Crystal clear: visualizing the intervention mechanism of the PD-1/PD-L1 interaction by two cancer therapeutic monoclonal antibodies

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ABSTRACT

Antibody-based PD-1/PD-L1 blockade therapies have taken center stage in immunotherapies for cancer, with multiple clinical successes. PD-1 signaling plays pivotal roles in tumor-driven T-cell dysfunction. In contrast to prior approaches to generate or boost tumor-specific T-cell responses, antibody-based PD-1/PD-L1 blockade targets tumor-induced T-cell defects and restores pre-existing T-cell function to modulate antitumor immunity. In this review, the fundamental knowledge on the expression regulations and inhibitory functions of PD-1 and the present understanding of antibody-based PD-1/ PD-L1 blockade therapies are briefly summarized. We then focus on the recent breakthrough work concerning the structural basis of the PD-1/PD-Ls interaction and how therapeutic antibodies, pembrolizumab targeting PD-1 and avelumab targeting PD-L1, compete with the binding of PD-1/PD-L1 to interrupt the PD-1/PD-L1 interaction. We believe that this structural information will benefit the design and improvement of therapeutic antibodies targeting PD-1 signaling.

KEYWORDS PD-1/PD-L1 interaction, checkpoint blockade, molecular basis, therapeutic antibody

INTRODUCTION

The host immune system is critical for defending against microbial pathogens and “non-self” malignant cells to maintain health. T-cell immune responses play pivotal roles in adoptive immune responses by directly killing target cells or indirect modulation via cytokines (Palucka and Coussens, 2016). Naïve T-cell activation involves both T-cell receptor (TCR)/peptide major histocompatibility complex (pMHC) interactions and co-stimulatory ligand-receptor interactions, the two-signal model proposed by Lafferty and Cunningham (Bretscher and Cohn, 1970; Lafferty and Cunningham, 1975; Cunningham and Lafferty, 1977; Gao and Jakobsen, 2000; Gao et al., 2002). Additionally, activated T cells also require...
co-stimulatory and co-inhibitory molecules to modulate TCR-mediated T-cell responses and self tolerance (Gao and Jakobsen, 2000; Gao et al., 2002). The most important co-stimulatory and co-inhibitory molecules involve B7-CD28 superfamily- and TNF-TNF receptor superfamily-related ligands and receptors. Programmed cell death 1 (PD-1) is a member of the CD28 superfamily and was first discovered as a gene upregulated in a T cell hybridoma undergoing cell death (Ishida et al., 1992). The negative regulatory function of PD-1 in T-cell activation was revealed in Pdcd1−/− mice that are genetically predisposed to systematic autoimmunity (Nishimura et al., 1999). PD-1 ligand 1 (PD-L1) and PD-1 ligand 2 (PD-L2) were identified to be the ligands (PD-Ls) of PD-1 in 2000 and 2001, respectively (Freeman et al., 2000; Latchman et al., 2001a, b; Tseng et al., 2001). Subsequently, exhausted T-cell function reversion was achieved through the blockade of the PD-1/PD-L1 interaction with antibodies that restored the exhausted CD8+ T-cell reactivity and regained their antitumor activity (Curiel et al., 2003; Hirano et al., 2005). Moreover, PD-1/PD-L1 signaling is important in the maintenance of T-cell exhaustion during chronic viral infection, and antibody blockade of the PD-1/PD-L1 interaction restores function in exhausted CD8+ T cells (Barber et al., 2006a). Other well-known co-inhibitory and co-stimulatory molecules include CTLA-4, LAG-3, CD226-TIGIT, CD96, TIM, and the TNF-TNF receptor (e.g., 4-1BB, OX-40, and GITR) families, etc. (Schildberg et al., 2016). Because T-cell activation or exhaustion depends strongly on the co-stimulatory and co-inhibitory signaling pathways, co-stimulatory and co-inhibitory molecules are also called “immune checkpoint” molecules (Tan and Gao, 2015; Callahan et al., 2016).

The breakthrough of antibody-based checkpoint blockade in cancer treatment in the last few years has given rise to a promising future for cancer immunotherapies (Callahan et al., 2016). Checkpoint blockade takes advantage of a monoclonal antibody (MAb) that blocks co-inhibitory signaling pathways to restore T-cell function (Barber et al., 2006b; John et al., 2013). Multiple PD-1/PD-L1 blockade antibodies have been approved for clinical use or have entered into clinical trials, such as pembrolizumab, nivolumab, and atezolizumab, and have shown great efficacies to treat multiple advanced-stage tumors (Powles et al., 2014; Chapman et al., 2015; Postow et al., 2015; Robert et al., 2015b). Previously, the molecular basis of PD-1/PD-L1 blockade and tumor immunotherapy has been thoroughly reviewed (Chen and Han, 2015; Li et al., 2016; Zou et al., 2016), we briefly overviewed the current understanding of the molecular mechanisms of the PD-1/PD-L1 interaction and focused on the recently defined structural basis of the therapeutic antibody-based PD-1/PD-L1 blockade in the present review.

**EXPRESSION AND INHIBITORY FUNCTIONS OF PD-1/PD-Ls**

**Tissue tropism of PD-1 and PD-L1/L2 expression and regulation**

As a co-inhibitory molecule of the B7/CD28 family, PD-1 negatively regulates T-cell responses to both internal and external antigens upon binding to its ligands PD-L1 or PD-L2 (Callahan et al., 2016). Inducible expression of PD-1 is observed in T and B lymphocytes, dendritic cells (DCs), natural killer cells, monocytes, and macrophages during immune activation and chronic inflammation (Nishimura et al., 1996; Petrovas et al., 2006; Chang et al., 2008; Liu et al., 2009). On T cells, PD-1 can be induced following TCR-mediated activation and/or cytokine stimulation (Agata et al., 1996; Kinter et al., 2008). The elevated PD-1 levels progressively render antigen-specific T cells susceptible to exhaustion or anergy during chronic infections or tumor development (Blank et al., 2006; Blackburn et al., 2009). Aside from immune cells, PD-1 expression has also been detected in tumor cells. Indeed, melanoma cell-intrinsic PD-1 promotes tumorigenesis by modulating downstream mTOR signaling (Kleffel et al., 2015).

The two PD-1 ligands also show distinct expression patterns. PD-L1 is widely expressed in a variety of hematopoietic and non-hematopoietic cells, while PD-L2 expression is restricted to antigen-presenting cells, macrophages, T helper 2 cells, and non-hematopoietic cells in the lung (Dong et al., 2002; Yamazaki et al., 2002; Ohigashi et al., 2005; Hamanishi et al., 2007; Nomis et al., 2007; Lesterhuis et al., 2011). Elevated PD-L1 expression on multiple tumor cells is also an important mechanism of tumor-induced immune escape (Iwai et al., 2002; Kataoka et al., 2016).

**PD-1 signaling and PD-1-induced T-cell exhaustion**

T-cell exhaustion is defined as dysfunction of T cells during chronic virus infection or cancer (Curiel et al., 2003; Barber et al., 2006b). Progressive loss of T-cell function occurs in a hierarchical manner, where T cells lose the distinct properties of IL-2 production and the ability to proliferate at the first step and then fail to produce TNF-α and IFN-γ at later stages (Wherry et al., 2003). The PD-1 pathway serves as a critical regulator of T-cell exhaustion state (Freeman et al., 2000). The cytoplasmic domain of PD-1 contains an immunoreceptor tyrosine-based inhibition motif (ITIM) and an immunoreceptor tyrosine-based switch motif (ITSM). Both of these motifs contribute to PD-1-mediated T-cell inhibition (Chatterjee et al., 2013). Binding of the PD-L1 or PD-L2 to PD-1 induces phosphorylation on ITIM (Y223) and ITSM (Y248) tyrosine residues, thus leading to recruitment of Src

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homology region 2 domain-containing protein tyrosine phosphatases (SHP-1 and SHP-2) and subsequent down regulation of TCR signaling through dephosphorylation of signaling intermediates such as CD3ζ, ZAP70, and PKCθ in T cells (Okazaki et al., 2001; Chemnitz et al., 2004; Shepard et al., 2004). However, it is unclear how the cytoplasmic domain interacts with these factors.

PD-1 and PD-L1 upregulation in the tumor microenvironment and tumor-induced immunosuppression

Studies show that co-inhibitory molecules such as PD-1 and PD-L1 induce immune suppression in the tumor microenvironment (Iwai et al., 2002; Blank et al., 2006; Blackburn et al., 2009; Kato et al., 2016). To date, expression of PD-L1 is detected in multiple solid tumors, including melanoma, lung, breast, and ovarian cancers, as well as in myeloma, T cell lymphoma, etc. (Brown et al., 2003; Wherry et al., 2003; Ghebeh et al., 2006; Hamanishi et al., 2007; Liu et al., 2007; Hino et al., 2010). Moreover, PD-L1 expression can be detected in myeloid DCs, which is induced by factors in the tumor microenvironment (Curiel et al., 2003). The PD-L1 expression levels on tumor cells tend to be associated with tumor progression and are predictive of unfavorable prognosis and better response to PD-1 blockade treatment, to a certain extent, in ovarian, kidney, pancreatic, and gastric cancers (Thompson et al., 2005; Wu et al., 2006; Hamanishi et al., 2007; Nomizu et al., 2007; Garon et al., 2015; Gandini et al., 2016). PD-1 expressed by T lymphocytes, particularly tumor-infiltrating lymphocytes (TILs), can lead to dysfunction of tumor-specific T cells to eliminate tumors (Tume et al., 2014). Elevated expression of PD-1 on CD4+ T cells in Hodgkin lymphoma negatively affects CD4+ T cells and is suspected to facilitate immune evasion of the tumor cells (Chemnitz et al., 2007). Elevated expression of PD-1 is also observed in CD4+ T cells rather than CD8+ T cells in adult T-cell leukemia/lymphoma (Shimauchi et al., 2007).

ANTIBODY-BASED PD-1/PD-L1 IMMUNE CHECKPOINT BLOCKADE FOR TUMOR THERAPY

The mechanism of PD-1/PD-L1 interaction interference for reactivating immune activity

Forced expression of PD-1 and PD-L1 by T cells and tumor cells underlies the rationale that blockade of the PD-1 pathway would restore tumor-specific T-cell function to eliminate tumor cells (Curiel et al., 2003). Targeting the PD-1 pathway may induce T-cell immune responses via the following: 1) Activation of T cells. The PD-1/PD-L1 interaction would block the TCR-driven "stop signal" that limits T-cell mobility and thereby interrupts T cell-DC contacts and T-cell activation, proliferation, and cytokine production (Benvenuti et al., 2004). Antibodies that block PD-1/PD-L1 interaction would result in alteration of T-cell motility and promotion of T cell-DC contacts. 2) Diminishment of T-cell exhaustion. Persistent PD-1 expression could result in T-cell exhaustion, which is reversible by blocking the PD-1 pathway. Upregulation of PD-1 on CD8+ T cells in the tumor microenvironment is suggested to reflect exhaustion or anergy of T cells accompanied by the reduction of cytokine production (Ahmadzadeh et al., 2009). 3) Inhibition of Treg cells. There is a recent report that PD-1 play critical roles in modulating the activation threshold and maintaining the balance between regulatory and effector T cells (Zhang et al., 2016). Further, infiltration of PD-1-positive Treg cells into tumors can hinder the proliferation and function of effector CD8+ T cells (Wang et al., 2004; Francisco et al., 2009). In summary, blockade of the PD-1 pathway can effectively induce anti-tumor immune responses by restoration of T-cell function and inhibiting intratumoral Treg cells within the tumor microenvironment.

It is noting that PD-L1 also interacts with CD80 to inhibit T cells, while PD-L2 binds to repulsive guidance molecule b (RGMb) to mediate respiratory tolerance (Butte et al., 2007; Xiao et al., 2014). Antibodies targeting PD-1 would block PD-1/PD-L1 or PD-1/PD-L2 interactions, leaving PD-L1/CD80 and PD-L2/RGMb signaling unaffected. On the other hand, though PD-1/PD-L1 signal would be blocked by PD-L1 targeted MAbs, the PD-1/PD-L2 interaction would not be abrogated during administration of anti-PD-L1 antibodies. Additionally, other inhibitory molecules also play important roles with similar or distinct inhibitory pathways compared to the PD-1 pathway. Combination therapies with different checkpoint blockade agents might improve tumor regression efficiency, and multiple combination therapies involving different checkpoint blockade agents are now in clinical trials (Mahoney et al., 2015).

Clinical findings of PD-1/PD-L1 immune checkpoint blockade therapy

The US Food and Drug Administration (FDA) has approved two PD-1-targeted MAbs, nivolumab from Bristol-Myers Squibb (Opdivo, also known as BMS-936558, MDX-1106, and ONO-4538) and pembrolizumab from Merck (Keytruda, also known as lambrolizumab and MK-3475), for advanced melanoma, non-small cell lung cancer (NSCLC), and kidney cancer. In 2016, the US FDA gave accelerated approval to atezolizumab from Genentech (Tecentriq, also known as MPDL-3280A) for the treatment of patients with locally advanced or metastatic urothelial carcinoma. Further, various MAbs targeting the PD-1 pathway are being developed and evaluated in numerous clinical trials involving thousands of patients (Table 1). Most of the PD-1-targeted therapeutic antibodies are IgG4 human or humanized MAbs that block the PD-1/PD-L1 or PD-1/PD-L2 interaction to restore tumor-specific T cell reactivity without mediating antibody-dependent cell-mediated cytotoxicity (ADCC). PD-1-L1 targeted therapeutic antibodies possess PD-1/PD-L1 blockade activity with or without ADCC activity.
| Target | Agent | NCT number | Antibody class | Stage of development | Targeted diseases | Developer | FDA approved (diseases) |
|--------|-------|------------|----------------|----------------------|------------------|-----------|-----------------------|
| PD-1   | Nivolumab (BMS-936558/MDX-1106/ONO-4538) | NCT01658878, NCT01844505, NCT02596035, NCT02017717, NCT02105636, etc. | Human IgG4 | Bristol-Myers Squibb | FDA approved (melanoma, NSCLC) | Non-small cell lung cancer (NSCLC), melanoma, renal cell carcinoma, colon carcinoma, hepatocellular carcinoma, kidney cancer | |
| PD-1   | Pembrolizumab (MK-3475) | NCT02834052, NCT01295827, NCT02444741, NCT02819518, NCT02231749, etc. | Humanized IgG4 | Merck & Co., Inc., USA | FDA approved (melanoma, NSCLC) | Non-small cell lung cancer (NSCLC), triple negative breast cancer, renal cell carcinoma, melanoma, colon cancer, etc. | |
| PD-1   | MEDI0680 (AMP-514) | NCT02118337, NCT02013804, NCT02271945 | Humanized IgG4 | Medimmune | | Advanced malignancies, relapsed/refractory NSCLC, advanced squamous cell carcinoma | |
| PD-1   | REGN2810 | NCT02760498, NCT02383212, NCT02596034, NCT02520245 | Humanized IgG4 | Regeneron/Sanofi | | Advanced cutaneous squamous cell carcinoma, advanced malignancies | |
| PD-1   | PDR001 | NCT02795429, NCT02829723, NCT02742935 | Humanized IgG4 | BeiGene | | Advanced hepatocellular carcinoma, melanoma, NSCLC, triple negative breast cancer, lymphomas, nasopharyngeal carcinoma, etc. | |
| PD-1   | Shr 1210 | NCT02492789, NCT02738489, NCT0283823 | Humanized IgG4 | Incyte/Jiangsu HengRui | | Melanoma, neoplasm, lung cancer, breast cancer | |
| PD-1   | Tsr-042 | NCT0215394 | Humanized mab | Tesaro | | Advanced or metastatic solid tumor | |
| PD-L1  | Avelumab (MSB001798C) | NCT02715984 | Humanized IgG1 | Medimmune/Adamant | | Gastric cancer, urothelial cancer, ovarian cancer, NSCLC, etc. | |
| PD-L1  | Avelumab (MSB001798C) | NCT02715984 | Humanized IgG1 | Medimmune/Adamant | | Gastric cancer, urothelial cancer, ovarian cancer, NSCLC, etc. | |
| PD-L1  | Durvalumab (MEDI4693) | NCT02536483, NCT02536634, NCT02125461, NCT02239891, etc. | Humanized IgG1 | Genentech/Roche | | Melanoma, urothelial cancer, NSCLC, triple negative breast cancer, etc. | |
| PD-L1  | PD-169091 | NCT02500751, NCT02340817, NCT02340818, NCT02340819, etc. | Humanized IgG1 | Tekmira | | Melanoma, NSCLC, urothelial cancer, head and neck cancer, bladder cancer, etc. | |
| PD-L1  | Avelumab (MSB001798C) | NCT02715984 | Humanized IgG1 | Medimmune/Adamant | | Gastric cancer, urothelial cancer, ovarian cancer, NSCLC, etc. | |
| PD-L1  | Avelumab (MSB001798C) | NCT02715984 | Humanized IgG1 | Medimmune/Adamant | | Gastric cancer, urothelial cancer, ovarian cancer, NSCLC, etc. | |
Nivolumab displays promising tumor suppressive activity in metastatic melanoma, NSCLC, and metastatic renal cell carcinomas (Brahmer et al., 2010; Topalian et al., 2012). The use of nivolumab has achieved an overall objective response rate (ORR) of 30-40% in multiple clinical trials in patients with melanoma (Topalian et al., 2014; Robert et al., 2015a). Pembrolizumab demonstrates similar efficacy in advanced melanoma. Data from phase III clinical trials on advanced melanoma indicates that patients receiving pembrolizumab show better survival benefits compared to ipilimumab, a MAb targeting CTLA-4 (Robert et al., 2015b). Pembrolizumab is also promising for the treatment of advanced NSCLC (with an ORR of 19%), advanced bladder cancer (with an ORR above 20%), head and neck cancer (with an ORR above 20%), classical Hodgkin’s lymphoma, and triple-negative breast cancer (Garon et al., 2015; Tanguy Y. Seiwert, 2015; Yung-Jue Bang, 2015; Peter H. O’Donnell, 2015).

PD-L1-targeting MAbs are also efficacious in multiple tumors. For instance, atezolizumab (Genentech/Roche) displays promising effects, with an ORR of 43% in PD-L1+ patients and an ORR of 11% in PD-L1- patients for the treatment of metastatic urothelial bladder cancer (Powles et al., 2014). In another clinical trial involving NSCLC, melanoma, renal cell carcinoma, etc., a response to atezolizumab has more frequently been observed in patients expressing high levels of PD-L1 in tumors, especially when PD-L1 is expressed in TILs (Herbst et al., 2014). Avelumab and durvalumab are also in multiple Phase III clinical trials involving NSCLC, gastric cancer, urothelial cancer, ovarian cancer, etc. (Table 1).

However, cases of ineffective PD-1 treatment have also emerged in the observation of clinical trials (Herbst et al., 2014; Tumeh et al., 2014; Rizvi et al., 2015). Considering the complex strategies developed by tumors to evade immune surveillance, pathological types of tumors, mutations of oncogenes and tumor suppressor genes, the stage of disease, and the number of TILs are all essential factors in determining the suitability of immunotherapy. Additionally, the intensity of PD-L1 expression by tumor cells is implicated to be a potential predictor of the efficacy of PD-1 pathway blockade (Topalian et al., 2012).

### STRUCTURAL BASIS OF THE PD-1/PD-L1/L2 RECEPTOR-LIGANDS INTERACTION

PD-1 is a type I membrane protein as a member of Ig superfamily with a single extracellular immunoglobulin variable (IgV) domain and is structurally and functionally a monomer (Zhang et al., 2004). On the other hand, its ligands PD-L1 and PD-L2 contain two extracellular Ig domains: the N-terminal IgV domain and C-terminal immunoglobulin constant (IgC) domain (Lazar-Molnar et al., 2008; Lin et al., 2008). The PD-1 extracellular domain adopts an anti-parallel β-sandwich IgV-type monomeric topology, including front
sheets (A’ CC’C’’ FG) and back sheets (ABED) with a disulfide bridge between Cys54 and Cys123 (Fig. 1A–C). Compared to other CD28 family molecules (CTLA-4, CD28, ICOS, etc.), PD-1 lacks a Cys in the stalk region, which prevents PD-1 homodimerization (Schwartz et al., 2001). Both monomeric and homodimeric human PD-L1 (hPD-L1) structures were reported by our group and the others, though additional functional evidence is still needed to support these findings (Chen et al., 2010; Zak et al., 2015).

The protein level sequence identity between murine and human PD-1 (mPD-1 and hPD-1) is 64%, while the identity between murine and human PD-L1 (mPD-L1 and hPD-L1) is 77% (Fig. 1D and 1E) (Lin et al., 2008). Cross-species binding has been demonstrated (i.e., mPD-1 can bind to
hPD-L1, and hPD-1 can bind to mPD-L1), and the cross-species binding affinities show no significant differences compared to the intra-species interactions (Freeman et al., 2000; Latchman et al., 2001a, b; Zhang et al., 2004; Nomi et al., 2007; Cheng et al., 2013). The amino acids of PD-1 and PD-L1 contributing to the PD-1/PD-L1 interaction are highly conserved between mice and humans, which explains the cross-species binding properties of these paired molecules (Fig. 1D and 1E). However, hPD-1 lacks a well ordered C'' strand like that found in the IgV fold of mPD-1, which is instead replaced with a flexible loop connecting the C’ and D strands. The flexibility of the C’D loop is supported by the NMR structure and complex structure of pembrolizumab/hPD-1 (discussed below) (Cheng et al., 2013; Na et al., 2016). Additionally, the interaction details of the interface are also quite different between the orthologs (Lin et al., 2008; Zak et al., 2015). Thus, despite the high similarity of the overall structures of human and murine PD-1/PD-L1 and the high conservation of the amino acids involved in the PD-1/PD-L1 interaction between the orthologs, the development and evaluation of hPD-1- or hPD-L1-targeting agents in mouse models deserves more consideration.

Three PD-1/PD-L1/L2 complex structures have so far been determined: mPD-1/hPD-L1, mPD-1/mPD-L2, and hPD-1/hPD-L1 (Lazar-Molnar et al., 2008; Lin et al., 2008; Zak et al., 2015). The interaction of PD-1 and PD-L1 involves both of the front β-sheet faces of their IgV domains (Fig. 1A). The interaction involves the FGCC’’ strands, CC’ loop, and FG loop of PD-1 and the AFGCC’’ strands of PD-L1 (Fig. 1A and 1C). In comparing the structure of apo-hPD-1 to hPD-1 from hPD-1/hPD-L1 complex structures, significant complex formation-associated conformational changes within hPD-1 are observed involving CC’ loop rearrangement to form hydrogen bonds with hPD-L1 (Zak et al., 2015). In contrast, only minor adjustments of side chains involved in the interaction surface are observed, without significant changes of the backbone, within hPD-L1.

The interaction of mPD-1 with mPD-L2 reveals a similar binding mode to that with PD-L1, which also involves both of the IgV domains with the front β sheet faces interacting with each other (Fig. 1B) (Lazar-Molnar et al., 2008). Most (17/18) of the mPD-1 amino acids that interact with PD-L2 are also involved in the PD-L1 interaction, indicating a similar binding mode of PD-L1 and PD-L2 to PD-1 (Fig. 1D). Thus, agents targeting PD-1 would abrogate the binding of both PD-L1 and PD-L2 to PD-1. However, the detailed interactions of the mPD-1/mPD-L2 interaction significantly differ from that of mPD-1/hPD-L1 (Lazar-Molnar et al., 2008; Lin et al., 2008), suggesting distinct structural basis for the development of PD-L1- and PD-L2-targeting agents.

The reported complex structures reveal the molecular basis of the PD-1/PD-L1/L2 interactions. However, how hPD-1 interacts with hPD-L2 remains undetermined. Moreover, PD-L1 also binds to CD80, which is a ligand of CTLA-4 and CD28, and PD-L2 also has an additional receptor, RGMb. Complex structures of these paired molecules would benefit our understanding of the PD-1/PD-L1/L2 interactions and the development of PD-1/PD-L1/L2 targeting agents in the future.

Based on the complex structure of mPD-1/hPD-L1, Maute et al. have taken advantage of directed evolution of the amino acids in hPD-1 which contributes to the binding with PD-L1 by yeast-surface display to engineer the PD-1 ectodomain as a high-affinity (110 pmol/L) competitive antagonist of PD-L1 (Maute et al., 2015). There are also some peptides, peptidomimetics and small drug-like molecules in preclinical or clinical investigations (Zhan et al., 2016). The recent report on the first nonpeptidic chemical inhibitors that target the PD-1/PD-L1 interaction suggesting that there are “hot spots” on PD-L1 for PD-L1 antagonist drug design (Zak et al., 2016). The structural basis of PD-1 or PD-Ls complexed with these small molecules are also important for drug discovery in the field.

**STRUCTURAL BASIS OF THERAPEUTIC ANTIBODY INTERVENTION**

Crystal structures of the anti-PD-1 pembrolizumab Fab fragment complexed with hPD-1 and the anti-PD-L1 avelumab single chain Fv fragment (scFv) complexed with hPD-L1 have been determined by Na et al. (2016) and our group, revealing the molecular basis of therapeutic antibody-based immune checkpoint therapy for tumors (Liu et al., 2016; Na et al., 2016). The interaction of pembrolizumab with hPD-1 is mainly located on two regions: the flexible C’D loop and the C, C’ strands. Unlike the C’’ strand observed in mPD-1, the corresponding region in hPD-1 contains a disordered C’D loop in solution (Fig. 2A left) (Cheng et al., 2013). Though the C’D loop is not involved in the interaction with hPD-L1, it contributes major contacts with pembrolizumab through polar, charged, and hydrophobic contacts. Both the heavy chain (VH) and light chain (VL) of pembrolizumab are involved in contacting the C’D loop of hPD-1 (Fig. 2A right). The other regions that pembrolizumab interacts with are located on the C and C’ strands of hPD-1, which contribute critical contacts with hPD-L1 (Fig. 2A right). Thus, the blockade of the hPD-1/hPD-L1 interaction by pembrolizumab occurs predominantly by binding to the C’D loop and overlaps binding to the C and C’ strands to compete with the binding of hPD-L1.

Structural analysis of the interaction of avelumab with hPD-1 reveals that avelumab utilizes both VH and VL to bind to the IgV domain of PD-L1 on its side (Liu et al., 2016). The VH of avelumab dominates the binding to hPD-L1 by all three complementarity determining regions (CDR) loops, while VL contributes partial contacts by the CDR1 and CDR3 loops, leaving VL CDR2 without any binding to hPD-L1 (Fig. 2B left). The binding epitope region of avelumab on hPD-L1 predominantly consists of the C, C’, F, and G strands and the CC’ loop of hPD-L1. The blockade binding of avelumab is mainly occupied by the VH chain, with minor contribution from VL chain (Fig. 2B right). The detailed analysis of the
buried surface on hPD-L1 reveals that the overlapping area of avelumab and hPD-1 is mainly located on the F and G strands, which are predominantly occupied by the HCDR2 loop of avelumab (Fig. 2B right). Therefore, the mechanism of avelumab blockade involves the protruding HCDR2 loop dominating the hPD1 binding region and competing for the binding of hPD-1/hPD-L1 interaction.

The binding affinities ($K_d$) of pembrolizumab to hPD-1 and avelumab to hPD-L1 are 27.0 pmol/L and 42.1 pmol/L, respectively (Na et al., 2016). On the other hand, the binding affinity between hPD-1 and hPD-L1 is 0.77–8.2 μmol/L (Collins et al., 2002; Butte et al., 2007; Cheng et al., 2013), which is much weaker than that of the antibodies. The strong binding of pembrolizumab to hPD-1 and avelumab to hPD-L1 would enable the binding priority of the therapeutic antibodies with checkpoint molecules and subsequent blockade of the hPD-1/hPD-L1 interaction.

There are yet more therapeutic antibodies targeting PD-1/PD-L1/L2 in clinical use or clinical trials (e.g., nivolumab, atezolizumab, and durvalumab). Whether these antibodies utilize the same blockade mode as pembrolizumab or avelumab remains undetermined. Moreover, whether there are hot-spots on PD-1 or PD-L1 to be targeted by different therapeutic antibodies requires further investigation. All of...
these findings would benefit the development of therapeutic agents targeting the PD-1 pathway to disrupt the PD-1/PD-L1 interaction.

CONCLUSION AND PERSPECTIVES

The success of checkpoint blockade therapy has brought immunotherapy from the corner to center stage in fighting against human cancers, especially for solid tumors. In contrast to other strategies that prime or boost cancer-specific immune responses, immune checkpoint blockade therapy targets tumor-induced immune defects and revives existing tumor-specific T cells to kill tumor cells. The PD-1/PD-L1 pathway has been taking the priority that single use of PD-1 or PD-L1 blockade antibodies can eliminate tumors in at least a portion of patients. Though clinical success with anti-PD-1 therapy has been achieved, the molecular basis of the PD-1/PD-L1/L2 interaction and PD-L1/L2 interaction with other receptors needs to be further investigated. The recently reported therapeutic antibody complex structures with PD-1 or PD-L1 make it clear how the therapeutic antibodies work, providing a new approach to modify these antibodies for the better effects. However, more antibody/PD-1 (or PD-L1, PD-L2) interaction details are still needed to define the antibody targeting hot-spots and to better design PD-1/PD-L1/L2 antagonists for tumor treatment. Such efforts will pave a way to improve the efficacy of antibody targeting the PD-1 pathway and prolong survival in advanced cancer patients.

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ABBREVIATIONS

CDR, complementarity determining regions; DCs, dendritic cells; ITIM, immunoreceptor tyrosine-based inhibition motif; IgC domain, immunoglobulin constant domain; IgV domain, immunoglobulin variable domain; ITSM, immunoreceptor tyrosine-based switch motif; hPD-1, human programmed cell death 1; hPD-L1, human programmed cell death 1 ligand 1; hPD-L2, human programmed cell death 1 ligand 2; mPD-1, murine programmed cell death 1; mAb, monoclonal antibody; mPD-L1, murine programmed cell death 1 ligand 1; mPD-L2, murine programmed cell death 1 ligand 2; NSCLC, non-small cell lung cancer; ORR, objective response rate; PD-1, programmed cell death 1; PD-L1, programmed cell death 1 ligand 1; PD-L2, programmed cell death 1 ligand 2; PD-Ls, programmed cell death 1 ligands; pMHC, peptide major histocompatibility complex; scFv, single chain Fv fragment; TCR, T-cell receptor; TILs, tumor-infiltrating lymphocytes.

COMPLIANCE WITH ETHICS GUIDELINES

Shuguang Tan, Danqing Chen, Kefang Liu, Mengnan He, Hao Song, Yi Shi, Jun Liu, Catherine W-H. Zhang, Jianxun Qi, Jinghua Yan, Shan Gao, George F. Gao declare that they have no conflict of interest.

This article does not contain any studies with human or animal subjects performed by the any of the authors.

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