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Original article

Discordance of high PD-L1 expression in primary and metastatic urothelial carcinoma lesions

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Abstract

Immune checkpoint inhibitors (ICI) targeting PD-(L)1 are effective in select patients with advanced urothelial carcinoma (UC). High PD-L1 expression enriches for response to ICIs; however, the predictive value of PD-L1 expression is limited, which may be due in part to dynamic expression of PD-L1 in the tumor environment. We sought to characterize PD-L1 expression in primary UC and paired metastatic lesions to gain insight into the potential discordance of tumor PD-L1 expression during the metastatic process. Materials and methods: Immunohistochemical staining for PD-L1 using the SP-142 antibody was performed on primary tumors and matched metastatic specimens in 77 evaluable subjects with advanced UC. Immunohistochemical staining was scored for the percentage of cells positive (<5%, ≥5%) in tumor cell (TC) and immune cell (IC) compartments. Correlation of PD-L1 expression in TCs and ICs was estimated using Spearman's correlation coefficients (rho, ρ). Cohen's kappa statistics (κ) were utilized to assess the agreement in PD-L1 expression between groups. Results: High (≥5%) PD-L1 expression in primary and metastatic biopsies, respectively, was observed in 6.0% and 7.7% of TCs and in 14.5% and 11.5% of ICs. IC PD-L1 expression in primary tumors was not correlated with IC PD-L1 expression in paired metastatic lesions ($\rho = 0.05$, $P = 0.67$) and there was poor agreement in high expression rates between primary and metastatic lesions in the IC compartment ($\kappa = 0.086$). Conclusion: High PD-L1 IC expression is temporally and spatially discordant between primary and metastatic UC lesions. Future studies of PD-(L)1 targeted therapies in patients with metastatic UC may benefit from use of fresh biopsies of metastatic lesions to define PD-L1 expression when feasible.

Keywords: PD-(L)1; SP142; Urothelial carcinoma; Bladder cancer; Immune checkpoint inhibitor; Atezolizumab

1. Introduction

Regulatory approval of immune checkpoint inhibitors (ICIs) targeting the programmed death-1 (PD-1) axis has established an additional standard of care for patients with advanced urothelial carcinoma (UC). To date, 5 therapeutic antibodies targeting PD-1 or PD-L1 (programmed death ligand 1) have been granted accelerated or full approval on the basis of established clinical benefit [1].

Observations from clinical studies with anti-PD-1/PD-L1 antibodies in patients with advanced urothelial carcinoma suggest that high intratumoral PD-L1 expression enriches for response to these agents, as would be expected [2–6]. However, these studies have also consistently shown that high expression of PD-L1 in the tumor environment is not required for a therapeutic response to these agents, and in some cases responses have been documented in tumors purportedly not expressing PD-L1. The limited predictive value of intratumoral PD-L1 expression may be due to dynamic spatial and temporal expression patterns and discordance between primary and metastatic lesions, which has been observed with other molecular markers [7].

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PD-L1 expression status determined from a single, archival tumor specimen may not accurately reflect tumor PD-L1 expression status at the time of therapeutic intervention. In clinical studies of ICIs in patients with advanced urothelial cancer, biopsy specimens from primary tumors were commonly utilized for characterization of PD-L1 expression [2,8]. Although analysis of primary diagnostic transurethral bladder tumor resection specimens is convenient due to abundance of tumor material, we hypothesized that PD-L1 expression in primary urothelial tumors may not accurately reflect the PD-L1 expression status in synchronous or metachronous metastatic lesions. Therefore, we sought to characterize PD-L1 expression in paired primary and metastatic biopsies from patients with advanced UC to assess the extent of discordance of PD-L1 expression between primary and metastatic lesions.

2. Materials and methods

2.1. Patients

Following institutional review board approval of our protocol, patients with metastatic UC diagnosed between January 2007 and February 2018 were retrospectively identified from an institutional tumor bank. Patients were selected for study participation if paired archival tumor specimens from both primary and metastatic lesions were available for immunohistochemical analysis. Hematoxylin and eosin stained slides from study cases were reviewed by 2 tumor pathologists (CL, AH) to confirm adequate tumor cellularity for analysis. Specimens were considered adequate for evaluation if they contained at least 50 viable tumor cells and tumor-associated stroma.

2.2. Immunohistochemistry

Formalin-fixed paraffin-embedded tumor blocks from cystectomy, transurethral resection, core and cell block biopsy specimens were sectioned at 4 microns on positively charged glass slides and stained with the Ventana PD-L1 SP142 assay. Human tonsil tissue was used as a positive and negative control for staining.

PD-L1 expression in tumor cells was assessed as the proportion of tumor cells showing membrane staining of any intensity (number of PD-L1-positive tumor cells/total number of PD-L1-positive and PD-L1-negative tumor cells). PD-L1 expression in immune cells (IC) was scored as the proportion of tumor area that was occupied by PD-L1 staining ICs of any intensity. Any IC staining irrespective of cell type or localization was included. Only IC staining in the tumor microenvironment was evaluated. Tumor area was defined as the area containing viable tumor cells, associated intratumoral stroma and contiguous peritumoral stroma. IC staining was classified as null (PD-L1 = 0%), low (0%< PD-L1 <5%) or high (PD-L1 ≥5%), based on the presence of staining in tumor-infiltrating ICs covering 0, 1 to 5% or ≥5% of tumor area. Tumor proportion score for PD-L1 was also scored using this scale. All stains were scored manually by a single pathologist with >15 years of experience in surgical pathology and immunohistochemistry.

2.3. Statistics

Specimens from 84 subjects were available, with 83 primary tumor specimens and 78 metastatic specimens, resulting in 77 matched primary/metastatic specimens. Patient characteristics were summarized with frequencies and proportions. Immunohistochemical staining was dichotomized as <5% vs. ≥5% for both tumor cell (TC) and IC compartments. The PD-L1 staining threshold of ≥5% of tumor-infiltrating ICs has been utilized with the Ventana SP142 assay in recent clinical trials with atezolizumab and therefore was selected to define the high IC PD-L1 expression threshold for this study [9]. To assess the degree of association of PD-L1 expression levels in TCs and ICs, Spearman's correlation coefficients (ρ) were estimated. We considered estimated correlation coefficients between 0.90 and 1 as very high degree correlation; values between 0.70 and 0.90 as high degree correlation; value between 0.50 and 0.70 as moderate degree correlation; values between 0.30 and 0.50 as low degree correlation; and values between 0 and 0.30 as little if any correlation [10]. Frequencies and proportions were utilized to summarize expression categories and, in addition to simple agreement rates, kappa statistics (κ) were also utilized to assess the agreement in PD-L1 expression category between groups, including null (PD-L1 = 0%), low (0%< PD-L1 <5%) and high (PD-L1 ≥5%) categories. Kappa statistics and corresponding confidence intervals were estimated to adjust simple agreement rates for chance agreement [11]. In this setting, the magnitude of the Kappa statistic adjustment relative to the simple agreement increases with the degree of skewness of the overall prevalence of PD-L1 expression categories. Kappa values that are zero or less are considered no agreement; values greater than 0−0.20 are considered slight agreement; values greater than 0.20−0.40 are considered fair agreement; values greater than 0.40−0.60 are considered moderate agreement; values greater than 0.60−0.80 are considered substantial agreement; values greater than 0.80 are considered almost perfect agreement [12].

3. Results

3.1. Patient characteristics

Patient characteristics are shown in Table 1. The majority (82.1%) of patients did not have metastatic disease at presentation, and the median time between primary and metastatic biopsies was 12.2 months (0.1−89.5 months). Seventeen (24.6%) patients with nonmetastatic disease at presentation received perioperative chemotherapy in

Table 1
Patient characteristics

All patients ($n = 84$)	n (%)
Gender	
Male	69 (82.1%)
Female	15 (17.9%)
Race	
Caucasian	65 (77.4%)
Black	15 (17.8%)
Other/unknown	4 (4.8%)
Metastatic at presentation?	
Yes	15 (17.9%)
No	69 (82.1%)
Perioperative chemotherapy?	
Yes	17 (20.2%)
No	42 (50.0%)
Unknown	25 (29.8%)
Patients with paired biopsies ($n = 77$)[a]	
	Median [Range]
Duration between primary and metastatic biopsies (months)	12.2 [0.1–89.5]
	n (%)
Metastatic biopsy type	
Surgical	28 (36.4%)
Core needle	26 (33.8%)
Fine needle aspirate	22 (28.6%)
Other	1 (1.3%)

[a] Only patients with adequate biopsy material from both paired specimens were included.

addition to radical cystectomy for curative intent. In patients with available paired biopsies, 70.5% of metastatic biopsies were obtained from either core needle or surgical biopsies. Characteristics of the 6 patients from this cohort who received ICI therapy in the metastatic setting are shown in Table 2.

3.2. PD-L1 expression

Intratumoral PD-L1 expression was spatially heterogenous within specimens. Representative IC PD-L1 staining is shown in Fig. 1. PD-L1 expression rates observed in this cohort are summarized in Table 3. We observed high (≥5%) tumor IC PD-L1 expression in primary and metastatic lesions in 14.5% and 11.5% of patients, respectively. High TC PD-L1 expression was noted in 6% of primary and 7.7% of metastatic samples. Coexpression of high PD-L1 in both IC and TC compartments was infrequent and observed only in 3.6% of primary lesions and 2.6% of metastatic lesions (Fig. 2). No PD-L1 expression was observed in the IC compartment of 56.6% and 79.5% of primary and metastatic lesions, respectively. A larger proportion of null PD-L1 expression was observed in the tumor cell compartment compared to the IC compartment in primary and metastatic biopsies (Table 3).

3.3. Correlation of PD-L1 expression between tumor cell and IC compartments

Analysis of PD-L1 coexpression in tumor cells and infiltrating ICs was performed to examine concordance of expression between cellular compartments. In primary tumors, a low positive correlation between the PD-L1 expression in the TC and IC compartments was observed ($\rho = 0.468$, $P < 0.001$), and fair agreement in PD-L1 expression categories between TC and IC compartments existed, adjusting for chance agreement (($\kappa = 0.293$, 95% CI −0.004, 0.590; simple agreement rate: 86.4%).

In metastatic lesions, little if any correlation between the PD-L1 expression in TC and IC compartments was observed ($\rho = 0.267$, $P = 0.018$), and slight agreement in PD-L1 expression categories between TC and IC compartments was observed ([$\kappa = 0.192$, 95% CI −0.118, 0.503]; simple agreement rate: 85.9%).

3.4. Correlation of PD-L1 expression between primary and metastatic biopsies

Comparison of PD-L1 expression levels between paired primary and metastatic biopsies was performed. IC PD-L1 expression in primary tumors did not correlate with IC PD-L1 expression in paired metastatic lesions ($\rho = 0.049$, $P = 0.673$). Importantly, only slight agreement existed between IC PD-L1 expression rates between primary and

Table 2
Characteristics of patients treated with immune checkpoint inhibitors

		PD-L1 staining (%)				
		Primary tumor		Metastatic lesion		
Patient	ICI	TC	IC	TC	IC	Response to ICI[a]
1	*Durvalumab*	*0%*	1-5%	>5%	*0%*	PD
2	*Ipilimumab + Nivolumab*	*0%*	*0%*	*0%*	*0%*	PD
3	*Nivolumab*	1-5%	>5%	*0%*	*0%*	PD
4	*Atezolizumab*	*0%*	*0%*	*0%*	*0%*	PD
5	*Atezolizumab*	*0%*	*0%*	*0%*	*0%*	PD
6	*Atezolizumab*	*0%*	1-5%	*0%*	*0%*	PD

[a] Investigator assessed; PD = progressive disease (radiographic or clinical).

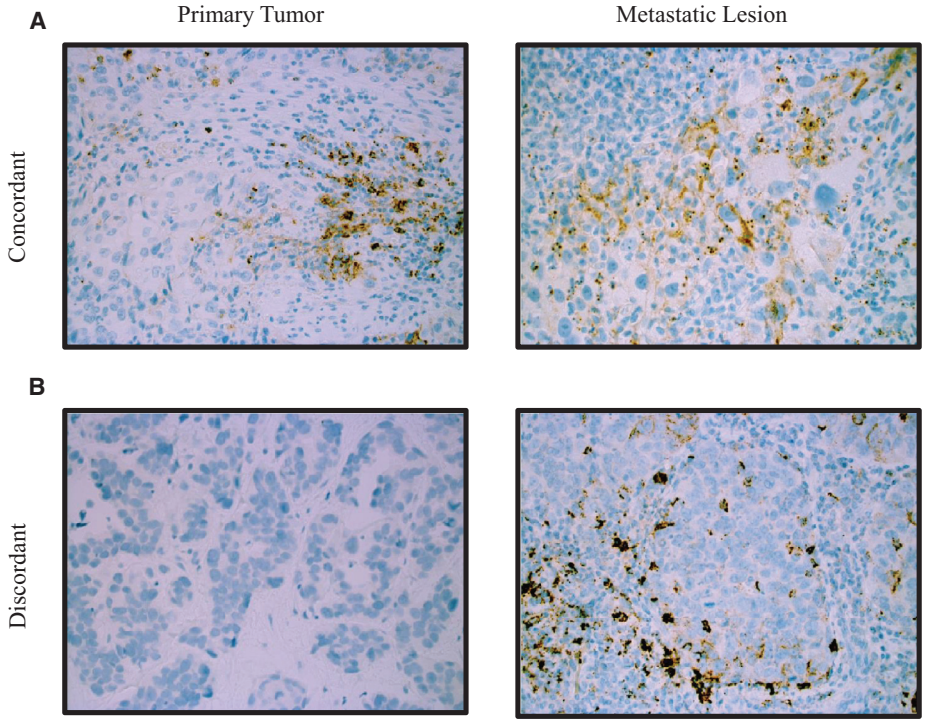

Fig. 1. PD-L1 expression from representative paired primary tumor and metastatic lesion biopsy samples are shown. Example patients with biopsies demonstrating concordant (A) and discordant (B) high IC PD-L1 expression between tumor sites.

metastatic lesions ($\kappa = 0.086$, 95% CI −0.169, 0.341; simple agreement rate: 79.2%). Characteristics of patient biopsies with high IC PD-L1 expression are shown in Table 4. Of the 12 patients with high IC PD-L1 expression observed in the primary tumor, only 2 of these patients were also found to have concordant high IC PD-L1 expression in the metastatic lesion. Similarly, 6 patients demonstrated high IC PD-L1 expression within the metastatic lesion and had no observed PD-L1 IC expression within the paired primary tumor sample (Table 4). Also, in evaluating IC PD-L1 expression as 3 categories in order to discern between low PD-L1 and no PD-L1 expression, almost no agreement was observed ($\kappa = 0.046$, 95% CI −0.115, 0.199; simple agreement rate: 50.6%).

For tumor cell PD-L1 expression in primary tumors, there was low positive correlation with TC PD-L1 expression in paired metastatic lesions ($\rho = 0.439$, $P < 0.001$). However, only slight agreement existed between TC PD-L1 expression rates between primary and metastatic lesions ($\kappa = 0.147$, 95% CI −0.198, 0.491; simple agreement rate: 89.6%).

4. Discussion

The use of ICIs targeting PD-1/PD-L1 has improved patient outcomes in patients with advanced UC; however, identification of highly accurate predictive biomarkers to

Table 3
PD-L1 expression levels in primary and metastatic biopsies

	PD-L1 expression					
	Null		Low		High	
	PD-L1 = 0%		0% < PD-L1 <5%		PD-L1 ≥ 5%	
	n	%	*n*	%	*n*	%
	Primary ($n = 83$)					
TC	74	89.2%	4	4.8%	5	6.0%
IC	47	56.6%	24	28.9%	12	14.5%
	Metastatic ($n = 78$)					
TC	70	89.7%	2	2.6%	6	7.7%
IC	62	79.5%	7	9.0%	9	11.5%

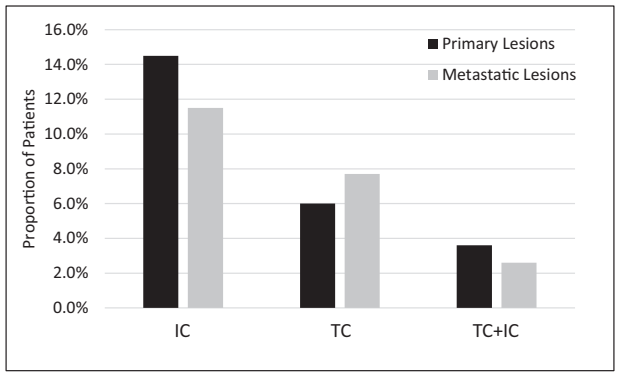

Fig. 2. High PD-L1 expression in immune cell (IC), tumor cell (TC) and co-expression in both (TC+IC) cellular compartments in primary and metastatic lesions.

Table 4
Characteristics of patients with IC high PD-L1 expression

		Primary tumor	Metastatic lesion	
Patient	Time between biopsies (months)	IC PD-L1 expression[a]	Biopsy type[b]	IC PD-L1 expression[a]
1	4.5	High	FNA	Null
2	36.0	High	FNA	High
3	0.2	High	CNB	High
4	0.5	High	SB	Null
5	0.3	High	CNB	Low
6	4.1	High	CNB	Null
7	24.1	High	FNA	Null
8	15.2	High	CNB	Null
9	0.9	High	SB	Null
10	10.2	High	CNB	Null
11	13.7	High	SB	Low
12	14.4	High	CNB	Null
13	7.2	Null	FNA	High
14	3.1	Null	CNB	High
15	31.9	Unknown	SB	High
16	53.5	Null	SB	High
17	75.1	Null	CNB	High
18	26.7	Null	SB	High
19	25.1	Null	SB	High

[a] Null: PD-L1 = 0%, Low: 0% < PD-L1 < 5%, High: PD-L1 ≥ 5%.
[b] FNA = Fine needle aspirate, CNB = Core needle biopsy, SB = Surgical biopsy.

optimize patient selection for these agents remains elusive. Dynamic temporally and spatially heterogeneous intratumoral PD-L1 expression may explain the poor predictive value of this biomarker.

Intratumoral IC PD-L1 expression, characterized by the SP142 antibody, correlated with increased potential for response to the anti-PD-L1 antibody atezolizumab in early phase studies of patients with advanced urothelial cancer [2,13,14]. However, a randomized phase III trial failed to confirm improved survival with the use of atezolizumab compared to chemotherapy in this setting [9]. This trial was designed with a hierarchal primary endpoint that was dependent upon the outcome from the subgroup of patients with high (≥5%) PD-L1 expression in the tumor IC compartment; therefore, any potential misclassification of patients' tumor PD-L1 status may have jeopardized the primary outcome. In this study, the subgroup of patients with high PD-L1 expression failed to show an improvement in survival or objective response rate with atezolizumab compared to chemotherapy. This finding contrasts with the results from a similar randomized phase III trial with the anti-PD1 antibody pembrolizumab in a similar clinical setting [6]. The latter study design did not rely upon the outcome of cohorts defined by PD-L1 status, which potentially explains the difference in trial outcomes and illustrates the potential limitation of utilizing a single assessment of PD-L1 status to guide clinical decision making.

Recent interim analyses of ongoing phase III studies investigating the use of ICIs in previously untreated patients with metastatic UC led regulatory bodies to issue warnings that low tumor PD-L1 scores were associated with inferior survival rates in patients treated with ICI monotherapy [15,16]. Our study findings are not incongruent with these unpublished observations supporting the predictive value of low tumor PD-L1 status, as we observed the greatest discordance of PD-L1 status between lesions with at least one high IC PD-L1 score. Since the observed rates of low or null PD-L1 IC expression exceeded the rates of high IC PD-L1 expression in our data set, the simple agreement rates of low or null PD-L1 expression between primary and metastatic sites was much more likely to be concordant than in patients with at least one biopsy demonstrating high IC PD-L1 expression. We observed discordance in the rates of high intratumoral IC PD-L1 expression between paired primary and metastatic UC lesions ($\kappa = 0.086$, 95% CI −0.169, 0.341). Our findings suggest that identifying high IC PD-L1 tumor status in primary UC tumors may not accurately reflect PD-L1 expression in the metastatic environment and further support the potential limitations of a single characterization of PD-L1 status in patients with metastatic disease.

In this cohort, only 6 patients received therapeutic ICIs. Curiously, primary tumors from 3 of these patients demonstrated IC PD-L1 expression (2 low, 1 high), though none of the patients had detectible IC PD-L1 expression within the biopsied metastatic lesions, and all patients progressed with ICI treatment. Although this sample size is too small to draw firm conclusions, the potential clinical significance of discordant PD-L1 expression between primary and metastatic lesions warrants further study to characterize the predictive value of discordant tumor site IC PD-L1 expression in patients receiving ICI therapeutic agents.

Our study has several limitations, although we believe that they do not vitiate the results of the study which were biologically focused and thus not likely to have been influenced by institutional case selection bias. This analysis was conducted in a retrospective fashion and limited to patient specimens from a single institution. The rates of high IC PD-L1 expression in our cohort were lower than observed in larger prospective clinical trials [2]. It is possible the use of needle biopsies to characterize PD-L1 status in metastatic lesions may under-represent the actual incidence of high PD-L1 expression due to under-sampling; however, the rate of high PD-L1 expression in all metastatic sites in our cohort is similar to a recent case series reporting high PD-L1 expression in nodal metastases from cystectomy specimens [17]. Furthermore, we feel the prospect of deriving a false negative assessment of PD-L1 status in metastatic sites due to sampling technique reinforces the limitations of using PD-L1 expression status for clinical decision making, since the observed discordance of PD-L1 expression between primary and metastatic sites may be due not only to dynamic expression kinetics but also under-sampling. In either case, clinicians are left with an inaccurate understanding of interlesional PD-L1 expression and thus should not rely upon this marker for therapeutic guidance.

The findings we report have significant clinical implications for the use of ICIs in patients with advanced UC. Unlike oncogenic molecular aberrations that drive the metastatic process and are present in both primary and metastatic lesions, PD-L1 expression is induced in response to inflammatory stimuli within the tumor microenvironment. Since therapeutic anti-PD-(L)1 antibodies act through disruption of intratumoral binding of PD-L1 to the PD-1 receptor on tumor infiltrating lymphocyte (TILs), accurate characterization of the intratumoral PD-L1 expression level contemporaneous with the use of therapeutic anti-PD-(L)1 agents is of paramount importance.

Our findings of discordant high IC PD-L1 expression between primary and metastatic lesions in patients with advanced UC support prior observations that PD-L1 expression is dynamic and establishes that PD-L1 levels in primary tumors may not predict for high PD-L1 status in metastatic lesions with accuracy. For this reason, fresh biopsies of metastatic sites should be considered when characterization of intratumoral PD-L1 expression status is planned to guide therapeutic use of ICIs in patients with advanced UC.

5. Conclusion

The results from our analysis provide an explanation for the poor predictive value of tumor PD-L1 status that has been observed in prior studies. Moreover, our results also suggest that the predictive value of PD-L1 expression may be improved when limited to fresh biopsies of metastatic lesions. The potential predictive impact of discordant intratumoral PD-L1 expression between primary and metastatic lesions warrants further investigation, and it may be prudent to obtain fresh biopsies of metastatic lesions for trials that test novel ICIs in view of the burgeoning expense of this treatment domain.

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