

PD-L1 Expression as a Predictive Biomarker in Cancer Immunotherapy

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Abstract

The resurgence of cancer immunotherapy stems from an improved understanding of the tumor microenvironment. The PD-1/PD-L1 axis is of particular interest, in light of promising data demonstrating a restoration of host immunity against tumors, with the prospect of durable remissions. Indeed, remarkable clinical responses have been seen in several different malignancies including, but not limited to, melanoma, lung, kidney, and bladder cancers. Even so, determining which patients derive benefit from PD-1/PD-L1-directed immunotherapy remains an important clinical question, particularly in light of the autoimmune toxicity of these agents. The use of PD-L1 (B7-H1) immunohistochemistry (IHC) as a predictive biomarker is confounded by multiple unresolved

issues: variable detection antibodies, differing IHC cutoffs, tissue preparation, processing variability, primary versus metastatic biopsies, oncogenic versus induced PD-L1 expression, and staining of tumor versus immune cells. Emerging data suggest that patients whose tumors overexpress PD-L1 by IHC have improved clinical outcomes with anti-PD-1-directed therapy, but the presence of robust responses in some patients with low levels of expression of these markers complicates the issue of PD-L1 as an exclusionary predictive biomarker. An improved understanding of the host immune system and tumor microenvironment will better elucidate which patients derive benefit from these promising agents. *Mol Cancer Ther*; 14(4): 847–56. ©2015 AACR.

Introduction

One of the initial observations of the important role that the host immune system plays in cancer control was made by Dr. William Coley at the turn of the century, when he described a patient with advanced sarcoma who achieved a complete remission (CR) following two severe bacterial skin infections (1). Over time, an improved understanding of immunobiology uncovered the importance of immune checkpoints in facilitating tumor escape, leading to the development of multiple novel therapeutics targeting the CTLA-4 (cytotoxic T-lymphocyte-associated protein 4, CD152) and PD-1/PD-L1 (programmed cell death protein 1, CD279; programmed death-ligand 1, CD274) immune checkpoints (2, 3, 4). In particular, melanoma, renal cell carcinoma (RCC), and non-small cell lung cancer (NSCLC) have demonstrated durable responses to immune checkpoint inhibition (3, 4). For example, in patients with metastatic melanoma treated with concurrent ipilimumab (anti-CTLA-4) and nivolumab (anti-PD-1), 17% of patients achieved a CR, with an overall survival (OS) rate for all patients of 79% at two years (5). Thus, immunotherapy holds promise not only in attaining CRs in refractory patients, but in generating long-term remissions as well. With the recent regulatory approval of nivolumab (anti-PD-1, Bristol-Myers Squibb) and pembrolizumab (anti-PD-1, Merck) in the US, the search for a predictive biomarker to select for patients who

would preferentially benefit from anti-PD-1/PD-L1 therapy becomes more pressing—both to spare patients ineffective therapy, and to limit the number of patients exposed to autoimmune side effects from agents targeting this axis (6).

Mitigation of autoimmunity via regulation of activated T cells is an important feature of immune homeostasis. T-cell antigen-presenting cell (APC, dendritic cell, or tumor cell) interactions and inhibitory checkpoints include (format: receptor on T cell ~ ligands on APC): CTLA-4~CD80/CD86, PD1~PD-L1/PD-L2, GAL9~TIM3 (galectin-9/T cell immunoglobulin and mucin domain 3), TCR~LAG3 (T-cell receptor/lymphocyte activation gene 3), and HVEM~BTLA (hepatitis virus entry mediator/B and T lymphocyte attenuator; ref. 7). Overexpression of these inhibitory checkpoints by tumors, recruitment of immunosuppressive cells and factors into the microenvironment, and decreased immunogenic antigen presentation by tumor sculpts the immune microenvironment towards an immunosuppressive state via a process termed "immunoediting" (8). This tumorigenic process involves three phases: (i) an initial immune elimination of tumor followed by (ii) an equilibrium state between the host immune system and tumor, and (iii) generation of a tumor that evades immune surveillance through multiple immunoinhibitory mechanisms. Thus, immunoediting is a maladaptive reciprocal process by which the interaction between the host immune system and tumor results in the host immune system selecting for a less immunogenic tumor, and a tumor selecting for a less immunologically adept host.

PD-1 (CD279) is a T-cell immune checkpoint involved in dampening autoimmunity in the peripheral effector phase of T-cell activation, leading to immune tolerance of cells expressing PD-L1 (B7-H1; CD274) and PD-L2 (B7-DC; ref. 9). PD-1, akin to its more centrally acting counterpart CTLA-4, is also preferentially expressed on the regulatory T cell, an immune cell that contributes to the immunosuppressive tumor microenvironment, as

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well as on activated B cells and NK cells (9). PD-1/PD-L1 activation results in peripheral immunologic tolerance in T cells. In PD-1 and PD-L1 knockout mice, the autoimmune phenotype is milder as compared to that in CTLA-4 knockouts (10, 11). This parallels the clinical severity of observed toxicities of anti-PD-1/anti-PD-L1 and anti-CTLA-4 therapeutic monoclonal antibodies, which are more pronounced with the latter agent (12).

PD-L1 is expressed on a variety of cell types, including placenta, vascular endothelium, pancreatic islet cells, muscle, hepatocytes, epithelium, and mesenchymal stem cells, as well as on B cells, T cells, dendritic cells, macrophages, and mast cells (13). PD-L2, a second ligand for PD-1, has a more restricted expression pattern, predominantly on dendritic cells, macrophages, and mast cells (14). Immune attack via IFN γ release leads to inducible upregulation of PD-L1 by mucosa creating an "immune shield" to protect against autoimmune attack in the setting of chronic inflammation or infection. Upregulated PD-L1 on these cells binds PD-1 on T cells, contributing to the development of T-cell exhaustion (15). Tumor cells have co-opted this PD-1/PD-L1 regulatory mechanism, designed to protect normal mucosa from autoimmune attack, and instead overexpress PD-L1 to avoid immunologic surveillance to facilitate cancer growth. We review here the early clinical experience and development of PD-L1 immunohistochemistry (IHC) as a predictive biomarker for anti-PD-1–directed therapy in human cancer.

PD-L1 Overexpression in Solid Tumors

Multiple solid tumor types including melanoma, RCC, NSCLC, thymoma, ovarian, and colorectal cancer co-opt this immune shield by expressing PD-L1 to generate an immunosuppressive tumor microenvironment and avoid T cell cytotoxicity (16, 17, 18). Tumor PD-L1 overexpression confers a poorer prognosis across multiple tumor histologies, making therapeutic intervention on this immunomodulatory axis enticing (19, 20, 21, 22). With the success of targeting HER2-overexpressing cancers, utilizing IHC-based approaches to measure levels of HER2 expression, substantial effort has been expended on similar immunohistochemical approaches to screen tumors for PD-L1 expression in order to select for patients who may benefit from anti-PD-1/PD-L1 directed therapies (23). However, to date, IHC-based detection of PD-L1 has been problematic in determining not only which tumor histologies are responsive to anti-PD-1/PD-L1 based immunotherapy, but also in determining which individual patients may benefit from therapy.

Tumor expression of PD-L1 in human cancer specimens is summarized by histology in Table 1. There is wide variability in the reported ranges of percent tumor samples overexpressing PD-L1 in a given histology, due to numerous factors that are discussed below. For the tumors classically associated with clinical responses to immune checkpoint inhibition with anti-PD-1 agents—melanoma, RCC, NSCLC, and more recently bladder cancer—the range of PD-L1 IHC expression on tumor ranges widely from 14% to 100%, highlighting the issues with PD-L1 as a predictive biomarker for this class of therapy (24, 25, 26). Among the hematologic malignancies, PD-L1 expression on FACS-separated malignant cells ranges from 37–58%, with higher expression in cancers driven by viruses such as EBV and HTLV (27). In comparison, tumor histologies currently thought to be less responsive to immune checkpoint inhibition, such as colorectal cancer and sarcoma, have tumor PD-L1 expression ranging

in the 12%–53%, indicating that tumor PD-L1 IHC expression is not the sole determinant of which tumors histologies respond to PD-1/PD-L1 directed inhibition (24, 28).

Hematologic Malignancies and PD-L1 Expression

Analysis of PD-L1 expression in hematologic malignancies as a predictive biomarker has also been studied. Flow cytometric measurement of PD-L1 expression has been utilized, and a general trend towards increased PD-L1 expression on virally mediated hematologic cancers is apparent (27). In addition, increased soluble PD-L1 (sPD-L1) in diffuse large B-cell lymphoma (DLBCL) is associated with inferior prognosis, particularly in patients with high-risk disease by International Prognostic Index (IPI), with 3-year OS of 40.9% in patients with elevated sPD-L1 and 82.1% in patients with non-elevated sPD-L1 (ref. 29; $P < 0.01$). However, analysis of PD-L1 expression as a predictive biomarker in hematologic malignancies has been limited to date, though a phase II study of pidilizumab (anti-PD-1) after autologous stem cell transplantation in DLBCL had a response rate of 51% in patients with measurable disease after transplant (30).

Classical Hodgkin lymphoma, particularly the nodular sclerosing variant, has a unique mechanism of immune suppression and PD-L1/PD-L2 upregulation, via chromosome 9p24.1 amplification, which contains an amplicon containing *PD-L1*, *PD-L2*, and *JAK2* (31). In a landmark phase I study of nivolumab in a highly refractory Hodgkin lymphoma population (23 patients treated; 78% with prior autologous stem cell transplant, 78% with prior brentuximab vedotin treatment), 87% had an objective response, including 17% with complete response (32). Exploratory biomarker analysis in 10 available biopsy specimens demonstrated 100% PD-L1 and PD-L2 expression on Reed–Sternberg cells by IHC, with PD-L1/PD-L2 copy number gain in 60% of samples, amplification in 40% of samples, and polysomy 9p in 80% of samples. Given these impressive results, there is intense clinical interest in immunomodulation for hematologic malignancies.

Tumor Molecular Phenotypes and PD-L1

Within a given tumor histology, immune checkpoints may play a differential role based on tumor molecular phenotype. Within breast cancer, there is increasing recognition that the immune microenvironment has prognostic and predictive implications for HER2-overexpressing and triple-negative breast cancers (TNBC). Analyses of HER2-overexpressing breast cancer specimens from the GeparQuattro trial, a phase III neoadjuvant trial assessing different combinations of chemotherapy with trastuzumab, demonstrated improved pathologic complete response (pCR, 47.4% vs. 31.7%, $P = \text{NR}$) in tumors with higher levels of tumor-infiltrating lymphocytes (TIL). A similar result was seen in the adjuvant FinHER trial, where a 10% increase in TILs was associated with a 16% increase in pCR ($P = 0.037$), and expression profiling of these samples demonstrated that trastuzumab was associated with improved outcomes in the setting of upregulated CTLA-4 and PD-1 expression (33). Experiments in a transgenic BALBc/c-MMTV-neu mouse model demonstrated synergy between trastuzumab and anti-PD-1 and anti-PD-L1 antibodies (34). Based on an analysis of TNBC samples from the BIG 02-98 trial, a phase III adjuvant study comparing sequential versus concurrent docetaxel with anthracycline-based chemotherapy in

Table 1. Prevalence of PD-L1 expression in human cancers

Tumor histology	Percent tumor samples expressing PD-L1	Tumor surface expression cutoff for positivity	PD-L1 detection antibody	References
Melanoma	45%	5%	28-8	(76)
	100%	>10%	5H1	(24)
	91%	NR	R&D B7-H1	(37)
	38%	5%	5H1	(54)
	77%	1%	NR	(70)
RCC	44% (primary tumor), 54% (metastasis)	10%	5H1	(77)
	24%	5%	5H1	(22)
	14%	5%	405.9A11	(78)
	24%	H-score >0	5H1	(79)
NSCLC	49%	5%	28-8	(76)
	52%	NR	R&D B7-H1	(37)
	95%	>10%	5H1	(24)
	50%	11%	MIH1	(80)
	21% (squamous only)	>1% vs. >5% vs. H-score	5H1	(50)
	60%	5%	DAKO IHC	(71)
Bladder	21% (37% squamous, 22% transitional)	5%	28-8	(38)
	28%	1%	5H1	(82)
	20%	5%	405.9A11	(83)
Head and neck	31%	5%	28-8	(38)
	66%	10%	5H1	(84)
Cervical	29%	5%	28-8	(38)
	19%	NR	5H1	(64)
Glioblastoma multiforme	25%	5%	28-8	(38)
	45%	50%	NR	(85)
Breast cancer	18% TNBC (0% ER/PR/HER2+)	5%	28-8	(38)
	43% overall (59% TNBC)	NR	R&D B7-H1	(37)
	50%	1%	MIH1	(86)
Gastric	42%	NR	2H11	(87)
Esophageal	20%	5%	28-8	(38)
	44% (squamous)	10%	MIH1	(88)
Hepatocellular carcinoma	15%	5%	28-8	(38)
	25%	NR	eBioscience anti-B7-H1	(89)
Pancreatic	39%	10%	MIH1	(90)
Colorectal	53%	>10%	5H1	(24)
	56% (MSI-H) vs. 21% (MSS)	NR	Caris	(42)
Thymic cancer	100% (thymoma), 88% (thymic carcinoma)	NR	29E (.5A9/.2A3)	(91)
	100% (thymoma and thymic carcinoma)	NR	Sino clone 15	(92)
Ovarian	87%	>10%	5H1	(24)
	89%	5%	27A2	(20)
Sarcoma	12% (27% GIST)	1%	DAKO PD-L1 IHC	(28)
Unknown primary	28%	5%	28-8	(38)
Acute myeloid leukemia	37%	5%	MIH1	(93)
Leukemias (various)	57%	NR	5H1	(94)
B-cell lymphomas	58%	5%	DICI 339.7G11	(27)
Multiple myeloma	93%	NR	N20 antihuman PD-L1	(59)

Abbreviations: ER, estrogen receptor; H-score, immunoreactivity score: (3 × percentage of strongly staining tumor cells) + (2 × percentage of moderately staining tumor cells) + (percentage of weakly staining tumor cells), range of 0 to 300; NR, not reported; PR, progesterone receptor.

breast cancer, a 10% increase in stromal TILs was associated with improved prognosis with a 15% reduction in relapse and a 17% reduction in death from TNBC (35, 36). Expression of PD-L1 by IHC appears to be increased in TNBC relative to that in hormone-driven tumors, and the prognostic importance of TILs in TNBC make the PD-1/PD-L1 axis appealing as a therapeutic target (37, 38). Recently, two phase 1 studies in metastatic TNBC have demonstrated preliminary signs of clinical activity. Among 27 patients treated with pembrolizumab, 1 patient had a complete response, 4 with a partial response, and 7 with stable disease (39). A similar study of 12 patients with TNBC treated with MPDL3280A resulted in 1 complete response, 2 partial responses, and 1 stabilization of disease (40). Further study is warranted, and an improved understanding of the unique biologic characteristics of the complete responders in the above study may help elucidate novel biomarkers.

In colorectal cancer, patients with microsatellite unstable (MSI-H) primary tumors have a superior prognosis, associated with increased TILs within tumor (41). In addition to increased TILs, MSI-H colorectal cancers have increased PD-L1 expression (56% vs. 21% in MSS tumors; $P = 0.007$), which may represent a unique molecular subset of patients with colorectal cancer that may benefit from PD-1/PD-L1 targeting therapy (42). One potential explanation for this series of findings is that MSI-H tumors, which are deficient in their ability to repair DNA mismatches, have an increased mutational burden resulting in increased neoantigen formation. These neoantigens are immunogenic, resulting in increased TILs in the MSI-H colorectal tumor microenvironment (43). Over time, an immunosuppressive tumor microenvironment is sculpted with overexpression of immune checkpoints such as PD-L1. Clinically, the potential benefit of immune checkpoint inhibition in MSI-H colorectal cancer is currently under active investigation (NCT02060188, NCT01876511; refs. 44, 45). Mutational burden appears to be correlated with response to immunotherapy between histologies as well. Tumor histologies with higher mutational burden—melanoma, NSCLC, and bladder cancer—are also those histologies currently associated with superior responses to immune checkpoint blockade, including combinatorial strategies, relative to histologies with fewer mutations per

megabase (46, 47). This may be due to a higher number of tumor mutations increasing the probability of generating a “high quality” immunogenic peptide, resulting in an improved anti-tumor response in the setting of checkpoint inhibitor therapy (48).

Technical Issues with PD-L1 IHC

As summarized in Table 1, interpretation of PD-L1 by IHC in human tumor specimens is marred by multiple complicating factors. A multitude of detection PD-L1 IHC antibodies have been utilized, including 28-8, 5H1, MIH1, and 405.9A11 (24, 38). In addition, a number of proprietary companion diagnostics are being developed in this area (Table 2). Comparative performance characteristics of these assays are not well known. The lack of a clear definition of “positive” tumor PD-L1 staining by IHC is problematic, with cut-off points for a positive result ranging from >1% to >50% based on percent tumor cells stained, compounding the existing issue of PD-L1 expression heterogeneity within the microenvironment (49, 50).

Furthermore, PD-L1 has limited binding sites for IHC detection antibodies, as it contains only two small hydrophilic regions, making immunohistochemical approaches classically used in formalin-fixed, paraffin-embedded (FFPE) specimens less effective (51, 52). Because of this lack of binding sites on PD-L1 amenable for IHC detection, IHC antibodies typically bind PD-L1 at structurally unique sites compared with therapeutic PD-L1 antibodies. In addition, the use of multiple IHC cutoffs and proprietary PD-L1 IHC detection systems represents a barrier to interpretation of clinical trial biomarker data across trials. As summarized in Table 3, multiple agents have been studied with varying detection antibodies and IHC cutoffs, with the most data currently available in melanoma and NSCLC. The nivolumab phase I melanoma study compared a 1% versus 5% cutoff for PD-L1 status, by Dako (Carpinteria, CA) IHC, in archival tissue (53). For the 41 patient samples available for analysis, at the 1% cutoff, 26 were positive (35% response rate) and 15 were negative (13% response rate), while for the 5% cutoff 18 samples were positive (44% response rate) and 23 were negative (13% response rate). The

Table 2. Examples of available PD-L1 detection and therapeutic antibodies

Therapeutic agent	Company	Companion PD-L1 detection antibody	Examples of trials by histology	Comments
Anti-PD-1				
Nivolumab	BMS	28-8	Melanoma, NSCLC, RCC, Hodgkin's lymphoma, other solid tumors	FDA (USA) approved for refractory unresectable/advanced melanoma (12/22/14); approved in Japan for unresectable melanoma (7/4/14)
Pembrolizumab (MK-3475, lambrolizumab)	Merck	Merck PD-L1 IHC assay	NSCLC, other solid tumors	FDA (USA) approved for ipilimumab-refractory melanoma (9/4/14)
Pidilizumab (CT-011)	Curetech		NHL, melanoma, RCC	
AMP-224, AMP-514	Amplimmune		Phase 1	
“Anti-PD-1”	Novartis/CoStim		Phase 1	
Anti-PD-L1				
BMS-946559	BMS	28-8		
MPDL3280A	Genentech/Roche	Roche PD-L1 IHC assay	NSCLC, melanoma, RCC	
MEDI4736	MedImmune/Astrazeneca	SP263 Ventana IHC	NSCLC, other solid tumors	
MSB0010718C	EMD Serono (Merck)		Phase 1	

Abbreviations: BMS, Bristol-Myers Squibb; NHL, non-Hodgkin lymphoma.

Table 3. Comparison of overall response rates to anti-PD-1 and anti-PD-L1 therapy based on histology and PD-L1 IHC expression

Therapeutic agent	Detection antibody; membrane staining cutoff (in percent of tumor cells)	Histology	PD-L1 IHC expression (% samples at IHC level)	Response rate for PD-L1-positive versus PD-L1-negative patients	PFS for PD-L1-positive versus PD-L1-negative patients	OS for PD-L1-positive versus PD-L1-negative patients	Ref(s)
Nivolumab	28-8; 5%	Melanoma (<i>n</i> = 38)	+ (45%) – (55%)	44% vs. 17% (<i>P</i> = NR)	9.1 vs. 2 months (median PFS, <i>P</i> = NR)	21.1 vs. 12.5 months (median OS, <i>P</i> = NR)	(76)
	DAKO; 5%	NSCLC (<i>n</i> = 20)	+ (60%) – (40%)	67% vs. 0% (<i>P</i> = NR)	NR	NR	(71)
	5H1; 5%	Melanoma, RCC, NSCLC, CRC, prostate	+ (60%) – (40%)	36% vs. 0% (<i>P</i> = NR)	NR	NR	(4)
Pembrolizumab	NR; 1%	Melanoma (<i>n</i> = 71)	+ (77%) – (23%)	51% vs. 6% (<i>P</i> = 0.0012)	12 vs. 3 months (median PFS; <i>P</i> = 0.0004)	84% vs. 69% (1-year OS rate; <i>P</i> = 0.2146)	(70)
	NR; 50%	NSCLC (<i>n</i> = 38)	+ (25%) – (75%)	67% vs. 0% (6-month irORR; <i>P</i> < 0.001)	67% vs. 11% (6-month PFS rate; <i>P</i> = 0.009)	89% vs. 33% (6-month OS rate; <i>P</i> = NR)	(49)
MPDL3280A	Roche/Genentech	NSCLC, RCC, melanoma, CRC, gastric cancer	NR	39% vs. 13% (<i>P</i> = NR)	NR	NR	(26, 73)
	Roche/Genentech	NSCLC (<i>n</i> = 37)	+ (13%) – (87%)	100% vs. 15% (<i>P</i> = NR)	NR	NR	(72)
	Roche/Genentech	Bladder (<i>n</i> = 20)	NR	52% vs. 11% (<i>P</i> = NR)	NR	NR	(25)

Abbreviations: CRC, colorectal cancer; NR, not reported.

response rate for the PD-L1–negative group remained unchanged (13%) when the 8 patients who were positive by the 1% cutoff were reclassified as negative by the 5% cutoff. The PD-L1–positive cohort had an improved response from 35% to 44% with an increase in the IHC cutoff, indicating the higher 5% IHC cutoff is reasonable in this analysis.

Biological Issues with PD-L1 IHC

Mechanistically, PD-L1 is biologically active only when expressed on the cell membrane, either through dynamic IFN γ expression or through constitutive oncogene activation (9, 54). Oncogene-driven PD-L1 expression represents a histopathologically and biologically distinct entity compared to inflammation-driven PD-L1 expression. While the latter occurs focally at sites of IFN γ -mediated immunologic attack, oncogene-driven PD-L1 expression is constitutive and diffuse (13). PD-L1 expressed during inflammation as a component of adaptive immune resistance is often associated with an immune infiltrate, while oncogene-driven PD-L1 expression is characterized by the lack of an immune infiltrate (54, 55). Numerous cell signaling perturbations have been associated with constitutive PD-L1 expression across histologies. PTEN loss has been associated with upregulation of PD-L1 in glioblastoma multiforme and colorectal cancer (56, 57). In glioblastoma multiforme, Akt and Ras activation in combination with PTEN loss led to the highest levels of PD-L1 expression by IHC, with concomitant reduction in T-cell killing *in vitro* (56). In colorectal cancer cell lines, PTEN knockdown by siRNA resulted in PD-L1 overexpression by IHC, independent of IFN γ stimulation (57). Notably, PD-L1 immunohistochemical staining in both studies was relatively diffuse within tumor, consistent with oncogene-dependent PD-L1 expression. Dysregulation in the JAK/STAT pathway can also

result in oncogene-driven PD-L1 overexpression. STAT3 upregulation, via upstream activation of ALK, results in induction of PD-L1 and tumor evasion in T-cell lymphoma (58). PD-L1 upregulation in these experiments was independent of PI3K, mTOR, or MEK/ERK signaling, demonstrating that multiple disparate signaling pathways are involved in PD-L1 regulation. In myeloma, both IFN γ as well as TLR signaling results in upregulation of the STAT1, MyD88, and the MEK/ERK pathways (59). EGFR pathway activation in NSCLC also leads to PD-L1 upregulation, in addition to overexpression of a number of other immunosuppressive factors, including IL6 and TGF β (60). Inhibition of PD-1 in this EGFR-driven chimeric mouse model resulted in tumor regression and improved survival. Overall, an increasing number of signaling pathways are associated with PD-L1 regulation, lending support to the pursuit of combination therapies involving TKIs and immunotherapeutics.

In contrast to oncogene-mediated PD-L1 expression, IFN γ -induced PD-L1 expression represents a dynamic biomarker and is present at sites of active inflammation, and biopsy samples represent a snapshot of the tumor immune microenvironment in space and time. Other factors in the tumor metabolic microenvironment, including hypoxia, can result in PD-L1 upregulation and are dependent on signaling via HIF1 α (61). Smaller tumor biopsies may miss the pertinent tumor–immune interface, or the biopsy may be performed after the biologically relevant PD-L1 overexpression has already taken place. PD-L1 itself is expressed at two potentially clinically relevant immunologic synapses—the tumor/T-cell interface, as well as the APC/T-cell interface (62). The majority of PD-L1 IHC biomarker development has been devoted to the former interface, but the latter likely plays a biologic role in tumor control as well, especially in light of the importance of the APC/T-cell interface for anti-CTLA-4 therapies (63). For the tumor/T-cell interface, biopsy capture of the tumor/immune

Table 4. PD-L1 expression in primary versus metastasis

Tumor histology	Detection antibody	Primary lesion (% tumor cells positive for PD-L1)	Metastatic lesion (% tumor cells positive for PD-L1)	Comment	References
RCC	5H1	44%	43%	Unmatched (different patient) primary and metastatic samples	(77)
	405.9A11	30%	27%	Matched (same patient) primary and metastatic samples	(66)
Melanoma	5H1	35%	43%	Unmatched (different patient) primary and metastatic samples	(54)
TNBC	NR	100%	92%	Matched (same patient) primary and metastatic samples	(95)

Abbreviations: H-score, immunoreactivity score: (3 × percentage of strongly staining tumor cells) + (2 × percentage of moderately staining tumor cells) + (percentage of weakly staining tumor cells), range of 0 to 300; NR, not reported.

interface is a key determinant in PD-L1 detection by IHC in melanoma. In a study assessing PD-L1 expression in patients with metastatic melanoma, 96% of PD-L1–overexpressing melanomas had a lymphocytic infiltrate, while the remaining 4% of PD-L1–overexpressing lacked TILs, possibly representing oncogene-driven PD-L1 expression (54). In addition, 22% of PD-L1 negative samples were associated with a lymphocytic infiltrate, indicating alternative mechanisms of tumor immune interference. The majority of PD-L1 expression was at the tumor interface, with immune cells secreting IFN γ , leading to the counterintuitive hypothesis that PD-L1 overexpression may be an initially protective response to successful tumor killing by TILs, which over time becomes co-opted into an immunosuppressive tumor environment. This parallels PD-1 biology, where PD-1 upregulation on T cells is initially associated with T-cell activation; however, over time PD-1 overexpression is a hallmark of T-cell exhaustion, likely due to expression of other immune checkpoints such as LAG-3, TIM-3, and others (9). Thus, the prognostic and predictive impact of PD-L1 IHC is temporally dependent on the time of biopsy in relation to the development of the lesion as well as in relation to antecedent therapies, which may help reconcile findings that PD-L1 may be associated with improved prognosis in melanoma and cervical cancer, and poorer prognosis in other histologies such as RCC (22, 64). In addition, the prognostic and predictive significance of PD-L1 expression on immune cells such as macrophages and lymphocytes may differ from tumor-associated PD-L1 expression in terms of the adaptive immune response (26). The role of anti-PD-1 and anti-PD-L1–directed therapies against oncogene-activated PD-L1–overexpressing tumors, in the absence of concomitant immune expression, will also require further study (65).

In addition to temporal considerations, numerous spatial considerations exist in terms of assessment of PD-L1 in cancer. Limited data exist comparing PD-L1 status in primary versus metastatic lesions, with few matched sets available from the same patient. As summarized in Table 4, there appears to be general parity between primary and metastatic lesions in terms of PD-L1 expression. However, among matched tumors in RCC, there was discordance in 5 of 33 samples, with 3 patients expressing PD-L1 in the primary only, and 2 in the metastasis only (66). Data on clinical response to PD-1/PD-L1–directed therapies in these patients are not available, but utilizing PD-L1 as a negative biomarker to exclude patients from PD-1/PD-L1–directed immunotherapy is concerning given these discordant results. In addition, selection of the appropriate site for biopsy for PD-L1 status remains enigmatic. While pretreatment FFPE primary tumor samples may be most readily available,

these samples may not reflect the overall immunologic slate that currently exists in a given patient, particularly if interim treatment has been administered. The absence of PD-L1 expression in a biopsied lesion may not reflect the systemic immunologic landscape, and may not capture the beneficial effect therapy is having on other sites of disease that are dependent on PD-L1 signaling. This phenomenon is recognized in NSCLC with driver mutations, where the overall systemic burden of disease is being controlled by the TKI. Abrupt cessation of targeted therapy in this situation may result in a dramatic tumor flare and current management of focal progression is to continue the TKI to treat the concerning lesion with local measures (67). Alternatively, an enlarging lesion may represent a growing immune infiltrate invading a previously quiescent tumor, which may represent the biologic basis for immune-related radiographic criteria in assessment of treatment response (68).

Clinical Experience with PD-L1 IHC

A number of PD-1/PD-L1 targeting immunotherapeutics are under clinical development across multiple histologies, though predominantly in melanoma, RCC, and NSCLC to date (Table 2). Many of these trials have not reported or are utilizing a proprietary PD-L1 IHC companion diagnostic, limiting generalized analysis of biomarker data. In addition, variation in IHC cut-off definitions complicates interpretation given the lack of a working standard. Despite these issues, preliminary data suggest that patients with higher levels of PD-L1 expression by IHC have an improved response rate, progression-free survival (PFS), and OS across histologies, as summarized in Table 3.

In melanoma clinical trials, PD-L1 overexpression by IHC is detected in approximately 45%–75% of samples. In the nivolumab study, 45% of patients were positive for PD-L1 expression based on a 5% cutoff using the 28-8 detection antibody (53, 69). The response rate for PD-L1–positive patients was 44%, compared with 17% in PD-L1–negative patients. PD-L1–positive melanoma patients treated with nivolumab had an OS of 21.1 months and a PFS of 9.1 months, versus 12.5 months and 2 months in PD-L1 negative patients. Of note, while the general trend was a higher response rate with nivolumab in patients who were PD-L1 positive at a population level, on an individual level those PD-L1–negative patients that did respond to nivolumab (13%) had as robust a radiographic response as the elite PD-L1–positive responders, with a 75%–100% reduction in measurable tumor. Pembrolizumab (anti-PD-1) has also been studied in advanced melanoma utilizing an IHC cutoff of 1% (70). PD-L1–positive patients (77%) had an ORR of 51%, while

PD-L1-negative patients had an ORR of 6%. PD-L1-positive patients treated with pembrolizumab had a PFS of 12 months and a 1-year OS rate of 84%, while PD-L1-negative patients had a PFS of 3 months and a 1-year OS of 69%. Of note, approximately 20% of PD-L1-negative patients had a durable response to pembrolizumab by PFS, and OS data between groups were not statistically significant, with approximately 65% of patients in both groups with sustained responses. Similar data were seen in the phase I study evaluating nivolumab in combination with ipilimumab in melanoma, where a 5% PD-L1 IHC cutoff was used (5). While 57% of PD-L1-positive patients responded to combination immunotherapy, 41% of PD-L1-negative patients responded as well, with similar reductions in tumor size in responders compared with PD-L1-positive responders. Conversely, patients that had disease progression on concurrent immunotherapy were those with PD-L1-negative tumors.

In NSCLC, a similar trend is seen as in melanoma, where PD-L1-positive patients seem to preferentially benefit from PD-1/PD-L1-directed therapy. Nivolumab was studied in patients with refractory NSCLC, and PD-L1 IHC was performed using a DAKO IHC assay with a 5% cutoff. On the basis of these criteria, 60% of patients were classified as positive for PD-L1, and the response rate in PD-L1-positive patients was 67% compared with 0% in PD-L1-negative patients (71). Pembrolizumab has also been investigated in NSCLC, utilizing a unique 50% IHC cutoff for PD-L1 expression with an unreported assay. On the basis of this cutoff, 25% of tumors were positive for PD-L1 and, at 6 months, PD-L1-positive patients had a 67% immune-related ORR (irORR), 67% PFS rate, and 89% OS rate compared to PD-L1-negative patients who had an 0% irORR, 11% PFS rate, and 33% OS rate (49). MPDL3280A, an anti-PD-L1 antibody, has also been studied in NSCLC utilizing a proprietary IHC platform with 0-3+ grading (3+ for >10% cells, 2+ for >5% cells, 0-1 for <5% cells; ref. 72). NSCLC patients with 3+ PD-L1 expression had an 83% response rate, compared with 46% in patients with either 2+ or 3+ expression. Patients with 1+/2+/3+ PD-L1 expression had a 31% ORR. On the basis of these limited data, it appears patients with NSCLC with higher levels of PD-L1 by IHC have superior responses to PD-1/PD-L1-directed therapy, but less is known about the nature of responses or survival outcomes in PD-L1-negative patients treated with immunotherapy. On initial evaluation, it appears as if select PD-L1-negative patients with melanoma can still obtain durable responses to anti-PD-1/PD-L1 therapy, while response rates in NSCLC in PD-L1-negative patients are rare. Whether this trend is reproduced in larger trials in these histologies remains to be seen and, if so, may represent a fundamental difference in the immunobiology between tumor types that can be exploited with novel immunotherapeutics, or a technical issue with IHC in different tissue types. For example in a phase I clinical trial of MPDL3280A in metastatic urothelial bladder cancer, PD-L1-positive patients had a 52% ORR at 12 weeks, compared with 11% in PD-L1-negative patients (25). The depth and duration of responses in PD-L1-negative patients remains to be seen.

PD-L1 IHC as a predictive biomarker has also been assessed in clinical trials involving multiple histologies. The nivolumab phase I study included patients with melanoma, RCC, NSCLC, metastatic colorectal cancer (mCRC), and metastatic castration-resistant prostate cancer (mCRPC; ref. 4). PD-L1 was detected by 5H1 antibody utilizing a 5% threshold, and 60% of tumors were positive by this criterion. Patients with PD-L1-positive tumors had a 36% response rate, while patients with PD-L1 negative

tumors had a 0% ORR. MPDL3280A has been studied in patients with melanoma, RCC, NSCLC, mCRC, and gastric cancer utilizing a proprietary PD-L1 IHC platform (73). PD-L1-positive patients had a 39% response rate, while PD-L1-negative patients had a 13% response rate.

Conclusions

Immunotherapy represents a paradigm shift in oncology therapy along multiple fronts. While targeted therapy ameliorates the underlying signaling defect resulting in oncogenesis within the tumor, immune checkpoint blockade is fundamentally a therapy directed at the patient's native immune system to tilt immune homeostasis away from self-tolerance towards cytotoxicity, with the goal of inducing antitumor immunity. On the basis of the paradigm of matched therapy, it is not surprising that HER2 IHC is predictive of response to HER2-targeting therapy, or that inhibition of overexpressed BCR-ABL with small-molecule inhibitors results in dramatic tumor responses, the underlying oncogenic defect is being detected and targeted by specific therapy (23, 74). With immune checkpoint blockade of CTLA-4, PD-1, and PD-L1, the primary target is the host immune system, not the underlying intratumoral signaling defect. PD-L1 overexpression is not the oncogenic driver of the tumor, but rather a co-opted and maladaptive immune shield that protects the tumor from its immune microenvironment.

Patients who have PD-L1 overexpression, based on different assays with varying cutoffs, tend to have more robust responses to anti-PD-L1-directed therapy. For instance, patients with PD-L1-overexpressing melanoma have a 44%–51% response rate to anti-PD-1-directed therapy, while patients with PD-L1-overexpressing NSCLC have a 67%–100% response rate (Table 3). In contrast, patients with PD-L1-negative melanoma have response rates of about 6%–17% (with some of these responses being durable); whereas for PD-L1-negative NSCLC, the response rate is about 0%–15%. Therefore, PD-L1 IHC at present is not a clear cut exclusionary predictive biomarker. This may not be surprising as few biomarkers represent binary variables. For instance, patients with a biomarker may not respond to a therapy because secondary biologic or molecular changes have occurred and drive the tumor. Similarly, patients negative for a biomarker may respond because activated biologic pathways may intersect and hence activate a pathway or signal, without the specific biomarker being activated or overexpressed. Technical factors and cut-off standards may also influence the utility of a biomarker. For example, even HER2, a *bona fide* oncologic driver with clear guidelines for HER2-directed therapy utilizing IHC and FISH assays, required a recent change in classification to lower the threshold for HER2 positivity (75). Overall, few biomarkers represent true dichotomous variables, with the utility of most being due to a statistically significant and clinically meaningful increase in response rate in patients harboring the biomarker.

Clinical responses to PD-1/PD-L1-directed therapy in refractory tumors such as melanoma, RCC, NSCLC are impressive and appear durable, similar to experience with other immunotherapeutics. The recent approval of nivolumab and pembrolizumab for ipilimumab-refractory metastatic melanoma in the United States likely represent the first of many future indications for anti-PD-1-directed therapy. The continued development of PD-L1 IHC, along with other immunologic assays, as a predictive biomarker for anti-PD-1/anti-PD-L1

therapy is an important endeavor to potentially spare patients from ineffective therapy with autoimmune toxicity. Ultimately, immunotherapy, and immune checkpoint inhibition in particular, has led to a paradigm shift in oncology, requiring development of new radiographic response criteria and altering expectations for durable responses with therapy. The promising efficacy of these agents combined with an increasing understanding of the complicated interactions between the immune system and tumor foster optimism for progress in biomarker development in this field.

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Disclosure of Potential Conflicts of Interest

R. Kurzrock has ownership interest (including patents) in RScueRX and is a consultant/advisory board member for Sequenom. No potential conflicts of interest were disclosed by S.P. Patel.

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