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"DILEMMA OF SCORING PD-L1 BY IMMUNOHISTOCHEMISTRY"

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ABSTRACT

Cancer therapeutics have evolved with the advent of newer drugs that target immune inhibitory checkpoints. Pembrolizumab and Nivolumab are FDA approved drugs with different diagnostic guidelines. Pembrolizumab, requires a companion diagnostic test PD- L1 immunohistochemistry (IHC) 22C3 pharmDx test, in non-small cell lung tumours. The FDA has acknowledged that the level of PD-L1 IHC expression may help to identify patients who are likely to have improved survival due to treatment with Nivolumab by approving a corresponding test. PD-L1 IHC test is being used as a "complementary diagnostic test" for Nivolumab. In future approvals for other similar anti–PD-L1 agents, such as Atezolizumab (Roche/Genentech, Basel, Switzerland), Durvalumab (Astra Zeneca, Cambridge, United Kingdom), and Avelumab (Pfizer/Merck Serono, Berlin/Darmstadt, Germany), may be anticipated. This article is an attempt to address present issues associated with PD-L1 by IHC method.

INTRODUCTION

Tumor cells express neoantigens that are recognized by host immune cells as non-self. They acquire immune editing mechanism to evade elimination by the immune system. The interaction of PD-1 expressed on the cell membrane of T lymphocytes and PD-L1 on antigen presenting cells is an example of immune inhibitory checkpoint (1). Studies have shown that surgically resected Non-small cell lung cancer (NSCLC) expressing PD-L1 has a poorer postoperative survival, implying PD-L1 expressed by tumor cells is a likely immune avoidance mechanism (2,3). Currently, very little is known regarding other factors that may be adding to or abrogating the immune inhibitory effect of PD-1–PD-L1 binding in cases of NSCLC. Blockade of this PD-1 & PD-L1 interaction by therapeutic monoclonal antibodies against either PD-1 (Nivolumab, Pembrolizumab) or PD-L1 (Atezolizumab, Durvalumab, Avelumab) is a commendable advancement in cancer therapeutics. In the future we may have to be more sophisticated about our testing approach, perhaps using several assays, to identify those patients who will benefit from immune-modulatory agents. Currently, PD-L1 IHC is the best-developed strategy. A positive PD-L1 IHC test has been proven to be a predictive marker of better response as well as better patient outcome.

Understanding the decision-making matrix of physician and pathologist is complicated considering issues regarding institutional cost and logistic concerns. Tandem decisions on Choice of test Vs. Choice of therapy is debatable. Medical community needs to overcome a long checklist of hurdles (Table-1). According to some studies, the mutational frequency serves as a 'global' measure across the tumours. In some situations where PD-L1 IHC expression with <1% of cells in the tumour microenvironment, it may still serve as a biomarker for non-response to checkpoint blockade when it is seen in a spatial relationship with host immune cells (4). Recent observations have shown that PDL2 expression may be discordant to PD-L1 and those PDL1- negative patients who respond to PD-1 inhibitors may have an active PD-L2 pathway (5). Herbst and colleagues have delineated those NSCLC patients who have high PD-L2 expression responded to Atezolizumab (6). As the newer targeted therapies enter the market with their companion or complementary diagnostic tests, additional clinical trials outcomes will provide guidance on how and when to use these diagnostics. Presently, FDA has mandated a comparison of the different PD-L1 diagnostics (7). A reliable and universal observation in trials of anti–PD-1 and anti–PD-L1 immune checkpoint inhibitors is better overall response rate and also improved progression-free and overall survival in patients who have showed positive test result for PD-L1 IHC test (8).

Table 1: Challenges in determining choice of test and choice of therapy

Sl.No	Challenges	Issues	Dilemma
1.	Technical	Multiple clones of primary antibody	Choice of clone
		Multiple cutoffs	Choice of therapy
		Preanalytical variables	Variability in Interlaboratory comparision
		Validation methodology	Universally accepted protocols and standards
2.	Biological	Expression by multiple cell type- Tumour cells, Macrophages, and lymphocytes	Universally accepted scoring system
		Dynamic heterogeneity - Intratumoral	Validation method employed
		Dynamic heterogeneity – Intertumoral	Control tissues to be used in validation method

IHC IN BIOMARKER STUDIES

Protein expression is a biological continuum from absent through low to intermediate to high levels of expression, and expression is heterogeneous among tumour cells. The protein is not either present or absent. When IHC is used in biomarker studies, scoring creates an apparently binary situation. Pathologists use statistically derived cut-off point for IHC expression, above which the tumour is deemed positive, and below as negative. Although, it is an artificial creation employed, it has been determined as the most efficient point for patient selection (9). However, even PD-L1 negative patients have shown some response to therapy attributed partly to the PD-L1 protein expression heterogeneity and also to the inducibility of PD-L1 expression following first line chemotherapy (10). Heterogeneity, therefore, leads to some patients being incorrectly placed in either positive or negative groups, and this risk is greater as the cut-off used is lower. The net result of this will be, less difference in average outcome measures among positive and negative cohorts. Given clone will determine epitope of the PD-L1 molecule to which primary antibody will bind and give particular staining intensity. Thus, one needs to understand that different clones may give different staining results. Staining results will also vary according to the chemistry used to detect and visualize the primary antibody bound to the tissue section. Multiple steps, namely, tissue fixation, handling, processing, and also treatment of sections being prepared for an IHC test, which includes antigen-retrieval steps, often influence the test results. All of these factors must be borne in mind while carrying out these tests. Furthermore, pathologists interpreting

the test outcomes must learn to differentiate between true staining and artefact for each particular assay, and also should be able to apply any scoring algorithm. Despite all of these variables, IHC is a powerful diagnostic tool capable of excellent accuracy, sensitivity, and specificity, provided that all aspects of the assay and its assessment are carefully controlled, executed and monitored.

MULTIFACTORIAL HURDLES

Rigorous test validation and reliable test performance is the biggest challenge. Studies have shown significant differences in staining performance of 2 commercially available clones (11). The Pathology Committee of the International Association for the Study of Lung Cancer has been interacting with 6 of the commercial stakeholders (Astra Zeneca, Bristol Myers Squibb, Dako, Merck Sharpe Dohme, Roche/Genentech Pharmaceuticals and Roche Ventana Diagnostics) to compare these tests (The Blueprint Project) (12). There is lack of data on comparative studies published on assays [laboratory developed tests (LDT)]. Although these LDT's are technically validated, there is no guarantee of equivalent clinical predictive performance. However, these studies might assess what we can expect when comparing the companion or complementary diagnostic assay (13, 14).

Transition from chemotherapy to molecular therapy, and recently to immune therapeutics will require multifaceted studies to determine role of biomarkers to response/ resistance to single or multi-agent therapeutic regimes. Presently, it is impractical for a single biomarker to represent the complex and dynamic nature of human immune system. In morphology based biomarker studies, validation of assay should include accuracy assessment, assay linearity, assay precision across multiple platforms. In case of laboratory developed tests, one needs to refine assay with regard to heterogeneous expression, antigen stability and fixation sensitivities, etc. Many studies have identified a relationship between the pre-treatment expression level of PD-L1 in the tumour microenvironment and the possibility of response to single-agent PD-1 pathway inhibitor pathway therapy. The key issue is whether laboratories should attempt to deliver the trial validated assays for one or more of these treatments, or introduce instead one or more laboratory developed tests, or attempt to provide a single PD-L1 immunohistochemistry assay for all possible anti–PD-1 and anti–PD-L1 treatments that may be used. IHC as a PD-L1 biomarker assay has aroused several confusions and criticisms in the oncology community (Table-2). However, it is now evident that a biomarker assay will have a role to play in the selection of patients for these clinical treatments, at least in some circumstances (15).

While reporting, the Pathologist will have to indicate which biomarker test was employed, the actual score assessment, and also indicate required minimum number of cells that was assessed (the 22C3-based assay for Pembrolizumab requires that a minimum of 100 tumor cells be assessed). Finally, the report should provide some comment regarding how this score stands with respect to the relevant cut-off for that test.

The mechanism of PD-L1 expression is intricate. Numerous factors appear to influence both PD-L1 expression and also response to therapy. For example, specific BRAF mutations when pretreated with Dabrafenib have been associated with reduced response to PD-1/PD-L1 inhibitory agents in melanoma (16-18). Nevertheless, when Dabrafenib was combined with MEK suppressor Tremelimumab, a better response to PD-L1 inhibition was noted (18,19). Similarly, blockade of mutated BRAF and MEK was related to improved response to PD-1/PD-L1 inhibition in NSCLC. Recent studies have proven that EGFR mutations and rearrangements in EML4-ALK are associated with up regulation of PD-L1 synthesis and expression in NSCLC. The presence of KRAS in the tumor also appears to be associated with increased expression of PD-L1 (20-22).Cigarette smoking in patients with NSCLC appears to increase the number of lymphocytes and the overall proportion of PD-L1 positivity (23). Platinum based chemotherapy also has been shown to affect the tumor microenvironment in a way similar to cigarette smoke (24). Since tumor cells gradually adopt an adaptive immune response, tumor tissue which may express slight PD-L1 at the moment of initial tissue sampling for IHC staining, may show considerable increase in PD-L1 expression at later point during the course of the disease (25).

Table 2: Cut-off scores for the use of different therapies and their respective staining platforms

Sl. no	Drug	Staining Platform	Antibody Clone	Cut-off Score
1.	Nivolumab	Dako Link 48 platform	28-8 clone	≥1% to ≥10%
2.	Nivolumab	Dako Link 48 platform	28-8 clone	
3.	Nivolumab	Dako Link 48 platform	28-8 clone	
4.	Pembrolizumab	Dako Link 48 platform	22C3 clone	≥1% to ≥50%
5.	Atezolizumab	Ventana automated platform	SP142 clone	≥1% to ≥ 50% ≥1% to ≥10%
6.	Durvalumab	Ventana automated platform	SP263 clone	≥25%

Table 3: Technical hurdles in biomarker testing:

Technical Issue	Reason
1. Choice of reagent kits RUO, In Vitro Vs Companion diagnostics	No standard guidelines by FDA or CAP
2. Different antibodies	Depends on physician choice of drug. No standard guidelines by FDA or CAP
3. Different staining protocols	No standard guidelines by FDA or CAP
4. Different target cell assessment (tumor cells, tumor-infiltrating immune cells, or both).	No standard guidelines by FDA or CAP
5. Different thresholds for defining a positive test result	No standard guidelines by FDA or CAP ***Nivolumab*** **(**Clone 28-8)**-** ≥1% to ≥10% ***Pembrolizumab*** **(**Clone22C3) - ≥1% to ≥50% ***Atezolizumab***(Clone SP142)- ≥1% to ≥50% (tumor cells) ≥1% to ≥10%(immune cells) ***Durvalumab*** **(**Clone SP263) - ≥25%

CONCLUSION

Alteration of specific checkpoints in anti-tumor immune response has resulted in significant improvement in the treatment of various malignancies. The association of tumor expression of PD-L1 and patient outcome has been established. Several tumor and patient characteristics has been shown to influence response to PD-1/PD-L1 inhibitor and should be considered while selecting patients for this therapy. Providing several tissue samples and obtaining tissue samples at different time intervals may allow for more accurate selection of appropriate patient for treatment. In the Blueprint PD-L1 IHC Assay Comparison Project, the Pathology Committee of the International Association for the Study of Lung Cancer collaborated with 6 of the commercial stakeholders (Astra Zeneca, Bristol Myers Squibb, Dako, Merck Sharpe Dohme,Roche/ Genentech Pharmaceuticals, and Roche Ventana Diagnostics) to correlate these tests (27). The interchangeability of the current assays is probably a challenge. PD-L1 IHC as a predictive assay for selecting patients for anti–PD-1 or anti–PD-L1 therapy. Our testing strategy could be improved, perhaps by additional IHC markers or incorporation of some assessment of the immune cellular environment in the tumor. Most validation-metric data available

for PD-L1 tests relate to the use of IHC test for lung cancer, and this evidence raises some crucial challenges that may influence the uptake of PD-L1 testing. In particular, standardization among available PD-L1 IHC tests is currently deficient with regard to antibodies used, cut-offs/thresholds for a particular antibody, and differences in scoring algorithm and test sites. Developing strategies to address this variability in the available IHC tests and establishing specific scoring algorithms will be important in order to improve the predictive value of this biomarker assay for patient selection for appropriate therapy.

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