Published in final edited form as:

Immunotherapy. 2009 January 1; 1(1): 129–139. doi:10.2217/1750743X.1.1.129.

Inhibitors of B7–CD28 costimulation in urologic malignancies

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Abstract

T-cell costimulatory molecules deliver positive or negative signals to govern the ultimate fate of immune responses. These ligands and receptors that negatively costimulate T cells (including cytotoxic T-lymphocyte antigen [CTLA]-4, B7-H1, programmed death [PD]-1, B7-H3 and B7x) have received significant interest recently owing to their proposed ability to form a molecular shield for tumor cells. CTLA-4 represents the most extensively studied receptor in the costimulatory pathway and functions as a potent inhibitor of T-cell-mediated immunity. Clinical trials with anti-CTLA-4 are ongoing, although numerous objective responses have been observed in heavily pretreated patients, albeit with autoimmune side effects. In renal cell carcinoma, B7-H1, PD-1 and B7x have been observed to be expressed on tumor cells or infiltrating lymphocytes and are individually associated with adverse pathologic features and poor clinical outcome. In prostate cancer, B7-H3 and B7x immunostaining intensity correlate with disease spread, clinical cancer recurrence and cancer-specific death. External validation and prospective studies are now needed to confirm these findings, while further development of humanized monoclonal antibodies, similar to the experience with anti-CTLA-4, are underway. Herein, we review the B7–CD28 family as it applies to urologic malignancies.

Keywords

lymphocyte activation; prostatic neoplasm; regulatory T lymphocyte; renal cell carcinoma; urinary bladder neoplasm

Within the last decade, there has been an upsurge of new insights into fundamental regulatory mechanisms governing host immune cell activation and function. As a result, a number of potent and relatively novel immunotherapeutic manipulations have recently emerged that hold great promise for the treatment of human malignancy, including cancers arising within the genitourinary tract. In general, immunotherapy encompasses targeted manipulation of components of the host immune system in order to promote effective immune-mediated tumor killing in the tumor microenvironment. The primary goal of antitumoral immunotherapy is to generate a systemic antigen-specific T-cell response that is capable of destroying a tumor, its metastases, or even the parent tissues from which tumors arise. Herein, we review the background and rationale for immune manipulation of inhibitory T-cell signaling in human malignancy, summarize the preclinical and clinical experience with antibody-mediated cytotoxic T-lymphocyte antigen (CTLA)-4 (CD152) blockade, and discuss other negative costimulatory molecules, including B7-H1 (CD274), programmed death (PD)-1 (CD279), B7-

H3 (CD276) and B7x (B7-H4, B7-S1), which have recently been observed to be potent predictors of clinical outcome and further represent attractive targets for immune manipulation.

The costimulatory pathway

Under normal situations, professional antigen-presenting cells (APCs; dendritic cells, monocytes and macrophages) play a central role in the induction of antigen-specific T-cell activation. Under the influence of proinflammatory cytokines, APCs scavenge tumor cell debris, undergo maturation and then migrate to lymphoid tissues in order to encounter naive and memory T cells. It is within these tissues that APCs can interact with CD4⁺ and CD8⁺ T cells to induce the activation of T cells capable of recognizing tumor-specific or tumor-associated antigens.

At the molecular level, T lymphocytes require two simultaneous but independent signals to become fully activated [1–3]. The first signal arises from interactions between the antigen/MHC, present on the surface of APCs, with their corresponding antigen-specific T-cell receptor. The second signal arises from B7-1 (CD80) and B7-2 (CD86) costimulatory molecules, also present on the surface of APCs, interacting with the T-cell costimulatory receptor CD28 (Figure 1). It is this second costimulatory signal, which is antigen independent, that is critical for facilitating T-cell activation, sustaining T-cell proliferation, allowing for cell-to-cell cooperation and the induction of differentiation from a naive to an effector or memory T-cell phenotype [4]. Underscoring the importance of costimulatory signaling, it has been demonstrated that transgenic mice lacking either the CD28 costimulatory receptor or B7 costimulatory ligands exhibit severe impairments in generating T-cell responses [5].

In addition to the B7–CD28 interaction, recent evidence suggests that the ultimate fate of an activated T cell is governed by a number of additional positive and negative costimulatory signals emanating from a variety of T-cell receptors interacting with their cognate ligands [6]. For example, OX-40 (CD134) [7,8], 4-1BB (CD137) [9], and inducible costimulator (ICOS; CD278) [4] represent positive costimulators that may further act to stimulate activated antigen-specific T cells (Figure 2). By contrast, CTLA-4, B7-H1, PD-1 and B7x represent negative costimulators that act to inhibit T-cell function and diminish T-cell survival; presumably to thwart self-antigen recognition and the subsequent development of autoimmune disease [10]. More recently, immunotherapeutic manipulations of the proposed negative T-cell costimulators have garnered significant attention in the fields of immunology and oncology. In the following sections, we discuss the background along with the translational investigations for the negative costimulatory molecules (CTLA-4, B7-H1, PD-1, B7-H3 and B7x) that have garnered the most interest to date.

CTLA-4

CTLA-4 background

Cytotoxic T-lymphocyte antigen-4 is one of the most extensively studied costimulatory pathway receptors reported in the current literature [1,3]. CD28 is typically expressed on the surface of all T cells. When engaged by B7-1 or B7-2, CD28 provides a vital positive signal to the T cell that stimulates its activation, proliferation and maturation [1]. By contrast, CTLA-4 appears on the surface of T cells only following their activation and binds to B7-1 or B7-2 with 50- to 200-fold higher affinity compared with CD28 [1]. When bound to B7-1 or B7-2, CTLA-4 has been shown to deliver a negative or inhibitory signal to T cells, perhaps to truncate ongoing T-cell responses in order to abort the induction of autoimmunity [1,3]. In support of this, knockout mice lacking the CTLA-4 receptor spontaneously develop an aggressive lymphoproliferative disorder that results in dramatic multiorgan, polyclonal lymphocytic infiltration and lethality by 1 month of age [11–13]. In addition, *in vivo* experiments further

demonstrate that engagement of CD28 stimulates T cells whereas engagement of CTLA-4 inhibits T-cell responses [1]. Thus, these observations collectively suggest that CTLA-4 inhibits T-cell activation not only by outcompeting CD28 for binding to B7 ligands, but also by actively suppressing positive costimulatory signals that typically arise from CD28 to mediate T-cell activation. Therefore, T-cell expression of CTLA-4 is thought to play a critical role in maintaining immune system homeostasis by limiting the generation of autoimmune disease. Moreover, CTLA-4 is capable of inhibiting the activity of antitumoral T cells and, therefore, represents an attractive target for cancer immunotherapy.

The first *in vivo* antibody-mediated blockade of the CTLA-4 receptor occurred approximately a decade ago, demonstrating that CTLA-4 blockade is capable of promoting T-cell-mediated regression of solid tumors in mice (Figure 3) [14]. Subsequently, using tumor cells derived from transgenic adenocarcinoma of the mouse prostate (TRAMP) mice, it became evident that *in vivo* blockade of CTLA-4 is capable of enhancing T-cell-mediated regression of subcutaneous prostate tumors in nearly all tumor-bearing mice [15]. In further studies, systemic CTLA-4 blockade was shown to markedly diminish metastatic outgrowth of TRAMP tumors (by ~50%) when administered as an adjunctive therapy following primary tumor extirpation by surgery [16]. Additional studies demonstrated that when combined with granulocyte-macrophage colony-stimulating factor (GM-CSF) tumor cell vaccination, CTLA-4 blockade could even generate potent T-cell-mediated antitumoral responses causing regression of poorly immunogenic murine tumors [17–19]. Based in part on these studies, two fully human anti-CTLA-4 antibodies, MDX-010 (ipilimumab) and CP-675,206 (ticilimumab) were developed for human use.

Anti-CTLA-4 in urologic cancer

Following development of a humanized monoclonal antibody to block CTLA-4, multiple Phase I trials to assess the safety of anti-CTLA-4 treatment were conducted in patients with advanced prostate cancer and melanoma. In brief, these Phase I trials established that a single dose of anti-CTLA-4 antibody is generally well tolerated, producing relatively few and minor side effects. In addition, these Phase I trials in melanoma and prostate cancer demonstrated some evidence that CTLA-4 blockade is capable of generating antitumoral activity [20–22]. Thus, Phase II trials to test the effectiveness of CTLA-4 blockade for the treatment of a number of forms of cancer, including prostate cancer, have recently been initiated. From these early Phase I/II clinical trials, some provocative observations can be drawn. First, anti-CTLA-4 is capable of inducing objective tumor responses in multiple different tumors, including prostate cancer, renal cell carcinoma (RCC), melanoma and lymphoma [3]. Despite the fact that most patients were heavily pretreated and refractory to multiple modalities, objective tumor responses following anti-CTLA-4 monotherapy have been demonstrated in approximately 15% of patients [3,23]. Second, objective responses have involved multiple visceral sites including brain metastases. Third, complete responses have been durable for nearly 3 years to date [24]. A summary of Phase I/II clinical trials using CTLA-4 blockade in urologic malignancies is demonstrated in Table 1. While the optimal dose and frequency of delivery have yet to be determined, evidence to date clearly demonstrates that anti-CTLA-4 can produce objective tumor responses in patients with refractory malignancy, including prostate cancer and RCC.

As might be anticipated, reported toxicities with CTLA-4 blockade have included autoimmune related events including dermatitis, uveitis, hypophysitis, nephritis and enterocolitis. Enterocolitis, mostly manifested by diarrhea, appears to be the most common major toxicity, occurring in approximately 20% of patients [23]. Importantly, most patients with grade 3/4 toxicities, including enterocolitis, respond to systemic steroids and the administration of steroids does not appear to negatively impact tumor response [23]. In fact, at the National Cancer Institute, Bethesda, MD, USA, objective tumor responses were significantly more

likely to occur in patients who also developed enterocolitis (35 vs 2%, $p = 0.0016$) compared with patients who did not develop enterocolitis [23]. Based on this, it has been suggested that in order to generate an effective antitumoral response during immunotherapy, it might also be necessary to induce some degree of auto immunity against the parent tissues from which a given tumor arises (i.e., tissue-specific auto immunity). Certainly, it has been demonstrated that many human tumor-associated antigens are simply overexpressed self-antigens. Thus, it remains possible that host tolerance to self-antigens must be broken or reduced in order to achieve an effective antitumoral response. Regardless, it is quite clear that the balance between achieving tumor regression and autoimmunity will require close scrutiny as CTLA-4 blockade continues to evolve as an immunotherapeutic treatment for cancer.

B7-H1

B7-H1 background

B7-H1 (PD-L1) was discovered in 1999 and is a cell surface glycoprotein within the B7 family of T-cell costimulatory molecules [25]. B7-H1 mRNA is widespread in human tissues, although constitutive cell surface protein expression is limited to a fraction of macrophage-lineage cells [25,26]. However, cell surface B7-H1 is also found on activated T lymphocytes and is aberrantly expressed by numerous human tumors [26–31]. These findings indicate that B7-H1 protein expression is controlled by post-transcriptional mechanisms, perhaps via proinflammatory cytokines such as $\text{INF-}\gamma$ or loss of tumor suppressors, including phosphatase and tensin homolog (PTEN) [10,26,32]. In addition, tumor expression of B7-H1 is high *in vivo* yet reduced following *ex vivo* culture, suggesting that the *in vivo* milieu upregulates B7-H1 expression. Experimental evidence suggests that B7-H1 can both positively and negatively costimulate T-cell responses depending on the location of expression [10,25,26,33]. While this dichotomous function is not completely understood and a consensus opinion is lacking, evidence suggests that B7-H1 participates in the activation of naive T cells in lymphoid tissue and areas of inflammation; however, in peripheral tissues including solid tumors, B7-H1 downregulates immune responses by inhibiting activated or memory T cells [10].

In murine models, tumor-associated B7-H1 has been shown to inhibit antitumor cell-mediated immunity by interacting with T-cell PD-1 to induce tumor-specific T-cell apoptosis or by impairing cytokine production and the cytotoxicity of activated T cells [26,30,34–36]. In addition, murine tumors expressing B7-H1 have been reported to abrogate immune-mediated tumor regression following adoptive transfer of tumor antigen-specific CD8^+ T-cell clones and treatment with agonistic costimulatory antibodies (4-1BB) that promote T-cell activation [37]. Consistent with this, *in vivo* blockade of B7-H1 can potentiate antitumor T cell responses directed against immunogenic murine cancers expressing B7-H1 either endogenously or following *B7-H1* gene transduction [26,34,37,38]. Thus, preclinical studies support that B7-H1 blockade can be used to enhance antitumor immunity in murine cancer models [31,37, 38].

B7-H1 in renal cell carcinoma

In 2004, the first investigation of B7-H1 in RCC was reported [39]. B7-H1 immunohistochemistry was performed on 196 fresh-frozen clear-cell RCC specimens and expression levels were correlated with pathologic features and clinical outcome. With early clinical follow-up, some important findings were observed in this study. First, B7-H1 protein is expressed by both RCC tumor cells (present in 66% of specimens) and tumor-infiltrating lymphocytes (present in 59% of specimens) [39]. This finding in itself is novel because B7-H1 is not expressed within normal human kidney tissues [25,26]. The fact that malignant cells of the kidney acquire the ability to express B7-H1 and are associated with B7-H1-positive lymphocytes suggest that perhaps B7-H1 is related to the immunogenicity of this immunogenic

tumor [40]. Second, patients with high levels of B7-H1, present on either tumor cells or lymphocytes, were significantly more likely to have adverse pathologic features including higher nuclear grade ($p < 0.001$), positive lymph nodes ($p < 0.001$), distant metastases ($p = 0.022$) and histologic tumor necrosis ($p < 0.001$) [39]. This effectively demonstrated that B7-H1-positive tumors are associated with aggressive tumor phenotypes. Third, RCC patients with high tumor-associated or lymphocyte-associated B7-H1 were at significant risk of cancer-specific death in a univariate model [39], which was later confirmed to retain statistical significance in a multivariate model [41,42]. Thus, B7-H1 became the first costimulatory molecule associated with mortality in any human malignancy. Additional studies in paraffin-embedded sections confirmed these observations demonstrating that tumor expression of B7-H1 is significantly associated with progression-free survival (risk ratio 3.46), cancer-specific survival (risk ratio 3.92), and overall survival (risk ratio 2.37) [43]. In fact, the 5-year cancer-specific survival rates were 42% for patients with B7-H1-positive tumors and 83% for patients with B7-H1-negative tumors (Figure 4) [43]. Additional investigations indicate that metastatic clear-cell RCC specimens express B7-H1 at a rate similar to that of primary specimens (Figure 5) [41]. This finding is notable because systemic therapy is often given to patients following cytoreductive nephrectomy. With B7-H1 clinically present in metastatic deposits, advanced RCC patients would remain candidates for B7-H1-targeted therapy even if the primary tumor was surgically extirpated [41].

B7-H1 in urothelial & prostate cancer

Immunohistochemistry for B7-H1 in urothelial cancer has recently been performed and reported by two groups in 2007 [44,45]. Both studies suggest that B7-H1 expression is associated with the primary tumor classification with levels of expression escalating from Ta tumors up to T4 tumors [44,45]. In addition, higher levels of B7-H1 expression were also associated with higher tumor grade [44]. One study evaluated B7-H1 expression in prostate cancer. Performing immunohistochemistry in 338 men, less than 1% demonstrated protein expression of B7-H1 [46].

Pd-1

PD-1 background

The PD-1 receptor was first described in 1992 as a member of the CD28 family of costimulatory molecules [47]. In contrast to CTLA-4, CD28 and ICOS, which are all obligate disulfide-linked homodimers, PD-1 is monomeric both on the cell surface and in solution [48,49]. The cytoplasmic region for PD-1 contains an immunoreceptor tyrosine-based inhibitory motif and an immunoreceptor tyrosine-based switch motif, indicating a negative regulatory function [48–50]. Cell-surface expression of PD-1 has been observed on activated T cells, stimulated macrophages, and mature dendritic cells [51]. PD-1 protein can also accumulate within the cytoplasm of natural regulatory T cells, becoming upregulated to the cell surface following T-cell receptor (TCR) activation [52]. In contrast to B7-H1 and B7-H3/B7x (described below), tumor-cell expression of PD-1 by nonhematopoietic solid neoplasms [53], has not been reported. The known ligands for PD-1 include B7-H1 and B7-DC (PD-L2) [10]. The broad distribution of these ligands suggests that the PD-1/B7-H1/B7-DC family may regulate immune responses in both lymphoid and nonlymphoid organs [10]. In addition, compared with other B7 costimulatory members, PD-1 is expressed at a relatively late phase following T-cell activation, suggesting that the PD-1 pathway may primarily function at sites of inflammation in peripheral organs [48,49].

In vitro and *in vivo* studies demonstrate that engagement of PD-1 by B7-H1 inhibits T-cell proliferation, survival or function whereas blockade of PD-1 removes inhibitory signals regulating tolerance of peripheral T cells facilitating expansion and activation [48,49]. In

addition, PD-1-deficient mice develop a troponin-specific antibody response that is associated with a dilated cardiomyopathy secondary to T- and B-cell dysregulation [48,49]. Additional studies demonstrate that patients with rheumatoid arthritis or Sjogren's syndrome harbor higher percentages of PD-1⁺ lymphocytes relative to healthy subjects [48]. Recently, Barber *et al.* reported that, in mice, chronic lymphocytic choriomeningitis infection produces 'exhausted' viral-specific CD8⁺ T cells that bear upregulated levels of PD-1 [54]. In other studies, PD-1 has been reported to be upregulated in HIV-specific CD8⁺ T cells, and blockade of the PD-1/B7-H1 pathway has been shown to enhance the capacity for these cells to proliferate and survive [55–57]. Collectively, the aforementioned studies support that PD-1 functions as a negative regulator of immune responses and that blockade of PD-1 may enhance antitumor and antiviral immunity.

PD-1 in renal cell carcinoma

To our knowledge, one study has evaluated PD-1 protein expression in RCC patients [58]. Using immunohistochemistry on 267 patients with clear-cell RCC, PD-1 was not detected on RCC tumor cells [58]. However, among the 136 patients with a lymphocytic infiltration, PD-1 was expressed by the immune cells in approximately 50% of the specimens. In this study, patients with PD-1-positive immune cells were significantly more likely to have B7-H1-positive tumors and other adverse pathologic features, including higher nuclear grade ($p = 0.001$), larger tumors ($p = 0.001$), advanced tumor, nodes, metastasis (TNM) stage ($p = 0.005$), coagulative tumor necrosis ($p = 0.027$) and sarcomatoid differentiation ($p = 0.008$) [58]. In addition, patients with infiltrating immune cells expressing PD-1 were significantly more likely to have associated tumor cells expressing B7-H1 ($p < 0.001$) [58]. In fact, 95% of patients with PD-1-positive immune cells had B7-H1-positive tumor cells. Univariate, patients with PD-1-positive immune cells were significantly more likely to die from RCC (risk ratio 2.24) although statistical significance was not demonstrated in a multivariate analysis probably owing to the short duration of follow-up (median 2.9 years) [58]. Collectively, these results suggest that the B7-H1/PD-1 signal pathway is present clinically in RCC patients, potentially fostering tumor progression in a setting of impaired immune surveillance. Thus, antibody-mediated blockade, similar to the development of anti-CTLA-4, remains a promising therapeutic approach in RCC.

B7-H3

B7-H3 background

B7-H3 was discovered in 2001 and serves as an accessory costimulatory molecule following initial antigen priming in cooperation with a putative counter-receptor [59]. The precise physiologic role for B7-H3 remains debatable as both stimulatory properties, including promotion of T-cell proliferation and IFN- γ production, along with inhibitory properties, including impairment of Th1 responses and protection from natural killer cell-mediated lysis, have been described [59–62]. B7-H3 protein expression has been described in numerous peripheral organs along with human malignancies of the lung, stomach and prostate [46,63, 64]. In the context of malignancy, the precise physiologic and pathologic role of B7-H3 expression has yet to be fully elucidated. Transfection of B7-H3 into experimental tumor lines (P815 mastocytoma, colon-26 and EL-4 lymphoma) have been reported to accelerate *in vivo* tumor rejection, supporting a net positive costimulatory effect [65–67]. In addition, observations of human gastric carcinoma suggest that B7-H3-positive tumor cells is associated with improved patient survival [64]. By contrast, tumor cell expression of B7-H3 in human lung cancer was reported to be associated with an increased risk of lymph node metastases [63]. However, two very recent studies of human patients with prostate carcinoma helped further define a negative costimulatory role for B7-H3.

B7-H3 in prostate cancer

Recently, Roth *et al.* evaluated B7-H3 in 338 human prostate cancer patients who underwent radical prostatectomy [46]. Performing immunohistochemistry for B7-H3, 100% of tumor specimens demonstrated aberrant B7-H3 expression. While B7-H3 was also found to be expressed in benign prostatic epithelial cells and in intraepithelial neoplasia, these levels were low compared with cancer cells, suggesting that greater expression is acquired as cells go from benign to *in situ* carcinoma to malignant [46]. Roth *et al.* also observed that strong staining intensity was associated with adverse clinicopathologic features and was an independent predictor for cancer progression after surgery (risk ratio 4.42) [46]. A second study by Zang *et al.* also evaluated prostate cancer in a similar fashion performing immunohistochemistry for B7-H3 (and B7x as described below) in 823 patients [68]. Remarkably, very similar results were observed. Zang *et al.* reported that 93% of tumors had aberrant B7-H3 protein expression with a median of 80% of tumor cells staining positive [68]. In this study, strong immunostaining intensity was also associated with disease spread at time of surgery (extracapsular extension, seminal vesicle involvement or lymph node metastases) and was also associated with clinical cancer recurrence ($p < 0.001$) and cancer-specific death ($p = 0.004$) [68].

What we found to be most interesting about the two aforementioned studies [46,68] was that despite unique patient populations, differences in antibodies utilized, variations in definitions of end points and different pathologists evaluating the specimens, both groups independently obtained remarkably similar results [46,68]. That is, most prostate cancer cells express B7-H3, absolute percentage of expression is not as predictive as intensity, and strong intensity is associated with extracapsular extension, seminal vesicle invasion, and clinical recurrence of cancer. One study was also able to demonstrate an association with cancer-specific survival although this may relate to the larger sample cohort [68]. Collectively, these studies support, at least in prostate cancer, that B7-H3 functions at the clinical level precluding immune containment and thereby fostering cancer recurrence. As more translational studies mature with time, the true mechanism of B7-H3 and prognostic significance of B7-H3 will be declared. Until then, B7-H3 remains an independent prognostic indicator in prostate cancer and represents a promising therapeutic target.

B7x

B7x (B7-H4, B7S1) was discovered in 2003 and is the newest member of the B7 family [69–71]. In contrast to B7-H3, B7x has consistently demonstrated a negative costimulatory mechanism via inhibition of CD4⁺ and CD8⁺ T-cell proliferation, cell-cycle progression, IL-2 production, and rendering tumor cells refractory to apoptosis [69–72]. Similar to B7-H3, B7x is a type I transmembrane protein and has a yet unidentified counter-receptor. However, B7x protein expression seems to be more restricted compared with B7-H3. Despite mRNA expression in various human tissues, immunohistochemistry for B7x fails to reveal detectable protein expression in any healthy human organs [73]. By stark contrast, aberrant expression of B7x has been observed in cancers of the breast [72,74], lung [63], ovary [72,75], uterus [76], prostate [68] and kidney [77]. In addition, a soluble form of B7x was recently reported to be elevated in ovarian cancer patients compared with control patients [78]. Thus, B7x has been proposed as potential therapeutic targets in multiple human malignancies.

B7x in renal cell carcinoma

Krambeck *et al.* recently performed immunohistochemistry for B7x in 259 patients with clear-cell RCC [77]. Using fresh frozen tissue, approximately 60% of patients had ectopic tumor cell expression of B7x. Patients with tumor expression of B7x were significantly more likely to demonstrate adverse pathologic features (including advanced tumor size, grade and stage) and an increased risk of death from disease (risk ratio 3.05) compared with tumors negative for

B7x [77]. Interestingly, B7x was found to be preferentially expressed on tumor vasculature (>80% of specimens had B7x expression on tumor vasculature compared with 7% of vessels to normal renal parenchyma), suggesting a potential role for initiating or maintaining tumor angiogenesis [77]. While this study needs external validation, B7x appears to be another predictor for RCC patients and represents a very attractive therapeutic target, especially with the potential association with tumor angiogenesis.

B7x in prostate cancer

B7x was recently evaluated in patients with prostate cancer treated surgically between 1985 and 2003. Using immunohistochemistry to evaluate B7x protein expression in 823 prostatectomy specimens, Zang *et al.* demonstrated that nearly all (99%) patients with prostate cancer have aberrant or ectopic expression of B7x. Similar to B7-H3, strong B7x intensity was associated with cancer spread (extracapsular extension, seminal vesical invasion or lymph node positivity), along with clinical cancer recurrence (hazard ratio 2.22) and cancer-specific death (hazard ratio 2.71). Similar to RCC, external validation of these observation is needed. However, B7x currently represents a potential therapeutic target in human prostate cancer while blockade may theoretically provide appropriate protection for effector lymphocytes.

Conclusion

Current applications of immunotherapy and vaccination for human malignancies are often capable of eliciting strong and functionally intact T-cell responses; however, these responses against tumor antigens do not necessarily correlate with tumor regression in clinical trials. In the last decade, there has been significant progress in the understanding of fundamental regulatory mechanisms directing host immune cell activation, with particular emphasis on T-cell costimulation. Ligands and receptors that negatively costimulate T cells (i.e., downregulate the immune responses), including CTLA-4, B7-H1, PD-1, B7-H3 and B7x, have garnered significant interest in recent years. CTLA-4 blockade is currently utilized in numerous clinical trials with objective responses in heavily pretreated patients. B7-H1 is the first T-cell costimulatory molecule reported to be significantly and independently associated with poor survival in a human malignancy. PD-1, B7-H3 and B7x have also been observed to be associated with adverse pathologic features and poor outcome in human malignancies. These findings suggest a tumor escape mechanism whereby cancer cells are capable of evading antitumor immunity with a molecular shield of immune resistance and manipulation of negative costimulatory molecules holds promise for patients with immunogenic malignancies.

Future perspective

With the rapid progress made in understanding the fundamental mechanisms governing immune system activation, and control thereof, we expect continued growth and interest in immune manipulation for a host of immune related states, including malignancy, autoimmune disease and transplantation. In the future, results from ongoing clinical trials with anti-CTLA-4 are anxiously awaited. Monoclonal antibodies to other inhibitory B7-CD28 ligands and receptors (including PD-1, B7-H1, B7-H3 and B7x) are expected to become fully humanized and investigated in clinical trials. We speculate that while manipulation of the immune system can facilitate potent T-cell responses, it will take a combinational approach, such as monoclonal antibody blockade of inhibitory immune receptors in conjunction with therapies such as radiation, radiofrequency ablation or targeted medications to facilitate antigen release. Such an approach will eventually lead to effective and lasting immune control of tumors, including cancers of the urinary tract.

Acknowledgments

James P Allison has financial interest with Medarex and is an inventor of relevant intellectual property that is held by the University of California, CA, USA. R Houston Thompson and Eugene D Kwon have filed for patents pertaining to B7-H1 and B7x prognostic ability in cancer.

The authors have no other relevant affiliations or financial involvement with any organization or entity with a financial interest in or financial conflict with the subject matter or materials discussed in the manuscript apart from those disclosed.

No writing assistance was utilized in the production of this manuscript.

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Figure 1. T-cell activation requires two independent signals

(A) First, antigen is presented to the TCR via an Ag/MHC. A second antigen-independent signal, termed costimulation, is required to govern the fate of the T cell. When the TCR CD28 interacts with its counter-receptor B7-1 or B7-2, a positive T-cell response is generated. (B) In the presence of CTLA-4, T-cell responses are abrogated and tolerance of presented antigen is allowed. Ag: Antigen; APC: Antigen-presenting cell; CTLA: Cytotoxic T-lymphocyte antigen; TCR: T-cell receptor. Redrawn with permission from [5].

Figure 2. Positive and negative costimulatory signals governing the fate of an activated T-cell
Although required for T-cell activation, the Ag–TCR interaction does not govern the fate of the T cell. Positive costimulatory molecules, such as CD28, 4-1BB, ICOS and OX40 can stimulate a T-cell response specific for the antigen presented. On the other hand, negative costimulatory molecules, such as CTLA-4, can inhibit T-cell activation and may induce anergy (tolerance) to the presented antigen. Ag: Antigen; CTLA: Cytotoxic T-lymphocyte antigen; TCR: T-cell receptor. Redrawn with permission from [5].

Figure 3. CTLA-4 blockade is capable of promoting T cell-mediated regression of solid tumors in mice

Monoclonal antibody blockade of CTLA-4 allows CD28 to interact with its B7-family counter-receptor, thus stimulating a tumor specific T-cell response. Ag: Antigen; APC: Antigen-presenting cell; CTLA: Cytotoxic T-lymphocyte antigen; TCR: T-cell receptor. Redrawn with permission from [5].

Figure 4. Cancer-specific survival in patients with renal cell carcinoma

Association of tumor B7-H1 expression with death from renal cell carcinoma (RCC) for 306 patients with clear-cell RCC (risk ratio: 3.92; 95% CI: 2.61–5.88; $p < 0.001$). Cancer-specific survival rates at 5 and 10 years following nephrectomy were 42 and 37%, respectively, for patients with positive tumor B7-H1 expression compared with 83 and 77%, respectively, for patients with negative tumor B7-H1 expression. Reprinted with permission from [43].

Figure 5. Metastatic clear-cell renal cell carcinoma specimens express B7-H1 at a rate similar to that of primary specimens

Photomicrograph at $\times 400$. Metastatic renal cell carcinoma specimen to lung with high B7-H1 expression on both tumor cells (white arrow) and infiltrating lymphocytes (black arrow). Reprinted with permission from [41].

Summary of published Phase I/II clinical trials using cytotoxic T-lymphocyte antigen-4 blockade in urologic malignancies.

Table 1

Antibody	Dose (mg/kg)	Urologic cancer	Patients (n)	Objective responses (n)	Ref.
Ipilimumab	3, then 1.5 every 4 weeks	Prostate	4	0	[79]
Ipilimumab	3	Prostate	14	2*	[80]
Ipilimumab + GVAX®	3	Prostate	6	5*	[3]
Ticilimumab	0.01–15	Kidney	4	0	[24]
Ipilimumab	1–3	Kidney	61	7	[23]

* Response defined as >50% reduction in serum prostate-specific antigen.