Review

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Human cancer immunotherapy with antibodies to the PD-1 and PD-L1 pathway

Kim C. Ohaegbulam[1*], Amer Assal[2*], Eszter Lazar-Molnar[3], Yu Yao[4], and Xingxing Zang[1,2]

[1] Department of Microbiology and Immunology, Albert Einstein College of Medicine, New York, NY 10461, USA
[2] Department of Oncology, Montefiore Medical Center, New York, NY 10467, USA
[3] Department of Pathology, University of Utah School of Medicine, Salt Lake City, UT 84132, USA
[4] Department of Neurosurgery, Huashan Hospital, Fudan University, Shanghai 200040, China

The programmed death 1 (PD-1) receptor and its ligands programmed death ligand 1 (PD-L1) and PD-L2, members of the CD28 and B7 families, play critical roles in T cell coinhibition and exhaustion. Overexpression of PD-L1 and PD-1 on tumor cells and tumor-infiltrating lymphocytes, respectively, correlates with poor disease outcome in some human cancers. Monoclonal antibodies (mAbs) blockading the PD-1/PD-L1 pathway have been developed for cancer immunotherapy via enhancing T cell functions. Clinical trials with mAbs to PD-1 and PD-L1 have shown impressive response rates in patients, particularly for melanoma, non-small-cell lung cancer (NSCLC), renal cell carcinoma (RCC), and bladder cancer. Further studies are needed to dissect the mechanisms of variable response rate, to identify biomarkers for clinical response, to develop small-molecule inhibitors, and to combine these treatments with other therapies.

Expression of PD-1 and its ligands

The PD-1 (CD279) (see Glossary) receptor can be detected at the cell surface of T cells during thymic development and in the periphery of several types of hematopoietic cell following T cell receptor (TCR) signaling and cytokine stimulation. PD-1 is expressed on $CD4^{-}CD8^{-}$ thymocytes and inducibly expressed on peripheral $CD4^{+}$ and $CD8^{+}$ T cells, B cells, monocytes, natural killer (NK) T cells, and some dendritic cells (DCs) [1,2]. Persistent expression of PD-1 on T cells induces T cell exhaustion [3]. Exhausted CD8 T cells lose their effector function, evidenced by their inability to secrete cytolytic molecules such as perforin and their failure to secrete proinflammatory cytokines, such as IL-2, interferon gamma (IFN-γ), and tumor necrosis factor alpha (TNF-α) [4,5].

$CD4^{+}Foxp3^{+}$ regulatory T cells (Tregs), a highly immunosuppressive subset of $CD4^{+}$ T cells that is critical in maintaining tolerance and attenuating immune responses, express cell-surface PD-1, which contributes to their development, maintenance, and functional response [6]. Ligand binding to the PD-1 receptor on Tregs in the presence of CD3 and transforming growth factor beta (TGF-β) leads to an increase in the *de novo* conversion of naive $CD4^{+}$ T cells to Tregs. This induction generates heightened suppressive function and maintenance of Foxp3 expression through inhibition of Akt–mammalian target of rapamycin (mTOR) signaling and increased phosphatase and tensin homolog (PTEN) activity [7,8]. This indicates that PD-1 pathway stimulation results not only in a reduction in effector T cell function, but also an increase in immunosuppressive Treg function. This allows proper control of immune homeostasis and creates a high threshold for T cell activation.

Although PD-1 has best been characterized in T cells, its function in other cell subsets have also become apparent. The regulation of PD-1 expression is tightly controlled during B cell differentiation. Levels are undetectable in pro-B cells, an early precursor in B cell development, and increase as B cell differentiation [9]. Additionally, surface levels of PD-1 can be greatly enhanced in mature B cells following stimulation with Toll-like receptor 9 (TLR9) agonists. Blockade of PD-1 on B cells has been shown to increase antigen-specific antibody responses,

Glossary

Cancer immunotherapy: treatments that use the host immune system to inhibit cancer.
Monoclonal antibody (mAb): antibodies generated by immune cells derived from a single parent cell.
Programmed death 1 (PD-1): a 288-amino acid cell-surface molecule, encoded in humans by the *PDCD1* gene, that functions to negatively regulate immune responses.
Programmed death ligand 1 (PD-L1): a 40-kDa type 1 transmembrane protein, encoded in humans by the *CD274* gene, that suppresses the immune system in cancer, pregnancy, tissue allografts, and autoimmune diseases.
T cell: a type of lymphocyte that has an important role in cell-mediated immunity, distinguished by its T cell receptor on the cell surface; referred to as T cells because they mature in the thymus.
T cell coinhibition: a signal required for inhibition of activated T cells in the presence of T cell receptor signal.

Corresponding author: Zang, X. (xing-xing.zang@einstein.yu.edu).
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*These authors contributed equally.

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suggesting that PD-1 plays a role in inhibiting B cell clonal responses [10].

PD-1 has two binding ligands, PD-L1 (B7-H1, CD274) [11,12] and PD-L2 (B7-DC, CD273) [13,14], with PD-L1 being the most prominent in regulation. PD-L1 is inducibly expressed on both hematopoietic cells and non-hematopoietic cells following cell-specific stimulation. Cytokines such as IFN-γ and TNF-α upregulate the expression of PD-L1 on T cells, B cells, endothelial cells, and epithelial cells, furthering its role in the maintenance of peripheral tolerance [1]. Data also link genetic changes seen in cancer cells to the induction of PD-L1, although this can vary by cancer type. PTEN dysfunction in human glioma cells induces Akt activation and subsequently PD-L1 expression, while human melanoma cells show no association between PTEN or Akt and PD-L1 induction [15,16]. Recent data show that PD-L1 binds to B7-1 (CD80) in addition to PD-1 [17]. While PD-L1 expression is induced on a wide array of both hematopoietic and non-hematopoietic cells, PD-L2 expression is restricted to inducible expression on DCs, macrophages, mast cells, and some B cells in response to IL-4 and IFN. The affinity of PD-L2 for PD-1 is three times greater than that of PD-L1, which indicates competition between the two ligands. Recent data confirm a second cognate receptor for PD-L2, repulsive guidance molecule B (RGMb) [18]. Despite recent research efforts surrounding PD-L2, little is known regarding the transcriptional regulation of the ligand.

Structures of PD-1 and its ligands

Structurally, PD-1 is a type I transmembrane receptor and belongs to the Ig superfamily (IgSF). Although it is functionally related to the costimulatory/coinhibitory receptors CD28, cytotoxic T lymphocyte-associated protein 4 (CTLA-4) and inducible T cell costimulator (ICOS), PD-1 has important structural and functional differences. Other receptors in the CD28 family are disulfide-linked dimers; however, structural and cell-surface studies demonstrated that PD-1 is a monomeric glycoprotein [19]. The crystal structure of the extracellular region of mouse PD-1 shows the presence of a typical Ig variable domain (IgV) comprising front sheets (A'GFCC'C") and back sheets (ABED) (Figure 1) stabilized by a disulfide bond linking the F and B strands [19]. This IgV domain is linked to transmembrane and cytoplasmic domains through a 20-amino acid stalk region. In contrast to other CD28 family receptors, the absence of an extracellular cysteine residue in the stalk region prevents PD-1 from covalent dimer formation.

Human and mouse PD-1 share around 60% overall identity at the protein level, which increases to 75% for the residues forming the IgV domain. It is unsurprising, therefore, that crystallographic [Protein Data Bank (PDB) code 3RRQ] and NMR structures [20] show a high degree of similarity between mouse and human PD-1. Overlay of the crystal structures of mouse and human PD-1 shows very similar arrangements (Figure 1). One notable difference between human and mouse PD-1 is the lack of the C" strand at the edge of the front GFCC' sheet in human PD-1, as shown by the NMR data [20]. This region instead presents as a highly flexible loop, consistent with the poor

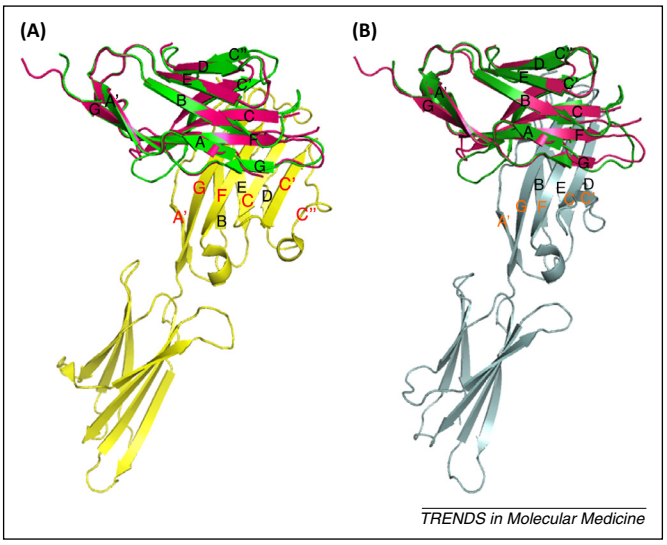

Figure 1. Crystal structures of programmed death 1 (PD-1) receptor/programmed death ligand 1 (PD-L1) and PD-1/PD-L2 complexes. **(A)** Overlay of the crystal structures of human PD-1 [Protein Data Bank (PDB) code 3RRQ] and the mouse PD-1/human PD-L1 complex (3BIK). **(B)** Overlay of human PD-1 with the mouse PD-1/PD-L2 complex (3BP5). Pink, human PD-1; green, mouse PD-1; yellow, human PD-L1; grey, mouse PD-L2.

electron density observed for that region in the crystallographic dataset, indicating a disordered arrangement (PBD code 3RRQ).

Another unique structural feature of PD-1 is that it lacks a consensus complementarity-determining region 3 (CDR3)-like conserved ligand-binding motif. The ligand-binding site comprises a hydrophobic patch on the front face contributed by multiple residues from several strands [19]. Crystal structures available for complexes of mouse PD-1 and human PD-L1 [21] and mouse PD-1 with mouse PD-L2 [22] show similar overall molecular architecture for these inhibitory complexes. Both PD-1 and its ligands interact with their respective surface residues distributed over their front beta sheets (front-to-front binding). By contrast, the FG loop of PD-1, which corresponds to the CDR3 variable region of the Ig structure, makes little or no contact with PD-L1 or PD-L2 (Figure 1). The crystal structures of the PD-1/PD-L complexes reveal that PD-1 binds its ligands with 1:1 stoichiometry and forms monomeric complexes. This indicates a distinct ligand-binding mode and signaling mechanism that differs from other coinhibitory receptor/ligand interactions such as CTLA-4/B7, where oligomerization plays an important role in signaling. Although crystal structures for the human PD-1/PD-L1 and PD-1/PD-L2 complexes remain to be solved, the overall similarity of the mouse and human PD-1 structures suggests that mouse and human PD-1 are likely to form similar complexes. Overlay of human PD-1 with the mouse complexes shows that human PD-1 may bind to its ligands in the same way as mouse PD-1; however, a recent study using NMR with binding data and mathematical modeling suggests that PD-1 may be engaged by its two ligands differently [20].

Importantly, the available crystal structures of PD-1 and the PD-1/PD-L1 and PD-1/PD-L2 complexes allow not only mapping of the ligand-binding sites and mAb blocking epitopes, but also the design of small-molecule inhibitors.

Table 1. Prognostic significance of PD-1 expression in human tumor-infiltrating lymphocytes

Tumor	Clinical correlation	Refs
Breast	High tumor-infiltrating PD-1$^+$ cell counts decreased patient survival	[54]
Breast	PD-1$^+$ TILs associated with tumor size, grade, LN status, and worse overall survival	[55]
Prostate	CD8$^+$ TILs expressed high levels of PD-1 and had restricted TCR Vbeta gene usage	[56]
Thyroid	PD-1$^+$ T cells in LNs were indicative of recurrent disease and correlated with Treg frequency	[57]
Melanoma	PD-1$^+$ TILs expressed CTLA-4, displayed an exhausted phenotype, and were functionally impaired compared with PD-1$^-$ TILs	[58]
Melanoma	PD-1 expression on CD4$^+$/CD8$^+$ T cells was found in primary tumor, with greater expression in distant metastases	[59]
Ovarian	CD8$^+$PD-1$^+$ T cells were impaired in IFN-γ/TNF-α secretion compared with CD8$^+$PD-1$^-$ T cells	[60]
RCC	Presence of PD-1$^+$ intratumoral immune cells associated with advanced stage and significant risk for cancer-specific death compared with PD-1$^-$ patients	[61]
NSCLC	CD8$^+$ TILs increased PD-1 expression resulting in reduced cytokine production and capacity to proliferate	[62]
HCC	CD8$^+$PD-1$^+$ TILs predicted disease progression and tumor recurrence	[63]

Prognostic relevance of PD-1 and its ligands in human malignancies

Persistent expression of PD-1 by T cells is highly indicative of an exhausted phenotype, noted by a decrease in effector function [4,5]. This phenotype has been observed in various types of tumor-infiltrating lymphocyte (TIL) and linked to poor prognosis and tumor recurrence, highlighting PD-1 as an important molecule in regulating antitumor activity (Table 1). Similar to PD-1, PD-L1 and PD-L2 also possess prognostic capacities in some human malignancies (Table 2). Some clinical studies associate high expression of PD-L1 in tumors to tumor size, lymph node (LN) involvement, grade, and overall survival, while PD-L2 has generally been tied only to a trend in decreased survival that is not of statistical significance (Table 2). PD-L1 generally has a much broader expression pattern compared with PD-L2. This indicates that the regulation of PD-L2 depends much more on environmental stimuli than that of PD-L1. Data from these studies provide a solid rationale for investigating the immunological mechanisms behind the clinical associations. The poor prognosis indicated by the expression of PD-1 on TILs and of PD-L1/2 on tumor cells supports the targeting of the pathway therapeutically.

Mechanisms of anti-PD-1 and anti-PD-L1 immunotherapy

Appreciating the consequences of the upregulation of the PD-1/PD-L1/2 axis aids our progress in manipulating an immunosuppressive cancer microenvironment. The cytoplasmic tail of PD-1 contains two signaling motifs. One is an immunoreceptor tyrosine-based inhibitory motif (ITIM) and the other is an immunoreceptor tyrosine-based switch motif (ITSM). Binding of PD-L1 or PD-L2 to PD-1 on activated T cells, along with TCR signaling, leads to phosphorylation of the cytoplasmic domain tyrosines and recruitment of a Src homology 2-containing tyrosine phosphatase (SHP-2) to the ITSM. Consequently, SHP-2 dephosphorylates TCR-associated CD-3ζ and zeta chain-associated protein kinase 70 (ZAP70), resulting in inhibition of downstream signaling including blocking phosphoinositide 3-kinase (PI3K) and Akt activity, disrupting glucose metabolism and IL-2 secretion [5,23].

mAbs have been developed for cancer immunotherapy by enhancing T cell function via blockade of the binding between PD-1 and PD-L1 or PD-L2 (Figure 2). Many of these studies have shown that blockade of PD-1 alone or PD-L1 leads to an increase in T cells and IFN-γ at the tumor site [24], along with decreases in the percentages of the highly immunosuppressive myeloid-derived suppressor cell (MDSC) population [25]. Increase in the effector-to-suppressor cell ratio usually supports an antitumor microenvironment. These results demonstrate that the neutralization of PD-1, PD-L1, or PD-L2 can be effective in

Table 2. Prognostic significance and pathological associations of PD-L1 and PD-L2 on human tumor cells

Tumor	Clinical correlation	Refs
Colon	PD-L1 expression was associated with TNM stage and predicted prognosis	[64]
Cervical	PD-L1 was expressed in only a minority of samples and influences patient survival	[65]
Pancreatic	PD-L1-positive patients had poorer prognosis than PD-L1-negative patients and PD-L1 was inversely correlated with CD8$^+$ TILs; PD-L2 showed no significant correlation with patient survival	[66]
Breast	PD-L1 expression was correlated with tumor size, grade, LN status, and significantly worse overall survival	[67]
Ovarian	PD-L1 expression on monocytes in ascites and blood from patients with malignant ovarian carcinoma was greater than in those with borderline/benign disease; PD-L1 expression led to poorer prognosis and was inversely correlated with intraepithelial CD8$^+$ T cells, while PD-L2 showed poorer prognosis but not a significant difference	[68,69]
RCC	Soluble PD-L1 was associated with larger tumors, worse stage, grade, and necrosis, and increased risk of death; PD-L1 was associated with poor prognosis	[70,71]
HCC	PD-L1 expression on hematoma cells enriched apoptotic CD8$^+$ T cells; greater expression of PD-L1 was associated with significantly poorer prognosis and was an independent predictor for recurrence, while PD-L2 expression correlated with poorer survival but not recurrence	[63,72]
NSCLC	PD-L1 was associated with EGFR[a] mutations and was a negative prognostic factor	[73]
Melanoma	Greater PD-L1 expression correlated with significantly lower overall survival and vertical growth of primary tumors; PD-L1 marks a subset of melanomas with shorter overall patient survival	[74,75]
Esophageal	PD-L1 and PD-L2 expression led to significantly poorer prognosis while only PD-L2 expression was inversely correlated with CD8$^+$ TILs	[76]

[a]Epidermal growth factor receptor.

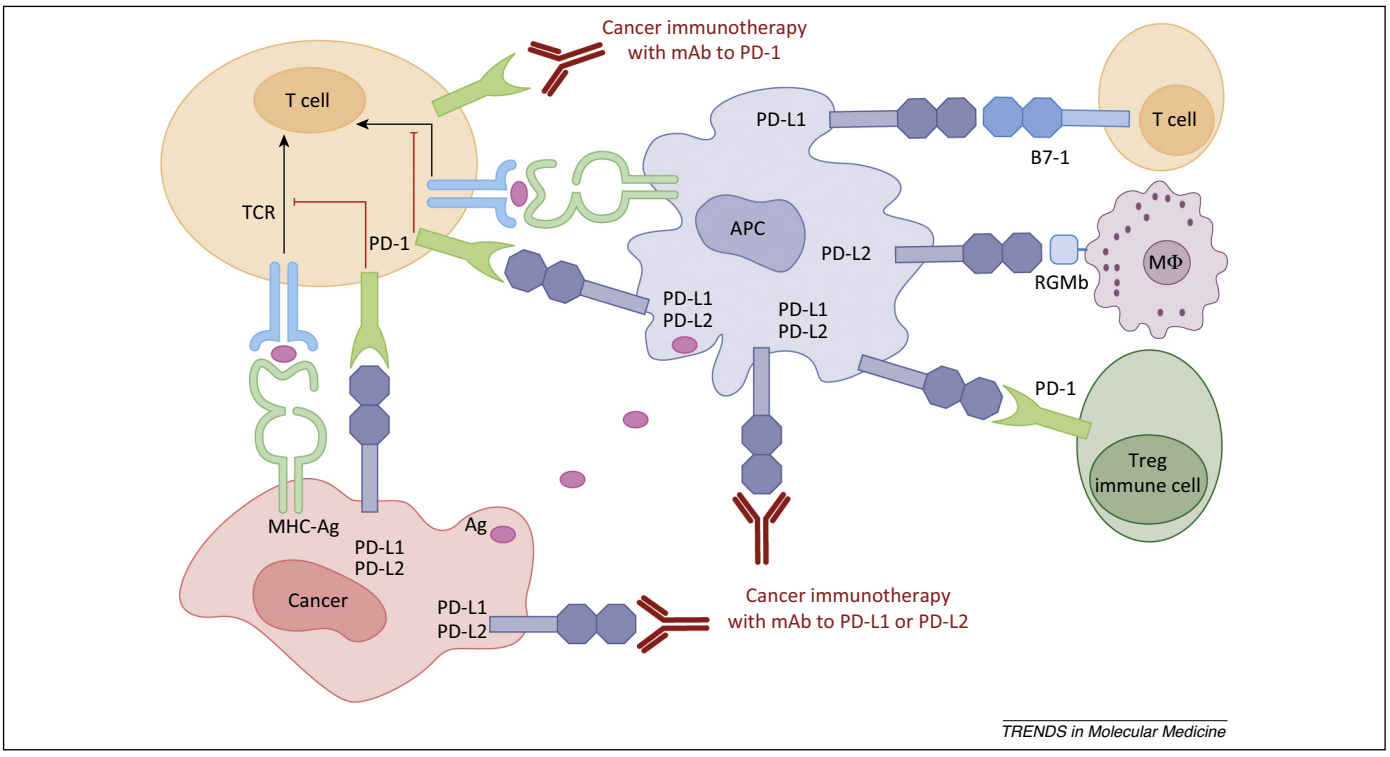

Figure 2. Human cancer immunotherapy with anti-programmed death 1 (PD-1) receptor and anti-programmed death ligand 1 (PD-L1)/L2 antibodies. Antigen-presenting cells (APCs) take up antigen (Ag) released from cancer cells and present it to T cells. Cancer cells can also present Ag to activated T cells in the context of the MHC. On T cell activation, PD-1 receptors are expressed on T cells and inhibit immune responses by engagement of PD-L1 and PD-L2 on APCs and PD-L1 on cancer cells. Therefore, monoclonal antibody (mAb)-mediated specific blockade of the PD-1/PD-L1/PD-L2 pathway can enhance antitumor immunity. In addition to binding to PD-1, PD-L1 and PD-L2 also bind B7-1 and repulsive guidance molecule B, respectively. In addition to T cells and APCs, PD-1 and PD-L1 can be induced on other immune cells.

controlling tumor growth by changing the dynamic of the tumor microenvironment.

Additional approaches generating synergy are the blockade of PD-1 or PD-L1 in combination with other therapeutic agents. Simultaneous blockade of both PD-1 and CTLA-4 leads to expansion of TIL populations while reducing the number of MDSCs within the tumor, leading to tumor regression and significant increases in IFN-γ and TNF-α in $CD8^+$ T cells [26]. Furthermore, chemotherapy and radiotherapy are being studied in combination with the blockade of the PD-1/PD-L1 pathway [27,28]. Together these results set the stage for an optimistic clinical outlook.

Various biological inhibitors of PD-1 and PD-L1 have been developed and are currently being tested in clinical trials with cancer patients (Table 3). These inhibitors include mAbs to PD-1 and PD-L1 as well as PD-L2 fusion protein.

Clinical trials of mAbs to PD-1

Pidilizumab (CT-011) was the first mAb to PD-1 to reach clinical trials [29] (Table 4). It was initially identified as a mAb binding to the B lymphoblastoid cell line that stimulated murine lymphocytes and showed antitumor activity in mice [30]. It stimulated human peripheral blood lymphocytes and enhanced cytotoxicity toward human tumor cell lines. The first Phase I trial with pidilizumab recruited patients with hematologic malignancies, including acute myeloid leukemia (AML), chronic lymphocytic leukemia (CLL), non-Hodgkin's lymphoma (NHL), Hodgkin's lymphoma (HL), and multiple myeloma (MM) [29]. Dose levels ranged from 0.2 to 6 mg/kg. A maximum tolerated dose (MTD) was not reached and the drug was well tolerated. Of the 17 patients enrolled in the study, one patient experienced a complete response, four had stable disease, and one had a mixed response, amounting to a 33% clinical benefit rate. Durable responses of greater than 60 weeks were

Table 3. Biological agents targeting PD-1 or PD-L1 in cancer clinical trials

Biological agent	Class	Target	Company	Refs
CT-011 (pidilizumab)	Humanized IgG1	PD-1	CureTech	[29]
MK-3475 (lambrolizumab, pembrolizumab)	Humanized IgG4	PD-1	Merck	[33]
BMS-936558 (nivolumab)	Human IgG4	PD-1	Bristol–Meyers Squibb	[37]
AMP-224	PD-L2 IgG2a fusion protein	PD-1	Amplimmune/GlaxoSmithKline	[52]
BMS-936559	Human IgG4	PD-L1	Bristol–Meyers Squibb	[44]
MEDI4736	Humanized IgG	PD-L1	MedImmune	[45]
MPDL3280A	Human IgG	PD-L1	Roche	[49]
MSB0010718C	Human IgG1	PD-L1	Merck	[51]

Table 4. Summary of reported clinical trials of mAbs that target PD-1 or PD-L1 as monotherapy, or in combination therapy with other agents, in human cancer

Antibody	Dose	Phase	Cancer	NCT[a] Number	Refs
Monotherapy					
Pidilizumab	0.2–6 mg/kg	I	AML, CLL, NHL, HL, MM	N/A	[29]
Pidilizumab	1.5 or 6 mg/kg	II	Malignant melanoma	NCT01435369	[77]
Pembrolizumab	1–10 mg/kg	I	Advanced solid tumors	NCT01295827	[33,34,36,78]
Nivolumab	0.3–10 mg/kg	I	Advanced solid tumors	NCT00441337	[39]
Nivolumab	1–10 mg/kg	I	Advanced solid tumors	NCT00730639	[40]
Nivolumab	1–20 mg/kg	I	Advanced solid tumors	N/A	[79]
Nivolumab	0.3–10 mg/kg	II	RCC	NCT01354431	[80]
Nivolumab	1 or 3 mg/kg	II	Platinum-resistant ovarian cancer	N/A	[81]
BMS-936559	0.3–10 mg/kg	I	Advanced solid tumors	NCT00729664	[44]
MPDL3280A	1–20 mg/kg	I	Advanced solid tumors and disease-specific cohorts	NCT01375842	[49,50,82–84]
MEDI4736	0.1–15 mg/kg	I	Advanced solid tumors	NCT01693562	[46]
MSB0010718C	1–20 mg/kg	I	Refractory malignancies	NCT01772004	[51]
Combination therapy					
Pidilizumab after ASCT	1.5 mg/kg	II	NHL	NCT00532259	[32]
Pidilizumab + rituximab	3 mg/kg	II	Follicular lymphoma	NCT00904722	[31]
Nivolumab + ipilimumab	1 mg/kg + 3 mg/kg	I	Malignant melanoma	NCT01024231	[43]
Nivolumab + platinum and nivolumab + ipilimumab	10 mg/kg and 1 mg/kg + 3 mg/kg	I	NSCLC	NCT01454102	[85,86]
Nivolumab + ipilimumab	3 mg/kg + 1 mg/kg	I	RCC	NCT01472081	[87]
Nivolumab + multipeptide vaccine	1–10 mg/kg	I/II	Malignant melanoma	NCT01176461	[88]

[a]National Clinical Trial.

noted. This was followed by two Phase II clinical trials [31,32]. Patients with diffuse large B cell lymphoma (DLBCL) or primary mediastinal B cell lymphoma (PMBCL) who underwent autologous hematopoietic stem cell transplantation (ASCT) and who had chemosensitive disease were treated with pidilizumab at 1.5 mg/kg every 42 days for three cycles starting 30–90 days post-transplantation [32]. The study enrolled 72 patients. Sixteen-month progression-free survival (PFS) for eligible patients was 72%, meeting the primary end point of the study. Intention-to-treat analysis revealed a 16-month PFS of 68%. The overall response rate for patients with measurable disease after ASCT was 51%. The most common grade 3 or 4 toxicities included neutropenia and thrombocytopenia. Correlative studies of select lymphocyte subsets revealed an increase in the number of activated $CD25^+PD\text{-}L1^+CD4^+$ T cells, $PD\text{-}L1^+PD\text{-}L2^+CD14^+$ monocytes, and circulating peripheral and central memory CD8 T cells as well as central memory CD4 T cells. These results suggest that pidilizumab may reverse PD-1-mediated inhibition of T cell survival and proliferation.

The second Phase II study with pidilizumab was a combined treatment with rituximab for follicular lymphoma [31]. Patients with rituximab-sensitive disease were treated with four doses of pidilizumab at 3 mg/kg every 4 weeks, with the option to continue therapy if they showed a response or stable disease. The study enrolled 32 patients. Patients were fairly well distributed across the three risk groups of the Follicular Lymphoma International Prognostic Index (FLIPI) 1 and 2. The objective response rate was 66%, which met the study end point of greater than 60% compared with a historical response rate of 40% with rituximab alone. The complete response rate was 52%. Responses were durable, with a median PFS of 18.8 months. FLIPI 1 or 2 score was not associated with response rate. The regimen was well tolerated, with no grade 3 or 4 adverse events. PD-L1 expression was significantly higher on $CD4^+$, $CD8^+$, and $CD14^+$ peripheral blood cells from responding patients but was not associated with PFS. Gene expression data suggested that intrinsic antilymphoma immunity might be predictive of a response to pidilizumab. Pidilizumab continues to be evaluated in various clinical trials, including solid tumors and hematologic malignancies, both as a single agent and in combination with other regimens including cellular therapies and cancer vaccines.

Pembrolizumab (MK-3475; previously known as lambrolizumab) (Table 4) is a humanized IgG4 PD-1-blocking mAb [33,34]. It is also the first mAb targeting PD-1 that has been granted accelerated FDA approval. A very high-affinity mouse antihuman PD-1 antibody was developed, the variable region of which was grafted to human IgG4 with a stabilizing S228P Fc mutation. The IgG4 subtype does not engage Fc receptors or activate complement, thus avoiding cytotoxic activity against T cells. Pembrolizumab was studied in a Phase I trial in patients with advanced solid tumors [33]. The dose range was 1–10 mg/kg and a MTD was not identified. In the nine patients enrolled in the study, no grade 3 or 4 toxicities were noted. One patient with melanoma experienced a partial response, with an additional three patients experiencing stable disease. Pembrolizumab activity and safety in melanoma was further explored by recruiting an expansion cohort at the 10 mg/kg dose level [34]. Doses ranged from 2 mg/kg every 3 weeks to 10 mg/kg every 2 weeks. Patients with advanced melanoma, including patients who had received prior treatment with ipilimumab, an FDA-approved mAb to CTLA-4, were allowed in this study. Results from 135 treated patients were reported. The response rate across all

dose cohorts was 38%, with the highest response rate observed in the cohort given 10 mg/kg every 2 weeks (52%). Responses were durable and overall PFS was longer than 7 months. Median overall survival (OS) was not reached. Treatment-related grade 3 or 4 adverse events were reported in 13% of patients. The highest incidence of treatment-related adverse events was in the group given 10 mg/kg every 2 weeks. Endocrine toxicities included hypothyroidism in 8.1% of patients, with one case being grade 3 or 4, grade 3 hyperthyroidism, and grade 2 adrenal insufficiency. Correlative studies on available tumor biopsies showed that regressing lesions were densely infiltrated with cytotoxic T lymphocytes (CTLs), which was consistent with the mechanism of action of the drug. PD-L1 expression by tumor cells was significantly associated with PFS and response rate but not with OS [35]. It is important to note here that, despite a very low cut-off for PD-L1 positivity (1% of stained cells), antitumor activity was noted in tumors with low PD-L1 expression. These results cast doubt on the utility of PD-L1 expression as a biomarker and suggest that the mechanism of action of PD-1-targeting antibodies remains to be fully elucidated.

To explore the efficacy of pembrolizumab in malignant melanoma patients who had progressed after ipilimumab or treatment with a BRAF or mitogen-activated protein kinase kinase (MEK) inhibitor, or both, an open-label, randomized, multicenter expansion cohort of the KEYNOTE-001 trial was performed [36]. The trial randomized 173 patients to receive pembrolizumab at 2 mg/kg or 10 mg/kg every 3 weeks. The overall response rate was 26% in both groups. Survival at 1 year was similar in the two treatment groups (58% and 63%). Pembrolizumab was well tolerated, with drug-related grade 3 or 4 adverse events reported in 12% of patients in both arms. Six patients (3%) discontinued treatment due to adverse events. Three patients experienced immune-related adverse events and were managed with dose interruption and corticosteroid treatment. Results from the KEYNOTE-001 trial served as the basis for the accelerated FDA approval of pembrolizumab for the treatment of patients with advanced or unresectable melanoma that progressed after therapy with ipilimumab or after therapy with a BRAF inhibitor in tumors carrying the BRAF V600 mutation. Of note in this trial is that the two dose levels exhibited similar antitumor activity and toxicity profiles. Pembrolizumab continues to be evaluated in Phase I trials in advanced solid tumors, head and neck cancers, and hematologic malignancies and in combination with lenalidomide and dexamethasone in relapsed/refractory MM, as well as in Phase II and III trials in microsatellite unstable tumors, in combination with pazopanib in renal cell cancer, and compared with docetaxel in NSCLC.

Nivolumab (MDX-1106, BMS-936558, ONO-4538) (Table 4) is a fully human IgG4 mAb to PD-1. In a humanized *in vitro* model of melanoma, addition of nivolumab to human vaccine-induced $CD8^+$ T cells specific to melanoma antigens allowed the expansion of these lymphocytes [37]. In another study of an *ex vivo* melanoma model, addition of the same antibody led to the unmasking of CTL inhibition by Tregs and stimulated their proliferation [38]. Nivolumab was first studied clinically in a Phase I trial in patients with advanced solid tumors. Thirty-nine patients with metastatic melanoma, colorectal cancer (CRC), castrate-resistant prostate cancer (CRPC), NSCLC, or RCC were enrolled [39]. Initial treatment entailed a single infusion of nivolumab in dose-escalating, six-patient cohorts at 0.3, 1, 3, or 10 mg/kg. This was followed by a 15-patient expansion cohort at the 10 mg/kg level. Patients who had shown clinical benefit at 3 months were eligible for repeated therapy. MTD was not reached. One durable complete response of greater than 21 months was noted in a patient with CRC and two partial responses were noted in a patient with melanoma and another with RCC. Two additional responses were noted in one melanoma and one NSCLC patient, but did not meet partial response criteria. Tumor biopsies were available from a few patients and PD-L1 expression appeared to correlate with response to therapy. A cut-off of 5% of tumor cells exhibiting membranous PD-L1 staining was used to define positivity for PD-L1 expression. Nivolumab was well tolerated, with one patient experiencing grade 3 inflammatory colitis treated with infliximab and steroids.

Nivolumab was further studied in another large Phase I study [40]. Patients were treated with escalating doses ranging from 1 to 10 mg/kg. Analysis of 304 patients, including expansion cohorts, revealed that nivolumab was well tolerated, with no MTD reached. Objective response rates for melanoma, NSCLC, and RCC were 31%, 16%, and 29%, respectively. No responses were noted in patients with CRC or CRPC. Responses were durable and lasted over 1 year. Updated results indicate that responses are durable in patients with NSCLC, with OS rates of 12–45% at 2 years [41]. Similarly, 2- and 3-year OS rates in patients with advanced melanoma were 48% and 41%, respectively [42]. Examination of PD-L1 expression revealed that responses were noted only in PD-L1-positive tumors (36%). Again, a cut-off of 5% of tumor cells staining positive for membranous PD-L1 expression by immunohistochemistry was used to define positivity. Grade 3 or 4 adverse events were noted in 15% of patients and 6% discontinued treatment. Adverse events with potentially immune-related etiology occurred in 45% of patients, being grade 3 or 4 in 6% of these. Three deaths due to pneumonitis were reported. Other studies of nivolumab have reported similar results, as detailed in Table 4.

Nivolumab was also studied in combination with ipilimumab in a Phase I trial [43]. The trial included a combined-regimen arm that enrolled 53 patients and a sequenced-regimen arm that enrolled 33. The MTD was determined to be at the 1 mg/kg nivolumab and 3 mg/kg ipilimumab dose level (cohort 2) and at 3 mg/kg for nivolumab and 1 mg/kg for ipilimumab (cohort 2a) in the combined regimen. Response rates were 40% in the combined regimen group and up to 53% in the MTD cohort. Complete responses were noted as well as 80% tumor reduction in 16 patients. Responses were durable, ranging from 6.1 to 72.1 weeks at the time of analysis. Treatment-related grade 3 or 4 adverse events were observed in 53% of patients and serious adverse events were reported in 49% of patients in the combined-regimen group; 21% of patients discontinued therapy due to adverse events. Adverse events were managed with immune suppressants, some requiring infliximab or mycophenolate mofetil. No

treatment-related deaths were reported. Regarding PD-L1 expression by tumor cells, 21 of 56 patients (38%) were positive for PD-L1 on immunohistochemistry, but this did not correlate with response. This study utilized a different anti-PD-L1 clone and a cut-off of 5% of tumor cells expressing PD-L1 was used to define positivity. Responses were noted in PD-L1-negative tumors.

Ongoing trials of nivolumab include Phase I trials in hematologic malignancies, hepatocellular carcinoma (HCC), and malignant melanoma, in combination with sunitnib, pazopanib, or ipilimumab in RCC, with ipilimumab in advanced solid tumors, in combination with IL-21 in advanced solid tumors, with a multipeptide vaccine in malignant melanoma, with lirilumab (anti-KIR; BMS-986015) in advanced solid tumors, and with dasatinib in relapsed chronic myelogenous leukemia (CML). Ongoing Phase II and Phase III trials with nivolumab are being conducted with or without ipilimumab versus bevacizumab in glioblastoma and with ipilimumab in malignant melanoma, compared with chemotherapy in NSCLC and malignant melanoma, and compared with everolimus in RCC.

Clinical trials of mAbs to PD-L1

BMS-936559 is a fully human monoclonal IgG4 antibody that blocks PD-L1 [44] (Table 4). This blockade was shown to augment T cell proliferation in response to allogeneic dendritic cells in a mixed lymphocyte reaction, as well as antigen-specific T cell responses to cytomegalovirus (CMV) antigen and antitumor peptide responses in subjects treated with melanoma antigen peptide vaccines. BMS-936559 can reverse *in vitro* Treg-mediated suppression. Use of BMS-936559 in clinical trials was supported by the ability of anti-PD-L1 antibodies to inhibit tumor growth in murine syngeneic tumor models and long-lived antitumor immunity was observed. BMS-936559 did not induce antibody-dependent cytotoxicity or complement-dependent cytotoxicity in PD-L1-positive cells. BMS-936559 was studied in a Phase I trial [44]. Patients with various solid tumors were enrolled, with disease-specific expansion cohorts enrolled in parallel and treated at the 10 mg/kg dose level. MTD was not reached and the discontinuation rate due to adverse events was 11%, 6% of which were treatment related. Objective responses were observed in patients treated with a dose of 1 mg/kg or higher. Responses were noted in patients with melanoma, NSCLC, RCC, or ovarian cancer but none were observed in CRC or pancreatic cancer. Treatment was well tolerated with the expected immune-related adverse events, which were mostly grade 1 or 2 and were managed by interruption of treatment or with glucocorticoids. Despite encouraging results, BMS-936559 does not seem to have been developed any further by the manufacturer.

MEDI4736 is a humanized PD-L1-blocking mAb [45] that showed T cell-dependent antitumor activity in an *in vivo* model where tumor cells were coimplanted with human T cells or when a surrogate antibody was used in a syngeneic mouse model (Table 4). Combination with oxaliplatin resulted in complete tumor regression in greater than 50% of treated animals. MEDI4736 is being studied in a Phase I trial in advanced solid tumors with expansion cohorts in several responding tumor types including NSCLC and melanoma [46,47]. It was noted to be well tolerated, with tumor shrinkage detectable in many tumor types. It is also being studied in combination with tremelimumab (a mAb to CTLA-4) in patients with advanced solid tumors [48] and with dabrafenib and trametinib or trametinib alone in patients with malignant melanoma.

Other mAbs to PD-L1 include MPDL3280A, which has shown impressive results shrinking tumors in 43% of patients in a Phase I clinical trial in metastatic urothelial bladder cancer [49] (http://www.roche.com/media/media_releases/med-cor-2014-05-31.htm) (Table 4). It has been granted FDA breakthrough designation due to its activity in urothelial bladder cancers. It has also shown acceptable tolerability and efficacy in several solid tumors, with no MTD reached [50]. Ongoing clinical trials include Phase I trials in advanced solid tumors and the phase II BIRCH trial and phase III OAK trial in NSCLC. MSB0010718C is another mAb to PD-L1. It is a fully human IgG1 and is expected to show antineoplastic activity by inhibiting the PD-1/PD-L1 interaction and by antibody-dependent cell-mediated cytotoxicity [51]. MSB0010718C is being studied in a Phase I trial in refractory malignancies.

Clinical trials of PD-L2 Ig fusion protein

As an alternative strategy to mAbs, AMP-224 (B7-DC-Ig) was developed as a chimeric fusion protein between the extracellular domain of PD-L2 and an Fc portion of IgG2a (http://www.prnewswire.com/news-releases/glaxosmithkline-and-amplimmune-form-global-strategic-collaboration-99938599.html). *In vivo* studies suggested that this fusion protein can ameliorate disease by inducing immune responses to pathogens. Furthermore, the murine form of AMP-224 can enhance the therapeutic efficacy of vaccination when combined with cyclophosphamide in a mouse model [52]. AMP-224 exerts its therapeutic effect via a mechanism distinct from the direct blocking of the PD-1/PD-L1 interaction. It is hypothesized that AMP-224 can deplete exhausted effector T cells that express high levels of PD-1 and the T cell pool is replenished with functional T cells [53]. Ongoing trials with AMP-224 include a Phase I trial with cyclophosphamide in advanced solid tumors that has shown no drug-related inflammatory adverse events other than infusion reactions, as well as preliminary tumor responses [53].

Concluding remarks

After success with ipilimumab in the treatment of malignant melanoma, the field of cancer immunotherapy continues to grow. Blockade of T cell inhibition allows restored antitumor immunity and has shown impressive results in clinical trials. Beyond the CTLA-4 pathway, T cell inhibition mediated by the PD-1/PD-L1 pathway is now the most studied and clinically developed cancer immunotherapy. Several mAbs to PD-1 or PD-L1 have been studied in Phase I trials and continue to be evaluated in Phase II and Phase III trials. These therapies have been well tolerated, with no MTD reached in most single-agent Phase I studies. Grade 3 or 4 adverse event rates have been generally acceptable, with low treatment-related discontinuation rates. Response rates have been impressive, particularly in melanoma, NSCLC, and RCC, with encouraging early reports from urothelial bladder cancer and platinum-resistant

Box 1. Outstanding questions

- Why are the response rates of anti-PD-1 and anti-PD-L1 variable among different cancers?
- Can clinical response biomarkers be identified and how can these be integrated into clinical practice?
- How can anti-PD-1 and anti-PD-L1 antibodies be integrated into current treatment regimens in upfront and relapsed settings?
- Does PD-1 expressed on immune cells other than T cells play a role in anti-PD-1/PD-L1 therapy?
- Can we develop small-molecule inhibitors of the PD-1/PD-L1 interaction?

ovarian cancer. PD-1/PD-L1 blockade has also shown efficacy in hematologic malignancies. What has been striking is the durability of responses. Potential biomarkers for efficacy of PD-1/PD-L1 blockade are being studied, to date mainly focusing on PD-L1 expression by the tumor. The data from most clinical studies are not yet mature, but preliminary data indicate that PD-L1 expression by tumor cells may correlate with higher response rate and PFS in some patients. It is encouraging that pembrolizumab has been granted accelerated FDA approval as second-line therapy in advanced melanoma. Integrating immunotherapy into current clinical practice remains to be studied and many outstanding questions remain (Box 1). The variability in response both by tumor subtype and by PD-L1 expression indicates that immune checkpoint inhibition remains a field that is open to study and that PD-1/PD-L1 blockade, like CTLA4 blockade before it, is only the beginning of immunomodulatory therapies.

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References

1 Keir, M.E. *et al.* (2008) PD-1 and its ligands in tolerance and immunity. *Annu. Rev. Immunol.* 26, 677–704
2 Ishida, Y. *et al.* (1992) Induced expression of PD-1, a novel member of the immunoglobulin gene superfamily, upon programmed cell death. *EMBO J.* 11, 3887–3895
3 Barber, D.L. *et al.* (2006) Restoring function in exhausted CD8 T cells during chronic viral infection. *Nature* 439, 682–687
4 Wherry, E.J. (2011) T cell exhaustion. *Nat. Immunol.* 12, 492–499
5 Hofmeyer, K.A. *et al.* (2011) The PD-1/PD-L1 (B7-H1) pathway in chronic infection-induced cytotoxic T lymphocyte exhaustion. *J. Biomed. Biotechnol.* 2011, 451694
6 Francisco, L.M. *et al.* (2010) The PD-1 pathway in tolerance and autoimmunity. *Immunol. Rev.* 236, 219–242
7 Francisco, L.M. *et al.* (2009) PD-L1 regulates the development, maintenance, and function of induced regulatory T cells. *J. Exp. Med.* 206, 3015–3029
8 Haxhinasto, S. *et al.* (2008) The AKT–mTOR axis regulates *de novo* differentiation of $CD4^+Foxp3^+$ cells. *J. Exp. Med.* 205, 565–574
9 Thibult, M.L. *et al.* (2013) PD-1 is a novel regulator of human B-cell activation. *Int. Immunol.* 25, 129–137
10 Nicholas, K.J. *et al.* (2013) B cell responses to HIV antigen are a potent correlate of viremia in HIV-1 infection and improve with PD-1 blockade. *PLoS ONE* 8, e84185
11 Dong, H. *et al.* (1999) B7-H1, a third member of the B7 family, co-stimulates T-cell proliferation and interleukin-10 secretion. *Nat. Med.* 5, 1365–1369
12 Freeman, G.J. *et al.* (2000) Engagement of the PD-1 immunoinhibitory receptor by a novel B7 family member leads to negative regulation of lymphocyte activation. *J. Exp. Med.* 192, 1027–1034
13 Latchman, Y. *et al.* (2001) PD-L2 is a second ligand for PD-1 and inhibits T cell activation. *Nat. Immunol.* 2, 261–268
14 Tseng, S.Y. *et al.* (2001) B7-DC, a new dendritic cell molecule with potent costimulatory properties for T cells. *J. Exp. Med.* 193, 839–846
15 Atefi, M. *et al.* (2014) Effects of MAPK and PI3K pathways on PD-L1 expression in melanoma. *Clin. Cancer Res.* 20, 3446–3457
16 Parsa, A.T. *et al.* (2007) Loss of tumor suppressor PTEN function increases B7-H1 expression and immunoresistance in glioma. *Nat. Med.* 13, 84–88
17 Butte, M.J. *et al.* (2007) Programmed death-1 ligand 1 interacts specifically with the B7-1 costimulatory molecule to inhibit T cell responses. *Immunity* 27, 111–122
18 Xiao, Y. *et al.* (2014) RGMb is a novel binding partner for PD-L2 and its engagement with PD-L2 promotes respiratory tolerance. *J. Exp. Med.* 211, 943–959
19 Zhang, X. *et al.* (2004) Structural and functional analysis of the costimulatory receptor programmed death-1. *Immunity* 20, 337–347
20 Cheng, X. *et al.* (2013) Structure and interactions of the human programmed cell death 1 receptor. *J. Biol. Chem.* 288, 11771–11785
21 Lin, D.Y. *et al.* (2008) The PD-1/PD-L1 complex resembles the antigen-binding Fv domains of antibodies and T cell receptors. *Proc. Natl. Acad. Sci. U.S.A.* 105, 3011–3016
22 Lazar-Molnar, E. *et al.* (2008) Crystal structure of the complex between programmed death-1 (PD-1) and its ligand PD-L2. *Proc. Natl. Acad. Sci. U.S.A.* 105, 10483–10488
23 Parry, R.V. *et al.* (2005) CTLA-4 and PD-1 receptors inhibit T-cell activation by distinct mechanisms. *Mol. Cell. Biol.* 25, 9543–9553
24 Zang, X. and Allison, J.P. (2007) The B7 family and cancer therapy: costimulation and coinhibition. *Clin. Cancer Res.* 13, 5271–5279
25 John, L.B. *et al.* (2013) Anti-PD-1 antibody therapy potently enhances the eradication of established tumors by gene-modified T cells. *Clin. Cancer Res.* 19, 5636–5646
26 Curran, M.A. *et al.* (2010) PD-1 and CTLA-4 combination blockade expands infiltrating T cells and reduces regulatory T and myeloid cells within B16 melanoma tumors. *Proc. Natl. Acad. Sci. U.S.A.* 107, 4275–4280
27 Ding, Z.C. *et al.* (2014) Immunosuppressive myeloid cells induced by chemotherapy attenuate antitumor $CD4^+$ T-Cell responses through the PD-1–PD-L1 Axis. *Cancer Res.* 74, 3441–3453
28 Deng, L. *et al.* (2014) Irradiation and anti-PD-L1 treatment synergistically promote antitumor immunity in mice. *J. Clin. Invest.* 124, 687–695
29 Berger, R. *et al.* (2008) Phase I safety and pharmacokinetic study of CT-011, a humanized antibody interacting with PD-1, in patients with advanced hematologic malignancies. *Clin. Cancer Res.* 14, 3044–3051
30 Hardy, B. *et al.* (1995) Activation of human lymphocytes by a monoclonal antibody to B lymphoblastoid cells; molecular mass and distribution of binding protein. *Cancer Immunol. Immunother.* 40, 376–382
31 Westin, J.R. *et al.* (2014) Safety and activity of PD1 blockade by pidilizumab in combination with rituximab in patients with relapsed follicular lymphoma: a single group, open-label, Phase 2 trial. *Lancet Oncol.* 15, 69–77
32 Armand, P. *et al.* (2013) Disabling immune tolerance by programmed death-1 blockade with pidilizumab after autologous hematopoietic stem-cell transplantation for diffuse large B-cell lymphoma: results of an international Phase II trial. *J. Clin. Oncol.* 31, 4199–4206
33 Patnaik, A. *et al.* (2012) Phase I study of MK-3475 (anti-PD-1 monoclonal antibody) in patients with advanced solid tumors. *ASCO Meeting Abstracts* 30, 2512
34 Hamid, O. *et al.* (2013) Safety and tumor responses with lambrolizumab (anti-PD-1) in melanoma. *N. Engl. J. Med.* 369, 134–144
35 Daud, A.I. *et al.* (2014) Antitumor activity of the anti-PD-1 monoclonal antibody MK-3475 in melanoma (MEL): correlation of tumor PD-L1 expression with outcome [abstract]. In *Proceedings of the 105th Annual Meeting of the American Association for Cancer Research*. Abstract nr CT104, AACR
36 Robert, C. *et al.* (2014) Anti-programmed-death-receptor-1 treatment with pembrolizumab in ipilimumab-refractory advanced melanoma: a randomised dose-comparison cohort of a Phase 1 trial. *Lancet* 384, 1109–1117

37 Wong, R.M. *et al.* (2007) Programmed death-1 blockade enhances expansion and functional capacity of human melanoma antigen-specific CTLs. *Int. Immunol.* 19, 1223–1234
38 Wang, W. *et al.* (2009) PD1 blockade reverses the suppression of melanoma antigen-specific CTL by $CD4^{+}CD25^{Hi}$ regulatory T cells. *Int. Immunol.* 21, 1065–1077
39 Brahmer, J.R. *et al.* (2010) Phase I study of single-agent anti-programmed death-1 (MDX-1106) in refractory solid tumors: safety, clinical activity, pharmacodynamics, and immunologic correlates. *J. Clin. Oncol.* 28, 3167–3175
40 Topalian, S.L. *et al.* (2012) Safety, activity, and immune correlates of anti-PD-1 antibody in cancer. *N. Engl. J. Med.* 366, 2443–2454
41 Brahmer, J.R. *et al.* (2014) Nivolumab (anti-PD-1, BMS-936558, ONO-4538) in patients (pts) with advanced non-small-cell lung cancer (NSCLC): survival and clinical activity by subgroup analysis. *ASCO Meeting Abstracts* 32, 8112
42 Hodi, F.S. *et al.* (2014) Long-term survival of ipilimumab-naive patients (pts) with advanced melanoma (MEL) treated with nivolumab (anti-PD-1, BMS-936558, ONO-4538) in a Phase I trial. *ASCO Meeting Abstracts* 32, 9002
43 Wolchok, J.D. *et al.* (2013) Nivolumab plus ipilimumab in advanced melanoma. *N. Engl. J. Med.* 369, 122–133
44 Brahmer, J.R. *et al.* (2012) Safety and activity of anti-PD-L1 antibody in patients with advanced cancer. *N. Engl. J. Med.* 366, 2455–2465
45 Beck, A. *et al.* (2011) 6th Annual European Antibody Congress 2010: November 29–December 1, 2010, Geneva, Switzerland. *MAbs* 3, 111–132
46 Lutzky, J. *et al.* (2014) A Phase 1 study of MEDI4736, an anti-PD-L1 antibody, in patients with advanced solid tumors. *ASCO Meeting Abstracts* 32, 3001
47 Segal, N.H. *et al.* (2014) Preliminary data from a multi-arm expansion study of MEDI4736, an anti-PD-L1 antibody. *ASCO Meeting Abstracts* 32, 3002
48 Callahan, M.K. *et al.* (2014) A Phase 1 study to evaluate the safety and tolerability of MEDI4736, an anti-PD-L1 antibody, in combination with tremelimumab in patients with advanced solid tumors. *ASCO Meeting Abstracts* 32, TPS3120
49 Powles, T. *et al.* (2014) Inhibition of PD-L1 by MPDL3280A and clinical activity in pts with metastatic urothelial bladder cancer (UBC). *ASCO Meeting Abstracts* 32, 5011
50 Herbst, R.S. *et al.* (2013) A study of MPDL3280A, an engineered PD-L1 antibody in patients with locally advanced or metastatic tumors. *ASCO Meeting Abstracts* 31, 3000
51 Heery, C.R. *et al.* (2014) Phase I open-label, multiple ascending dose trial of MSB0010718C, an anti-PD-L1 monoclonal antibody, in advanced solid malignancies. *ASCO Meeting Abstracts* 32, 3064
52 Mkrtichyan, M. *et al.* (2012) B7-DC-Ig enhances vaccine effect by a novel mechanism dependent on PD-1 expression level on T cell subsets. *J. Immunol.* 189, 2338–2347
53 Infante, J.R. *et al.* (2013) Clinical and pharmacodynamic (PD) results of a phase I trial with AMP-224 (B7-DC Fc) that binds to the PD-1 receptor. *ASCO Meeting Abstracts* 31, 3044
54 Sun, S. *et al.* (2014) $PD\text{-}1^{+}$ immune cell infiltration inversely correlates with survival of operable breast cancer patients. *Cancer Immunol. Immunother.* 63, 395–406
55 Muenst, S. *et al.* (2013) The presence of programmed death 1 (PD-1)-positive tumor-infiltrating lymphocytes is associated with poor prognosis in human breast cancer. *Breast Cancer Res. Treat.* 139, 667–676
56 Sfanos, K.S. *et al.* (2009) Human prostate-infiltrating $CD8^{+}$ T lymphocytes are oligoclonal and $PD\text{-}1^{+}$. *Prostate* 69, 1694–1703
57 French, J.D. *et al.* (2012) Programmed $death\text{-}1^{+}$ T cells and regulatory T cells are enriched in tumor-involved lymph nodes and associated with aggressive features in papillary thyroid cancer. *J. Clin. Endocrinol. Metab.* 97, E934–E943
58 Ahmadzadeh, M. *et al.* (2009) Tumor antigen-specific CD8 T cells infiltrating the tumor express high levels of PD-1 and are functionally impaired. *Blood* 114, 1537–1544
59 Chapon, M. *et al.* (2011) Progressive upregulation of PD-1 in primary and metastatic melanomas associated with blunted TCR signaling in infiltrating T lymphocytes. *J. Invest. Dermatol.* 131, 1300–1307
60 Matsuzaki, J. *et al.* (2010) Tumor-infiltrating NY-ESO-1-specific $CD8^{+}$ T cells are negatively regulated by LAG-3 and PD-1 in human ovarian cancer. *Proc. Natl. Acad. Sci. U.S.A.* 107, 7875–7880
61 Thompson, R.H. *et al.* (2007) PD-1 is expressed by tumor-infiltrating immune cells and is associated with poor outcome for patients with renal cell carcinoma. *Clin. Cancer Res.* 13, 1757–1761
62 Zhang, Y. *et al.* (2010) Programmed death-1 upregulation is correlated with dysfunction of tumor-infiltrating $CD8^{+}$ T lymphocytes in human non-small cell lung cancer. *Cell. Mol. Immunol.* 7, 389–395
63 Shi, F. *et al.* (2011) PD-1 and PD-L1 upregulation promotes $CD8^{+}$ T-cell apoptosis and postoperative recurrence in hepatocellular carcinoma patients. *Int. J. Cancer* 128, 887–896
64 Shi, S.J. *et al.* (2013) B7-H1 expression is associated with poor prognosis in colorectal carcinoma and regulates the proliferation and invasion of HCT116 colorectal cancer cells. *PLoS ONE* 8, e76012
65 Karim, R. *et al.* (2009) Tumor-expressed B7-H1 and B7-DC in relation to $PD\text{-}1^{+}$ T-cell infiltration and survival of patients with cervical carcinoma. *Clin. Cancer Res.* 15, 6341–6347
66 Nomi, T. *et al.* (2007) Clinical significance and therapeutic potential of the programmed death-1 ligand/programmed death-1 pathway in human pancreatic cancer. *Clin. Cancer Res.* 13, 2151–2157
67 Muenst, S. *et al.* (2014) Expression of programmed death ligand 1 (PD-L1) is associated with poor prognosis in human breast cancer. *Breast Cancer Res. Treat.* 146, 15–24
68 Maine, C.J. *et al.* (2014) Programmed death ligand-1 over-expression correlates with malignancy and contributes to immune regulation in ovarian cancer. *Cancer Immunol. Immunother.* 63, 215–224
69 Hamanishi, J. *et al.* (2007) Programmed cell death 1 ligand 1 and tumor-infiltrating $CD8^{+}$ T lymphocytes are prognostic factors of human ovarian cancer. *Proc. Natl. Acad. Sci. U.S.A.* 104, 3360–3365
70 Frigola, X. *et al.* (2011) Identification of a soluble form of B7-H1 that retains immunosuppressive activity and is associated with aggressive renal cell carcinoma. *Clin. Cancer Res.* 17, 1915–1923
71 Thompson, R.H. *et al.* (2006) Tumor B7-H1 is associated with poor prognosis in renal cell carcinoma patients with long-term follow-up. *Cancer Res.* 66, 3381–3385
72 Gao, Q. *et al.* (2009) Overexpression of PD-L1 significantly associates with tumor aggressiveness and postoperative recurrence in human hepatocellular carcinoma. *Clin. Cancer Res.* 15, 971–979
73 Azuma, K. *et al.* (2014) Association of PD-L1 overexpression with activating EGFR mutations in surgically resected non-small cell lung cancer. *Ann. Oncol.* 25, 1935–1940
74 Hino, R. *et al.* (2010) Tumor cell expression of programmed cell death-1 ligand 1 is a prognostic factor for malignant melanoma. *Cancer* 116, 1757–1766
75 Massi, D. *et al.* (2014) PD-L1 marks a subset of melanomas with a shorter overall survival and distinct genetic and morphological characteristics. *Ann. Oncol.* Published online September 15, 2014. (http://dx.doi.org/10.1093/annonc/mdu452)
76 Ohigashi, Y. *et al.* (2005) Clinical significance of programmed death-1 ligand-1 and programmed death-1 ligand-2 expression in human esophageal cancer. *Clin. Cancer Res.* 11, 2947–2953
77 Atkins, M.B. *et al.* (2014) Phase 2, multicenter, safety and efficacy study of pidilizumab in patients with metastatic melanoma. *ASCO Meeting Abstracts* 32, 9001
78 Garon, E.B. *et al.* (2014) Safety and clinical activity of MK-3475 in previously treated patients (pts) with non-small cell lung cancer (NSCLC). *ASCO Meeting Abstracts* 32, 8020
79 Felip, E. *et al.* (2012) Developmental therapeutics. *Ann. Oncol.* 23, ix152–ix174
80 Motzer, R.J. *et al.* (2014) Nivolumab for metastatic renal cell carcinoma (mRCC): results of a randomized, dose-ranging Phase II trial. *ASCO Meeting Abstracts* 32, 5009
81 Hamanishi, J. *et al.* (2014) Efficacy and safety of anti-PD-1 antibody (nivolumab: BMS-936558, ONO-4538) in patients with platinum-resistant ovarian cancer. *ASCO Meeting Abstracts* 32, 5511
82 Cho, D.C. *et al.* (2013) Clinical activity, safety, and biomarkers of MPDL3280A, an engineered PD-L1 antibody in patients with metastatic renal cell carcinoma (mRCC). *ASCO Meeting Abstracts* 31, 4505
83 Hamid, O. *et al.* (2013) Clinical activity, safety, and biomarkers of MPDL3280A, an engineered PD-L1 antibody in patients with locally

advanced or metastatic melanoma (mM). *ASCO Meeting Abstracts* 31, 9010

84 Spigel, D.R. *et al.* (2013) Clinical activity, safety, and biomarkers of MPDL3280A, an engineered PD-L1 antibody in patients with locally advanced or metastatic non-small cell lung cancer (NSCLC). *ASCO Meeting Abstracts* 31, 8008

85 Rizvi, N.A. *et al.* (2013) A Phase I study of nivolumab (anti-PD-1; BMS-936558, ONO-4538) plus platinum-based doublet chemotherapy (PT-doublet) in chemotherapy-naive non-small cell lung cancer (NSCLC) patients (pts). *ASCO Meeting Abstracts* 31, 8072

86 Antonia, S.J. *et al.* (2014) Nivolumab (anti-PD-1; BMS-936558, ONO-4538) and ipilimumab in first-line NSCLC: interim phase I results. *ASCO Meeting Abstracts* 32, 8023

87 Hammers, H.J. *et al.* (2014) Phase I study of nivolumab in combination with ipilimumab in metastatic renal cell carcinoma (mRCC). *ASCO Meeting Abstracts* 32, 4504

88 Weber, J.S. *et al.* (2013) Phase I/II trial of PD-1 antibody nivolumab with peptide vaccine in patients naive to or that failed ipilimumab. *ASCO Meeting Abstracts* 31, 9011