

PD-L1

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ABSTRACT

Programmed death ligand 1 (PD-L1) is the principal ligand of programmed death 1 (PD-1), a coinhibitory receptor that can be constitutively expressed or induced in myeloid, lymphoid, normal epithelial cells and in cancer. Under physiological conditions, the PD-1/PD-L1 interaction is essential in the development of immune tolerance preventing excessive immune cell activity that can lead to tissue destruction and autoimmunity. PD-L1 expression is an immune evasion mechanism exploited by various malignancies and is generally associated with poorer prognosis. PD-L1 expression is also suggested as a predictive biomarker of response to anti-PD-1/PD-L1 therapies; however, contradictory evidence exists as to its role across histotypes. Over the years, anti-PD-1/PD-L1 agents have gained momentum as novel anticancer therapeutics, by inducing durable tumour regression in numerous malignancies including metastatic lung cancer, melanoma and many others. In this review, we discuss the immunobiology of PD-L1, with a particular focus on its clinical significance in malignancy.

INTRODUCTION

Programmed death ligand 1 (PD-L1), otherwise known as B7-H1 or CD274, is the first functionally characterised ligand of the coinhibitory programmed death receptor 1 (PD-1). Together with its cognate ligand PD-L2, PD-L1 plays a key role in maintaining peripheral and central immune cell tolerance through binding to the PD-1 receptor.¹

STRUCTURE

PD-L1 is encoded by the PDCDL1 gene and is found on chromosome 9 in humans at position p24.1.² First described by Dong *et al* in 1999 as B7-H1, PD-L1 was recognised as the third member of the B7 protein family, displaying a 15%–20% homology with B7.1 and B7.2 proteins.³ The full length of PD-L1 is encoded within seven exons, corresponding to a 40 kDa protein of 290 amino acids. PD-L1 is a type 1 transmembrane protein and consists of IgV-like and IgC-like extracellular domains, a hydrophobic transmembrane domain and a short cytoplasmic tail made from 30 amino acids, with unclear signal transduction properties.^{3,4}

EXPRESSION OF PD-L1

PD-L1 expression can be constitutive or inducible. Constitutive, low-level PD-L1 expression can be found, on resting lymphocytes, antigen-presenting cells (APCs) and in corneal, syncytiotrophoblastic and Langerhans' islet cells where it contributes to tissue homeostasis in proinflammatory responses.¹ PD-L1 confers certain tissues such

as placenta, testis and the anterior chamber of the eye an 'immune privileged' status, where inoculation of exogenous antigens is tolerated without induction of an inflammatory/immune response.⁵ In the context of inflammation and/or infection, PD-L1 is induced as a suppressive signal on haematopoietic, endothelial and epithelial cells.⁶ PD-L1 expression is primarily influenced by toll-like receptors (TLRs), a subtype of non-catalytic receptors, highly expressed in APCs and activated by pathogen-associated molecular patterns. TLR-mediated regulation of PD-L1 relies on the activation of the MEK/ERK kinases, which enhance PD-L1 messenger RNA (mRNA) transcription via nuclear factor kappa B. Interferon- γ (IFN- γ) receptors 1 and 2 are also implicated in regulating PD-L1 expression, largely through Jak/STAT-mediated activation of IRF-1. Interferon-mediated activation of Jak/STAT can also up-regulate PD-L1 expression through the MEK/ERK and the phosphatidylinositol 3 kinase (PI3K)/AKT pathway, which exerts a permissive role on PD-L1 transcription through phosphorylation of mammalian target of rapamycin.⁷

In carcinogenesis, PD-L1 can be overexpressed as a result of driver oncogenic events. Epidermal growth factor receptor (EGFR) mutations, for instance, positively correlate with PD-L1 expression in lung cancer, with EGFR inhibitors acting as repressors of PD-L1 transcription.⁸ In phosphatase and tensin homolog (PTEN)-mutant tumours, PD-L1 overexpression is sustained by unrestrained activation of the PI3K/AKT pathway.⁹ In T cell lymphoma, the nucleophosmin (NPM)/anaplastic lymphoma kinase (ALK) fusion gene up-regulates PD-L1 via constitutive STAT3 activation.¹⁰

PD-L1/PD-1 ACTIVATION AND SIGNAL TRANSDUCTION

The biological functions of PD-L1 depend on binding with PD-1 (CD279), a 288 amino acid long type 1 transmembrane receptor encoded by the PDCD1 gene and physiologically expressed on lymphocytes and myeloid cells (figure 1). PD-1 is composed of an extracellular IgV-like domain and a transmembrane region. Its intracellular tail is composed of tyrosine based switch motif (ITSM) and immune receptor tyrosine based inhibitory motif sequences.¹¹

On ligation with PD-L1, recruitment of Src homology 2 domain containing phosphatases 1 and 2 (SHP-1/SHP-2) to the ITSM causes dephosphorylation of signalling kinases such as CD3 ζ , PKC θ and ZAP70 resulting in a global inhibitory action of T cell expansion.¹² Such inhibitory response is secondary to inactivation of the



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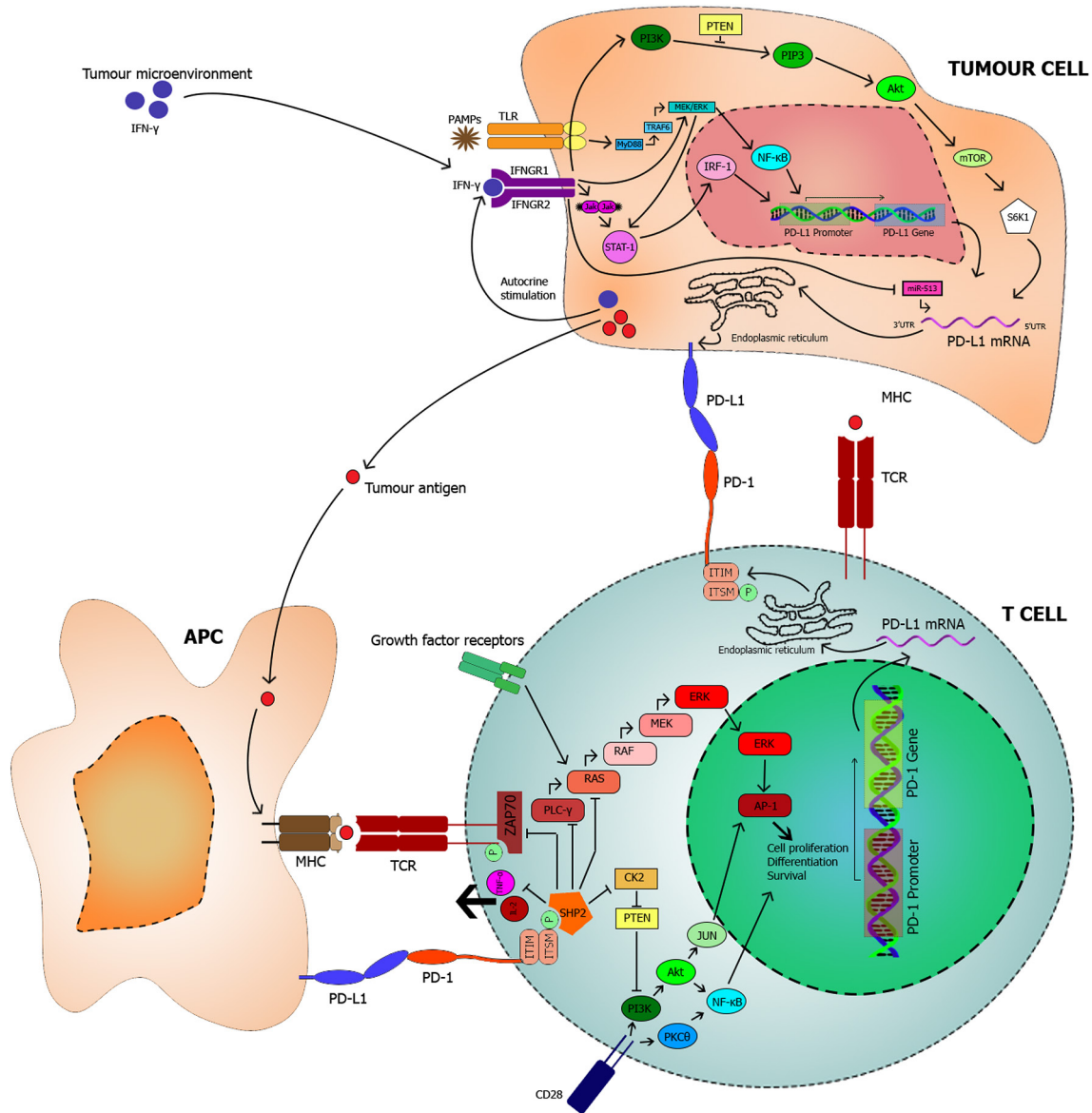


Figure 1 A schematic representation illustrating the signalling molecules that are linked with or influenced by the programmed death 1 (PD-1)/programmed death ligand 1 (PD-L1) interaction, as well as the cellular processes they affect.

PI3K-Akt and Ras-MEK-ERK cascades.¹⁰ Casein kinase 2 (CK-2) is a target of SHP-2. Casein kinase 2 (CK-2) dephosphorylation leads to unrestrained activation of PTEN, a physiological PI3K-Akt signalling antagonist.¹³ The inhibitory effect of PD-1 on the Ras-MEK-ERK cascade mostly depends on direct inhibition of Ras and dephosphorylation of phospholipase Cγ.^{14–16}

FUNCTIONS OF PD-L1

Central and peripheral tolerance

The PD-1/PD-L1 pathway is crucial for the development of immune tolerance, a process of negative selection of auto-reactive lymphocytes taking place in primary (central tolerance) and secondary lymphoid organs (peripheral tolerance).¹⁷ High PD-L1 expression is in fact demonstrated within the thymus and on dendritic cells, where the PD-L1/PD-1 interaction prevents the proliferation and differentiation of naïve T cells.^{18 19} Knock-out of PD-1/PD-L1 leads to autoimmunity in animal models with lupus-like arthritis, glomerulonephritis and diabetes.^{20 21} In humans, immune-related toxicity is a

recognised class effect of anti-PD-1/PD-L1 antibodies, where colitis, endocrinopathy and immune/inflammatory dermatoses are common complications.²²

Immune exhaustion

Immune exhaustion, that is, the progressive impairment of effector T cell function following persistent antigen presentation, is a physiological mechanism that prevents tissue destruction in chronic infection.²³ A cardinal feature of T cell exhaustion includes the induction of various coinhibitory pathways including PD-1/PD-L1.²³ HIV-specific CD4/CD8 cells coexpress PD-1, and a similar role for PD-1/PD-L1 has been found in viral hepatitis and tuberculosis,^{24–26} where impairment of effector T cell function is induced through apoptosis, inhibition of T cell replication and maturation^{27–29} as well as parallel induction of regulatory T cells.³⁰

Regulation of the anticancer immune response

Persistent up-regulation of PD-1 is commonly found in tumour-infiltrating lymphocytes, where PD-L1 expression is

Table 1 The principal PD-1/PD-L1 checkpoint inhibitors currently approved and in clinical development

	Nivolumab (BMS-936558)	Pembrolizumab (MK-3475)	Atezolizumab (MPDL3280A)	Durvalumab (MED14732)	Avelumab (MSB0010718C)	Pidilizumab (CT-011)
Target	PD-1	PD-1	PD-L1	PD-L1	PD-L1	PD-1
Monoclonal antibody class	Fully human IgG4	Humanised IgG4k	Humanised IgG1	Engineered IgG1k	Fully human IgG1	Humanised IgG1k
Stage of clinical development	FDA approved Phase III	FDA approved Phase III	FDA approved Phase III	FDA approved Phase III	FDA approved Phase III	Phase II
Approved indication	Melanoma (2014), NSCLC (2015), RCC (2015), urothelial carcinoma (2017), MMR-d colorectal cancer (2017)	Melanoma (2014), NSCLC (2016), HNSCC (2016), Hodgkin's lymphoma (2017), MMR-d tumours (2017)	Urothelial carcinoma (2016), NSCLC (2016)	Urothelial carcinoma (2017)	Merkel cell carcinoma (2017)	
Companion PD-L1 assay	Dako 28-8 (rabbit)	Dako 22c3 (mouse)	Ventana SP142 (rabbit)	Ventana SP263 (rabbit)		NA
Target cells	TC	TC IC	TC IC	TC IC		
Cut-off for positivity	NSCLC >1%–5% RCC >5%	NSCLC >1% TC any IC (as second-line therapy)	Urothelial >5% IC NSCLC >10% IC or >50% TC	Urothelial: ≥25% TC or IC if IC present in >1% of specimen >25% TC or 100% IC if IC present in <1% of specimen NSCLC: ≥25% TC		

FDA, Food and Drug Administration; HNSCC, head and neck squamous cell carcinoma; IC, infiltrating cells; MMR-d, mismatch repair deficient; NSCLC, non-small cell lung cancer; PD-1, programmed death 1; PD-L1, programmed death ligand 1; RCC, renal cell carcinoma; TC, tumour cells.

exploited by malignant cells to avoid immune destruction.^{31 32} Interestingly, PD-1 activation by PD-L1 up-regulates Slug, Snail and Twist through the MAPK/ERK pathway suggesting a link between tumour invasiveness and antitumour immune control.^{33–36} PD ligands are also regulated by hypoxia-inducible factor-1 α implying an interplay with neoangiogenesis, an independent hallmark of cancer progression.^{4 37}

PD-L1 EXPRESSION IN MALIGNANCY

Expression of PD-L1 either in tumour or in infiltrating immune cells has been verified predominantly by immunohistochemistry (IHC) in a variety of tumours, suggesting a role for the PD-1/PD-L1 axis as a prognostic trait and therapeutic target across multiple histotypes. However, IHC-based detection of PD-L1 expression is constrained by preanalytical and analytical variability including heterogeneity in antibody clones, scoring methodology and intrinsic biological variation in PD-L1 expression due to the type of specimen analysed (surgical resection vs biopsy, primary tumour vs metastasis, archival vs fresh frozen) as well as prior treatment status.^{38 39} The complex interplay between these factors plays a major role in the diffusion and clinical application of PD-L1 IHC assays as predictive biomarkers of response to PD-1/PD-L1 inhibitors.

NSCLC

Approximately 20%–30% of non-small cell lung cancer (NSCLC) express PD-L1 in >50% of the sampled tumour and infiltrating immune cells.^{40 41} PD-L1-positive NSCLCs are characterised by a fainter lymphocytic infiltrate⁴² and shorter disease-free survival.⁴³ However, in a large study of 982 patients prospectively accrued in three adjuvant chemotherapy trials, PD-L1 expression in either tumour or stroma did not predict survival despite the use of different thresholds.⁴⁴

PD-L1 expression enriches for responses to anti-PD-1/PD-L1 antibodies. In a study of 184 NSCLC cases treated with

atezolizumab, clinical responses correlated with the presence of PD-L1-positive infiltrating immune cells.⁴⁵ In the KEYNOTE-001, 010 and 024 studies of pembrolizumab in advanced NSCLC, higher tumoural PD-L1 expression predicted for better progression-free, overall survival and response rates across lines of treatment, with similar results observed in non-squamous NSCLC treated with nivolumab.^{40 46–48} While a number of studies have suggested interassay and biological heterogeneity in PD-L1 expression, IHC testing has, nevertheless, rapidly emerged as a stratifying biomarker in patients receiving PD-1/PD-L1-targeted checkpoint inhibitors, where harmonisation efforts are underway to promote interassay reliability and reproducibility.^{49 50}

Melanoma

The prevalence of PD-L1 expression in melanoma ranges from 24% to 49%,^{51–53} being highest (~60%) in tumours arising from chronic sun-damaged skin and lowest in uveal melanoma (10%).⁵⁴ PD-L1 independently predicts for poorer prognosis, being strongly correlated to tumour thickness, lymphatic and visceral spread, and in BRAF-mutant melanoma, PD-L1 overexpression is an adaptive feature of resistance to BRAF inhibitors.^{55 56} In the KEYNOTE-001 trial, patients with PD-L1-overexpressing tumours had response rates >50% and longer progression-free and overall survival.⁵⁷ However, the durable responses observed in PD-L1-negative tumours led to unrestricted licensing of anti-PD-1/PD-L1 therapies irrespective of PD-L1 status.

Epithelial ovarian cancer (EOC)

PD-L1 expression is common to 70% of EOC and predicts for worse 5-year survival rates (53%) compared with PD-L1-negative tumours (80%). PD-L1 inversely correlated with CD8+ T cell infiltrate suggesting its role in impairing the antitumour cytotoxic response, a renowned positive prognostic trait in EOC.^{58 59} Mechanistic studies have shown induction of PD-L1 expression to attenuate the cytolytic activity of CD8+ T cells in vitro and

promote the peritoneal spread of EOC.⁶⁰ PD-L1 expression strongly depends on IFN- γ release within the tumour microenvironment: genetic silencing of the IFN- γ receptor 1 decreases tumoural PD-L1 expression and improves survival in animal models.⁶¹

Breast cancer

PD-L1 expression is observed in invasive lobular and ductal breast cancer, where it is associated with local recruitment of PD-L1-positive CD8+ T lymphocytes.^{62,63}

Analysis of RNA-sequencing datasets has confirmed PD-L1 mRNA overexpression to be associated with a number of adverse prognostic factors such as negative hormone receptor status, Her-2-positive status, higher tumour grade, stage and proliferative index.⁶⁴ PD-L1 expression is typical of 20% of triple-negative breast cancer (TNBC) as a result of constitutive transcriptional activation secondary to PTEN loss.⁶⁵ PD-L1-overexpressing TNBC is molecularly defined by abundant cytotoxic T cell infiltrate and higher complete response rates to neoadjuvant chemotherapy,⁶⁴ findings that are in support of the development of anti-PD-1/PD-L1 inhibitors in TNBC.⁶⁶

Gastrointestinal malignancies

In gastro-oesophageal cancers, PD-L1 status is a negative predictor of outcome and is associated with nodal and visceral metastases and a more intense regulatory T cell infiltrate.^{67,68} Response rates to pembrolizumab in PD-L1-overexpressing gastro-oesophageal tumours approach 20%.⁶⁹

In colorectal cancer, tumoural expression of PD-L1 is infrequent (5%) and strongly associated with PD-1-positive lymphocytic infiltrate and mismatch-repair deficiency (MMR-d), features precluding to high immunogenicity and responsiveness to anti-PD-1/PD-L1 therapies.^{70,71}

In cholangiocarcinoma, PD-L1 expression ranges from 11% to 30% and is linked to worse prognosis.^{72,73} The prevalence of PD-L1 expression is 20% in hepatocellular cancer and correlates with higher alpha-fetoprotein levels, vascular invasion, poor differentiation and hepatic reserve.^{74,75}

Pancreatic cancer is poorly immunogenic due the presence of a dense immunosuppressive desmoplastic microenvironment. PD-L1 expression is scarce, and responses to single agent PD-1/PD-L1 targeted inhibitors are low.^{76–78}

Other malignancies

The range of tumours where the PD-1/PD-L1 pathway is emerging as a potential therapeutic target is rapidly expanding. PD-L1 overexpression has been shown to identify a group of 15%–20% of head and neck squamous cell carcinomas (HNSCCs) with poorer prognosis and enhanced chemoresistance.^{79,80} In urothelial malignancies, PD-L1 expression is low in tumour cells (4%) but higher in infiltrating lymphocytes (34%), a trait that predicts for improved survival in metastatic patients.^{81–83} B cell lymphomas rely heavily on the PD-1/PD-L1 immune checkpoint as a tumorigenic mechanism. In Hodgkin lymphoma (HL), Reed-Sternberg cells are commonly characterised by PD-L1 gene amplification, justifying the response rates in excess of 85% observed in chemorefractory HL treated with nivolumab.^{84,85}

PD-L1 is involved in avoidance of tumour rejection in non-HL and in different subtypes of leukaemia.⁸⁵ Blast cells are PD-L1 immunopositive in acute myeloid leukaemia, where PD-L1 expression attenuates antitumour cytotoxicity and predicts for a higher risk of relapse.⁸⁶

PD-1/PD-L1 INHIBITORS

The PD-1/PD-L1 interaction is an established therapeutic target in immuno-oncology which led to 'Breakthrough of the Year' status in 2013.⁸⁷ Selective inhibition of PD-1 or PD-L1 is not biologically identical due to the distinct spectrum of molecular interactions that characterise the ligand and receptor. Inhibition of PD-1, for instance, halts immunosuppressive signals derived from PD-L1 and PD-L2, whereas blockade of PD-L1 exerts inhibitory effects on PD-1 and B7.1 receptors.⁸⁸ In terms of clinical efficacy, therapeutic equivalence between the two approaches is presumed but not definitely proven.

As shown in table 1, on the basis of the significant survival benefit and durable responses observed in phase II/III studies, antibodies inhibiting PD-1/PD-L1 have become, to date, clinically approved therapies in seven oncological indications.

However, a number of challenges still exist in optimising the delivery of PD-1/PD-L1 inhibitors and expanding their use as safe and effective therapies across indications.

In cancer, responses are limited to a fraction of patients. Combined inhibition of PD-1 and CTLA-4 has resulted in doubling of response rates at the price, however, of increased toxicity.⁵¹ These results have paved the way to a number of combination studies with other systemic anticancer therapies and locoregional treatments.⁴⁸

An improved characterisation of predictive correlates of response to PD-1/PD-L1 inhibitors is expected to improve patient selection and facilitate the delivery of personalised immunotherapy. Besides harmonisation of PD-L1 IHC testing, prediction of response will require multitechnology integration to comprehensively evaluate tumour-intrinsic and tumour-extrinsic factors, including somatic mutational load, MMR-d status, proinflammatory signatures and many other factors.⁸⁹

Lastly, the non-oncological development of PD-1/PD-L1 inhibitors in disease areas with a paucity of effective therapeutic targets including chronic infection and immune pathology might further expand the clinical relevance of PD-L1 as a therapeutic target in human disease.⁹⁰

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