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PD-L1 in breast cancer: comparative analysis of 3 different antibodies☆

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Summary The interaction of programmed cell death-1 and its ligand-1 (PD-L1) serves as a regulatory check against excessive immune response to antigen and autoimmunity. We compared the performance of 3 different PD-L1 antibodies (Ventana SP263, Dako 22C3, and BioCare RbMCAL10 antibodies) in 136 invasive ductal carcinoma specimens including 43 primary, 48 locally metastatic, and 46 distantly metastatic diseases. PD-L1 expression was correlated with clinicopathologic parameters including tumor size, grade, lymphovascular invasion, estrogen receptor, progesterone receptor, HER2, Ki67, molecular type, and triple-negative status. There was excellent agreement between the 3 antibodies, with highly significant κ values ($P \leq .001$). PD-L1 expression was more likely to be associated with higher tumor grade and estrogen receptor–negative, progesterone receptor–negative, triple-negative, and highly proliferative tumors ($P < .001$). When we studied PD-L1 expression at 0, 1%-9%, 10%-49%, and ≥50% cutoff points by the 3 antibodies, there were 20 discordant cases between the antibodies. Sixteen were of inconsequential impact as far as low and high PD-L1 expression. The 4 differences between antibodies did exhibit an interesting pattern of expression, where there was a general agreement between the BioCare and Ventana antibodies with consistently higher PD-L1 expression compared with the Dako antibody. Given the high concordance, it is not surprising that all 3 antibodies demonstrated the same associations with all pathologic and clinical parameters studied. Standardization studies to identify reliable biomarkers that help in patient selection for immune therapy to improve the risk-benefit ratio for these drugs are still needed.

1. Introduction

Cancers use multiple mechanisms to evade the immune response. Programmed death-1 (PD-1) is an inhibitory signaling receptor on the surface of activated T and B cells [1]. Its ligand programmed cell death ligand-1 (PD-L1) has been reported to be expressed on tumors cells and stromal tumor-infiltrating lymphocytes (TILs). The normal physiological role of this protein is to bind to PD-1 receptors expressed on the surfaces of

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Table 1 Protocols for immunohistochemistry

Antibody	Vendor	Titer	Time	Epitope retrieval	Method of detection
ER	BioCare Medical	1:1000	30 min	BioCare Nuclear Decloaker	Envision + LP, mouse (Dako)
PgR	Dako	1:5000	30 min	Citrate, pH 6	Envision + LP, mouse (Dako)
Ki-67	Dako	1:1000	30 min	BioCare Reveal	Envision + LP, mouse (Dako)
HercepTest	Dako	P.D. HER2	30 min	Per kit instructions	Kit Components (K5204, Dako)
PD-L1 RbM CAL10	BioCare Medical	1:100	30 min	BioCare Decloaker	BioCare Mach 2 Rabbit HRP-Polymer
PD-L1 22C3 pharmDx	Dako	Kit	30 min	Flex TRS Low PT Link	Dako pharmDx Kit Visualization Reagent
PD-L1 SP263	Ventana Roche	Kit	16 min	Ultra CC1 64 Min	Ventana OptiView Kit

Abbreviation: HRP, horseradish peroxidase; P.D., prediluted.

activated cytotoxic T cells [2]. This PD-1/PD-L1 interaction serves as a regulatory check against excessive immune response to antigen and autoimmunity. Recent data suggest that the PD-1 pathway may be an active immune checkpoint in a variety of cancers. Targeting the PD-1/PD-L1 pathway may prevent inhibitory T-cell signaling and reactivate T cells to mediate tumor cell killing. Recent exciting studies have highlighted the therapeutic potential of agents that target the PD-1/PD-L1 pathway in patients with advanced cancers such as melanoma, non–small cell lung cancer, pancreatic cancer, esophageal cancer, squamous cell carcinoma of the head and neck, renal cell carcinoma, and urothelial carcinoma [2-4]. New classes of drugs either singly or in combination such as pembrolizumab provide cancer patients with a chance for a long-term and durable response [2-7]. Pembrolizumab is a humanized monoclonal antibody that binds to PD-1. Several recent clinical trials using pembrolizumab highlighted its value as a new option for first-line treatment or in combination for patients with advanced non–small cell lung cancers [5-7]. Moreover, recent studies have highlighted the potential value of evaluating PD-L1 expression as a predictive marker in breast cancer immunotherapy, particularly for triple-negative (TN) molecular type [8-12].

The value of PD-L1 detection by immunohistochemistry (IHC) as a valuable marker is confounded by many unresolved issues such as different detecting antibodies, different staining protocols and platforms, and different cutoff points in addition to variable tissue preparations and variable tumors with different characteristics. Some studies have agreed upon PD-L1 expression from low (≥1%-49%) to high (≥50%-100%) as an accepted standard in lung cancer. It is not clear, however, whether cutoffs using frequency of positive cells and/or intensity of PD-L1 expression are of value in predicting the response to immune therapy in other cancers such as breast cancer. Given the concerns surrounding the analytic and clinical validity of PD-L1 testing, it is possible that a negative test result with one antibody might be changed to a positive test result using a different assay and antibody.

The aim of this study is to compare the performance of 3 commercially available PD-L1 antibodies (Dako 22C3, Agilent, Santa Clara, CA, USA, Ventana SP263, Ventana Medical Systems, Roche, Tucson, AZ, USA and BioCare RbM CAL10, Pacheco, CA, USA) in breast cancer.

Table 2 Distribution of clinical and histopathologic parameters in breast cancer patients

Clinical feature or frequency of biomarker expression	Number
Age at diagnosis	n = 94
<50 y	33 (35%)
≥50 y	61 (65%)
Surgical procedure	n = 136
Breast core needle biopsies	42 (31%)
Lymph node core needle biopsies	48 (35%)
Distant metastasis biopsy/excision	46 (34%)
Grade	n = 90
II	36 (40%)
III	33 (37%)
Mets with grade unknown in primary	21 (23%)
Lymphovascular invasion	n = 48
Not identified	31 (65%)
Suspicious	2 (4%)
Present	15 (31%)
Molecular subtype	n = 136
Luminal A	40 (29%)
Luminal B	55 (40%)
TN	25 (18%)
HER2	6 (4%)
Unknown	10 (7%)
ER positivity, ≥1%	n = 88
No	19 (22%)
Yes	59 (67%)
Not known	10 (11%)
PgR positivity, ≥1%	n = 88
No	37 (42%)
Yes	41 (47%)
Not known	10 (11%)
HER2 positivity (3+)	n = 88
No	67 (76%)
Yes	11 (13%)
Not known	10 (11%)
High Ki-67 (expression ≥20%)	n = 88
No	34 (39%)
Yes	40 (45%)
Not known	14 (16%)
TN	n = 88
No	63 (72%)
Yes	15 (17%)
Not known	10 (11%)

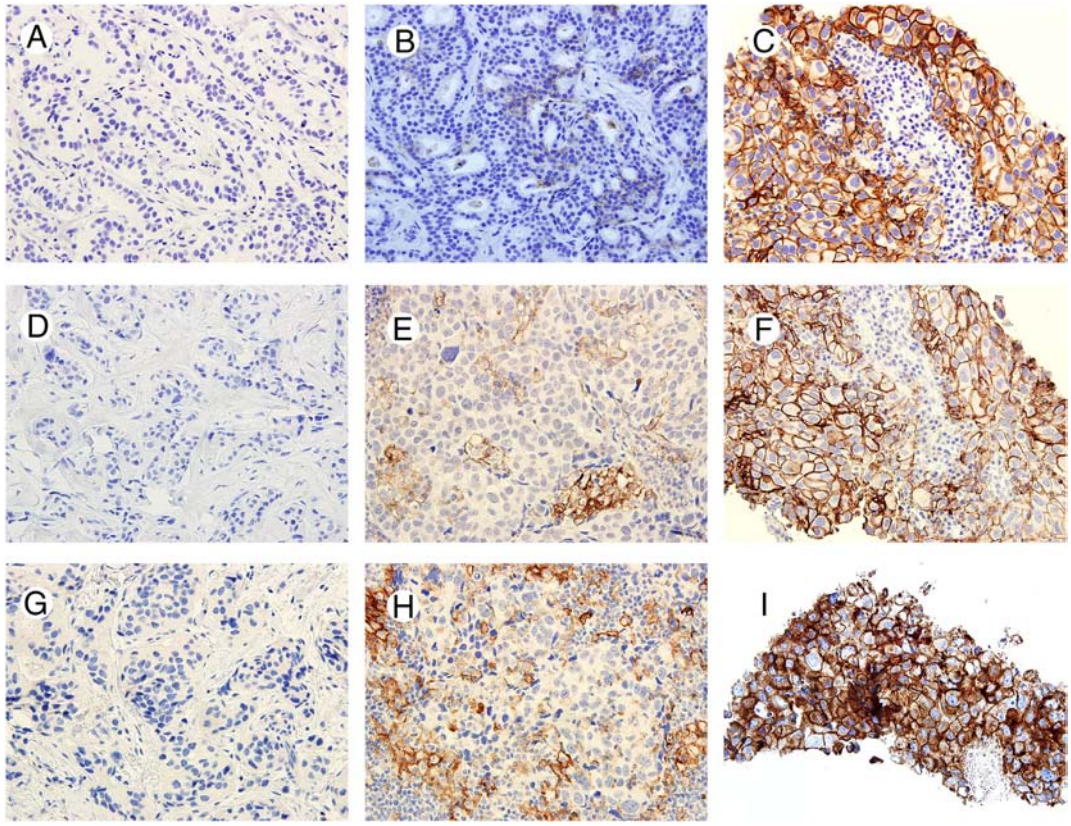

Figure Representative examples of PD-L1 expression in breast cancer using 3 different antibodies: Dako 22C3, Ventana SP263, and BioCare RbM CAL10. Examples of negative, low, and high expression for BioCare antibody are shown in panels A, B, and C, respectively; examples for the Dako antibody are shown in panels D, E and F, respectively; and examples for the Ventana antibody are shown in panels G, H, and I. PD-L1 scoring was divided into 3 groups: those with zero staining were considered "negative," those with 1%-49% positive cells were considered "low PD-L1 expression," and those with 50%-100% positive cells were considered "high PD-L1 expression" (A, B, D, and G, original magnification ×200; C, E, H, and I, ×400).

2. Materials and methods

2.1. Patient cohort

This study was approved by the institutional review committee at the University of Kansas Medical Center. A total of 136 specimens including 42 primary breast cancers, 48 metastatic diseases in regional Lymph nodes (LNs) (42 paired to the primary tumors), and 46 nonpaired distant metastases (15 paired to the primary tumors) diagnosed between 2007 and 2016 were examined. The samples were taken from 42 breast core needle biopsy specimens, 48 lymph node core needle biopsy specimens, and 46 excisions of metastases. All the specimens were diagnosed as invasive ductal carcinomas. All tumors were graded using the modified "Nottingham" histological scoring system. Histopathologic parameters, including histologic grade and type; estrogen receptor (ER), progesterone receptor (PgR), and HER2 status; and proliferation index, were extracted from patient pathology records. Additional parameters including patients' age, tumor size, local and distant metastases, and lymphovascular invasion for primary tumor and LN specimens were also recorded.

2.2. Immunohistochemistry

At diagnosis, tissue blocks of tissue fixed with 10% neutral buffered formalin containing the most representative and well-preserved tumor were selected for immunohistochemical analysis of tumor proliferation index (determined by Ki-67 immunostaining), ER, PgR, and HER2. To determine Ki-67 labeling, the percentage of nuclei with immunopositivity was

Table 3 Agreement between 3 PD-L1 antibodies

Biomarker expression parameter	BioCare *vs* Dako	Ventana *vs* Dako	BioCare *vs* Ventana
Percent positive cells (absolute values)	0.581	0.600	0.650
Level of expression: negative, low, high	0.845	0.841	0.940
Status: negative or positive	0.872	0.902	0.969

NOTE. Cohen κ statistic depended on which 2 antibodies were being compared and the parameter being assessed.

Table 4 PD-L1-1 expression in primary, locally, and distantly metastatic breast cancer: comparative analysis of 3 different antibodies

Site	Dako (22C3)			Ventana (SP263)			BioCare (RbM CAL10)		
	Negative	Positive		Negative	Positive		Negative	Positive	
		Low	High		Low	High		Low	High
Primary	35 (83%)	5 (12%)	2 (5%)	34 (81%)	5 (12%)	3 (7%)	34 (81%)	5 (12%)	3 (27)
Local metastasis	40 (83%)	7 (15%)	1 (2%)	39 (81%)	5 (10%)	4 (8%)	38 (79%)	7 (15%)	3 (8%)
Distant metastasis	44 (96%)	2 (4%)	0 (0%)	45 (98%)	1 (2%)	0 (0%)	45 (98%)	1 (2%)	0 (0%)
Total	119	17		118	18		117	19	

determined using the Automated Cellular Imaging System (San Juan Capistrano, CA). For ER and PgR, both a CAS-200 (Cell Analysis System) image analyzer (Bacus Laboratory, Chicago, IL) and the Automated Cellular Imaging System were used for scoring. Positivity for ER and/or PgR was defined as greater than 1% nuclear staining. Positive (3+) HER2 staining was defined as greater than 10% strong membranous staining, per scoring instructions included in the HercepTest kit (DAKO, Carpinteria, CA). HER2 positivity being considered as complete, intense, circumferential membrane staining in >10% of invasive tumor cells according to current American Society of Clinical Oncology and College of American Pathologists guidelines. Fluorescence in situ hybridization testing for *HER2* amplification was performed whenever equivocal results (2+) were rendered.

Immunohistochemical analysis for PD-L1 expression in tumor cells was performed using 3 commercially available PD-L1 antibodies: the Roche Biomedical Ventana (SP263) antibody, the Dako (22C3) pharmDX antibody, and BioCare Medical RbMCAL 10 antibody. PD-L1 Ventana and Dako assays were performed per manufacturer specifications on the Dako Link AS-48 autostainer system and the Ventana Ultraview system, respectively. The BioCare assay was performed on the BioCare autostainer. Table 1 highlights the different IHC protocols with the different vendors, antibody titers, incubation time, epitope retrieval, and detection methods.

Tumor cells with partial or complete cell membrane PD-L1 staining were considered positive. Cytoplasmic PD-L1 staining, although occasionally noted, and staining intensity were ignored while scoring the percent of tumor cell positivity for PD-L1. For PD-L1 scoring, the results were divided into 3 groups: those with zero staining were considered "negative," those with 1%-49% positive cells were considered "low positive," and those with 50%-100% positive cells were considered "high positive." In addition, a 4-category grouping was evaluated using 0%, 1%-9%, 10%-49%, and 50%-100% positive cells.

Table 5 Expression of PD-L1 in breast cancer and its correlation with tumor hormonal status

Hormonal status		PD-L1 [a]	
		Negative n = 79	Positive n = 9
ER positive	No	11 (58%)	8 (42%)
	Yes	58 (98%)	1 (2%)
	Unknown	10 (100%)	0 (0%)
PgR positive	No	29 (78%)	8 (22%)
	Yes	40 (98%)	1 (2%)
	Unknown	10 (100%)	0 (0%)
HER2 positive	No	60 (90%)	7 (10%)
	Yes	9 (82%)	2 (18%)
	Unknown	10 (100%)	0 (0%)
Ki67 >20%	No	34 (100%)	0 (0%)
	Yes	31 (78%)	9 (22%)
	Unknown	14 (100%)	0 (0%)
Triple negative	No	60 (95%)	3 (5%)
	Yes	9 (60%)	6 (40%)
	Unknown	10 (100%)	0 (0%)

[a] Specific associations for the Dako (22C3) antibody are shown, but the same overall results were obtained with the other 2 antibodies.

2.3. Statistical analysis

The results of IHC assessment for ER, PgR, Her-2, and PD-L1 were analyzed either using the percent of positive cells, dichotomized into negative versus positive, or after clustering into discrete ranges of frequency of expression. Comparison of numerical values for specimens from different tissues (primary, lymph node, distant metastasis) was performed by nonparametric Mann-Whitney test (for unpaired specimens) or Wilcoxon signed-rank test for paired specimens. Categorical variables were assessed by Fisher exact test. Agreement between the various PD-L1 antibodies was evaluated using Cohen κ statistic. All tests were 2 sided, with $P < .05$ considered statistically significant. Because of the exploratory nature of all analyses, no corrections were made for multiple comparisons.

3. Results

3.1. Distribution of clinical and histopathologic parameters in breast cancer

Table 2 summarizes the clinicopathologic parameters for the 136 specimens from 90 patients included in our study. Sixty-five percent of the patients were 50 years or older. Forty percent of the specimens were grade II, and 37% were grade

Table 6 Comparative analysis of the concordant-discordant PD-L1 expression between the 3 antibodies at various cutoff points

Percent cutoff values for positive tumor cells	PD-L1 expression (concordance/discordance)		
	PD-L1 BioCare	PD-L1 Dako	PD-L1 Ventana
0% (complete agreement)	118	118	118
0%	1 [a]	3	2
2%-9%	6 [a]	6	6
10%-49%	7 [a]	8	5
50%-100%	6 [a]	3	7

[a] Number of discordant specimens between the 3 antibodies where there is not a universal result of zero at various cutoff points.

III. The remaining 23% were distant metastases with unknown grades of their primary tumors. Sixty-seven percent and 47% of the tumor plus metastatic lesions were ER and PgR positive, respectively. A significant percentage (45%) of this cohort of samples had a high Ki-67 expression of ≥20%. Based on these findings, further molecular subtyping revealed 29% luminal A, 40% luminal B, 18% TN, and 4% HER2 subtypes of breast cancer. Seven percent of the tumors/specimens had no molecular data available.

3.2. Differential expression of PD-L1 in breast cancer and its correlation with tumor aggression

Generally, the 3 antibodies performed equally well. Of the 136 specimens, 119 (88%) were negative by the Dako antibody, 118 (87%) by Ventana antibody, and 117 (86%) by BioCare antibody. The Figure highlights examples of the staining patterns of the 3 different antibodies when we used the following cutoffs: PD-L1 negative (0% staining), low PD-L1 expression (1%-49%), and high expression (50%-100% staining). Of the 136 comparisons between the 3 antibodies, only in 4 instances was there a discrepancy in terms of classification as PD-L1 negative (0% staining) versus PD-L1 positive (1%-100% staining). There was generally excellent agreement between the 3 antibodies, with all values of κ being highly statistically significant ($P \leq .001$) (Table 3). The exact value of κ was dependent upon which 2 antibodies were being compared and the expression parameter being tested.

This high concordance is qualified by the fact that 86%-88% of specimens were uniformly negative (Table 4). The proportions of the negative PD-L1 primary, locally metastatic, and distantly metastatic tumors were nearly identical for the 3 antibodies. There was a trend in finding higher percentages of negative tumors in the metastatic compared with the primary or local lymph node specimens (Table 4). PD-L1 expression was more likely to be associated with grade 3, with no grade 2 specimen being PD-L1 positive ($P < .001$ for all 3 antibodies). Likewise, PD-L1 expression was more likely in ER−, PgR−, TN, and highly proliferative specimens ($P < .001$) (Table 5). In contrast, there was significantly near total absence of PD-L1 expression in distant metastases compared with Breast Cancer (BC) and LNs (2%-4% in distant metastases versus 17%-20% in BC and LN, $P = .009$). When PD-L1 expression at 0, 1%-9%, 10%-49%, and ≥50% cutoff points by the 3 antibodies there were a total of 20 discordant cases between the different antibodies studied (Table 6). One hundred eighteen specimens studied were uniformly negative by the 3 antibodies (Table 6). Sixteen discordant samples were of inconsequential impact as far as low and high PD-L1 expression. The 4 differences between antibodies did exhibit an interesting pattern of expression, where there was a general agreement between the BioCare and Ventana antibodies with consistently higher PD-L1 expression compared with the Dako antibody. Two of these samples showed high PD-L1 expression by the BioCare and Ventana antibodies with no expression by the Dako antibody. One from a primary TN subtype and 3 from regional LN specimens including one from a HER2 primary and 2 from TN primary subtypes.

4. Discussion

At least 5 therapeutic anti–PD-1 or PD-L1 monoclonal antibodies are currently available for evaluating PD-L1 expression in many types of cancer [13-18]. Unfortunately, each clinical trial evaluating the PD-1/PD-L1 status has elected to use a custom primary antibody along with a specific staining platform and unique scoring criteria, making it very difficult for investigators to accurately compare results correlating staining patterns with treatment selection. This is very well illustrated in the case of lung cancer and PD-L1 testing. Pharmaceutical companies partnering with outstanding investigators went into great detail in describing their preferred guidelines. There are now published guidelines for what antibody to use, criteria for appropriate tissue handling, where to conduct the test, what platform, how to evaluate the tumor cells and report results, and whether to consider these tests as companion or complementary diagnostics.

This chaotic environment has created confusion for patients, clinicians, and pathologists alike. One has to consider the economic issues related to the highly expensive FDA-cleared assays in an era where there is a trend of decreasing reimbursements in pathology. It is inconceivable to perform a unique FDA-cleared assay for each marker and disease following the recommendation of a certain biopharmaceutical-sponsored or investigator-driven study. Indeed, many investigators have recently recommended an urgent need to harmonize approaches for PD-L1 testing independent of biopharma for realistic economic and practice expectations in PD-L1 assessment for targeted therapy [13-18].

Similar to Scheel et al [14], our study compared 2 well-studied PD-L1 monoclonal antibodies with staining and reporting performed according to the FDA-approved manufacturer guidelines, plus a third antibody not used in any of the clinical trials studied. The results for lung cancer demonstrated

that pulmonary adenocarcinoma and squamous carcinoma cells could be reproducibly scored by 4 different PD-L1 assays including 28-8, 22C3, SP142, and SP263 using an integrated scoring system. Different scoring cutoffs for the different antibodies were independently compared by 2 pathologists. For our integrated approach in breast cancer, we elected to only score tumor cells, excluding the immune cells. Our results have shown that the 3 studied antibodies (Dako 22C3, Ventana SP263, and BioCare RbM CAL10) exhibited similar if not identical performance with high κ values. PD-L1 expression is heterogeneously expressed in tumor cells. PD-L1 expression was more prevalent among more aggressive tumors with a higher histologic grade ($P < .001$ for all 3 antibodies) and/or a TN status (Table 6, $P < .001$). Locally metastatic breast cancer cells showed the same pattern of PD-L1 expression as primary tumors. Eighty-six percent of specimens were uniformly negative by the 3 antibodies. Ninety-six percent of the positive specimens were TN tumors. Sixteen discordant specimens were of inconsequential impact as far as low and high PD-L1 expression. Similar findings were noted by the Scheel et al [14] team where the 4 tested PD-L1 assays did not show comparable staining patterns in all cases. Only 4 of the studied specimens showed a significant discordant pattern of PD-L1 expression by the 3 antibodies, where there was a general agreement between the BioCare and Ventana antibodies with consistently higher PD-L1 expression compared with the Dako antibody. Two of these specimens showed high PD-L1 expression by the BioCare and Ventana antibodies with no expression by the Dako antibody.

In the blueprint PD-L1 IHC Assay Comparison Project, Hirsch et al [15] similarly compared the performance of 4 PD-L1 IHC assays (22C3, 28-8, SP142, and SP263) on 39 NSCLC cases. Analytical comparison demonstrated that the percentage of PD-L1–stained tumor cells was comparable when the 22C3, 28-8, and SP263 assays were used, whereas the SP142 assay exhibited fewer stained tumor cells overall [15]. However, when they compared the assays' cutoffs, the study indicated that despite similar analytical performance of PD-L1 expression for 3 assays, interchanging assays and cutoffs would lead to "misclassification" of PD-L1 status for some patients. The authors recommended that more studies are needed for standardized therapy-related PD-L1 cutoffs [15]. Similarly, in the AstraZeneca study, Ratcliffe et al [16] have demonstrated excellent correlation of 3 PD-L1 IHC assays (22C3, 28-8, and SP263) across multiple protein expression cutoffs in non–small cell lung cancer with excellent interobserver reproducibility. There was greater than 90% overall percentage agreement between the different assays at $\leq$1%, 10%, 25%, and $\geq$50% cutoffs [16]. In a study sponsored by Bristol Myers Squibb, the Yale Cancer Center team also compared the performance of the 4 available PD-L1 assay tests including 22C3, 28-8, SP142, and E1L3N. They found that the SP142 assay failed to reveal comparable levels of PD-L1 compared with the rest [17]. They also reported that the 28-8 and E1L3N assays were very comparable, whereas the 22C3 assay had slightly lower sensitivity [17]. Recently, Sun et al [18] have similarly shown an adequate concordance among reviewers evaluating PD-L1 expression in TN breast cancer and immune cells by 3 different antibodies including 28-8, E1L3n, and SP142 at various cutoff values. The concordance rate between 28-8 and E1L3N was high in both cancer cells and immune cells, whereas there were low concordance rates between SP142 and the other 2 antibodies. This is suggestive that concordance between the different PD-L1 antibodies could vary not only among tumor types but within a single tumor type at various cutoffs. There are several variables that impact the analytical validity of PD-L1 testing, and to date, there is no single validated antibody for this testing. One critically important variable could be related to the epitope identified by the different antibodies. The Dako 22C3 antibody only identifies the extracellular domain of the PD-L1 receptor, whereas the Ventana and BioCare antibodies bind to the intracellular domain of the receptor.

Studies have shown that tumors express PD-L1 through cytokine-driven and intrinsic pathways. The former appears to be dependent on the presence of tumor-infiltrating lymphocytes [19], whereas the latter is not. Multiple mechanisms could lead to PD-L1 expression including chromosomal amplification, activation in EGFR driven, PI3K/AKT/mTOR or the JAK-STAT pathways [20,21]. These studies indicate that PD-L1 is at least in part regulated at both the transcriptional and translational levels.

Recent studies have shown that PD-L1 is also expressed in breast cancer, however, with considerable heterogeneity across breast cancer subtypes and stages [8-10,18,22-24]. Our results are similar to the ones reported in the literature where PD-L1 expression was shown to be associated with a variety of adverse features such as higher grade, negative hormonal status, positive HER2 status, lymphovascular invasion, and TN status [25-27]. PD-L1 is expressed in approximately 20% to 58% of TN tumors, and it is not clear whether PD-L1 expression is predictive of response to immune therapy in TN tumors [9,22,27-30]. Most studies have shown an overall 10%-20% PD-L1 expression in all types of breast cancer combined, including TN tumors [8,10]. There is now a growing body of emerging evidence on the clinical efficacy of agents targeting PD-1/PD-L1 in TN tumors [31], and they have shown that binding of PD-1 to its ligands results in the downregulation of lymphocyte activation and that inhibition of the interaction between PD-1 and its ligands promotes immune responses. A single-arm study has recently demonstrated that in PD-L1–expressing advanced pretreated TN tumors, pembrolizumab produced response rates of 18% [31].

In conclusion, all 3 studied antibodies exhibited similar if not identical performance. Given the high concordance, it is not surprising that all 3 antibodies demonstrated the same associations with all pathologic and clinical parameters studied. Thus, as in the case with quantitation of PD-L1 in lung cancer and melanoma, pathologists might have the option of using less expensive reagents for the evaluation of this marker in breast cancer. Standardization studies to identify reliable biomarkers which would aid patient selection for immune

therapy and further improve the risk-benefit ratio for these drugs are still needed. On rare occasions, however, certain markers for PD-L1 alone as a marker of selection and response to these agents might exclude some patients who have the potential of benefiting. In our quest to fight cancer, we still need to learn more about its molecular characteristics, the host immune system, as well as the environmental factors and their continuous evolving interactions with each other. Even with the increase in the number of drugs in this new class of cancer treatment, challenges do exist. Just as bacterial organisms evolve resistance to antibiotics, cancer cells, by using numerous tactics, many of them still unknown, have the capability of knowing how to escape the killing by the immune system.

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