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A Quantitative Comparison of Antibodies to PD-L1

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Abstract

Importance—There are at least four immunohistochemistry assays for PD-L1 at various stages of interaction with the FDA as companion or complementary diagnostic tests for benefit from PD-1 axis therapies. The performance of each assay for selection of patients that respond to therapy has been published, but no data has been published that compares the assays to one another or to direct measurements of PD-L1.

Objective—To determine whether the antibody reagents are interchangeable, we quantitatively compared expression of PD-L1 protein using six monoclonal antibodies (SP142, E1L3N, 9A11, SP263, 22c3 and 28-8).

Design—To test for protein measurement, rather than clinical utility, we created a PD-L1 index tissue microarray including cell line and tissue controls, in addition to 30 NSCLC cases with full dynamic range of PD-L1 expression. We then validated our results on a commercially available, genetically defined PD-L1 engineered cell line array with a range of controlled protein expressing cell lines. Protein levels were measured by both quantitative immunofluorescence and quantitative chromogenic assessment.

Results—Concordance between 4 antibodies showed regression (R^2 values) between 0.42-0.91 for tumor tissue cores and 0.83-0.97 for cells line cores by QIF in the PD-L1 index tissue microarray. All six antibodies showed high levels of concordance (R^2 ranging from 0.76 to 0.99) when using chromogenic staining in isogenic cell lines.

Conclusions and Relevance—Since the antibodies are highly concordant, these results suggest that assays based on the use of these antibodies could yield concordant results. They further suggest that previously described differences in PD-L1 expression in tissue is independent of the antibody utilized and likely due to tumor heterogeneity, assay/platform-specific variables or other factors.

To identify responders and guide treatment, assessment of PD-L1 expression by immunohistochemistry (IHC) has been used as a predictive diagnostic test in trials of PD-1 axis inhibitors nivolumab, atezolizumab, durvalumab and pembrolizumab in NSCLC (1-4).

The PD-L1 IHC 22C3 PharmDx kit (Dako North America) was recently approved by the FDA as a companion diagnostic for pembrolizumab in NSCLC while PD-L1 28-8 PharmDx kit (Dako North America) was approved as a “complementary” diagnostic for nivolumab.

The definition of “PD-L1 positive” lacks standardization, and prediction of response by IHC is additionally limited by the subjective nature of the technique. Variable cutoffs for defining positive cases across trials have been utilized, measuring either tumor and/or immune cells in the stroma (4-6). While some PD-L1 antibodies have been rigorously validated in the published literature, including 28-8 and E1L3N, others are less documented and specific epitope sequences remain proprietary (7, 8).

Significant differences in case classification has been observed with two validated PD-L1 antibodies; concluding that discordance seen was either a function of tissue heterogeneity or variability between antibodies (9). To examine the effect of epitope targeting or potential non-specific binding, we developed a tissue microarray with a range of positive and negative specimens including tumor, normal tissue and cell lines (eFigure 1). We analyzed five PD-L1 monoclonal antibodies (intracellular and extracellular domain specific) to determine the concordance between antibodies.

Retrospectively collected formalin fixed paraffin embedded tissue blocks from 30 patients were obtained from Yale Pathology Archives with a range of expression of PD-L1 as assessed in previous studies. All cases obtained had signed consent or waiver of consent for tissue use under approved Yale Human Investigation Committee protocol #9505008219. Tissue and cell line blocks, processed identically, were prepared in a tissue microarray format containing 0.6 mm representative FFPE cores in two-fold redundancy. Horizon Discovery's gene editing technology was used to develop a genetically defined 15 spot cell line microarray (CLMA) PD-L1 IHC Reference standard with a range of controlled protein expression levels (negative, low, medium and high protein expressing cell lines). Individual cell lines in the 15 spot CLMA were extensively characterized and verified using molecular assays, IHC and quantitative digital pathology.

PD-L1 expression was evaluated by chromogenic immunohistochemistry (IHC) and quantitative immunofluorescence (QIF) using six monoclonal antibodies raised against PD-L1 as summarized in Table 1. Antibodies were titrated at a range of concentrations and optimal assay concentration was determined using an algorithm that utilizes both signal to noise ratio and dynamic range. QIF was performed successfully for four of the six antibodies using AQUA as previously described(9). Chromogenic IHC was quantified using the Aperio Positive Pixel Count based on the intensity of membrane staining of respective PD-L1 antibodies using a modified HER2 algorithm (10). A detailed description of these methods and immunostaining examples are included in supplemental materials. (eFigure 2 and 3).

Correlation between antibodies was measured by linear regression. Tumor cores showed lower concordance than cell lines with the lowest being E1L3N showing a correlation coefficient of 0.42, 0.68 and 0.58 to SP263, 9A11 and SP142 respectively (eFigure 4A). In cell lines, the concordance increased to 0.83, 0.90 and 0.86 for SP263, 9A11 and SP142 respectively (eFigure 4B). To confirm this concordance, we tested the four antibodies in the

15 Spot CLMA (eFigure 5 contains a schematic of this array and representative immunostaining images). Regression between the antibodies tested on the 15 spot CLMA showed high concordance with R^2 values 0.89 and 0.94 (eFigure 6A) confirming the results in Yale index TMA. The antibody 28-8 results were reproducible only under DAB staining conditions (eFigure 7). The antibody 22c3 was also tested using the FDA approved platform and included in the study analysis. For all chromogenic assessment, we utilized the Aperio Positive Pixel counter and assessed 3-3 diaminobenzidine (DAB) staining of the 15 Spot CLMA. All staining was carried out in a single run on the Bond Rx as described in supplemental material except 22c3. Using this method, all six antibodies (E1L3N, SP142, 9A11, SP263, 28-8 and 22c3) showed high levels of concordance between one another with R^2 values of 0.76 to 0.99 in agreement with QIF results. (eFigure 6B).

At the time of submission, there are multiple assays under investigation or approved as companion/complementary diagnostic tests for PD-L1. It is concerning that the FDA clearance of an “assay” on the basis of “assay performance” appears to have become more important than the accurate and reproducible measurement of the target. As a result, at least four separate antibodies have been included in assays that are part of separate FDA submissions. This creates a challenge for the pathologist that may need to perform 4 different assays rather than assess PD-L1 expression. Imagine a situation where a pathologist required separate assays for the dozen or so drugs that target the estrogen receptor in breast cancer.

Lung cancer cell lines when tested with the four antibodies tested by QIF showed high concordance for PD-L1 binding. The Abcam 28-8 antibody only produced reproducible results when tested on the isogenic cell lines. This suggests 28-8 recognizes PD-L1 similarly to other antibodies tested. Furthermore, reproducibility in our hands was only seen within a single vial, despite the purchase of four vials from the same manufacturer's lot. The E1L3N antibody appears to be the only outlier with concordance levels as low as 0.42 on tumor specimens. However, high concordance in cell lines and DAB assessments and extensive previous validation of E1L3N support the hypothesis that low regressions are a function of tumor heterogeneity. The possibility that E1L3N recognizes either a PD-L1 variant or another B7 family member cannot be definitively excluded (11). Overall this result demonstrates the need for a tissue series of the tumor type under investigation to be included in antibody validation.

Limitations of this study include the relatively small patient series utilized, but high levels of concordance and reproducibility between the antibodies suggests this does not adversely affect the results except as noted above. Another limitation was our inability to compare 28-8 and 22c3 by QIF. Finally, this work does not define the positive-negative threshold for PD-L1 or the reproducibility of assessment around that threshold. Future work will address the issue of assay threshold reproducibility.

In summary, we believe this study provides good evidence that the majority of the antibodies used for PD-L1 studies are highly similar in their ability to bind PD-L1. Our previous study (9) showed about 25% discordance between E1L3N and SP142 on tissue specimens quantified in over 500 fields of view. In that work, we concluded: “Objective determination

of PD-L1 protein levels in NSCLC reveals heterogeneity within tumors and prominent inter-assay variability". This new data allows us to suggest that the discordance seen in that study is likely to be due to the high levels of heterogeneity of expression of PD-L1 that has also been described in other works (9, 12, 13) rather than antibody-based inter-assay variability. This result suggests that assays based on the use of antibodies tested here should be concordant, barring the substantial effects of the heterogeneity of PD-L1 expression or subjective scoring systems or other assay or platform-specific variables.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Figure 1. Concordance of PD-L1 antibodies in NSCLC index

TMA Concordance of four PD-L1 antibodies as determined by QIF score comparison in YTMA337 in A) tumor cases and B) cell lines

Figure 2. Concordance of PD-L1 antibodies in HDx PD-L1 reference TMA

Concordance of four PD-L1 antibodies as determined by A) QIF in immunostaining and B) six PD-L1 antibodies as determined by DAB quantified membrane intensity staining in 15 Spot PD-L1 IHC reference TMA.

Antibody Characteristics and concentrations tested to determine optimal concentration for QIF**Table 1**

Antibody	PD-L1	PD-L1	PD-L1	PD-L1	PD-L1
Clone	E1L3N	SP142	405.9A11	SP263	28-8
Source	Cell Signaling Technology Inc.	Spring Bioscience Corp.	Cell Signaling Technology Inc.	Ventana Medical Systems Inc.	Abcam Plc.
Isotype and Host Species	Rabbit IgG	Rabbit IgG	Mouse	Rabbit IgG	Rabbit IgG
Binding Site	Intracellular	Intracellular	Intracellular	Intracellular	Extracellular
Neat Antibody Concentration	1010 µg/ml	77 µg/ml	100 ug/ml	1.61 µg/ml	967 µg/ml
Optimal Antibody Concentration	1 µg/ml	0.1 µg/ml	1 µg/ml	Pre-dilute	U
					NP
					NP