

The B7 Family and Cancer Therapy: Costimulation and Coinhibition

Xingxing Zang and James P. Allison

Abstract The activation and development of an adaptive immune response is initiated by the engagement of a T-cell antigen receptor by an antigenic peptide-MHC complex. The outcome of this engagement is determined by both positive and negative signals, costimulation and coinhibition, generated mainly by the interaction between the B7 family and their receptor CD28 family. The importance of costimulation and coinhibition of T cells in controlling immune responses is exploited by tumors as immune evasion pathways. Absence of the expression of costimulatory B7 molecules renders tumors invisible to the immune system, whereas enhanced expression of inhibitory B7 molecules protects them from effective T cell destruction. Therefore, the manipulation of these pathways is crucial for developing effective tumor immunotherapy. Translation of our basic knowledge of costimulation and coinhibition into early clinical trials has shown considerable promise.

Extended B7 Family and CD28 Family

Mature naïve T lymphocytes emerge from the thymus and migrate to peripheral lymphoid organs such as the spleen and lymph nodes. There, the clonally distributed T-cell antigen receptors (TCR) of naïve CD4 or CD8 T cells detect antigenic peptide-MHC II or MHC I complexes, respectively, on antigen-presenting cells (APC). Although TCR signal transduction is necessary for the activation of naïve T cells, TCR ligation alone is not sufficient to initiate an immune response. For optimal activation of naïve T cells, a secondary signal, known as costimulation, is needed. Activation through the TCR in the presence of such costimulatory signals results in clonal T cell expansion and differentiation. Upon activation in lymphoid organs, T cells differentiate and migrate to the periphery where they carry out their effector functions. After the initial activation, coinhibitory molecules are engaged in order to restrain T cell function. Expression of costimulatory and coinhibitory molecules at the appropriate times and locations positively and negatively control the priming, growth, differentiation, and functional maturation of a T cell response.

In 1987, the first definitive experiment showed that TCR engagement was insufficient for T cell activation (1). In the same year, coincidentally, CD28 was cloned as a T cell-specific surface protein by expression cloning (2), whereas CTL antigen-4 (CTLA-4) was discovered in the search for novel genes expressed in activated CD8 T cells (3). B7-1 was originally cloned as a

B-cell activation antigen (4), and was subsequently identified as a ligand for CD28 (5) and CTLA-4 (6). The suggestion that B7-1 plays an important role in costimulation came from *in vitro* experiments showing that CD28 engagement by B7-1 could enhance T cell proliferation and interleukin-2 (IL-2) production (7–9). Subsequently, it was shown that an anti-CD28 antibody could provide the costimulatory signal required to block T cell anergy induction (10). The observations that CTLA-4 could bind to a non-B7-1 ligand and that mice deficient in B7-1 could still induce costimulation, resulted in the discovery of B7-2, a second ligand for CD28 and CTLA-4 receptors (11, 12). Thus, the B7-1, B7-2/CD28, CTLA-4 pathway was established in 1993 as the first pathway of T cell costimulation and coinhibition, and has been the subject of intense study over the following decade. Six years later, two new members of the B7 family, B7h (13) and B7-H1 (14), and a new member of the CD28 family, inducible costimulator (ICOS; ref. 15), were discovered. B7h (also called B7RP-1, GL50, B7H2, and LICOS; refs. 16–19, respectively) is the costimulatory ligand for ICOS. Thus, the B7h/ICOS pathway was acknowledged in 1999. Subsequently, it was shown that B7-H1 is the ligand for programmed death 1 (PD-1) and was thus renamed as PD-L1 (20). Although PD-1 was originally isolated from apoptotic cells in 1992 (21), it was not until its ligand was found to be homologous to the B7 family members that it was recognized as a member of the CD28 family. The second PD-1 ligand, PD-L2 (22), also known as B7-DC (23), was then quickly cloned. Thus, the PD-L1, PD-L2/PD-1 pathway was confirmed in 2001. More recently, by homology search of genome databases, B7-H3 was cloned in 2001 (24), and B7x (25), also called B7-H4 (26) or B7S1 (27), was cloned in 2003. The receptors for B7-H3 and B7x remain to be identified.

By phylogenetic analysis, we divided the B7 family into three groups (Table 1; refs. 25, 28). Group I includes B7-1, B7-2, and B7h. The B7-1, B7-2/CD28 pathway provides critical costimulatory signals for naïve T cells as CD28 knockout mice exhibit severe diminution of most T cell responses (29). Conversely, the B7-1, B7-2/CTLA-4 pathway acts as a key negative regulator of CD28-dependent T cell activation as CTLA-4 knockout mice

Authors' Affiliation: Howard Hughes Medical Institute, Immunology Program, Ludwig Center of Cancer Immunotherapy, Memorial Sloan-Kettering Cancer Center, New York, New York

Received 4/30/07; revised 6/11/07; accepted 6/27/07.

Grant support: Howard Hughes Medical Institute and the NIH.

Requests for reprints: James P. Allison, Howard Hughes Medical Institute, Memorial Sloan-Kettering Cancer Center, 415 East 68th Street, Box 470, New York, NY 10021. Phone: 646-888-2332; Fax: 646-422-0470; E-mail: allisonj@mskcc.org.

© 2007 American Association for Cancer Research.

doi:10.1158/1078-0432.CCR-07-1030

Table 1. Expression and function of the B7 family ligands and their receptors of the CD28 family

The B7 family ligands			The CD28 family receptors		
Expression	Name		Name	Expression	Function
			<u>Group I</u>		
APC	B7-1		CD28	T cells	Costimulation
APC	B7-2		CTLA-4	Activated T cells	Coinhibition
APC, tissue	B7h		ICOS	Activated T cells	Costimulation
			<u>Group II</u>		
APC, tissue, tumors	PD-L1		PD-1	Activated T, B, and myeloid cells	Coinhibition
APC	PD-L2		PD-1	Activated T, B, and myeloid cells	Coinhibition
			<u>Group III</u>		
APC, tissue, tumors	B7-H3		Unidentified receptor	Activated T cells	Coinhibition
Tissue, tumors	B7x		Unidentified receptor	Activated T cells	Coinhibition

succumb to a lethal lymphoproliferative disorder at an early age (30–32). The B7h/ICOS plays an important role in T-cell and B-cell interactions (33–35). Group II consists of PD-L1 and PD-L2. The PD-L1/PD-1 pathway is involved in the induction and maintenance of peripheral immune tolerance and plays a crucial role in the functional impairment of antigen-specific exhausted CD8 T cells during chronic viral infections (36–38). PD-1 knockout mice slowly develop spontaneous lupus-like disease and autoimmune dilated cardiomyopathy. Intriguingly, PD-L1 and PD-L2 double knockout mice do not exhibit spontaneous autoimmune diseases (39). Group III consists of B7-H3 and B7x. As the newest members of the B7 family, their precise roles in T cell regulation remain to be defined. Initial studies showed that B7-H3 fusion protein stimulated T cell proliferation and IFN- γ production (24). However, several recent studies have shown that B7-H3 has an inhibitory function *in vitro* (40, 41). The phenotype of B7-H3 knockout mice also supports an inhibitory function for B7-H3 *in vivo* (42). We and others have shown that B7x inhibits TCR-mediated CD4 and CD8 T cell proliferation, cell cycle progression, and IL-2 production *in vitro* (25–27). Intriguingly, B7x protein is detected in peripheral organs but not in the lymphoid compartment. The discovery of new B7 molecules and counter-receptors suggests that T cell costimulation and coinhibition is more complex than previously thought.

Enhancement of Costimulation: B7-1, B7-2, B7h – Transfected Tumor Cell Vaccines

Tumors may be capable of delivering antigen-specific signals to T cells as long as they express MHC. However, according to the two-signal model for T cell activation, tumors may not be able to deliver the costimulatory signals necessary for full activation of T cells as the expression of B7-1 and B7-2 are generally limited to APC and are not on most tumors. Therefore, ectopic expression of costimulatory B7 molecules on tumor cells could render tumor cells capable of effective T cell activation, leading to their eradication *in vivo* (Fig. 1). This hypothesis was first tested by three laboratories with two mouse

tumor models (43–45). Initial experiments used transplantable murine melanoma and sarcoma tumors. Induction of B7-1 expression on tumors by transfection was sufficient to induce CD8 T cell-mediated rejection of melanoma and CD4 T cell-mediated rejection of sarcoma. This rejection generated a memory response and consequent immunity to rechallenge with wild-type tumors in the absence of additional treatment. Subsequent work showed that the expression of B7-1 or B7-2 on carcinoma tumor cells rendered tumors equally susceptible to rejection (46). The same approach was used to show that ectopic B7h expression also provides costimulation and promotes the rejection of fibrosarcoma and plasmacytomas by CD8 T cells (47, 48). However, optimal B7h-induced clonal expansion of tumor antigen-recognized CD8 T cells required costimulation by B7-1 and B7-2 on endogenous host APC. Although many studies have shown that conferring B7-1 or B7-2 expression on tumor cells augmented immune response against tumors of varied origins, it was apparent that B7-1 or B7-2 expression alone was insufficient to induce immunity against poorly immunogenic tumors (49).

At least three early phase I clinical trials have been conducted to test the efficacy of B7-1-transfected tumor cell vaccines. In a phase I trial of metastatic renal cell carcinoma, in which 15 patients were vaccinated with B7-1-transfected autologous tumor cells in combination with systemic IL-2, one patient showed a 70% reduction in the size of a bone metastasis whereas another patient had a 90% regression of multiple pulmonary nodules, and another two patients had 17% and 18% decreases in the sizes of their metastatic lesions, considered to be stable disease (50). In a trial of advanced non-small cell lung cancer, 19 patients were vaccinated with an adenocarcinoma line transfected with B7-1 and HLA-A1 or A2. Overall, one patient had a partial response, and five had stable disease (51). In an ongoing phase I trial, patients with acute myeloid leukemia will be vaccinated with autologous acute myeloid leukemia transfected with B7-1 and IL-2 combined with allogeneic hematopoietic stem cell transplantation (52). This study hopes to enhance the immune eradication of residual acute myeloid leukemia cells in poor prognosis

patients that have achieved donor chimerism. Although such studies are currently in their infancy, one needs to keep in mind that in addition to CD28, both B7-1 and B7-2 bind CTLA-4 with much higher affinity; therefore, in some circumstances, it is possible that B7-1- or B7-2-transfected tumor cell vaccines could attenuate activated T cells by CTLA-4 engagement. Indeed, low surface expression of B7-1 on tumor cells is an immunoescape mechanism in a colon carcinoma mouse model (53).

Blockade of Coinhibition: Anti-CTLA-4 Therapy

It seems that the cross-priming process is critical for the initiation of T cell responses to tumors (54). During this process, bone marrow-derived APC, most likely dendritic cells, can pick up antigens released from tumor cells, and process and present them to naïve T cells in the context of B7-1 and B7-2 costimulation. Upon T cell activation, CTLA-4 is expressed and it starts to carry out its inhibitory function. Therefore, specific blockade of CTLA-4 signals, whereas leaving TCR and CD28 signals intact, is a very attractive approach for tumor immunotherapy (Fig. 1). This idea was first tested in mouse models 10 years ago (55). Administration of antibodies that block CTLA-4 interactions with B7-1 and B7-2 resulted in the rejection of colon carcinoma and fibrosarcoma, including pre-established tumors. Furthermore, this rejection was accompanied by a long-lasting immunity to a secondary exposure to tumor cells in the absence of additional treatment. This work proved the principle that CTLA-4 blockade could enhance tumor-elicited weak immune responses to a level that could mediate tumor rejection. Subsequent work showed that anti-CTLA-4 monotherapy could also promote the rejection of other transplantable tumors, including prostatic carcinoma, lymphoma, renal cell carcinoma, and colon carcinoma (56). However, this approach does not seem to be effective for poorly immunogenic tumors.

Based on the results of anti-CTLA-4 therapy in mouse tumor models, two human anti-CTLA-4 antibodies, MDX-010 (Ipilimumab) and CP-675,206 (Ticilimumab), were developed and have entered into clinical trials at multiple centers (Table 2; refs. 57–70). In addition to prostate, ovarian, breast, and colon carcinoma, these antibodies have been mainly tested in metastatic melanoma and in renal cell cancer in which immune responses are believed to play important roles. From these early phase I/II clinical trials, some provocative observations can be drawn. Anti-CTLA-4 monotherapy is capable of inducing objective tumor responses, partial response, or even complete response in patients with melanoma, renal cell, prostate carcinoma, and non-Hodgkin's lymphoma (Table 2). In heavily pretreated melanoma patients, objective tumor responses to anti-CTLA-4 monotherapy were observed to be as high as 20% (59, 71). Responses have involved multiple visceral sites such as the lung and brain. Moreover, response durations can be significant, most from 18 to 35 months. It seems likely that anti-CTLA-4 monotherapy is able to enhance preexisting antitumor responses and generate *de novo* antitumor responses. In addition, CTLA-4 blockade is unlikely to function through effects on T regulatory cells because anti-CTLA-4

treatment in patients with cancer or in mice does not show inhibition or depletion of T regulatory cells *in vivo* (72, 73). Although a few different dosing schedules in patients showing clinical benefit have been explored, the optimal dosing schedule for anti-CTLA-4 remains to be determined. The most common adverse events in anti-CTLA-4 trials involved the skin (rash and pruritus) and the gastrointestinal tract (diarrhea and colitis), and there have been several reports of hypopituitarism. If severe, these adverse events may require the cessation of anti-CTLA-4 therapy or treatment with steroids or infliximab.

The failure of anti-CTLA-4 monotherapy against poorly immunogenic tumors in mouse models led to the exploration of its use in combination with other therapies such as tumor vaccine with granulocyte macrophage colony-stimulating factor-transfected irradiated tumor cells (GVAX; ref. 74) and regulatory T cell depletion (75). In clinical trials, anti-CTLA-4 has been used with other treatments such as peptide vaccine, IL-2, and the chemotherapy drug dacarbazine. The majority of these studies have focused on coadministration with peptides from melanoma antigens. However, the objective tumor responses and durations seen in those studies seem to be similar to anti-CTLA-4 monotherapy studies (60). GVAX elicits a dense local infiltrate of dendritic cells, granulocytes, and macrophages, which promotes the cross-priming process (76). A phase I trial studying the combination of anti-CTLA-4 and GVAX showed better antitumor responses in patients with metastatic hormone-refractory prostate cancer (70). Of the six patients receiving GVAX plus higher doses of anti-CTLA-4, antitumor activities were observed in five patients, including prostate-specific antigen declines of >50% that were maintained in four of these patients for at least 6 months, with the longest response ongoing for >1 year. Importantly, three of the five prostate-specific antigen responders had regression of multiple sites of prostate cancer in the bone and the abdominal lymph nodes, and improvement of pain due to bone metastases. As a logical next step, new clinical trials will compare objective responses and durations between anti-CTLA-4 monotherapy and combinatorial therapies.

PD-L1/PD-1: Tumor Evasion Pathway

Many human cancers have been reported to aberrantly express PD-L1, including glioblastoma, melanoma, and cancers arising from the lung, ovary, colon, head and neck, and breast (77). More interestingly, several long-term follow-up investigations have established an inverse correlation between PD-L1 expression in tumor cells and poor prognosis of patients (Table 3; refs. 78–85). Two initial studies revealed that PD-L1 was up-regulated in both renal cell carcinoma and non-small cell lung cancer (78, 79). However, the relationship between tumor-associated PD-L1 level and clinical progression was observed only in renal cell carcinoma. Sixty-six percent of renal cell carcinomas were positive for PD-L1 expression. In contrast, proximal tubules of the renal cortex, from which clear cell tumors are believed to arise, failed to express PD-L1. Patients harboring high intratumoral expression levels of PD-L1,

contributed by tumor cells alone or tumor-infiltrating lymphocytes alone, exhibited aggressive tumors and were at markedly increased risk of death from renal cell carcinoma. Moreover, the combination of increased tumor cell PD-L1 and

lymphocyte PD-L1 was an even stronger predictor of patient outcome. This combined feature was also significantly associated with regional lymph node involvement, distant metastases, nuclear grade, and histologic tumor necrosis, all of which

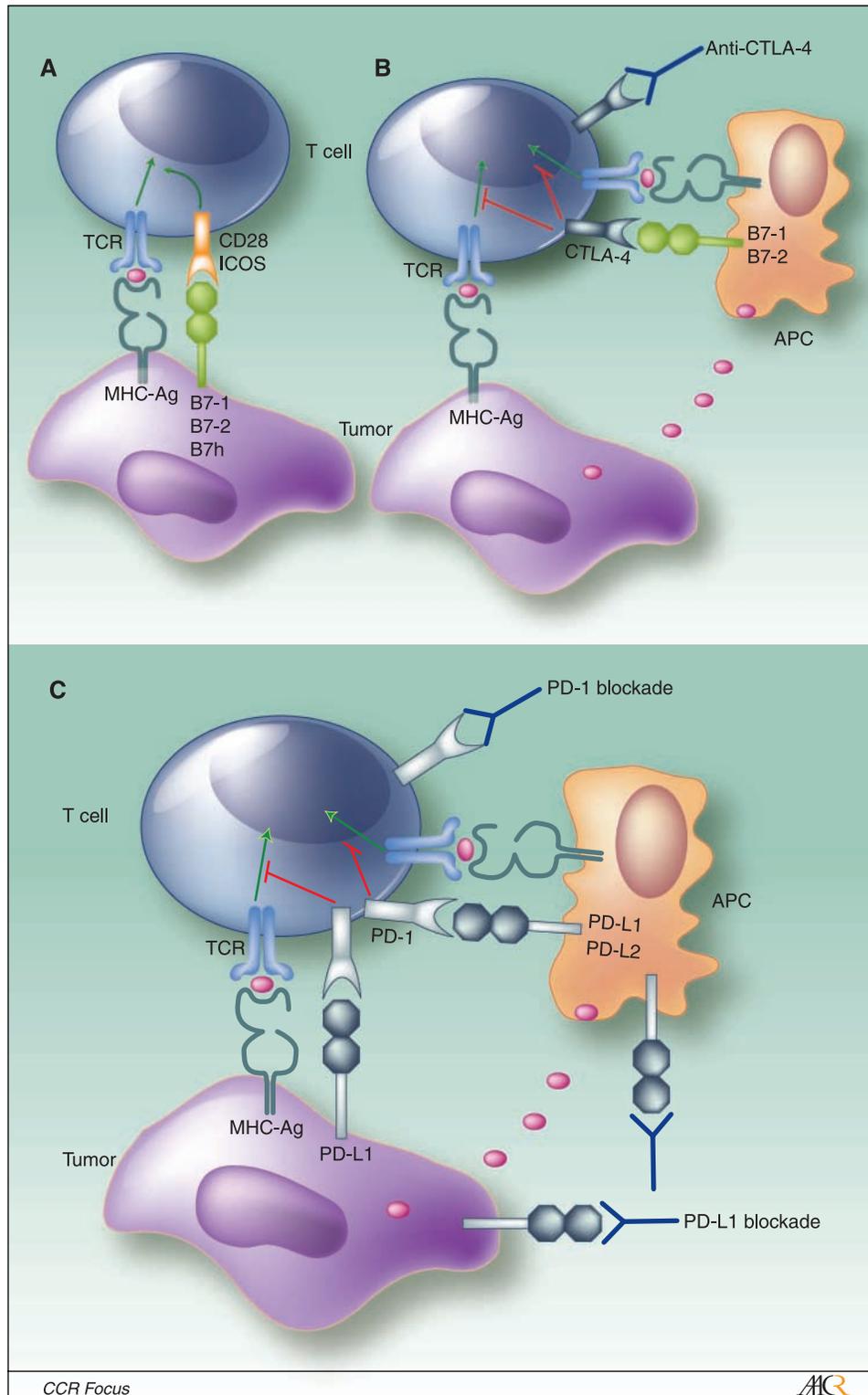


Fig. 1. The B7 family and cancer therapy. *A*, enhancement of costimulation: B7-1, B7-2, B7h-transfected tumor cell vaccines. Absence of expression of costimulatory B7 molecules renders tumors invisible to the immune system. Therefore, transduction of tumor cells with B7-1, B7-2, and B7h on tumor cell could render tumor cells capable of effective T cell activation if tumor cells also express the appropriate MHC-peptide complex. *B*, blockade of coinhibition: Anti-CTLA-4 therapy. APC pick up antigens released from tumor cells and present them to naive T cells in the context of B7-1 and B7-2 costimulation. On T cell activation, CTLA-4 is expressed and it begins to perform its inhibitory function. Therefore, specific blockade of CTLA-4, while leaving signals to TCR and CD28 intact, leads to enhanced antitumor immunity and tumor eradication. *C*, PD-L1/PD-1. Tumor evasion pathway. There is an inverse correlation between tumor PD-L1 expression and poor prognosis of cancer patients. It is a very attractive possibility for tumor immunotherapy by blocking the PD-L1/PD-1 pathway between PD-L1 on tumors and PD-1 on CD8 T cells as well as PD-L1 on APC or activated T cells and PD-1 on T cells.

have been shown to portray a poor prognosis in renal cell carcinoma (78). Focal PD-L1 and PD-L2 expression was observed in almost all non-small cell lung cancer tumor specimens, but no relationship was found between the expression of PD-L1 or PD-L2 and clinical outcome (79). Other studies also showed that intratumor expression of PD-L1 was significantly associated with clinical outcome in breast cancer (80), gastric carcinoma (82), bladder urothelial carcinoma (83, 84), ovarian cancer (81), and pancreatic cancer (85). Thirty-four percent of breast cancers had intratumor expression of PD-L1, associated with histologic grade III, estrogen and progesterone receptors. In addition, 41% of tumor-infiltrating lymphocytes expressed PD-L1, which was associated with a large tumor size and Her2/neu-positive status (80). More recently, it was found that ovarian cancer patients with a higher expression of PD-L1 had a significantly poorer prognosis than patients with lower expression (81). A significant inverse correlation was also observed between PD-L1 expression and intraepithelial CD8 T lymphocyte count. In contrast to PD-L1, expression of PD-L2 in tumors was much less frequent, which is due to the fact that PD-L2 is generally limited to dendritic cells and macrophages.

The molecular mechanisms regulating PD-L1 expression are unclear. Inflammatory mediators such as IFN- γ and tumor necrosis factor- α up-regulated PD-L1 expression on the surface of several tumor cell lines, and PD-L1 expression was more frequent in freshly isolated tumor tissue specimens than in cultured tumor cell lines (86). These observations suggest that

cytokines in the tumor microenvironment induce the expression of PD-L1 on tumor cells. Recent work has revealed that PD-L1 expression increased post-transcriptionally in human glioma after the loss of phosphatase and tensin homologue and activation of the phosphatidylinositol-3-OH kinase pathway (87). Therefore, IFN- γ may regulate PD-L1 by activating the components of the phosphatidylinositol-3-OH kinase-Akt-mTOR-S6K1 pathway. Understanding the regulation of PD-L1 in cancer may elucidate the links between oncogenesis and cancer immune evasion, whereas helping to refine immunotherapy.

Although the extent to which PD-L1 protein expression directly affects tumor progression remains to be determined, it is generally accepted that aberrant expression of PD-L1 on tumor cells impairs antitumor immunity—resulting in the immune evasion of the tumor cells. Therefore, blockade of the PD-L1/PD-1 pathway would be a very attractive possibility for tumor immunotherapy (Fig. 1). Initial studies found that murine myeloma cell lines naturally expressed PD-L1, and that their growth *in vivo* was inhibited significantly, although transiently, by the administration of antibody to PD-L1 antibodies (88). Subsequent studies have revealed that ectopic expression of PD-L1 on squamous cell carcinomas or mastocytomas did not affect either tumorigenicity or immunogenicity in immunocompetent mice, but tumor eradication can be accelerated by PD-L1-blocking antibodies (89, 90). Two metastatic tumor models have been shown to be sensitive to PD-1 blockade. Administration of

Fig. 1 Continued. *D*, tumor-associated B7-H3 and B7x: Last-ditch mechanism. B7-H3 and B7x are the newest members of the B7 family. Both B7-H3 and B7x inhibit T cell functions and are overexpressed on some tumor cells and tumor blood vessels. Consequently, blockade of tumor-associated B7-H3 and B7x could offer a new dual beneficial therapy: enhancement of T cell-mediated antitumor immunity (immunotherapy) and destruction of tumor vessels (antiangiogenesis).

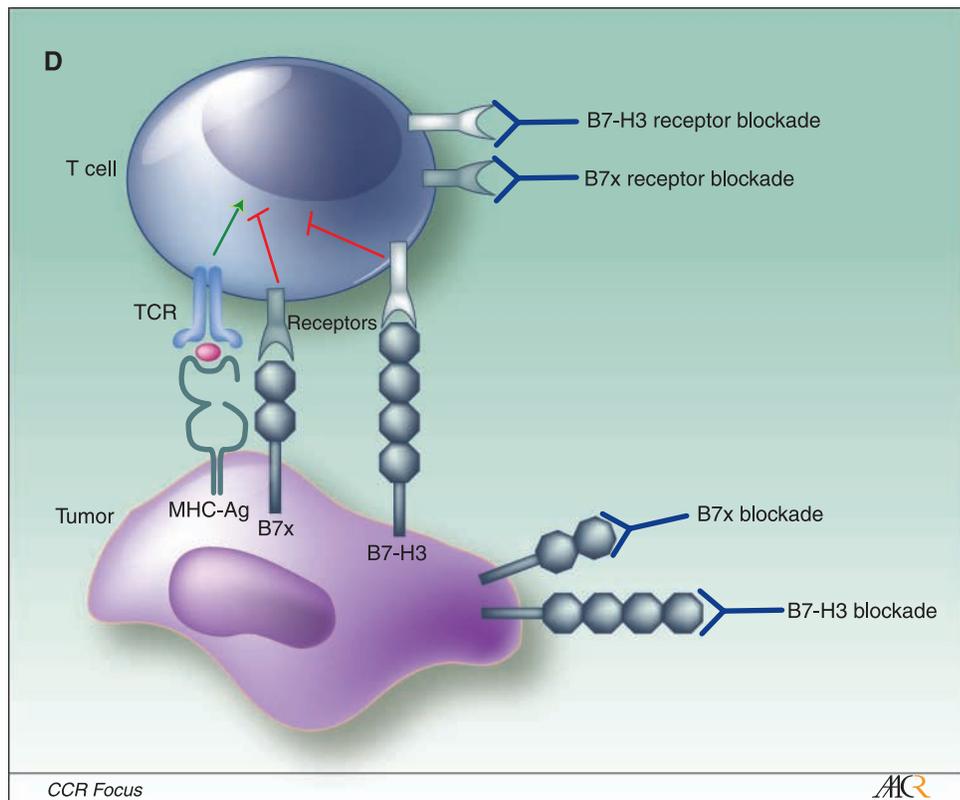


Table 2. Phase I/II clinical trials of CTLA-4 blockade

Antibodies	Dose	Cancer	No. of cases	No. of objective responses	Reference
MDX-010	3 mg/kg, 1.5 mg/kg every 4 weeks	Lymphoma	4	2	(63)
		Colon	3	0	
		Prostate	4	0	
MDX-010	3 mg/kg	Prostate	14	2 (≥50% decrease of prostate-specific antigen)	(58)
MDX-010	3 mg/kg every 3 weeks	Renal	21	1 partial response	(66)
	3 mg/kg, 1 mg/kg every 3 weeks	Renal	38	5 partial responses	
MDX-010	3 mg/kg	Melanoma	7	0	(57)
		Ovarian	2	0	
MDX-010	3 mg/kg every 4 weeks	Melanoma	37	2 partial responses	(69)
MDX-010 + dacarbazine		Melanoma	35	1 complete response 5 partial responses	
MDX-010	1-3 mg/kg	Renal	61	7	(64)
MDX-010 ± gp100 peptide	1-9 mg/kg	Melanoma	137	20	
MDX-010 + gp100 peptide	3 mg/kg every 3 weeks	Melanoma	14	2 complete responses 1 partial response	(61)
MDX-010 + gp100 peptide	3 mg/kg every 3 weeks	Melanoma	29	2 complete responses 2 partial responses	(60)
	1-3 mg/kg, 1 mg/kg every 3 weeks	Melanoma	27	3 partial responses	
MDX-010 + IL-2	1 mg/kg × 3-6	Melanoma	6	1 partial response	(65)
	2 mg/kg × 3-7	Melanoma	3	1 complete response	
	3 mg/kg × 1-9	Melanoma	27	2 complete responses 4 partial responses	
MDX-010 + peptide	0.3-3 mg/kg every 3 weeks	Melanoma	19	7	(62)
MDX-010 + GVAX	3 mg/kg	Prostate	6	5 (≥50% decrease of prostate-specific antigen)	(70)
CP-675,206	0.01-15 mg/kg × 1	Melanoma	34	2 complete responses 2 partial responses	(59)
		Renal	4	0	
		Colon	1	0	
CP-675, 206	3-10 mg/kg every 4 weeks	Melanoma	14	1	(67)
CP-675, 206	10 mg/kg every 4 weeks	Melanoma	16	3	(68)
	15 mg/kg every 12 weeks	Melanoma	10	2	

PD-1 blocking antibodies markedly inhibited both CT26 colon carcinoma metastasis to the lung and B16 melanoma metastasis to the liver (91). In addition, PD-1-deficient CD8 TCR transgenic T cells caused tumor rejection in an adoptive transfer model in which wild-type and CTLA-4-deficient T cells failed (92). Both APC and activated T cells express PD-L1; therefore, enhanced antitumor immunity by blockade of the PD-L1/PD-1 pathway is more likely the result of inhibition of interaction between PD-L1 on tumor and PD-1 on CD8 T cells as well as interaction between PD-L1 on APC or activated T cells and PD-1 on T cells. Due to the striking inverse correlation between tumor PD-L1 expression and poor prognosis in patients with cancer, we expect to see clinical trials of PD-L1/PD-1 pathway blockade for cancer therapy soon. PD-L1 is expressed widely, not only in tumors, but also in immune cells and other tissue cells. Therefore, potential autoimmune diseases induced by the blockade of PD-L1/PD-1 for cancer therapy needs to be carefully monitored in the future.

Tumor-Associated B7-H3 and B7x: Last-ditch Mechanism

Although the B7 family provides critical costimulatory and coinhibitory signals that regulate T cell-mediated antitumor immunity, the role of B7-H3 and B7x, the newest members of the B7 family, in cancer has not yet been established. However, more than half of the B7x expressed-sequence tags in databases were originally found from tumors and that B7x mRNA was highly expressed in a number of tumor cell lines (25). Therefore, we have hypothesized that expression of B7x by tumors represents a previously unrecognized mechanism of down-regulating antitumor T cell responses at the level of the effector cell (25). This hypothesis may have important implications for the development of new strategies for tumor immunotherapy (93).

We are just beginning to examine the role of B7-H3 and B7x expression in human cancer. Both B7-H3 and B7x have been observed to be overexpressed in non-small-cell lung

Table 3. Expression of PD-L1 and PD-L2 in tumors and clinical significance

Cancer	No. of samples	PD-L1 (%)	PD-L2 (%)	Clinical association	Reference
Renal cell carcinoma	196	66		3-fold increased mortality	(78)
Tumor-infiltrating lymphocytes		59		3.6-fold increased mortality	
Non-small cell lung cancer	52	100	100	None	(79)
Breast cancer	44	50		Associated with grade III, and estrogen/ progesterone receptors	(80)
Tumor infiltrating lymphocytes		34		Associated with tumor size, grade III, Her2/neu positive status	
Gastric carcinoma	102	42.2		Associated with tumor size, invasion, metastasis, and survival	(82)
Bladder urothelial carcinoma	65	100		Associated with high recurrence and poor survival	(84)
Bladder urothelial carcinoma	280	36.1		Associated with high-grade tumor infiltrating mononuclear cells	(83)
Ovarian cancer	70	68.6		Poor prognostic factor for survival Inversely associated with intraepithelial CD8 cells	(81)
Pancreatic cancer	51	38.2		None	
			37.1	Associated with poor survival Inversely associated with tumor-infiltrating CD8 cells	(85)
			27.5	None	

cancers (94), ovarian and prostate cancers.¹ In addition, B7x was also expressed in breast cancer and renal cell carcinoma (95–97). In renal cell carcinoma, tumor cell B7x expression was associated with adverse clinical and pathologic features, including constitutional symptoms, tumor necrosis, and advanced tumor size, stage, and grade. Patients with tumors expressing B7x were thrice more likely to die from cancer compared with patients lacking B7x (97). In contrast with PD-L1, which is expressed on both tumor cells and immune cells, B7x is not expressed on ovarian tumor-infiltrating lymphocytes. This phenomenon is due to inherent lack of B7x expression in lymphocytes rather than the influence of the tumor microenvironment. Structurally, both B7-H3 and B7x are type I transmembrane proteins. However, the majority of these proteins are in the cytoplasm of tumor cells. Factors that regulate B7-H3 and B7x mRNA translation and protein access to the cell surface can spatially and temporally determine the extent to which tumor-associated B7-H3 and B7x regulate T cell function. We have also found that approximately half of ovarian and prostate cancer patients have soluble B7x in the blood,² but the mechanism of production and the function of soluble B7x are unknown.

Both B7x and B7-H3 inhibit T cell function (25–27, 40–42), therefore, B7x- and B7-H3-positive tumor cells could have an advantage over the negative tumor cells by down-regulating T cell-mediated antitumor immunity. Consequently, the blockade of tumor-associated B7-H3 and B7x could offer a new therapeutic opportunity for the enhancement of antitumor immunity (Fig. 1). Interestingly, we have observed that B7-H3 expression in ovarian tumor vessels was associated with poor

clinical outcome. Endothelial B7x expression on tumor vasculature was also found in some renal cell carcinomas. It remains unknown what the signals trigger B7x and B7-H3 expression in tumor vessels, but one most likely source could be the tumor microenvironment. Tumor blood vessels are prime targets for suppressing tumor growth because they are distinct from normal resting blood vessels and can be selectively destroyed without significantly affecting normal vessels. Thus, we suggest that blockade and/or destruction of tumor vasculature-associated B7x and B7-H3 could provide a dual beneficial therapy: enhancement of T cell-mediated antitumor immunity (immunotherapy) and destruction of tumor vessels (antiangiogenesis).

Conclusion

The intense efforts towards understanding T costimulatory and coinhibitory molecules over the past decade has shaped much of our understanding regarding the immune system and its interaction with tumors. It is now established that members of the B7 family ligands play a central role in the positive and negative regulation of antigen-specific T cell-mediated immune responses. Whereas the B7-1, B7-2/CD28, CTLA-4 pathway serves as the main switches regulating the clonal composition of activated naïve T cells, other B7 family members fine-tune the expansion and properties of activated T cells. T cell costimulation and coinhibition extensively influence the outcome of interactions between tumor and the immune system. Blockade of the CTLA-4 checkpoint has shown clinical benefit for patients with cancer, including long-term durable complete responses. These clinical responses prompted the initiation and completion of a phase III randomized clinical trial that will provide data as to the use of this novel therapy as a standard treatment for patients with cancer. Our knowledge of the newer costimulatory and coinhibitory pathways remain

¹ X. Zang, et al., manuscript in preparation.

² X. Zang, et al., unpublished data.

far more rudimentary, but they will no doubt offer other avenues for augmenting immune responses in the treatment of cancer, either in combination with other immunologic approaches or with more conventional therapies.

Acknowledgments

We thank Joyce Wei, Tyler Simpson, and Neil Segal for critical reading of the manuscript.

References

- Jenkins MK, Schwartz RH. Antigen presentation by chemically modified splenocytes induces antigen-specific T cell unresponsiveness *in vitro* and *in vivo*. *J Exp Med* 1987;165:302–19.
- Aruffo A, Seed B. Molecular cloning of a CD28 cDNA by a high-efficiency COS cell expression system. *Proc Natl Acad Sci U S A* 1987;84:8573–7.
- Brunet JF, Denizot F, Luciani MF, et al. A new member of the immunoglobulin superfamily—CTLA-4. *Nature* 1987;328:267–70.
- Freeman GJ, Freedman AS, Segal JM, Lee G, Whitman JF, Nadler LM. B7, a new member of the Ig superfamily with unique expression on activated and neoplastic B cells. *J Immunol* 1989;143:2714–22.
- Linsley PS, Clark EA, Ledbetter JA. T-cell antigen CD28 mediates adhesion with B cells by interacting with activation antigen B7/BB-1. *Proc Natl Acad Sci U S A* 1990;87:5031–5.
- Linsley PS, Brady W, Urnes M, Grosmaire LS, Damle NK, Ledbetter JA. CTLA-4 is a second receptor for the B cell activation antigen B7. *J Exp Med* 1991;174:561–9.
- Linsley PS, Brady W, Grosmaire L, Aruffo A, Damle NK, Ledbetter JA. Binding of the B cell activation antigen B7 to CD28 costimulates T cell proliferation and interleukin 2 mRNA accumulation. *J Exp Med* 1991;173:721–30.
- Gimmi CD, Freeman GJ, Gribben JG, et al. B-cell surface antigen B7 provides a costimulatory signal that induces T cells to proliferate and secrete interleukin 2. *Proc Natl Acad Sci U S A* 1991;88:6575–9.
- Koulova L, Clark EA, Shu G, Dupont B. The CD28 ligand B7/BB1 provides costimulatory signal for alloactivation of CD4+ T cells. *J Exp Med* 1991;173:759–62.
- Harding FA, McArthur JG, Gross JA, Rautel DH, Allison JP. CD28-mediated signalling co-stimulates murine T cells and prevents induction of anergy in T-cell clones. *Nature* 1992;356:607–9.
- Freeman GJ, Gribben JG, Boussiotis VA, et al. Cloning of B7-2: a CTLA-4 counter-receptor that costimulates human T cell proliferation. *Science* 1993;262:909–11.
- Azuma M, Ito D, Yagita H, et al. B70 antigen is a second ligand for CTLA-4 and CD28. *Nature* 1993;366:76–9.
- Swallow MM, Wallin JJ, Sha WC. B7h, a novel costimulatory homolog of B7.1 and B7.2, is induced by TNF α . *Immunity* 1999;11:423–32.
- Dong H, Zhu G, Tamada K, Chen L. B7-1, a third member of the B7 family, co-stimulates T-cell proliferation and interleukin-10 secretion. *Nat Med* 1999;5:1365–9.
- Hutloff A, Dittrich AM, Beier KC, et al. ICOS is an inducible T-cell co-stimulator structurally and functionally related to CD28. *Nature* 1999;397:263–6.
- Yoshinaga SK, Whoriskey JS, Khare SD, et al. T-cell co-stimulation through B7RP-1 and ICOS. *Nature* 1999;402:827–32.
- Ling V, Wu PW, Finnerty HF, et al. Cutting edge: identification of GL50, a novel B7-like protein that functionally binds to ICOS receptor. *J Immunol* 2000;164:1653–7.
- Wang S, Zhu G, Chapoval AI, et al. Costimulation of T cells by B7-2, a B7-like molecule that binds ICOS. *Blood* 2000;96:2808–13.
- Brodie D, Collins AV, Iaboni A, et al. LICOS, a primordial costimulatory ligand? *Curr Biol* 2000;10:333–6.
- Freeman GJ, Long AJ, Iwai Y, et al. Engagement of the PD-1 immunoinhibitory receptor by a novel B7 family member leads to negative regulation of lymphocyte activation. *J Exp Med* 2000;192:1027–34.
- Ishida Y, Agata Y, Shibahara K, Honjo T. Induced expression of PD-1, a novel member of the immunoglobulin gene superfamily, upon programmed cell death. *EMBO J* 1992;11:3887–95.
- Latchman Y, Wood CR, Chernova T, et al. PD-L2 is a second ligand for PD-1 and inhibits T cell activation. *Nat Immunol* 2001;2:261–8.
- Tseng SY, Otsuji M, Gorski K, et al. B7-DC, a new dendritic cell molecule with potent costimulatory properties for T cells. *J Exp Med* 2001;193:839–46.
- Chapoval AI, Ni J, Lau JS, et al. B7-H3: a costimulatory molecule for T cell activation and IFN- γ production. *Nat Immunol* 2001;2:269–74.
- Zang X, Loke P, Kim J, Murphy K, Waitz R, Allison JP. B7x: a widely expressed B7 family member that inhibits T cell activation. *Proc Natl Acad Sci U S A* 2003;100:10388–92.
- Sica GL, Choi IH, Zhu G, et al. B7-H4, a molecule of the B7 family, negatively regulates T cell immunity. *Immunity* 2003;18:849–61.
- Prasad DV, Richards S, Mai XM, Dong C. B7S1, a novel B7 family member that negatively regulates T cell activation. *Immunity* 2003;18:863–73.
- Zang X, Allison JP. To be or not to be B7. *J Clin Invest* 2006;116:2590–3.
- Shahinian A, Pfeffer K, Lee KP, et al. Differential T cell costimulatory requirements in CD28-deficient mice. *Science* 1993;261:609–12.
- Waterhouse P, Penninger JM, Timms E, et al. Lymphoproliferative disorders with early lethality in mice deficient in Ctla-4. *Science* 1995;270:985–8.
- Tivol EA, Borriello F, Schweitzer AN, Lynch WP, Bluestone JA, Sharpe AH. Loss of CTLA-4 leads to massive lymphoproliferation and fatal multiorgan tissue destruction, revealing a critical negative regulatory role of CTLA-4. *Immunity* 1995;3:541–7.
- Chambers CA, Sullivan TJ, Allison JP. Lymphoproliferation in CTLA-4-deficient mice is mediated by costimulation-dependent activation of CD4+ T cells. *Immunity* 1997;7:885–95.
- McAdam AJ, Greenwald RJ, Levin MA, et al. ICOS is critical for CD40-mediated antibody class switching. *Nature* 2001;409:102–5.
- Dong C, Juedes AE, Temann UA, et al. ICOS costimulatory receptor is essential for T-cell activation and function. *Nature* 2001;409:97–101.
- Tafuri A, Shahinian A, Blatt F, et al. ICOS is essential for effective T-helper-cell responses. *Nature* 2001;409:105–9.
- Barber DL, Wherry EJ, Masopust D, et al. Restoring function in exhausted CD8 T cells during chronic viral infection. *Nature* 2006;439:682–7.
- Nishimura H, Nose M, Hiai H, Minato N, Honjo T. Development of lupus-like autoimmune diseases by disruption of the PD-1 gene encoding an ITIM motif-carrying immunoreceptor. *Immunity* 1999;11:141–51.
- Nishimura H, Okazaki T, Tanaka Y, et al. Autoimmune dilated cardiomyopathy in PD-1 receptor-deficient mice. *Science* 2001;291:319–22.
- Keir ME, Liang SC, Guleria I, et al. Tissue expression of PD-L1 mediates peripheral T cell tolerance. *J Exp Med* 2006;203:883–95.
- Ling V, Wu PW, Spaulding V, et al. Duplication of primate and rodent B7-H3 immunoglobulin V- and C-like domains: divergent history of functional redundancy and exon loss. *Genomics* 2003;82:365–77.
- Prasad DV, Nguyen T, Li Z, et al. Murine B7-H3 is a negative regulator of T cells. *J Immunol* 2004;173:2500–6.
- Suh WK, Gajewska BU, Okada H, et al. The B7 family member B7-H3 preferentially down-regulates T helper type 1-mediated immune responses. *Nat Immunol* 2003;4:899–906.
- Townsend SE, Allison JP. Tumor rejection after direct costimulation of CD8+ T cells by B7-transfected melanoma cells. *Science* 1993;259:368–70.
- Chen L, Ashe S, Brady WA, et al. Costimulation of antitumor immunity by the B7 counterreceptor for the T lymphocyte molecules CD28 and CTLA-4. *Cell* 1992;71:1093–102.
- Baskar S, Ostrand-Rosenberg S, Nabavi N, Nadler LM, Freeman GJ, Glimcher LH. Constitutive expression of B7 restores immunogenicity of tumor cells expressing truncated major histocompatibility complex class II molecules. *Proc Natl Acad Sci U S A* 1993;90:5687–90.
- Hodge JW, Abrams S, Schlom J, Kantor JA. Induction of antitumor immunity by recombinant vaccinia viruses expressing B7-1 or B7-2 costimulatory molecules. *Cancer Res* 1994;54:5552–5.
- Wallin JJ, Liang L, Bakardjiev A, Sha WC. Enhancement of CD8+ T cell responses by ICOS/B7h costimulation. *J Immunol* 2001;167:132–9.
- Liu X, Bai XF, Wen J, et al. B7h costimulates clonal expansion of, and cognate destruction of tumor cells by, CD8+ T lymphocytes *in vivo*. *J Exp Med* 2001;194:1339–48.
- Chen L, McGowan P, Ashe S, et al. Tumor immunogenicity determines the effect of B7 costimulation on T cell-mediated tumor immunity. *J Exp Med* 1994;179:523–32.
- Antonia SJ, Seigne J, Diaz J, et al. Phase I trial of a B7-1 (CD80) gene modified autologous tumor cell vaccine in combination with systemic interleukin-2 in patients with metastatic renal cell carcinoma. *J Urol* 2002;167:1995–2000.
- Raez LE, Cassileth PA, Schlesselman JJ, et al. Allogeneic vaccination with a B7.1 HLA-A gene-modified adenocarcinoma cell line in patients with advanced non-small-cell lung cancer. *J Clin Oncol* 2004;22:2800–7.
- Chan L, Hardwick NR, Guinn BA, et al. An immune edited tumour versus a tumour edited immune system: prospects for immune therapy of acute myeloid leukaemia. *Cancer Immunol Immunother* 2006;55:1017–24.
- Tirapu I, Huarte E, Guiducci C, et al. Low surface expression of B7-1 (CD80) is an immunoescape mechanism of colon carcinoma. *Cancer Res* 2006;66:2442–50.
- Huang AY, Golumbek P, Ahmadzadeh M, Jaffee E, Pardoll D, Levitsky H. Role of bone marrow-derived cells in presenting MHC class I-restricted tumor antigens. *Science* 1994;264:961–5.
- Leach DR, Krummel MF, Allison JP. Enhancement of antitumor immunity by CTLA-4 blockade. *Science* 1996;271:1734–6.
- Egen JG, Kuhns MS, Allison JP. CTLA-4: new insights into its biological function and use in tumor immunotherapy. *Nat Immunol* 2002;3:611–8.

57. Hodi FS, Mihm MC, Soiffer RJ, et al. Biologic activity of cytotoxic T lymphocyte-associated antigen 4 antibody blockade in previously vaccinated metastatic melanoma and ovarian carcinoma patients. *Proc Natl Acad Sci U S A* 2003;100:4712–7.
58. Small EJ, Tchekmedyian NS, Rini BI, Fong L, Lowy I, Allison JP. A pilot trial of CTLA-4 blockade with human anti-CTLA-4 in patients with hormone-refractory prostate cancer. *Clin Cancer Res* 2007;13:1810–5.
59. Ribas A, Camacho LH, Lopez-Berestein G, et al. Antitumor activity in melanoma and anti-self responses in a phase I trial with the anti-cytotoxic T lymphocyte-associated antigen 4 monoclonal antibody CP-675,206. *J Clin Oncol* 2005;23:8968–77.
60. Attia P, Phan GQ, Maker AV, et al. Autoimmunity correlates with tumor regression in patients with metastatic melanoma treated with anti-cytotoxic T lymphocyte antigen-4. *J Clin Oncol* 2005;23:6043–53.
61. Phan GQ, Yang JC, Sherry RM, et al. Cancer regression and autoimmunity induced by cytotoxic T lymphocyte-associated antigen 4 blockade in patients with metastatic melanoma. *Proc Natl Acad Sci U S A* 2003;100:8372–7.
62. Sanderson K, Scotland R, Lee P, et al. Autoimmunity in a phase I trial of a fully human anti-cytotoxic T lymphocyte antigen-4 monoclonal antibody with multiple melanoma peptides and montanide ISA 51 for patients with resected stages III and IV melanoma. *J Clin Oncol* 2005;23:741–50.
63. O'Mahony D, Morris JC, Quinn C, et al. A pilot study of CTLA-4 blockade after cancer vaccine failure in patients with advanced malignancy. *Clin Cancer Res* 2007;13:958–64.
64. Beck KE, Blansfield JA, Tran KQ, et al. Enterocolitis in patients with cancer after antibody blockade of cytotoxic T-lymphocyte-associated antigen 4. *J Clin Oncol* 2006;24:2283–9.
65. Maker AV, Phan GQ, Attia P, et al. Tumor regression and autoimmunity in patients treated with cytotoxic T lymphocyte-associated antigen 4 blockade and interleukin 2: a phase I/II study. *Ann Surg Oncol* 2005;12:1005–16.
66. Yang JC, Beck KE, Blansfield JA, Tran KQ, Lowy I, Rosenberg SA. Tumor regression in patients with metastatic renal cancer treated with a monoclonal antibody to CTLA-4 (MDX-010) [Abstract 2501]. *Am Soc Clin Oncol* 2005.
67. Ribas A, Bozon VA, Lopez-Berestein G, et al. Phase I trial of monthly doses of the human anti-CTLA-4 monoclonal antibody CP-675,206 in patients with advanced melanoma [Abstract 7524]. *Am Soc Clin Oncol* 2005.
68. Reuben JM, Lee BN, Shen DY, et al. Therapy with human monoclonal anti-CTLA-4 antibody, CP-675,206, reduces regulatory T cells and IL-10 production in patients with advanced malignant melanoma [Abstract 7505]. *Am Soc Clin Oncol* 2005.
69. Fischkoff SA, Hersh E, Weber J, et al. Durable responses and long-term progression-free survival observed in a phase II study of MDX-010 alone or in combination with dacarbazine in metastatic melanoma [Abstract 7525]. *Am Soc Clin Oncol* 2005.
70. Gerritsen W, Van Den Eertwegh A, De Gruij T, et al. A dose-escalation trial of GM-CSF-gene transduced allogeneic prostate cancer cellular immunotherapy in combination with a fully human anti-CTLA antibody (MDX-010, Ipilimumab) in patients with metastatic hormone-refractory prostate cancer (mHRPC) [Abstract 2500]. *Am Soc Clin Oncol* 2006.
71. Korman A, Yellin M, Keler T. Tumor immunotherapy: preclinical and clinical activity of anti-CTLA4 antibodies. *Curr Opin Investig Drugs* 2005;6:582–91.
72. Maker AV, Attia P, Rosenberg SA. Analysis of the cellular mechanism of antitumor responses and autoimmunity in patients treated with CTLA-4 blockade. *J Immunol* 2005;175:7746–54.
73. Quezada SA, Peggs KS, Curran MA, Allison JP. CTLA4 blockade and GM-CSF combination immunotherapy alters the intratumor balance of effector and regulatory T cells. *J Clin Invest* 2006;116:1935–45.
74. van Elsas A, Hurwitz AA, Allison JP. Combination immunotherapy of B16 melanoma using anti-cytotoxic T lymphocyte-associated antigen 4 (CTLA-4) and granulocyte/macrophage colony-stimulating factor (GM-CSF)-producing vaccines induces rejection of subcutaneous and metastatic tumors accompanied by autoimmune depigmentation. *J Exp Med* 1999;190:355–66.
75. Suttmuller RP, van Duivenvoorde LM, van Elsas A, et al. Synergism of cytotoxic T lymphocyte-associated antigen 4 blockade and depletion of CD25⁺ regulatory T cells in antitumor therapy reveals alternative pathways for suppression of autoreactive cytotoxic T lymphocyte responses. *J Exp Med* 2001;194:823–32.
76. Dranoff G, Jaffee E, Lazenby A, et al. Vaccination with irradiated tumor cells engineered to secrete murine granulocyte-macrophage colony-stimulating factor stimulates potent, specific, and long-lasting anti-tumor immunity. *Proc Natl Acad Sci U S A* 1993;90:3539–43.
77. Thompson RH, Dong H, Kwon ED. Implications of B7-1 expression in clear cell carcinoma of the kidney for prognostication and therapy. *Clin Cancer Res* 2007;13:709–15s.
78. Thompson RH, Gillett MD, Chevillat JC, et al. Costimulatory B7-1 in renal cell carcinoma patients: indicator of tumor aggressiveness and potential therapeutic target. *Proc Natl Acad Sci U S A* 2004;101:17174–9.
79. Konishi J, Yamazaki K, Azuma M, Kinoshita I, Dosaka-Akita H, Nishimura M. B7-1 expression on non-small cell lung cancer cells and its relationship with tumor-infiltrating lymphocytes and their PD-1 expression. *Clin Cancer Res* 2004;10:5094–100.
80. Ghebeh H, Mohammed S, Al-Omair A, et al. The B7-H1 (PD-L1) T lymphocyte-inhibitory molecule is expressed in breast cancer patients with infiltrating ductal carcinoma: correlation with important high-risk prognostic factors. *Neoplasia* 2006;8:190–8.
81. Hamanishi J, Mandai M, Iwasaki M, et al. 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* 2007;104:3360–5.
82. Wu C, Zhu Y, Jiang J, Zhao J, Zhang XG, Xu N. Immunohistochemical localization of programmed death-1 ligand-1 (PD-L1) in gastric carcinoma and its clinical significance. *Acta Histochem* 2006;108:19–24.
83. Inman BA, Sebo TJ, Frigola X, et al. PD-L1 (B7-H1) expression by urothelial carcinoma of the bladder and BCG-induced granulomata: associations with localized stage progression. *Cancer* 2007;109:1499–505.
84. Nakanishi J, Wada Y, Matsumoto K, Azuma M, Kikuchi K, Ueda S. Overexpression of B7-H1 (PD-L1) significantly associates with tumor grade and post-operative prognosis in human urothelial cancers. *Cancer Immunol Immunother* 2007;56:1173–82.
85. Nomi T, Sho M, Akahori T, et al. Clinical significance and therapeutic potential of the programmed death-1 ligand/programmed death-1 pathway in human pancreatic cancer. *Clin Cancer Res* 2007;13:2151–7.
86. Dong H, Strome SE, Salomao DR, et al. Tumor-associated B7-H1 promotes T-cell apoptosis: a potential mechanism of immune evasion. *Nat Med* 2002;8:793–800.
87. Parsa AT, Waldron JS, Panner A, et al. Loss of tumor suppressor PTEN function increases B7-H1 expression and immunoresistance in glioma. *Nat Med* 2007;13:84–8.
88. Iwai Y, Ishida M, Tanaka Y, Okazaki T, Honjo T, Minato N. Involvement of PD-L1 on tumor cells in the escape from host immune system and tumor immunotherapy by PD-L1 blockade. *Proc Natl Acad Sci U S A* 2002;99:12293–7.
89. Strome SE, Dong H, Tamura H, et al. B7-H1 blockade augments adoptive T-cell immunotherapy for squamous cell carcinoma. *Cancer Res* 2003;63:6501–5.
90. Hirano F, Kaneko K, Tamura H, et al. Blockade of B7-H1 and PD-1 by monoclonal antibodies potentiates cancer therapeutic immunity. *Cancer Res* 2005;65:1089–96.
91. Iwai Y, Terawaki S, Honjo T. PD-1 blockade inhibits hematogenous spread of poorly immunogenic tumor cells by enhanced recruitment of effector T cells. *Int Immunol* 2005;17:133–44.
92. Blank C, Brown I, Peterson AC, et al. PD-L1/B7H-1 inhibits the effector phase of tumor rejection by T cell receptor (TCR) transgenic CD8⁺ T cells. *Cancer Res* 2004;64:1140–5.
93. Pure E, Allison JP, Schreiber RD. Breaking down the barriers to cancer immunotherapy. *Nat Immunol* 2005;6:1207–10.
94. Sun Y, Wang Y, Zhao J, et al. B7-H3 and B7-H4 expression in non-small-cell lung cancer. *Lung Cancer* 2006;53:143–51.
95. Salceda S, Tang T, Kmet M, et al. The immunomodulatory protein B7-H4 is overexpressed in breast and ovarian cancers and promotes epithelial cell transformation. *Exp Cell Res* 2005;306:128–41.
96. Tringler B, Zhuo S, Pilkington G, et al. B7-H4 is highly expressed in ductal and lobular breast cancer. *Clin Cancer Res* 2005;11:1842–8.
97. Krambeck AE, Thompson RH, Dong H, et al. B7-H4 expression in renal cell carcinoma and tumor vasculature: associations with cancer progression and survival. *Proc Natl Acad Sci U S A* 2006;103:10391–6.