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Review article

A review of the PD-1/PD-L1 checkpoint in bladder cancer: From mediator of immune escape to target for treatment[1]

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Abstract

Purpose: Recent observations have focused attention on the means that human tumors employ to evade host defense systems critical to immune surveillance. The concepts of immunotherapy are familiar to urologists because of the use of bacillus Calmette-Guérin in bladder cancer. Research demonstrating the importance of checkpoint inhibitors in suppressing immune responses against tumors has heightened interest in immunotherapy at a time when there is a need for alternatives to bacillus Calmette-Guérin. We review the literature on the application of immunotherapeutic agents targeting a key checkpoint pathway, programmed death 1 (PD-1) and its ligand (PD-L1), in the field of bladder cancer.

Materials and methods: A comprehensive literature review was performed using Medline/Pubmed and Embase.

Results: The PD-1/PD-L1 pathway may be manipulated by cancer cells to subvert the immune system. PD-1/PD-L1 blockade has been tested in clinical trials for various malignancies including metastatic urothelial carcinoma, with significant response rates and limited side effects. PD-L1 expression has also been proposed as a prognostic marker for bladder cancer with mixed results.

Conclusions: PD-1 is one of several key receptors mediating immune escape, and agents targeting its ligand PD-L1 have already been successfully applied to patients with metastatic urothelial cancer. More research is needed to standardize criteria for PD-L1 positivity, explore its use as a biomarker, and optimize its use in the treatment for bladder cancer. © 2017 Elsevier Inc. All rights reserved.

Keywords: PD-1; PD-L1; Bladder cancer; BCG failure; Immunotherapy; Biomarker

1. Introduction

Bladder cancer is the fifth most common malignancy in the United States, with an estimated 74,000 new cases every year accounting for 4.5% of all cancer diagnoses and 16,000 deaths annually [1]. Up to 75% of patients present with non–muscle-invasive bladder cancer and are managed with transurethral resections with or without adjuvant intravesical therapy. For individuals with high-risk non–muscle-invasive bladder cancer (i.e., stage T1, high-grade papillary tumors, and carcinoma in situ), intravesical bacillus Calmette-Guérin (BCG) has been the only agent shown to reduce recurrence and possibly risk of progression to muscle-invasive disease [2]. Treatment failure, treatment-associated toxicity, and a recent shortage of BCG have led to a search for alternative agents. This need may soon be met by the recent resurgence in the field of immunotherapy and the emergence of checkpoint inhibition as a therapeutic approach.

2. Materials and methods

A comprehensive search of MEDLINE/Pubmed and Embase was conducted to identify conference abstracts, basic science, original, and review articles in the English

language before December 2015 (updated in June 2016) using keywords including BCG-refractory bladder cancer, programmed death 1 (PD-1), programmed death ligand 1 (PD-L1), B7-H1, CD274, tuberculosis, and granuloma. For example, we searched Medline through PubMed using the following MESH terms: "BCG vaccine/therapeutic use" (MESH) AND "granuloma" (MESH); "Antigens, CD274" (Mesh) AND "Urinary Bladder Neoplasms" (Mesh). Embase was then searched using the Emtree queries "bladder cancer"/exp AND "PD-L1"/exp. Inclusion criteria included English language and accessibility to full article.

3. Results

3.1. Birth of immunotherapy

In 1892, William B. Coley reported regression of an inoperable sarcoma in a young girl who developed a superficial streptococcal infection [3]. He pioneered the field of cancer immunotherapy by injecting various antigens, "Coley's toxins," into patients with unresectable soft tissue sarcomas in the hope of stimulating a therapeutic immune response against malignant tumors. A possible link between mycobacterial infection and development of resistance to cancer was also suggested by Dr. Raymond Pearl [4], who observed a lower rate of tuberculosis in autopsies of patients who died of cancer compared with a higher rate of tuberculosis infection in a control group who died of other causes. Dr. Lloyd Old [5] then demonstrated that mice inoculated with BCG, a strain of attenuated *Mycobacterium bovis* previously developed as a vaccine against tuberculosis, showed resistance to tumors possibly by host immune stimulation. These experiments provided an experimental foundation for the clinical use of BCG first in the treatment of melanoma, and subsequently for bladder cancer [6].

3.2. BCG immunotherapy for NMIBC

In the 1970s, Alvaro Morales described the use of intravesical BCG for non–muscle-invasive bladder cancer (NMIBC) and demonstrated a reduction in tumor recurrence in 7 of 10 patients [7]. As a consequence, BCG became a standard of care in the treatment of high-risk NMIBC after transurethral resection. A recent meta-analysis of randomized trials has shown a 32% reduction in risk of recurrence with BCG maintenance when compared with mitomycin C [8]. Furthermore, maintenance BCG has been shown to result in a 28% decreased risk of recurrence when compared with BCG induction therapy alone [8,9].

Although the exact mechanism by which BCG exerts its therapeutic effect remains poorly understood, several theories relating to immune modulation have emerged. The latest evidence suggests that there is complex interplay between malignant urothelial cells and the host immune system in the context of BCG therapy. It has been proposed that attachment of live BCG to bladder cancer cells is followed by internalization, a process mediated by the oncogenes phosphatase and tensin homolog and RAS that activate pinocytosis [10]. BCG internalization up-regulates major histocompatibility complex (MHC) class II molecules and intercellular adhesion molecule 1 adhesion molecules by the tumor cell to mediate attachment of immune cells, and triggers cytokine release to recruit effector cells to the area. A predominantly T helper type 1 (Th1) response then activates cytotoxic natural killer cells, neutrophils, and macrophages to eliminate malignant cells.

Despite its success, several issues make BCG a suboptimal therapy. Up to 30% of patients fail to respond initially and, of the responders, 74% eventually relapse [11]. For BCG-refractory disease, radical cystectomy remains the gold standard with limited data for bladder-sparing options (e.g., intravesical interferon and valrubicin) [12]. Maintenance of BCG therapy has been advocated as an essential regimen for maximizing BCG efficacy, but up to a third of patients do not complete their course owing to local or systemic toxicity. Recent problems associated with the BCG manufacturing process have furthermore complicated matters by creating a worldwide shortage and limiting access to the most effective intravesical agent for high-risk NMIBC [13]. Consequently, there is an unmet need for a well-tolerated therapy to reduce disease recurrence and progression.

3.3. Mechanisms of tumor evasion

The host immune system plays a critical role in detecting and controlling the proliferation of tumor cells. In response to antigens presented by MHC molecules, T-cell receptors on T cells activate a cascade of signals in both CD4$^+$ and CD8$^+$ cells resulting in destruction of target cells [14]. Malignancies may adopt a variety of mechanisms at different points along this pathway, including evading detection. Some lung and prostate cancers down-regulate MHC class I molecules resulting in altered antigen presentation [15,16]. Melanoma has been shown to decrease expression of adhesion molecules leading to impaired effector cell migration to the tumor site [17]. Tumors may also develop resistance to the T-cell–mediated killing mechanism. For example, melanoma, breast, and cervical cancers have been shown to exhibit a serine protease inhibitor that interferes with the granzyme-mediated apoptotic pathway [18].

3.4. Checkpoint inhibitors

An alternative regulatory pathway that limits immune response in diseases including cancer has been recently elucidated [19]. Inhibitory coreceptors called immune checkpoint inhibitors have been found to play a major role in maintaining peripheral T-cell tolerance.

Cytotoxic T-lymphocyte-associated protein 4 (CTLA-4) is an example of an immune checkpoint that has been manipulated for immunotherapy [20]. Expressed on T cells, CTLA-4 competes with the costimulatory receptor CD28 for the B7 family ligands (B7–1 and B7–2). Once bound to a B7 ligand, the activated CTLA-4 complex down-regulates T-cell activity. A human monoclonal antibody directed against CTLA-4, ipilimumab, therefore, blocks the interaction between CTLA-4 and its 2 B7 ligands and has demonstrated antitumor activity in patients with stage III and IV melanoma who have failed prior systemic therapy [21]. In the largest anti-CTLA-4 trial involving patients with a urologic malignancy, 799 men with castration-resistant bone-metastatic prostate cancer who had progressed after receiving docetaxel underwent bone radiotherapy followed by either ipilimumab or placebo [22]. Although no significant difference in overall survival was observed between the 2 groups, the investigators did identify reductions in prostate-specific antigen and improvements in progression-free survival (4 mo vs. 3.1 mo, $P < 0.0001$) suggestive of ipilimumab antitumor activity. There was significant toxicity attributable to ipilimumab administration, including patients deaths. Other ipilimumab studies have demonstrated nontrivial side effects including vitiligo, rash, pruritis, anorexia, fatigue, diarrhea, and a small number of immune-related adverse events requiring prompt administration of steroids [23].

3.5. Immune checkpoint PD-1

The PD-1 (CD279) pathway functions similarly to CTLA-4 by activating a cascade of events that limits immune activity, leading to decreased autoimmunity and cytokine secretion, which ultimately prevents collateral tissue damage [19]. The pathway consists of the receptor PD-1 and its 2 ligands, PD-1 ligand 1 (PD-L1, B7-H1, and CD274), and PD-1 ligand 2 (PD-L2, B7-DC, and CD273), that are cell surface glycoproteins within the B7 family of costimulatory and coinhibitory molecules. PD-L1 is induced on human antigen-presenting cells, T cells, and natural killer cells, but also by stem cells and a variety of nonhematopoietic cells [24]. PD-L2 is expressed by a more limited population of cells at baseline, but its expression is inducible under certain conditions and remains poorly studied in the context of tumor and viral immunology.

PD-L1 and PD-L2 bind the receptor PD-1, which is expressed on activated and exhausted T cells, and also induced on antigen-presenting cells such as macrophages, dendritic cells, and B cells (Fig.) [25]. PD-L1 interaction with its receptor triggers phosphorylation of the immunoreceptor tyrosine-based switch motif, a segment of the intracellular domain of PD-1, which recruits phosphatases SHP-1 and SHP-2 [26]. These phosphatases further modulate kinases associated with the T-cell antigen receptor, reducing cytokine production, T-cell activation, and target cell lysis. There is also limited evidence suggesting that PD-
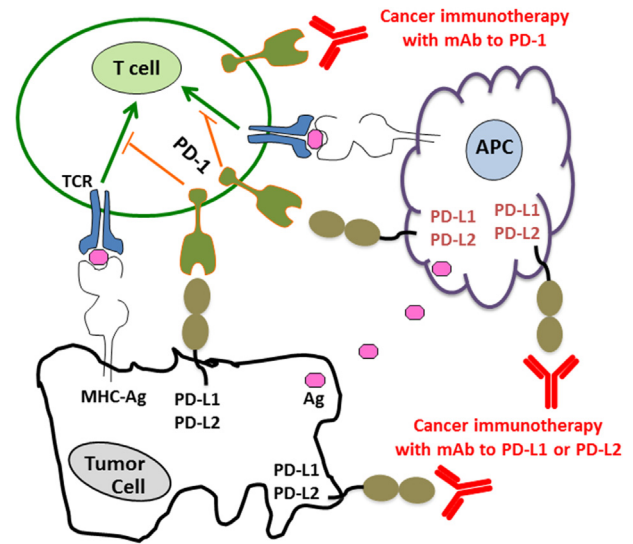

Fig. PD-1 pathway. MHC molecules expressed by tumor cells, antigen-presenting cells (APCs) and other immune cells present antigen to T cells. Subsequent activation of the T-cell receptor complex results in expression of PD-1 receptor on the T-cell surface. PD-L1 and PD-L2 ligands on APC's and tumor cells may then engage the PD-1 receptors resulting in suppression of T-cell mediated immune response. Antibody-mediated blockade of the PD-1/PD-L1 pathway may, therefore, enhance antitumor immunity.

1 limits effector T-cell function and regulatory T cells may have a role, although the connection remains tenuous and Treg cells are just single player in a dynamic immunological milieu [27].

This physiologic mechanism may be manipulated by viral infections or cancer cells to subvert immune detection and its blockade may, therefore, serve as a therapeutic target [28]. PD-1 function in chronic viral infections was first studied in a mouse model of lymphocytic choriomeningitis virus [29]. High expression of PD-1 served as a signature of exhausted CD8 T cells, although mice that cleared the infection had undetectable levels of PD-1. Subsequent PD-1 blockade in the exhausted CD8 T-cell population resulted in clonal expansion, cytokine expression, reduction in viremia, and overall restoration of function to the exhausted T cells.

The PD-1 pathway has also been implicated as a contributor to the persistent viremia and immunosuppression of human immunodeficiency virus (HIV). In patients with chronic HIV infection naïve to highly active antiretroviral therapy, PD-1 expression was positively associated with HIV viral load and inversely related to CD4 T-cell count [30]. In vitro blockade of the PD-1 pathway not only resulted in proliferation of HIV-specific CD4 T cells but also restored function to a dormant CD4 T-cell population. These data provided the first evidence linking PD-1 pathway activation and T-cell impairment in the context of HIV disease status and progression. Although further studies are needed, PD-1 blockade remains a viable therapeutic target in the treatment of chronic infections including HIV,

hepatitis B and C, and even tumors, which may be associated with chronic inflammation.

3.6. Success of PD-L1/PD-1 blockade for treatment of metastatic urothelial cell carcinoma

Melanoma, renal cell carcinoma (RCC), non–small-cell lung cancer, and gastric cancers are all associated with an immunosuppressive tumor microenvironment [31–34]. The potential of PD-1/PD-L1 blockade in bladder cancer has been observed in 2 clinical trials. A phase 1b study of anti-PD-1 pembrolizumab (Pembro; MK-3475) enrolled 33 patients with recurrent or metastatic urothelial cancer and >1% PD-L1 expression in tumor cells [35]. Through a median follow-up duration of 11 months, a 24% overall response rate was observed with 10% achieving complete response and a median overall survival duration of 9.3 months. Overall, 61% of patients reported an adverse event, most commonly fatigue ($n = 6$), peripheral edema ($n = 4$), and nausea ($n = 3$), with grade 3 to 4 adverse events occurring in 4 patients.

Another phase 1 trial reported on the efficacy and safety profile of MPDL3280A (atezolizumab), a human anti-PD-L1 monoclonal antibody, in a cohort of 68 heavily pre-treated subjects [36]. Of these patients with metastatic urothelial cell carcinoma, 93% received cisplatin-based chemotherapy with 72% having previously failed multiple systemic treatments. By 6-week follow-up, a 50% objective response rate was observed in the cohort with high PD-L1 expression by tumor-infiltrating immune cells, whereas only 8.3% of subjects who were PD-L1 negative responded. Nevertheless atezolizumab, in appropriately selected patients, compared favorably with other salvage therapies [37].

Atezolizumab toxicity should be placed in context of the poor pretreatment status of subjects, including 33% with creatinine clearances <60 ml/min, 75% with visceral metastases, and 42% of those received chemotherapy within 3 months of starting PD-L1 blockade. Overall, 57% experienced grades 1 to 3 adverse events, without any grade 4 or 5 complications. These events consisted primarily of decreased appetite, fatigue, nausea, weakness, and chills and were comparable with side effects seen in RCC trials of nivolumab, an anti-PD-1 antibody [38].

A recent phase 2 multicenter single-arm trial involving atezolizumab was completed to furthermore assess safety and efficacy (NCT02108652) [39]. A total of 310 patients with inoperable locally advanced or metastatic urothelial carcinoma with Eastern Cooperative Oncology Group performance status of 0 or 1 were recruited to receive atezolizumab. PD-L1 status of tumor-infiltrating mononuclear cells (TIMCs) as well as tumor cells were prospectively collected using their SP142 assay and classified in the following manner: IC0 (<1% expression), IC1 (≥1% and <5%), and IC2/3 (≥5%). SP142 was recently approved as a complementary assay to identify patients

likely to respond to treatment with atezolizumab and is the subject of an ongoing randomized study (NCT02302807). When compared with a historical control response rate of 10%, atezolizumab treatment resulted in significantly improved overall objective response rate of 15% and complete response rate in 5% according to Reponse Evaluation Criteria In Solid Tumors criteria. Reponses were more common in patients with higher levels of PD-L1 expression on TIMCs: IC2/3 (26%), IC 1/2/3 (18%), and 15% overall. During a median survival follow-up of 11.7 months, median progression-free survival time was 2.1 months regardless of PD-L1 status. Overall, 69% of patients had a treatment-related adverse event of any grade, with 16% of patients experiencing a grade 3 or 4 adverse event attributed to atezolizumab. Most events were mild including fatigue, nausea, anorexia, pruritus, fever, diarrhea, rash, and arthralgia. Pneumonitis and dyspnea were more severe complications and no treatment-related deaths were reported. Based on these results, atezolizumab was approved by the FDA in May 2016 for the treatment of patients with locally advanced or metastatic urothelial carcinoma who have disease progression during or after platinum-based chemotherapy.

3.7. Evolving role of anti-PD-1/PD-L1 therapy

In limited clinical trials, PD-1/PD-L1 blockade has shown promise as salvage therapy for metastatic chemotherapy-refractory urothelial carcinoma. An understanding of PD-1 and PD-L1 as checkpoints in metastatic disease suggests that its blockade may be useful in the treatment of patients with earlier-stage disease. Boorjian et al. observed that the high expression of a related glycoprotein B7-H3 in urothelial tumors may be an early event in the up-regulation of PD-1. The expression patterns of PD-L1, B7-H3, and PD-1 matched closely between primary bladder tumor and metastatic nodal tissue [40]. If metastases respond to systemic immunotherapy, then so should a histologically similar primary tumor. In NMIBC, PD-1 blockade may enhance BCG activity by subverting a mechanism of resistance. In muscle-invasive disease, it may serve as a better-tolerated neoadjuvant or adjuvant treatment than traditional chemotherapy for select patients.

3.8. PD-L1 as a biomarker

Using PD-L1 for prognostication has been suggested in other urologic malignancies. Overall, 24% of patients who underwent nephrectomy for clear cell RCC demonstrated PD-L1 positivity in pathologic specimens. These patients were found to be at significantly increased risk of death from RCC, even after multivariate adjustment for TNM stage, grade, and performance status [32]. Tumor over-expression of PD-L1 also portends a poor outcome for patients with melanoma, ovarian cancer, and lung cancers,

but the role of PD-L1 in bladder cancer as a predictive biomarker remains less clear [41].

In 56 consecutive radical cystectomy specimens, 17% of which stained positive ($\geq 5\%$ expression) for the PD-L1, PD-L1 up-regulation was postulated to lead to a reduction in the number of tumor-killing CD8 T cells [42]. Although high density of intratumoral CD8 T cell predicted favorable overall and disease-specific survival in the cohort, the expected inverse relationship was not observed between CD8 T cells and PD-L1 expression, nor between PD-L1 expression and overall survival [42]. Bellmunt et al. [43] retrospectively identified pathology specimens from 160 patients who had undergone TURBT or radical cystectomy. PD-L1 tumor cell positivity was defined by presence of $\geq 5\%$ of tumor cells, whereas PD-L1 expression by TIMCs was recorded subjectively as absent, focal, mild, moderate, or severe. Within a subgroup of 100 patients with metastatic disease who ultimately received platinum-based chemotherapy, PD-L1 expression by TIMCs, but not by tumor cells appeared to be associated with longer overall survival (18 vs. 11 mo). There was no further correlation between stage and PD-L1 expression by either TIMCs or tumor cells (Table).

Other analyses offer mixed results between PD-L1 expression and clinical outcomes. In a large cohort of 318 consecutive radical cystectomy specimens, $\geq 5\%$ expression of PD-L1 by urothelial tumor cells and moderate to marked expression of PD-1 by tumor-infiltrating

lymphocytes were significantly associated with increased pathologic stage, and tumor PD-L1 expression independently predicted all-cause, but not disease-specific mortality after cystectomy for organ-confined tumors [40]. An external validation study was performed in a group of 302 consecutive patients who underwent radical cystectomy with lymphadenectomy [44]. Although PD-L1 and its receptor PD-1 were expressed in significantly higher amounts by tumor cells than normal adjacent urothelium, there was no association with pathologic staging, disease recurrence, cancer-specific mortality, or overall mortality when evaluated in all patients undergoing radical cystectomy. In a subset of patients with organ-confined disease, PD-L1 expression was associated with a significantly increased risk of all-cause mortality on univariate analysis, with a nonsignificant trend toward increased risk of death on multivariate analysis [44].

A few studies offer PD-L1 as a reliable prognostic biomarker. In 65 patients treated for both upper and lower tract urothelial cancer with radical nephroureterectomy, radical cystectomy, or transurethral resection, $>12.2\%$ PD-L1 expression was associated with tumors of higher grade and lower rate of recurrence-free survival, and was the most significant prognostic factor after stage [45]. Another study provided compelling evidence of a clinically significant role for the PD-1 pathway in which the proportion of PD-L1 expression by urothelial cells in resection specimens increased with tumor stage. Overall,

Table
Urothelial PD-L1 Expression and Clinicopathologic Outcomes

Authors	n	Tissue source	Tissue preservation	PD-L1 antibody	PD-L1 positivity threshold (%)	Expression of PD-L1 by tumor cells (%)	Association between PD-L1 status[a] and pathologic findings/outcomes?
Bellmunt et al.	160	Bladder	FFPE	405.9a11	>5	20	PD-L1 expression by tumor cells not associated with stage or OS. Longer median OS associated with PD-L1 expression by tumor-infiltrating mononuclear cells in patients with metastatic disease
Boorjian et al.	318	Bladder	FFPE	5H1	>5	12	PD-L1 expression by tumor cells and PD-1 by TIMCs significantly associated with higher stage. PD-L1 expression associated with all-cause mortality in organ-confined tumors
Faraj et al.	56	Bladder	FFPE	5H1	>5	18	No
Inman et al.	280	Bladder	FFPE	5H1	>1	28	PD-L1 expression associated with higher grade and stage. No outcomes analysis
Nakanishi et al.	65	Bladder, ureter, and renal pelvis	Frozen	M1H1	>12	71	PD-L1 expression associated with higher grade, but not overall stage. PD-L1 positivity associated with shorter OS and DSS
Wang et al.	60	Bladder	FFPE	Pdcd-1L1 (H-130)	>10	72	PD-L1 expression associated with higher grade, muscle-invasion, recurrence, and shorter OS
Xylinas et al.	302	Bladder	FFPE	5H1	>5	25	No significant correlation between PD-L1 and stage, recurrence, or OS

FFPE = formalin-fixed paraffin-embedded; 5H1,M1H1 = murine antihuman PD-L1 monoclonal antibodies; OS = overall survival; DSS = disease-specific survival.
[a]PD-L1 expression by tumor cells except for Boorjian and Bellmunt et al. who also assessed TIMC's.

7% of pTa, 16% of pT1, 23% of pT2, 30% of pT3 and 4, and 45% of carcinoma in situ samples stained positive ($\geq 1\%$) for PD-L1, implying that PD-L1 expression is linked to aggressive tumor biology and may predict clinical outcomes although this was not explicitly studied. A multivariate regression model further confirmed that higher tumor grade and greater PD-L1 positivity correlated with increasing tumor stage [46]. Wang et al. compared pathology specimens from 50 patients with bladder cancer to 10 controls. They determined that a PD-L1 expression $> 10\%$ was associated with higher grade, muscle-invasion, recurrence, and shorter overall survival [47].

The variable and somewhat conflicting results from previous studies have highlighted the difficulty in elucidating a connection between the PD-L1 expression and clinicopathologic outcomes (Table). Beyond the retrospective design of studies, the main limitation lies with IHC technique. PD-L1 expression in melanoma, lung cancer, and RCC is highly variable and ranges from 14% to 100% depending on staining technique, although a more reasonable range of 20% to 28% is found in urothelial cell carcinoma [48]. The studies also reported on a heterogeneous group of subjects: patients with metastatic disease, those who underwent radical cystectomy for high-grade or muscle-invasive disease, NMIBC, and nephroureterectomy samples from patients with upper tract disease [40,45,46]. The IHC protocol varied as well, with some being formalin fixed and paraffin embedded, and others with frozen tissue specimens. Some studies used different clones of anti-PD-L1 antibody (e.g., 5H1 and M1H1). Even more ambiguity exists when interpreting PD-L1 expression. Some defined PD-L1 positivity as more than 1% expression of tumor cells, whereas others set a higher threshold of $> 12\%$ [45,46]. Bellmunt et al. were the first to evaluate PD-L1 expression by tumor-infiltrating mononuclear cells and correlate this with outcomes, but even their study used subjective evaluation of PD-L1 expression ranging from absent to focal, mild, moderate, and severe. The SP142 complementary PD-L1 assay used in atezolizumab trial NCT02108652 suggests that higher PD-L1 expression by immune cells predicts favorable atezolizumab efficacy.

Owing to this environment Meng et al. [49] have recognized that PD-L1 status is an important, but limited biomarker for response to PD-1 blockade and posit a role for tumor-infiltrating immune cells and additional molecules, which may better predict clinical response. Huang et al. [50] have also recognized the limits of immunohistochemistry and instead quantified mRNA expression of the PD-1 pathway. They extracted clinical and microarray gene expression data from 3 independent bladder cancer datasets within the Gene Expression Omnibus database. High tumor cell expression of PD-L1 mRNA, defined as the top quartile, was significantly associated with reduced overall survival ($P < 0.001$). They conclude that the assessment of gene expression at the transcriptional level by quantitative tests may remove the subjectivity of interpreting immunohistochemistry results.

4. Conclusions

PD-1/PD-L1 provides a mechanism of immune escape, the blockade of which has already been accomplished successfully in various cancers and has reinvigorated interest in the treatment of metastatic urothelial cancer. With 2 phase 1 trial and 1 phase 2 trial completed, the data on PD-1/PD-L1 blockade in metastatic bladder cancer should be considered very intriguing, but preliminary. To facilitate accurate interpretation of future studies, PD-L1 positivity criteria must first be standardized to eliminate ambiguity in tissue preparation and classification. Once this is achieved, the following questions can be addressed: (1) Can PD-L1 serve as a reliable biomarker for disease progression? (2) Given both PD-L1 positive and PD-L1 negative patients appear to respond to therapy, should all patients be treated regardless of PD-L1 status? (3) Does PD-1 blockade halt progression of disease or offer significant advantages in overall survival in early localized cancer? (4) What is the durability of an immunotherapy-based treatment? and (5) is there any role for combination CTLA-4 and PD-1 blockade to target complementary pathways?

For years, BCG has potentiated immune responses in patients living with NMIBC. Yet in the current era of BCG shortages, more available and effective targeted therapies are required. A promising new age of immunotherapy has arrived in the form of checkpoint inhibition. We must strive to understand the complex immune milieu responsible for its success and implications for clinical management.

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