nab*-paclitaxel in PD-L1–positive patients according to their respective scoring algorithms. The SP142 and SP263 assays use the PD-L1 on IC cutoff of at least 1%, and the 22C3 assay uses the CPS of at least 1 cutoff. The prevalence of patients with PD-L1–positive tumors was 46% (SP142), 75% (SP263), and 81% (22C3) (57). The overall percentage agreements between the 22C3 and SP263 assays with SP142 were 64% and 69%, respectively, showing that they are not analytically equivalent to the SP142 assay (57). The 22C3 (CPS $\geq$1) and SP263 (1% IC) assays both identified a larger PD-L1–positive patient population, of which the SP142-positive ($\geq$1% IC) population was a subgroup. This SP142 subgroup had the greatest clinical benefit of atezolizumab plus *nab*-paclitaxel, with an OS hazard ratio of 0.71 for SP142 positivity. The patients who were SP142 negative but 22C3 positive or SP263 positive experienced less benefit, with OS hazard ratios of 0.92 (95% CI = 0.64 to 1.31) and 0.87 (95% CI = 0.58 to 1.29), respectively. This suggests that the survival benefit in patients who were PD-L1 positive with the 22C3 or SP263 assay was driven by the subgroup with SP142 positivity.

A follow-up study attempted to determine the cutoffs for the SP263 and 22C3 assays that would harmonize the analytical and clinical results with those obtained with the SP142 assay at the IC cutoff of at least 1% (88). Overall percentage agreement of 22C3 using the CPS and SP263 using IC scoring was subpar at less than 90% for all cutoffs evaluated. A statistical model determined that cutoffs of CPS of at least 10 and IC of at least 4% were optimal for 22C3 and SP263 analytical concordance, respectively. However, patients identified by the 22C3 CPS of at least 10 cutoff omitted 22% of SP142-positive patients and did not achieve a benefit similar to the SP142-positive patients. Patients identified by the SP263 of at least 4% cutoff achieved a PFS and OS benefit similar to that of SP142-positive patients. However, 26% of the SP142-positive patients were omitted. Overall, the 22C3 and SP263 assays were not analytically or clinically interchangeable with SP142, and currently, the SP142 remains the only clinical validated companion assay to select patients with mTNBC for treatment with atezolizumab plus *nab*-paclitaxel.

Laboratory-developed tests (LDTs) are expected to have the same drawbacks with regard to lack of training, inconsistencies in procedures, and validation of scoring algorithms. Real-world data evaluating the accuracy of LDTs in determining PD-L1 status compared with established algorithms have shown variable concordance (86,89). LDTs continue to be developed and explored but require additional validation before they should be used to stratify patients for treatment. Clinicians should pay special attention to the type of IHC clone and assay vs LDT performed and the scoring system used, because they are not fully interchangeable.

Understanding the Role of PD-L1 Expression in TNBC

The SP142 assay was optimized to score PD-L1 based on IC. Therefore, its algorithm and staining are particularly suited to measuring PD-L1 expression in TNBC, which has a strong involvement of IC in the TME. The 22C3 assay also incorporates IC into its scoring algorithm. SP142 is being used in routine clinical practice, and the SP142 and 22C3 assays are now being used in ongoing clinical studies in breast cancer, especially TNBC (13,49-52,90). Overall, the calculated prevalence of PD-L1–positive tumors can vary, even in the same patient population, because of differences in the assay platform, the inclusion of amplification of the immunohistochemical signal, and the method used to calculate PD-L1 positivity.

Overview of Breast Cancer Guidelines for Testing

Current American Society of Clinical Oncology–College of American Pathologists and National Comprehensive Cancer Network guidelines recommend that a diagnostic workup for all invasive breast cancers and breast cancer recurrences include IHC to determine ER and PR status and IHC and ISH assays to assess HER2 expression and *HER2* gene copy number, respectively (17-19,91). The introduction of the low-ER and low-PR categories adds complexity to deciding which patients should be considered for treatment that is at present recommended only for TNBC.

After the approval of atezolizumab plus *nab*-paclitaxel, published guidelines have since recommended testing for PD-L1 in recurrent TNBC or mTNBC (20,58,59,91), and clinical studies that include evaluation of PD-L1 expression on TC and IC are accumulating to present a clearer picture of decision making for anti–PD-L1/PD-1 therapies in different types of breast cancer (93-98). IMpassion130 showed that PD-L1 positivity on IC per the SP142 assay can assist oncologists in the decision to add atezolizumab to a *nab*-paclitaxel treatment regimen in the first-line setting (49).

Given these recent advancements, medical oncologists and pathologists will need to become familiar with PD-L1 as a predictive biomarker in mTNBC and develop a workflow for IHC testing. For laboratories already using the relevant platform for other tumor types, pathologist training may be the primary issue for adopting PD-L1 testing for mTNBC. The scoring algorithms that include IC or are IC only incorporate key differences from TC scoring, as discussed earlier. Digital pathology may provide some assistance in this regard by increasing the availability while maintaining the consistency of training programs (99). Laboratories without the relevant staining platforms will have the additional consideration of whether their expected

volume of samples will be worth the capital expense of adding the platform and the ongoing reagent costs. For many of these laboratories, the solution may be to send samples out to a reference laboratory that provides the test, although this has its own costs and may cause delays in treatment for patients whose prognosis is often measured in months.

Currently, several points need to be considered when developing a workflow, and several questions remain to be answered. The first of these is whether PD-L1 testing should use primary or metastatic tissue. Most comparisons of PD-L1 expression in primary and metastatic tissues have been done in lung cancer; some of these comparisons reported approximately 90% concordance and were similar across the cutoff range (100), although others have found discrepancies as high as 48% (101,102).

Results in TNBC have shown different concordance rates. Discordance rates as high as 40% for PD-L1 IC expression between primary and metastatic sites have been documented, with approximately equal proportions of cases (20%) converted from negative to positive and vice versa (103). PD-L1 IC expression was determined using the SP263 assay with a scoring algorithm based on membranous staining. Furthermore, in patients with a PD-L1–negative primary tumor, the finding of PD-L1–positive metastasis was prognostic of better survival (64,103-105). Using the SP142 assay and a cutoff of at least 1%, discrepancy between core biopsy and excision biopsy of chemotherapy-naive TNBC has been reported to be as high as 30% (106). This has been attributed to the relatively small amount of tissue present in the core biopsy. Retesting of PD-L1–negative core biopsies using larger tissue samples from the excision specimen is now in clinical practice in some laboratories.

PD-L1 expression in primary vs metastatic tissue from the IMpassion130 trial demonstrated that the prevalence of PD-L1 IC expression was higher in primary TNBC tissue than in metastatic samples from various organs. Nevertheless, the benefit from atezolizumab plus *nab*-paclitaxel was observed in patients with PD-L1 expression, regardless of the tissue measured (57). However, in light of the slightly lower prevalence of PD-L1–positive IC seen in metastatic tissue, especially with liver metastases, a metastatic biopsy result that is negative would justify evaluation of the primary tumor, which may be more representative of overall PD-L1 IC status. Nevertheless, PD-L1 expression of at least 1% in IC from any tissue site is adequate to indicate PD-L1-positive status.

PD-L1 expression in primary sites vs lymph node metastases has been shown to be heterogeneous using an LDT. In fact, the differences in expression may have some prognostic or predictive value. Elevated PD-L1 expression in lymph node TC and lymphocytes was associated with worse outcomes, suggesting that both primary and lymph node PD-L1 status may be useful for indicating whether a patient is appropriate for PD-L1–targeted therapy (107). Conversely, data on PD-L1 expression in bone metastases are limited, and assays have not been validated to analyze tissues of this nature.

The rate of conversion from PD-L1–negative status to PD-L1–positive status (or the opposite) over time is still being explored; however, reports in NSCLC and TNBC suggest that conversion of PD-L1 status may occur during treatment with PD-L1–targeted treatment. Therefore, assessing PD-L1 expression in resected specimens and re-biopsy specimens may provide relevant information (68,108).

In view of the diverse nature of the tests used and assessment criteria, more work is needed to assess the true extent of any discrepancy between TNBC tumor at presentation and recurrence, between primary and metastatic sites, and among various metastatic sites. Taken together, PD-L1 has demonstrated its utility as a biomarker that can now be used to identify patients most likely to respond to treatment with atezolizumab plus *nab*-paclitaxel and pembrolizumab plus chemotherapy for mTNBC, joining the list of key breast cancer biomarkers such as *BRCA*, ER, PR, and HER2.

Looking Forward: Novel Biomarkers for Immunotherapy in TNBCs

Tumor-Infiltrating Lymphocytes

Various subtypes of TILs have inhibitory and stimulatory effects on the TME, with concomitant effects on prognosis and progression. A meta-analysis of 37 TNBC studies showed that high TIL levels predicted better pCR, with each 10% increase in percentage of TILs leading to an increase in the pCR rate by a factor of 1.09. A corresponding improvement in OS (HR = 0.90) and DFS (HR = 0.92) was observed. High CD4+ TIL levels were associated with better OS (HR = 0.49) and DFS (HR = 0.54), but CD8+ and FOXP+ TIL levels were associated only with DFS (HR = 0.55 and 0.50, respectively) (109).

Understanding the evolution of the TME as TNBC evolves from early-stage TNBC to mTNBC may help inform prognosis and selection of immunotherapy. Evidence appears to show that TNBC becomes less immunogenic as the disease evolves from early stage to metastatic. A study of longitudinally collected tumor samples from patients with TNBC (n = 33) used multipanel immunofluorescence to analyze TILs (110). Stromal T cells (P = .008), PD-1+ T cells (P = .02), and the total of stromal and tumor PD-1+ T cells (P = .04) statistically significantly decreased as the disease advanced from primary to metastatic. Good outcomes were associated with higher levels of stromal or tumor PD-1+ CD8+ T cells, PD-1+ CD4+ T helper cells and all PD-1+ T cells, as well as higher levels of tumor myeloid cells, CD4+ T helper cells, regulatory T cells, and PD-1+ regulatory T cells. Another study performed targeted exome sequencing and whole-transcriptome sequencing on paired primary and recurrent TNBC specimens (n = 43) (111). The proportion of basal-like immune-activated molecular subtypes decreased from 42.2% to 17.2%. A modest but statistically significant (P = .03) decrease in stromal TIL levels was also observed. Finally, gene signatures for interferon-ɣ (P = .013), T-cell inflamed (P = .004), helper T-cell response activating (P = .049), and immune activating (P = .035) were statistically significantly decreased in metastatic samples.

Tumor Mutational Burden

The somatic evolution of TC drives oncogenesis and may lead to the transcription of altered proteins, some of which may result in immunogenic neoantigens (112). Cancer immunoediting is a process by which somatic mutations can enable cancer cells to evade the host's mounting immune response. Thus, high tumor mutational burden (TMB) is seen in many immunogenic tumors (113). Tumors that harbor impairment of DNA damage repair are characterized by an increase in the number of somatic mutations and high TMB. Furthermore, TMB has been shown to increase sensitivity to immunotherapy in NSCLC (114). TMB levels in breast cancer overall are lower compared with melanoma or NSCLC (113). Publicly available genomic data from 3969 patients with breast cancer from 6 different cohorts showed that metastatic tumors had high TMB (≥10 mut/Mb) more frequently than primary tumors (8.4% vs 2.9%). Median TMB in patients with TNBC was higher than for other subtypes;

approximately 3.8% of TNBCs had high TMB. In patients with mTNBC (n = 62) who received anti–PD-L1/PD-1 monotherapy or in combination with targeted agents or chemotherapy, high vs low TMB was associated with longer PFS (12.5 vs 3.7 months; $P = .04$). No benefit for high TMB was seen in those receiving chemotherapy or noncheckpoint inhibitor regimens (115). In IMpassion130, the PFS benefit of atezolizumab when combined with *nab*-paclitaxel for mTNBC was highest in the top TMB quartile (HR = 0.56, 95% CI = 0.38 to 0.81) and was not correlated to PD-L1 expression (116,117).

Conclusion

With the advent of atezolizumab and the likely approval of pembrolizumab for mTNBC, patients with a more aggressive disease with poor outcomes, despite receiving conventional treatments, now have a personalized, effective immunotherapy option. Integrating PD-L1 testing into the current workflows for these patients is needed to offer this treatment option to patients. Research on the TME continues to shed light on the factors predicting response and resistance to immunotherapy in TNBC and may eventually guide personalized selection of targeted combination therapies.

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Data Availability

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