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Programmed Cell Death-Ligand 2: A Neglected But Important Target in the Immune Response to Cancer?

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ABSTRACT

Programmed cell death-ligand 2 (PD-L2) is one of the two ligands of the programmed cell death-1 (PD-1) receptor, an inhibitory protein mainly expressed on activated immune cells that is targeted in the clinic, with successful and remarkable results. The PD-1/PD-Ls axis was shown to be one of the most relevant immunosuppressive pathways in the immune microenvironment, and blocking this interaction gave rise to an impressive clinical benefit in a broad variety of solid and hematological malignancies. Although PD-L2 has been historically considered a minor ligand, it binds to PD-1 with a two- to six-fold higher affinity as compared to PD-L1. PD-L2 can be expressed by immune, stromal, or tumor cells. The aims of this narrative review are to summarize PD-L2 biology in the physiological responses of the immune system and its role, expression, and clinical significance in cancer.

Contents

Introduction

The role of the immune microenvironment in determining the development of tumors [1] and influencing outcomes and responses to treatments [2–5] gave rise to a new era for the treatment of cancer. Indeed, the immune response has been efficiently manipulated through the use of a variety of novel immunotherapeutic agents, the immune checkpoint blockade, given alone or in combination with other drugs, with the goal to rescuing and/or boosting the activation of a pre-existing antitumor immune response. A new focus of investigation is the role played by stromal cells, which are able to prevent an immune infiltration into tumors [6], representing potential targets that can be manipulated in order to induce more "fighters" (e.g., T cells) into the tumor bed.

Among these cells, cancer-associated fibroblasts (CAFs) [7] and tumor-associated macrophages (TAMs) [8] were shown to express the ligands [programmed cell death-ligand 1 (PD-L1) and PD-L2) for the well-known

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targetable inhibitory immune checkpoint molecule programmed cell death-1 (PD-1) and to regulate the activity of the cells of innate and adaptive immunity in the tumor microenvironment (TME). Furthermore, CAFs are able to determine T cell dysfunction by inducing the expression of a variety of alternative inhibitory immune checkpoint molecules [e.g., PD-1 [9], cytotoxic T lymphocyte antigen-4 (CTLA-4), T-cell immunoglobulin and mucin-domain containing-3 (TIM3) [10], and lymphocyte activation gene-3 (LAG3) [11]] on their surface [7], contributing to curb the immune response. While the clinical significance of PD-L1, the main ligand for PD-1, has been widely investigated in cancer, the role of PD-L2 expressed by immune, stromal, and tumor cells has received less attention and has been considered less relevant in predicting responses to immune checkpoint blockade with anti–PD-1/PD-L1 agents [12].

In this era of cancer immunotherapy, the evaluation of responses to treatments [13,14], including rarely described abscopal responses [15], the diagnosis and management of toxicities [16–18], and the selection of patients represent some of the most important focuses of interest. In addition, it is becoming more evident that a multiparametric approach is needed to optimize patient selection for a more personalized cancer immunotherapy strategy. The biomarkers that have shown an association with benefit from immune checkpoint blockade are: immune-related [e.g., PD-L1 expression particularly in non–small cell lung cancer, the extent of tumor-infiltrating lymphocytes (TILs) as consistently shown in breast cancer [19–24], the presence of $CD8^+$ T cells [24], the detection of immune gene signatures, etc.]; the levels of circulating biomarkers [e.g., lactate dehydrogenase (LDH) [25]]; the presence of genomic parameters, like the tumor mutational burden (the number of somatic mutations) [26]; and other clinical criteria such as the line(s) of treatment received [19] and the gender of treated patients [27,28].

Remarkably, tumors responding to single-agent immune checkpoint blockade are usually the most infiltrated, whereas the immune-excluded tumors are characterized by stromal reactions that prevent immune infiltration into intratumoral areas [6]. The latter are those tumors that might benefit from combinational treatments aiming to increase the priming and activation and to bring activated T cells into tumors [6].

The aims of this narrative review are to summarize PD-L2 biology in the physiological responses of the immune system and its role, expression, and clinical significance in cancer.

PD-L2: Biology

The PD-1/PD-Ls pathway plays a fundamental role in manipulating the magnitude of T cell responses, regulating their activation and generating immune tolerance in the TME and in peripheral tissues [29]. Furthermore, the PD-1 pathway controls humoral responses, where the activity of B cells is modulated by follicular helper T cells [30] and follicular regulatory T cells that were found positive to both PD-1 [31] and CTLA-4 [32,33]. Indeed B cells can express PD-L1 [34–36] and PD-L2 [36–39] but also cluster of differentiation (CD)80 and CD86 [40], which are the respective ligands for PD-1 and CTLA-4.

PD-1 is an inhibitory receptor found on a variety of immune cells, principally T lymphocytes [9], representing an efficient target for cancer immunotherapy. The role of PD-L2 in modulating antitumor responses has historically received less attention with respect to PD-L1. Nevertheless, PD-L2 binds to PD-1 with a higher (two- to six-fold) affinity as compared to PD-L1 [41]. Another receptor that binds to PD-L2 is Rgmb, and this interaction can conversely activate T cells [42].

Furthermore, PD-L2 expression was identified in a variety of tumor types even in the absence of PD-L1 expression [43,44], and a recent study revealed the presence of PD-L2 specific T lymphocytes able to recognize their targets expressed by either tumor or immune cells and able to induce the release of T helper (Th)-1 cytokines, like interferon-gamma (IFN-γ) and tumor-necrosis factor-alpha (TNF-α) [45]. PD-L2 can be found on immune cells, including B lymphocytes (where it can bind to PD-1 on follicular helper T cells, resulting in reduced long-lived plasma cell number [36,46]), and on dendritic cells as well as on other types of hematopoietic and nonhematopoietic cells [47].

Physiologically, PD-L2, together with PD-L1, plays a role in the regulation of mucosal $CD4^+$ T cell responses when expressed by colonic myofibroblasts, a cell population that is major histocompatibility complex class II positive ($MHC\text{-}II^+$). These $PD\text{-}L2^+$ cells exert suppressive functions with respect to activated $CD4^+$ Th cell proliferation by inhibiting interleukin (IL)-2, whose production can be increased after PD-1/PD-L1 or PD-1/PD-L2 blockade [48].

In addition, PD-L2 can be expressed on macrophages, on bone marrow derived mast cells, on more than 50% of peritoneal B1 lymphocytes [49], and on intestinal stromal cells (exerting a role in immune tolerance) [50]. PD-L2 expression on dendritic cells is induced by IL-4 and granulocyte-monocyte colony stimulating factor (GM-CSF) [51].

In rhesus macaques, human-like B1 cells ($CD11b^+$ B1) were observed in the spleen and were able to express PD-L2 and the immunosuppressive IL-10. These cells induced PD-1 expression on $CD4^+$ T cells, contributing to enhancing T cell exhaustion [52]

PD-L2: Expression in Cancer

Figure 1 depicts the various cell subsets that express PD-L2 in the TME. Table 1 summarizes the clinical significance of PD-L2 expression in a variety of tumor types.

PD-L2 expression was found on activated $CD1a^+$ dendritic cells in patients with cutaneous squamous cell carcinoma [53]. It was usually associated with the presence of PD-L1 and with the size of the tumor. Further, PD-L2 was detected on either $CD4^+$- or $CD8^+$-specific T lymphocytes that did not cross-react with $PD\text{-}L1^+$–specific T lymphocytes in melanoma [45]. These PD-L2–specific T cells were able to react to autologous target cells depending on PD-L2 expression, directly supporting antitumor immunity by killing of target cells and, indirectly, by releasing proinflammatory cytokines in the TME in response to PD-L2–expressing immunosuppressive cells [45].

In clear cell renal cell carcinoma, high expression of PD-L2 together with PD-1, LAG3, and PD-L1 was associated with a more immunosuppressive TME [54].

In breast cancer, membrane immunohistochemical (IHC) expression of PD-L2 by immune cells, detected with the monoclonal antibody (clone = D7U8C) from Cell Signaling Technology, was found almost negative, with these findings being confirmed also by flow cytometry data [here, the monoclonal antibody (clone = MIH18) used was from Miltenyi] [36]. In prostate cancer, deficient mismatch repair mutational signatures (found in 8.1% of the cohort) were associated with an increased extent of immune cells, immune checkpoint molecule detection, and T lymphocyte–associated transcripts, including the expression of PD-L2 by antigen-presenting cells [55].

In B cell lymphomas, PD-L2 is usually detected on protumor M2 TAMs [56], and similar findings can be observed in classical Hodgkin lymphoma where high expression of PD-L2 (and PD-L1) by TAMs was associated with the suppression of the function of natural killer cells [56].

In peripheral T lymphocytes from non–small cell lung cancer patients, PD-L2 and PD-L1 expression was linked with higher levels of IL-2 and TNF-α and with a worse overall survival (OS) [57].

In addition, tumor cells are able to express PD-L2 [58–60]. Preclinical models of renal cell carcinoma and lung squamous cell carcinoma reveal that tumor expression of PD-L2, through the inhibition of the activity of $CD8^+$ T cells, has a protumor role in the TME and could be involved in the resistance to anti–PD-1 antibodies, which could be overcome by the combined use of anti–PD-1 or anti–PD-L2 immune checkpoint blockade [61]. Its significance was also confirmed in the clinical setting.

A recent study identified a key super-enhancer, PD-L1L2-SE, located between the encoding regions for PD-L1 and PD-L2. The activation of PD-L1L2-SE is required for PD-L1 and PD-L2 expression on tumor cells and genetic deletion of PD-L1L2-SE and causes tumor cell loss of immune evasion and increased sensitivity to T cell killing. Interestingly, this mechanism of induction of PD-L1 and PD-L2 is independent of IFN-γ [62].

In humans, PD-L2 expression by tumor cells is probably associated with a Th2 response, mediated by IL-4 and IL-13, as shown in esophageal cancer

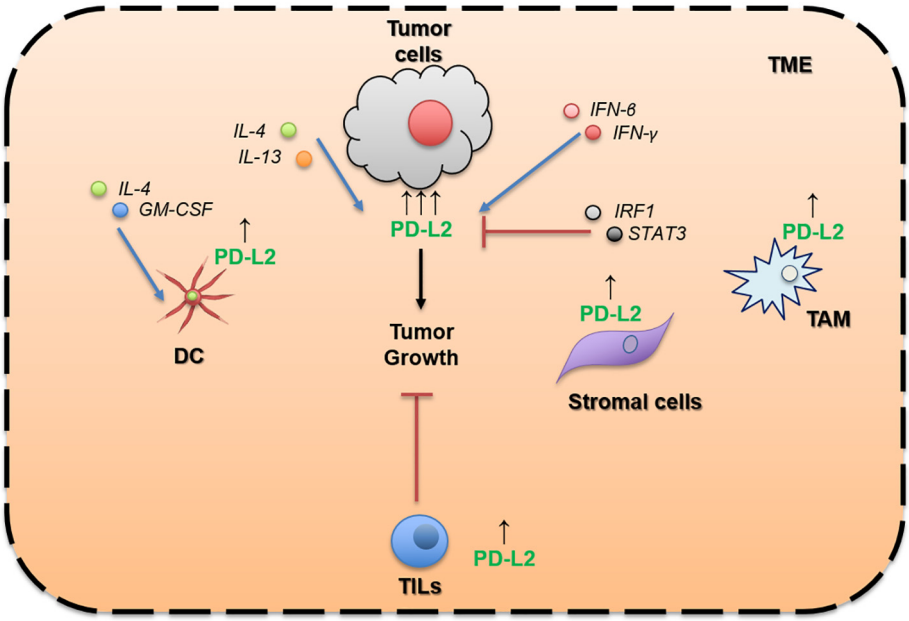

Figure 1. Expression of PD-L2 by the cells of the tumor microenvironment. In the TME, PD-L2 expression is inducible on dendritic cells (DCs), tumor cells, TAMs, stromal cells, and TILs. Various cytokines (e.g., IL-4, GM-CSF, IFN-γ and IFN-β) modulate this expression. Legend: *IRF*, interferon regulatory transcription factor; *STAT*, signal transducer and activator of transcription.

[59] and by IFN-γ in colorectal cancer [63]. In melanoma cells, PD-L2 is responsive to IFN-beta (β) and IFN-γ and is regulated through the transcription factors IRF1 and STAT3 that bind to the PD-L2 promoter [44,64].

PD-L2 presence is inversely associated with a Crohn-like lymphoid reaction in colorectal cancer [58] probably inhibiting the development of tertiary lymphoid tissues [60]. In small intestine and pancreatic neuroendocrine tumors, while the expression of PD-1 or PD-L1 was rare, that of PD-L2 was common. No clear associations between PD-L2, immune infiltrates, and specific mutational profiles within each tumor type were found [65]. In gallbladder cancer, PD-L2 was more common than PD-L1 expression [66]. In endometrial cancer, PD-L2 positivity was found on tumor cells in 64.4% and on immune cells in 93.2% of cases [67]. In bladder cancer, 67% of the specimens exhibited PD-L2 positivity on tumor cells [68]. PD-L2 expression by tumor cells was also observed in lung cancer [69], where it is usually associated with the detection of other immune checkpoint molecules (PD-L1, indoleamine-pyrrole 2,3-dioxygenase, others) [70]. However, membrane PD-L2 found on tumor cells was not associated with baseline peripheral blood markers (white blood cells, absolute neutrophil, lymphocyte, monocyte and eosinophil counts, serum C-reactive protein, and serum LDH levels) in lung adenocarcinoma patients who underwent primary surgery [69]. In a retrospective series of lung adenocarcinoma patients, including all the stages of the disease (from early to metastatic), PD-L2 was evaluated by IHC. Around 50% of the cases were stained PD-L2^{+} on tumor cells, with the 30% resulting PD-L2^{high} ($\geq$50% positive cells) and being associated with an *EGFR* mutation and a lower stage of the tumor. A longer progression free survival was observed in patients defined PD-L2^{+} (>1% of positive cells) [71] . Different results were obtained in another more homogeneous retrospective cohort of patients (only those with resected lung adenocarcinoma were included), where the expression of PD-L2 by tumor cells (1% cut-off) was associated with worse outcomes (shorter disease-free survival and OS) [72], probably related to the different patient population evaluated. In addition, in patients with resected squamous cell lung carcinoma, PD-L2 expression on tumor cells at two different cuts-off (>5% and >10% positive cells) predicted better outcome [73].

In renal cell carcinoma, PD-L2 on tumor cells (together with PD-1 and PD-L1) was less expressed in primary tumors (16%) with respect to metastases (24%) even though this difference was not statistically significant [74]. In histotypes with sarcomatoid transformation, amplifications of the *JAK2/PD-L1/PD-L2* genes were detected [75]. PD-L2 expression by tumor cells was further associated with better prognosis in oropharyngeal squamous cell carcinoma [58] and melanoma [44,76] and with a worse prognosis in colorectal cancer [50], malignant salivary gland tumors [77], and chromophobe renal cell carcinoma [78]. Different from PD-L1, cytoplasmic PD-L2 expression by IHC was found in 42% of patients with esophageal squamous cell carcinoma with no impact on disease-free survival.

In breast cancer, PD-L2 expression by tumor cells was found in around 29% of the cases analyzed by IHC, with no clinically significant associations [79], findings that were conflicting with another study [36] probably due to the different antibodies employed.

Concerning the last subset of cells from the TME expressing PD-L2, the stromal cells, a contemporaneous overexpression of either PD-L2 or PD-L1 by CAFs was observed as shown in human pancreatic carcinoma [7]. These fibroblasts showed higher levels of PD-L1 and particularly of PD-L2 with respect to the skin fibroblasts from healthy donors, playing an inhibitory role on the activity of TILs [7]. Remarkably, these CAFs had higher levels of PD-L2 with respect to PD-L1, and blockade of the pathway through the use of anti–PD-L1 and anti–PD-L2 immune checkpoint blockade restored CD4^{+} (and with less frequency CD8^{+}) T cell proliferation. In mouse models, CAFs were shown to suppress T lymphocyte function through the interaction of immune checkpoint pathways, like FAS/FASL and PD-1/PD-L2. FASL and PD-L2 were more abundant in CAFs with respect to normal fibroblasts, and when these ligands were blocked, the capacity of T cells to exert their cytotoxic activity was rescued in an antigen-dependent manner [80]. In lung tumors, there was an enrichment of FASL and PD-L2 [80] in stromal areas, revealing the potential relevance of CAFs in the human TME and probably explaining some mechanisms of immune evasion that are mediated by stromal cells. PD-L1 and PD-L2 were constitutively expressed but also upregulated through IFN-γ in non–small cell lung cancer [81]. Also in head and neck cancer, PD-L1 and PD-L2 were observed in fibroblasts [82], with inhibitory actions on T lymphocytes. PD-L2 can also be expressed by mast cells [83–85] with immunosuppressive effects.

At the transcriptomic level, high expression of the gene for PD-L2 (*PDCD1LG2*) was associated with *CDKN2A* loss in non–small cell lung [86,87] and thyroid cancers [88]. Detection of *PDCD1LG2* in lung cancer was one of the best predictors for the expression of *PDCD1LG1* mRNA and vice versa [89], and a stronger correlation between PD-L2 and active

Table 1
Prognostic Value of PD-L2 in Tumors

Reference	Cancer Type	No. of Patients	Disease Stage	Trial Design	Cut-off for Positive PD-L2	Method (Antibody)	Outcomes	HR	*P* Value	Conclusions
Wang et al. [107]	Gliomas (WHO II-IV)	1107 (total) 310 (training cohort) 797 (validation cohort)	NA	PC	>10% positive cells	IHC (clone: 18251-1-AP)	OS: PD-L2 high (vs. low) No. of patients (training cohort) 163 (147) No. of patients (validation cohort) 258 (539)	1.68 (95% CI, NA) 1.28 (95% CI, NA)	.017 .023	Increased PD-L2 expression conferred a worse outcome in gliomas
Xue et al. [108]	Ovarian cancer	77	• FIGO stage I-II (16) • FIGO III-IV stage (61)	PC	Scores: 1-2-3	IHC (clone: NA; mouse monoclonal antibody by R&D system)	OS: PD-L2 high (vs. low) No. of patients 17 (60)	2.204 (95% CI, 1.037-4.682)	.0105	Increased PD-L2 expression conferred a worse outcome in ovarian cancer
Derks et al. [59]	Esophageal adenocarcinoma	352	• Stage I (116) • Stage II (53) • Stage III (171) • Stage IV (12)	RC	≥10% positive cells	IHC (clone: 366C.9E5)	OS: PD-L2 high (vs. low) No. of patients 70 (282)	0.75 (95% CI, 0.54-1.03)	.078	Increased PD-L2 expression showed a trend toward an improved outcome in esophageal adenocarcinoma
Jung et al. [109]	Hepatocellular carcinoma	85	• Stage I (5) • Stage II (47) • Stage III (29) • Stage IV (4)	RC	Scores: 3-5	IHC (clone: NA)	OS: PD-L2 low (vs. high) No. of patients 65 (20) DFS: PD-L2 low (vs. high) No. of patients 65 (20)	1.904 (95% CI, 1.035-3.503) 1.337 (95% CI, 0.745-2.401)	.039 .331	Increased PD-L2 expression conferred a worse outcome in hepatocellular carcinoma No statistically significant difference for DFS in hepatocellular carcinoma patients with increased PD-L2 expression was found
Gao et al. [110]	Gastric adenocarcinoma	119	• Stage II- IIIA (12) • Stage IIIb-IIIc (107)	RC	> Median values	IHC (clone: MAB1224)	OS: PD-L2 high (vs. low) No. of patients 34 (85)	2.362 (95% CI, 1.342-4.157)	.003	Increased PD-L2 expression conferred a worse outcome in gastric adenocarcinoma
Pinato et al. [111]	Pheochromocytomas Paragangliomas	64 36	• Benign (90) • Malignant (10)	PC	≥5% positive cells	IHC (clone: NA; Sigma Aldrich)	OS: PD-L2 high (vs. low) No. of patients 16 (84)	HR: NA OS was significantly shorter in patients overexpressing PD-L2 with a median of 13.5 y (95% CI 8-19 years) compared with 25 years in patients with PD-L2 negative (95%CI 20-30 years)	.029	Increased PD-L2 expression conferred a worse outcome in pheochromocytomas and paragangliomas
Wang et al. [63]	Colorectal cancer	124	• Stage I (6) • Stage II (64) • Stage III (49) • Stage IV (4)	RC	Scores: 2-3	IHC (clone: NA; Abcam)	OS: PD-L2 high (vs. low) No. of patients 48 (76) DFS: PD-L2 high (vs. low) No. of patients 48 (76)	2.778 (95% CI, 1.668-4.627) 12.31 (95% CI, 1.04-145.53)	<.0001 .0463	Increased PD-L2 expression conferred a worse outcome in colorectal cancer Increased PD-L2 expression may moderately accelerate cancer relapse or metastasis in colorectal cancer
Wu et al. [112]	Gastric cancer	340	• Stage I-II (96) • Stage III-IV (244)	PC	Scores: 3-6	IHC (clone: 176611)	OS: PD-L2 low (vs. high) No. of patients 117 (223)	1.16 (95% CI, 0.85-1.60)	.352	Increased PD-L2 expression was not related to the prognosis of gastric cancer

Table 1 (*continued*)

Reference	Cancer Type	No. of Patients	Disease Stage	Trial Design	Cut-off for Positive PD-L2	Method (Antibody)	Outcomes	HR	*P* Value	Conclusions
Tanaka et al. [113]	Esophageal squamous carcinoma	180	• Stage I (19) • Stage II (53) • Stage III (60) • Stage IV (48)	RC	Scores: 4-9	IHC (clone: 176611)	OS: PD-L2 low (vs. high) No. of patients 117 (223)	1.1524 (95% CI, 0.7527-1.7789)	.0237	Increased PD-L2 expression conferred a worse outcome in esophageal squamous carcinoma
Shin et al. [114]	Clear cell renal cell carcinoma	91	• Stage IV	RC	Scores: 2-3	IHC (clone: 176611)	OS: PD-L2 high (vs. low) No. of patients 36 (55)	1.231 (95% CI, 0.769-1.970)	.387	Increased PD-L2 expression was not related to the prognosis of clear cell renal cell carcinoma in terms of OS
							PFS: PD-L2 high (vs. low) No. of patients 36 (55)	1.057 (95% CI, 0.665-1.681)	.814	Increased PD-L2 expression was not related to the prognosis of clear cell renal cell carcinoma in terms of PFS
Baptista et al. [115]	Breast cancer	192	• Completely resected stage I, II, and III	RC	> Median values	IHC (clone: NA; Abcam)	OS: PD-L2 low (vs. high) No. of patients 94 (97)	1.72 (95% CI, 0.57-5.17)	.32	Increased PD-L2 expression was not related to the prognosis of breast cancer in terms of OS
							DFS: PD-L2 low (vs. high) No. of patients 94 (97)	1.35 (95% CI, 0.67-2.71)	.39	Increased PD-L2 expression was not related to the prognosis of breast cancer in terms of PFS
Dong et al. [116]	Gastric cancer (Epstein-Barr virus negative)	796	• Stage I-II (327) • Stage III-IV (469)	PC	> Median values	IHC (clone: NA; ab170675)	OS: PD-L2 high (vs. low) No. of patients 507 (58)	1.162 (95% CI, 0.720-1.874)	.538	Increased PD-L2 expression was not related to the prognosis of Epstein-Barr virus–negative gastric cancer
Erlmeier et al. [78]	Chromophobe renal cell carcinoma	81	• Localized (70) • Advanced (11)	RC	> Median values	IHC (clone: 176611)	OS: PD-L2 low (vs. high) No. of patients 58 (23)	4.7 (95% CI, 0.86-25.25)	.074	Increased PD-L2 expression showed a trend toward a worse outcome in chromophobe renal cell carcinoma
Gao et al. [117]	Hepatocellular carcinoma	240	• Stage I (106) • Stage II (76) • Stage III (58)	RC	> Median values	IHC (clone: NA; R&D Systems)	OS: PD-L2 low (vs. high) No. of patients 180 (60)	1.10 (95% CI, 0.68-1.79)	.71	Increased PD-L2 expression was not related to the prognosis of hepatocellular carcinoma in terms of OS
							DFS: PD-L2 low (vs. high) No. of patients 180 (60)	0.96 (95% CI, 0.60-1.53)	.86	Increased PD-L2 expression was not related to the prognosis of hepatocellular carcinoma in terms of DFS
Kogashiwa et al. [118]	Oral squamous cell carcinoma	84	• Stage III (13) • Stage IVa (71)	RC	>5% positive cells	IHC (clone: 80380)	OS: PD-L2 low (vs. high) No. of patients 64 (20)	0.442 (95% CI, 0.132-1.486)	.187	Increased PD-L2 expression was not related to the prognosis of hepatocellular carcinoma in terms of OS
							PFS: PD-L2 low (vs. high) No. of patients 64 (20)	1.01 (95% CI, 0.431-2.37)	.978	Increased PD-L2 expression was not related to the prognosis of hepatocellular carcinoma in terms of PFS

(continued on next page)

Table 1 (*continued*)

Reference	Cancer Type	No. of Patients	Disease Stage	Trial Design	Cut-off for Positive PD-L2	Method (Antibody)	Outcomes	HR	*P* Value	Conclusions
Kim et al. [119]	Pleomorphic lung carcinoma	41	• Stage I (12) • Stage II (15) • Stage III (12) • Stage IV (2)	RC	Scores: 2-3	IHC (clone: 176611)	PFS: PD-L2 low (vs. high) No. of patients 5 (36)	Not applicable	.370	Increased PD-L2 expression was not related to the prognosis of hepatocellular carcinoma in terms of PFS
Zhang et al. [120]	Non–small cell lung cancer (adenocarcinoma)	143	• Stage I (66) • Stage II-III (77)	PC	> Median values	IHC (clone: HPA013411)	OS: PD-L2 high (*vs.* low) No. of patients 71 (62)	2.328, (95% CI, 1.201-4.512)	.012	Increased PD-L2 expression conferred a worse outcome in patients with lung adenocarcinomas
Shinchi et al. [71]	Non–small cell lung cancer (adenocarcinoma)	231	• Stage 0-I (178) • Stage II-IV (53)	RC	>1% positive tumor cells	IHC (clone: D7U8C)	PFS: PD-L2 positive (vs. negative) No. of patients 122 (109)-	0.388, (95% CI, 0.216-0.672)	.0006	PD-L2$^+$ cases had an improved PFS (univariate analysis) No correlation found between PD-L2 expression and OS
Takamori et al. [72]	Non–small cell lung cancer (adenocarcinoma)	433	• Stage I (319) • Stages ≥ II (114)	RC	>1% positive tumor cells	IHC (clone: 176611)	DFS and OS: PD-L2 Positive (*vs.* negative) No. of patients 306 (127)	1.63, (95% CI 1.10-2.50) and 2.01, (95% CI 1.16-3.72) (univariate analysis)	.015 and .011	PD-L2 expression conferred a worse outcome in patients with resected lung adenocarcinoma
Matsubara et al. [73]	Non–small cell lung cancer (squamous cell carcinoma)	211	• Stage I (114) • Stages ≥ II (97)	RC	>5% (low) and >10% (high) positive tumor cells	IHC (clone: NA)	OS: PD-L2 negative (vs. low) No. of patients 48 (163) Negative (vs. high) No. of patients 69 (142)	1.68, (95% CI 1.03-2.66) and 1.66, (95% CI 1.07-2.54) (multivariate analysis)	.0170 and .0500	PD-L2 expression conferred a better outcome in patients with resected squamous cell lung carcinoma

CI, confidence interval; *DFS*, disease-free survival; *HR*, hazard ratio; *IHC*, immunohistochemistry; *NA*, not available; *OS*, overall survival; *PC*, prospective cohort; *PFS*, progression-free survival; *RC*, retrospective cohort; *WHO*, World Health Organization.

IFN-signaling as compared to PD-L1 was observed in adenocarcinomas and squamous cell carcinomas [90]. Indeed, expression of PD-L1 is usually coupled with at least another immune checkpoint, such as PD-L2 and indoleamine-pyrrole 2,3-dioxygenase [70]. Similar findings were observed in breast cancer [36].

Elevated expression of both *PD-L1* and *PD-L2* genes was observed in virus-associated tumors such as human papilloma virus$^+$ head and neck squamous cell cancer and Epstein-Barr virus$^+$ gastric cancer, together with the upregulation of T and B lymphocyte signatures, suggesting that viral infections recruit immune effector cells, with an upregulation of immunosuppressive pathways. Interestingly human papilloma virus integration sites were observed close to *PD-L1* and *PD-L2* genes. Integration of *PD-L2* was much more complicated, having multiple integration sites in or after intron 3 of *PD-L2* [91].

In hepatocellular carcinoma, gene expression of *PD-L1* and *PD-L2* together with other genes significantly correlated with a high-risk signature [92], and an altered expression of *PD-L2* was associated with a worse OS (HR: 1.52, 95% CI: 1.02-2.26) in The Cancer Genome Atlas cohort of 361 patients. In esophageal adenocarcinoma, gene expression of *PD-L1* and *PD-L2* was associated with a worse OS and with a poor response to neoadjuvant chemotherapy [68].

Recently, a study on digital gene expression, T cell receptor repertoire analysis, flow cytometry, multispectral immunofluorescence, and next-generation sequencing was performed in patients with early- and advanced-stage follicular lymphoma, revealing that high PD-L2 expression was associated with high immune infiltration (by macrophages and expanded populations of T cell clones) and improved outcomes [93]. Intriguingly, the extent of immune infiltration and its effect on outcome were independent of the mutational profile.

Concerning the responses to immune checkpoint blockade, in one of the earliest studies investigating PD-1, PD-L1, and PD-L2 in tumor tissue sections of patients treated with anti–PD-1 agents, it was shown that while the expression of PD-L1 on tumor cells was associated with a response to the treatment, that of other molecules, including PD-1 and PD-L2, was not [12]. In contrast, clinical responses to the anti–PD-1 pembrolizumab in recurrent or metastatic head and neck squamous cell carcinoma patients have been partially linked to the blockade of the PD-1/PD-L2 pathway. Indeed, PD-L2 IHC expression detected on stromal, immune, and tumor cells was independently associated with a higher likelihood of response and better survival outcomes [43]. Expression of PD-L2 was positively correlated with that of PD-L1. Responses were greater in patients positive for both PD-L1 and PD-L2 (27.5%) as compared to those positive for PD-L1 only (11.4%).

To sum up the principal findings arising from the literature, PD-L2 expression was associated with certain genetic characteristics, such as the *JAK/STAT* pathway amplification and the *CDKN2A* loss. At the moment, it is not clear whether the upregulation of PD-L2 is linked to the anatomical origin of the tumor (it is the case for hematological vs. solid tumors) or whether, most probably, it is the type of cells expressing this inhibitory ligand that influences prognosis, with PD-L2$^+$ immune cells associated with a better outcome, whereas PD-L2$^+$ tumor cells and PD-L2$^+$ stromal cells were probably linked with a worse outcome.

Indeed, from what has been shown in the literature, the expression of PD-L2 by immune cells, particularly by T-TILs, was associated with a

potentially efficient antitumor immune response even though these findings need further confirmation. In contrast, in clear cell renal cell carcinoma, the expression of multiple immune checkpoint molecules and ligands, including PD-L2, characterizes an immunosuppressive TME. Further, PD-L2 in circulating T lymphocytes from patients with non–small cell lung cancer has been linked with a worse outcome, signifying that probably the compartment where T lymphocytes are present (TME vs. peripheral blood) might determine different behaviors (e.g., anti- vs. protumor) or less probably showing that the tumor type could influence the outcomes. In addition, PD-L2 expression by TAMs in hematological malignancies seems to be associated with a better outcome and an extensive immune infiltration that positively impacts on the outcome.

Globally, the expression of PD-L2 by tumor cells was almost linked to a worse prognosis in the majority of tumor types, with conflicting results observed in esophageal carcinoma. Intriguingly, some tumor cells express more frequently PD-L2 rather than PD-L1.

When looking at PD-L2 expression by stromal cells, data consistently show that particularly PD-L2$^+$ CAFs might predict a worse prognosis, reflecting the presence of an immunosuppressive stroma that prevents an immune infiltration.

Current literature data show a better response to anti–PD-1 agents in PD-L2–enriched head and neck squamous cell carcinomas, requiring further confirmation and validation.

PD-1/PD-L2 Pathway: Functional Outcomes

The PD-1/PD-L2 pathway is a negative regulator of immune responses taking place particularly in peripheral tissues. The inhibitory receptor PD-1 is able to transduce signals only when cross-linked together with the B cell receptor or with the T cell receptor, and this signaling inhibits lymphocyte glucose consumption, cytokine production, proliferation, and survival. The PD-1 inhibition can be overcome through CD28 co-stimulation [94] or IL-2 availability [95].

Further PD-L2 blockade generates significant increase in the frequency of CD4$^+$FoxP3$^+$ regulatory T cells in the draining lymph nodes of mice that had PD-L1 deleted on dendritic cells, together with a significant increase in the percentage of follicular regulatory T cells in the draining lymph node and blood of PD-L1F/F CD11c Cre$^+$ mice. No differences were found in the percentages of follicular regulatory T cells in mice that lacked PD-L1 specifically on B cells and controls, or in follicular helper T cells in the draining lymph node of CD11cCre$^+$ PD-L1F/F mice after immunization compared to controls, with heightened percentages of follicular helper T cells in the blood of CD11cCre$^+$ mice compared to control mice [96].

One possible explanation for the lack of appreciation for PD-L2 in cancer is due to the extensive use of murine preclinical cell lines that minimally express PD-L2. When cell lines overexpressing PD-L2 (MC38 and CT26-NY-ESO1) were implanted in mice, a reduced effector function of CD8$^+$ TIL*s* was observed. This study demonstrated the relevant role of PD-L2 in diminishing antitumor cytotoxicity [61].

PD-L2: Clinical Trials

In recent years, the surprising therapeutic success obtained with the use of monoclonal antibodies specifically designed to inhibit the PD-1/PD-L1 axis (e.g., nivolumab, pembrolizumab, atezolizumab, durvalumab, etc.) for the treatment of solid and hematological malignancies has generated an increasing interest in the possibility of exploring other molecular mechanisms implicated in the regulation of the immune response against tumor cells, to be exploited to modulate the immune system and achieve new therapeutic options [97].

In this context, in addition to the aforementioned pharmacological strategies targeting the immune checkpoint molecules CTLA-4, TIM3, and LAG3 in human cancers, the scientific evidence about the use in the clinical setting of drugs blocking the PD-1/PD-L2 pathway is progressively growing. Table 2 summarizes ongoing and completed clinical trials of PD-L2–based therapies in cancer.

Most of these studies are represented by phase I or II trials and are still ongoing (NCT03381768, NCT03939234, NCT02812875, and NCT02528682).

The NCT03381768, NCT03939234, and NCT02528682 investigate the safety and immunological effects of peptide or dendritic cell vaccinations directed against PD-L1 and PD-L2 in patients with several hematological malignancies, such as lymphomas or acute and chronic leukemias. This therapeutic strategy is based on the evidence that the TME and the immune-escape mechanisms play a crucial role in the persistence of these hematological neoplasms, and one of the most important escape mechanisms is the inhibition of T lymphocytes by PD-L1 and PD-L2 expressed in the TME. By preventing the interaction between PD-1$^+$ T lymphocytes with PD-L1$^+$/PD-L2$^+$ cells, an inhibition of the immunosuppressive TME and a control of the tumor by the immune response are hopefully accomplished.

The NCT02812875 is a multicenter, open-label, phase I trial of CA-170 conducted on adult patients with advanced solid tumors or lymphomas who have progressed or are nonresponsive to available therapies and for which no standard therapy exists. CA-170 is an orally available small molecule specifically targeting PD-L1, PD-L2, and the V-domain immunoglobulin suppressor of T cell activation immune checkpoint, resulting in T lymphocyte proliferation and cytokine production. The main objectives of this early trial are toxicity and the pharmacokinetic and preliminary antitumor activity of CA-170.

Up to now, the NCT00658892 is the only study that has been completed. This clinical trial explored the side effects and best dose of rHIgM12B in treating patients with stage IV melanoma. The PD-L2 cross-linking antibody rHIgM12B is a recombinant monoclonal IgM antibody M12 isolated from a Waldenstrom macroglobulinemia patient which binds and crosslinks to PD-L2 on dendritic cells and on antigen-presenting cells, resulting in an enhanced activation of dendritic cells and antigen-presenting activity and an increased production of immunomodulatory cytokines (especially IL-12). Results from all of these trials are eagerly awaited.

The immune checkpoint inhibitor toripalimab deserves separate mention; it is a recombinant, humanized PD-1 monoclonal antibody designed to be directed against the PD-1 receptor with the aim to prevent its interaction with PD-L1 and PD-L2, developed by Shanghai Junshi Bioscience Co. Ltd. in China for the treatment of various types of tumors. In December 2018, toripalimab received its first *conditional* approval in China for patients with unresectable or metastatic melanoma who progressed to standard systemic therapies. This approval was based on the encouraging activity of toripalimab observed in a single-arm, phase II trial conducted in this setting [98]. Treatment with toripalimab resulted in an objective response rate (ORR) of 17.3% ($n = 127$; full analysis set) with a disease control rate (complete response + partial response + stable disease) of 57.5%. With a median follow-up of 12.4 months (range 0.9-20.5 months), the median progression-free survival was 3.6 months, while the median duration of response and the median OS were not reached [99]. The subgroup analysis showed that ORR was 38.5% in patients with PD-L1$^+$ tumors ($\geq 1\%$ cut-off with SP142; $n = 26$) compared with 11.9% in those with a PD-L1$^-$ melanoma ($n = 84$). The PD-L1 status was unknown in 17 patients. Moreover, ORR was 32.4% in patients with a *BRAF* mutation ($n = 34$) and 9.3% in those with a *BRAF*-negative melanoma. The full approval of toripalimab in patients with advanced melanoma is conditioned by results of an ongoing, confirmatory, randomized phase III trial vs. dacarbazine (NCT03430297). Furthermore, toripalimab showed promising activity in several early studies on solid malignancies, such as nasopharyngeal carcinoma [100], urothelial cancers [101], esophageal squamous cell cancer [102], gastric adenocarcinoma [103], triple-negative breast cancer [104], soft tissue sarcoma [105], and nonfunctional neuroendocrine neoplasms [106], and it is currently under investigation in all of these settings. In particular, three phase III trials are being conducted to assess the efficacy of toripalimab in advanced nasopharyngeal carcinoma (NCT03581786), melanoma (NCT03430297), and advanced/metastatic esophageal squamous cell

Table 2
Clinical Trials of PD-L2–Based Therapies in Cancer

Reference	Drug(s)	Sponsor	Phase	Tumor Type	Main Objectives	Status
NCT03381768	100 μg PD-L2 peptide dissolved in DMSO and water mixed with 500 μl montanide. 100 μg PD-L1 peptide dissolved in DMSO and water mixed with 500 μl montanide.	Lars Møller Pedersen, Herlev Hospital (Denmark)	Phase I	Follicular lymphoma	Primary outcome: adverse events evaluated by CTCAE 4.03 (1-year follow-up) Secondary outcome: immune responses (1-year follow-up) T-cell cytokine release towards target antigens	Active, not recruiting
NCT03939234	PD-L1: 19 amino acid sequence from the PD-L1 protein; PD-L2: 21 amino acid sequence from the PD-L2 protein; the peptides are dissolved in DMSO and mixed with Montanide.	Lars Møller Pedersen, Herlev Hospital (Denmark)	Phase II	Chronic lymphocytic leukemia	Primary outcome: clinical response according to International Working Group on CLL (IW-CLL) (1-year follow-up) Secondary outcomes: immune response by ELISpot (1-year follow-up) T-cell responses measured by enzyme-linked immunospot assay (ELISpot) counting the number of spots with cytokine release. Adverse events evaluated by CTCAE 4.03 (1-year follow-up)	Recruiting
NCT02812875	CA-170: orally available, small molecule that directly targets the PD-L1/PD-L2, and V-domain Ig suppressor of T cell activation immune checkpoints	Curis, Inc. (USA)	Phase I	Advanced solid tumors Lymphomas	Primary outcomes: DLT in the first treatment cycle (24-month follow-up) MTD of CA-170 (24-month follow-up) RP2D of CA-170 (24-month follow-up) Secondary outcomes: PK profile of CA-170 maximum concentration (Cmax) PK profile of CA-170 AUC Preliminary antitumor activity of CA-170 based on RECIST and irRC for solid tumors or Cheson for lymphoma (36-month follow-up)	Active, not recruiting
NCT02528682	MiHA-loaded PD-L-silenced DC vaccination: a DC-based vaccine composed of PDL1/L2-silenced DCs and loaded with the recipient's MiHA, with potential use for GVT induction following allogeneic stem cell transplantation.	Radboud University (Holland)	Phase I Phase II	Hematological malignancies	Primary outcomes: Evaluation of toxicity Development of GVHD The generation and magnitude of an immunological response When after DC vaccination >1.0% of all $CD8^+$ lymphocytes at any time point are specific $CD8^+$ T cells to the used MiHA vaccine target, a complete response will be considered to be present. When the percentage is between 0.02% and 1% but has been doubled for 2 weeks, a partial immune response will be considered to be present. Secondary Outcomes: changes in chimerism When chimerism changes towards donor or disease load decreases according to objective standard clinical criteria after vaccination, this will be considered as a clinical response. Disappearance of residual disease In the case of presence of detectable residual or persistent disease before DC vaccination, clinical effects will be investigated by monitoring residual disease by quantitative real-time bcr-abl PCR (CML, Ph+ ALL), WT1-specific PCR (AML, MDS), M-protein (MM), immunophenotyping (CLL, AML, ALL, MDS) and radiological examination (NHL) after vaccination. When disease load decreases according to objective standard clinical criteria after vaccination, this will be considered as a clinical response.	Recruiting
NCT00658892	• B7-DC cross-linking antibody rHIgM12B7: A recombinant monoclonal IgM antibody M12 isolated from a Waldenstrom macroglobulinemia patient (rHIgM12) which binds and crosslinks the B7 co-stimulatory family member B7-DC (PD-L2) on DCs and APCs resulting in enhanced activation of DCs, antigen-presenting activity and increased production of immunomodulatory cytokines (especially interleukin 12);	Mayo Clinic (USA)	Phase I	Melanoma	Primary Outcome: evaluation of safety/toxicity Secondary outcomes: • Evaluation of immunological changes (Th1/Th2 balance, frequency of tumor-specific cytotoxic T lymphocytes, and plasma cytokine profiles) in treated patients. • Evaluation of treatment impact on tumor growth (objective response, time to progression).	Completed

ALL, acute lymphocytic leukemia; *AML*, acute myeloid leukemia; *APCs*, antigen-presenting cells; *AUC*, area under the curve; *CLL*, chronic lymphocytic leukemia; *DLT*, dose-limiting toxicity; *DMSO*, dimethyl sulfoxide; *GVHD*, graft-versus-host disease; *GVT*, graft-versus-tumor; *irRC*, immune-related response criteria; *MDS*, myelodysplastic syndrome; *MiHA*, minor histocompatibility antigens; *MTD*, maximum tolerated dose; *NHL*, non-Hodgkin lymphoma; *PK*, pharmacokinetic; *RP2D*, recommended phase 2 dose.

carcinoma (NCT03829969); seven phase II trials are further investigating the activity of toripalimab in bladder urothelial cancer (NCT03113266), non–small cell lung cancer (NCT03513666), mucosal melanoma (NCT03178123), melanoma (NCT03013101), biliary tract cancer (NCT03796429), *POLE* or *POLD-1* mutated and non–microsatellite unstable high (MSI-H) advanced solid tumors (NCT03810339), and

advanced small cell carcinoma of the esophagus (NCT03811379). Lastly, there are several ongoing phase I/II or phase I trials in gastric adenocarcinoma, esophageal squamous cell carcinoma, nasopharyngeal carcinoma or head and neck squamous cell carcinoma (NCT02915432), mucosal melanoma (NCT03086174), and advanced tumors (NCT02836834).

Conclusions

PD-L2 is an evolving and critical immune checkpoint molecule, which could play a crucial role in the regulation of antitumor immune responses even if the exact mechanisms underlying the immunological functions of this ligand have yet to be fully understood in all malignancies.

On this regard, the available scientific evidences about the prognostic value of PD-L2 expression by tumor, immune, and stromal cells among patients with solid tumors are increasing, although still controversial.

Some studies have reported that PD-L2 overexpression is a negative predictor for OS and progression-free/disease-free survival in patients with solid cancers, especially after surgery. Remarkably, it seems that PD-L2 positivity is associated with unfavorable prognosis in terms of OS for hepatocellular carcinoma and progression-free/disease-free survival for hepatocellular and renal cell carcinomas. On the contrary, other studies concluded that PD-L2 has a not significant or inconsistent prognostic value in several tumor histotypes and in some types of cancer (e.g., esophageal cancer) where its overexpression seems to be related to a better prognosis trend. These conflicting findings underline the possibility that PD-L2 has a distinctive role for immunosuppression in relation to the different malignancies. For this reason, there is an urgent need for further studies reporting the prognostic value of PD-L2 in specific cancer histotypes, with more homogeneous patient populations and with adequate antibodies (or other products) employed to reliably detect its expression.

In recent years, the pharmacological use of monoclonal antibodies against immune checkpoint molecules and in particular those targeting the PD-1 pathway has become a practice-changing therapy in cancer treatment. The activity and efficacy of anti–PD-1 and anti–PD-L1 drugs seem to be similar across the biggest trials performed in the main tumors, although in none of them has a direct comparison between anti–PD-1 and anti–PD-L1 been performed. However, since they block the PD-1/PD-L1 pathway only, it seems that anti–PD-L1 agents generate less adverse events, while preserving the activity of the PD-1/PD-L2 pathway, thus protecting healthy tissues from a hyperactivation of the immune response generated by these drugs.

On the basis of these successes, immunotherapies dedicated to the blockade of PD-L2 have also been developed with the aim to expand the antitumor therapeutic possibilities. Small molecules specifically targeting PD-L2, monoclonal antibodies blocking the PD-1/PD-L2 pathway, and novel PD-L2–based vaccines, alone or in combination with other immune checkpoint blockade agents, are at various stages of development. Results from these clinical trials are awaited to find out the therapeutic potential of PD-L2 blockade and to further investigate the different spectrum of adverse events.

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Authors' Contribution Statement

C. S. and M. A. equally contributed to the conception, design, data collection, and drafting of the work.

E. R., M. L., and K. W.-G. contributed to the data collection and critical revision of the article.

E. M. contributed to the data collection, critical revision, and drafting of the work.

All authors have approved the submitted version of the work.

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