Antagonist Antibodies to PD-1 and B7-H1 (PD-L1) in the Treatment of Advanced Human Cancer

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Abstract

The immune suppressive molecule programmed death-1 (PD-1) is upregulated in activated T lymphocytes and inhibits T-cell function upon binding to its ligands B7-H1 (PD-L1, CD274) and B7-DC (PD-L2, CD273). Substantial experimental data from *in vitro* cell culture systems and animal models, and more recently from clinical trials, indicate that PD-1/PD-1-ligand interactions are a major mechanism of immune suppression within the tumor microenvironment. Initial clinical studies of antibodies directed against PD-1 and B7-H1 showed both an encouraging safety profile and remarkable antitumor activity in subsets of patients with metastatic disease, including malignancies—such as lung cancer—which were previously thought to be unresponsive to immunotherapy. Preliminary data have suggested a correlation between tumor membrane B7-H1 expression and clinical response to anti-PD-1 antibodies. Several key challenges remain to optimize development of PD-1/B7-H1 pathway blockade, including defining the biologic significance of all potential ligand–receptor interactions in the tumor microenvironment, developing more accurate predictive biomarkers of response, determining the breadth of activity in human malignancies, and developing rational combinations of therapy that address key mechanisms involved in positive and negative regulation of antitumor immune responses. *Clin Cancer Res; 19(5); 1021–34. ©2013 AACR.*

Introduction

Antigen-specific T-cell responses are controlled positively and negatively by costimulatory and coinhibitory molecules, respectively. Coinhibitory molecule signaling prevents inappropriately directed immunity and limits the size and duration of immune responses. Among the key coinhibitory molecules, broadly categorized as "checkpoint molecules," are CTL antigen-4 (CTLA-4), which controls early stages of T-cell activation, and programmed death-1 (PD-1; ref. 1). PD-1 (CD279) is a member of the B7-CD28 family that regulates T-cell activation, peripheral tolerance, and the prevention of bystander tissue damage during immune responses (2–4).

Induction and Expression of PD-1 and Its Counter-Receptors

PD-1, so named for its involvement in classical programmed cell death (1), is expressed on activated $CD4^+$ and $CD8^+$ T cells, natural killer (NK) T cells, B cells, and activated monocytes and dendritic cells (DC; ref. 4). PD-1 protein is not detectable on resting T cells but is found on the cell surface within 24 hours of T-cell activation (4). The known counter-receptors of PD-1, B7-H1 (also called PD-L1; ref. 5) and B7-DC (also called PD-L2; ref. 6)—both of which have been observed on cancer cells (7, 8)—have distinct expression profiles. Low levels of B7-H1 mRNA are found in virtually all normal tissues and cell types examined thus far (7). However, constitutive expression of B7-H1 cell-surface protein in normal tissues is rare and has been found [via immunohistochemistry (IHC)-based analysis] only in a fraction of tissue macrophages within lung, liver, tonsil, and placenta (9). These findings indicate the existence of 1 or more posttranscriptional mechanisms controlling B7-H1 cell-surface protein expression.

The biologic consequences of B7-H1 expression depend on cell membrane localization because it is presumed that B7-H1 is functional only when it ligates a counter-receptor. B7-H1 cell-surface protein can be induced by various inflammatory mediators, including IFN-α, -β, and -γ, bacterial lipopolysaccharide, granulocyte-macrophage colony-stimulating factor, VEGF, and the cytokines interleukin-4 (IL-4) and IL-10 (9–12). In particular, the IFN family of cytokines are potent inducers of B7-H1 mRNA and protein on cultured $B7\text{-}H1^-$ cells. In addition to binding PD-1, B7-H1 can also bind CD80 on activated T cells, thus inhibiting T-cell activation and production of cytokines (4).

B7-DC is expressed on myeloid dendritic cells, activated T cells, and some nonhematopoietic tissues (including lung;

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doi: 10.1158/1078-0432.CCR-12-2063

ref. 6), although only on a minority of patient tumors (6, 8, 13–15). Further studies are required to define the role of B7-DC expression, induction, and signaling on T-cell activation and function. Results from studies of B7-DC-knockout mice and *in vitro* studies have been inconsistent and show either increased or decreased response to antigens (14–16). These results are consistent with an as-yet unrecognized second receptor for B7-DC. Several studies in the literature have provided evidence for a preferential inhibitory role of B7-DC on Th2 responses (17), which in addition to the known binding between B7-H1 and CD80, could explain potential differences in clinical activity and toxicity of antibodies targeted against B7-H1 versus those directed against PD-1.

Role(s) of the PD-1/B7-H1 Pathway in Healthy Hosts

In a healthy host, PD-1 signaling in T cells regulates immune responses to minimize damage to bystander tissue and prevents the development of autoimmunity by promoting tolerance to self-antigens. Ligation of PD-1 results in the formation of PD-1/T-cell receptor (TCR) inhibitory microclusters that recruit SHP2 molecules, which dephosphorylate multiple members of the TCR signaling pathway, effectively turning off T-cell activation (18). Inhibition of RAS and PI3K/AKT pathways was also shown, resulting in downstream suppression of cell-cycle progression and T-cell activation (ref. 19; Fig. 1). PD-1 ligation by B7-H1 on macrophages, other antigen-presenting cells (APC), or endothelium inhibits production of several cytokines, including IFN-γ, IL-2 (which protects against T-cell apoptosis), and TNF-α (4, 13), and promotes T-cell apoptosis via inhibition of survival factor Bcl-xL (4).

B7-H1 can also serve as a receptor. It has been shown that reverse signaling through B7-H1 in T cells and dendritic cells regulates cytokine production and inhibits dendritic cell maturation and survival of activated T cells (20, 21). B7-H1 signaling also inhibits tumor cell apoptosis induced by antigen-specific $CD8^+$ T cells and in response to various stimuli (22). During chronic viral infection, B7-H1 expression in hematopoietic cells reduces the induction and functionality of virus-specific T-cell responses, whereas B7-H1 expressed in the nonhematopoietic compartment regulates tissue immunopathology and viral clearance (23). Thus, the functional consequences of B7-H1 interaction with its receptors are determined by the location of its expression.

Although PD-1 and its counter-receptors primarily control the function of effector cells, this pathway may also have a role in modulating T-cell priming (24). The varied regulatory roles of PD-1 signaling are supported by preclinical studies showing spontaneous, late-onset development of autoimmune disease in PD-1–deficient mice (25, 26). B7-H1–deficient mice do not display spontaneous autoimmunity (3, 27) but have increased T-cell accumulation in select peripheral organs and impaired apoptosis of $CD8^+$ T cells (27). Several reports also suggest that PD-1/B7-H1 signaling contributes to regulatory T cell (Treg) induction, maintenance, and suppressive function (28) via the downregulation of mTOR and AKT phosphorylation (29).

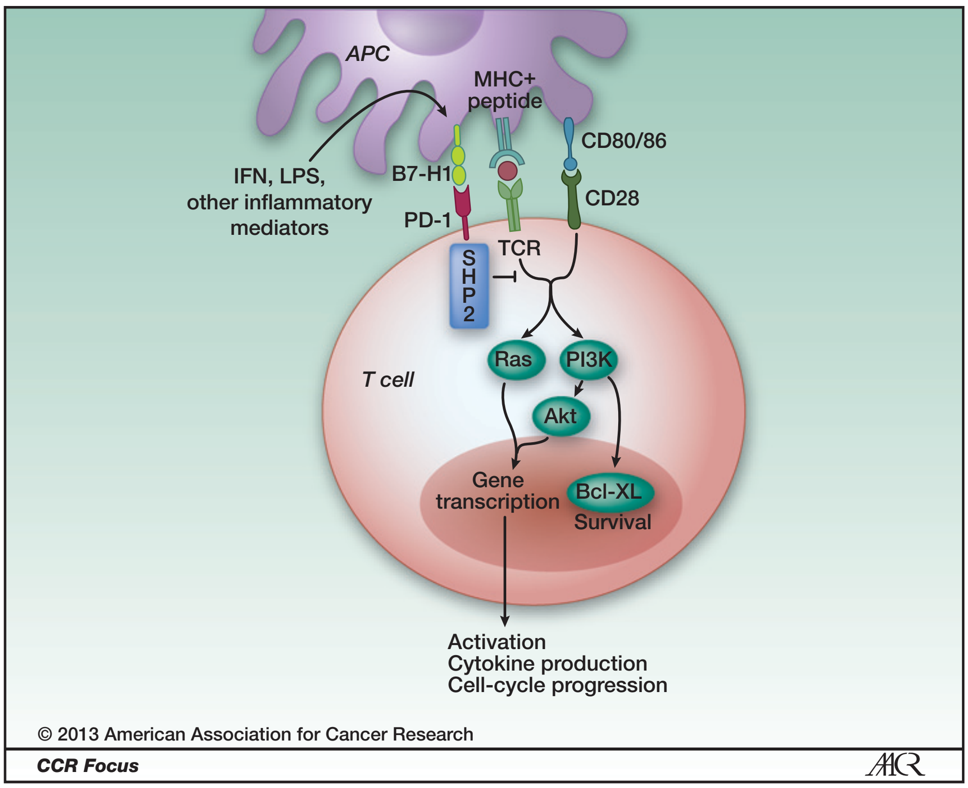

Figure 1. PD-1 signaling in inflamed tissue. Ligation of T-cell PD-1 by B7-H1 on APC leads to the formation of PD-1/TCR microclusters and the subsequent recruitment of SHP2 phosphatases, which dephosphorylate multiple members of the TCR signaling pathway. This abrogates downstream effects of T-cell activation, including cytokine production, cell-cycle progression, and the expression of survival proteins. By preventing T-cell activation, PD-1 signaling contributes to the maintenance of tolerance to self-antigens and prevents immune-mediated damage of healthy tissue during the resolution of infection and other inflammatory responses.

Preclinical Studies of PD-1/B7-H1 Blockade in Antitumor Therapy

Multiple *in vivo* mouse experiments showed tumor regression or prolonged host survival after abrogation of PD-1 pathway signaling alone (9, 30, 31) or in combination with other agents, including cancer vaccines (32–37). Tumor regression was accompanied by increased effector T-cell (Teff) function and cytokine production. However, the exact sequence of events within the tumor microenvironment that culminates in tumor regression after PD-1 pathway blockade is unknown. Although it is reasonable to assume that PD-1 pathway blockade reactivates PD-1–expressing tumor-infiltrating lymphocytes (TIL; Fig. 2), studies of T-cell function in chronic viral infection suggest that the population of T cells expressing high levels of PD-1 are "exhausted" and may not be reactivated with PD-1 blockade alone (38). Exhausted T cells result from chronic antigen exposure (caused by persistent viral infections or progressive tumor), express high levels of inhibitory molecules, have poor effector functionality and a distinct transcriptional profile, and may, in fact, constitute a discrete stage of T-cell differentiation. In patients with metastatic melanoma (mMEL), $CD8^+$ TIL can express several cell-surface molecules associated with exhaustion including TIM-3, LAG-3, and PD-1 (with some cells expressing all markers), which may be the cause of resistance to PD-1 pathway blockade in some patients and may provide the rationale for future combinations of checkpoint/coinhibitory molecule antagonists (39, 40). Recently, Mkrtichyan and colleagues reported that treating mice with a B7-DC-Ig fusion protein in combination with cyclophosphamide and a vaccine resulted in a significant decrease (via effects on B7-DC/PD-1 signaling and cell proliferation) in the number of $PD\text{-}1^{hi}$ $CD4^+$ and $CD8^+$ T cells within the tumor, allowing infiltration by and/or expansion of nonexhausted $PD\text{-}1^{lo}$ T cells into the tumor microenvironment (41). Whether this phenomenon occurs in human patients as a result of administration of the other PD-1 pathway-targeted immunotherapies presented later also remains to be determined.

Expression and Prognostic Significance of PD-1, B7-H1, and B7-DC in Human Cancer

B7-H1 is expressed by various tumor tissues and high PD-1 expression is often present on TILs. Peripheral blood $CD4^+$ and $CD8^+$ T cells in patients with cancer can also express PD-1, in some cases in a large percentage of cells, which makes it unlikely that $PD\text{-}1^+$ T cells exclusively represent tumor-specific T cells (42). These findings are consistent with recent data showing that PD-1 can be upregulated by common γ-chain cytokines including IL-2, IL-7, IL-15, and IL-21 (43).

Table 1 summarizes PD-1, B7-H1, and B7-DC expression in several different malignancies and correlates expression with patient prognosis. Most large retrospective studies show that B7-H1 expression correlates with poor prognosis and/or more aggressive disease; however, several reports indicate a lack of association (44–46) or even that B7-H1 expression is associated with improved survival and influx of lymphocytes into the tumor microenvironment (47). Although there may be biologic reasons to explain the discrepant outcomes, reviews of the literature correlating B7-H1 expression with prognosis should nevertheless be interpreted with caution, because of the heterogeneity in expression within tumor tissue, the requirement to assess membrane B7-H1 protein rather than intracellular protein or mRNA, the lack of specificity of several commercially available antibodies, and the substantial difficulty in developing reagents and methods for detection of B7-H1 expression in formalin-fixed, paraffin-embedded tissue (FFPE). B7-H1 protein contains only 2 small linear hydrophilic

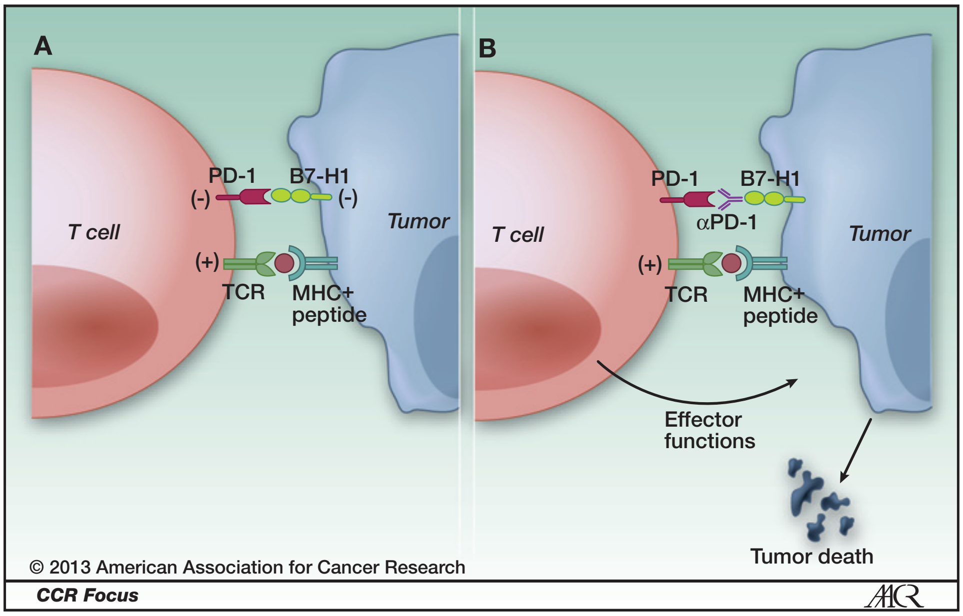

Figure 2. PD-1 and cancer. A, ligation of T-cell PD-1 by tumor B7-H1 results in the downregulation of T-cell effector functions that destroy tumor tissue. B, blockade of this pathway by anti-PD-1 antibodies prevents this downregulation, and allows T cells to maintain their antitumor functionality and ability to mediate tumor cell death.

Table 1. PD-1, B7-H1, and B7-DC expression and prognostic significance in cancer patients

Disease	Citation	n	Detection method/Ab clones	Location of PD-L Expression	Notes on T-cell Infiltrate	Pathologic Observations	Prognosis
Malignant brain tumors	(80)	83	FACS; anti-PD-1, B7-H1, and B7-DC (BD Pharmingen)	N/A	Variable; 6 of 23 tumors with TIL had PD-1^+ $CD4^+$ lymphocytes, with PD-1 expression significantly higher than on the intratumoral Tregs of these patients ($P = 0.005$)	61% of brain tumors (but no WHO grade 1 tumors) expressed PD-L1 and none expressed PD-L2	N/A
Cervical cancer	(44)	115	Paraffin IHC; anti-B7-H1 (5H1), B7-DC and PD-1 (R&D)	PD-L1/2 on tumor cell membrane and throughout tumor bed	PD-L1 expression was associated with higher intraepithelial infiltration by $Foxp3^+$ T cells ($P = 0.022$) but not with $CD8^+$ T cells, and more intraepithelial PD-1^+ T cells (not significant)	19% of tumor samples expressed PD-L1 and 29% expressed PD-L2; in patients with PD-$L1^+$ or PD-$L1^-$ tumors, more than half of the infiltrating $CD8^+$ T cells and half of the $Foxp3^+$ T cells expressed PD-1; PD-L2 expression did not correlate with prognosis but did correlate with adenocarcinoma subtype ($P = 0.012$) and more advanced disease ($P = 0.013$)	No direct effect of PD-L1 expression on prognosis; OS of patients with PD-$L1^+$ tumors and a low $CD8^+$/$FoxP3^+$ T-cell ratio was better than in patients with a PD-$L1^-$ tumor and a low $CD8^+$/$FoxP3^+$ T-cell ratio ($P = 0.033$)
Pancreatic cancer	(50)	51	Frozen IHC; anti-B7-H1 (MIH1); anti-B7-DC (MIH18)	B7-H1 and B7-DC were expressed in plasma membrane and cytoplasm of cancer cells; also found in some TILs and stromal cells	PD-L1 expression was inversely correlated with TILs ($CD4^+$ T cells, $P = 0.019$; $CD8^+$ T cells, $P < 0.0001$)	20 of 51 tumor samples were B7-$H1^+$ and 14 of 51 tumor samples were B7-DC^+; no correlation between tumor B7-H1 status and tumor/nodal/metastatic status or pathologic stage	B7-$H1^+$ patients had poorer prognosis than the B7-$H1^-$ negative patients ($P = 0.016$); no correlation of tumor B7-DC expression with patient survival; B7-H1 was an independent prognostic factor ($P = 0.022$)
Urothelial cancer	(81)	65	Frozen IHC; anti-B7-H1 (MIH1)	B7-H1 present on plasma membrane and/or cytoplasm of urothelial cancer cells in a focal pattern	In 13 cases selected for examination, most TILs expressed high levels of PD-1	B7-H1 expression correlated with WHO grade ($P < 0.001$) and primary tumor classification ($P = 0.031$); no association between B7-H1 expression and primary node or stage classification	Increased B7-H1 expression was associated with poor survival ($P = 0.021$) and increased likelihood of postresection recurrence ($P = 0.026$)
Gastric cancer	(82)	102	Paraffin IHC; anti-B7-H1 (2H11)	B7-H1 was expressed predominantly in the cytoplasm; some nuclear membrane localization was also present	N/A	42.2% of gastric carcinoma tissues were B7-$H1^+$; B7-H1 correlated with tumor size, invasion, and lymph node metastasis ($P < 0.01$, 0.05, 0.01, respectively)	B7-H1 expression was an independent prognostic factor ($P = 0.040$) and correlated with reduced patient survival ($P < 0.01$)
Esophageal cancer	(49)	41	Frozen IHC, mRNA analysis; anti-B7-H1 (MIH1), anti-B7-DC (MIH18)[c]	Cytoplasmic or membranous	PD-L2 mRNA expression inversely correlated with presence of tumor-infiltrating $CD8^+$ T cells ($P = 0.011$)	N/A	PD-L1 and PD-L2 mRNA expression were associated with decreased OS ($P = 0.025$ and $P = 0.003$, respectively) and were independent predictors of worse prognosis
RCC	(48)	306	Paraffin IHC; anti-B7-H1 (5H1)	Tumors were considered B7-$H1^+$ if ≥5% of tumor cells had cell-surface staining	N/A	B7-$H1^+$ tumors were associated with 2002 TNM stage III or IV, tumor size of ≥5 cm, nuclear grade 3 or 4, and coagulative tumor necrosis (all $P < 0.001$)	Patients with B7-$H1^+$ tumors had increased risk of death from RCC [risk ratio (RR), 3.92; $P < 0.001$] and overall mortality (RR, 2.37; $P < 0.001$), and decreased 5-year survival (41.9% for patients with B7-$H1^+$ tumors vs. 82.9% for patients with B7-$H1^-$ tumors)

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Table 1. PD-1, B7-H1, and B7-DC expression and prognostic significance in cancer patients (Cont'd)

Disease	Citation	n	Detection method/Ab clones	Location of PD-L Expression	Notes on T-cell Infiltrate	Pathologic Observations	Prognosis
NSCLC	(83)	109	Paraffin IHC; anti-B7-H1 (clone not specified)	PD-L1 on membrane and in cytoplasm of tumor cells, in cluster and scattered patterns within tumors	$CD1a^+$ TIDC were increased in PD-$L1^+$ sections of tumor[a] and had higher expression of PD-L1 than $CD83^+$ DC	PD-$L1^+$ cells in adenocarcinoma were more numerous than those in squamous cell carcinoma (65.2% vs 44.4%, $P = 0.032$)	PD-L1 positivity correlated with survival shorter than 3 years after lobectomy ($P = 0.034$)
	(45)	52	Frozen IHC; anti-B7-H1 (MIH1), anti-B7-DC (MIH14)	Cytoplasmic, membranous, or cytoplasmic and membranous B7-H1 and B7-DC staining was observed in focal or scattered patterns in all 52 specimens of NSCLC	There were fewer TILS overall and fewer PD-1^+ TILs in B7-$H1^+$ regions of tumor than in B7-$H1^-$ regions of tumor ($P = 0.01$, 0.02) in a subset of 5 patients	No correlation of B7-H1 or B7-DC expression with clinicopathologic characteristics	No correlation between B7-H1 or B7-DC expression and patient survival
Glioma	(84)	10	Frozen IHC; anti-B7-H1 (5H1)	B7-H1 expression was detected in all 10 glioma samples examined; B7-$H1^+$ cells were scattered evenly throughout the specimens[b]	N/A	N/A	N/A
Hepatocellular carcinoma	(51)	240 and 125	Paraffin IHC; anti-B7-H1 (eBioscience) and B7-DC (R&D)	Both ligands present on membrane and/or cytoplasm of tumor cells, in scattered (most cases) or focal patterns	Positive correlation between B7-H1 expression and $FoxP3^+$ Treg infiltration ($P = 0.009$) as well as between B7-DC expression and Treg infiltration ($P = 0.002$)	B7-$H1^+$ patients harbored more tumors with vascular invasion, whereas B7-DC^+ patients had more tumor vascular invasion and advanced TNM stage	Patients with PD-$L1^+$ tumors had poorer DFS and OS than patients with PD-$L1^-$ tumors; tumor PD-L1 status was an independent prognostic factor for DFS, and PD-$L1^+$ patients were nearly 2 times more likely to suffer from relapse after resection than PD-$L1^-$ patients
	(85)	26	Frozen IHC; anti-PD-1 (J116), B7-H1 (MIH1), and B7-DC (MIH18, eBiosciences)	Focal or scattered	PD-1^+ T cells accumulated within tumors and in peritumoral areas	PD-L expression was restricted mainly to Kupffer cells and liver sinusoidal endothelial cells; 24 of 26 HCC specimens expressed PD-L1, whereas 23 of 26 expressed PD-L2; PD-L1 expression was associated with earlier tumor stage ($P = 0.018$)	N/A
	(86)	56	Paraffin IHC, FACS; anti-PD-1 (R&D), B7-H1 (Biolegend); FACS with PE-conjugated PD-1 and B7-H1 (eBiosciences)	Seems to be both cytoplasmic and membranous[b]	PD-1 expression was increased on TILs in comparison with PBMC and noninfiltrating lymphocytes ($P <$ 0.001, 0.001, respectively)	$CD8^+$ T cells were mainly distributed around the PD-$L1^+$ portion of tumor nest	Patients with high levels of intratumoral PD-1^+ $CD8^+$ T cells had shorter DFS than those with low levels ($P < 0.001$)
Melanoma	(87)	59	Paraffin IHC; anti-B7-H1 (clone 27A2, MBL)	N/A	In 2 patients, PD-1 expression on $CD8^+$ cells increased as disease progressed	BTT in the PD-$L1^{hi}$ expression group was higher than in the PD-$L1^{lo}$ expression group ($P = 0.0298$); T3–T4 tumors had higher PD-L1 expression than T0–T2 tumors ($P = 0.0072$); PD-L1 expression in primary tumors from patients with LN metastasis was higher than that in patients without LN metastasis ($P = 0.0375$); PD-L1 expression in metastatic LNs was higher than in nonmetastatic LNs ($P < 0.0001$)	OS and PFS rate were lower in the PD-$L1^{hi}$ expression group compared with the PD-$L1^{lo}$ expression group ($P = 0.0402$, 0.0522 respectively), indicating that PD-L1 expression is an independent predictor of OS and DFS

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Table 1. PD-1, B7-H1, and B7-DC expression and prognostic significance in cancer patients (Cont'd)

Disease	Citation	n	Detection method/Ab clones	Location of PD-L Expression	Notes on T-cell Infiltrate	Pathologic Observations	Prognosis
	(47)	150	Paraffin IHC; anti-B7-H1 mAb (5H1) or anti-B7-H1 polyclonal Ab (4059, ProSci)	Membranous PD-L1 expression by melanocytes within the tumors had 3 patterns: no PD-L1; regional expression of PD-L1 on melanocytes colocalized with TILs (most common); and PD-L1 expression in the absence of TILs	Almost all PD-L1^{+} tumors were associated with TILs, whereas only 28% of PD-L1^{-} tumors were associated with TILs; a sample of PD-L1^{+} tumors with TIL were shown to contain IFN-γ, whereas no PD-L1^{-} tumors examined contained IFN-γ	PD-L1 was expressed on a proportion (57 of 150) of various MEL lesions, most commonly in close juxtaposition to TILs; when an inflammatory response to the tumor was detected, it was likely that both the tumor and infiltrating cells were PD-L1^{+}; PD-L1 expression was associated with the superficial spreading and nodular MEL subtypes ($P = 0.033$) and not with MEL stage	Patients with PD-L1^{+} mMEL had longer survival than those with PD-L1^{-} mMEL ($P = 0.032$); patients with mMEL with TILs had significantly improved survival compared with those without TILs ($P = 0.017$)
	(88)	35	Paraffin IHC, FACS; anti-B7-H1 3.1 (D Olive); FACS with B7-H1, B7-DC, PD-1 mAbs (BD Pharmingen)	B7-DC was present predominantly on myeloid cells, whereas B7-H1 was present on the surface of tumor cells, myeloid cells, or both	PD-1 expression was upregulated on T-cell populations extracted from primary tumors and metastases	66% of mMEL biopsies were PD-L2^{+}, whereas 58% of mMEL biopsies were PD-L1^{+}	N/A
Head and neck squamous cell carcinoma (HNSCC)	(89)	24	Frozen IHC; anti-B7-H1 (5H1)	11 of 24 specimens had intracytoplasmic staining, 11 of 24 tumors had membrane reactivity; 10 of 24 had both	N/A	16 of 24 specimens (66%) had PD-L1 staining	N/A
	(90)	N/A	Anti-B7-H1		Majority of CD8^{+} TILs in HPV-HNSCC express PD-1	Association between expression of PD-L1 on tumor cells and tumor-associated macrophages with the presence of TILs; tumor-cell PD-L1 and CD68^{+} APCs were found at the periphery of tumor beds in opposition to fronts of TILs	N/A
Leukemia	(91)	30	FACS, functional assays, frozen IHC; anti-B7-H1 (5H1)	17/30 samples of human leukemia cells were B7-H1^{+}	N/A	N/A	N/A
Various	(57)	42	Paraffin IHC; anti-B7-H1 (5H1)	25 of 42 patients had tumor-cell surface PD-L1 staining	N/A	N/A	Of the 25 patients with PD-L1^{+} tumors, 9 experienced an objective response after treatment with anti-PD-1 antibody
	(9)	85	Frozen IHC; anti-B7-H1 (5H1)	B7-H1 present on majority of melanoma and carcinoma tissue and a few pulmonary macrophages in LC samples; B7-H1 observed in the plasma membrane, cytoplasm or both; in most cases, B7-H1 was expressed in a focal pattern	N/A	N/A	N/A
	(13)	130	FACS, functional assays, frozen and paraffin IHC; anti-B7-H1 (29E.2A3, 29E.5A9 for FACS, functional assays and frozen IHC; 29E.2A3 for paraffin IHC)	B7-H1 present on high percentage of thymic neoplasms, multiple carcinomas, and primary T-cell lymphomas but not B-cell non-Hodgkin lymphoma[b]	N/A	N/A	N/A

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Table 1. PD-1, B7-H1, and B7-DC expression and prognostic significance in cancer patients (Cont'd)

Disease	Citation	n	Detection method/Ab clones	Location of PD-L Expression	Notes on T-cell Infiltrate	Pathologic Observations	Prognosis
Ovarian cancer	(52)	70	Paraffin IHC; anti-PD-1 (NAT, Abcam), anti-B7-H1 (27A2, MBL), anti-B7-DC (polyclonal, R&D)	N/A	N/A	TIL PD-1 expression correlated with $CD8^+$ ($P = 0.002$), $CD4^+$ ($P = 0.011$), and $CD57^+$ cell infiltration ($P = 0.002$) in the tumor; negative correlation between $CD8^+$ cell infiltration and PD-L1 tumor expression; PD-L2 tumor expression was associated with $FoxP3^+$ cell infiltration	High PD-L1 expression was an independent negative prognostic factor; patients with tumors with high immune cell infiltration (characterized by high PD-1, high $CD4^+$ and $CD8^+$ expression, among others) had better 5-year survival than patients with tumors without high TIL infiltration (84.6% vs. 55.2%, $P = 0.041$)
	(12)	N/A	FACS, functional assays; anti-B7-H1 (BD Pharmingen for FACS, 5H1 for functional assays)	B7-H1 present on nearly all myeloid dendritic cells from tumor ascites and from tumor-draining lymph nodes	N/A	N/A	N/A
Prostate cancer	(92)	7	FACS; anti-PD-1 (A. Korman, Medarex, Inc.)	N/A	$CD8^+$ T-cell PD-1 was upregulated on cells infiltrating the prostate gland of patients with cancer (compared with those in peripheral blood, $P < 0.0001$); in some, almost 90% of prostate-infiltrating $CD8^+$ T cells were $PD\text{-}1^+$	N/A	N/A
Multiple myeloma	(67)	82	FACS, Western blot analysis, mRNA analysis; anti-B7-H1 (M1H1 for FACS, N20 for Western blot analysis)	B7-H1 present in most multiple myeloma plasma cell samples examined	N/A	N/A	N/A
Breast cancer	(93)	44	FACS, frozen IHC; anti-B7-H1 (MIH1, eBiosciences)	B7-H1 present in 22/44 breast cancer tissues examined (15/44 in tumor cells, 18/44 in TIL); staining was both membranous and cytoplasmic	N/A	Intratumoral B7-H1 expression was associated with histologic grade III–negative ($P = 0.012$), estrogen receptor–negative ($P = 0.036$), and progesterone receptor–negative ($P = 0.040$) patients; TIL B7-H1 was associated with large tumor size ($P = 0.042$), histologic grade 3 ($P = 0.015$), positivity of Her2/neu status ($P = 0.019$), and increased tumor lymphocyte infiltration ($P = 0.001$)	N/A
Bladder cancer	(94)	280	Frozen IHC; anti-B7-H1 (5H1)	B7-H1 present in 28% of specimens examined; sample considered positive if ≥1% of tumor cells had membrane staining	B7-H1 expression associated with presence of TIL ($P = 0.004$)	B7-H1 expression associated with high-grade tumors ($P = 0.009$)	N/A

NOTE: Table does not include discussion of PD-1 on circulating T cells in these patients.

Abbreviations: BTT, Breslow tumor thickness; DFS, disease-free survival; FACS, fluorescence-activated cell sorting; HCC, hepatocellular carcinoma; LN, lymph node; N/A, not applicable; OS, overall survival; PBMC, peripheral blood mononuclear cells; TIDC, tumor-infiltrating dendritic cells; TNM, tumor–node–metastasis; WHO, World Health Organization.

[a]No significance measurement.

[b]Report does not distinguish cytoplasmic from membrane staining.

[c]Because of positive correlations between protein and mRNA, clinicopathologic correlations in this report were based on mRNA expression data.

regions, and therefore, a limited number of antibody-binding sites (5). Because FFPE tumor tissue is generally available in patients with metastatic cancer but frozen tissue is not, a customized protocol was subsequently developed to renature B7-H1 in FFPE tissues (47, 48) permitting reproducible and specific immunohistochemical staining of B7-H1 with the 5H1 monoclonal antibody (mAb; ref. 9).

Thus far, only a small number of studies have examined the prognostic significance of B7-DC expression on tumor tissue and other cells within the tumor microenvironment. Tumor-cell B7-DC seems to be a negative predictor of survival for patients with esophageal carcinoma (49); other studies have identified either no relationship (50) or a correlative trend (although not significant) between B7-DC expression and reduced survival (51, 52). It should be noted that in many of these studies, B7-DC was expressed by only a minority of patient tumors (8).

Clinical Development of Antibodies Targeting the PD-1/B7-H1 Pathway

In human *ex vivo* studies of $CD4^+$ or $CD8^+$ T-cell activation, addition of an anti-B7-H1 or anti-PD-1 antibody augmented T-cell expansion and proliferation, increased cytokine production, and enhanced cytolytic activity (9, 53–55; Fig. 2). These data supported the clinical development of human antibodies blocking either PD-1 or B7-H1, several of which are currently being evaluated in clinical trials.

Nivolumab (MDX-1106, BMS-936558), a fully human monoclonal immunoglobulin (Ig)G4 antibody that binds PD-1 with high affinity and blocks its interaction with both B7-H1 and B7-DC, was initially evaluated in a standard phase I dose-escalation trial in which patients received intravenous doses from 0.3 to 10 mg/kg (56). Patients could be retreated with up to 2 courses, each course consisting of 2 doses spaced 4 weeks apart, at 12-week intervals. Among 39 patients, including 21 treated at 10 mg/kg, treatment produced a complete response (CR) lasting more than 21 months in a patient with colorectal cancer, partial responses in a patient with melanoma and a patient with renal cancer, and mixed responses in a patient with lung cancer and a patient with melanoma. PD-1 receptor occupancy was maintained beyond 60 days at all dose levels and beyond the time when drug levels were no longer detectable in the blood. In a subsequent, much larger phase I trial accruing 304 patients (as of July 2012), nivolumab was administered in doses ranging from 0.1 to 10 mg/kg i.v. every 2 weeks (57, 58). Small expansion cohorts were accrued in metastatic renal cell carcinoma (mRCC), melanoma, non–small cell lung carcinoma (NSCLC), prostate, and colorectal cancers. Including all dose levels, at the time of data analysis, objective responses were observed in 33 of 106 (31%), 20 of 122 (16%), and 10 of 34 (29%) of previously treated patients with mMEL, NSCLC, and mRCC, respectively. In NSCLC, objective response rate (ORR) and progression-free survival (PFS) rate at 24 weeks appeared higher at the 3- and 10-mg/kg dose levels compared with 1 mg/kg, but there was no clear dose–response relationship for activity in other diseases or for toxicity across the trial. No objective responses were observed in patients with prostate or colorectal cancer. Objective responses were durable (>6 months) in the majority of patients and several with sufficient follow-up remain progression-free even beyond 2 years. Thirteen patients showed nonconventional patterns of tumor response (such as prolonged reduction of tumor burden in the presence of new lesions) that were suggestive of clinical benefit but were not included in the calculation of ORR.

Nivolumab was well tolerated at all dose levels. Common drug-related adverse events included pruritus, rash, diarrhea, fatigue, nausea, and decreased appetite. Only 14% of the patients developed grade 3 or 4 drug-related adverse events. Pneumonitis was observed in 3% of all patients and 3 (2 NSCLC and 1 RCC; 1%) died from grade 3 or 4 pneumonitis-related events, leading to more proactive implementation of an early identification and intervention protocol including monitoring and administration of glucocorticoids. Gastrointestinal and hepatic adverse events were managed with treatment interruption or corticosteroids as necessary and endocrine adverse events were managed with replacement therapy, similar to the algorithms developed for management of ipilimumab immune-related adverse events. In a subset of 42 patients in which pretreatment paraffin-embedded tumor tissue was assessed for B7-H1 expression using the 5H1 antibody, no patients with $B7\text{-}H1^-$ tumors experienced an objective response, whereas 9 of 25 (36%) patients with $B7\text{-}H1^+$ tumors (defined as $\geq$5% membrane expression) had an objective response ($P = 0.006$). Although these data support a role for tumor cell B7-H1 expression as a potential predictive biomarker of response to PD-1 pathway blockade, further confirmation will be required in larger patient populations.

Two other PD-1 antagonist antibodies are currently being evaluated in clinical trials. MK-3475, a humanized IgG4 with high affinity for PD-1, was well tolerated in doses ranging from 1 to 10 mg/kg administered i.v. 4 weeks apart and subsequently every 2 weeks (59). IL-2 production from *ex vivo* stimulated lymphocytes was increased at all 3 dose levels. No grade $\geq$3 drug-related adverse events were observed, although 1 patient developed pneumonitis-requiring corticosteroids. For the 13 patients with melanoma treated in the dose-escalation phase and 10-mg/kg expansion cohort, 7 confirmed or unconfirmed objective responses were observed. One of 4 patients with NSCLC also achieved a partial response (unconfirmed at the time of the presentation). Cohorts of patients with melanoma were subsequently treated at 10 mg/kg i.v. every 2 to 3 weeks or 2 mg/kg every 3 weeks (60). Among 83 evaluable patients at the time of presentation, including 25 previously treated with ipilimumab, the ORR was 47% and 40% in patients with prior ipilimumab therapy. Although follow-up was relatively short, responses were ongoing in most patients. The latter data confirmed the high rate of activity in mMEL observed with nivolumab and also showed substantial activity of anti-PD-1 even in patients with prior exposure to another checkpoint inhibitor (anti-CTLA-4). It remains unclear if the higher reported rates of response for MK-3475 are due to differences in patient selection or the antibody characteristics.

Another anti-PD-1 mAb, CT-011, is a humanized IgG1 antibody shown to enhance human NK and T-cell function *in vitro*. In a phase I trial in advanced hematologic malignancies examining doses from 0.2 to 6 mg/kg, only 1 patient experienced drug-related adverse events, and antitumor activity was observed in 1 patient with follicular lymphoma and 1 patient with acute myelogenous leukemia (AML; ref. 61). Development of CT-011 has been focused primarily in hematopoietic malignancies and in combination with select chemotherapies, although a large, single-agent phase II trial is being conducted in patients with mMEL. In addition, a phase I trial of a third PD-1 antagonist, the B7-DC-Fc fusion protein AMP-224, is ongoing.

Other antibodies blocking B7-H1 are also in clinical development. BMS-936559, a high-affinity human IgG4 that blocks B7-H1 binding to PD-1 and CD80, was evaluated at doses ranging from 0.3 to 10 mg/kg administered i.v. every 2 weeks (62). Among the 207 enrolled patients with advanced cancer, only 9% developed grade 3 or 4 treatment-related adverse events, similar to those observed with nivolumab. Across all dose levels and expanded cohorts, objective responses were observed in 9 of 52 (17%) patients with mMEL, 2 of 17 (12%) with mRCC, 5 of 49 (10%) with NSCLC, and 1 of 17 (6%) with ovarian cancer. A subset of responses was durable. Although the activity of this drug seems to be less than that observed with nivolumab, sample sizes were smaller and the patient populations were heterogeneous. It is also possible that lower response rates were caused by differences in the antibodies administered or in the functions of their molecular targets. Data from the phase I trial of anti-B7-H1 antibody MPDL3280A/RG7446 are not yet publicly available. Recently, Medimmune, the Ludwig Institute for Cancer Research (New York, NY), and the Cancer Research Institute (New York, NY) announced a partnership to pursue development of multiple anticancer immunotherapies, including the anti-B7-H1 mAb MEDI4736 (63). Table 2 presents ongoing trials with PD-1 pathway-targeted agents.

Perspectives and Future Directions

The promising clinical data available for agents blocking PD-1 and B7-H1 validate the PD-1/B7-H1 pathway as a critical anticancer and immunotherapy target and extend the potential for immunotherapy activity beyond melanoma and RCC to other solid tumors. ORRs in melanoma, RCC, and NSCLC for the anti-PD-1 antibody nivolumab and in melanoma for MK-3475 were sufficiently high to pursue registration trials as a single agent and phase I to III trials of more traditional empiric combinations with other approved chemotherapy or targeted agents. In the absence of definitive predictive biomarkers or clear understanding of tumor–host immune relationships in most malignancies (including the role of B7-DC), the activity observed to date may merit empiric phase II exploration of PD-1 pathway blockade in multiple types of solid tumors. Preclinical data showing the expression of PD-1 on B- and T-cell lymphomas, the role of B7-H1/B7-DC in blunting graft-versus-leukemia effect in a murine model of chronic phase chronic myeloid leukemia (64), and the role of the pathway in suppressing CTL activity in a murine leukemia model (65) also support exploration of PD-1 pathway-targeted agents in hematologic malignancies, and indeed, preliminary evidence of activity was observed with the IgG1 CT-011.

There are valid reasons to expect that differences will emerge in activity or toxicity of the different PD-1/B7-H1 antagonists, because of differences in binding affinity, target, or antibody isotype. Antibody isotype can influence the ability to mediate antibody- and/or complement-dependent cell-mediated cytotoxicity [antibody-dependent cell-mediated cytotoxicity (ADCC), complement-dependent cellular cytotoxicity (CDCC)]. An IgG1 anti-PD-1 antibody that mediates strong ADCC or CDCC could result in the death of PD-1–expressing T cells, thus eliminating the cell population responsible for mediating antitumor effects. Conversely, ADCC and CDCC by an anti-PD-1 might be advantageous in eliminating intratumoral Treg or malignant hematopoietic cells expressing PD-1, and could also be desirable functions in anti-B7-H1 antibodies, by predominantly targeting cancer cells (although T cells can also express B7-H1). Human IgG4 isotype antibodies have a reduced capacity to mediate ADCC and CDCC in comparison with IgG1 isotype antibodies. Agents with lower binding affinity to the target may affect the adequacy or duration of pathway blockade and blocking PD-1 versus B7-H1 may lead to differing outcomes because of other unblocked coreceptor interactions in this pathway as discussed previously. Predicted differences in potential toxicity profiles (i.e., lower pulmonary toxicity) by blocking B7-H1 versus PD-1 because of unblocked expression of B7-DC in lung have not been verified yet in clinical trials. Additional data, including possibly head-to-head trials, will be required.

The key challenge for further clinical development of PD-1 pathway blockade is to understand the tumor–host immune relationship(s) which is/are permissive for clinical activity without other interventions, and the tumor–host immune relationships which require other therapeutic interventions in addition to PD-1 pathway blockade to produce optimal antitumor effects. A recent study showed that B7-H1 was expressed on a proportion (57 of 150) of various melanocytic lesions including primary, mMEL, and various types of nevi, most commonly only at the edges of the tumor bed or in opposition to TILs (47). Four distinct groups of tumors were identified and described as having the presence of both B7-H1 and TILs, presence of TILs without B7-H1, B7-H1 expression without TILs, or absence of both TILs and B7-H1 expression (Fig. 3). As analyzed by laser-capture microdissection of the tumor edge, selective expression of IFN-γ mRNA, a potent inducer of membrane B7-H1, was shown to correlate with tumor B7-H1

Table 2. Ongoing trials with PD-1 pathway-targeted immunotherapy

Indication	Compound	NCT#	Phase	Sponsor	Description or control arm	Expected patient enrollment	Expected completion date
Metastatic BRAFV600+ melanoma	MPDL3280A/RG7446 with vemurafenib	NCT01656642	1	Genentech	3 Arms, no dose info	44	November, 2014
mMEL	Nivolumab with ipilimumab	NCT01024231	1	Bristol-Myers Squibb	Varying doses of nivolumab + ipilimumab or nivolumab only	64	August, 2014
Resected melanoma	Nivolumab with vaccine	NCT01176474	1	H. Lee Moffitt Cancer Center	Nivolumab dose-escalation + vaccine	30	July, 2013 (primary)
Unresectable melanoma	Nivolumab with vaccine	NCT01176461	1	H. Lee Moffitt Cancer Center	Nivolumab dose-escalation + vaccine	85	June, 2014 (primary)
Melanoma	Nivolumab (biomarker identification)	NCT01621490	1	Bristol-Myers Squibb	3 mg/kg, single arm	80	December, 2015
Melanoma	CT-011	NCT01435369	2	CureTech, LTD.	1.5 vs. 6 mg/kg	100	March, 2014
Hematologic malignancies	Nivolumab	NCT01592370	1	Bristol-Myers Squibb	Dose-escalation	110	February, 2015
Multiple myeloma after SCT	CT-011 with DC/MM vaccine	NCT01067287	2	Beth Israel Deaconess Med Ctr	CT-011 alone	35	March, 2013
Relapsed follicular lymphoma	CT-011 with rituximab	NCT00904722	2	CureTech, LTD.	3 mg/kg, single arm	30	June, 2013
Diffuse large B-cell lymphoma after ASCT	CT-011	NCT00532259	2	CureTech, LTD.	1.5 mg/kg, single arm	70	August, 2011 (but final data not yet reported)
Acute myelogenous leukemia	CT-011 with DC/AML vaccine	NCT01096602	2	Beth Israel Deaconess Med Ctr	DC/AML vaccine alone	35	May, 2013
RCC	Nivolumab (biomarker identification)	NCT01358721	1	Bristol-Myers Squibb	0.3, 2, and 10 mg/kg (one group no previous treatment)	80	August, 2015
RCC	Nivolumab with sunitinib or pazopanib	NCT01472081	1	Bristol-Myers Squibb	Nivolumab + pazopanib vs. nivolumab + sunitinib	72	August, 2013
RCC	CT-011 with DC/RCC vaccine	NCT01441765	2	Beth Israel Deaconess Med Ctr	CT-011 alone	44	November, 2013
RCC	Nivolumab	NCT01354431	2	Bristol-Myers Squibb	0.3, 2, and 10 mg/kg arms	150	November, 2012
Advanced or mRCC	Nivolumab	NCT01668784	3	Bristol-Myers Squibb	Everolimus	822	February, 2016
Multiple solid tumors	MPDL3280A/RG7446 with bevacizumab	NCT01633970	1	Genentech	Dose-escalation with or without chemotherapy	68	March, 2015
Multiple solid tumors	MK-3475	NCT01295827	1	Merck	1, 3, and 10 mg/kg arms	439	March, 2015
Multiple solid tumors	AMP-224	NCT01352884	1	Amplimmune, GlaxoSmithKline	Dose-escalation	63	July, 2013
Multiple solid tumors	MPDL3280A/RG7446	NCT01375842	1	Genentech	Dose-escalation	88	July, 2013
Multiple solid tumors	BMS-936559	NCT00729664	1	Bristol-Myers Squibb	Dose-escalation	286	September, 2013
Multiple solid tumors	Nivolumab	NCT00730639	1	Bristol-Myers Squibb	Dose-escalation	290	October, 2015
Multiple solid tumors	Nivolumab	NCT00836888	1	Ono Pharmaceutical Co.	Dose-escalation	24	September, 2010 (primary)
Multiple solid tumors	MEDI4736	NCT01693562	1	MedImmune LLC.	Dose-escalation	110	November, 2014
Multiple solid tumors	Nivolumab	NCT01629758	1	Bristol-Myers Squibb	IL-21 dose-escalation, with 3 mg/kg nivolumab	160	September, 2015
NSCLC	Nivolumab with various chemotherapies	NCT01454102	1	Bristol-Myers Squibb	Nivolumab alone vs. with 1/5 chemotherapies	108	December, 2013
Squamous NSCLC	Nivolumab	NCT01642004	3	Bristol-Myers Squibb	Docetaxel	264	August, 2014
Nonsquamous NSCLC	Nivolumab	NCT01673867	3	Bristol-Myers Squibb	Docetaxel	574	November, 2014
Hepatocellular carcinoma (±HCV or HBV)	Nivolumab	NCT01658878	1	Bristol-Myers Squibb	Dose-escalation; no infection vs. HCV vs. HBV	72	April, 2014
Pancreatic cancer	CT-011 with Sipuleucel-T and cyclophosphamide	NCT01420965	2	Georgia Health Sciences University	Sipuleucel-T vs. Sipuleucel-T plus CT-011 vs. Sipuleucel-T plus CT-011 plus cyclophosphamide	57	December, 2017
Pancreatic cancer	CT-011 with gemcitabine	NCT01313416	2	Georgia Health Sciences University	Single arm	29	February, 2017
Metastatic colorectal cancer	CT-011 with FOLFOX	NCT00890305	2	CureTech, LTD.	FOLFOX alone	168	September, 2012

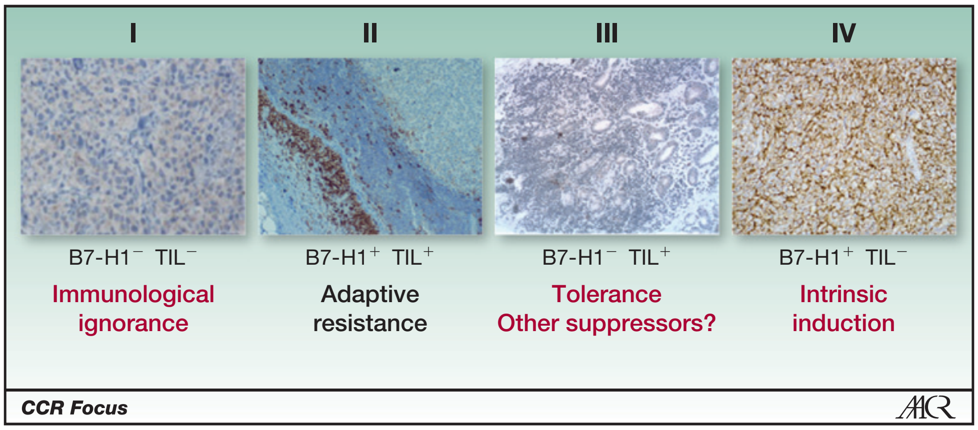

Figure 3. B7-H1 expression and inflammation: implications for mechanisms and therapy. I, B7-H1$^-$ tumor without TILs. II, B7-H1$^+$ melanoma with TILs at advancing edge. III, B7-H1$^-$ tumor with TILs present. IV, B7-H1$^+$ tumor without TILs. Panel IV reprinted with permission from ref. 47.

expression. These data suggest that a subset of patients have an ongoing immune response within the tumor microenvironment and that B7-H1 expression is an adaptive method of tumor resistance to cytokine-producing TILs. Another patient subset likely upregulates B7-H1 expression through aberrant cell signaling, for example, via PTEN loss (66) or via activation of the MEK/ERK or MyD88 signaling pathways, which participate in the upregulation of B7-H1 in response to IFN-γ or Toll-like receptor signaling (67–69). The data also raise important questions about the function and specificity of TIL in B7-H1$^-$ tumors and the biologic basis for tumors lacking TIL. In the latter group, it is appropriate to ask whether the lack of T-cell infiltration reflects the absence of tumor antigen-specific responses or an as-yet unidentified process that excludes TIL from the microenvironment.

The findings in the study by Taube and colleagues may have important implications for B7-H1 as a prognostic and predictive biomarker as well as a target for therapy. The subgroup with both B7-H1 expression and TILs may have improved prognosis compared with the group with B7-H1 expression without TILs; indeed, multiple literature studies have shown improved prognosis associated with the presence of TIL (70, 71). Consistent with this hypothesis, patients with B7-H1$^+$ mMEL, which is more commonly associated with TIL, had significantly longer survival than those with B7-H1$^-$ mMEL ($P = 0.032$; ref. 47). Current data also raise concern for using tumor B7-H1 expression as a predictive biomarker to select individual patients for treatment with PD-1/B7-H1 blockade or to select certain tumor types for development. Designation of positive B7-H1 expression remains confounded by tumor heterogeneity, variability in the assays, location of intratumoral expression, and clear cutoff values for positive versus negative expression. Moreover, the association of TIL with B7-H1 expression, or perhaps simply the presence of TIL, may be more important for predicting response than B7-H1 expression alone. Intriguingly, immune cells that infiltrate colorectal cancer have been observed to organize into intratumoral structures, which resemble lymph nodes; this associates with a particular chemokine signature, which may be useful in identifying immunotherapy-responsive patients, especially if similar phenomena are observed in other tumor types (72). The association of intratumoral lymphocytes and chemokine expression with TIL PD-1 expression and/or tumor B7-H1 expression has not yet been defined. Ascierto and colleagues discuss biomarkers for immunostimulatory antibody therapy in this issue of *Clinical Cancer Research* (73).

Substantial therapeutic advances for the B7-H1$^+$ TIL$^+$ subgroup may follow further characterization of other coinhibitory ligand–receptor interactions or immune suppressive factors in the tumor microenvironment. Recent murine studies suggest that concurrent blockade of other exhaustion/coinhibitory molecules (TIM3 and LAG3) and PD-1 can result in synergistic antitumor activity, and 1 preclinical study suggested incremental activity by blocking both PD-1 and B7-H1 (74–76). TIL and/or peripheral blood lymphocyte CTLA-4 expression increases in response to PD-1 blockade in both animal tumors and in patients, and assuming that CTLA-4 ligands are present in the tumor microenvironment, these data provide a rationale for the ongoing phase I trial of anti-PD-1 and anti-CTLA-4 (J. Weber, personal communication). For noninflamed tumors, strategies that induce and expand tumor-antigen–specific T cells and drive them into the tumor microenvironment may be necessary before or concurrent with PD-1 pathway blockade. Potential strategies to drive T cells into the tumor microenvironment include anti-CTLA-4, IFNs (which also induce surface B7-H1 expression), and signaling antagonists such as B-raf inhibitors (e.g., vemurafenib; refs. 71, 77, 78). Compared with pretreatment tumor biopsies, TIL populations (especially CD8$^+$ T cells) increased in biopsies taken after patients with mMEL were treated with vemurafenib or dabrafenib, suggesting that the combination of B-raf inhibitors and immunotherapies such as PD-1-pathway inhibitors might complement one another and lead to improved patient outcomes (78). As mentioned earlier, preclinical studies have also supported combinations of anti-PD-1 antibodies with a variety of agents including chemotherapy, cytokines, and costimulatory agents such as anti-CD137 (32); CD40 is another promising immunostimulatory target (79). Although combinations with chemotherapy will be preferred in diseases where chemotherapy is standard of care, it will also be important to assess the activity of PD-1 blockade alone and in combination with other immune therapies. If additional data confirm that tumor B7-H1 expression at a predefined level is a predictive biomarker for activity of PD-1 blockade alone, the lack of expression should not preclude testing combinations of PD-1 with other agents that could drive T cells into the tumor microenvironment. Finally, combinations of PD-1 blockade with other agents such as anti-CTLA-4 antibodies would be predicted to produce greater levels of immune-related adverse events. In the setting of potentially increased activity of such combinations, the observation of unique and increased toxicities should not preclude continued development, because of the capacity to develop effective management algorithms as already shown in clinical studies of anti-CTLA-4 antibodies.

Disclosure of Potential Conflicts of Interest

M. Sznol is a consultant/advisory board member of Bristol-Myers Squibb. L. Chen has a commercial research grant from Amplimmune Inc. and is a consultant/advisory board member of Amplimmune.

Authors' Contributions

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Analysis and interpretation of data (e.g., statistical analysis, biostatistics, computational analysis): M. Sznol, L. Chen
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Acknowledgments

The authors thank Cailin Moira Wilke, PhD (StemScientific, funded by Bristol-Myers Squibb) for professional medical writing and editorial assistance.

Grant Support

This study is partially supported by Melanoma Research Alliance and NIH grants CA142779 and CA121974.

Received October 16, 2012; revised December 19, 2012; accepted January 10, 2013; published online March 4, 2013.

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