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REVIEW ARTICLE

Predictive potential and need for standardization of PD-L1 immunohistochemistry

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Abstract

Checkpoint inhibitors targeting the PD-1/PD-L1 axis are a promising treatment option in several tumor types. PD-L1 expression detected by immunohistochemistry is the first clinically validated predictive biomarker for response to PD-1/PD-L1 inhibitors, though its predictive value varies significantly between tumor types. With the approval of pembrolizumab monotherapy for treatment-naïve, advanced non-small cell lung cancer, PD-L1 testing has to become broadly available in pathology laboratories. When PD-L1 testing started to be introduced in routine pathology practice, there were several open issues, which needed to be addressed in order to provide accurate results. This review will discuss the complex biological background of PD-L1 as predictive biomarker, summarize relevant clinical trials in NSCLC illustrating the origin of different PD-L1 expression cutoffs and scorings, and address issues important for PD-L1 testing including the analytical comparability of the different clinical trial-validated PD-L1 immunohistochemistry assays, the potential of laboratory-developed tests, and an overview of the different scoring algorithms.

Keywords PD-L1 · Immunohistochemistry · Predictive biomarker · Immunotherapy

Introduction

Immunotherapy by checkpoint blockade targeting programmed death 1 (PD-1) or its ligand programmed death ligand 1 (PD-L1) has emerged as a new pillar in cancer treatment. Since 2014, five PD-1 (pembrolizumab and nivolumab) or PD-L1 inhibitors (atezolizumab, durvalumab, and avelumab) have been approved for a wide range of malignancies, including for example malignant melanoma, numerous carcinoma types, and Hodgkin lymphoma [1–9].

Much enthusiasm surrounds this new class of drugs as a subset of patients, notably in advanced fatal disease, experience remarkable and durable efficacy with fewer side effects than conventional chemotherapy. However, a significant subset of patients do not benefit from this expensive treatment and are at risk of severe immune-related adverse events. Response rates to PD-1 or PD-L1 inhibitors, for example in unselected melanoma and non-small cell lung cancer (NSCLC) range from 30 to 40 and 20%, respectively [10–12]. Identification of predictive biomarkers is therefore in the focus of research and drug development in order to improve selection of patients for treatment.

PD-L1 expression, determined by immunohistochemistry (IHC) on tumor tissue, is the first clinically validated predictive biomarker, which has translated into clinical practice (Table 1). Since the approval of pembrolizumab monotherapy for first-line treatment of metastatic NSCLC with high PD-L1 expression, pathologists have to provide valid PD-L1 IHC testing [14]. IHC in general is a well-established method. However, lack of standardized PD-L1 testing caused by various clinical trial-validated PD-L1 assays and different indication-specific scoring algorithms has led to a Babylonian confusion.

Within the rapidly evolving field of PD-1/PD-L1-targeted immunotherapy in terms of drug approval and predictive biomarker development, this review will summarize the current significance of PD-L1 IHC as predictive marker emphasizing NSCLC and address important issues of PD-L1 IHC testing, including the performance of available tests and IHC evaluation and scoring.

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Table 1 Companion or complementary PD-L1 testing using FDA-approved or CE-marked assays by tumor type and approved drug [13]

Tumor type	Drug	Indication	Type of testing	PD-L1 assay	PD-L1 scoring	Clinically relevant PD-L1 expression levels
NSCLC	Pembrolizumab [14–16]	1 and 2 line for advanced stage	Companion (by FDA and EMA)	22C3, SP263*	TPS	≥ 50% - first line ≥ 1% - second line
	Nivolumab [17, 18]	2 line for advanced stage	Complementary (only for non-squamous)	28–8, SP263*	TPS	≥ 1%, ≥ 5%, ≥ 10%
	Atezolizumab [19, 20]	2 line for advanced stage	Complementary	SP142	TC/IC	≥ 50% TC or ≥ 10% IC
Melanoma	Nivolumab in combination with ipilimumab [18, 21]	1 line, unresectable or metastatic	Companion** (by EMA) complementary (by FDA)	22–8	TPS	<1%
Gastric/gastroesophageal adenocarcinoma	Pembrolizumab [16, 22]	3 line, recurrent, locally advanced or metastatic***	Companion	22C3	CPS	≥ 1%
Urothelial carcinoma	Atezolizumab [20, 23]	2 line, locally advanced or metastatic	Complementary	SP142	IC	≥ 5%
	Nivolumab [18, 24]	2 line, locally advanced or metastatic	Complementary	22-8	TPS	≥ 1%
	Durvalumab [25, 26]	2 line, locally advanced or metastatic	Complementary	SP263	TC/IC	≥ 25% of either TC or IC
Squamous cell carcinoma of the head and neck	Nivolumab [18, 27]	2 line, recurrent or metastatic	Complementary	28-8	TPS	≥ 1%

FDA, US Food and Drug Administration; *EMA*, European Medicines Agency; *22C3*, PD-L1 IHC 22C3 pharmDx (Dako); *SP263*, VENTANA PD-L1 (SP263) Assay (Ventana); *28-8*, PD-L1 IHC 28-8 pharmDx (Dako); *VENTANA*, PD-L1 (SP142) Assay (Ventana)

*SP263 is CE-IVD-marked, not FDA-approved, for PD-L1 testing to select patients for pembrolizumab or nivolumab; all other assays are CE-IVD-marked and FDA-approved for the listed tumor-specific indications

**Wording of the EMA authorization: Relative to nivolumab monotherapy, an increase in progression-free survival and overall survival for the combination of nivolumab with ipilimumab is established only in patients with low tumor PD-L1 expression (TPS < 1%). [4]

***Approved by FDA, not by EMA.

PD-L1 immunohistochemistry as predictive biomarker: biological background and predictive potential

In contrast to chemotherapy and targeted treatment of tumors with oncogenic driver mutations, immunotherapy by checkpoint blockade does not attack tumor cells (TC) directly, but can reactivate a specific antitumoral immune response, from which the tumor has managed to escape by becoming immune resistant [28, 29]. The level of such an induced antitumoral immune response depends on tumor intrinsic factors and is driven by genetic alterations, which are very diverse across and within tumor types [29, 30]. Though the interaction between cancer and the immune system is highly complex, PD-L1 expression on TC and immune cells (IC) in the tumor microenvironment with its ability to suppress primed effector T cells is a powerful escape mechanism from immune surveillance.

As PD-1 and PD-L1 inhibitors target the PD-1/PD-L1 interaction between effector T cells and TC—unleashing the immune response—investigating PD-L1 expression by IHC as a predictive biomarker was obvious. IHC as a predictive biomarker testing method is widely available, cost-effective, fast, and well-established, for example for the detection of oncogenic HER2, ALK, or ROS1 alterations. However, PD-L1 expression differs from predictive oncogenic alterations in many ways [31, 32]. Biomarker tests for oncogenic driver alterations usually provide a black and white answer (positive or negative), which usually remains stable during tumor progression and treatment as the oncogenic alteration is the main driver of the tumor. In contrast, PD-L1 IHC will provide a continuous variable as expression levels can range from 1 to 100% without a natural gap between a positive and negative result. Additionally, PD-L1 expression is not restricted to TC, but also expressed by IC. Different clinical trials used different PD-L1 IHC scoring systems and expression levels as cutoffs to stratify PD-L1-selected patient's subgroups (Table 1) [14, 15, 17, 19, 21–25, 27]. Moreover, the immune system is dynamic, its interaction with TC may change during tumor progression and treatment, and its ability to attack the tumor is obviously crucial for treatment success.

Therefore, data on the predictive value of PD-L1 IHC are inconsistent—due to both, the complex biology and clinical trial design. The degree of the association between PD-L1 expression and response to treatment varies between different tumor types, lines of treatment, scoring systems, and cutoffs defining a PD-L1 positive result. Additionally, lack of PD-L1 expression is generally an insufficient negative predictor of response, and for most tumors and approved drugs, a negative PD-L1 IHC result will not disqualify a patient from treatment.

Thus, PD-L1 expression cannot be expected to be a perfect stand-alone predictive biomarker. Yet, clinical trials have established a general association between PD-L1 expression levels and efficacy of PD-1/PD-L1-targeted immunotherapy in several tumor types (the higher the better) [33]. In clinical practice, PD-L1 testing is most relevant in metastatic NSCLC, as pembrolizumab treatment—so far, the only checkpoint inhibitor approved for initial stand-alone treatment of this common cancer type—depends on the PD-L1 status [14]. In general, as for June 2018, companion PD-L1 IHC testing is required only for one drug—pembrolizumab—and two tumor types—NSCLC and gastric or gastroesophageal junction adenocarcinoma (G/GEJ) (Table 1) [14, 22].

For all other drugs and tumor types, PD-L1 testing is not required for prescription. However, the PD-L1 expression level might provide information on the expected degree of treatment response and help oncologists assess the benefit-risk ratio in order to tailor the best treatment option for the patient. For this setting, the FDA has approved complementary PD-L1 IHC testing for NSCLC, melanoma, and urothelial carcinoma (UC) (Table 1) [13].

PD-L1 testing in advanced non-small cell lung cancer: mandatory for first-line treatment

The last decades' improvement in treatment of advanced lung cancer was mainly restricted to adenocarcinomas and driven by targeted therapy of oncogenic driver mutations, like EGFR, ALK, and ROS1, which affect only a minor subset of patients. Recently, immunotherapy targeting the PD-1/PD-L1 axis has become standard of care for a subset of NSCLC patients, notably with squamous and non-squamous histology, adding much needed treatment options [34]. As for June 2018, pembrolizumab (first and second line), nivolumab (second line), atezolizumab (second line), and, more recently, durvalumab (second line) are approved for treatment of advanced NSCLC [14, 15, 17, 19, 35–37].

As already mentioned, companion PD-L1 testing is required in metastatic squamous and non-squamous NSCLC to select patients for pembrolizumab monotherapy and has therefore become part of the predictive biomarker testing algorithm [34]. For metastatic NSCLC with high PD-L1 expression (at least 50% of TC) and negative for targetable oncogenic alterations in EGFR, ALK, and ROS1, pembrolizumab is the first-line treatment of choice and has replaced chemotherapy [14, 34]. Prescription of second-line pembrolizumab monotherapy also depends on the PD-L1 status, though the cutoff differs from the first-line setting (≥ 1% of TC). The PD-L1 cutoff selecting for patients with treatment benefit for second-line pembrolizumab versus chemotherapy is significantly lower as further-line chemotherapy is much less effective compared to the first-line setting [15]. In contrast, second-line treatment with nivolumab, atezolizumab, or durvalumab has been approved irrespective of PD-L1 status.

In the randomized phase III nivolumab trials, which led to the FDA approval of second-line nivolumab for metastatic

NSCLC, PD-L1 testing was performed with the co-developed PD-L1 IHC 28-8 pharmDx assay (Dako) (in the pembrolizumab trials the Dako PD-L1 IHC 22C3 pharmDx assay was used) [17, 36]. Since the treatment benefit of nivolumab was associated with increasing percentage of PD-L1-stained TC (tumor proportion score, TPS) in these trials but not restricted to PD-L1-expressing NSCLC, the FDA has approved the PD-L1 IHC 28-8 pharmDx assay as complementary test for second-line nivolumab in metastatic non-squamous NSCLC [17]. In squamous cell carcinoma, no association between PD-L1 expression levels and efficacy was observed [36].

In the randomized phase III atezolizumab trial (OAK), PD-L1 expression was assessed prospectively with the co-developed VENTANA SP142 PD-L1 IHC assay (Ventana) [19, 38]. PD-L1 scoring differed from pembrolizumab and nivolumab trials, as not only PD-L1 expression on TC, but also on IC was included to stratify patients into PD-L1 expression subgroups. Compared to chemotherapy, overall survival (OS) was improved by atezolizumab across all prespecified PD-L1 expression subgroups, though the proportion of objective response was lower in the < 1% PD-L1 subgroup. Patients with high PD-L1 expression (on ≥ 50% of TC or ≥ 10% IC) had the greatest OS benefit, and the FDA has therefore approved the VENTANA SP142 PD-L1 IHC assay as complementary test for second-line atezolizumab in advanced NSCLC.

In the randomized, placebo-controlled phase III trial (PACIFIC), which led to the approval of durvalumab for unresectable stage III NSCLC after chemoradiation, PD-L1 testing was performed in only 64% of patients using the co-developed VENTANA SP263 assay (Ventana) and again TPS for evaluation [37]. Retrospective analysis with a PD-L1 TPS cutoff of 25% suggested a correlation between response to durvalumab and high PD-L1 expression (TPS ≥ 25%). Since the benefit from durvalumab in terms of progression-free survival (data on OS were immature) was independent of PD-L1 status, the FDA did not co-approve the complementary VENTANA SP263 PD-L1 test for NSCLC. However, the assay is approved as complementary test for second line durvalumab treatment of advanced urothelial carcinoma (Table 1). Additionally, the assay was recently CE-IVD marked as companion and complementary test for pembrolizumab and nivolumab (see below).

Though the treatment landscape has become complex for NSCLC, the task for pathologists is straightforward: All treatment-naïve metastatic NSCLCs, irrespective of histological subtype, need PD-L1 testing.

PD-L1 testing by immunohistochemistry: as standardized as possible

As outlined above, the co-development of different PD-L1 IHC diagnostics using proprietary antibodies resulted in four FDA-approved and CE-IVD-marked assays, each linked to a specific drug and scoring system, as their predictive value at a given PD-L1 expression cutoff was clinically validated in the respective trials (Table 1). These assays are highly standardized, automated, ready to use kits, providing a primary antibody, optimized reagents, and a software protocol for a specified staining platform. Apart from the primary monoclonal antibodies, the assays differ in the platforms and the detection systems used. The Dako assays 22C3 and 28-8 are optimized for the Dako Autostainer Link 48 and the Ventana assays SP142 and SP263 for the VENTANA BenchMark platform.

When these clinical trial assays became commercially available, it was unknown if their analytical performance is comparable and if one assay could cover predictive PD-L1 testing for all approved drugs. A scenario where several assays would be required to test for one protein, commonly on limited tumor material, which might need to suffice for analysis of a whole range of predictive markers, would pose significant practical challenges for pathology laboratories [31, 32]. Even the fact that only the 22C3 Dako assay is a mandatory companion test, neither the Dako nor the Ventana platforms are universally available, and in several countries, the expensive assays are not sufficiently reimbursed. Therefore, far less costly laboratory-developed tests (LDT) for different platforms are a need to make PD-L1 testing broadly available. And finally, pathologists have to adopt various scoring algorithms, which differ in terms of cutoffs, evaluated cells (TC and/or IC), and in algorithms including IC, also in the method by which PD-L1 staining IC are scored.

PD-L1 testing by standardized, clinical trial-validated immunohistochemistry assays

Data are accumulating, mainly from comparison studies performed on NSCLC, that the analytical performance of the 22C3, 28-8, and SP263 assays are comparable for PD-L1 staining on TC [39–45]. Based on a study performed by AstraZeneca, which showed an overall percentage agreement of > 90% between these three assays at multiple expression cutoffs (1%, 10%, 25%, and 50% TPS), the SP263 assay has been CE-IVD-marked for durvalumab, nivolumab, and pembrolizumab, which provides a standardized PD-L1 test for the commonly used Ventana platforms in Europe [41]. The SP142 assay is less sensitive and stains a significant lower portion of TC, which is in line with the lower prevalence of NSCLC with high PD-L1 expression in the atezolizumab compared to the pembrolizumab trials (14% with ≥ 50% of TC or ≥ 10% of IC versus 23–30% with TPS > 50%) [14, 15, 19, 40, 43, 44, 46]. The recently published comparability study of the Blueprint phase 2 project is further consolidating these findings [44]. The study analyzed the analytical comparability of the 22-C3, 22-8, SP263, and SP142 assays and the investigational assay 73-10 used in clinical trials for avelumab on 81 formalin-fixed, paraffin-embedded (FFPE) lung cancer

specimens (resections, biopsies, and cell blocks), including the full range of PD-L1 expression levels. The staining was performed in a central laboratory and scored by 24 expert pulmonary pathologists. As part of the study, all pathologists received a study-specific training for the scoring of PD-L1 IHC on TC and IC. A special emphasis was put on IC scoring, and a pattern recognition approach was developed with the goal to better reproduce the proportion of tumor area that is occupied by PD-L1 staining IC. The study confirmed the comparable analytical performance of the 22C3 and 28-8 assays for PD-L1 staining on TC. For both clinically relevant cutoffs (TPS ≥ 1% and ≥ 50%, respectively) 3/59 cases showed a discordant result with a 22C3 and 22-8 TC staining concordance rate of 94.9%. The SP263 assay had a slightly greater sensitivity. Compared to 22C3, the SP263 assay showed discordant results in 8/59 (TPS ≥ 1%) and 5/59 (TPS ≥ 50%) cases with a concordance rate of 86.4 and 91.5%, respectively. Compared to 22-8, 6/59 (TPS ≥ 1%) and 2/59 (TPS ≥ 50%) cases were discordant, resulting in a concordance rate of 89.8 and 96.6%, respectively.

A greater sensitivity of SP263 compared to 22C3 and 28-8 was also previously reported [40, 47, 48]. In one of these studies, the overall analytical concordance between the three assays was still high and only 2–3% of the NSCLCs would have been classified differently using the first-line PD-L1 expression threshold of 50% [47]. Also, Hendry et al. found that SP263 classified significantly more cases as positive at the threshold of 1%; however, the difference was not significant at the PD-L1 expression threshold of 50% [48]. Though the analytical performance in general seems to be comparable for the 22C3, 22-8, and SP263 assays (here at least for the 50% PD-L1 cutoff), individual cases might be classified into different treatment groups and only a clinical validation study around clinically relevant cutoffs will provide evidence if such patients respond differently to PD-1/PD-L1-targeted treatment.

The Blueprint phase 2 study also confirmed that the SP142 assay is less sensitive. The 73-10 assay showed higher sensitivity compared to the other assays. Further, the study showed that the 22C3, 28-8, and SP263 assays had a comparable distribution of scores for IC. Similar to the findings for TC, SP142, and 73-10 differed and stained less and more IC, respectively. However, despite special effort on IC training, the overall agreement for assessment of PD-L1 staining on IC was only slight to fair, notably among expert pathologists. In contrast, the agreement for assessment of PD-L1 staining on TC was excellent. These findings are well in line with several studies, which reported on interobserver variability, mostly between expert pathologists, with generally good results for TC and poor results for IC scoring [41–43, 49]. This difficulty of scoring IC challenges PD-L1 scoring algorithms, which include IC (Table 1, see also the "PD-L1 interpretation and scoring" section below).

PD-L1 testing by laboratory-developed immunohistochemistry tests

Numerous analytical variables influence the performance of IHC, including the sensitivity and specificity of the primary antibody, the antibody concentration, the epitope retrieval conditions, the sensitivity of the detection method, and the calibration of staining using appropriate positive controls. The most commonly studied commercialized antibodies available for the development of LDT are the 22C3, 28-8, SP263, SP142, and the E1L3N clones. LDT using these antibodies with various detection systems and platforms can achieve PD-L1 staining on TC, which are concordant with the 22-C3, 22-8, or SP263 reference assays [45, 50–53]. It has been shown that all these antibodies have a similar ability to detect PD-L1 and that differences in PD-L1 IHC test performance are due to other variables in the IHC protocols [54]. In contrast to the ready to use IHC assay kits, establishing a LDT requires rigorous protocol development and extensive initial validation. Validation of PD-L1 LDT is still not standardized, and IHC validation practices in general are commonly inconsistent with significant interlaboratory variations. PD-L1-specific recommendations are lacking; however, general recommendations for analytic validation of predictive IHC LDT have been proposed in order to provide an accurate and reproducible IHC staining [55]. These recommendations include that for initial analytical validation of a new predictive LDT protocol, a minimum of 20 positive and 20 negative tissue controls, fixed and processed in the same manner as the clinical cases, should be tested. The PD-L1 positive controls in the validation set should represent the whole range of PD-L1 staining intensities at different expression levels (including weak staining and low proportion of stained cells) to ensure an adequate sensitivity of the test. As there is no gold standard for PD-L1 expression, a new LDT result should ideally be compared with the result of a validated assay kit on the same tissue validation set (if not available in house, then in another laboratory) [55, 56]. This comparison should achieve an overall concordance of at least 90% [55].

Extensive initial validation using appropriate control tissues are key for establishing a new LDT as highlighted by the recently published multicenter French harmonization study [45]. In this study, 27 new PD-L1 LDT were developed using FFPE tonsil tissue as the sole positive and negative control. Tonsil tissue is generally recommended as positive and negative tissue control for PD-L1 testing, as it provides the complete range of PD-L1 staining (epithelial crypt cells with moderate to strong, and germinal center macrophages with weak to moderate staining; no staining of endothelium, fibroblasts, and surface epithelium). The LDT protocols were not optimized on a tumor tissue validation set. When performed on 41 FFPE NSCLC specimens, 14/27 LDT (51.8%) failed to sufficiently correlate with the reference assays, highlighting the suboptimal validation.

When implementing PD-L1 testing, timely identification of appropriate tumor positive controls can be challenging and collaboration between laboratories can solve this issue, provided, that preanalytical steps are standardized.

Several PD-L1 LDT protocols, including 22-C3 and E1L3N on the commonly used Ventana and Leica platforms, have been published [52, 53, 57]. Adoption of protocols already validated by another laboratory can simplify establishing a local LDT protocol. However, as the local conditions may differ (even the water supply can matter), validation is necessary to verify that the LDT is performing as expected and to optimize the protocol if needed.

Once a PD-L1 test has been introduced in diagnostic service internal quality control, prospective monitoring of expected PD-L1 expression levels (e.g., expected prevalence of NSCLC with PD-L1 TPS ≥ 50% in Caucasian patients: 23–30%) and regular proficiency testing is essential in order to detect changes in analytical test performance and ensure accurate and reproducible PD-L1 staining [55].

Proficiency testing can lead to significant improvement of the analytical PD-L1 testing performance as recently shown by the PD-L1 module of the international external quality assurance program NordiQC. About half of the participating pathology laboratories used LDT for PD-L1 testing. Compared to the first run of the module in 2017, the pass rate for LDT improved significantly in the second and third runs 2018, from 20 to 73 and 93%, respectively [58]. The combined pass rates for the 22C3, 28-8, and SP263 assay kits also improved from 80 to 95%, highlighting that ready to use assays do not guarantee accurate staining in every laboratory. Therefore, even when introducing a PD-L1 assay kit, validation with a positive and negative tumor tissue control is necessary as local preanalytical conditions and slight local variations in machine calibration can influence staining.

All PD-L1 assay kits were validated for FFPE tissues using fixation in 10% neutral buffered formalin. This is of particular relevance to NSCLC, which is commonly diagnosed by cytology alone. Preanalytical processing of cytology specimens is less standardized and includes conventional alcohol-, air-dried-, or liquid-based preparations as well as FFPE cell blocks (CB) and even for CB several preparation protocols are in use [59]. In general, cytology specimens are well-established for diagnostic and predictive IHC. As preanalytical processing varies significantly from histology specimens, especially for conventional cytology specimens, cytology specific PD-L1 protocols need to be established and validated [60]. CB can be processed according to FFPE histology specimens in the IHC laboratory, though preanalytical variables do differ. Nevertheless, first studies show that CB achieve concordant PD-L1 staining results on TC with matched FFPE tissue specimens [16, 61–64].

PD-L1 interpretation and scoring

One major challenge for pathologists is to keep pace with the different indication-specific interpretation and scoring algorithms, which vary between drugs and tumor types (Table 1). These different interpretation algorithms basically reflect the difficulty of scoring IC. Evaluation of PD-L1 expression on TC is standardized across all indications [18, 20, 26, 65]. A TC is positive for PD-L1 expression when partial or complete membrane staining, irrespective of staining intensity, is present (Fig. 1). Necrotic TC and cytoplasmic TC staining are excluded from scoring. A minimum of 100 viable TC must be present for evaluation [66]. In the pembrolizumab and durvalumab trials for NSCLC, and in all nivolumab trials, only TC were included in scoring using the TPS. The TPS is the percentage of PD-L1-positive TC relative to all TC present in the sample. As the expression cutoff values are indication-specific, TPS is best reported as percentage value, including all relevant cutoffs (< 1%, 1–4%, than in 5% increments until

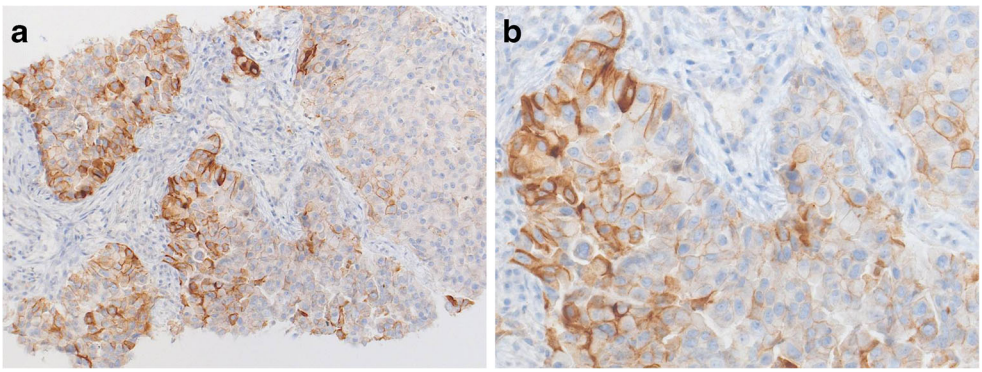

Fig. 1 Solid adenocarcinoma with a tumor proportion score (TPS) of 60% and heterogeneous PD-L1 expression. **a**, **b** Membranous staining intensity on tumor cells ranges from strong to weak to absent with partial and complete staining. Any membranous staining, partial or complete regardless of intensity, is included in the TPS. Cytoplasmic staining is excluded (PD-L1 immunohistochemistry with the VENTANA SP263 assay, original magnification × 200 and × 400, respectively)

50% and in 10% increments until 100%) without interpretation into positive and negative results.

In addition to TC, IC were included for PD-L1 scoring in all atezolizumab trials; in the pembrolizumab trials for G/GEJ, UC, and cervical cancer; and in the durvalumab trial for UC. Unfortunately, the scoring methods differ not only between the drugs but for pembrolizumab also between the tumor types (Table 1). TC are evaluated as outlined above.

For atezolizumab, IC are scored as the percentage of tumor area that is occupied by PD-L1 staining IC of any intensity (membrane and cytoplasmic) [67]. Any IC staining irrespective of type of cells is included. The tumor area is defined as the area occupied by viable TC and its associated intratumor and continuous peritumor stroma.

G/GEJ, UC, and cervical cancer tested for pembrolizumab are scored based on the combined positive score (CPS) [18]. The CPS is the ratio of PD-L1 staining cells (TC, lymphocytes, macrophages) at any intensity, relative to all viable TC [68]. Any staining in lymphocytes and macrophages in the tumor area should be included in scoring. Neutrophils, eosinophils, plasma cells (and BCG-induced granulomas in UC), and stromal cells (including fibroblasts) are excluded. The cutoffs are a CPS ≥ 1 for G/GEJ and cervical cancer and a CPS ≥ 10 for UC. Patients with advanced G/GEJ and a CPS of ≥ 1 are eligible for third-line treatment with pembrolizumab. If PD-L1 expression is not present in an archival G/GEJ specimen, a re-biopsy should be considered, as there was a significantly higher proportion of PD-L1 expression in newly obtained compared to archival biopsies in the Keynote-059 trial, which led to the approval of pembrolizumab for G/GEJ [22]. This is in contrast to NSCLC, where no such difference was observed and re-biopsy is not required if an archival tumor specimen lacks PD-L1 expression [15].

And finally, yet another scoring method has been developed for UC and complementary PD-L1 testing for durvalumab [69]. PD-L1 expression is determined by the percentage of PD-L1 staining TC (TPS) or by the percentage of staining tumor-associated IC (IC+). The percent of tumor area occupied by any tumor-associated IC (Immune Cell Present, ICP) is used to determine the IC+. A high PD-L1 expression is defined as TPS or IC+ ≥ 25%.

Conclusion

Predictive PD-L1testing by IHC has become standard of care in advanced NSCLC and can guide treatment decisions in several other tumor types. Current evidence suggests that with the exception of SP142, the clinical trial validates assays are interchangeable for TC staining. In general, IC scoring is challenging, which is further complicated by various scoring algorithms. Introduction of LDT needs standardized and careful validation and can achieve high-quality PD-L1 staining at lower costs irrespective of IHC platforms used. PD-L1 scoring algorithms can vary significantly between drugs and tumor types, and therefore, training and ongoing education for accurate PD-L1 interpretation are crucial.

Even the fact that there are intensive ongoing efforts to identify further predictive biomarkers, PD-L1 IHC will most likely remain to support patient's selection in several tumor types. However, a combination of biomarkers might better capture the complex biological background, allowing to further improve identification of patients who are most likely to benefit from PD-1/PD-L1-targeted immunotherapy.

Author contribution S.S.: assembly of the literature, conception, and writing of the manuscript

L.B.: assembly of the literature, conception, and writing of the manuscript

Compliance with ethical standards

Conflict of interest S.S. attended advisory board meetings of MSD and Astra Zeneca, has a consulting role for MSD, and received speaker's honoraria from MSD and Roche. L.B. attended advisory board meetings of Roche, MSD, Bristol-Myers Squibb, and Astra Zeneca and received financial research support from Roche and MSD. The manuscript is in compliance with ethical standards.

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