HHS Public Access

Author manuscript

J Thorac Oncol. Author manuscript; available in PMC 2017 March 16.

Published in final edited form as:

J Thorac Oncol. 2016 July ; 11(7): 964–975. doi:10.1016/j.jtho.2016.04.014.

PD-L1 Expression in Lung Cancer

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Abstract

Immunotherapies targeted against programmed death ligand 1 (PD-L1) and its receptor (PD-1) have improved survival in a subset of patients with advanced lung cancer. PD-L1 protein expression has emerged as a biomarker that predicts which patients are more likely to respond to immunotherapy. The understanding of PD-L1 as a biomarker is complicated by the history of use of different immunohistochemistry platforms with different PD-L1 antibodies, scoring systems, and positivity cut-offs for immunotherapy clinical trials with different anti-PD-L1 and anti-PD-1 drugs. Herein, we summarize the brief history of PD-L1 as a biomarker and describe the challenges remaining to harmonize PD-L1 detection and interpretation for best patient care.

Keywords

Lung cancer; Biomarker; PD-L1; Immunotherapy

Introduction

Immunotherapy with antibodies to prevent the interaction of the programmed death ligand-1 (PD-L1) with the programmed death ligand-1 (PD-1) receptor, also known as cluster of differentiation 274 (CD274), has dramatically improved the survival of some patients with lung cancer.[1–5] Binding of PD-1 ligand to its receptors, PD-L1 or programmed death

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Disclosure: Dr. Boyle an honorarium from Bristol-Meyers Squibb outside the submitted work. Dr. Rimm reports grants from Genoptix and Gilead Sciences during conduct of the study and personal fees from Bristol-Meyer Squibb and AstraZeneca outside the submitted work.

The remaining authors declare no conflict of interest.

ligand-2 (PD-L2), can prevent an innate cytotoxic T-cell response against tumor by inhibiting kinases that are involved in T-cell activation.[6–8] Immunotherapy with anti–PD-L1 or anti–PD-1 antibodies unleashes the innate immune system to react to the tumor growth. With the recent U.S. Food and Drug Administration (FDA) regulatory approval of nivolumab (anti–PD-1, Bristol-Myers Squibb, Lawrenceville, NJ) and pembrolizumab (anti–PD-1, Merck, Kenilworth) in the United States, the search for a predictive biomarker to select for patients who would preferentially benefit from anti–PD-1/PD-L1 therapy becomes more urgent—both to spare patients from ineffective therapy and to limit the number of patients exposed to potential autoimmune side effects from agents targeting this axis.[2–4,9]

Pembrolizumb is FDA approved with a PD-L1 immunohistochemical (IHC) “companion diagnostic” (the 22C3 pharmDx assay [Dako, Carpinteria, CA]), whereas nivolumab is FDA approved without a “companion diagnostic” assay but instead with a “complementary diagnostic” assay (the 28-8 pharmDx assay [Dako]).

PD-L1 Expression Emerges as a Biomarker in Early Studies

Initial studies of PD-L1 protein expression in tumors were performed in only a small subsets of patients but showed promising evidence for the utility of PD-L1 protein as a predictive biomarker for response to anti–PD-L1/PD-1 immunotherapy. PD-L1 expression was first reported in solid tumor specimens from 14 patients participating in a single-dose pilot phase I study of anti–PD-1 (nivolumab).[10] Membranous expression of PD-L1 on tumors was assessed by IHC analysis using the clone 5H1.[11] Four of nine patients had tumors positive for PD-L1 expression, and three of the four responded to nivolumab (75% response rate). In contrast, there was no response to nivolumab in five patients with tumors that lacked PD-L1 expression (three of four versus none of five). In a follow-up multidose, phase I nivolumab trial, nine of 25 patients with PD-L1–expressing tumors responded to treatment versus no response in any of 17 patients with tumors that lacked PD-L1 expression (nine of 25 versus none of 17).[1] Although in subsequent studies, clinical responses have been observed in patients with PD-L1–negative tumors, they are still observed at a lower rate than in patients with PD-L1–positive tumors.[12,13] Analysis of several anti–PD-1/PD-L1 phase I–II trials demonstrated a two to three times higher objective response rate in patients with PD-L1–positive tumors than in those with PD-L1–negative tumors despite a wide range of detection assays used.[14]

The prognostic role of PD-L1 is unclear. Different studies have found that the expression of PD-L1 is associated with better prognosis, worse prognosis, or no prognostic significance.[15–19] One explanation for these discordant results may be the current use of non-standardized IHC techniques for measuring PD-L1 levels in tissue. Another possibility is that PD-L1 expression may truly differ between different lung cancer cohorts.

Challenges and Pitfalls in Detecting PD-L1 by IHC Analysis

The prevalence of PD-L1 expression in the population of patients with non–small cell lung cancer (NSCLC) ranges from 24% to 60%, even with a cutoff for positivity set at 5% (Table 1 [clinical trials] and Table 2 [prevalence and outcomes]). Because few studies have

compared assays in the same specimens, it is difficult to understand whether differences in results are caused by differences in patient demographics, therapies, assay antibodies, or methods. Standardized methods and definitions of PD-L1 positivity are clearly needed to facilitate studies of PD-L1 as a predictive biomarker. Without standardization, comparisons of clinical results across studies in biomarker-defined subsets of patients have limited value and may even be misleading.[14]

Comparison of Different Antibodies

Several different primary antibodies for detecting PD-L1 proteins by IHC analysis have been developed by different companies in isolation. These antibodies include the clone E1L3N (Cell Signaling Technology, Danvers, MA), the clone SP142 (Spring Bioscience, Pleasanton, CA) (atezolizumab [Genentech, San Francisco, CA]), the clone 22C3 (Merck) (pembrolizumab, Merck), the clone 28-8 (Dako) (nivolumab [Bristol-Myers Squibb]), and the clone SP263 (Ventana Medical Systems, Tucson, AZ) (durvalumab [AstraZeneca, London, United Kingdom]). A study by Toplian et al.[1] in 2012 compared the performance of two anti–PD-L1 human antibodies, the clone 5H1 (noncommercial, from L. Chen, John Hopkins University, Baltimore, MD) and the clone 28-8. The membranous staining of tumor and tumor-infiltrating immune cells (TIICs) for PD-L1 protein was evaluated in formalin-fixed paraffin-embedded (FFPE) tissue specimens with NSCLC, melanoma, and renal cell carcinoma. The clone 28-8 demonstrated stronger staining in general with higher histoscores than those of 5H1, although both clones had similar binding affinities to PD-L1.

The ability of four PD-L1 antibodies to detect PD-L1 protein in PD-L1–transfected cells (Mel624), placenta, and known PD-L1–positive NSCLC cases was evaluated by Velchetti et al. using a quantitative immunofluorescence (QIF) approach.[39] Only one antibody, clone 5H1, attained the status of a “validated antibody,” with requirements for specific binding to PD-L1 protein in the PD-L1–transfected cells, no expression in parental cells, membranous staining of the syncytiotrophoblast layer of the placenta, and no staining in the stromal and vascular regions of the placenta. Using the same specimens and approach, Velcheti et al. validated an RNAscope in situ method (Advanced Cell Diagnostics, Hayward, CT) for specific detection of PD-L1 messenger RNA (mRNA).

A comparison of the prevalence of PD-L1 protein expression in small cell lung cancer (SCLC) has varied dramatically, from 0% to 83%, in different studies (Table 3). In two separate studies by Ishii et al.[42] and Komiya et al.,[43] a high prevalence of PD-L1 positivity in SCLC (71.6% and 82.8% with a 5% cutoff for positivity) was reported with the use of two different anti–PD-L1 antibodies from Abcam (Cambridge, United Kingdom) but without rigorous validation of the antibodies. Schultheis et al. compared staining of two different PD-L1 antibodies (5H1 and E1L3N) to stain specimens from another SCLC cohort and reported a complete lack of PD-L1 protein positivity in tumor, although an 18.5% PD-L1 positivity rate was observed in TIICs.[41]

One concern in comparing PD-L1 antibodies that have been used for IHC analysis is that different antibodies bind to different extracellular and cytoplasmic domains on the PD-L1 protein. This is not yet well characterized, but it could possibly alter the sensitivity and

specificity of the detection assay. The antibodies SP142 and E1L3N bind to the cytoplasmic domain of PD-L1. Other antibodies, including 28-8 and 22C3, SP263, and the E1J2J (Cell Signaling Technologies) all bind to the extracellular domain of the PD-L1. The wide range of prevalence rates with different antibodies may be at least partially due to differences in binding domains and the variable antibody affinity and signal to noise, most of which have not yet been published.

The lack of published work on antibodies that are FDA cleared (22-C3 pharmDx as a class III in vitro diagnostic companion diagnostic assay and 28-8 pharmDx as a complementary diagnostic assay) and those that are commercially available (SP142 and SP263 as investigational use–only assays) is surprising for a biomarker that could lead to prescription of more than $100,000 of therapy. A companion diagnostic assay is designed by a diagnostic company and investigated in conjunction with a biopharma company in a clinical trial. A companion diagnostic assay segregates a patient population into positive and negative groups, and only the “marker-positive” group can be treated with the drug. A complementary diagnostic assay is intended to guide therapy but is not required for the patient to receive a particular drug. In an ongoing effort initiated by the American Association for Cancer Research (AACR) and the International Association for the Study of Lung Cancer (IASLC) together with four pharmaceutical companies (Bristol-Meyers Squibb, Merck, Genentech/Roche, and AstraZeneca) and two diagnostic companies (Dako and Ventana), four PD-L1 assays are being compared on the same set of tumors (the Blueprint Project, an AACR–International Association for the Study of Lung Cancer joint effort) to better understand the similarities and differences between antibodies and platforms. The goal is to create comparison data on the same tissue stained with each of the different antibodies and platforms to pave the way for eventual postmarket standardization and guideline development. The phase I results from this study were presented at the AACR meeting (April 16–20, 2016). The scores from staining of 39 of the same lung cancer specimens was similar for the 22C3, 28-8, and SP263 PD-L1 antibodies, and the scores for the SP142 antibody were generally lower. This difference may be explained by the binding of the extracellular domain of PD-L1 for the three antibodies with similar scores and the intracellular domain of PD-L1 for SP142. When the results for the different assays were dichotomized into “positive” and “negative” by predetermined cut-offs, only 50% (19/39), had the same result for all four assays. At this point in time, it is recommended that results from PD-L1 IHC testing should be interpreted independently by antibody and platform used.[44]

Definitions of Positivity

Many different definitions for result categories and cutoffs for PD-L1 “positivity” have been explored. In an early study of PD-L1 protein expression, Dong et al. defined PD-L1 staining categories as: negative (−), with staining in less than 10% of tumor cells; weak positive (+), with staining in 10% to 40% of tumor cells; moderate positive (++), with staining in 40% to 80% of tumor cells; and strong positive (+++), with diffuse staining in more than 80% of cancer tissues.[7] The initial report by Topalian et al., which described a higher frequency of response to anti–PD-1 therapy in patients with tumors that were positive versus negative for PD-L1 protein expression, used a threshold of 5% for positivity, and many subsequent

studies have also used a 5% threshold for positivity.[1] Although the rationale for selection of 5% as a cutoff for positivity was not fully described in these reports, it was likely selected to detect protein expression greater than background “noise” or “artifact.” In a clinical trial to predict response to pembrolizumab in patients with NSCLC, a cutoff of 50% tumor cells positive for PD-L1 expression was selected on the basis of receiver operating curve analysis in a first small cohort and validated in a second larger cohort.[4] However, subsequent analyses have been performed with other cutoffs, and although the 50% cutoff remains the approved cutoff for the FDA companion diagnostic 22C3 pharmDx assay for second-and third-line settings, lower cutoff values for “positivity” are being evaluated in first-line settings.

Several clinical trials evaluating the response to MPDL3280A (atezolizumab) demonstrated that responses were observed in patients with multiple types of cancers expressing high levels of PD-L1, especially when PD-L1 was expressed by TIICs.[27,46,47] In the MPDL3280A clinical trials, in which the clone SP142 (Spring Bioscience/Ventana) and the Ventana platform were used, all patients involved were scored as TIIC 0, 1, 2, or 3 if less than 1%, 1% to less than 5%, 5% to less than 10%, or 10% or more of TIICs, respectively, stained positive for PD-L1. All types of TIICs, including macrophages, dendritic cells (DCs), and lymphocytes, were included in the estimate. Because of such different scoring methods for different PD-L1 antibodies, including differences in scoring of tumor cells versus TIICs, these PD-L1 assays are not interchangeable. Currently, these assays can be evaluated only in the context of the companion PD-1 or PD-L1 antibody and/or platform used in each particular trial. There are essentially no studies in which the cut point has been validated for reproducibility by multiple pathologists in multiple laboratories. Collaborative studies involving multiple pathologists who have received the same training for scoring PD-L1 with analysis of interobserver and intraobserver variability of scores are much needed.

From a pathologist’s perspective, it is more difficult to clearly distinguish between “true positive” protein staining and “false-positive” artifact in specimens with lower percentages of positive cells, especially if the staining is faint. With lower cutoffs, such as 1% and even 5% of tumor cells positive, there is a greater risk for inconsistent results than with a higher cutoff such as 10%. The downside of higher thresholds for positivity though is that more patients who may benefit from immunotherapy may be designated as negative. Also, because PD-L1 can stain heterogeneously, a specimen with a lower percentage of staining may be called negative even though it might have higher PD-L1 expression in a different region.[48] Although staining sections from different tumor blocks could help avoid false-negative results owing to heterogeneity, this is not a practical solution because, oftentimes, only one tumor block is created. Another consideration is that if a cutoff is too low, patients with a low likelihood of benefit may be treated with immunotherapy and suffer from unnecessary adverse effects or miss the opportunity for a more beneficial therapy regimen. Much work remains to be done to determine optimal cut points for different antibodies, both from the perspective of sensitivity and specificity for response and from the perspective of reproducibility between clinical studies. Once PD-L1 assays are approved by regulatory authorities, it becomes much more difficult to adjust for optimal scoring methods and cut points. Validated antibodies and standardized definitions of PD-L1 positivity will be critical for the development of PD-L1 as a predictive biomarker.

Challenges with Characterization of PD-L1 Expression by Cell Type

PD-L1 is expressed broadly in hematopoietic cells, including DCs, macrophages, mast cells, T cells, and B cells, and in nonhematopoietic cells, including endothelial, epithelial, and tumor cells.[49] However, the function and mechanism of PD-L1 expression on TIICs is less clear. One possible mechanism by which tumor inhibits the host immune response is through upregulation of PD-L1 on tumor cells and interaction with PD-1 on antigen-specific CD8-positive T cells (termed *adaptive immune resistance*).[50] TIICs may reflect a preexisting T-cell–inflamed tumor microenvironment and be important to the PD-1/PD-L1 pathway. In a report describing treatment of metastatic melanoma with pembrolizumab, tumor regression after therapeutic PD-1 blockade required preexisting CD8-positive T cells negatively regulated by PD-1/PD-L1–mediated adaptive immune resistance.[51] Several clinical trials of atezolizumab (MPDL3280A) observed a particularly strong response in patients with tumors that expressed high levels of PD-L1, especially when PD-L1 was also expressed by TIICs.[27,46]

As described by Kerr et al.,[52] any biomarker test that is dependent on distinguishing protein expression in lymphoid or other TIICs from expression in tumor cells may be particularly challenging in small biopsy or cytology samples. Many lung cancer biopsy and cytology specimens are so small and disaggregated in architecture that it is difficult with immunohistochemically stained slides to confidently differentiate protein expression on lymphoid or other immune effector cells versus tumor cells.

Development of standardized multiplexed methods to microscopically evaluate several markers on a single slide with accurate assessment of protein expression by cell type may be crucial for guiding immunotherapy.[53,54]

Heterogeneity and Associated Risks for False-Positive and False-Negative Results

In a study of the heterogeneity of PD-L1 protein expression by Velcheti et al., PD-L1 expression was assessed with automated QIF in FFPE tumor tissue microarrays (TMAs) from two cohorts. QIF with the automated quantitative analysis (AQUA) method was used to objectively measure the quantity of PD-L1 present in a defined compartment of the tumor. The AQUA score for PD-L1 was calculated by dividing the PD-L1 compartment pixel intensities by the area of the compartments within which they were measured. AQUA scores were normalized to the exposure time and bit depth at which the images were captured, allowing comparison of scores collected at different exposure times. Regressions were calculated by comparison of histospots from different tissue microarray cores taken at least 3 mm apart from the same tumor. The linear regression coefficients were 0.53 and 0.59, reflecting some degree of heterogeneity in PD-L1 expression.[39] In a follow-up study by McLaughlin et al.,[48,55] PD-L1 expression was assessed using the E1L3N (Cell Signaling Technology) and SP142 (Spring Bioscience) antibodies on 49 NSCLC whole-tissue sections and a corresponding microarray with the same cases. When analyzed with QIF, overall discordance between positive and negative results for the two antibodies was approximately

25%. Further studies are underway to more carefully characterize heterogeneity of expression both within a block and between blocks on the same cases.

Human placenta and tonsil tissue are often used as positive controls for endogenous PD-L1 staining. PD-L1 expression also can occur in the stromal surrounding of native tissue (for example, alveolar macrophages in lung specimens and DCs in nonneoplastic lymph node parenchyma), providing an internal staining control. Positive xenograft control specimens for PD-L1 IHC analysis have been created by transfecting cultured human melanoma Mel624 cells with a recombinant plasmid encoding full-length human programmed death ligand-1 gene (PD-L1I) DNA. Cell surface PD-L1 expression on the cultured Mel624 cells was confirmed with flow cytometry. NOD-SCID IL2Rgamma^{null} mice were inoculated with the PD-L1–transfected Mel624 cells. The subsequent xenografted tumors were harvested and preserved for use as a PD-L1 positive control.[18,39,56] Nonspecific isotype controls and PD-L1 knockout mouse tissue have also been used as a negative control for PD-L1 IHC analysis in some studies.[18,57]

Effect of Tissue Processing on PD-L1 Expression

Tissue processing and storage may alter the ability to detect PD-L1 in tumor. A comparison of the prevalence of PD-L1 protein positivity in a renal cancer cohort with fresh frozen tissue versus in FFPE tissue demonstrated a higher PD-L1 positivity rate in the cohort with fresh frozen tissue (37% versus 24%, respectively).[11,58,59] Both groups of tissue samples were stained with the 5H1 antibody and analyzed with a positive cutoff of ≥5% membranous tumor staining. The decreased prevalence in the FFPE tissue may be caused by PD-L1 protein denaturation with formalin fixation and a loss in PD-L1 antigenicity.

Several other factors are known to alter immunore-activity in FFPE tissue. PD-L1 protein antigenicity may decay over time in a tissue block and likely more rapidly in unstained tissue slides because of increased exposure of the tissue to the environment. Storage conditions such as light, temperature, and humidity may also play a role in the timing of loss of protein antigenicity. Most likely, the storage of slides or age of specimens results in loss of detection of PD-L1. Experiments that evaluate the timing and degree of decay of PD-L1 protein antigenicity in both tumor tissue blocks and unstained slides are needed. In an unpublished study, an attempt to stain tissue from four separate cohorts with tissue blocks more than 10 years old was made and there was no measurable staining. Although high-temperature heating is a critical factor for PD-L1 protein exposure, detailed procedures and reagents have not been standardized for PD-L1 epitope retrieval.[39,60–63] The updated guidelines for breast biomarker analysis by the American Society of Clinical Oncology/College of American Pathologists in 2013 address significant preanalytic factors with recommendations about promptly placing specimens into standard fixative types to minimize exposure time, as well as about maintenance of standard time lines for gross sectioning of tissue, transfer of sections to cassettes, and fixation times.[64,65] As PD-L1 expression develops as a biomarker, it will become increasingly important to develop guidelines that address preanalytical factors.

The Impact of Different Therapies on the Expression of PD-L1

Chemotherapy or targeted therapy may induce PD-L1 expression; consequently, PD-L1 expression in the original “chemonaive” diagnostic sample may not represent the PD-L1 expression of the tumor at the time that immunotherapy is introduced. The impact of first-line treatment (usual chemotherapy) on the expression of PD-L1 has not been elucidated because frequently posttreatment samples are not obtained as part of the standard of care for patients with lung cancer. If PD-L1 protein expression is to be used as a biomarker to guide immunotherapy, fresh specimens may need to be collected and evaluated for PD-L1 expression after other lines of therapy and before the start of immunotherapy.

Role of Other PD-L1 Biomarkers

To date, PD-L1 protein expression by IHC analysis has been the main predictive biomarker explored for response to immunotherapy. Nonetheless, researchers are investigating different biomarkers and methods to predict response to anti–PD-1/PD-L1 immunotherapy. In one study, PD-L1 mRNA expression was positive in 52.4% of NSCLC specimens (255 of 487) and associated with better outcome.[39] In a different study, concordance of PD-L1 protein expression by IHC analysis and expression of PD-L1 mRNA by mRNA in situ hybridization was demonstrated in squamous cell lung cancer.[66] The prevalence of PD-L1 mRNA positivity was higher than that of PD-L1 protein positivity. Although this difference may be caused by different cutoffs for positivity, mRNA in situ hybridization may identify patients who would benefit from immunotherapy that would otherwise be negative for PD-L1 protein expression by IHC analysis.

A study of the CD274 molecule gene (*CD274*) (PD-L1) locus (9p24.1) with fluorescence in situ hybridization demonstrated that the *CD274* gene is specifically rearranged and amplified in primary mediastinal large B-cell lymphoma (more frequently than in diffuse large B-cell lymphoma, follicular lymphoma, and Hodgkin’s lymphoma). Rearrangement was significantly correlated with overexpression of CD274 transcripts.[67] More research is needed to explore genetic alterations of the *CD274* locus in relation to protein expression in patients with lung cancer.

Evidence is accumulating to suggest that tumor neoantigen profile and mutation burden may also be important predictors of response to anti–PD-1/PD-L1 immunotherapy. In a study by Hellman et al., the response rate to nivolumab was significantly higher in former and current smokers compared with in never-smokers or minimal smokers with advanced NSCLC. Because smoking is associated with higher immunogenicity and mutational burden, it was postulated that these may be potential biomarkers for response to nivolumab.[68] In a different study by Rizvi et al., whole-exome sequencing of NSCLC in two independent cohorts revealed that patients with tumors having a higher nonsynonymous mutation burden had an improved objective response, durable clinical benefit, and progression-free survival after immunotherapy with pembrolizumab.[69] Another study concluded that mismatch-repair deficiency detected by microsatellite instability analysis predicted clinical benefit from immunotherapy with pembrolizumab in patients with progressive metastatic colorectal carcinoma.[70] Recently, a study of atezolizumab therapy in 310 patients with locally

advanced and metastatic urothelial carcinoma showed that mutation load may be an important biomarker of response to immune checkpoint inhibition in advanced urothelial carcinoma.[71] In this study, exploratory analyses showed that The Cancer Genome Atlas subtypes and mutation load were predictive for response to atezolizumab independent of PD-L1 expression status in TIICs.

Biomarkers to evaluate immune checkpoints other than the PD-L1/PD-1 checkpoint may provide clues about which patients will respond to PD-L1/PD-1 inhibitors. In essence, patients may not respond to PD-L1/PD-1 inhibitors if their innate immune response is inhibited by a non–PD-L1/PD-1 checkpoint such as the cytotoxic T-lymphocyte antigen-4 (CTLA-4)/B7 ligand checkpoint. CTLA-4 inhibitors have been used as an immunotherapy to block the interaction of the CTLA-4 receptor on T-cells with the B7 ligand on DCs. The B7 ligand is then free to bind to the CD28 receptor and activate an immune response against tumor. Although CTLA-4 inhibitor therapy has been associated with adverse side effects, it has been effectively used alone and in combination with PD-1 blockade for melanoma.[72–74]

PD-L2 is the second known ligand for the PD-1 T-cell coreceptor.[75] It is a transmembrane protein encoded by programmed cell death 1 ligand 2 gene (*PDCD1LG2*) and is structurally similar to PD-L1. Although PD-L1 is the dominant ligand for PD-1, PD-L2 can compete with PD-L1 with a twofold to sixfold higher affinity to PD-1 than PD-L1.[76] PD-L2 is expressed in relatively few cells and tissues but is upregulated on activated antigen-presenting cells, including monocytes, macrophages, and DCs.[77] However, the role of PD-L2 in mediating immunosuppression in the human tumor microenvironment, and as a marker for clinical characteristics, has not been clearly established.

Recently, several groups have investigated the possible correlation between tumor PD-L2 expression and clinical outcome in retrospective patient cohorts using IHC staining with different antibodies. Shin et al.[78] analyzed the expression of PD-L2 in renal cell carcinoma using IHC analysis with mouse monoclonal anti–PD-L2 (#176611 [R&D Systems, Minneapolis, MN]). The authors found that PD-L2 expression predicted poor prognosis in clear cell renal cell carcinoma. The same antibody was used in another study detecting PD-L2 expression in pleomorphic carcinomas of the lung and showed that PD-L2 expression had no prognostic implications in their cohort.[79] In a study involving 114 patients with Kirsten rat sarcoma viral oncogene homolog–mutant NSCLC, PD-L2 expression was detected by IHC staining in 47% of patients independent of smoking status by using mouse monoclonal anti–PD-L2 (clone 366C.9E5 from Gordon Freeman’s laboratory, Dana-Farber Cancer Institute).[80]

Of note, anti–PD-1 therapies can block the interaction between either PD-L1 or PD-L2 and PD-1, whereas anti–PD-L1 antibodies leave PD-L2 free to interact with PD-1.[27,81] A better understanding of the relationship between PD-L1 protein expression and the expression of other proteins involved in immune response, particularly in patients who do not respond to PD-L1/PD-1 inhibitors, may lead to better therapies for PD-L1/PD-1 nonresponders.

Conclusion

PD-L1 protein expression detected by IHC analysis has been the main predictive biomarker explored for response to anti–PD-1/PD-L1 immunotherapy. Comparative studies of PD-L1 detection methods and antibodies will be important for guiding the use of immunotherapy for patient care and development of immunotherapy biomarker guidelines. The development of standardized methods from the preanalytical stages of specimen processing to scoring of PD-L1 expression will benefit from a collaborative approach. Other methods of detection of PD-L1 expression, such as detection of mRNA expression and the use of multiplex platforms to detect PD-L1 expression by cell type and in relation to other immune checkpoints, may contribute to a deeper understanding of PD-L1 as a biomarker. In the future, PD-L1 protein expression may be evaluated in the context of other information, such as other immune checkpoints or mutational load, to more accurately guide the clinical use of immunotherapy.

Acknowledgments

Dr. Boyle reports grants from the National Institutes of Health (Lung SPORE, E SPORE P1-H, and H 5P50CA058187-20), and Dr. Hirsch reports grants from NIH (Lung SPORE P50 CA058187) during the conduct of the study.

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Table 1

Clinical trials with PD-L1 protein expression as a biomarker by immunohistochemistry (IHC)

Reference	Drug	Phase	Tumor Type	Sample Size with PD-L1 Data	Antibody (IHC platform)	Cell Location	Cutoff	Prevalence %	Objective Response Rate (ORR)		Clinical Trial ID
									PD-L1 (+)	PD-L1 (−)	
Brahmer et al.[2]	Nivolumab	III	Stage IIIB or IV squamous NSCLC	225 (total) 117 (nivolumab arm)	28-8 (Dako automated Link48)	TC membrane	≥ 1% ≥ 5% ≥ 10%	53% 36% 31%	17% 21% 19%	17% 15% 16%	NCT01642004 CheckMate017
Borghaei et al.[3]	Nivolumab	III	Stage IIB or IV nonsquamous NSCLC	231	28-8 (Dako)	TC membrane	≥ 1% ≥ 5% ≥ 10%	54% 40% 36%	31% 36% 37%	9% 10% 11%	NCT01673867, Checkmate 057
Rizvi et al.[20]	Nivolumab	II	Stage IIIB or IV squamous-cell NSCLC	117 (treated);76 with PD-L1 data	28-8 (Dako)	TC membrane	≥ 1% ≥ 5% ≥ 10%	59% 33% 33%	20%[a] 24%[a] 24%[a]	13%[a] 14%[a] 14%[a]	NCT01721759 CheckMate063
Gettinger et al.[21]	Nivolumab	I	Pretreated advanced NSCLC	68	28-8 (Dako)	TC membrane	≥ 5%	49%	15%	14%	NR
Gettinger et al.[22]	Nivolumab	I	Chemotherapy naïve advanced NSCLC	47	NR	TC	≥ 5%	55%	31%	10%	NCT01454102, Checkmate 012
Rizvi et al.[23]	Nivolumab	I	Chemotherapy naïve advanced NSCLC	17	28-8 (Dako)	TC	≥ 5%	59%	50%	0%	NR
Antonia et al.[24]	Nivolumab + Ipilimumab	I	Chemotherapy naïve advanced NSCLC	38	28-8 (Dako)	TC	≥ 5%	42%	19%	14%	NR
Ramalingam et al.[25]	Nivolumab	II, single-arm study	Advanced refractory squamous NSCLC	76	NR	NR	≥ 5%	33%	20%	10%	NR
Garon et al.[4]	Pembrolizumab	I	Advanced NSCLC	824 screened; 325 treated	22C3 (Dako)	TC membrane	≥ 50% ≥ 1%	23% 61%	41% 26%	13% 10%	NCT01295827 KEYNOTE-001
Herbst et al.[26]	Pembrolizumab	II/III	Previously treated NSCLC	2222 screened; 1034 assigned to therapy; 980 treated with pembrolizumab	22C3 (Dako)	TC	≥ 1% 1%–49% ≥ 50%	66% 38% 28%	18% NR 30%	NR NR NR	NCT01905657; KEYNOTE-010
Herbst et al.[27]	Atezolizumab	I	Locally advanced or metastatic solid tumor	184	SP142 (Ventana)	TC IC	≥ 5% ≥ 5%	24% 26%	46%	18%	NCT01375842
Spira et al.[28]	Atezolizumab	II	Previously treated NSCLC	287	SP142 (Ventana)	TC and/or IC	TC0 and IC0[b] TC1/2/3 or IC1/2/3[b] TC2/3 orIC2/3[b] TC3 or IC3[b]	32% 68% 37% 16%	18% 22% 38%	8%	NCT01903993
Spigel et al.[29]	Atezolizumab	II	Chemo-naïve Stage IIIB/IV NSCLC ≥ 2 lines of therapy with no brain metastasis ≥ 2 lines of therapy with treated asymptomatic brain metastasis	31 71 12	SP142 (Ventana), in archival or fresh tumor biopsies (required for the latter two cohorts)	TC and/or IC	TC3 or IC3[b] TC3 or IC3 TC3 or IC3[b]	23% 37% 67%	29% 27% 25%		NCT01846416
Horn et al.[30]	Atezolizumab	IA	NSCLC, majority of pts has 3 lines of therapy	78	SP142 (Ventana)	TC and/or IC	TC3 or IC3	26%	45%	14%	NCT01375842
Liu et al.[31]	Atezolizumab	IB	Chemotherapy-naive locally advanced or metastatic NSCLC	37	SP142 (Ventana)	NR	NR		NR		NCT01633970
Rebelatto et al.[32]	Durvalumab	I/II	NSCLC	81	SP263, (Ventana automated BenchMark ULTRA platform)	TC membrane	≥ 25% (clinical response)	NR	PD-L1 + patients had a higher response rate than PD-L1 - patients		NCT01693562
Gulley et al.[33]	Avelumab	IB	Advanced NSCLC progressing after platinum-based chemotherapy	138	NR	TC	≥ 1%	86%	14%	10%	NCT01772004

[a] The percentage was partial response rate only in Rizvi's study.

[b] The numbers after TCs and ICs of 0, 1, 2, or 3 mean less than 1%, 1% to less than 5%, 5% to less than 10%, or 10% or more of the TCs or ICs, respectively, stained positive for PD-L1.

PD-L1, programmed death ligand-1; IHC, immunohistochemistry; ID, identifier; NSCLC, non-small cell lung cancer; NR, not reported; RECIST, Response Evaluation Criteria in Solid Tumors; TC, tumor cells; ICs, tumor-infiltrating immune cells.

Table 2

The Association between PD-L1 Protein Expression and Clinical Outcome in Patients with Lung Cancer

Reference	Tumor Type	Sample Size	Method	Antibody/Platform (Anti–PD-L1)	Cell Location	Cutoff	Prevalence	Outcome Associated with PD-L1 Positivity	Treatment
Kim et al.[15]	Squamous carcinoma	331	IHC analysis	E1L3N (Cell Signaling Technologies)/Ventana Benchmark XT automated staining system	Tumor cell membrane	≥2+ (weak to moderate or strong staining in 10% tumor cells)	26.9%	No significant association with prognosis	
Schmidt et al.[16]	Squamous carcinoma	149	IHC analysis	E1L3N (Cell Signaling Technologies), #13684 clone, rabbit IgG1	Tumor cell membrane	5% of the tumor cells displayed at least moderate staining	28%	Improved OS	Adjuvant therapy
Zhang et al.[34]	NSCLC	109	ELISA	Beijing Keyingmei Science and Technology Ltd	Peripheral blood	0.636 ng/mL	56% above the threshold	Worse prognosis	NR
Tang et al.[17]	EGFR wild-type advanced NSCLC	56	IHC analysis	E1L3N (Cell Signaling Technologies), 1:200	Tumor cell membrane	H-score ≥5	57.1%	Worse prognosis	
Lin et al.[35]	EGFR mutated adenocarcinoma	56	IHC analysis	#ab58810 (Abcam)	Tumor cell membrane and cytoplasm	≥Mean H-score	53.6%	Improved OS	EGFR-targeted TKI therapy
Zhang et al.[36]	Adenocarcinoma	143	IHC analysis	#SAB2900365 (Sigma-Aldrich)	Tumor cell membrane and cytoplasm	Semiquantitative quick score≥8 (median quick score)	50%	Worse OS	Surgical resection
Schmidt et al.[16]	NSCLC	321	IHC analysis	E1L3N (Cell Signaling Technologies)	Tumor cell cytoplasm	≥5% with at least moderate staining	24%	Improved prognosis in squamous cell carcinoma subset	Curative resection
Azuma et al.[37]	NSCLC	164	IHC analysis	Lifespan Biosciences/Ventana BenchMark XT platform	Tumor cell membrane and/or cytoplasm	>Median value of H-score (30)	50%	Worse OS	Surgical resected
Yang et al.[38]	Stage I lung adenocarcinoma	163	IHC analysis	Proteintech Group	Tumor cell membrane staining	≥5% with at least moderate staining	39.9%	Improved relapse-free survival	Surgical resection
Velcheti et al.[39]	NSCLC (Yale) NSCLC (Greece)	204 340	QIF	5H1 (Dr. Lieping Chen's laboratory)		AQUA quantitative score higher than signal intensity from normal lung and negative controls	25% 36%	Improved OS	
Chen et al.[40]	NSCLC	120	IHC analysis		Tumor cell membrane and cytoplasm		57.5%	Worse OS	

PD-L1, programmed death ligand-1; IHC, immunohistochemical; NSCLC, non–small cell lung cancer; ELISA, enzyme-linked immunosorbent assay; EGFR, epidermal growth factor receptor; IgG1, immunoglobulin G1; OS, overall survival; NR, not reported; TKI, tyrosine kinase inhibitor; QIF, quantitative immunofluorescence; AQUA, automated quantitative analysis.

Table 3

Prevalence of PD-L1 Protein Expression in Small Cell Lung Cancer by IHC Analysis Using Different Antibodies

Reference	Histologic Diagnosis	Stage	Antibody for IHC Analysis	Cellular Compartment Scored	Cells Scored for PD-L1 Expression	Percentage Positive ≥1%	≥5%
Schultheis et al.[41]	Small cell neuroendocrine carcinomas	Primary tumors and metastases	5H1; E1L3N (Cell Signaling Technologies)	Membranous	Tumor Tumor-associated macrophages	0% (0 of 92) 18.5% (17 of 92)	
Ishii et al.[42]	Small cell lung cancer (SCLC)	Limited and extensive stage	Unknown (Abcam)	Membrane and/or cytoplasm	Tumor		71.6% (73 of 102)
Komiya and Madan[43]	SCLC	Unknown	EPR1161(2) (Abcam)	Membranous	Tumor		82.8% (82 of 99)
Ott et al.[45]	SCLC	Unknown	Unknown	Unknown	Tumor or stroma	27% (37 of 135)	

PD-L1, programmed death ligand-1; IHC, immunohistochemical; SCLC, small cell lung cancer.