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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%)