Small Molecules as PD-1/PD-L1 Pathway Modulators for Cancer Immunotherapy

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Abstract: Blockade of PD-1/PD-L1 interactions using PD-1/PD-L1 pathway modulators has shown unprecedented clinical efficacy in various cancer models. Current PD-1/PD-L1 modulators approved by FDA are exclusively dominated by therapeutic antibodies. Nevertheless, therapeutic antibodies also exhibit several disadvantages such as low tumor penetration, difficulty in crossing physiological barriers, lacking oral bioavailability, high manufacturing costs, inaccessible to intracellular targets, immunogenicity, immune-related adverse events (irAEs). Modulation of PD-1/PD-L1 pathway using small molecules may be an alternative approach to mobilize immune system to fight against cancers. In this review, we focus on summarizing the recently disclosed chemical structures and preliminary structure-activity relationships (SARs) of small molecules as PD-1/PD-L1 modulators for cancer immunotherapy.

Keywords: Immune checkpoint modulators, sulfonamides, thiadiazoles, oxadiazoles, biphenyl derivatives, molecular docking.

1. INTRODUCTION

Mobilizing immune system by immune checkpoint modulators to fight against cancers has demonstrated unprecedented clinical efficacy in more than 20 cancer types [1-3]. This approach shows at least three unique advantages including antigenic specificity, immunologic memory, and tumor heterogeneity, which elicits durable responses and substantial clinical benefits [4]. Programmed cell death-1 (PD-1) and programmed cell death ligand-1 (PD-L1) represent the central pillar of immune checkpoints in cancer immunotherapy. Since the identifications of these two proteins in 1990s [5, 6], a new revolution is taking place with the theme of mobilizing the immune system to fight against cancers [7-9]. PD-1 is often expressed by activated immune cells such as T cells, B cells, natural killer cells (NKs), dendritic cells (DCs), and tumor-associated macrophages (TAMs) in tumor microenvironment, suggesting that expression of PD-1 is a common mechanism for the inhibition of immune responses in the innate and adaptive immune systems [10-12]. PD-L1, a ligand for PD-1, is usually expressed on the surface of activated immune cells, vascular endothelial cells, mesenchymal stem cells, various tumor cells as well as their exosomes [13-15]. Although PDL1 mRNA is widely expressed in various tissues, human normal tissues seldom express PD-L1 protein. This suggests that PDL1 mRNA is under rigorous posttranscriptional regulation [16]. Importantly, most human tumor tissues freshly isolated from patients express high levels of PD-L1 despite the majority of cultured tumor cell lines are PD-L1 negative [17]. This discrepancy was explained by the finding that cancer cells could up-regulate the expression of PD-L1 after encountering T cells in the actual tumor microenvironment (TME), mostly via interferon-γ (IFN-γ) [18, 19]. Recent studies have shown that PD-L1 also plays an inhibitory role during the naïve-to-effector CD8 T cell transition [20]. PD-1/PD-L1 interactions lead to negative regulation of T cell activation in TME and play central roles in tumor-induced immune escape (Fig. 1) [21, 22]. Furthermore, PD-1/PD-L1 interactions also lead to cancer cells escaping from immune surveillance in tumor microenvironment by inducing T cell apoptosis, T cell exhaustion, IL-10 induction, Treg-cell-mediated immune suppression, and restraining TAMs as well as NKs from attacking cancer cells [23-25].

Blockade of PD-1/PD-L1 interactions using PD-1 or PD-L1 antibodies has shown durable clinical benefits and long-term remissions where patients exhibit no clinical cancer signs for many years after treatment [26-28]. Recent results of nivolumab in previously treated advanced non-small-cell lung cancer showed that 5-year overall survival (OS) rate was 16% for all treated patients. In patients who had ≥50% PD-L1 expression, the 5-year OS rate was up to 43% [27]. Six therapeutic antibodies have received the Food and Drug Administration (FDA) approvals, and over 15 antibodies are in clinical development for treatments of patients with various cancers (Table 1). However, the durable clinical benefits and long-term remissions by therapeutic antibodies have been limited to a small fraction of patients with certain cancer types [29-31]. Combinations of PD-1/PD-L1 modulators with kinases inhibitors, chemotherapeutics, and other immune checkpoint modulators may produce durable antitumor responses in patients who would not benefit from monom-immune checkpoint therapy [29, 32]. Other disadvantages such as low tissues penetration, difficulty in crossing physiological barriers (such as the blood-brain barrier), lacking oral bioavailability, immunogenicity, immune-related adverse events (irAEs), high manufacturing costs, inaccessible to intracellular targets, and sex-based disparities are quite evident [33-37]. Therefore, modulation of PD-1/PD-L1 pathways by small molecules may be an alternative approach to mobilize the immune system to fight against cancers [38].

2. SMALL MOLECULES AS PD-1 MODULATORS

Harvard scientists disclosed the earliest small molecules as PD-1 modulators (Fig. 2) in 2011 [39]. Because it is believed that the interaction of PD-1 with its ligands can inhibit IFN-γ secretion to a greater extent than T cell proliferation, IFN-γ secretion was chosen as the readout. Screening results showed that both Harvard-001 and Harvard-002 rescued PD-1 mediated inhibition of IFN-γ secretion, which indicated that these two compounds could bind to PD-1 and disrupt PD-1/PD-L2 interaction. Further structure-activity relationships (SARs) studies demonstrated that sulfamethizoles bearing phenyl (Harvard-003 and Harvard-004) showed higher IFN-γ fold...
Anticancer mechanism of PD-1/PD-L1 modulators based on T cell responses. T cells are activated under TCR-Ag-MHC and co-stimulatory signaling. However, the interactions of PD-1/PD-L1 deliver co-inhibitory signal to T cells, thus protect tumor cells from cytotoxic lysis. Blocking PD-1/PD-L1 interactions using antibodies or small molecules leads to immune normalization and eradication of tumor cells in TME.

Table 1. PD-1/PD-L1 pathway modulators approved or under clinical development.

<table>
<thead>
<tr>
<th>No.</th>
<th>Drug</th>
<th>Target</th>
<th>Class</th>
<th>$K_D$ (pM)$^a$</th>
<th>$IC_{50}$ (nM)$^b$</th>
<th>Indication$^c$</th>
<th>Stage$^d$</th>
<th>Investigator</th>
<th>NCT number</th>
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<tr>
<td>1</td>
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<td>29</td>
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<td>Melanoma, NSCLC</td>
<td>Approved</td>
<td>Merck Sharp &amp; Dohme</td>
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<tr>
<td>2</td>
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<td>IgG4κ</td>
<td>42</td>
<td>1</td>
<td>Melanoma, SNSCLC</td>
<td>Approved</td>
<td>Bristol-Myers Squibb</td>
<td>-</td>
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<tr>
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<td>IgG4</td>
<td>628</td>
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<td>Approved</td>
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<tr>
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<td>Melanoma</td>
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<td>-</td>
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<td>7</td>
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<td>-</td>
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<td>-</td>
<td>-</td>
<td>OC</td>
<td>Phase 3</td>
<td>Tesaro</td>
<td>NCT03602859</td>
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(Table 1) Contd....
change at 3200 nM compared to sulfamethizoles bearing pyridyl (Harvard-005). Although the activities of Harvard compounds are not good enough, these compounds might be utilized as important lead compounds in the discovery of novel PD pathway modulators.

Aurigene researchers disclosed a series of thiadiazole, oxadiazole and cyclic compounds as PD-1 modulators (Fig. 3) [40-44]. Splenocyte proliferation rescue assay was used to quantitatively measure their activities. Since PD-L1 could inhibit the proliferation of splenocytes stimulated with anti-CD3/CD28 via interacting with PD-1 expressed by splenocytes, compounds that could effectively disrupt the PD-1/PD-L1 interaction would rescue splenocyte proliferation. Percent rescue of splenocyte proliferation was positively correlated with the activity of the test compound. Screening results indicated that 1,3,4-thiadiazoles (Aurigene-001 and Aurigene-002, Fig. 3A) showed poor activities with only 48.4-60% rescue of
spleenocyte proliferation [40]. Replacement of sulfur with oxygen in 1,3,4-thiadiazoles results in the generation of 1,3,4-oxadiazoles (Aurigene-003 and Aurigene-004, Fig. 3B). However, these compounds still did not show acceptable activities with only 53-92% rescue of spleenocyte proliferation [40]. Further modifications of 1,3,4-oxadiazoles lead to the discovery of 1,2,4-oxadiazoles as effective PD-1 modulators (Fig. 3C). For example, Aurigene-005 and Aurigene-006 showed 97% and 119% rescue of spleenocyte proliferation, respectively [45, 46]. Furthermore, 3-substituted 1,2,4-oxadiazoles (Aurigene-005) were found to be dual inhibitors of PD-1 and V domain-containing Ig suppressor of T-cell activation (VISTA) pathways [45, 47]. Recently, CA-170, an Aurigene compound that targets to these two proteins and results in activation of T cell proliferation and cytokine production, has been evaluated in patients with advanced tumors and lymphomas (Phase I) [20]. Aurigene researchers also disclosed a series of cyclic compounds containing a 17- to 77-membered heterocyclic ring as PD-1 modulators [41]. The most effective compound, Aurigene-008, showed 91% rescue of spleenocyte proliferation (Fig. 3D).

Scientists from Augusta University (AU) randomly screened several compounds that modulated PD-1 signal transduction (Fig. 4) [48]. These compounds could bind to PD-1, inhibit or prevent ligands from binding to PD-1, suppress PD-1 inhibitory signal transduction, and thereby promote or induce a T cell activating signal. In addition, these compounds could be administered in conjunction or combination with, or as a component of, a vaccine composition, chemotherapeutic agents, potentiating agents, and tumour-infiltrating lymphocytes.

Researchers from Jilin University (JU) investigated a series of amino- and dimethylcarbamate-substituted resorcinol as PD-1 modulators (Fig. 5) [49]. PD-1 and PD-L1 were labeled by anti-Tag2-XL665/d2 and anti-Tag1-EuK respectively. When PD-1 and PD-L1 engaged with each other, they bring the labeling agents into close proximity and lead to the fluorescence intensities (AU) at the emission wavelengths of 665 and 620 nm. If a compound could actually disrupt the engagement of PD-1 with PD-L1, the ratio of AU between 665 and 620 nm will decrease. Screening results indicated that a V-type configuration with respect to resorcinol was important for the blockage of PD-1/PD-L1 interactions. Compounds JU-003 and JU-004 were proven to be the most effective compounds with inhibitory percent being 41.9-43% at 500 μM, which suggested that the introduction of hydrophobic groups into the amine moiety or electron-withdrawing groups into phenyl contributed to activity enhancement of PD-1 inhibitors.

3. SMALL MOLECULES AS PD-L1 MODULATORS

Bristol-Myers Squibb (BMS) chemists disclosed a series of biphenyl derivatives as PD-L1 modulators (Fig. 6A) in 2015 [50-53]. PD-1/PD-L1 homogenous time-resolved fluorescence (HTRF) binding assay was used to evaluate the ability of BMS compounds to bind to PD-L1. In brief, the extracellular domains of PD-1 and PD-L1 were expressed as fusion proteins with detection tags. The tag for PD-1 was Fc domain of Immunoglobulin (PD-1-Ig) and tag for PD-L1 was 6 histidine motif (PD-L1-His). PD-L1-His in assay buffer were pretreated with BMS compounds, followed by the addition of PD-1-Ig in assay buffer and further incubation. HTRF detection was achieved using europium cryptate-labeled anti-Ig and allophycocyanin (APC) labeled anti-His. The reaction mixture was allowed to equilibrate and signal was obtained using an EnVision fluorometer at 665/620 nm. Preliminary screening results demonstrated that BMS compounds generally had hydrophobic and hydrophilic moieties. Hydrophobic moiety was selected from biphenyl substituted with methyl, cyano, halo, halomethyl, dihalomethyl, and trihalomethyl. Hydrophilic moieties were mostly selected from amino acids and cyano containing heterocycles. Compared to BMS-001, BMS-002 was approximately 10-fold more potent in the inhibition of PD-1/PD-L1 interactions, suggesting that the introduction of amino acids should be at the para position. The subsequent activities of disclosed compounds demonstrated this result. Further optimizations of BMS compounds lead to the discovery of more potent PD-L1 modulators, BMS-009 and BMS-010 (Fig. 6B). Since PD-L1 is over-expressed by most cancer cells in TME, small molecules specifically targeting PD-L1 hold great promise as PD-1/PD-L1 pathway modulators for cancer immunotherapy. Scientists from Jagiellonian University in Poland revealed that BMS compounds directly bound to PD-L1, induced PD-L1 dimerization in solution, thus occluded the PD-1 interaction surface of PD-L1 and disrupted PD-1/PD-L1 or CD-80/PD-L1 interactions [54-57]. Our Lab performed the docking study of BMS-008 with PD-L1 dimer (Fig. 7). Results showed that the biphenyl moiety of BMS-008 was inserted in the hydrophobic pocket of PD-L1 dimer; the cyano pyridyl and 2-methylsiline moieties were anchored in the hydrophilic pockets; and seven hydrogen bonds were formed between BMS-008 and PD-L1.

Incyte chemists disclosed another series of biphenyl derivatives containing amides as PD-L1 modulators (Fig. 8) [58, 59]. The mechanism of Incyte compounds to block PD-1/PD-L1 interactions was similar to BMS compounds. Fragments including pyridin-2-one, pyridine, oxazole, thiazole and pyrazole as the linker of hydrophobic (biphenyl) and hydrophilic (ethanolamino) moieties were...
Fig. (4). Compounds disclosed by Augusta University scientists as PD-1 modulators via random screening.

Fig. (5). Examples of amino- and dimethylcarbamate-substituted resorcinol investigated by Jilin University researchers as PD-1 modulators. Inhibitory percents of PD-1/PD-L1 interaction were assessed at a compound concentration of 500 μM.
Fig. (6). Examples of biphenyl derivatives disclosed by BMS chemists as PD-L1 modulators.

Fig. (7). Docking study of BMS-008 with PD-L1 dimer in our Lab. A) The surface of PD-L1 dimer was shown in line manner; B) The surface of PD-L1 dimer was shown in lipophilic potential manner (green, hydrophilic; brown, hydrophobic; PDB code: 5j8o).
Fig. (8). Examples of biphenyl derivatives disclosed by Incyte chemists as PD-L1 modulators.

Further investigated in the blockade of PD-1/PD-L1 interactions. Screening results indicated that compounds containing these six fragments blocked PD-1/PD-L1 interactions in the nM range. Compared to Incyte-005 and Incyte-003, Incyte-006 was less active suggesting that the introduction of large or hydrophilic moieties such as N,N-dimethylamino group in the 3 positions of pyridine might decrease their activities. Incyte-008 was more active than Incyte-007 demonstrating that biphenyl was essential for the blockade of PD-1/PD-L1 interaction, as replacement of phenyl (Incyte-008) by cyclohexyl (Incyte-007) led to the significant increase of IC$_{50}$ value. Since BMS compounds (BMS-009 and BMS-010) could significantly block the PD-1/PD-L1 interaction, their analogs (In-
cyte-013 and Incyte-014) might be alternative lead compounds as PD-1/PD-L1 inhibitors for cancer immunotherapy.

Polaris scientists disclosed a series of symmetric biphenyl derivatives as PD-L1 modulators (Fig. 9) [60]. The ability of Polaris compounds to block PD-1/PD-L1 complex formation was assessed using biochemical interaction assay based on the ELISA platform. Briefly, PD-L1-Fc-biotin treated with Polaris compounds was added to PD-1-Fc coated plates (EIA/RIA high binding 96-well plates) and incubated for 2 hours. Unbound PD-L1-Fc-biotin was washed away with PBST. The bound PD-L1-Fc-biotin was detected with streptavidin-HRP and TMB substrate. Screening results indicated that the size of groups on biphenyl fragment seemed to be important for the inhibition of PD-1/PD-L1 interaction, as replacement of hydrogen (Polaris-001) by chlorine (Polaris-003) or methyl (Polaris-004) led to the enhancement of inhibitory activity. The piperidine ring in piperidine-2-carboxylic acid seemed to be inessential and allowed to be modified, as its replacement with pyrrolidine (Polaris-006) or disruption (Polaris-005) did not cause the significant decrease of inhibitory activity. In contrast, the carboxyl in piperidine-2-carboxylic acid was essential and un-modifiable, as its replacement with amide (Polaris-007) and methyl ester (Polaris-008) resulted in the significant decrease of inhibitory activity.

Fig. (9). Examples of symmetric biphenyl derivatives disclosed by Polaris scientists as PD-L1 modulators.

CONCLUSION

Blockade of PD-1/PD-L1 interaction using therapeutic antibodies has demonstrated impressive clinical efficacy in various cancer types. However, this impressive clinical efficacy has been limited to a small fraction of patients (10%) [27] with certain cancer types. Due to the long half-life and strong target occupancy, therapeutic antibodies have also shown immune-related adverse events (irAEs) in a fraction of patients. As a result, small molecules may be an alternative approach to mobilize immune system to fight against cancers. However, small molecules as immunomodulators are still in the stage of infancy. This may be explained by the fact that the interaction surfaces of human PD-1 or PD-L1 proteins are relatively flat, lacking deep pockets, making small molecules difficult to bind to them. Fortunately, chemists have found their way to discover effective small molecules as PD-1/PD-L1 or VISTA pathway modulators by designing new classes of protein-protein interaction modulators. In this review, we focus on addressing the recently disclosed chemical structures and structure-activity relationships (SAR) of small molecules as PD-1/PD-L1 modulators, which is urgently needed for the design of new generation of PD-1/PD-L1 modulators for cancer immunotherapy. Review results indicated that both symmetric and asymmetric biphenyl derivatives had
shown potent activities to disrupt PD-1/PD-L1 interactions in solution. However, whether these compounds work very well on cancer cell surface remain unknown. Although the IC\textsubscript{50} values of small molecules as PD-L1 modulators were fractionally disclosed in patents, their detailed SARs and comparison with therapeutic antibodies remained unknown. Additionally, their molecular weights are generally high, which may hinder their absorptions and distributions in vivo. In brief, small molecules have already shown feasibilities as PD-1/PD-L1 pathway modulators in cancer immunotherapy, but there is still a long way to go before as bedside pills.

CONSENT FOR PUBLICATION

Not applicable.

CONFLICT OF INTEREST

The authors declare no conflict of interest, financial or otherwise.

ACKNOWLEDGEMENTS

This work is supported by A Project of Shandong Province Higher Educational Science and Technology Program (J17KA101) and Shandong Province Higher Educational Reform Program (M2018X087), China.

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