Immune checkpoint inhibitors of PD-L1 as cancer therapeutics

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Keywords: PD-1, PD-L1, immune checkpoint inhibitors, clinical trials, adverse events, monoclonal antibody.

Abstract: Early preclinical studies suggested that PD-1 and PD-L1 inhibition may be utilized as a cancer immunotherapy Antibodies that inhibit programmed cell death 1 receptor (PD-1) and programmed cell death receptor ligand 1 (PD-L1) for a subpopulation of cancer patients have piqued researchers' attention since the discovery of immune checkpoint proteins. Early-phase trials in advanced solid tumors using various humanized monoclonal IgG4 antibodies targeting PD-1 and PD-L1 opened the path for the development of the first PD-1 inhibitors, nivolumab and pembrolizumab, which were authorized by the FDA in 2014. With treatment indications spanning a wide variety of malignancies, the number of FDA-approved medications in this class is rapidly increasing. The focus of this review will be on the mechanism of action of PD-1/PD-L1 inhibitors, as well as clinical trials of currently approved PD-1/PD-L1 targeted drugs and the occurrence of related adverse reactions, allowing clinicians to pay closer attention to these side effects and better formulate intervention and resolution strategies.

1. Introduction

Cancer treatment has been a hard topic in the clinical field because it involves treating the malignant tumor with the high incidence and mortality in the world. In the past few decades, the only treatment options available for advanced cancer patients have been targeted therapy or chemotherapy, but these therapies are inevitably tolerated by tumors.

As a key node in the process of tumorigenesis and development, immune checkpoints such as PD-1/PD-L1, CTLA-4, TIM-3, LAG-3 and others provide us with a new therapeutic target [1]. One of the checkpoints that controls the immune response is PD1. When PD1 binds to its ligands PDL1 and PDL2, negative signals are transmitted to T-cells. The expression of PD1 is a major factor in the exhausted effector T-cell phenotype. Tumor cells can escape anti-tumor immunity by expressing PD1 on effector T-cells and PDL1 on neoplastic cells. Blocking PD1 is a significant cancer immunotherapeutic approach. Therefore, the discovery of PD-1 immune checkpoints that mediate the immune escape of tumor cells have been promoting a series of immune checkpoint inhibitors to be used in cancer treatment and achieved great results [2]. T-cell function is regulated by the T-cell PD-1 receptor and the PD-L1 displayed by targeted cells via the immune system checkpoint PD-1/PD-L1 [3] CD28 signaling is largely inactivated by interactions between PD-1 and PD-L1, which suppresses T cell activation [4]. In order to generate an immune response against cancer cells, several licensed immunotherapies block PD-1/PD-L1 interactions [3]. This paper is focusing on the mechanisms of PD1/PD-L1 pathway and the clinical treatments using the immune checkpoint inhibitors.

2. Overview of PD-1/PD-L1 pathway

The interaction between PD-1 and PD-L1 is crucial for maintaining a balance between peripheral tolerance and autoimmunity, but it also compromises viral and tumor immunity, increasing persistent infection and tumor development [5].

2.1 The discovery of PDL1 and its mechanism

Lieping Chen's group, then at Mayo Clinic, identified PD-L1, or B7-H1, as a molecule with homology to B7-1 and B7-2 (Dong H). They reported that B7-H1 co stimulates T cells via a receptor different from CD28, CTLA4, or ICOS and delivers an activation signal to T cells, which leads to IL-10 production. Gordon Freeman at Dana–Farber Cancer Institute later named it PD-L as a ligand for PD-1 [6].

PD-L1 is a 33-kDa type 1 transmembrane glycoprotein with 290 amino acids with Ig- and IgC domains in its extracellular region that belongs to the B7 class [7]. Macrophages, certain activated T cells and B cells, DCs, and some epithelial cells all express PD-L1, especially under inflammatory circumstances [8].

In the presence of a tumor or in the tumor microenvironment on non-transformed cells, PD-L1 is overexpressed, promoting immune evasion and tumor development by inhibiting T-cell response [9]. PD-L1 produced on tumor cells interacts with PD-1 receptors on activated T cells, causing the cytotoxic T cells to be inhibited [7]. Interactions between the extracellular domains of PD-L1 and PD-1 can cause PD-1 to alter conformation, allowing Src family kinases to phosphorylate ITIM and ITSM [10]. These phosphorylated tyrosine motifs then bind the protein tyrosine phosphatases SHP-2 and SHP-1 to reduce T cell activation signals. In the tumor microenvironment, these deactivated T lymphocytes stay blocked [7]. Thus, tumor cells produce PD-L1 as an "adaptive immune strategy" to escape anti-tumor responses.

In addition to interacting with PD-1, PD-L1 can also interact with CD80, which may give inhibitory signals to activated T cells [11]. T cell proliferation, survival, cytokine generation, and other effector activities are all inhibited when PD-1 is engaged by PD-L1 [12].

2.2 Mechanism of PD1/PD-L1 Immune Checkpoint Inhibitors

In normal conditions, the immune system passes through a variety of steps which leads to immune response to cancer cell death. Tumor cells first produce modified antigens, which are absorbed by dendritic cells. The dendritic cells then proliferate with the tumor antigen and stimulate the activation of cytotoxic T cells, so t The activated T cells move to the tumor. The cancer cells can get identified by and connected with the activated T cells. Finally, the connected effector T cells release cytotoxins that trigger apoptosis to their target cancer cells [12].

The PD-1 and PD-L1 antibody inhibitors have been designed to block either the PD-1 or the PD-L1 side and turn on T-cell mediated immunity. PD-1 is majorly expressed on the T cells of the immune system, whereas PD-L1 is on the cancer cells and antigen-presenting cells (APC). The PD-1 and PD-L1 are receptor-ligand systems that are attached to each other in the tumor microenvironment, resulting in blockade of anti-tumor immune responses. Therefore, the inhibitors that block the interaction of PD-1 and PD-L1 will cause resurrection of T-cell mediated anti-tumor immune effect. Therefore, anti-PD1 pathway can thereby prevent autoimmunity of the host [13].

3. Effects of PD-1/L1 Inhibition on T cell Receptor (TCR) Signaling

3.1 Inhibition of PI3K/AKT signaling pathway

The phosphoinositide 3kinase/Akt (PI3K/AKT) pathway is one of the primary pathways of TCR signaling that is impacted by PD-1.

The PI3K/Akt pathway controls cell proliferation, cell cycle regulation, apoptosis, and other tumor-developed processes [14]. The regulatory mechanisms and biological activities of the PI3K/AKT/mTOR signaling pathway are important causes of a few human disorders, including ischemic brain injury, neurodegenerative diseases, and malignancies [15].

One of the ways of triggering cancer is by loss of PTEN. In PI3K/Akt pathway, PTEN is the lipid phosphatase tumor suppressor that inhibits PI3K signaling. When the negative regulator PTEN is down regulated, it is linked to increased PI3K-AKT pathway activation which triggers a variety of tumor types. For example, loss of PTEN occurs in up to 30% of melanomas [16].

PD-1 can target and block CK2-mediated PTEN phosphorylation and increase its degradation, suppressing PI3K/Akt signaling [17]. In gastrointestinal stromal tumors (GIST), Zhao discovered that inhibiting PD-1/PD-L1 reduces CD8+ T cell death by weakening the PI3K/AKT/mTOR pathway. Zhao discovered that CD8+ T cells rescued by the PD-1/PD-L1 blockade exhibited higher PI3K/Akt/mTOR levels than CD8+ T cells not treated with the PD-1/PD-L1 blockade using western blotting. PD-L1 knockdown in GIST cells has also been shown to reduce the expression of PI3K, p-AKT, and p-PI3K [18]. Therefore, in T cells, PD-1's inhibitory impact on the PI3K/Akt signaling pathway becomes an essential target to suppress cancer cells, promoting anti-apoptosis, cell proliferation, and metastatic downstream targets.

3.2 Suppression of the Ras/MEK/ERK Pathway

The Ras/MEK/ERK pathway is another signaling pathway regulated by PD-1 [19]. It is commonly activated in human cancers, in most cases due to abnormal upstream signaling initiated by amplification or activating mutations in receptor tyrosine kinases, the RAS GTPase, or BRAF [20].

This pathway begins with the PLC1 activity, which hydrolyzes PI2P into two DAG and IP3 molecules. Calcium and diacylglycerol activate RasGRP1 downstream of PLC-1 [21]. RasGRP1 activation is required for Ras and downstream MEK/ERK MAP kinase activation [21]. Thus, PD-1 inhibits the MEK/ERK pathway via inhibiting PLC-1 and Ras activity [17].

MEK-ERK signaling can regulate PD-L1 gene expression via interacting with inflammatory signaling. Chemical or genetic suppression of MEK reduces IFN-g-induced STAT1 phosphorylation and PD-L1 transcription in multiple myeloma cells. However, stimulation of MEK-ERK signaling promotes PD-L1 expression, which may be reversed by MEK inhibition [22].

JUN cooperates with STAT3 in the transcriptional control of PD-L1 expression and is a major target of MAPK signaling. In melanoma cells and many NSCLC cells, it has been hypothesized that suppressing MEK leads to reduced PD-L1 expression via inactivation of JUN and STAT3 [23]. MEK inhibition can also diminish the elevated PD-L1 expression found in multiple myeloma, bladder cancer, lymphoma, and dendritic cells when TLR ligands are stimulated [22].

3.3 Inhibition of JAK-STAT signaling pathway

Last but not least, The JAK-STAT pathway is a signaling pathway regulated by PD-1. It is discovered that NK cells increase the PD-L1 expression on the surface of the tumor cells by secretion of the IFN-γ through the JAK1, JAK2, and STAT1 pathways [24]. Studies conducted on melanoma cells indicated that IFN-γ secreted by T cells via JAK1/JAK2-STAT1/STAT2/STAT3-IRF1 pathway could regulate the expression of the PD-L1 gene since it in turn induces PD-L1 expression on the target cell surface including tumor cells [25].

The JAK/STAT pathway may be useful in cancer treatment since it can promote PD-L1 expression. AG490, a JAK2 inhibitor, reduced PD-L1 expression at both the mRNA and protein levels, according to Toshifumi et al [26]. These findings demonstrated that the JAK/STAT pathway controls PD-L1 expression. Furthermore, fibroblast growth factor receptor (FGFR)2 signaling efficiently stimulated the JAK/STAT3 signaling pathway in vitro, resulting in enhanced PD-L1 expression. Overexpression of FGFR2 boosted PD-L1 expression and increased tumor growth in colorectal cancer xenograft models. JAK inhibitors may be used to prevent PD-L1 expression by inhibiting the JAK/STAT3 pathway [27].

4. New development in clinical applications of PD-1 and PD-L1 antibodies

The US Food and Drug Administration (FDA) has approved six immune checkpoint inhibitors for the PD-1/PD-L1 pathway since May 2006, with three for PD-1 (pembrolizumab, nivolumab, and cemiplimab) and three for PD-L1 (atezolizumab, avelumab and durvalumab) [28].

4.1 Pembrolizumab

4.1.1 General mechanism

Pembrolizumab is a humanized monoclonal antibody that binds selectively to the PD-1 molecules on the surface of T cells. When pembrolizumab precisely binds to PD-1, steric hindrance effects can prevent PD-1 and its ligands, PD-L1 or PD-L2, from combining to restore the normal anti-tumor immune response inhibited by the PD-1 pathway [29].

4.1.2 Applied diseases

On September 4, 2014, pembrolizumab became the first PD-1 inhibitor to receive approval for patients with advanced or unresectable melanoma based on the findings from the KEYNOTE-001 study [30].

In a study that compares the efficacy of chemotherapy and the efficacy of pembrolizumab, 305 patients with previously untreated advanced NSCLC with PD-L1 expression on at least 50% of tumor cells and no sensitizing mutation of the epidermal growth factor receptor (EGFR) gene received either pembrolizumab (at a fixed dose of 200 mg every 3 weeks) or chemotherapy. The primary end point, progression-free survival, was assessed by means of blinded, independent, central radiologic review [31].

In Figure 1, it was found that the response rate was higher in the pembrolizumab group than in the chemotherapy group (44.8% vs. 27.8%), the median duration of response was longer (not reached [range, 1.9+ to 14.5+ months] vs. 6.3 months [range, 2.1+ to 12.6+]), and treatment-related adverse events of any grade were less frequent (occurring in 73.4% vs. 90.0% of patients), as were grade 3, 4, or 5 treatment-related adverse events (26.6% vs. 53.3%).

Therefore, pembrolizumab was associated with significantly longer progression-free and overall survival and with fewer adverse events than was platinum-based chemotherapy.

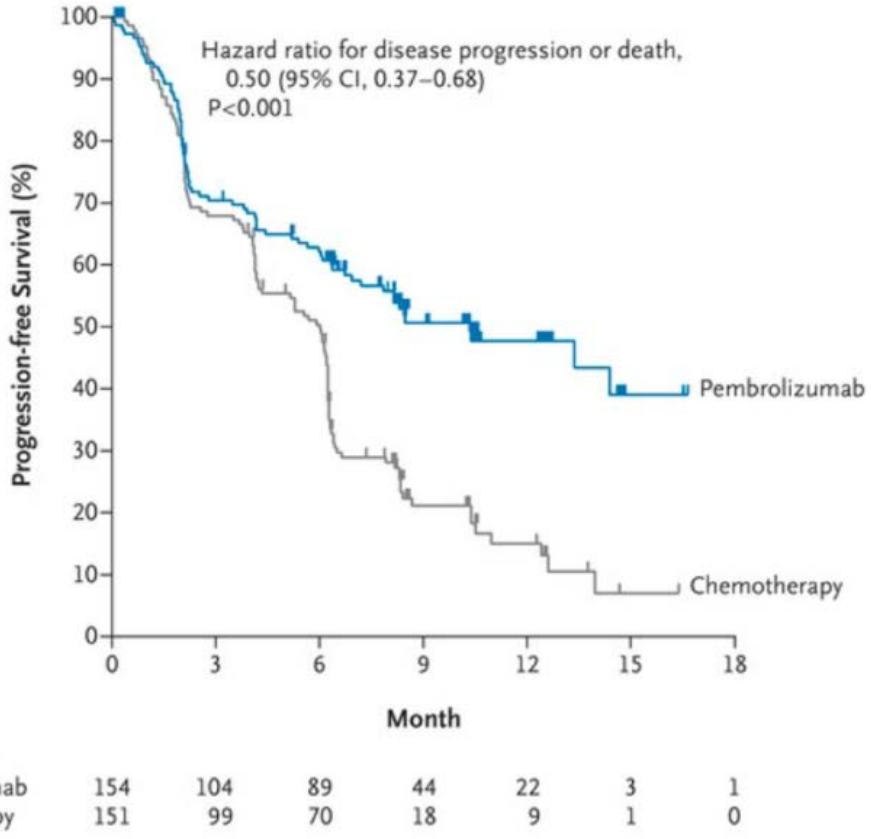

Figure 1. Progression-free survival in selected melanoma patients [31]

4.1.3 Adverse events

The most common adverse reactions (reported in at least 10% of pembrolizumab-treated patients) were diarrhea, pruritus, nausea, arthralgia, hypothyroidism, cough, rash, asthenia, influenza-like illness, weight loss, and hyperthyroidism.

Nosaki and colleagues discovered that older individuals receiving Pembrolizumab had fewer treatment-related adverse events (TRAEs) than those receiving chemotherapy (68.5 percent vs. 94.3 percent), 24.2 percent vs. 61.0 percent grade 3 AEs, and 16.1 percent vs. 26.7 percent severe TRAEs (Table 1). Immune-related adverse events (iAEs) and infusion reactions occurred in around 25% of all patients treated with Pembrolizumab, independent of age. Patients over 75 years old who received Pembrolizumab had a greater proportion of grade 3 TRAEs (24.2 percent vs. 16.9%), whereas grade 3 or higher iAEs were only marginally higher (9.4% vs. 7.1%) in the older patient group [32].

Table 1. Overall survival of Pembrolizumab versus Chemotherapy in NSCLC patients [32]

Reference	Study name	Path	Line	Treatment	≥65 years N (%)	Overall survival			All Grade 3–4 TRAE: ICI *vs.* Chemo
						Overall HR (95% CI)	HR (95% CI) for younger	HR (95% CI) for older	
Nosaki [2019	KEYNOTE -010, -024, -42	NSCLC	>1L or 1L[b]	Pembrolizumab *vs.* Chemo[c]	264		<75 yrs: 0.76 (0.69–0.84) (mOS 14.6 *vs.* 11.1 mo)	≥75 yrs: 0.76 (0.56–1.02) (mOS 15.7 *vs.* 11.7 mo)	<75 yrs 16.9 *vs.* 39.1 ≥75 yrs 24.2 *vs.* 61% (Grade 3–5)

4.2 Nivolumab

4.2.1 Mechanism

Nivolumab is an IgG4 fully human antibody targeting PD1 that binds to PD-1 and blocks PD-1/PD-L1 signaling. By delivering tumor antigens and B7 molecules, antigen-presenting cells stimulate and activate T lymphocytes. When T cells recognize malignancies, IFN-γ is produced, and PD-1 ligands are upregulated on tumors and antigen-presenting cells. T-cell activation is inhibited, and antitumor immune responses are dampened when PD-1 is engaged on T cells with PD-1 ligands. T-cell suppression is prevented, and antitumor immune responses are reactivated when PD-1 is blocked by nivolumab. As a result, it interferes with the negative signal regulating the activation and proliferation of T cells and releasing the immune response inhibition mediated by PD-1 pathway, including anti-tumor immune response [33]. Nivolumab is approved to treat melanoma,155 Hodgkin's lymphoma, 156 and non-small-cell lung cancer, among other malignancies (NSCLC) [34].

4.2.2 Melanoma

Immune checkpoint blockade was developed as a new avenue of immunotherapy with the approval of ipilimumab in 2011 for the treatment of metastatic or unresectable melanoma. Immune checkpoint blocking works by reducing inhibitory signals and re-establishing the patient's natural tumor-specific T-cell-mediated immune responses.

Nivolumab has been authorized in Japan and the United States for the treatment of advanced melanoma patients. Patients with advanced non-small-cell lung cancer, melanoma, and renal cell carcinoma had overall objective response rates of 17, 32, and 29 percent, respectively, in a Phase I study that included many severely pretreated patients. Non-small-cell lung cancer, melanoma, and renal cell carcinoma had 1-/2-year overall survival rates of 42 percent/24 percent, 63 percent/48 percent, and 70 percent/50 percent, respectively. In a Phase III study, nivolumab dramatically outperformed dacarbazine in previously untreated patients with metastatic melanoma.

4.3 Atezolizumab

4.3.1 Mechanism

Atezolizumab prevents PD-L1 from interacting with CD80 receptors and programmed cell death protein 1 (PD-1) (B7-1Rs) [35]. Certain cancers have high levels of PD-L1, which is considered to contribute to a reduction in the activation of immune cells (particularly cytotoxic T-cells) that would otherwise identify and kill the malignancy [35]. Atezolizumab inhibits PD-L1 to reverse this inhibitory effect, resulting in an anti-tumor response.

4.3.2 Clinical trial

Atezolizumab was also FDA approved in October 2016 for metastatic NSCLC whose disease progressed during or following platinum-containing chemotherapy. The randomized phase II study (POPLAR) observed that atezolizumab met its primary endpoint and showed a statistically significant survival benefit compared to docetaxel ($HR = 0.54$; $p = 0.014$) in people with recurrent NSCLC whose tumors expressed medium and high levels of PD-L1 expression (Fehrenbacher L, et al). Likewise, a phase II study (BIRCH) met its primary endpoint and showed that atezolizumab shrank tumors with ORR of up to 22% in patients with previously untreated and patients whose disease had progressed on prior one or more chemotherapy including platinum-containing chemotherapy [36].

Compared to the traditional treatment docetaxel, it showed a statistically significant OS advantage with atezolizumab compared to docetaxel in intention-to-treat (ITT) and PD-L1 expression population (IHC 1/2/3) with median OS of 13.8 months vs 9.6 months and 15.7 months vs 10.3 months, respectively.

4.3.3 Adverse events

Two hundred and seventy-seven patients were enrolled and received intravenous atezolizumab every 3 weeks (q3w) at doses of 10, 15, or 20 mg/kg of body weight until disease progression (PD) or unacceptable toxicity. Overall, atezolizumab was well tolerated up to the maximum administered dose of 20 mg/kg [29]. The most common adverse effects (AEs) were fatigue, decreased appetite, nausea, pyrexia, diarrhea, rash, pruritus, arthralgia, and headache [37].

5. Conclusion

In recent years, cancer immunotherapy has had a lot of success. Immune checkpoint inhibitors have gotten a lot of attention in the immunotherapy world, and the FDA has authorized them for cancer treatment. Immune checkpoint drugs that target the protein programmed cell death-1 (PD-1) have showed promise in the treatment of a number of solid and hematologic malignancies. Despite the favorable results, only a tiny number of patients respond well, and there is currently no single curative therapy method. To increase therapeutic efficacy, researchers are combining anti-PD-1/PD-L1 medicines with other inhibitors, including as vaccines, mitogen-activated protein kinase (MEK) inhibitors, and cytotoxic T lymphocyte-associated antigen-4 (CTLA-4) inhibitors.

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