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Review article

The expanding repertoire of targets for immune checkpoint inhibition in bladder cancer: What lies beneath the tip of the iceberg, PD-L1

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Abstract

Over the last decade, a new understanding of tumor-immune system interplay has been ushered in, lead in large part by the discovery of immune checkpoints mediated through B7-CD28 family interactions. Therapeutic blockade of the PD-L1 immune checkpoint pathway has already shown great success as a cancer immunotherapy for advanced urothelial carcinoma, leading to durable clinical remissions in an otherwise incurable disease. There are newly described members of the B7-CD28 family including B7-H3, B7x, and HHLA2. These ligands are thought to play an essential role in suppressing T-cell response, leading to immune tolerance of tumors. This feature makes them attractive targets for novel immunotherapy treatment paradigms. Here, we review the literature of current strategies and future directions of immune checkpoint blockade therapy for bladder cancer.

1. Historical perspective of immunotherapy for bladder cancer

The first suggestion of interplay between tumor biology and host immunity was in the 19th century when William Coley observed that infections were associated with tumor regression [1]. This antineoplastic effect of concomitant infection was again observed by Raymond Pearl at Johns Hopkins in 1929. He performed autopsies on patients who died of tuberculosis and noticed that there was a surprisingly low rate of underlying malignancy in these patients [2]. Alvaro Morales was the first to introduce immunotherapy to bladder cancer via intravesical treatment with bacillus Calmette-Guerin (BCG). BCG is a live attenuated strain of *Mycobacterium bovis* [3] that is reconstituted in solution and instilled into the bladder following transurethral resection (TUR) of non–muscle-invasive bladder tumors. Morales originally treated 9 patients with intravesical BCG and reported a significant reduction in bladder tumor recurrence [4]. This was the foundation for subsequent randomized controlled trials comparing patients undergoing TUR followed by adjuvant BCG to patients undergoing TUR alone [5,6]. These studies convincingly demonstrated that BCG lowers the risk of tumor recurrence and may delay tumor progression. Dr Morales established the currently used regimen of 6 weekly bladder instillations. Although the precise mechanism of action of BCG immunotherapy remains the subject of continued investigation, it is believed to function by activating both the innate and adaptive immune systems [7]. Intravesical BCG has been validated by multiple randomized controlled trials as a superior therapy to intravesical chemotherapy including mitomycin and epirubicin [8–11]. Since its introduction nearly 4 decades ago, BCG is still considered to be one of the most successful immunotherapy agents for any solid malignancy [12]. It would not be until the 21st century that our understanding of the relationship between cancer and immunity would make a significant leap forward by the discovery of new tumor-immune evasion pathways via immune checkpoints.

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2. Overview of immune checkpoint receptors and pathways

Immune evasion is considered to be one of the hallmarks of cancer and is an essential step in the evolution of a tumor [13]. Tumor-immune evasion is achieved through multiple mechanisms: (1) selective evolution of tumors with down-regulated expression of neoantigens; (2) decrease or loss of expression of class I MHC molecules; (3) resistance to T-cell–mediated cytolytic killing; (4) presence of immune suppressing cells—regulatory T cells (T regs), myeloid-derived suppressor cells (MDSCs), and secretion of immunosuppressive cytokines in the tumor microenvironment; and (5) expression of coinhibitory ligands and induction of T-cell exhaustion/anergy [14]. Regulation of T-cell activation requires 2 signals: (1) engagement of the T-cell receptor (TCR) through recognition of a peptide MHC complex, and (2) presence of a second (costimulatory or coinhibitory) signal delivered by the interaction of the family of CD28 receptors and B7 ligands [15]. If the second signal delivered is a costimulatory signal, then T-cell activation takes place leading to cytolysis of the cell, whereas if it is a coinhibitory signal, this results in T-cell exhaustion. The CD28 family of receptors are CD28 (costimulatory), CTLA-4 (coinhibitory), ICOS (costimulatory), PD-1 (coinhibitory) and TMIGD2 (unclear function), whereas the B7 family of ligands are B7-1, B7-2, PD-L1, PD-L2, B7-H3, B7x, and HHLA2. This pathway is essential for regulating the T-cell response, and tumors can induce T-cell suppression by expressing B7 coinhibitory ligands on the surface of tumor cells or by stimulating their expression on antigen presenting cells (APCs). The more extensively studied pathways are the CTLA-4/B7-1/B7-2 pathway and the PD-1/PD-L1/PD-L2 pathway. The characterization of these pathways has led to many important therapeutic advances. In this section, we discuss these pathways and review the relevant clinical trials.

2.1. CTLA-4/B7-1/B7-2 pathway

CD28 is expressed constitutively on naïve and activated T cells, whereas CTLA-4 is constitutively expressed only on T regs [16]. After an antigenic stimulus, CD28 interacts with the B7-1/B7-2 ligands on the APC and colocalizes with the TCR (Fig. 1). This results in phosphorylation of TCR-dependent kinases and also activates a distinct signaling program including increased production of IL-2 and high expression of the IL-2 receptor, which is needed for clonal expansion of naïve T cells [17]. Activation of the T cell through the CD28/B7-1/B7-2 pathway also results in movement of CTLA-4 from the intracellular compartment to the cell surface. As CTLA-4 has a higher binding affinity for B7-1/B7-2 than CD28, this results in suppression of costimulation, and the amount of CTLA-4 that translocates to the cell surface is proportional to the strength of the antigenic stimulus. CTLA-4 then recruits phosphatases such as SH-2 that decrease CD28 and TCR-dependent signaling of the T cell via dephosphorylation of TCR and other upstream signaling molecules. This balance between CD28 and CTLA-4 signaling is essential in controlling the T-cell immune response. Most normal tissues do not express B7-1 or B7-2, and thus cannot activate naïve T cells. In the absence of costimulation, activation of TCR by antigenic stimulus alone will result in anergy or functional inactivation of these cells. The physiological function of this pathway is the maintenance of self-tolerance; however, when tumor cells present an antigen in the absence of costimulatory molecules, this also leads to anergy and can contribute to immune evasion.

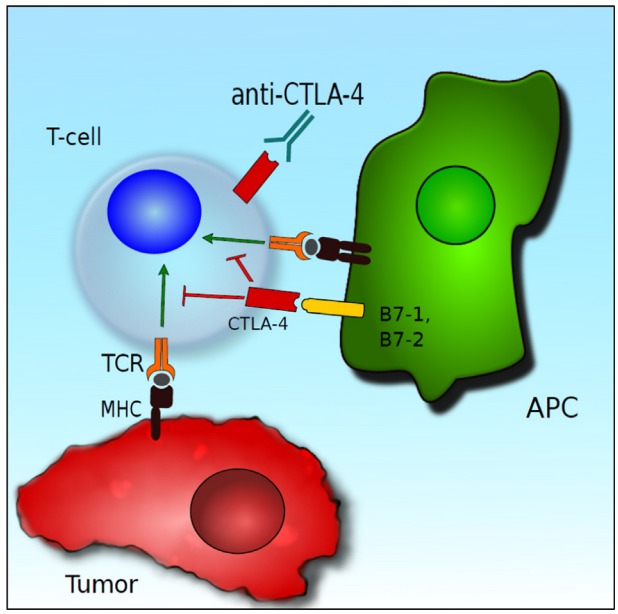

Fig. 1. Antigen presenting cells (APC) present tumor cell antigens to T cells alongside costimulation via B7-1 and B7-2; however, when these ligands bind CTLA-4, this leads to T-cell coinhibition. CTLA-4 can be blocked with anti-CTLA-4 antibodies, leading to enhanced antitumor immunity. (Color version of the figure available online.)

2.2. PD-1/PD-L1/PD-L2 pathway

PD-L1 is widely expressed in a variety of tissues including vascular endothelium and APCs, whereas PD-L2 is predominantly expressed in immune cells such as macrophages and dendritic cells. IFN-g stimulates PD-L1 expression, and IL-4 induces PD-L2 expression. PD-1 is not constitutively expressed on naïve T cells but can be up-regulated on T cells, B cells, and other myeloid cells [18]. Such wide expression of PD-L1 suggests that it is important for tolerance. After binding to PD-L1 or PD-L2, the PD-1 receptor similar to the CD28 receptor colocalizes with the TCR and inhibits the phosphorylation of CD3-E and Zap-70, resulting in blockage of TCR-generated antigenic signals (Fig. 2). In addition, phosphorylation of intracellular immunoreceptor tyrosine-based inhibitory motif of PD-1 leads to SHP-2 activation, which in turn inhibits the

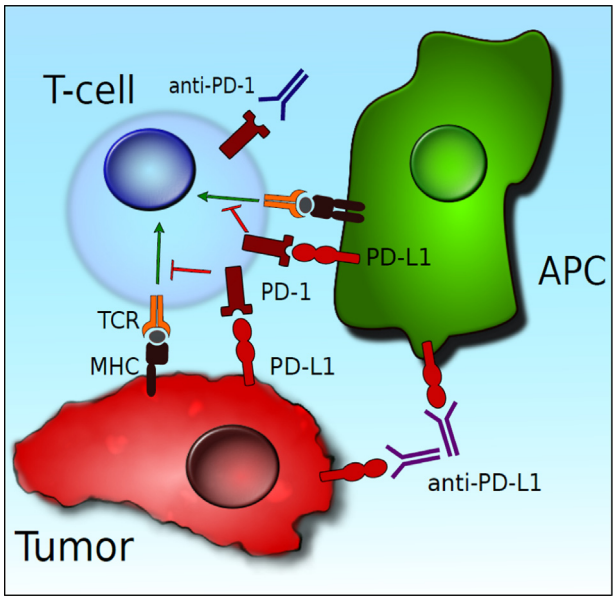

Fig. 2. PD-L1 can be expressed on both APCs and tumor cells. PD-L1 binds to PD-1 on the T-cell surface, leading to coinhibition of T-cell activity. The PD-1/PD-L1 pathway can be blocked with anti-PD-1/PD-L1 antibodies, leading to immune-mediated tumor destruction. (Color version of the figure available online.)

PI3K/Akt pathway activated by CD28 signaling. Similar to CTLA-4, PD-1 activation can block TCR and CD28 signaling, but their mechanisms of actions are different. In addition, PD-1 expression plays an important role in chronic viral infections and cancers. Persistent antigenic stimulation leads to a T-cell exhaustion phenotype characterized by high levels of coinhibitory receptors such as PD-1 on CD8+ T cells. T cells with an exhaustion phenotype have poor proliferative, cytokine producing, and cytolytic capabilities. Such an exhaustion phenotype can be found in chronic viral infections and malignancies, and reversal of the exhaustion phenotype results in improved control of infections and disease. PD-L1 expression has been shown to be prognostic in several cancers including melanoma, colon, cervical, renal cell cancer, and breast and ovarian cancer [19]. Response to PD-1/PD-L1 therapy depends on the mutational landscape of malignancies with malignancies having higher mutational burden demonstrating better responses [20].

3. Bladder tumors as immunogenic targets

Bladder cancers express high levels of PD-L1 [21]. Inman et al. demonstrated PD-L1 expression in bladder tumors of all stages, with particularly high levels in advanced stage tumors (30%) and tumors with carcinoma in situ (CIS) (45%). Furthermore, PD-L1 expression was abundant in tumors refractory to BCG treatment, suggesting that PD-L1 may play a role in shielding the tumor from immune-mediated tumor destruction. Boorjian et al. [22] observed that increased PD-L1 expression was associated with advanced urothelial cancer and independently predicted all-cause mortality.

Urothelial carcinoma genomes harbor a large number of somatic mutations. In a recent comprehensive genomic analysis by Lawrence et al. [23], a high frequency of somatic mutations was identified in exome sequences of human bladder tumors. Of the solid tumors evaluated to date, lung cancer, melanoma, and bladder cancer are characterized by the highest observed levels of mutational burden. Mutated cellular transcripts are processed into tumor neoantigens that are presented on the surface of APCs. This can lead to enhanced host immune recognition, a critical first step in generating a robust antitumor response.

4. Clinical trials of checkpoint inhibitors in urothelial carcinoma

Until 2016, there had been no new systemic treatments for advanced urothelial carcinoma approved by the FDA in 3 decades [12]. Recent clinical trials have demonstrated considerable therapeutic benefit of anti-CTLA-4 and anti-PD-1/PD-L1 agents in metastatic melanoma [24–26], leading to durable clinical remissions in an otherwise incurable disease.

The first phase I study of an anti-PD-L1 antibody (atezolizumab) in urothelial carcinoma was reported by Powles et al. [27]. Study participants had metastatic urothelial carcinoma who had failed prior systemic therapy. Sixty-seven patients received treatment and were stratified by their tumor PD-L1 expression status. Of these, 30/67 (45%) had high levels of PD-L1 expression and 35/67 (52%) had low levels of PD-L1 expression (2 patients had unknown expression). The objective response rate was 43% in PD-L1 high expression tumors and 11% in PD-L1 low expression tumors. Additionally, the study drug was tolerated remarkably well, with only 4% of the cohort experiencing grade 3 or greater toxicity. Immune-related adverse events (AEs) were the most common toxicities experienced. These preliminary data demonstrated a promising safety and efficacy profile for atezolizumab. In an effort to accelerate clinical trial expansion and increase patient access to the study drug, the FDA granted atezolizumab breakthrough designation in June 2014. The phase II study IMVigor 210 demonstrated an objective response rate of 18% in PD-L1+, platinum-pretreated tumors, and a grade 3/4 treatment-related AE rate of 16%, further supporting the safety and efficacy profile of atezolizumab for advanced urothelial carcinoma [28]. Based on these encouraging data, the FDA approved atezolizumab for treatment of metastatic or locally advanced bladder cancer in patients who have failed prior platinum-based chemotherapy in May 2016. More recently, the phase III study Keynote-045 demonstrated a survival benefit with anti-PD-1 antibody pembrolizumab vs. second-line chemotherapy in platinum refractory metastatic bladder cancer (10.3 vs. 7.4 mo, respectively) [29]. There were significantly less grade 3 to 5 AEs observed in the pembrolizumab arm compared to the

chemotherapy arm (15% vs. 49%, respectively). The most common immune-related AEs of any grade associated with checkpoint inhibitor therapy is transaminitis, hyper/hypothyroidism, pneumonitis, and colitis [30].

Multiple additional checkpoint inhibitors targeting PD-1/PD-L1 have been evaluated and have demonstrated activity in urothelial carcinoma (Table 1). Based on these encouraging early results, multiple phase III trials are planned or are underway (Table 2). Additional clinical trials are currently exploring the use of immune checkpoint inhibitors in the perioperative setting, in combination with radiotherapy, and in non–muscle-invasive disease. There are also several clinical trials studying combined checkpoint blockade targeting both CTLA-4 and PD-1/PD-L1 for advanced urothelial carcinoma [31,32]. Although there are no data published yet regarding outcomes for combination immunotherapy in urothelial carcinoma, combination therapy has led to clinical remission in metastatic melanoma, although there is significant cumulative toxicity associated with dual therapy [33]. Initial results exploring the combination of nivolumab (anti-PD-L1) with ipilimumab (anti-CTLA-4) from the phase I/II CheckMate 032 study was presented at the Society for Immunotherapy of Cancer meeting in November 2016. Among 103 patients with urothelial carcinoma treated with nivolumab 3 mg/kg and ipilimumab 1 mg/kg IV every 3 weeks for 4 cycles followed by nivolumab 3 mg/kg every 2 weeks, the overall response rate was 26%. Among the 26 patients treated with nivolumab 1 mg/kg and ipilimumab 3 mg/kg IV every 3 weeks for 4 cycles followed by nivolumab 3 mg/kg every 2 weeks, the response rate was 38.5%. In the 78 patients treated with single-agent nivolumab at 3 mg/kg IV every 2 weeks, the response rate was 25.6% [34]. Final results of CheckMate 032 are eagerly anticipated. A randomized phase III clinical trial (DANUBE) is underway comparing combination immunotherapy (anti-CTLA-4 + anti-PD-L1) vs. single-agent immunotherapy (anti-PD-L1) vs. standard combination, platinum-based chemotherapy in the first-line metastatic or locally advanced setting (NCT02516241). DANUBE will be the first reported phase III trial evaluating combination immunotherapy in urothelial carcinoma.

5. New immune checkpoints B7-H3, B7x, and HHLA2

The evolution of the B7 family members can be divided into 3 groups based on amino acid similarity. B7-H3 [35], B7x [36–38], and HHLA2 [39] form the third group of molecules [36] (Fig. 3). These molecules are widely expressed in many urologic malignancies, and their expression is associated with a poor prognosis [22,40]. Tumor cell surface overexpression of these ligands is thought to play an essential role in suppressing T-cell response, leading to immune tolerance of malignancies. This feature makes them attractive targets for novel immunotherapy strategies. In this section, we review the new immune checkpoint ligands B7-H3, B7x, and HHLA2.

5.1. B7-H3

B7-H3 (also known as CD276) is a ligand of the B7 family with a complex set of functions that is still being explored. Initial in vitro studies indicated that it stimulates proliferation, increases cytokine production of IFNγ [35], and promotes antitumor immunity [41]. Later studies demonstrated the opposite, however, showing that it inhibits proliferation, cytokine production, and T-cell immunity [42]. These apparently conflicting results may not be contradictory, but could instead entail multiple receptors (as in B7-1 with CD28 and CTLA-4), distinct functional isoforms, or posttranslational regulation. As of yet, the receptor for B7-H3 has not been identified.

B7-H3 mRNA is widely transcribed in somatic tissues; however, its protein is only found on certain cell types. It is constitutively expressed at low levels on nonlymphoid cells such as fibroblasts [43] and osteoblasts [44]. It is not constitutively expressed on immune cells, but can be induced on T cells, natural killer cells, dendritic cells, and monocytes [35]. This limited expression pattern suggests posttranscriptional regulation, which is at least partly due to RNA interference mediated by microRNA such as miR-29 [45]. Although B7-H3 expression is limited in normal tissues, it is found on many cancer types and in tumor vasculature [46]. As with the early in vitro experiments, some studies of human pancreatic [47] and gastric [48] cancer specimens seemed to indicate that B7-H3 is associated with improved patient survival. Conversely, other analyses of pancreatic [49], colorectal [50], breast [51], lung [52], liver [53], kidney [54] and bladder cancers show that it is associated with a greater risk of progression and worse prognosis. In urothelial cancer of the bladder, preoperative bacillus Calmette-Guerin treatment is associated with B7-H3 overexpression [22]. Subsequent studies on urothelial cell tumors confirmed that B7-H3 overexpression is present in bladder tumors across all pathologic stages [22,55] and is likely a consequence of increased B7-H3 mRNA expression in urothelial carcinoma cells [56].

Therapeutics targeting B7-H3 are being actively explored. The anti-B7-H3 monoclonal antibody Enoblituzumab (MGA271) was shown to reduce growth of renal cell and bladder carcinoma xenografts in mice [57]. It is currently being tested as a monotherapy in a phase I dose-escalation study in patients with refractory cancers (NCT01391143), and interim results show that it is well tolerated and has antitumor activity [58]. It is also being evaluated in combination with ipilimumab (anti-CTLA-4 mAb) or pembrolizumab (anti-PD-1 mAb) in safety studies of refractory cancers (trial NCT02381314 and NCI201501495). The murine mAb 8H9 was found to target an unknown antigen on many solid tumors, and only recently has this antigen been discovered to be B7-H3

Table 1
Data reported from phase I to III clinical trials of immune checkpoint inhibitors in urothelial carcinoma

NCT Identifier, Trial Name	Agent	Target	Phase	Population	Response, OR% (CR%)	OS (mo)	AEs	PD-L1 + definition	Refs.
JAVELIN Solid Tumor, NCT01772004	Avelumab	PD-L1	Ib	Multiple tumor types, UC = 44. Metastatic, postplatinum	18.2 (4.5), PD-L1+: 50	12mo OS: 50.9%	Gr ≥ 3 trAE: 11	PD-L1+ ≥5% IC	[85]
NCT02108652 IMvigor 210	Atezolizumab	PD-L1	II	C 1: first-line cisineligible (n = 119)	C 1: 24 (7)	C 1: 14.8	Cohort 1: Gr 3–4 trAE: 15	PD-L1 IC0: <1% IC	C 1: [86]
				C 2: Postplatinum (n = 311)	PD-L1+: 25 (6) C 2: 15 (5) PD-L1+: 18 (6)	C 2: 7.9	Gr 5 trAE: 1 Cohort 2: G3–4 trAE: 16	PD-L1 IC1: ≥1 ≤ 5% IC PD-L1 IC2/3: ≥5% IC	C 2: [28]
NCT01928394 CheckMate 032	Nivolumab Nivolumab + Ipiliumab (3/1 and 1/3)	PD-1 CTLA-4	I/II	Multiple tumor types (UC = 78) Metastatic, postplatinum	24.4 (6.4)	9.7	G3–4 trAE: 20.5 G5 trAE: 2.6	PL-L1+ if expression ≥ 1% TC	[34,87]
				N3/I1 (n = 104)	26.0	NR	31.7		
				N1/I3 (n = 26)	38.5	NR	30.8		
NCT02387996 CheckMate 275	Nivolumab	PD-1	II	Postplatinum, metastatic or locally advanced UC (n = 265)	19.6 (2) PD-L1 ≥ 5%: 28.4 PD-L1 ≥ 1%: 23.8 PD-L1-: 16.1	8.7	G3–4 trAE: 18 G5 trAE: 1.1	PL-L1+ if expression ≥1% TC (also reported outcomes for PD-L1 ≥ 5%)	[88]
NCT01693562	Durvalumab	PD-L1	I/II	Multiple tumor types (UC = 61) Metastatic (57 > first line)	31.0% PD-L1+: 46.4 PD-L1-: 0	NR	G3 trAE: 4.9 G4–5 trAE: 0	PD-L1+ if ≥ 25% of TC or IC	[89]
NCT01848834 KEYNOTE-012	Pembrolizumab	PD-1	I	Multiple tumor types (UC = 33) Metastatic, 76% > first line	27.6 (10.3) PD-L1+: 29–33 PD-L1-: 0–9	12.7	G3 trAE: 9.1 G4–5 tRAE: 0	PD-L1+ if staining in stroma or ≥ 1% TC	[90]
NCT02335424 KEYNOTE-052	Pembrolizumab	PD-1	II	First-line cisplatin-ineligible metastatic or locally advanced (n = 349)	24.0 (6.0) PD-L1 ≥ 10%: 36.7 (13.3) PD-L1 ≥ 1%: 25.4 (6.3)	NR	Gr ≥ 3 trAE: 16%	PD-L1 + if: ≥ 10% combined TC and IC (also reported response if ≥ 1%)	[91]
NCT02256436 KEYNOTE-045	Pembrolizumab	PD-1	III	Second-line postplatinum pembrolizumab (n = 270) vs. paclitaxel, docetaxel, or vinflunine (n = 272)	21.1 (7.0) vs. 11.4 (3.3)	10.3 vs. 7.4 (HR = 0.73, P = 0.002)	Gr ≥ 3 trAE: 15 vs. 49	PD-L1+ if: ≥ 10% combined TC and IC	[29]

BSC = best supportive care; C 1 = cohort 1; C 2 = cohort 2; Chemo = chemotherapy; Cis = cisplatin; CR = complete response; DFS = disease free survival; GC = gemcitabine + cisplatin; GCa = gemcitabine + carboplatin; MIBC = muscle-invasive bladder cancer; IC = immune cells; NR = not reported; OR = objective response; OS = overall survival; PC = placebo-controlled; PFS = progression free survival; Ref = reference; RP III = randomized, phase III trial; SD = stable disease; TC = tumor cells; trAE = treatment-related adverse events; UC = urothelial carcinoma.

Table 2
Selected ongoing phase III trials of immune checkpoint inhibitors in urothelial carcinoma

NCT Identifier Trial Name	Treatment arms	Design	Population	Notes	Status	Primary endpoint
NCT02853305 KEYNOTE-361	(1) Pembrolizumab (2) Pembrolizumab + Chemo (3) Chemo	RP III	(1) Metastatic (2) First line	Chemo will be GC or GCa	Not yet open Accruing	PFS OS
NCT02632409 CheckMate 274	(1) Nivolumab (2) Placebo	RP III PC	Adjuvant	(1) Includes upper tract UC (2) PD-L1 status is not entry criteria		DFS
NCT02603432 Javelin Bladder 100	(1) Avelumab + BSC (2) BSC	RP III	(1) Metastatic (2) Maintenance	Patients with SD or better after first-line chemo	Accruing	OS
NCT02450331 IMvigor010	(1) Atezolizumab (2) Observation	RP III	(1) MIBC Adjuvant (2) PD-L1 positive	Does not include upper tract UC	Accruing	DFS
NCT02807636 IMvigor130	(1) Atezolizumab + GCa (2) Placebo + GCa	RP III PC	(1) Metastatic (2) First line (3) Cisineligible	Randomization is 2:1	Accruing	OS PFS AEs
NCT02302807 IMvigor211	(1) Atezolizumab (2) Chemotherapy	RP III	(1) Metastatic (2) Postplatinum	Investigator's choice in chemo arm: vinflunine, paclitaxel or docetaxel	Completed Accrual	OS
NCT02516241 DANUBE	(1) Durvalumab + Tremelimumab (anti-CTLA-4 mAb) (2) Durvalumab (3) GC or GCa	RP III	(1) Metastastic (2) First-line	(1) PD-L1 status must be known but is not entry criteria (2) GC is cis-eligible. GCa if cisineligible	Accruing	OS

BSC = best supportive care; Chemo = chemotherapy; Cis = Cisplatin; DFS = disease free survival; GC = gemcitabine + cisplatin; GCa = gemcitabine + carboplatin; mAb = monoclonal antibody; MIBC = muscle-invasive bladder cancer; OS = overall survival; PC = placebo-controlled; PFS = progression free survival; RP III = randomized, phase III trial; SD = stable disease; UC = urothelial carcinoma.

[59]. Radioimmunoconjugated 8H9 with cytotoxic I131 is currently in phase I trials for central nervous system and peritoneal tumors (NCT01099644 and NCT01502917), and other groups are testing 8H9 with other toxic conjugates [60] in murine models. Targeting B7-H3 in combination with other treatments has synergistic effects in animal models, as seen in pancreatic tumor grafts treated with gemcitabine [61] and lymphoma xenografts with idarubicin and cytarabine [62]. Thus, targeting B7-H3 has been effective in preclinical models, and depending on the results of ongoing clinical trials, may be a viable treatment option for bladder cancer in the future.

5.2. *B7x*

B7x (also known as B7-H4, VTCN1, or B7S1) is a B7 family member discovered in 2003 that is shown to inhibit T-cell–mediated immunity. This coinhibitory ligand inhibits T-cell proliferation, reduces cytokine production [36], and induces cell-cycle arrest [37] in vitro. Further, it promotes the development of immunosuppressive immune cells such as regulatory T cells [63], tumor-associated macrophages [64], and MDSCs [65], which in turn inhibit T-cell function. B7x also has a role in the innate immune system: In a *Listeria* model of infection, it inhibited the proliferation of neutrophil progenitors [66]. The receptor for B7x has not yet been identified, though in vitro binding assays indicate that it must be distinct from known members of the B7 family [36] and is found on T cells [36] and MDSCs [65].

The B7x gene is widely transcribed, and its mRNA can be detected in tissues and cell types across the body [36]. Conversely, protein expression of B7x is fairly limited and found on tissues of lung [67]; pancreas [68]; breast, gynecological tract, and placenta [67], suggesting posttranscriptional regulation similar to B7-H3. Despite its restricted expression on healthy somatic tissues, B7x is frequently expressed in many cancer types including those of the breast, lung [52], colon [69], ovary [70], endometrium [71], kidney [72], pancreas [73], prostate [40], and bladder. B7x expression in renal cell carcinoma is associated with a greater cancer progression and decreased overall survival [72]. Likewise, in prostate cancer, strong intensity of staining with immunohistochemistry for B7x is associated with greater cancer spread, recurrence, and risk of death [40]. In urothelial carcinoma, B7x overexpression is associated with increased TNM stage, pathological grade, and poorer outcomes [74]. Serum B7x is also elevated in patients with urothelial carcinoma, suggesting that it may be useful as a diagnostic marker [75].

Considering the immunosuppressive effects of B7x and its expression profile in human cancers, it is a prime target for immunotherapy. Some strategies to target B7x include

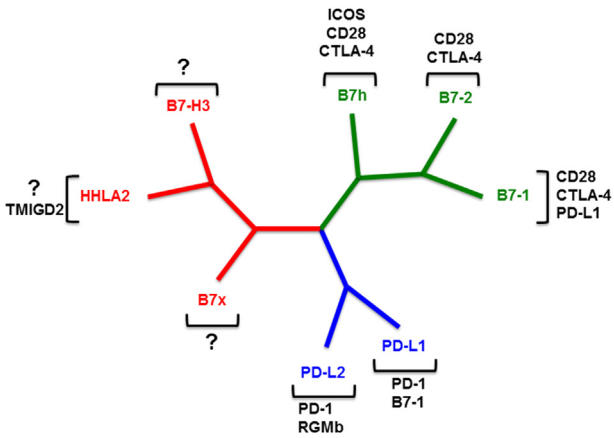

Fig. 3. Phylogenetic tree of the human B7 and CD28 families. Group I (green) includes B7-1/B7-2/CD28/CTLA-4, and B7h/ICOS/CD28/CTLA-4. Group II (blue) consists of PD-L1/PD-L2/PD-1, PD-L1/B7-1, and PD-L2/RGMb. Group III (red) contains B7-H3, B7x, and HHLA2/TMIGD2. (Color version of the figure available online.)

monoclonal antibodies, single-chain fragment variables (scFVs, that is, recombinant antibodies with single binding sites), and chimeric antigen receptor (CAR) T cells. Anti-B7x monoclonal antibodies reduce lung metastases of B7x-expressing colon cancer cells in a mouse experimental metastasis model [76]. Similarly, anti-B7x scFVs reduced the growth of ovarian tumor xenografts [77]. B7x-targeted CAR T-cell therapy proved to be effective in eliminating xenografts of B7x-expressing ovarian tumors, but also caused lethal, delayed toxicity 6 to 8 weeks postengraftment [78]. Thus, for the CAR T-cell strategy to be clinically feasible, a suicide-gene or comparable "off" switch would need to be incorporated to prevent long-term adverse effects. Taken together, B7x shows promise as an anti-cancer target, but further research is needed to refine the treatment strategies.

5.3. HHLA2

The newest member of the B7 family, HHLA2 (also known as B7-H5 or B7-H7) is unique among B7 family molecules in that it is found in humans but not in murine organisms. Within the B7 family, it shares the greatest amino acid similarity to B7-H3 and B7x [39]. In vitro experiments with human immune cells indicate that it inhibits T-cell function by reducing proliferation and cytokine release [39], although it was also reported that it can act as a costimulatory molecule to promote T-cell function [79]. Like B7-H3, HHLA2 may also have multiple receptors or functional isoforms that mediate distinct effects. Based on the consensus of in vitro and clinical data, HHLA2 has a largely inhibitory role on the immune system.

In humans, it can be found constitutively expressed on monocytes and can be induced on B cells [39]. It has limited expression in somatic tissues, but it can be found in the placenta, gut, kidney, and breast [80]. A receptor for HHLA2 is TMIGD2 (CD28H or IGPR-1), a protein present on endothelial cells believed to have a role in angiogenesis [81]. TMIGD2 mRNA is broadly transcribed in somatic tissues [81], and the protein can be found on resting T cells as well as monocytes, dendritic cells, and B cells but not on activated T cells [79], despite recombinant HHLA2 protein binding activated T cells in vitro. This suggests that there is a yet undiscovered receptor for HHLA2 that is responsible for its inhibitory effects.

HHLA2 is overexpressed in many cancers, including lung, breast, kidney, prostate, and bladder [80]. The prognostic significance of HHLA2 overexpression has been identified for triple-negative breast carcinoma, non–small cell lung carcinoma, and osteosarcoma. In triple-negative breast cancer, HHLA2 overexpression is associated with lymph node metastasis and higher stage [80]. Similarly, EGFR mutational status and high tumor-infiltrating lymphocyte density are associated with HHLA2 expression in non–small cell lung carcinoma [82]. In osteosarcoma, HHLA2 expression is associated with advanced disease and almost universally found in metastatic specimens, and confers a poorer prognosis [83]. HHLA2 is also overexpressed in over half of high-grade urothelial tumors [84]. Taken together, these data suggest that HHLA2 may be an important therapeutic target for cancer immunotherapy, but further work is needed to elucidate its value as a diagnostic or prognostic tool, and to develop therapeutics that can target it.

6. Summary

The understanding of the regulation of T-cell activation and function along with the discovery of immune checkpoints with respect to the CD28 and B7 family has led to major advances scientifically and therapeutically in cancer. Clinical trials of agents targeting the PD-L1 checkpoint pathway have demonstrated durable clinical remissions in patients with otherwise untreatable advanced bladder cancer. Future studies should aim to further characterize the role of the newest B7 family molecules B7-H3, B7x, and HHLA2 in the mechanisms of bladder tumor-immune evasion pathways. This will surely lead to the development of new therapeutic strategies to enhance immune-mediated tumor destruction.

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