Predictive biomarkers of response for PD-1/PD-L1 inhibitors: a cumbersome gold rush

Andrea Vingiani¹, Massimo Barberis¹, Elena Guerini-Rocco¹,²

Abstract
Programmed cell death protein 1 (PD-1) and its ligand programmed cell death-ligand 1 (PD-L1) are overexpressed in a number of human malignancies. More interestingly, their expression has been associated with patient survival in non-small cell lung cancer (NSCLC), melanoma, renal cell carcinoma, esophageal, pancreatic and colorectal carcinoma, with the data commonly suggesting a negative prognostic role. In this review, we summarize the pros and cons regarding the predictive role of PD-L1 expression in candidate patients for checkpoint inhibitors. Furthermore, we discuss the potential predictive role of other biomarkers, such as tumor mutational burden, microsatellite instability, mismatch repair deficiency and tumor infiltrating lymphocytes. We conclude that PD-L1 testing probably represents simply a “snapshot” of an intricate, fluctuating and dynamic process, that in turn represents the interplay between the immune system and cancer. The PD-L1 assay can be considered more useful for response stratification than in patient selection.

Key words: biomarkers, immunotherapy, PD-L1

Introduction
Programmed cell death protein 1 (PD-1 or CD279) and its ligand programmed cell death-ligand 1 (PD-L1 or CD274 or B7-H1) are cell surface transmembrane molecules playing a pivotal role in down-regulating the immune system, thus promoting immune tolerance by suppressing T cell inflammatory activity and preventing autoimmunity in physiological circumstances [1-3].

Activation of the PD1 – PD-L1 axis results in increased apoptosis of T lymphocytes, reduced apoptosis in regulatory T cells (Tregs), reduced T cell proliferation and interferon (IFN)-γ secretion [1, 4-6].

The expression of PD1 and PDL-1 on cancer cells and on immune cells in the tumor microenvironment is assumed to represent a crucial player of the negative feedback loop ultimately leading to the so-called “immune escape”, and resulting in uncontrolled cancer growth and progression [7-9].

PD1 and PD-L1 are overexpressed in a number of human malignancies; more interestingly, their expression has been associated with patient survival in non-small cell lung cancer (NSCLC), melanoma, renal cell carcinoma, esophageal, pancreatic and colorectal carcinoma, with the data commonly suggesting a negative prognostic role [10-17].

In this scenario, a number of PD1 inhibitors, namely nivolumab and pembrolizumab, and PD-L1 inhibitors, such as atezolizumab and avelumab, have demonstrated promising therapeutic activity and are currently available for the treatment of several advanced-stage neoplasms, such as NSCLC, melanoma, urothelial carcinoma, renal cell carcinoma, head and neck cancer and Hodgkin lymphoma [18-28].

Despite the unprecedented survival benefit obtained with checkpoint inhibition, nearly 40–60% of patients will not gain advantage from these therapies, that are furthermore costly and not free from toxicities. In this context, reliable and cost-effective predictive biomarkers are therefore highly desirable, and the assessment of PD-L1 expression by immunohistochemistry (IHC) seems to represent the most widely used and validated biomarker thus far.

PD-1/PD-L1 expression by ICH and the NSCLC paradigm
A number of different IHC assays have been developed to assess the expression of PD-1 and PD-L1, and different
clones, staining protocols, platforms, scoring systems, and thresholds have been introduced for and linked to specific inhibitors (Table 1). In patients with NSCLC, data stemming from clinical trials and large pooled analyses have shown a positive correlation between PD-L1 expression and the clinical benefit deriving from PD-1/PD-L1 inhibition [29-33].

The PD-L1 IHC 22C3 PharmDx assay has been approved by the US Food and Drug Administration (FDA) as the exclusive companion diagnostic for pembrolizumab (Figure 1).

In the KEYNOTE 024 trial, 305 patients with untreated advanced NSCLC, with PD-L1 expression on at least 50% of tumor cells were randomized to receive either pembrolizumab or platinum-based chemotherapy. Investigators showed that pembrolizumab was associated with significantly longer progression-free (hazard ratio [HR] 0.50; 95% confidence interval [CI] 0.37-0.68; p<0.001) and overall survival (HR 0.60; 95% CI 0.41-0.89; p=0.005) and with fewer adverse events than with platinum-based chemotherapy [34].

Similarly, PD-L1 IHC 28-8 PharmDx (Agilent Technologies, Santa Clara, CA/Dako Carpinteria, CA) has been approved as the companion test for nivolumab, given the clinical evidence of patients affected by NSCLC showing positivity for PD-L1 in 1% or more tumor cells. Notably, data coming from pivotal clinical trials showed that a significant subset of PD-L1-negative patients clearly gained an advantage from treatment with nivolumab [35-37]. Consequently, the FDA label for nivolumab did not specify any cut-off for PD-L1 positivity. The phase III Checkmate 026 study similarly compared the anti-PD-1 antibody nivolumab as a single agent with platinum-based chemotherapy in first-line PD-L1 >5% positive NSCLC. The primary endpoint of progression-free survival (PFS) in the population with PD-L1 >5% was not met (HR 1.15, p=0.25) [38].

The complementary diagnostic Ventana PD-L1 SP142 antibody (Ventana Medical Systems, Tucson, AZ) has been approved for therapy with atezolizumab, in consideration of the enhanced survival observed in patients with NSCLC presenting at least 50% of tumor cells expressing PD-L1 or at least 10% of the tumor area occupied by PD-L1-expressing tumor-infiltrating immune cells. Although these assays may provide useful information to clinicians as complementary diagnostics, neither the PD-L1 IHC 28-8 PharmDx nor the Ventana PD-L1 (SP142) assay are strictly required for treatment with nivolumab or atezolizumab, respectively. Finally, the Ventana SP263 clone is still under the FDA regulatory process and has been developed for treatment with durvalumab by using a positivity cutoff of 25% or more tumor cells [31].

From a practical point of view, what emerges is the number and heterogeneity of the antibody clones and platforms used, thereby giving rise to concerns about the reproducibility and robustness of different assays evaluating the expression of the same molecule and aimed at predicting clinical response to therapies sharing the same mechanism.

Table 1. Immunohistochemistry assays for assessing the expression of PD-1 and PD-L1.

<table>
<thead>
<tr>
<th>Manufacturer</th>
<th>Anti-PD-1/ PD-L1 Drug</th>
<th>IVD Partner</th>
<th>Ab Clone</th>
<th>Cutoffs</th>
<th>Cell Scored</th>
</tr>
</thead>
<tbody>
<tr>
<td>AstraZeneca</td>
<td>Durvalumab</td>
<td>Ventana</td>
<td>SP263</td>
<td>25%</td>
<td>Tumor cells</td>
</tr>
<tr>
<td>Bristol Myers Squibb</td>
<td>Nivolumab</td>
<td>Dako</td>
<td>28-8</td>
<td>1%, 5%, 10%</td>
<td>Tumor cells</td>
</tr>
<tr>
<td>Merck</td>
<td>Pembrolizumab</td>
<td>Dako</td>
<td>22C3</td>
<td>1%, 50%</td>
<td>Tumor cells</td>
</tr>
<tr>
<td>Merck KGaA and Pfizer</td>
<td>Avelumab</td>
<td>Dako</td>
<td>–</td>
<td>–</td>
<td>Tumor cells and TILs</td>
</tr>
<tr>
<td>Roche</td>
<td>Atezolizumab</td>
<td>Ventana</td>
<td>SP142</td>
<td>50% of tumor cells, 5% in TILs</td>
<td>Tumor cells and TILs</td>
</tr>
</tbody>
</table>

Ab: antibody; IVD: in vitro diagnostic; PD-1: programmed cell death protein 1; PD-L1: programmed cell death-ligand 1; TILs: tumor-infiltrating lymphocytes.
of action. For example, according to its FDA label, pembrolizumab administration is linked to an approved IHC test with the 22C3 antibody, run on the DAKO platform, that in turn not all laboratories around the world are provided with. The “Blueprint PD-L1 IHC Assay Comparison Project” compared the performances of 4 assays, namely SP263, 28-8, SP142 and 22C3, and revealed that three of the four assays were closely aligned on tumor cell staining whereas the fourth showed lower sensibility [39]. A number of harmonization studies followed, suggesting that some of IHC assays could be interchangeable, but these data still lack clinical validation [40-43]. Interestingly, Marchetti and colleagues found a high correlation between PD-L1 expression data obtained with the Agilent PD-L1 IHC 22C3 PharmDx and the Ventana PD-L1 (SP263) tests in NSCLC, thus suggesting that the two assays could potentially be utilized interchangeably.

Another drawback of note is the vast heterogeneity of the threshold used. To date, there is no consensus about the relevance of patterns of expression (on cancer cells, membranous or cytoplasmic, and immune cells) and different quantitative cutoffs have been variably used and approved by regulatory authorities. The Blueprint study highlighted that, despite similar analytical performance of PD-L1 expression for three assays, interchanging assays and cutoffs could lead to “misclassification” of PD-L1 status for a not-negligible subset of patients. Moreover, data stemming from clinical trials showed how some thresholds used could lead to the risk of exclusion of a considerable number of responders. In this context, it may make more sense to consider PD-L1 expression as a continuous variable rather than a “on” or “off” indicator. Probably this assay can be considered more useful for response stratification than in patient selection.

**PD-1/PD-L1 testing in other malignancies**

Checkpoint inhibition is one of the currently approved standards of care for advanced stage patients with malignant melanoma (MM). To date, no validated predictive biomarker of response exists [44, 45]. Nevertheless, the absence of PD-L1 expression on malignant and immune cells has been shown to predict a poor, non-existent response to PD-1 inhibition [46]. In their meta-analysis, Gandini et al. showed that objective response rates in patients with MM were significantly higher in PD-L1-positive than in PD-L1-negative tumors (45% vs 27%, respectively) [47]. Furthermore, recent evidence suggests that low PD-L1 expressing MM patients could derive greater benefit from dual checkpoint inhibition with nivolumab plus ipilimumab [48, 49]. Nivolumab has been registered for the treatment of advanced renal cell carcinoma (RCC); in this setting, the expression of PD-L1 was prognostic but still not predictive of clinical benefit deriving from checkpoint inhibition [50, 51].

With regard to urothelial carcinoma, the predictive value of PD-L1 expression varied across different studies. Rosenberg et al. showed that the overexpression of PD-L1 was predictive of clinical benefit in a second-line trial randomizing patient to receive atezolizumab [52], while in the first-line setting, no differences in efficacy according to PD-L1 expression were noted [53]. Several other checkpoint inhibitors have been validated in urothelial cancer, and again, evidence on the putative predictive value of PD-L1 expression is far from exhaustive.

**Alternative biomarker assays**

Recent evidence suggests that mutational burden could be positively associated with response to checkpoint inhibitors. In patients with NSCLC, Rizvi et al. demonstrated that tumors with high numbers of non-synonymous mutations showed better PFS and objective response rate in contrast to tumors with low mutational burden [54, 55]. Similarly, copy number variations, expressed as a quantitative chromosomal number instability (CNI) in tumor cell-free DNA obtained from liquid biopsy have been shown to predict benefit from immunotherapy [56].

Mismatch repair deficiency (MMRD)-driven tumors are associated with high mutational load, and therefore with overt immunogenicity due to a large amount of “neo-epitopes”. In this regard, Le et al. found MMRD to be predictive of response to pembrolizumab in patients with colorectal cancer [57]. These findings, along with data from 149 patients enrolled in five clinical trials, led to the FDA approval of pembrolizumab for any MMR-deficient solid cancer, thus perhaps representing the first example in the history of oncology of a therapeutic indication relying on a biological biomarker rather than the location of the neoplasm [58].

Tumor-infiltrating lymphocytes (TILs) have been positively correlated with prognosis and treatment response, especially in the neoadjuvant setting, in a large number of neoplasms. Interestingly, in a pivotal study by Tumeh et al., it was demonstrated in MM that the prevalence of CD8+ T cytotoxic lymphocyte was predictive of anti PD-1 therapy benefit, outperforming the assessment of PD-1 expression [59], thus suggesting a possible role for TILs as a biomarker in PD-1/PD-L1 inhibition. From a speculative point of view, the presence of immune cells in the tumor microenvironment could mirror the immunogenicity of the neoplasm itself, being able therefore to pinpoint tumors amenable to anti-tumor immune response restoration through checkpoint inhibition. The identification of the lymphocytic subpopulation and the evaluation of the actual state of the activation/exhaustion of
the immune system could provide valuable information. Two independent groups have demonstrated that T cell exhaustion is characterized by a specific genetic landscape, consisting of peculiar patterns of accessibility of gene regulatory elements to transcriptional factors [60-62]. These data led to the hypothesis that the existence of a window between an “initial” and reversible state of T cell exhaustion and a “permanent” state, during which patients could potentially take advantage of immune reactivation by anti-PD-1 drugs. Along this line, in the era of transcriptomics and of gene expression profiling, the assessment of PD-1/PD-L1 expression and of other markers of T cell activation and/or in tumor-associated T lymphocytes and/or tumor cells (such as CTLA 4, TIM3, LAG3, TIGIT and so on) by more and more comprehensive “immune signatures” might be of great value [63]. For example, Ribas et al. described an interferon-inflammatory immune gene expression signature associated with both enhanced overall response rates and PFS in advanced MM patients receiving pembrolizumab [64]. Assessing immune-related blood parameters has shown some interesting results, especially in MM. Ferrucci et al. demonstrated that high absolute neutrophil count and derived neutrophil to lymphocyte ratio were significantly related to poorer overall survival and PFS in 720 patients with advanced MM treated with ipilimumab [65], while other groups showed that absolute lymphocyte count was associated with a better outcome [66, 67]. Similarly, high serum lactate dehydrogenase levels have been demonstrated to be a reliable predictor of poor patient outcomes in response to CTLA 4 and/or PD-1 blockade in patients with MM [68].

Encouraging data surrounds the putative predictive role of the identification of tumor antigen-specific antibodies, antigen-reactive T-cells [69], the assessment of circulating lymphocyte subtypes [70] and the microbiome profile [71].

Conclusions

Checkpoint inhibitors have entered forcefully into the clinical arena for the treatment of several human malignancies, showing notable results. Still, only a fraction of the treated population gain advantage from these expensive therapies, which are not without side effects. The identification of reliable biomarkers of response is therefore crucial for selecting the target population. To date, PD-1/PD-L1 testing by IHC represents the most widely used biomarker, although it suffers from obvious limitations. PD-L1 testing is mainly performed on small biopsy samples, sometimes on fine needle biopsy, with the risk of not being representative of the entire tumor or of the numerous metastatic localizations. As well as being inadequate in deciphering the topographical heterogeneity of human malignancies, PD-L1 testing represents simply a “snapshot” of an intricate, fluctuating and dynamic process that in turn represents the interplay between the immune system and cancer. The status of the PD-1/PD-L1 axis can, furthermore, be influenced by several factors, namely disease stage, previous lines of therapy, and any eventual concomitant therapy such as immunosuppressive drugs. In this scenario, it is, therefore, reasonable to apply our efforts in seeking for biomarkers capable of pinpointing tumor actually amenable to immune activity restoration, taking into consideration the biology of the tumor cells themselves, the status of the immune system, and their protean interaction.

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Conflicts of Interest

The Authors declare there are no conflicts of interest in relation to this article.

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