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ABSTRACT: The ligation of programmed cell death-1 (PD-1) to its ligands PD-L1 and PD-L2 counteracts T-cell activation, which is critical in immune tolerance. The persistent high expression of PD-1 and PD-L1 are also observed on tumor-infiltrating lymphocytes and various tumor cells, maintaining the highly suppressive microenvironment in tumor sites and promoting tumor malignancies. The blockade of PD-1 axis with PD-L2 fusion protein or monoclonal antibodies against either PD-1 or PD-L1 has been clinically evaluated in various tumor types. This short review summarizes the progress of PD-1 axis blockade in clinical trials to evaluate its effectiveness in the antitumor immunotherapy.

KEYWORDS: PD-1, PD-L1, PD-L2, blockade, antitumor immunotherapy, clinical trials

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Programmed cell death-1 (PD-1) is encoded by *PDCD1* gene, which was first identified by exploring genes involved in stimulation-induced programmed cell death in murine T- and B-cell lines in 1992.¹ The human homolog *PDCD1* was isolated using murine PD-1 probe to screen a human T-cell cDNA library in 1994.² PD-1 possesses three domains: an N-terminal extracellular binding domain, a transmembrane domain, and a C-terminal cytoplasmic domain bearing an immunoreceptor tyrosine-based switch motif (ITSM) and an immunoreceptor tyrosine-based inhibitory motif (ITIM).³

PD-1 can bind two ligands, PD-L1 (B7-H1)^{4,5} and PD-L2 (B7-DC),^{6,7} to attenuate phosphorylation signaling and to further suppress immune cell activation.⁸ The expression of PD-L1 and PD-L2 are unregulated upon stimulation. PD-L1 is broadly expressed on both hematopoietic and non-hematopoietic cells. In contrast, PD-L2 expression is restricted to antigen-presenting cells and T_H2 cells.^{8,9} Although PD-L1 is the dominant ligand for PD-1, PD-L2 can compete with PD-L1 as the affinity of PD-L2 to PD-1 is two- to six-fold higher than that of PD-L1.¹⁰ In addition to PD-1, PD-L1 and PD-L2 serve as binding partners for B7-1¹¹ and repulsive guidance molecule B,¹² respectively, indicating the complexity associated with the regulation of costimulatory signals for T-cells.

Despite the name of “programmed cell death-1,” the main function of PD-1 is not involved in the cell death,

instead, to counteract T- and B-cell activation at different levels. First, PD-1 can directly affect immunological synapse (IS) formation of T-cells, which is the very early event in T-cell activation.^{13,14} Second, the ligation of PD-1 and PD-L1/2 attenuates TCR and essential costimulatory signaling in activated T-cells. The cytoplasmic tail of PD-1 will be phosphorylated. Subsequently, the phosphatase SHP-2 can be recruited to the phosphorylated ITSM, dephosphorylating CD3zeta, ZAP-70, PI3K, and PKC θ that are essential for T-cell activation.^{15,16} Although ITIM domain of PD-1 is shared by most of the inhibitory receptors, the exact contribution of ITIM to PD-1-mediated immune suppression is still not clear. Third, in addition to PD-1, PD-L1 could dampen T-cell activation by interacting with B7.1, blocking CD28-B7.1 costimulatory signaling.¹¹ Fourth, PD-1 is highly expressed on regulatory T-cells, a cell subset essential in immune suppression. In the presence of TCR stimuli and transforming growth factor beta (TGF- β), PD-1 ligation induces the conversion of naïve T-cells into functional induced regulatory T-cells.¹⁷ Therefore, PD-1 axis functions as an immune checkpoint, playing an important role in the immune tolerance and suppression.

The microenvironment in tumors are highly immune suppressive. It is evident that the PD-1/PD-L1 pathway contributes to immune suppression.¹⁸ It has been noted that



PD-1 expression is highly upregulated on tumor-infiltrating lymphocytes (TILs) in breast cancer, prostate cancer, ovarian cancer, melanoma, non-small cell lung cancer (NSCLC), and hepatocellular carcinoma (HCC). The upregulation of PD-1 expression on TILs has been functionally evaluated. Compared to PD-1 negative TILs, PD-1 positive TILs possess an exhausted phenotype, illustrated by blunt TCR signaling, defective calcium flux, and reduced cytokine (IL-2 and INF- γ) production.^{19–26} It is evidenced that a higher expression level of SHP-2 could be found in PD-1⁺ TILs, resulting in the impaired T-cell activation and Tc1/Th1 skewing through PD-1/SHP-2/STAT-1/T-bet signaling axis.²⁰ More importantly, growing evidences confirm that PD-1 expression on TILs correlates positively with tumor grade, size, lymph node status, and metastasis in breast cancer and melanoma, signifying the role of PD-1 in tumor malignancies.^{19,23} A large proportion of TILs express high levels of PD-1. Correspondingly, PD-L1 is highly expressed on many tumor cells. As mentioned above, PD-1 ligation may induce de novo Treg cell formation in the presence of TGF- β . All these factors contribute to the highly immune-suppressive tumor microenvironment, resulting in an exhausted phenotype of lymphocytes in the tumor sites. Similar to PD-1, the ligands PD-L1 and PD-L2 are also clinically relevant to tumor prognosis, recurrence, and patient survival in pancreatic cancer, breast cancer, ovarian, HCC, NSCLC, and melanoma.^{27–33}

Considering the importance and relevance of PD-1 and PD-L1/2 in tumor malignancies and patient survival, it has been hypothesized that PD-1 or PD-L1/2 blockade may provide a promising immunotherapy for patients with cancer. This is supported by the positive preclinical data. PD-1 knockout leads to delayed onset and organ-specific autoimmunity in

mice from different genetic backgrounds, providing strong evidence for the negative regulation of immune responses by PD-1.^{34,35} Likewise, the deficiency of PD-L1 results in an autoimmune phenotype.³⁶ In the cancer scenario, PD-1/PD-L1 deficiency or blockade augments effector T-cell function and accumulation at tumor sites.^{37,38} The promising pre-clinical data have paved the way for the application of PD-1/PD-L1 blockade in clinical trials.

The principle of PD-1/PD-L1 blockade in clinical trials is developing humanized antibodies or human IgG to bind either PD-1 or PD-L1, thereby blocking the ligation of PD-1 and PD-L1 and the downstream inhibitory signaling events. So far, several anti-PD-1 or PD-L1 monoclonal antibodies have been developed by various pharmaceutical companies.³⁹ Some of the blockade agents, which are summarized in Table 1, have been in different stages of clinical trials against various types of tumor. The clinical outcomes differ between antibodies, which could be because of the discrepancies in antibody sources, antibody isotypes (IgG, IgG1, or IgG4), and antibody affinities. CT-011 (pidilizumab), a humanized IgG1, is the first biological inhibitor of PD-1 in clinical trials.⁴⁰ The objective response rates (ORR) of CT-011 to follicular lymphoma and advanced melanoma are 66% and 5.9%, respectively, indicating that different mechanisms might be involved in hematologic malignancies and solid tumors. The treatment-associated severe adverse event (SAE) in melanoma is 4%, showing a good tolerance.^{41,42} MK-3475 (pembrolizumab), a humanized IgG4, is the first FDA approved anti-PD-1 mAb to treat metastatic melanoma.⁴³ The ORR of MK-3475 to advanced melanoma and NSCLC are 38% and 21%, respectively. The drug-related SAEs are acceptable (13% in melanoma).^{43,44} BMS-936558

Table 1. Summary of current PD-1 and PD-L1 blockade agents in clinical trials.

TARGET	BLOCKADE AGENT	MOLECULAR PROPERTY	PHASE	EVALUATED CANCER	COMPANY	REF
PD-1	CT-011 (pidilizumab)	Humanized IgG1	II	Hematologic cancer, melanoma	CureTech	40–42
	MK-3475 (pembrolizumab)	Humanized IgG4	III	Advanced solid tumors, Melanoma, NSCLC	Merck & Co	43, 44
	BMS-936558 (nivolumab)	Fully human IgG4	III	Melanoma, RCC, NSCLC, HNSCC, Advanced solid tumors	Bristol-Myers Squibb	45, 46
	AMP-224	PD-L2 fusion protein	I	Advanced solid tumors	Amplimmune/ GlaxoSmithKline	47
PD-L1	BMS-936559	Fully human IgG4	I	Advanced solid tumors	Bristol-Myers Squibb	48
	MEDI4736	Humanized IgG	I/III	Advanced solid tumors NSCLC	MedImmune	49, 50
	MPDL3280A	Fully human IgG4	I/II	Melanoma, RCC, NSCLC, Bladder cancer, Advanced solid tumors	Roche	51, 52
	MSB0010718C	Fully human IgG4	I/II	Advanced solid tumors, Merkel cell carcinoma	Merck & Co	53

Abbreviations: NSCLC, non-small cell lung cancer; RCC, renal cell carcinoma; HNSCC, head and neck squamous-cell carcinoma.



(nivolumab) is a human IgG4 mAb against PD-1 developed by Bristol-Myers Squibb and currently in phase III clinical trials in various tumors. The use of nivolumab has achieved an improved outcome in metastatic melanoma and NSCLC. A study reported that compared to dacarbazine (a drug used in chemotherapy), the ORR could be increased from 13.9% to 40% in the nivolumab arm. Similarly, the overall survival (OS) was increased from 42.1% to 72.9%. Moreover, the drug-related SAEs were decreased in the nivolumab arm (11.7% vs 17.6%).⁴⁵ In NSCLC, the OS was significantly better in the nivolumab arm when compared to docetaxel (9.2 vs 6 months).⁴⁶ The efficacy of AMP-224, a PD-L2 IgG2a fusion protein, has also been evaluated in clinical trials. As opposed to the direct blocking PD-1/PD-L1 interaction, PD-L2 promotes the apoptosis of lymphocytes with high PD-1 expression.⁴⁷ To block PD-1/PD-L1 axis, an alternative approach is to block the predominant ligand, PD-L1. Blockade of PD-L1 may cause less side effects and toxicity as PD-L2 can serve as an alternate ligand for PD-1. However, it may also compromise the antitumor effects, as PD-L2 on tumor cells might be sufficient to induce and maintain the exhausted phenotype of PD-1⁺ TILs. So far, four different anti-PD-L1 mAbs (BMS-936559, MEDI4736, MPDL3280A, MSB0010718C) are in clinical trials with the rationale being the high PD-L1 expression on various tumor cells.^{48–53} The efficacy of BMS-936559 has been evaluated in melanoma, renal cell cancer, NSCLC, and ovarian cancer. The ORR and progression-free survival (PFS) are 6%–17% and 12%–41%, respectively. The SAEs are acceptable.⁴⁸ MEDI4736, a humanized IgG antibody, was tested in NSCLC patients, showing 38.5% ORR and very low SAEs.^{49,50} MPDL3280A, a fully human IgG4 antibody, showed 21% ORR and 44% PFS at 24 weeks in advanced solid tumors.^{51,52} MSB0010718C, a fully human IgG4 antibody developed by Merck & Co., is recruiting participants with Merkel cell carcinoma or advanced solid tumors.⁵³

It has to be noted that PD-1 and PD-L1 blockade are effective only in patients with high PD-1 or PD-L1 expression. Topalian and colleagues conducted a phase I clinical trial to evaluate the efficacy of BMS-936558 (nivolumab) in tumor malignancies. Nine out of the 25 patients with PD-L1⁺ tumors exhibited a strong response to BMS-936558.⁵⁴ In contrast, no response to PD-1 blockade was observed in the 17 patients with PD-L1⁻ tumors.⁵³ This observation suggests that PD-L1 expression on tumor cells can be used as a potential biomarker to further stratify patients with cancer and to predict the efficacy of immunotherapy using PD-1 axis blockade. Further efforts are required to standardize the measurement of PD-L1 on tumor cells, which is critical for the prediction and evaluation of treatment efficacy.

To break the highly immune-suppressive microenvironment in tumors and potentiate antitumor immune responses mediated by T-cells, the concurrent therapy combining PD-1/PD-L1 blockade with antiangiogenic therapy, radiation

therapy, chemotherapy, or chimeric antigen receptor T-cell therapy or other immune checkpoint inhibitors may enhance the immune priming, effector/memory phase, and improve the clinical outcome. CTLA-4, similar to PD-1, is another important immune checkpoint.⁵⁵ Ipilimumab, a blocking antibody for CTLA-4, is currently in phase II/III clinical trials.⁵⁶ A recent study reported that the efficacy of the concurrent therapy using ipilimumab and nivolumab is significantly greater than ipilimumab alone in patients with melanoma.⁵⁷ However, we do not know if this concurrent therapy is superior to nivolumab alone yet. We also do not know if PD-L1 blockade is superior to PD-1 blockade in practice. Future efforts should be made to compare the efficacies of different regimens (concurrent therapy vs PD-1 axis blockade alone; PD-1 blockade vs PD-L1 blockade) to advance the improvement of treatment using PD-1 axis blockade.

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Wrote the first draft of the manuscript: PZ. Contributed to the writing of the manuscript: ZZ. Agreed with manuscript results and conclusions: PZ and ZZ. Jointly developed the structure and arguments for the paper: PZ and ZZ. Made critical revisions and approved the final version: PZ and ZZ. All the authors reviewed and approved the final manuscript.

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