Programmed Death-Ligand 1 Expression in Muscle-Invasive Bladder Cancer Cystectomy Specimens and Lymph Node Metastasis: A Reliable Treatment Selection Biomarker?

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Abstract

The aim of this study was to establish the frequency of PD-L1 expression in muscle-invasive bladder cancer cystectomy specimens and associated lymph node metastasis using immunohistochemistry. PD-L1 was overexpressed in the tumor cells of 5/52 (9.6%) of cystectomy specimens in this cohort with 17/52 (32.7%) of cases showing PD-L1 overexpression in tumor-infiltrating immune cells. Discordance was observed between PD-L1 expression in lymph node metastasis and the primary tumor.

Background: The programmed death-1 (PD-1) pathway negatively regulates T-cell activation and has an important role in regulating antitumor host immunity. Monoclonal antibodies directed against PD-1 or the PD-1 ligand (PD-L1) have shown activity in several tumor types with preliminary data suggesting a relationship between PD-L1 expression and response. The aim of this study was to establish the frequency of PD-L1 expression in muscle-invasive bladder cancer and associated lymph node metastasis using immunohistochemistry and to investigate the feasibility of using PD-L1 expression as a biomarker to select patients for PD−1-directed therapy. **Patients and Methods:** Cases of radical cystectomy for muscle-invasive bladder cancer with no exposure to previous chemotherapy were identified and representative slides from archived paraffin-embedded blocks stained with anti−PD-L1 antibody (5H1 clone) were identified. PD-L1 positivity was defined by a 5% expression threshold. **Results:** Fifty-two radical cystectomy specimens were reviewed. PD-L1 was overexpressed in the tumor cells of 5/52 (9.6%) of cystectomy specimens in this cohort with 17/52 (32.7%) of cases showing PD-L1 overexpression in tumor-infiltrating immune cells. Discordance was observed between PD-L1 expression in lymph node metastasis and the primary tumor. **Conclusion:** Standard assays for PD-L1 expression have yet to be established. The observation of discordance between PD-L1 expression in metastatic sites and primary tumors suggests that prospective biomarker studies should aim to acquire material immediately before treatment initiation rather than archived tissue from resected specimens that might not reflect the current immune-active microenvironment.

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Introduction

Overcoming cancer immune tolerance through the modulation of T-cell responses is a treatment strategy of great interest for several tumor types including urothelial bladder cancer. Molecular characterization of tumors from a cohort of patients with bladder cancer has shown this disease to exhibit high mutational complexity with no evidence of high-prevalence driver mutations[1]; novel treatment approaches are urgently required.

Programmed death-1 (PD-1) belongs to the B7-CD28 family of costimulating molecules that regulate T-cell responses.[2] Inhibition

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Table 1 Patient Demographic Characteristics (n = 52)

Characteristic	Value
Median Age, Years	68 (range, 30-88)
Sex	
Male	48 (92)
Female	4 (8)
Pathologic Stage	
Primary tumor	
pT2	15 (28.8)
pT3	27 (51.9)
pT4	10 (19.2)
Lymph Nodes	
pN0	34 (65.4)
pN1	11 (21.1)
pN2	7 (13.5)
High Grade	52 (100)

Data are presented as n (%) except where otherwise stated.

of the interaction between PD-1 and its ligand, PD-L1 (B7-H1), can enhance T-cell responses in vitro and mediate preclinical antitumor activity.[3] Several monoclonal antibodies targeting the PD-1 pathway are in clinical development with the anti−PD-1 monoclonal antibodies nivolumab (BMS-936558) and pembrolizumab (MK-3475) recently approved by the US Food and Drug Administration for the treatment of advanced melanoma and non−small-cell lung cancer.

Preliminary reports from early phase studies suggested that pretreatment tumor expression of PD-L1 could be a useful predictive biomarker for patient selection.[4,5]

Early clinical data from studies using the anti−PD-L1 monoclonal antibody pembrolizumab and the anti−PD-L1 monoclonal antibody MPDL3280A in advanced bladder cancer have shown promising efficacy signals.[6,7] Initial eligibility criteria for study enrollment into the phase I study of MPDL3280A included documentation of PD-L1 expression in tumor-infiltrating immune cells using a proprietary diagnostic antibody, however, the protocol was subsequently amended to include all patients. PD-L1 status was assessed on resected specimens in 59% of patients and biopsies in 27%; in most cases the tissue sample was acquired more than 1 year before the start of the study and more than 5 years in 4 cases. An overall response rate (ORR) at 6 weeks of 43% was seen in 30 patients with PD−L1-positive tumors compared with an ORR of 11% in 35 patients with PD−L1-negative tumors.[6]

Tumor cell or infiltrating immune cell PD-L1 expression appears to correlate with response to PD-1 pathway inhibitors, however, there is evidence that immune responses including PD-L1 expression are dynamic with evidence of adaptive PD-L1 upregulation with treatment.[8]

The aim of this study was to establish the frequency of PD-L1 expression in muscle-invasive bladder cancer and associated lymph node metastasis using immunohistochemistry and to investigate the feasibility of using PD-L1 expression as a biomarker to select patients for PD-1/PD-L1 directed therapy.

Patients and Methods

Patient Selection

The pathology database at the American University of Beirut Medical Center was reviewed from 1997 to 2013 after institutional review board approval. All cases of cystectomy for muscle-invasive urothelial cancer without previous chemotherapy were included. Clinicopathologic features including age, sex, histopathology, lymph node status, pathologic stage, and presence of chronic inflammation were recorded.

Table 2 Tumor PD-L1 Expression in Cystectomy Specimens and Associated Lymph Node Metastasis

	PD-L1 Expression in Lymph Node Metastasis	
	Positive	Negative
PD-L1 Expression in Primary Tumor Specimen Positive	2/18	1/18
PD-L1 Expression in Primary Tumor Specimen Negative	0/18	15/18

Abbreviation: PD-L1 = programmed death ligand 1.

Immunohistochemistry

Anti−PD-L1 immunohistochemistry, with the PD-L1 antibody clone 5H1 (kindly provided by Dr Chen, Mayo Clinic), was performed according to the previously described protocol by Thompson et al.[9] Paraffin-embedded tissue blocks were well preserved and freshly cut slides directly stained with the 5H1 monoclonal antibody. A dilution of 1:100 was used for the PD-L1 antibody with tonsil tissue serving as a positive control. PD-L1 expression, for tumor cells and tumor-infiltrating immune cells (histiocytes and lymphocytes) was defined as positive with a >5% threshold membranous staining and an intensity of mild (1+), moderate (2+), and strong (3+). Scoring was performed independently by 2 pathologists (M.N.J., M.S.) and reviewed by a third (A.N.T.).

Statistical Analysis

The association of immune cell infiltrates and tumor characteristics were evaluated using Fisher exact test. Correlation between the presence of chronic inflammation and PD-L1 staining was examined using the Kruskal−Wallis rank test. Statistical analyses were performed using STATA version 13 (StataCorp LP). All tests were 2-sided and P values $< .05$ were considered significant.

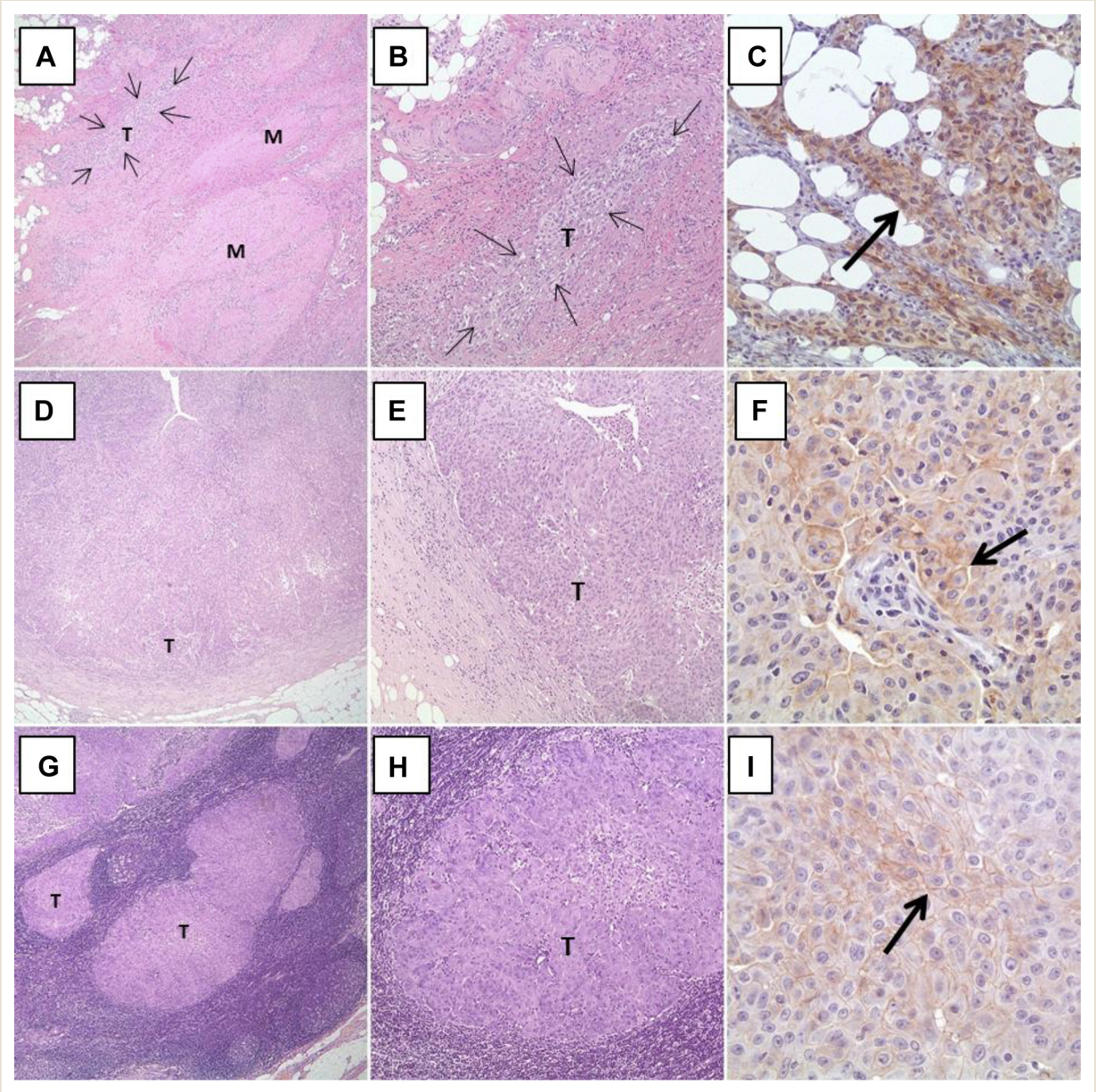
Results

Fifty-two cases of cystectomy for muscle-invasive bladder cancer were identified; clinicopathologic features are outlined in Table 1. Tumor PD-L1 expression was identified in 5 of 52 cases (9.6%) with mild to moderate focal staining (range, 10%-40%; Table 2). PD-L1 expression in lymph nodes was noted in 2 of 4 positive cases with associated lymph node metastasis with mild focal immunoexpression (range, 20%-40%; Table 3, Figure 1). Examination of PD-L1

Table 3 Programmed Death Ligand 1 Expression in Tumor Cells and Tumor-Associated Immune Cells

	Programmed Death Ligand 1 Expression	
	Tumor Cells	Histiocytes and Lymphocytes
Primary Tumor	5/52 (9.6%)	17/52 (32.7%)
Lymph Node Metastasis	2/18 (11.8%)	10/18 (55.5%)

Figure 1 (A) and (B) High-Grade Urinary Bladder Carcinoma (T) Infiltrating Through Muscularis Propria (M) Into Pericystic Fat (Hematoxylin and Eosin [H&E] Stain, Magnification [A] ×40 and [B] ×100). (C) The Same Case Showing Moderately Intense and Mainly Cytoplasmic Staining for Anti-PD-L1 (Magnification, ×400). (D) and (E) Another Case of High-Grade Muscle-Invasive Urinary Bladder Carcinoma (H&E Stain, Magnification [D] ×40 and [E] ×100) With (F) Weak Membranous Anti-PD-L1 Expression (Magnification, ×400). (G) and (H) The Corresponding Pelvic Lymph Node Metastasis (T) (H&E Stain, Magnification [G] ×40 and [H] ×100) With a Weak Membranous Anti-PD-L1 Expression Pattern Similar to That Shown in (I); Magnification, ×400). Arrows show anti-PDL1 staining of tumor cells

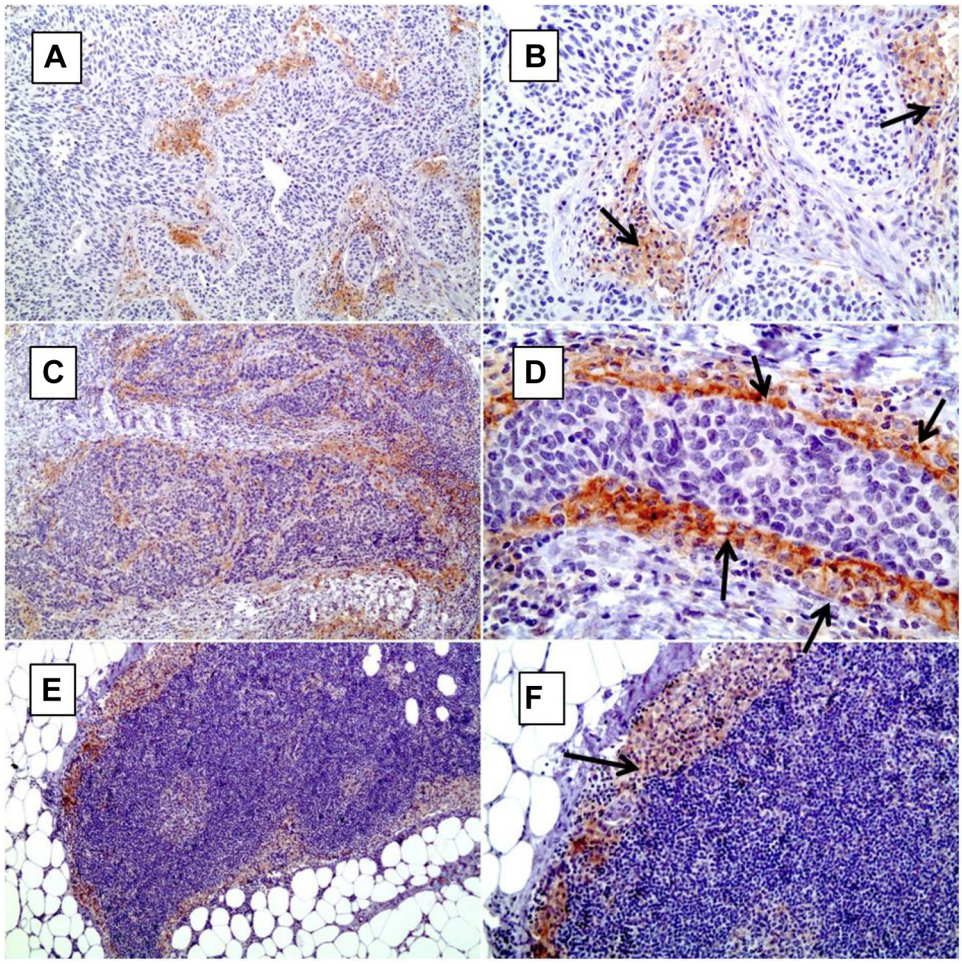


Abbreviation: PD-L1 = programmed death ligand 1.

expression in tumor-infiltrating immune cells, mainly histiocytes and lymphocytes, revealed mild to moderate focal immunostaining (range, 10%-40%) in 17 cases (32.7%). The distribution of PD-L1 immunoexpression within tumor-infiltrating immune cells ranged from peritumoral histiocytes and lymphocytes to isolated foci of histiocytic and lymphocytic germinal centers (Figure 2). PD-L1 expression in tumor-infiltrating immune cells associated with lymph node metastasis was weakly cytoplasmic and confined to the subcapsular histiocytes and sinus histiocytes in 10 of 18 lymph node-positive cases (55.5%; Table 4).

Whereas both of the lymph node metastasis cases staining positive for PD-L1 were associated with PD-L1 staining in the

Figure 2 (A) and (B) PD-L1 Expression in Groups of Tumor-Associated Histiocytes (Arrows) Scattered Between Sheets of High-Grade Invasive Urothelial Carcinoma (T). (C) and (D) PD-L1 Expression in Histiocytes Rimming Sheets of High-Grade Invasive Urothelial Carcinoma. (E) and (F) PD-L1 Expression in Lymph Nodes Within Histiocytes Mainly in the Subcapsular Sinus. Hematoxylin and Eosin Stain; Magnification, (A), (C), and (E) ×100; (B) and (F) ×200; (D) ×400



Abbreviation: PD-L1 = programmed death ligand 1.

corresponding cystectomy specimens, no significant association between PD-L1 expression in lymph node tumor-infiltrating immune cells and cystectomy specimens was observed ($P = 1.0$). In 8 cases, PD-L1 expression in lymph node tumor-infiltrating immune cells was observed in the absence of PD-L1 expression in the corresponding cystectomy specimen. There was no correlation observed between the presence of chronic inflammation (defined as a lymphoplasmacytic infiltrate within the urinary bladder wall) and PD-L1 staining of the tumor cells ($P = .84$). Furthermore, there was no significant association between PD-L1 staining in the tumor and any associated tumor-infiltrating histiocytes ($P = .37$).

Discussion

The aim of this study was to determine the feasibility of using archived tumor PD-L1 expression as a biomarker to select patients with advanced urothelial carcinoma for future use of PD-1 pathway targeting agents. To date there are no standardized assays available for evaluating tumor PD-L1 expression and in this study we used the 5H1 clone used by Topalian and colleagues.[4,5] In our cohort of muscle-invasive bladder cancer specimens with no previous exposure to chemotherapy, tumor PD-L1 expression was low both in intensity and distribution (5/52 cases, 9.6%) with a higher proportion of cases showing PD-L1 expression in tumor-associated immune cells (17/52 cases, 32.7%). These data are consistent with other studies of PD-L1 expression in urothelial cancer using alternative assays.[10,11] In contrast to a previous study by Boorjian et al,[10] we found significant discordance between PD-L1 expression in lymph node metastasis and corresponding cystectomy specimens. Limitations of our study include its exploratory nature secondary to the small sample size, however, unlike previous series in which the sample was heterogeneous; this sample included only muscle-invasive bladder cancer with no previous exposure to neoadjuvant chemotherapy. Although the age of the tissue samples might potentially decrease the intensity of tissue staining, no correlation

Table 4 Tumor-Associated Immune Cell PD-L1 Expression in Cystectomy Specimens and Associated Lymph Node Metastasis

	PD-L1 Expression in Lymph Node Histiocytes and Lymphocytes	
	Positive	Negative
PD-L1 Expression in Cystectomy Specimen Tumor Infiltrating Histiocytes and Lymphocytes Positive	2/18	1/18
PD-L1 Expression in Cystectomy Specimen Tumor Infiltrating Histiocytes and Lymphocytes Negative	8/18	7/18

Abbreviation: PD-L1 = programmed death ligand 1.

was observed between sample age and immunohistochemistry results. Several studies investigating PD-L1 expression in renal cell carcinoma have also shown discordance between primary tumors and metastatic sites.[12]

Despite the fact that PD-L1 expression according to immunohistochemistry has been shown to correlate with response to PD-1 pathway targeted therapy in several tumor types,[4,6] lack of a standardized assay and the observation of activity in PD−L1-negative patients mean that PD-L1 expression cannot yet be considered a reliable treatment selection biomarker. The observation of discordance between PD-L1 expression in metastatic sites and primary tumors suggests that prospective biomarker studies should aim to acquire samples immediately before treatment initiation rather than use archived tissue from resected specimens that might not reflect the current immune-active microenvironment.

Conclusion

Programmed death-ligand 1 was overexpressed in the tumor cells of 5/52 (9.6%) of muscle-invasive bladder cancer cystectomy specimens in this cohort with 17/52 (32.7%) of cases showing PD-L1 overexpression in tumor-infiltrating immune cells. Discordance was observed between PD-L1 expression in lymph node metastasis and the primary tumor. Prospective biomarker studies using fresh biopsy specimens are likely to provide a more accurate assessment of the immune-active microenvironment in the search for reliable treatment selection biomarkers for immune checkpoint targeted therapy.

Clinical Practice Points

- Data supporting the role of PD-L1 expression as a predictive biomarker for response to PD-1 pathway-directed therapy are not consistent.
- In bladder cancer, immunotherapy trials and translational studies are still in the early phases and there currently exists a need for further progress in this setting.
- The present study aimed at establishing the frequency of PD-L1 expression in muscle-invasive bladder cancer and associated lymph node metastasis using immunohistochemistry and to investigate the feasibility of using PD-L1 expression as a biomarker to select patients for PD−1- directed therapy.
- PD-L1 was overexpressed in the tumor cells of 5/52 (9.6%) of cystectomy specimens in this cohort with 17/52 (32.7%) of cases showing PD-L1 overexpression in tumor-infiltrating immune cells. Discordance was observed between PD-L1 expression in lymph node metastasis and the primary tumor.
- Prospective biomarker studies using fresh biopsy specimens are likely to provide a more accurate assessment of the immune-active microenvironment in the search for reliable treatment selection biomarkers for immune checkpoint targeted therapy.

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Disclosure

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