Programmed Death Ligand-1 (PD-L1) Expression in the Programmed Death Receptor-1 (PD-1)/PD-L1 Blockade

A Key Player Against Various Cancers

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• ***Context.***—**Immune checkpoint pathways, including programmed death receptor-1/programmed death ligand-1 (PD-1/PD-L1) signaling pathway, which are important in mediating self-tolerance and controlling self-damage, can sometimes be manipulated by cancer cells to evade immune surveillance. Recent clinical trials further demonstrate the efficacy of PD-1/PD-L1–targeted therapy in various cancers and reveal a new era of cancer immunotherapy.**

Objective.—**To review the mechanism of the PD-1/PD-L1 signaling pathway, the regulation of this pathway, PD-1/PD-L1 as a predictive and/or prognostic marker in various cancers, and strategies of measuring PD-L1 expression.**

Data Sources.—**Representative medical literature regarding PD-L1 expression in various cancers, including the preliminary results of the Blue Proposal, which compares different immunohistochemical stains for PD-L1 reported in the recent American Association of Cancer Research (AACR) Annual Meeting (April 16–20, 2016).**

Conclusion.—**Either PD-1/PD-L1–targeted therapy alone or in combination with other treatment modalities provides benefit for patients with advanced cancers. Because of the complexity of cancer immunity, we still do not have a reliable biomarker to predict the response of PD-1/PD-L1–targeted therapy. Future studies, including methods beyond immunohistochemical stains, are needed to develop reliable biomarker/biomarkers for pathology laboratories to aid in selecting patients who will benefit most from PD-1/PD-L1–targeted therapy.**

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During the past several decades, the efforts of antitumor immune therapy have been focused on identification of specific tumor antigens to augment antitumor immunity. However, this approach has been constantly challenged by the complexity and diversity of gene mutation and protein modification presented in human cancers, resulting in limited success.[1]

Recently, it has become well known that immune checkpoint pathways, including the programmed death receptor-1/programmed death ligand-1 (PD-1/PD-L1) signaling pathway,[2] which are important in mediating self-tolerance and controlling self-damage, can sometimes be manipulated by cancer cells to evade immune surveillance (as illustrated in Figure 1).[3,4] The fact that PD-1 is highly upregulated in tumor-infiltrating lymphocytes, as well as the correlation between PD-L1 expression and clinical outcome of patients in many types of solid cancers, has resulted in some seminal clinical trials. These trials further demonstrate the efficacy of PD-1/PD-L1–targeted therapy and reveal the new era of cancer immunotherapy.[5]

In this review, we will discuss the mechanism of the PD-1/PD-L1 signaling pathway, the regulation of this pathway, PD-1/PD-L1 as a predictive and/or prognostic marker in various cancers, and strategies for measuring PD-L1 expression.

MECHANISM

The PD-1/PD-L1 pathway belongs to the immune checkpoint signaling pathways regulating T-cell–mediated local inflammatory reactions and self-tolerance.[6] The importance of the PD-1/PD-L1 axis in cancer immunity has been demonstrated extensively in both animal models and clinical studies.[7]

PD-1, a cell surface protein belonging to the CD28 family, is encoded by *PDCD1* gene located in chromosome 2q37.[2] PD-1 is expressed on activated T cells, with particularly high expression by tumor-infiltrating T lymphocytes.[2] PD-1 is also expressed on activated non-T cells, including B cells, natural killer cells, and monocytes, implying that PD-1 may also modulate immunity in a T-cell–independent manner.[8] PD-1 has 2 major ligands: PD-L1/CD274 (encoded by

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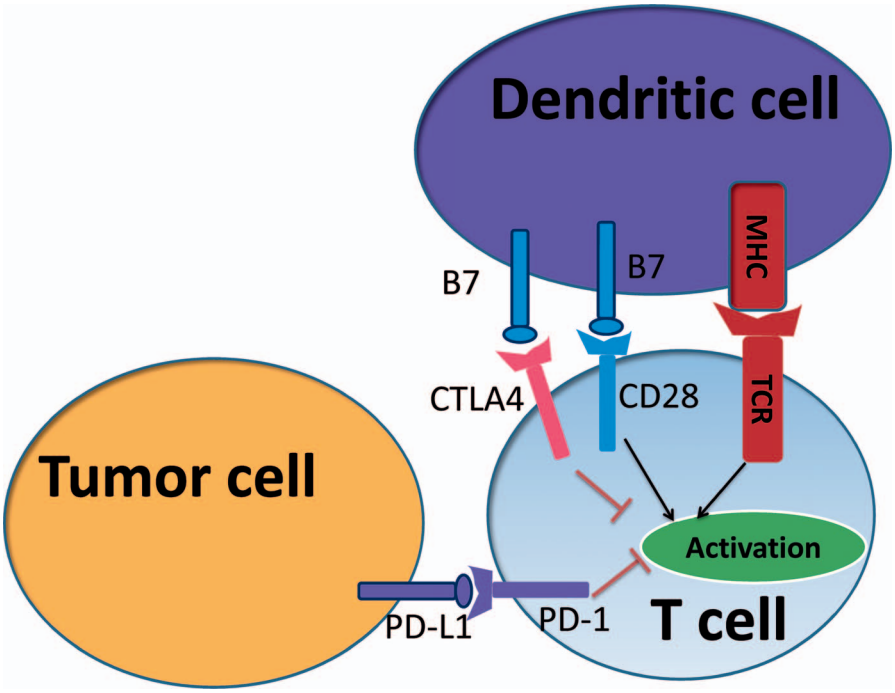

Figure 1. *Immune checkpoint signaling pathway in cancer cells. Activation of immune checkpoint signaling pathway including both cytotoxic T-lymphocyte–associated antigen 4 (CTLA4) and programmed death receptor-1/programmed death ligand-1 (PD-1/PD-L1) attenuates the amplitude of T-cell receptor (TCR)–mediated immunity. Abbreviation: MHC, major histocompatibility complex.*

PDCD1LG1 in chromosome 9) and PD-L2/CD273 (encoded by *PDCD1LG2* in chromosome 9).

Binding of PD-1 to either PD-L1 or PD-L2 results in the activation of inhibitory kinases involved in T-cell proliferation, adhesion, and cytokine production/secretion via phosphatase SHP2.[2] PD-1–PD-L interaction has been shown to play an important role in limiting the initial response of T cells upon antigen exposure and inducing T-cell tolerance. However, there are differences between PD-L1 and PD-L2.[2] PD-L1 is broadly expressed on many immune cell types, such as T cells,[9] B cells, macrophages, regulatory T (Treg) cells, and dendritic cells, as well as some non–immune cell types, such as vascular endothelial cells and pancreatic cells. On the contrary, PD-L2 is largely limited to antigen-presenting cells, such as macrophages and dendritic cells.

Overexpression of PD-L1 in murine tumor models results in inhibition of T-cell–mediated immune response via the PD-L1/PD-1 axis, indicating that blocking this axis has an important role in immunotherapy against cancers. PD-L1 is expressed in several solid tumors, including melanoma, glioblastoma, lung cancer, renal cancer, gastric cancer, colorectal cancer, pancreatic cancer, breast cancer, and cervical and ovarian cancers (please see Table 1 for percentage of PD-L1 expression in various tumors). PD-L1 is also found in hematologic malignancies, such as multiple myeloma, lymphoma, and various leukemia types. In contrast, PD-L2 is preferentially expressed in hematopoietic tumors, like B-cell lymphomas.[10] Additionally, strong association between PD-1/PD-L1 expression and poor prognosis has been reported in several tumor types, including gastric, lung, and renal carcinomas (details in Figure 1).

REGULATION OF PD-L1 EXPRESSION IN CANCERS

Many pathways have been suggested to be involved in PD-L1 upregulation in cancers (as illustrated in Figure 2).[6] For instance, activation of oncogenic signaling, such as through the phosphatase and tensin homolog–phosphoinositide 3-kinase–protein kinase B (PTEN-PI3K-AKT) pathway, upregulates PD-L1 on glioblastomas.[11] Similarly, activation of the anaplastic lymphoma kinase (ALK) signal transducer and activator of transcription 3 (STAT3) pathway is also able to induce PD-L1 expression in ALK-carrying T-cell lymphoma and chemoresistant non–small cell lung cancer (NSCLC).[12,13] In melanoma, the upregulation of PD-L1 is partially mediated by recruitment of histone deacetylase 6 (HDAC6) onto STAT3 to the PD-L1 promoter region.[14] Furthermore, interferons, especially interferon γ, are the most common inducer in adaptive immune-mediated PD-L1 upregulation on tumor cells or on tumor-infiltrated leukocytes, including melanoma[15] and ovarian cancer.[2,16] Other inflammatory signaling pathways, including interleukin 4, interleukin 10, vascular endothelial growth factor, granulocyte colony–stimulating factor, and bacterial lipopolysaccharide, have also been shown to induce the expression of PD-L1.[17] This may have contributed to the association of chronic inflammation and cancer development.

CURRENT THERAPEUTIC ANTIBODIES, APPROACHES, AND TOXICITIES

Encouraged by the success of preclinical studies in which PD-1 blockade via monoclonal antibodies attenuated the remote metastasis of melanoma cells or colon cancer cells, several agents to block the PD-1/PD-L1 axis have been developed and applied in different types of tumors. The journey started from a small pilot phase 1 study in which nivolumab (a humanized anti–PD-1 antibody; Table 2), manufactured by Bristol-Myers Squibb (New York, New York), was shown to be safe and to have antitumor activity in treatment-refractory patients with solid tumors (melanomas, renal cell carcinomas [RCCs], and NSCLCs) in 2010. A large trial with 296 patients then revealed that nivolumab

induced an objective response in 18% to 28% of patients with refractory solid tumors (26 of 94 in melanoma, 14 of 76 in NSCLC, and 9 of 33 in RCC), and the duration of the therapeutic response was greater than 1 year for most of these patients.[5] Nivolumab was shown to be effective in refractory classical Hodgkin lymphoma, with an objective response rate of 87% (20 of 23) reported.[18] Nivolumab soon became the first Food and Drug Administration (FDA)–approved PD-1 inhibitor for metastatic melanoma (2014), NSCLC (2014), and RCC (2015).

Another PD-1 antibody, pembrolizumab (lambrolizumab), manufactured by Merck (Kenilworth, New Jersey), was demonstrated to have antitumor effects in patients with advanced melanoma in an initial phase 1 study.[19] In the KEYNOTE-006 study, the response rate was 33.7% (94 of 279) in patients with advanced melanoma receiving pembrolizumab every 2 weeks.[20] A 19.4% (96 of 495) objective response rate and 1 year of median duration of overall response were also reported in pembrolizumab-treated patients with metastatic NSCLC.[21] Pembrolizumab became the second FDA-approved PD-1 inhibitor for metastatic melanoma and NSCLC. A recent report expanded the application of pembrolizumab further in advanced Merkel cell carcinoma. The objective response rate was as high as 56% (14 of 25) in patients with advanced Merkel cell carcinoma, including patients testing positive and those testing negative for Merkel cell polyomavirus.[22] Another promising PD-1 inhibitor, pidilizumab, manufactured by CureTech (Yavne, Israel), was tested in patients with solid tumors and hematologic tumors, particularly lymphomas.[23] More recent data from a study that evaluated the use of a combination of pidilizumab with rituximab in patients with relapsed follicular lymphoma showed a complete response rate of 52% (15 of 29) and a partial response rate of 14% (4 of 29).[24]

PD-L1–targeted therapy is also currently under investigation for use in both solid and hematologic tumors.[25,26] Atezolizumab, manufactured by Roche/Genentech (Basel, Switzerland), was listed as the Breakthrough Drug of 2014 by the FDA, given its significance in treating metastatic bladder cancer. In a recent phase 1a study in patients with RCC, atezolizumab treatment resulted in a 28.9-month median overall survival, even in some patients previously exposed to anti–vascular endothelial growth factor therapy.[27] Successful results have also been reported using atezolizumab in the treatment of bladder cancer.[28] MEDI4736 is another exciting PD-L1 antibody that is currently being tested in phase 3 trials for stage 3 NSCLC. Another exciting, newly emerged antibody is durvalumab, manufactured by AstraZeneca (London, United Kingdom), which is a more PD-L1–specific inhibitor minimizing the toxicity associated with PD-L2 inhibition.[29] There was a phase 1b study conducted in which a dual-antibody therapy including durvalumab and tremelimumab, manufactured by AstraZeneca, was tested in patients with advanced-stage NSCLC; the therapy demonstrated an objective response of more than 20% (6 of 26) and disease control in more than 30% (9 of 26), in both PD-L1–positive and PD-L1–negative groups.[30] Table 2 summarizes anti–PD-1 and anti–PD-L1 agents in clinical use or in the early phases of development.

Furthermore, the synergistic benefits of the combination of PD-1/PD-L1 inhibitors with other agents—such as vaccine, other checkpoint inhibitors, chemotherapy, and targeted inhibitors (epidermal growth factor receptor [EGFR], ALK, BRAF, and vascular endothelial growth factor inhibitors)—have been confirmed by several preclinical tests. Recently, these novel drug combinations have been tested in several tumors in various phases of clinical trials.

The most common nonspecific systemic side effects of PD-1/PD-L1–targeted therapy are fatigue and fever. Imbalance of immune tolerance/immunity induced by PD-1/PD-L1–targeted therapy sometimes leads to side effects mimicking autoimmune diseases. Toxicity associated with PD-1/PD-L1–targeted therapy occurs with less frequency than the toxicity associated with anti–cytotoxic T-lymphocyte–associated protein 4 (anti-CTL4) therapy, and grades 3 to 4 adverse events occur in only 7% to 12% of patients. The most affected organs/tissues in these immune-related adverse events are the skin, gastrointestinal system, liver, and lungs. Maculopapular rash is the most common skin pathology. Diarrhea/colitis and asymptomatic elevation of alanine aminotransferase/aspartate aminotransferase are the most common digestive system side effects. The incidence of pneumonitis is more common in patients with lung cancer or those treated with combinations of PD-1 inhibitors and conventional chemotherapeutic agents. Patients with pneumonitis may present with shortness of breath, cough, fever, and/or chest pain, as well as the associated radiologic findings mimicking acute interstitial pneumonia or acute respiratory distress syndrome. The onset of pneumonitis is usually seen in the range of 7 to 24 months following the exposure to PD-1/PD-L1 inhibitors. In general, patients with mild symptoms are normally treated by discontinuing the PD-1/PD-L1 inhibitors. Steroids and other immunosuppressants are sometimes necessary for patients with severe symptoms.

PD-1/PD-L1 EXPRESSION AS A PROGNOSTIC MARKER IN VARIOUS CANCERS

The correlation between the PD-L1 expression of cancer cells and prognosis remains to be determined. As shown in Table 1, most of the studies have indicated that expression of PD-L1 in tumor cells served as an adverse prognostic marker. However, a few studies revealed that expression of PD-L1 in tumor cells may imply better prognosis. Additionally, several studies beyond those noted in Table 1 showed that PD-L1 expression in tumor cells and tumor-infiltrating lymphocytes is correlated with poor prognosis in such cancers as NSCLC,[31] melanoma,[32] RCC,[33] and esophageal and gastric cancers.[34] A meta-analysis conducted by Wang et al[31] in 1157 patients with NSCLC showed that PD-L1 expression was significantly associated with poor differentiation of tumors (poor versus well: odds ratio, 1.91; 95% CI, 1.33–2.75; $P = .001$) and with worse overall survival (pooled hazard ratio, 1.75; 95% CI, 1.40–2.20; $P < .001$).[31] In another meta-analysis of RCC, high level of PD-L1 expression was a negative prognostic factor that increased the risk of death by 81% (hazard ratio, 1.81; 95% CI, 1.31–2.49; $P < .001$).[33] In gastric carcinoma, PD-L1 expression by immunohistochemistry was associated with larger tumor size, invasion into the deep muscular layers, lymph node metastasis, and decreased survival time of patients (<2 years).[34] Moreover, multivariate analysis demonstrated that PD-L1 immunodetection could be used as an independent factor to evaluate the prognosis of gastric carcinoma.[34] Additionally, PD-L1 expression has been associated with an aggressive melanoma phenotype characterized by a fibroblast-like morphology and invasive proper-

Table 1. Prevalence of Programmed Death Ligand-1 (PD-L1) Expression in Various Cancers by Different Cutoffs and Status of Anti–Programmed Death Receptor-1/PD-L1 Treatment

Type of Cancer	PD-L1$^+$ Cases, % (No.)	Cutoff Studied	Clone Name (Manufacturer, If Applicable)
Melanoma	24 (223 of 945)	≥5% cancer cells	Clone 5H1, homemade
	43 (10 of 23)		
	76 (35 of 46)	≥1% cancer cells	Clone 22C3 (Dako North America Inc, Carpinteria, California)
Non–small cell lung cancer	23.2 (191 of 824)	≥50% membrane staining	Clone 22C3 (Dako)
	Both squamous and nonsquamous		
	44 (119 of 272)	≥1% membrane staining	Clone 28-8 (Dako)
	Squamous cell		
	54 (455 of 582)	>1% membrane staining	Clone 28-8
	Nonsquamous		
Lung small cell carcinoma	71.6 (73 of 102)	Not defined	Polyclonal antibody (Abcam, Cambridge, United Kingdom)
	20.6 (24 of 116)	>1% tumor cells	Clone 28-8
Merkel cell carcinoma	56 (14 of 25)	>1% tumor cells	22C3 (Dako)
Breast cancers	34 (15 of 44)	Not defined	MIH1 clone (eBioscience, San Diego, California)
	23 (152 of 650)	Modified H-score ≥100[a]	Rabbit anti-human PD-L1 polyclonal antibody (Abcam)
	19 (20 of 109)	≥5% tumor cells	5H1
Ovarian cancers	68.6 (48 of 70)	≥Moderate expression (see reference)	27A2
	86.9 (20 of 23)		5H1
Renal clear cell carcinoma	59.1 (153 of 259)	≥5% cancer cells	5H1
	24 (181 of 756)	>1% tumor cells	28-8
	23.9 (73 of 306)	≥5% tumor cells	5H1
Non–clear cell renal cancer	10.9 (11 of 101)	≥5% tumor cells	405.9A11
Urothelial carcinoma	20 (10 of 56)	≥5% expression	5H1
	32.2 (100 of 310)	≥5% tumor-infiltrating immune cells	SP142
	20 (32 of 160)	≥5% tumor cells	5H1
	70.7 (46 of 65)	>12.2%	MIH1
Glioblastoma	88 (103 of 117)	≥5% tumor cells	5H1
	50	Not defined	MIH1 (eBioscience)
Colorectal carcinoma	44.8 (64 of 143)	Not defined	Ab58810 (Abcam)
Gastric carcinoma	12 (4 of 34)	≥5% tumor cells	5H1
	30.1 (140 of 465)	≥1% tumor cells	E1L3N (Cell Signaling Technology)
Endometrial carcinoma	83 (24 of 29)	≥1% tumor cells	Not indicated
	83 (24 of 29)	≥1% tumor cells	Not indicated
Classical Hodgkin lymphoma	74.5 (79 of 106)	Not defined	405.9A11
	75 (82 of 109)	≥20% Reed-Sternberg cells	E1L3N (Cell Signaling Technology)
	100 (10 of 10)	Not defined	PD-L1 (405.9A11)
Diffuse large B-cell lymphoma	11 (132 of 1253)	≥30 tumor cells	EPR1161/ab174838 (Abcam)
	26 (43 of 163)	≥30% tumor cells	E1L3N (Cell Signaling Technology)
Cutaneous T-cell lymphoma	100 (9 of 9)	>2% lymphocytes	Not indicated
Acute myeloid leukemia	37 (22 of 60)	≥5% tumor cells	MIH1

Abbreviations: FDA, Food and Drug Administration; H-score, histo-score; N/A, not applicable; TIMC, tumor-infiltrating mononuclear cells.

[a] H-score: assigned using the following formula: $[1 \times (\%\ \text{cells}\ 1^+) + 2 \times (\%\ \text{cells}\ 2^+) + 3 \times (\%\ \text{cells}\ 3^+)]$.[92]

ties leading to enhanced aggressiveness and invasiveness.[32] Also, in a meta-analysis of breast cancer patients, PD-L1 expression was associated with aggressive features, such as lymph node metastasis, poor nuclear grade, and negative estrogen receptor status. It is associated with a higher total risk of mortality risk ratio of 1.64 (95% CI, 1.14–2.34) and a higher 10-year risk of mortality risk ratio of 2.53 (95% CI, 1.78–3.59) after surgery.[35] In contrast, there are some studies

Table 1. Extended

Prognostic Marker	Predictive Marker	FDA Approval Status	Source, y
N/A	N/A	Keytruda (pembrolizumab, Merck, Kenilworth, New Jersey) Opdivo (nivolumab, Bristol-Myers Squibb, New York, New York)	Rodic et al,[62] 2015; and Larkin et al,[63] 2015
Good prognostic marker	N/A		Madore et al,[64] 2015
N/A	Positive predictive marker	Keytruda (pembrolizumab) 2015	Garon et al,[21] 2015
No correlation	N/A	Opdivo (nivolumab) 2015	Brahmer et al,[40] 2015
No correlation	Positive predictive marker	Opdivo (nivolumab) 2015	Borghaei et al,[65] 2015
Good prognostic marker	Positive predictive marker		Ishii et al,[66] 2015
N/A	No correlation		Antonia et al,[67] 2016
N/A	No correlation		Nghiem et al,[22] 2016
Adverse prognostic marker	N/A		Ghebeh et al,[68] 2006
Adverse prognostic marker	N/A		Muenst et al,[69] 2014
N/A	N/A		Mittendorf et al,[70] 2014
Adverse prognostic marker	N/A		Hamanishi et al,[71] 2007
N/A	N/A		Dong et al,[72] 2002
Adverse prognostic marker	N/A	Opdivo (nivolumab) 2015	Krambeck et al,[73] 2006
Adverse prognostic marker	N/A	Opdivo (nivolumab) 2015	Motzer et al,[74] 2015
Adverse prognostic marker	N/A		Thompson et al,[75] 2006
Adverse prognostic marker	N/A		Choueiri et al,[76] 2014
No correlation	N/A	Tecentriq (atezolizumab, Genentech, San Francisco, California)	Faraj et al,[77] 2015
NA	Positive predictive marker	Tecentriq (atezolizumab)	Rosenberg et al,[28] 2016
No correlation between tumor cell PD-L1 and prognosis. But PD-L1 in TIMCs is a good prognostic marker.	N/A		Bellmunt et al,[78] 2015
Adverse prognostic marker	N/A		Nakanishi et al,[79] 2007
No correlation			Berghoff et al,[80] 2015
N/A	N/A		Yao et al,[81] 2009
Adverse prognostic marker	N/A		Shi et al,[82] 2013
Adverse prognostic marker	N/A		Thompson et al,[83] 2016
Good prognostic marker	Good predictive marker		Boger et al,[84] 2016
N/A	N/A		Liu et al,[85] 2015
N/A	N/A		Vanderstraeten et al,[86] 2014
Adverse prognostic marker	N/A		Roemer et al,[58] 2016
Adverse prognostic marker	N/A		Koh et al,[87] 2015
Adverse prognostic marker	Good predictive marker	Opdivo (nivolumab)	Ansell et al,[18] 2015
Adverse prognostic marker	N/A		Kiyasu et al,[88] 2015
N/A	N/A		Georgiou et al,[89] 2016
N/A	N/A		Kantekure et al,[90] 2012
Adverse prognostic marker	Good predictive marker		Chen et al,[91] 2008

that showed that PD-L1 upregulation served as a positive prognostic marker in breast cancer and high-grade serous ovarian carcinoma. This is likely due to an increased T-cell cytotoxic immune response in these cancers.[36–38]

The mechanisms leading to these discrepancies are uncertain. However, the current use of nonstandardized immunohistochemistry (IHC) methodologies using different monoclonal antibodies (Tables 1 and 3) for measuring PD-

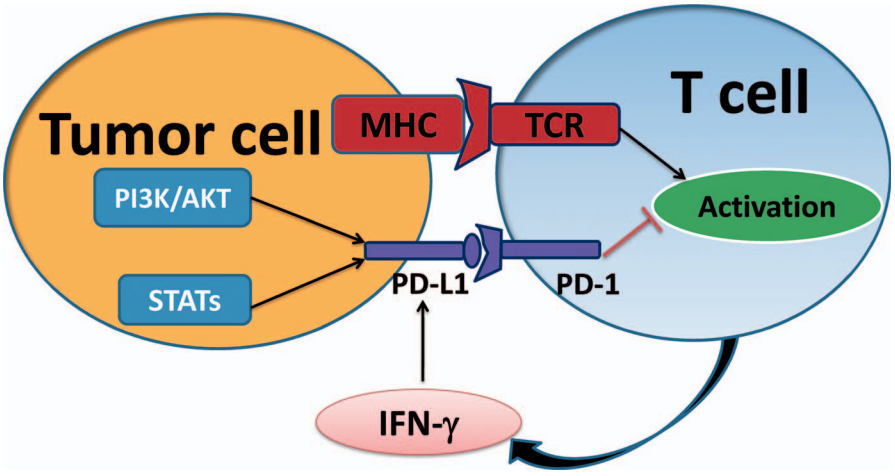

Figure 2. *Two pathways used by cancer cells to upregulate programmed death receptor-1 (PD-1) ligand and thus avoid immunity. The first one normally involves an innate immune response. The upregulation of programmed death ligand-1 (PD-L1) can be caused by active oncogenic signaling, such as via protein kinase B (AKT), which is independent of inflammatory response. The alternative one is mainly seen in adaptive immune responses. The regulation of PD-L1 is induced by inflammatory response, such as via interferon (IFN). Abbreviations: MHC, major histocompatibility complex; PI3K, phosphoinositide 3-kinase; STAT, signal transducer and activator of transcription.*

L1 levels in these studies may have partially contributed to these conflicting results.

PD-1/PD-L1 AS A PREDICTIVE MARKER FOR ANTI–PD-1/PD-L1 TREATMENT

A predictive marker for predicting treatment response to anti–PD-1/PD-L1 treatment is highly desired because the treatment is associated with certain toxicity as described above, although less common and less severe than conventional chemotherapy. PD-L1 expression has been investigated as a potential predictive biomarker for selecting responders to anti–PD-1/PD-L1 antibody treatment. A study conducted by Topalian et al[5] showed an objective response to nivolumab in 36% (9 of 25) of the cancer (advanced melanoma, NSCLC, castration-resistant prostate cancer, RCC, and colorectal cancer) patients who tested positive for PD-L1 (monoclonal antibody 5H1) expression, whereas none of the patients who tested negative for PD-L1 demonstrated a response. Similarly, another recent study showed that about 22% (191 of 824) of the NSCLC tumors (both squamous and nonsquamous) tested expressed PD-L1 in at least 50% of tumor cells, and these patients had a significantly higher response rate to anti–PD-1/PD-L1 treatment (41%), compared with those with PD-L1 expressed in less than 50% of tumor cells.[21] The study data showed a clear link between PD-L1 expression and the efficacy of pembrolizumab for patients with NSCLC, which was also reflected in the indication for use by the FDA. The companion diagnostic PD-L1 IHC 22C3 pharmDx test (Dako North America Inc, Carpinteria, California) is indicated as an aid in identifying NSCLC patients for treatment with pembrolizumab.[32] By definition, a companion diagnostic test is required and essential for the safe and effective use of the corresponding targeted therapy.

Studies have shown that atezolizumab treatment results in a median overall survival of 28.9 months in patients with metastatic RCC, with benefit more obvious in the group with strong PD-L1 IHC staining (Table 3, SP142 assay).[27] Similar results were also reported using atezolizumab in

Table 2. Anti–Programmed Death Receptor-1 (Anti-PD-1) and Anti–Programmed Death Ligand-1 (Anti–PD-L1) Agents in Clinical Use or in Early Phase of Development

Target	Name	Company	Characteristic	Approved by FDA/ Clinical Trial	Cancer Types
PD-1	Pembrolizumab (Keytruda; MK-3475 or Lambrolizumb)	Merck, Kenilworth, New Jersey	Humanized IgG4	Approved by FDA	Melanoma, NSCLC
	Nivolumab (Opvido; DMX1106 or BMS-936558)	Bristol-Myers Squibb, New York, New York	Fully human IgG4	Approved by FDA	Melanoma, NSCLC, RCC, and HL
	AMP-224	GlaxoSmithKline, Brentford, United Kingdom	Fusion human PD-L2	Phase 1	Solid tumors
	AMP-514 (MEDI0680)	AstraZeneca, London, United Kingdom	Humanized IgG4. mAb	Phase 1	Solid tumors
	CT-011 (pidilizumab)	CureTech, Yavne, Israel	Humanized IgG1	Phase 2	Lymphoma or solid tumors
	MDX1105 (BMS936559)	Bristol-Myers Squibb	Fully human IgG4	Phase 1b/2a	Solid tumors
PD-L1	Atezolizumab (MPDL3280A)	Genentech/Roche, San Francisco, California	Fc-modified human IgG1	FDA approved	Metastatic uroepithelial carcinoma
	Durvalumab (MEDI4736)	AstraZeneca, MedImmune, London, United Kingdom	Fully human IgG	Phase 1	Solid tumors
	MESB001078C	Merck	Fully human IgG1	Phase 1–2	Solid tumors

Abbreviations: FDA, Food and Drug Administration; HL, Hodgkin lymphoma; IgG, immunoglobulin G; NSCLC, non–small cell lung cancer; RCC, renal cell carcinoma.

Table 3. Current Programmed Death Ligand-1 (PD-L1) Immunohistochemistry (IHC) Assays With Coupled Treatment Agents

	PD-L1 IHC 28-8 pharmDx	PD-L1 22C3 IHC pharmDx	Ventana SP142	Ventana SP263
Coupled treatment agent	Nivolumab (Bristol-Myers Squibb, New York, New York)	Pembrolizumab (Merck, Kenilworth, New Jersey)	Atezolizumab (Roche/Genentech, San Francisco, California)	Durvalumab (AstraZeneca, London, United Kingdom)
mAb alone	28-8 (Abcam, Cambridge, United Kingdom)	22C3 (Dako, Carpinteria, California)	SP142 (Spring Bioscience, Pleasanton, California)	SP263 (Spring Bioscience)
Diagnostic platform, FDA status	Dako	Dako	Ventana/Roche (Tucson, Arizona)	Ventana/Roche
	FDA-approved complementary test for metastatic melanoma[a] and nonsquamous NSCLC[b]	FDA-approved companion diagnostic test for NSCLC[b]	FDA approved for metastatic uroepithelial cancer	Currently Investigation Use Only (IUO)
Staining location scored	Membrane	Membrane	Membrane	Membrane
Cell types scored	TCs	TCs	TCs and TIIC	TCs
Cutoff(s) tested	≥%1, 5%, or 10% of TCs	≥1%, or 50% of TCs	TCs: ≥1%, 5%, or 50%; TIICs: ≥1%, 5%, or 10%	≥25% of TCs
FDA-approved thresholds	N/A[c]	≥50% of TCs	N/A[d]	Not FDA-approved test

Abbreviations: FDA, Food and Drug Administration; mAb, monoclonal antibody; N/A, not applicable; NSCLC, non–small cell lung cancer; TCs, tumor cells; TIIC, tumor-infiltrating immune cells.

[a] For treatment-naive patients.
[b] For previously treated patients (ie, second-line therapy).
[c] All patients are eligible for treatment regardless of results.
[d] All patients are eligible for treatment regardless of results; however, patients with ≥5% of PD-L1+ TIICs may be associated with increased objective response rate.

treating bladder cancer, with a 61% overall reduction in tumor burden, a 26% (26 of 100) overall response rate, and a median overall survival of 11.4 months in the IC2/3 subgroup based on an IHC assay, compared with a 45% reduction in tumor burden, an 11% (11 of 107) objective response rate, and a median overall survival of 6.7 months in the IC1 subgroup (Table 3). Additionally, a systemic review and meta-analysis from 20 trials including patients with metastatic melanoma, NSCLC, and RCC receiving anti–PD-1/PD-L1 antibodies (4230 metastatic melanoma, 1417 NSCLC, and 312 RCC patients) showed that PD-L1 expression is associated with lower mortality and better clinical response to anti–PD-1/PD-L1 antibodies in patients with metastatic melanoma and is associated with a better clinical response in patients with nonsquamous NSCLC.[39]

However, a study by Brahmer et al[40] observed that an objective response to nivolumab could still occur in patients with squamous carcinoma of lung who test negative for PD-L1 (PD-L1 antibody [clone 28-8, Dako North America]).[40] Consequently, nivolumab has recently received FDA and European Medicines Agency approval for NSCLC, regardless of the PD-L1 expression status (for NSCLC in the United States and in European Union). Of interest, the FDA might have had some reservations about the clinical data submitted for review. The FDA indicated in the premarket approval that patients with PD-L1 expression, as detected by the PD-L1 IHC 28-8 pharmDx assay (Dako North America) in previously treated metastatic nonsquamous NSCLC, may experience enhanced survival when treated with nivolumab. Currently, this assay has been used as a complementary test based on the clinical trial results.

Additionally, in a meta-analysis of cancer patients treated with Nivolumab, pembrolizumab or atezolizumab, Carbognin et al[41] reported a clinical response in 239 of 702 patients (34%) with PD-L1–positive cancers and in 154 of 773 patients (20%) with PD-L1–negative cancers. The significant difference in response based on PD-L1 IHC was observed for NSCLC and melanoma but not for RCC or bladder carcinoma. Thus, using PD-L1 IHC as a predictive marker remains undetermined. Again, the use of different nonstandardized IHC techniques for measuring PD-L1 levels in tissue may have contributed to these differences.

STRATEGIES TO MEASURE PD-L1/PD-1 EXPRESSION

Immunohistochemistry

Assessment of PD-L1 expression through immunohistochemical staining has been advocated as one potential biomarker, as discussed above. Immunohistochemistry has several advantages: (1) the wide availability of formalin-fixed, paraffin-embedded tissue; (2) visualization of expression in various cell populations (tumor versus immune/stromal cells) to some extent based on morphology; (3) relative rapidity of the test; and (4) the test's relatively low cost and widespread use in pathology laboratories, particularly in contrast to molecular pathology–based methods. Different clinical trials have used different IHC assays from different pharmaceutical manufacturers to measure PD-L1 expression (Table 3). These assays use different monoclonal antibody clones recognizing various epitopes of PD-L1. Various systems for amplification and detection of the signal are used for IHC, leading to different thresholds of detecting PD-L1 expression. Additionally, 3 of these assays evaluate

the PD-L1 expression in the tumor cells of NSCLC only. The Ventana SP142 assay, manufactured by Spring Bioscience (Pleasanton, California), measures the PD-L1 expression in both tumor cells and tumor-infiltrating immune cells in metastatic uroepithelial cancers. Of note, studies using different PD-L1 antibodies to evaluate PD-L1 expression of tumor-infiltrating immune cells in various cancer types (head/neck squamous cell carcinoma, melanoma, and bladder cancers) are undergoing further evaluation to discern the impact of tumor-infiltrating immune cells in treatment response.

In 2015 a workshop by the Food and Drug Administration, the American Association for Cancer Research (AACR), and the American Society of Clinical Oncology led to a Blueprint Proposal developed by 4 pharmaceutical companies (Bristol-Myers Squibb Co, Merck & Co Inc, AstraZeneca PLC, and Genentech Inc), 2 diagnostic companies (Agilent Technologies Inc/Dako Corp and Roche/Ventana Medical Systems Inc), 2 professional societies (AACR–International Association for the Study of Lung Cancer), and 2 regulatory agencies (the European Medicines Agency and the FDA) to evaluate the analytic similarities of the 4 PD-L1 assays for use in NSCLC. The goal of this effort is to harmonize companion diagnostics for PD-L1 and to assess the possibility of interchangeable use of these assays. There are 2 phases in this proposal: phase 1 will evaluate analytic components by measuring PD-L1 expression on tumor or immune cells and predefine cutoffs in order to evaluate how these assays would compare using clinical samples; and phase 2 will design a statistically powered study with a large sample size based on the findings of phase 1. The preliminary data of phase 1, using 500 samples, was presented at the recent AACR annual meeting (April 16–20, 2016).[42] The preliminary results have indicated that 3 antibodies (22C3, 28-8, and SP263) have similar analytic performance in measuring the percentage of PD-L1–expressing tumor cells. The dynamic range reported for these 3 antibodies was between 1% and 100%. A fourth antibody, SP142, constantly labeled fewer tumor cells.[42] However, there is less precision in analytic performance when labeling immune cells compared with tumor cells. There is also less agreement between observers when evaluating immune cells compared with cancer cells. Additionally, the patient population defined by Ventana SP263, manufactured by Spring Bioscience, at the 25% cutoff point is similar to the group identified by the Dako 28-8 and Dako 22C3, manufactured by Dako, at the 1% cutoff. However, about 37% of the cases studied revealed discrepant results for PD-L1 expression between assays. This suggests the possibility of assignment into different diagnostic categories according to the key clinical cutoffs if assays and algorithms are mismatched. A recent study further suggested that the inherent tumor heterogeneity, or assay- or platform-specific variables may also contribute to the discordant results of these companion diagnostic tests.[43]

These results suggest the challenges of extrapolating the results from one test to that of another test. This is reillustrated by Blueprint Chair Dr Fred Hirsch (professor of medicine at the University of Colorado), who said in an interview, "when pathologists used each assay in combination with its own prescribed cutoff, the assays did sometimes disagree on whether samples were PD-L1-positive."[44] However, these results are preliminary and the planned phase 2 study is needed to further evaluate how to use these antibodies most efficiently in clinical practice. Additionally, other large-scale projects to investigate harmonization of PD-L1 antibodies are planned. For example, the National Comprehensive Cancer Network will soon begin a harmonization project in collaboration with Bristol Myers Squibb, MD Anderson Cancer Center, and Yale University. These results will be important for the efficient use of PD-L1 assay in pathology laboratories.

Several challenges remain regarding standardization of IHC beyond the Blueprint study. Preanalytically, the time for fixation in formaldehyde can modify the level of expression of PD-L1 and needs to be controlled. Additionally, there is heterogeneous PD-L1 expression in different regions of the same tumor specimen. Therefore, the absence of PD-L1 expression on small biopsies may not reflect the systemic immunologic landscape. This may have contributed to some patients responding to anti–PD-1 or anti–PD-L1 therapy independent of PD-L1 expression. Furthermore, it remains to be clarified whether the PD-L1 expression test should be performed on the primary tumor site or metastatic sites. PD-L1 expression in tumor cells is dynamic, influenced by interferon γ, hypoxia, and previous treatments, including chemotherapy, radiation therapy, and targeted therapy.[45] Additional comments regarding PD-L1 IHC as a predictive marker have been recently published.[46,47]

Flow Cytometry, Real-Time Quantitative Polymerase Chain Reaction, and Enzyme-Linked Immunosorbent Assay

As shown in Table 4, the first study with human peripheral blood cells demonstrating the feasibility of detecting PD-L1 expression with a flow cytometry method was published in 2009.[48] The authors showed that cryopreservation actually decreased the expression of both PD-1 and PD-L1 in peripheral blood cells. Using a similar approach with different antibodies, a study showed a higher expression of PD-1/PD-L1 in CD4/CD8 T lymphocytes from chronic lymphocytic leukemia patients when compared with age-matched controls.[49] In addition to peripheral blood cells or lymphocytes, Gowrishankar et al[15] have shown that interferon γ induces the upregulation of PD-L1 in a nuclear factor-κB–dependent fashion in 5 different melanoma cell lines and melanoma patient–derived cells using flow cytometry.[15] Furthermore, Andorsky et al[50] documented that PD-L1 is widely expressed by anaplastic large cell lymphoma, whereas it only has limited expression in diffuse large B-cell lymphoma (DLBCL).

Flow cytometry studies, compared with IHC-based methods, have the advantage of simultaneously measuring the expression of PD-1 and PD-L1 in malignant cells and various types of immune cells. This is accomplished by using combinations of phenotypic markers for immune cells (cytotoxic T cells [CD8, TIA1], natural killer cells [CD56], T-reg cells [CD4, forkhead box P3], and dendritic cells/macrophages [CD68]), tumor cells (epithelial cell adhesion molecule, epithelial membrane antigen, lymphoma/leukemia markers), and antibodies to PD-1 and PD-L1. This will likely provide a more comprehensive understanding of PD-1/PD-L1 interactions between tumor cells and the immune environment. In theory, the efficacy of anti–PD-L1 treatment relies on the blockade of the inhibitory action of anticancer cytotoxic lymphocytes induced by the interaction between PD-1, expressed on immune cells (particularly lymphocytes), and PD-L1, expressed mainly on cancer cells. Therefore, it is reasonable to hypothesize that the higher the expression of PD-1 on immune cells, the more efficient the anti–PD-L1 treatment. This method may prove to be useful

Table 4. Application of Nonimmunohistochemistry Methods to Determine Programmed Death Ligand-1 (PD-L1) Expression in Human Samples

Methods	Antibodies	Specimen/Cell Types	Source, y
Flow cytometry	PD-L1 (eBioscience; Biolegend [clone 29E.2A3]; BD Pharmingen, San Jose, California)	Cryopreserved PBMCs, lymphocytes, macrophages	Campbell et al,[48] 2009; Brusa et al,[49] 2013; Fang et al,[53] 2015; Pan et al,[93] 2015; Rodriguez-Garcia et al,[94] 2011
Immunomagnetic selection and CellTracks analyzer	PD-L1 (R&D-FAB1561, R&D Systems, Minneapolis, Minnesota)	Metastatic breast cancer cells	Mazel et al,[51] 2015
ELISA	PD-L1 (mAb, 2H11)	Serum	Chen et al,[54] 2011
Real-time PCR	Not applicable	Whole blood; osteosarcoma surgical specimens	Shen et al,[52] 2014; Fang et al,[53] 2015

Abbreviations: ELISA, enzyme-linked immunosorbent assay; mAb, monoclonal antibody; PBMC, peripheral blood mononuclear cells; PCR, polymerase chain reaction.

for predicting PD-1/PD-L1–targeted therapy, particularly for hematologic malignancies in which flow cytometry studies have been routinely used for diagnostic purposes. Similar to flow cytometry, another method that allows us to analyze PD-1 or PD-L1 expression is immunomagnetic selection using the CellTracks analyzer (Janssen Diagnostics LLC, Raritan, New Jersey). Using this method, researchers were able to identify the expression of PD-L1 in circulating tumor cells from patients with breast cancer with a sensitivity of 68.8%, indicating the promise of this noninvasive rapid screen in the future.[51]

Both SYBR dye–based and Taqman probe–based real-time polymerase chain reaction methods have been employed to detect PD-1/PD-L1 mRNA expression in whole-blood samples, cell lines, and surgical specimens.[52,53] In a recent report from Shen et al,[52] it was found that there is no uniform upregulation of PD-L1 expression in cancer cell lines compared with noncancer cell lines. However, authors identified a significant correlation between the expression of PD-L1 in human osteosarcoma and prognosis (median overall survival: 89 months in PD-L1–low patients versus 28 months in PD-L1–high patients).

An enzyme-linked immunosorbent assay–based method aimed at detecting soluble PD-L1 (sPD-L1) was developed in 2011.[54] Using this approach, researchers were able to demonstrate that sPD-L1 is a good prognostic marker in multiple myeloma and DLBCL.[55,56] In a small study with 81 multiple myeloma patients, the mean concentration of sPD-L1 was 2.851 ng/mL compared with 0.716 ng/mL sPD-L1 in matched controls.[55] Additionally, a lower sPD-L1 in patients with multiple myeloma is associated with higher progression-free survival.[55] In a multicenter, randomized phase 3 clinical trial in patients with DLBCL lymphoma, sPD-L1 was found to be higher in patients with DLBCL compared with healthy individuals, and the levels dropped after complete response. Moreover, cutoff of sPD-L1 at 1.52 ng/mL can be used to stratify patients with DLBCL into 2 groups with respect to 3-year overall survival. This results in 2 distinct populations: 1 with a 3-year overall survival of 76% and 1 with a 3-year overall survival of 89%.[56] These results support the possibility of using PD-1/PD-L1 blockade as a treatment option in these cancers.

Other Surrogate Markers for PD-L1 Expression

Using a fluorescence in situ hybridization probe targeting the gene locus encoding PD-L1 located at *9p24.1*, 1 study has shown that *9p24.1* amplification was associated with increased PD-L1 expression via the janus kinase 2 pathway in nodular sclerosing Hodgkin lymphoma and primary mediastinum large B-cell lymphoma.[57] Another study using fluorescence in situ hybridization demonstrated that the incidence of *9p24.1* amplification (at least $\geq$3 copies) was present in 24% of patients with early-stage Hodgkin lymphoma, compared with a 50% presence in advanced-stage (stages 3–4) Hodgkin lymphoma.[58] Additionally, the patients with Hodgkin lymphoma harboring *9p24.1* amplifications had a shorter progression-free survival. These results suggest that fluorescence in situ hybridization studies may be another potential method for predicting the response to PD-1/PD-L1 treatment.

The expression level of PD-L1 has been reported to be associated with other genetic alternations. The NSCLC cell lines with epidermal growth factor receptor (EGFR) mutations tend to have higher PD-L1 expression on the cell surface.[59] In a phase 2 trial studying pembrolizumab in multiple solid metastatic tumor, patients with mismatch repair–deficient (ie, microsatellite instability–high) colorectal cancer are more likely to benefit from PD-1 blockade (pembrolizomab) than those with mismatch repair–proficient tumors. Interestingly, PD-L1 expression is also elevated in mismatch repair–deficient colorectal cancer patients compared with those with mismatch repair–proficient tumors. However, the PD-L1 expression is not significantly associated with progression-free survival or overall survival.[60] In another study, higher nonsynonymous mutation burden was found to be associated with PD-L1–positive tumors and better melanoma-specific survival in patients with stage 3 melanoma. In addition, NF-1 mutation was shown to be limited to PD-L1–positive melanoma, but BRAF and NRAS mutations are distributed equally in PD-L1–positive or PD-L1–negative melanoma.[61]

OUTLOOK

PD-1/PD-L1–targeted therapy has demonstrated the impressive power of cancer immunotherapy. PD-1/PD-L1–targeted therapy either alone or in combination with other treatment modalities will benefit patients with advanced cancers. Because of the complexity of cancer immunity, we still have not yet completely understood the mechanism by which PD-1/PD-L1–targeted therapy improves survival in cancer patients. Many questions remain to be answered, particularly a better prediction system for predicting the response of PD-1/PD-L1–targeted therapy.

References

1. Melero I, Gaudernack G, Gerritsen W, et al. Therapeutic vaccines for cancer: an overview of clinical trials. *Nat Rev Clin Oncol*. 2014;11(9):509–524.
2. Pardoll DM. The blockade of immune checkpoints in cancer immunotherapy. *Nat Rev Cancer*. 2012;12(4):252–264.
3. Gubin MM, Zhang X, Schuster H, et al. Checkpoint blockade cancer immunotherapy targets tumour-specific mutant antigens. *Nature*. 2014; 515(7528):577–581.
4. Hodi FS, O'Day SJ, McDermott DF, et al. Improved survival with ipilimumab in patients with metastatic melanoma. *N Engl J Med*. 2010;363(8): 711–723.
5. Topalian SL, Hodi FS, Brahmer JR, et al. Safety, activity, and immune correlates of anti-PD-1 antibody in cancer. *N Engl J Med*. 2012;366(26):2443–2454.
6. Topalian SL, Drake CG, Pardoll DM. Immune checkpoint blockade: a common denominator approach to cancer therapy. *Cancer Cell*. 2015;27(4):450–461.
7. Sunshine J, Taube JM. PD-1/PD-L1 inhibitors. *Curr Opin Pharmacol*. 2015; 23:32–38.
8. Postow MA, Callahan MK, Wolchok JD. Immune checkpoint blockade in cancer therapy. *J Clin Oncol*. 2015;33(17):1974–1982.
9. Mueller SN, Vanguri VK, Ha SJ, et al. PD-L1 has distinct functions in hematopoietic and nonhematopoietic cells in regulating T cell responses during chronic infection in mice. *J Clin Invest*. 2010;120(7):2508–2515.
10. Rosenwald A, Wright G, Leroy K, et al. Molecular diagnosis of primary mediastinal B cell lymphoma identifies a clinically favorable subgroup of diffuse large B cell lymphoma related to Hodgkin lymphoma. *J Exp Med*. 2003;198(6): 851–862.
11. Parsa AT, Waldron JS, Panner A, et al. Loss of tumor suppressor PTEN function increases B7-H1 expression and immunoresistance in glioma. *Nat Med*. 2007;13(1):84–88.
12. Marzec M, Zhang Q, Goradia A, et al. Oncogenic kinase NPM/ALK induces through STAT3 expression of immunosuppressive protein CD274 (PD-L1, B7-H1). *Proc Natl Acad Sci U S A*. 2008;105(52):20852–20857.
13. Fujita Y, Yagishita S, Hagiwara K, et al. The clinical relevance of the miR-197/CKS1B/STAT3-mediated PD-L1 network in chemoresistant non-small-cell lung cancer. *Mol Ther*. 2015;23(4):717–727.
14. Lienlaf M, Perez-Villarroel P, Knox T, et al. Essential role of HDAC6 in the regulation of PD-L1 in melanoma. *Mol Oncol*. 2016; 10(5):735–750.
15. Gowrishankar K, Gunatilake D, Gallagher SJ, Tiffen J, Rizos H, Hersey P. Inducible but not constitutive expression of PD-L1 in human melanoma cells is dependent on activation of NF-kappaB. *PLoS One*. 2015;10(4):e0123410.
16. Abiko K, Matsumura N, Hamanishi J, et al. IFN-gamma from lymphocytes induces PD-L1 expression and promotes progression of ovarian cancer. *Br J Cancer*. 2015;112(9):1501–1509.
17. He J, Hu Y, Hu M, Li B. Development of PD-1/PD-L1 pathway in tumor immune microenvironment and treatment for non-small cell lung cancer. *Sci Rep*. 2015;5:13110.
18. Ansell SM, Lesokhin AM, Borrello I, et al. PD-1 blockade with nivolumab in relapsed or refractory Hodgkin's lymphoma. *N Engl J Med*. 2015;372(4):311–319.
19. Robert C, Ribas A, Wolchok JD, et al. Anti-programmed-death-receptor-1 treatment with pembrolizumab in ipilimumab-refractory advanced melanoma: a randomised dose-comparison cohort of a phase 1 trial. *Lancet*. 2014;384(9948): 1109–1117.
20. Robert C, Schachter J, Long GV, et al. Pembrolizumab versus ipilimumab in advanced melanoma. *N Engl J Med*. 2015;372(26):2521–2532.
21. Garon EB, Rizvi NA, Hui R, et al. Pembrolizumab for the treatment of non-small-cell lung cancer. *N Engl J Med*. 2015;372(21):2018–2028.
22. Nghiem PT, Bhatia S, Lipson EJ, et al. PD-1 Blockade with pembrolizumab in advanced Merkel-cell carcinoma. *N Engl J Med*. 2016;374(26):2542–2552.
23. Armand P, Nagler A, Weller EA, et al. Disabling immune tolerance by programmed death-1 blockade with pidilizumab after autologous hematopoietic stem-cell transplantation for diffuse large B-cell lymphoma: results of an international phase II trial. *J Clin Oncol*. 2013;31(33):4199–4206.
24. Westin JR, Chu F, Zhang M, et al. Safety and activity of PD1 blockade by pidilizumab in combination with rituximab in patients with relapsed follicular lymphoma: a single group, open-label, phase 2 trial. *Lancet Oncol*. 2014;15(1): 69–77.
25. Herbst RS, Soria JC, Kowanetz M, et al. Predictive correlates of response to the anti-PD-L1 antibody MPDL3280A in cancer patients. *Nature*. 2014; 515(7528):563–567.
26. Powles T, Eder JP, Fine GD, et al. MPDL3280A (anti-PD-L1) treatment leads to clinical activity in metastatic bladder cancer. *Nature*. 2014;515(7528):558–562.
27. McDermott DF, Sosman JA, Sznol M, et al. Atezolizumab, an anti-programmed death-ligand 1 antibody, in metastatic renal cell carcinoma: long-term safety, clinical activity, and immune correlates from a phase Ia study. *J Clin Oncol*. 2016;34(8):833–842.
28. Rosenberg JE, Hoffman-Censits J, Powles T, et al. Atezolizumab in patients with locally advanced and metastatic urothelial carcinoma who have progressed following treatment with platinum-based chemotherapy: a single-arm, multicentre, phase 2 trial. *Lancet*. 2016;387(10031):1909–1920.
29. Stewart R, Morrow M, Hammond SA, et al. Identification and characterization of MEDI4736, an antagonistic anti-PD-L1 monoclonal antibody. *Cancer Immunol Res*. 2015;3(9):1052–1062.
30. Antonia S, Goldberg SB, Balmanoukian A, et al. Safety and antitumour activity of durvalumab plus tremelimumab in non-small cell lung cancer: a multicentre, phase 1b study. *Lancet Oncol*. 2016;17(3):299–308.
31. Wang A, Wang HY, Liu Y, et al. The prognostic value of PD-L1 expression for non-small cell lung cancer patients: a meta-analysis. *Eur J Surg Oncol*. 2015; 41(4):450–456.
32. Massi D, Brusa D, Merelli B, et al. PD-L1 marks a subset of melanomas with a shorter overall survival and distinct genetic and morphological characteristics. *Ann Oncol*. 2014;25(12):2433–2442.
33. Iacovelli R, Nole F, Verri E, et al. Prognostic role of PD-L1 expression in renal cell carcinoma: a systematic review and meta-analysis. *Target Oncol*. 2016; 11(2):143–148.
34. Wu C, Zhu Y, Jiang J, Zhao J, Zhang XG, Xu N. Immunohistochemical localization of programmed death-1 ligand-1 (PD-L1) in gastric carcinoma and its clinical significance. *Acta Histochem*. 2006;108(1):19–24.
35. Guo Y, Yu P, Liu Z, et al. Prognostic and clinicopathological value of programmed death ligand-1 in breast cancer: a meta-analysis. *PLoS One*. 2016; 11(5):e0156323.
36. Baptista MZ, Sarian LO, Derchain SF, Pinto GA, Vassallo J. Prognostic significance of PD-L1 and PD-L2 in breast cancer. *Hum Pathol*. 2016;47(1):78–84.
37. Sabatier R, Finetti P, Mamessier E, et al. Prognostic and predictive value of PDL1 expression in breast cancer. *Oncotarget*. 2015;6(7):5449–5464.
38. Darb-Esfahani S, Kunze CA, Kulbe H, et al. Prognostic impact of programmed cell death-1 (PD-1) and PD-ligand 1 (PD-L1) expression in cancer cells and tumor-infiltrating lymphocytes in ovarian high grade serous carcinoma. *Oncotarget*. 2016;7(2):1486–1499.
39. Gandini S, Massi D, Mandala M. PD-L1 expression in cancer patients receiving anti PD-1/PD-L1 antibodies: a systematic review and meta-analysis. *Crit Rev Oncol Hematol*. 2016;100:88–98.
40. Brahmer J, Reckamp KL, Baas P, et al. Nivolumab versus docetaxel in advanced squamous-cell non-small-cell lung cancer. *N Engl J Med*. 2015;373(2): 123–135.
41. Carbognin L, Pilotto S, Milella M, et al. Differential activity of nivolumab, pembrolizumab and MPDL3280A according to the tumor expression of programmed death-ligand-1 (PD-L1): sensitivity analysis of trials in melanoma, lung and genitourinary cancers. *PLoS One*. 2015;10(6):e0130142.
42. Marianne J, Ratcliffe AS, Midha A, Barker C, Scorer P, Walker J. A comparative study of PD-L1 diagnostic assays and the classification of patients as PD-L1 positive and PD-L1 negative. *Cancer Res*. 2016;76(14)(suppl):LB-094.
43. Gaule P, Smithy JW, Toki M, et al. A quantitative comparison of antibodies to programmed cell death 1 ligand 1 [published online ahead of print August 18, 2016]. *JAMA Oncol*. 2016. doi: 10.1001/jamaoncol.2016.3015.
44. Yandell K. AACR Annual Meeting 2016: Can We Simplify Diagnostic Testing? Cancer Today. *American Association for Cancer Research. April 22, 2016*. http://www.cancertodaymag.org/EventCoverage/Pages/AACR-Annual-Meeting-2016-Can-We-Simplify-Diagnostic-Testing.aspx. Accessed November 16, 2016.
45. Ilie M, Hofman V, Dietel M, Soria JC, Hofman P. Assessment of the PD-L1 status by immunohistochemistry: challenges and perspectives for therapeutic strategies in lung cancer patients. *Virchows Arch*. 2016; 468(5):511–525.
46. Bernicker E. Next-generation sequencing and immunotherapy biomarkers: a medical oncology perspective. *Arch Pathol Lab Med*. 2016;140(3):245–248.
47. Kerr KM, Nicolson MC. Non-small cell lung cancer, PD-L1, and the pathologist. *Arch Pathol Lab Med*. 2016;140(3):249–254.
48. Campbell DE, Tustin NB, Riedel E, et al. Cryopreservation decreases receptor PD-1 and ligand PD-L1 coinhibitory expression on peripheral blood mononuclear cell-derived T cells and monocytes. *Clin Vaccine Immunol*. 2009; 16(11):1648–1653.
49. Brusa D, Serra S, Coscia M, et al. The PD-1/PD-L1 axis contributes to T-cell dysfunction in chronic lymphocytic leukemia. *Haematologica*. 2013;98(6):953–963.
50. Andorsky DJ, Yamada RE, Said J, Pinkus GS, Betting DJ, Timmerman JM. Programmed death ligand 1 is expressed by non-hodgkin lymphomas and inhibits the activity of tumor-associated T cells. *Clin Cancer Res*. 2011;17(13):4232–4244.
51. Mazel M, Jacot W, Pantel K, et al. Frequent expression of PD-L1 on circulating breast cancer cells. *Mol Oncol*. 2015;9(9):1773–1782.
52. Shen JK, Cote GM, Choy E, et al. Programmed cell death ligand 1 expression in osteosarcoma. *Cancer Immunol Res*. 2014;2(7):690–698.
53. Fang M, Meng Q, Guo H, et al. Programmed death 1 (PD-1) is involved in the development of proliferative diabetic retinopathy by mediating activation-induced apoptosis. *Mol Vis*. 2015;21:901–910.
54. Chen Y, Wang Q, Shi B, et al. Development of a sandwich ELISA for evaluating soluble PD-L1 (CD274) in human sera of different ages as well as supernatants of PD-L1+ cell lines. *Cytokine*. 2011;56(2):231–238.
55. Wang L, Wang H, Chen H, et al. Serum levels of soluble programmed death ligand 1 predict treatment response and progression free survival in multiple myeloma. *Oncotarget*. 2015;6(38):41228–41236.
56. Rossille D, Gressier M, Damotte D, et al. High level of soluble programmed cell death ligand 1 in blood impacts overall survival in aggressive

diffuse large B-cell lymphoma: results from a French multicenter clinical trial. *Leukemia*. 2014;28(12):2367–2375.

57. Green MR, Monti S, Rodig SJ, et al. Integrative analysis reveals selective 9p24.1 amplification, increased PD-1 ligand expression, and further induction via JAK2 in nodular sclerosing Hodgkin lymphoma and primary mediastinal large B-cell lymphoma. *Blood*. 2010;116(17):3268–3277.

58. Roemer MG, Advani RH, Ligon AH, et al. PD-L1 and PD-L2 genetic alterations define classical Hodgkin lymphoma and predict outcome. *J Clin Oncol*. 2016; 34(23):2690–2697.

59. Azuma K, Ota K, Kawahara A, et al. Association of PD-L1 overexpression with activating EGFR mutations in surgically resected nonsmall-cell lung cancer. *Ann Oncol*. 2014;25(10):1935–1940.

60. Le DT, Uram JN, Wang H, et al. PD-1 blockade in tumors with mismatch-repair deficiency. *N Engl J Med*. 2015;372(26):2509–2520.

61. Madore J, Strbenac D, Vilain R, et al. PD-L1 negative status is associated with lower mutation burden, differential expression of immune-related genes, and worse survival in stage-III melanoma. *Clin Cancer Res*. 2016; 22(15):3915–3923.

62. Rodic N, Anders RA, Eshleman JR, et al. PD-L1 expression in melanocytic lesions does not correlate with the BRAF V600E mutation. *Cancer Immunol Res*. 2015;3(2):110–115.

63. Larkin J, Chiarion-Sileni V, Gonzalez R, et al. Combined nivolumab and ipilimumab or monotherapy in untreated melanoma. *N Engl J Med*. 2015;373(1): 23–34.

64. Madore J, Vilain RE, Menzies AM, et al. PD-L1 expression in melanoma shows marked heterogeneity within and between patients: implications for anti-PD-1/PD-L1 clinical trials. *Pigment Cell Melanoma Res*. 2015;28(3):245–253.

65. Borghaei H, Paz-Ares L, Horn L, et al. Nivolumab versus docetaxel in advanced nonsquamous non-small-cell lung cancer. *N Engl J Med*. 2015;373(17): 1627–1639.

66. Ishii H, Azuma K, Kawahara A, et al. Significance of programmed cell death-ligand 1 expression and its association with survival in patients with small cell lung cancer. *J Thorac Oncol*. 2015;10(3):426–430.

67. Antonia SJ, Lopez-Martin JA, Bendell J, et al. Nivolumab alone and nivolumab plus ipilimumab in recurrent small-cell lung cancer (CheckMate 032): a multicentre, open-label, phase 1/2 trial. *Lancet Oncol*. 2016;17(7):883–895.

68. Ghebeh H, Mohammed S, Al-Omair A, et al. The B7-H1 (PD-L1) T lymphocyte-inhibitory molecule is expressed in breast cancer patients with infiltrating ductal carcinoma: correlation with important high-risk prognostic factors. *Neoplasia*. 2006;8(3):190–198.

69. Muenst S, Schaerli AR, Gao F, et al. Expression of programmed death ligand 1 (PD-L1) is associated with poor prognosis in human breast cancer. *Breast Cancer Res Treat*. 2014;146(1):15–24.

70. Mittendorf EA, Philips AV, Meric-Bernstam F, et al. PD-L1 expression in triple-negative breast cancer. *Cancer Immunol Res*. 2014;2(4):361–370.

71. Hamanishi J, Mandai M, Iwasaki M, et al. Programmed cell death 1 ligand 1 and tumor-infiltrating CD8+ T lymphocytes are prognostic factors of human ovarian cancer. *Proc Natl Acad Sci U S A*. 2007;104(9):3360–3365.

72. Dong H, Strome SE, Salomao DR, et al. Tumor-associated B7-H1 promotes T-cell apoptosis: a potential mechanism of immune evasion. *Nat Med*. 2002;8(8): 793–800.

73. Krambeck AE, Thompson RH, Dong H, et al. B7-H4 expression in renal cell carcinoma and tumor vasculature: associations with cancer progression and survival. *Proc Natl Acad Sci U S A*. 2006;103(27):10391–10396.

74. Motzer RJ, Escudier B, McDermott DF, et al. Nivolumab versus everolimus in advanced renal-cell carcinoma. *N Engl J Med*. 2015;373(19):1803–1813.

75. Thompson RH, Kuntz SM, Leibovich BC, et al. Tumor B7-H1 is associated with poor prognosis in renal cell carcinoma patients with long-term follow-up. *Cancer Res*. 2006;66(7):3381–3385.

76. Choueiri TK, Fay AP, Gray KP, et al. PD-L1 expression in nonclear-cell renal cell carcinoma. *Ann Oncol*. 2014;25(11):2178–2184.

77. Faraj SF, Munari E, Guner G, et al. Assessment of tumoral PD-L1 expression and intratumoral CD8+ T cells in urothelial carcinoma. *Urology*. 2015;85(3):703 e1–e6.

78. Bellmunt J, Mullane SA, Werner L, et al. Association of PD-L1 expression on tumor-infiltrating mononuclear cells and overall survival in patients with urothelial carcinoma. *Ann Oncol*. 2015;26(4):812–817.

79. Nakanishi J, Wada Y, Matsumoto K, Azuma M, Kikuchi K, Ueda S. Overexpression of B7-H1 (PD-L1) significantly associates with tumor grade and postoperative prognosis in human urothelial cancers. *Cancer Immunol Immunother*. 2007;56(8):1173–1182.

80. Berghoff AS, Kiesel B, Widhalm G, et al. Programmed death ligand 1 expression and tumor-infiltrating lymphocytes in glioblastoma. *Neuro Oncol*. 2015;17(8):1064–1075.

81. Yao Y, Tao R, Wang X, Wang Y, Mao Y, Zhou LF. B7-H1 is correlated with malignancy-grade gliomas but is not expressed exclusively on tumor stem-like cells. *Neuro Oncol*. 2009;11(6):757–766.

82. Shi SJ, Wang LJ, Wang GD, et al. B7-H1 expression is associated with poor prognosis in colorectal carcinoma and regulates the proliferation and invasion of HCT116 colorectal cancer cells. *PLoS One*. 2013;8(10):e76012.

83. Thompson ED, Zahurak M, Murphy A, et al. Patterns of PD-L1 expression and CD8 T cell infiltration in gastric adenocarcinomas and associated immune stroma [published online ahead of print January 22, 2016]. *Gut*. 2016. doi: 10.1136/gutjnl-2015-310839.

84. Boger C, Behrens HM, Mathiak M, Kruger S, Kalthoff H, Rocken C. PD-L1 is an independent prognostic predictor in gastric cancer of Western patients. *Oncotarget*. 2016;7(17):24269–24283.

85. Liu J, Liu Y, Wang W, Wang C, Che Y. Expression of immune checkpoint molecules in endometrial carcinoma. *Exp Ther Med*. 2015;10(5):1947–1952.

86. Vanderstraeten A, Luyten C, Verbist G, Tuyaerts S, Amant F. Mapping the immunosuppressive environment in uterine tumors: implications for immunotherapy. *Cancer Immunol Immunother*. 2014;63(6):545–557.

87. Koh YW, Jeon YK, Yoon DH, Suh C, Huh J. Programmed death 1 expression in the peritumoral microenvironment is associated with a poorer prognosis in classical Hodgkin lymphoma. *Tumour Biol*. 2015; 37(6):7507–7514.

88. Kiyasu J, Miyoshi H, Hirata A, et al. Expression of programmed cell death ligand 1 is associated with poor overall survival in patients with diffuse large B-cell lymphoma. *Blood*. 2015;126(19):2193–2201.

89. Georgiou K, Chen L, Berglund M, et al. Genetic basis of PD-L1 overexpression in diffuse large B-cell lymphomas. *Blood*. 2016;127(24):3026–3034.

90. Kantekure K, Yang Y, Raghunath P, et al. Expression patterns of the immunosuppressive proteins PD-1/CD279 and PD-L1/CD274 at different stages of cutaneous T-cell lymphoma/mycosis fungoides. *Am J Dermatopathol*. 2012; 34(1):126–128.

91. Chen X, Liu S, Wang L, Zhang W, Ji Y, Ma X. Clinical significance of B7-H1 (PD-L1) expression in human acute leukemia. *Cancer Biol Ther*. 2008;7(5):622–627.

92. McCarty KS Jr, Miller LS, Cox EB, Konrath J, McCarty KS Sr. Estrogen receptor analyses. Correlation of biochemical and immunohistochemical methods using monoclonal antireceptor antibodies. *Arch Pathol Lab Med*. 1985;109(8):716–721.

93. Pan T, Liu Z, Yin J, Zhou T, Liu J, Qu H. Notch signaling pathway was involved in regulating programmed cell death 1 expression during sepsis-induced immunosuppression. *Mediators Inflamm*. 2015;2015:539841.

94. Rodriguez-Garcia M, Porichis F, de Jong OG, et al. Expression of PD-L1 and PD-L2 on human macrophages is up-regulated by HIV-1 and differentially modulated by IL-10. *J Leukoc Biol*. 2011;89(4):507–515.