Impact of Neoadjuvant Chemotherapy on Concordance of PD-L1 Staining Fidelity between the Primary Tumor and Lymph Node Metastases in Bladder Cancer

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OBJECTIVE To evaluate programmed death ligand 1 (PD-L1) staining fidelity between the primary tumor and associated lymph node metastases in bladder cancer. To secondarily evaluate whether neoadjuvant chemotherapy (NAC) affects this relationship.

METHODS Sixty-seven subjects with residual bladder cancer on cystectomy and associated positive lymph nodes were identified between 2008 and 2015. PD-L1 staining of tumor cells was evaluated using H score and 49 specimens were also evaluated using combined positive score (CPS). Univariable and multivariable logistic regression analysis were used to assess how various clinical variables affected odds of PD-L1 fidelity between primary and metastatic tumors.

RESULTS Tumor PD-L1 staining was concordant in 79.1% of cases and CPS was concordant in 79.6% of cases. NAC did not significantly impact odds of PD-L1 or CPS fidelity (OR 1.974, 95% CI 0.673-5.784, OR 0.500, 95% CI 0.093-2.700). Among clinical variables analyzed on univariable analysis of tumor PD-L1 fidelity, H-score, and PD-L1 staining intensity were associated with significantly increased odds of PD-L1 fidelity and the association with staining intensity was confirmed on multivariable analysis.

CONCLUSION PD-L1 fidelity between primary bladder tumors and nodal metastases was observed in >75% of cases in this study. Additionally, NAC was not shown to diminish this propensity to maintain PD-L1 staining status. Further standardization of immunohistochemistry of tumor and infiltrating imsmune cells in metastatic bladder cancer is needed to improve application of therapeutics. UROLOGY 131: 150–156, 2019. © 2019 Elsevier Inc.

BACKGROUND

Recent advances in immunotherapeutics have brought an exciting new array of treatment targets to light. One such target is programmed death ligand 1 (PD-L1) which complexes with programmed death-protein 1 (PD-1) and inhibits native T cell responses to the tumor.[1] Five agents inhibiting this complex have rapidly come to market, and these immune cell checkpoint inhibitors have quickly evolved as an effective treatment option for a number of patients with advanced urothelial cell carcinoma. Optimal measures to predict treatment response have yet to emerge beyond PD-L1 staining positivity, which was recently demonstrated to be positively associated with aggressive clinical features and poorer recurrence-free and overall survival.[2] The evaluation of fidelity of PD-L1 staining between the primary and metastatic deposits is of interest since loss of staining in the lymph node sites may demonstrate a mechanism of therapeutic escape from these agents. Evaluation of this potential phenomenon has been limited.

Additionally, level 1 evidence exists for the use of neoadjuvant chemotherapy (NAC) prior to cystectomy and its implementation has grown significantly over the past several decades. It is unclear if this has an effect on PD-L1

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staining in general, or if there is a modulating effect on primary tumor and lymph node staining fidelity. The objective of this study therefore is to quantitate fidelity between the primary tumor and lymph node metastases with respect to presence or absence of staining. The secondary outcome is to evaluate any change in expression attributable to clinical or pathologic factors, notably NAC.

MATERIALS AND METHODS

Study Population

With institutional review board approval, we performed a retrospective review of multi-institutional, prospectively maintained bladder cancer databases including 770 patients who underwent radical cystectomy for bladder cancer between 2006 and 2017. After including only patients with lymph node-positive metastases and residual disease in the bladder at the time of cystectomy, 67 patients were included for analysis. Patients who did not have a lymph node dissection performed at the time of cystectomy or who had no residual malignancy in the bladder (pT0) were excluded.

Pathologic Data

The pathology was centrally re-reviewed by a genitourinary pathologist (PL). The pathologist was blinded to NAC status and other clinical variables relating to each case. One block most representative of the primary urothelial carcinoma within the cystectomy specimen and the corresponding lymph node metastasis were identified from each case. Immunohistochemistry was performed on whole sections of formalin-fixed paraffin-embedded tissue using Leica Bond-IIITM and Bond Polymer Refine Detection System (Leica Microsystems DS9800). Heat-induced epitope retrieval was done for 20 minutes with ER2 solution (Epitope Retrieval2 [ER2] #AR9640). PD-L1, Clone E1J2J, from Cell Signaling (15165BF) at a dilution of 1:2000 was used. PD-L1 membranous staining was evaluated on tumor cells in a semiquantitative manner on an intensity scale of 0 to 3+ where 0= negative; 1 = weak membranous; 2 = intermediate strength membranous and 3 = strong membranous staining. In cases where the tumor intensity varied, an average was assigned. The data were normalized by calculating an H-score (intensity × percentage) such that the minimum score was zero and the maximum score was 300. Since there are no current standards of evaluation of PD-L1 staining for bladder cancer, staining of >1% of tumor cells at any intensity was deemed "positive." Cohen's kappa coefficient was used to assess correlation of positive and negative staining status between the primary and metastatic tumors. The interclass correlation coefficient was used to assess the correlation between primary and metastatic tumor staining by average H-score. In a secondary analysis, 49 available paired specimens were evaluated for combined positive score (CPS). This score accounts for both the PD-L1 status of the tumor as well as the surrounding infiltrating immune cells and has been used in a number of clinical trials to more completely describe the PD-L1 staining pattern of a specimen.[3] CPS is calculated by the following formula: (PD-L1 positive tumor cells + PD-L1 positive infiltrating immune cells)/(total tumor cells). Specimens were divided into CPS categories of 0%, <10% and >10% (Fig. 1). Cohen's kappa coefficient was again used to assess correlation of CPS category between primary and metastatic specimens. Pathologic data collected included tumor histology, presence of lymphovascular invasion, and carcinoma in situ. Specimens were also divided by pathologic stage into pTis, pT1, pT2, pT3, and pT4 disease. NAC was defined as receipt of multiagent platinum-based chemotherapy prior to surgery.

Outcome of Interest

The primary outcome of interest was PD-L1 staining fidelity between the bladder primary and metastatic lymph nodes. The secondary outcome of interest was change in fidelity or intensity of staining between patients who did and did not undergo NAC.

Statistical Analysis

Demographic and baseline characteristics were described as frequencies (with percentiles). Univariable logistic regression analysis was used to determine the association of demographic, pathologic, and clinical variables with PD-L1 staining fidelity and CPS fidelity. Multivariable logistic regression was then performed to determine the association between baseline demographics and pathologic data with PD-L1 staining fidelity. SAS Version 9.4 was used for statistical analysis.

RESULTS

Study Cohort and Descriptive Analyses

Clinicopathologic data are shown in Table 1. The median age was 71 years: 85% were Caucasian, 64% were male, and 30% had received NAC. PD-L1 staining fidelity was observed between the primary bladder cancer and its corresponding lymph node metastasis in 79.1% of cases analyzed. Among the discordant minority, it was observed that 23.8% of PD-L1 positive bladder tumors had negative lymph node metastases and 16.0% of PD-L1 negative bladder tumors had positive lymph node metastases (Table 2). The kappa value associated with the staining status of the primary and metastatic tumors was 0.56, demonstrating a trend toward maintaining PD-L1 fidelity. The interclass correlation coefficient between the average bladder and lymph node H-scores was 0.85 (95% CI 0.75-0.91), demonstrating significant agreement between primary tumor and metastatic staining characteristics. CPS concordance was observed in 79.6% of cases analyzed (Table 3). The kappa value associated with the CPS of the primary and metastatic tumors was 0.694, demonstrating moderate agreement.

Univariable Logistic Regression for Odds of Tumoral PD-L1 Staining and CPS Fidelity

On univariable analysis, male sex was associated with significantly decreased odds of PD-L1 fidelity (OR 0.243, 95% CI 0.079-0.744). Among pathologic variables analyzed on univariable analysis, bladder H-score (OR 1.014, 95% CI 1.004-1.025), lymph node H-score (OR 1.038, 95% CI 1.007-1.071), bladder PD-L1 staining intensity (OR 6.253, 95% CI 2.918-13.401), and lymph node PD-L1 staining intensity (OR 14.365, 95% CI 4.509-45.762) were associated with significantly increased odds of PD-L1 fidelity (Supplementary Table 1). Receipt of NAC did not significantly impact odds of PD-L1 staining fidelity (OR 1.974, 95% CI 0.673-5.784). A similar univariable analysis was performed for CPS. Odds of CPS fidelity was not significantly impacted by age, race, sex, receipt of intravesical therapy, presence of lymphovascular invasion, or presence of carcinoma in situ. Receipt of NAC did not significantly influence odds of CPS concordance (OR 0.500, 95% CI 0.093-2.700).

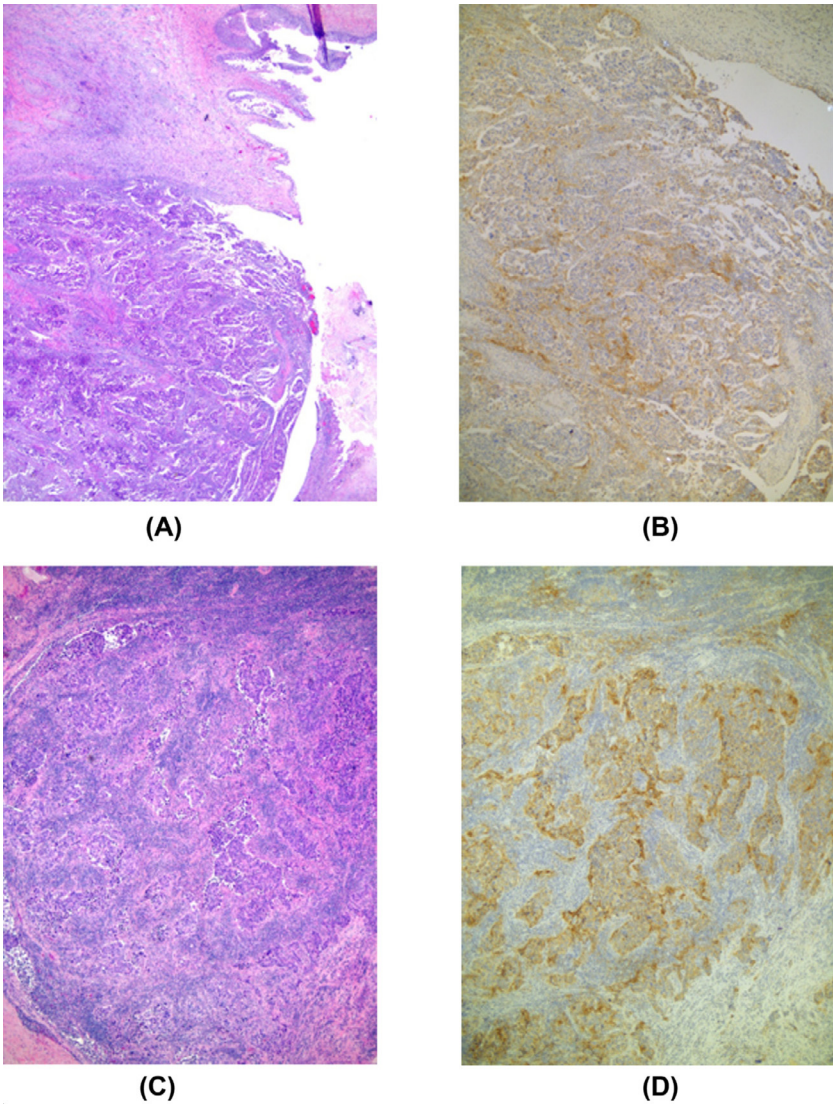


Figure 1. Representative stained primary bladder tumor and lymph node metastatic specimens by combined positive score.
Case 1: Figures A, B, C, and D: Combined positive score >10%.
A. H&E stain demonstrating urothelial carcinoma in the cystectomy specimen.
B. PD-L1 stain demonstrating more than 10% staining in the combined tumor and immune cell compartment of the primary tumor.
C. H&E stain demonstrating metastatic urothelial carcinoma in the lymph node specimen.
D. PD-L1 stain demonstrating more than 10% staining in the combined tumor and immune cell compartment of the lymph node metastasis.
Case 2: Figures E, F, G, and H: Combined positive score <10%.
E. H&E stain demonstrating urothelial carcinoma in a cystectomy specimen.
F. PD-L1 stain demonstrating less than 10% staining in the combined tumor and immune cell compartment of the primary tumor.
G. H&E stain demonstrating metastatic urothelial carcinoma in the lymph node specimen
H. PD-L1 stain demonstrating less than 10% staining in the combined tumor and immune cell compartment of the lymph node metastasis.
Case 3: Figures I, J, K, and L: Combined positive score 0.
I. H&E stain demonstrating urothelial carcinoma in the cystectomy specimen.
J. PD-L1 stain demonstrating negative staining in the combined tumor and immune cell compartment.
K. H&E stain demonstrating metastatic urothelial carcinoma in the lymph node specimen.
L. PD-L1 stain demonstrating negative staining in the combined tumor and immune cell compartment.
Control.
M. Tonsil PD-L1 control. (Color version available online.)

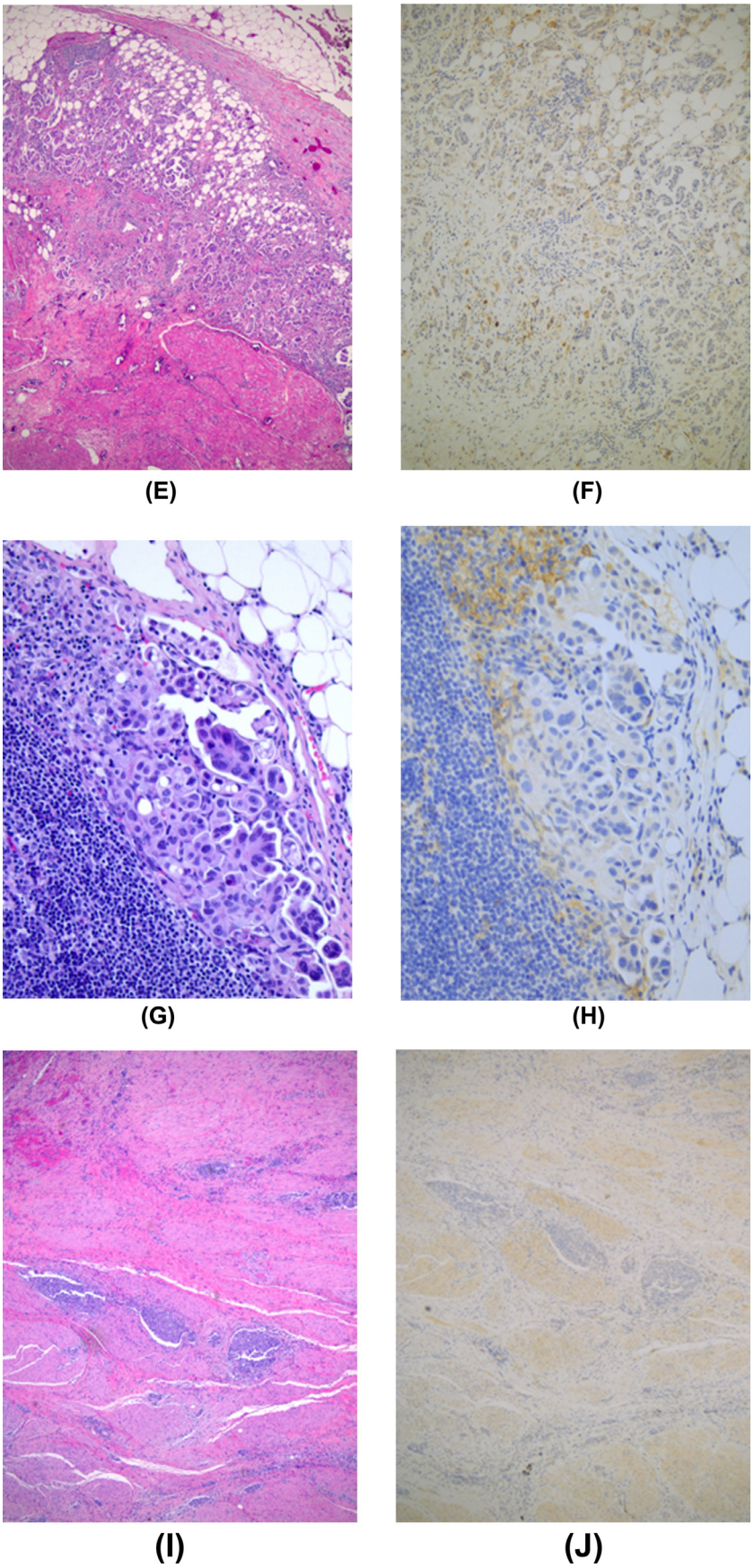
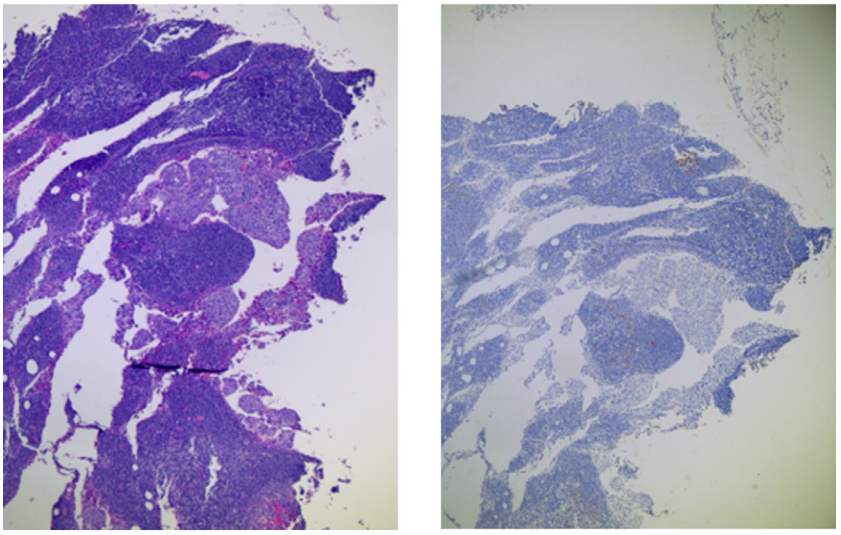


Figure 1. Continued



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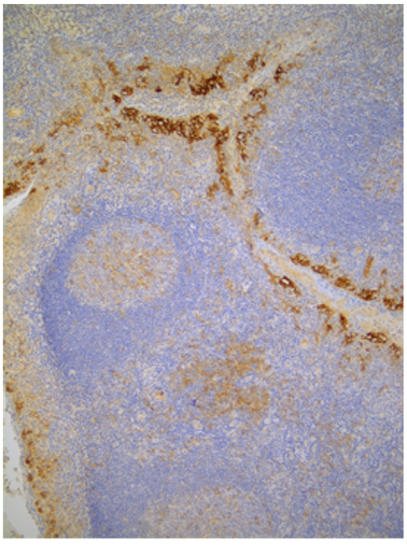

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Figure 1. Continued

Multivariable Logistic Regression for Odds of PD-L1 Staining Fidelity

Bladder PD-L1 staining intensity (OR 25.8, 95% CI 2.11-315) and lymph node PD-L1 staining intensity (OR 50.4, (95% CI 2.59-981) were significantly associated with PD-L1 fidelity on multivariable logistic regression (Supplementary Table 2).

DISCUSSION

The principal finding of this study was the high degree of fidelity in staining of the primary tumor and lymph node metastases with respect to PD-L1. Staining concordance of tumor cells was >75% with matched intensity of the stain as demonstrated by the H-score. CPS was used to include infiltrating lymphocytes in the analysis and similarly demonstrated >75% concordance between matched specimens. Additionally, those patients treated with NAC demonstrated no appreciable change in primary tumor—lymph node fidelity of PD-L1 status compared to those patients not receiving platinum based chemotherapy.

These findings are consistent with earlier, smaller studies assessing PD-L1 fidelity. One study examined 18 cystectomy and lymph node specimens from chemotherapy-naïve patients with lymph node metastatic urothelial cell carcinoma (UCC). They found no correlation between primary tumor and metastatic tumor cell PD-L1 status, and also reported discordance in PD-L1 status of associated lymphocytic infiltrates.[4] However, this study used the B7-H1 antibody and a >5% staining threshold to determine PD-L1 positivity, making it difficult to compare to our cohort. Another study with 27 chemotherapy-naïve cystectomy specimens with metastasis demonstrated significant PD-L1 staining fidelity of tumor cells (discordance rate of 22.2%) and no significant

Table 1. Demographic and clinical characteristics

Subjects—no.	67
Median Age—y (range)	71 (45-87)
Race—no. (%)	
Caucasian	57 (85.1)
African American	6 (9.0)
Other	4 (6.0)
Sex—no. (%)	
Female	24 (35.8)
Male	43 (64.2)
Neoadjuvant treatment—no. (%)	
Received intravesical therapy	17 (25.4)
Received neoadjuvant chemotherapy	21 (31.3)
Primary Histology—no. (%)	
Urothelial carcinoma	61 (91.0)
Adenocarcinoma	4 (6.0)
Squamous cell carcinoma	2 (3.0)
Variant urothelial histology—no. (%)	
Glandular, micropapillary, sarcomatoid, squamous	23 (37.7)
Pathologic features—no. (%)	
Presence of lymphovascular invasion	27 (40.3)
Presence of carcinoma in situ	10 (14.9)

Table 2. PD-L1 staining status of primary bladder tumor and lymph node metastases after cystectomy

	Bladder PD-L1 (+)	Bladder PD-L1 (−)	Total
LN PD-L1 (+)	32	4	36
LN PD-L1 (−)	10	21	31
Total	42	25	67

Table 3. PD-L1 combined positive score of primary bladder tumor and lymph node metastases after cystectomy

	Bladder CPS = 0%	Bladder CPS <10%	Bladder CPS >10%	Total
LN CPS = 0%	12	0	2	14
LN CPS <10%	1	14	1	16
LN CPS >10%	3	3	13	19
Total	16	17	16	49

correlation in PD-L1 staining status of immune cells (discordance rate of 44.4%) suggesting dynamic changes to the lymphocytic infiltrate at metastasis.[5] However, the majority of these trials were small and single center in nature with limited analysis examining the impact of clinical variables on concordance. More recently, a larger study trialing several antibodies on 79 pairs of specimens were published with similar results to these, with 89.9% PD-L1 concordance between primary and metastatic tumors. They demonstrated relative equivalence of the 4 assays studied; however, effects of NAC were not addressed.[6] A recent retrospective analysis of 77 matched primary tumor and metastatic pairs reported 89.6% concordance of PD-L1 staining in tumor cells, which is consistent with our findings. They concluded that there was poor overall concordance in PD-L1 staining percentage in tumor and immune cells, however; the median time between biopsy of the primary tumor and metastatic tumors in this study population was 12.2 months, demonstrating possible temporal changes based on evolution of the tumor microenvironment or responses to therapy.[7] Our study sought to look at primary tumors and corresponding metastases resected at the time of cystectomy to assess PD-L1 concordance earlier on in the metastatic process.

Second, on univariable analysis, neither NAC nor intravesical therapy impacted the odds of tumoral PD-L1 or CPS fidelity. In this cohort, 30.4% of patients underwent NAC and 24.6% underwent intravesical therapy prior to cystectomy. Studies specifically correlating NAC with PD-L1 status are limited. One small study examined the PD-L1 expression of tumor cells in the setting of NAC and found that 13/14 cases preserved PD-L1 status between bladder biopsy and cystectomy specimens.[8] Although this study did not have a non-NAC group to compare fidelity to over a comparable time period, the authors did evaluate survival and found that it was independent of staining status. Additionally, for this study, positivity was determined by >10% staining, making it difficult to compare to our cohort. Overall, current data are lacking for measuring or predicting the effect of NAC on PD-L1 expression, though the data in this study suggests that it does not correlate with PD-L1 fidelity. Further prospective studies with controlled intravesical therapy regimens and standardized NAC are needed to confirm these findings.

Finally, among clinicopathologic variables, tumor cell staining intensity was significantly associated with PD-L1 staining fidelity. Though PD-L1 intensity and H-score are semiqualitative measures, immunohistochemistry (IHC) staining systems have been incorporated into diagnostic and treatment algorithms for other malignancies (such as lung cancer), and could serve a role in bladder cancer. Most existing studies evaluating PD-L1 in bladder tumor cells employ arbitrary percentages of stained cells without evaluation of intensity, making data difficult to compare, and benefits conferred by checkpoint inhibitor therapy have been shown to persist irrespective of PD-L1 expression.[9,10] CPS is a useful tool to evaluate a larger picture of the microenvironment in addition to tumor cells, though further validation and application in the setting of checkpoint inhibitor therapy is needed to fully delineate its clinical utility.

The limitations of this study include small cohort size and its retrospective nature. Given the large confidence intervals on multivariable analysis, a larger sample size would be helpful in supporting these findings. Additionally, 6 of the cases consisting of nonurothelial primary bladder cancer were included in the analysis, since many of these agents are used to treat various histology types in other cancers. Outside of a prospective clinical trial, the utility of checkpoint inhibitor therapy on nonurothelial primary bladder cancer remains unknown. Our cohort included 23 cases featuring variant urothelial histology. Further pathologic analysis of specific subtypes in relation to PD-L1 staining fidelity is warranted, however we lacked sufficient power to draw specific conclusions about these subgroups. As many of these patients had

their initial endoscopic resection at outside institutions, there was insufficient presurgical specimen available to help elucidate a temporal change in PD-L1 staining from time of diagnosis to cystectomy. Additionally, this study could be improved in its clinical application by correlating PD-L1 fidelity with cancer specific survival. Unfortunately, we lacked sufficient data regarding the outcomes of the subjects in this retrospective study.

CONCLUSIONS

PD-L1 staining fidelity between the primary bladder tumor and metastatic lymph node deposit was observed in >75% of cases in this study. Standard NAC did not impact PD-L1 concordance of tumor cells though increased tumor staining intensity was associated with increased odds of fidelity. PD-L1 staining, while useful, is not an absolute indicator for response, since the effectiveness of immunotherapeutics is likely mediated by multiple aspects of the tumor microenvironment. Better understanding of the tumorigenic mechanisms associated with metastasis and response to PD-L1 inhibition will further tailor medical treatment to the individual characteristics of each patient.

SUPPLEMENTARY MATERIALS

Supplementary material associated with this article can be found in the online version at https://doi.org/10.1016/j.urology.2019.05.039.

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