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Association of PD-L1 expression on tumor-infiltrating mononuclear cells and overall survival in patients with urothelial carcinoma

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Background: Programmed death-1 (PD-1) receptor/PD-1 ligand (PD-L1) pathway negatively regulates T-cell-mediated responses. The prognostic impact of PD-L1 expression needs to be defined in urothelial carcinoma (UC).

Patients and methods: Formalin-fixed paraffin-embedded tumor samples from 160 patients with UC were retrieved. PD-L1 expression was evaluated by immunohistochemistry using a mouse monoclonal anti-PD-L1 antibody (405.9A11). PD-L1 positivity on tumor cell membrane was defined as ≥5% of tumor cell membrane staining. The extent of tumor-infiltrating mononuclear cells (TIMCs) as well as PD-L1 expression on TIMCs was scored from 0 to 4. A score of 2, 3, or 4 was considered PD-L1-positive. Clinico-pathological variables were documented. The Cox regression model was used to assess the association of PD-L1 expression with overall survival (OS) in patients who developed metastases.

Results: TIMCs were present in 143 of the 160 patient samples. Out of 160 samples, 32 (20%) had positive PD-L1 expression in tumor cell membrane. Out of 143 samples with TIMCs, 58 (40%) had positive PD-L1 expression in TIMCs. Smoking history, prior BCG use and chromosome 9 loss did not correlate with PD-L1 expression in either tumor cell membrane or TIMCs. PD-L1 positivity was not different between non-invasive or invasive UC. In patients who developed metastases (M1) and were treated with systemic therapy ($n = 100$), PD-L1 positivity on tumor cell membrane was seen in 14% of patients and did not correlate with OS ($P = 0.45$). Out of 89 M1 patients who had evaluable PD-L1 on TIMCs, PD-L1 expression was seen in 33% of patients and was significantly associated with longer OS on multivariate analysis ($P = 0.0007$).

Conclusion: PD-L1 is widely expressed in tumor cell membrane and TIMCs in UC. PD-L1 in tumor cells was not predictive of OS. However, positive PD-L1 expression in TIMCs was significantly associated with longer survival in those patients who developed metastases.

Key words: urothelial carcinoma, PD-1, PD-L1, immunotherapy, prognosis, overall survival

introduction

Metastatic urothelial carcinoma (UC) remains largely incurable and the mortality rates have not changed substantially over the past two decades [1] with the median overall survival (OS) of 14–15 months seen with the use of cisplatin-based chemotherapy. Targeted therapies have produced limited clinical activity and when responses occur, they are usually transient.

In the metastatic setting, clinical factors, such as performance status, visceral metastases, hemoglobin level, or liver metastases, have been used to predict clinical outcome in both first- and second-line [2]. Although The Cancer Genome Atlas (TCGA) has provided insights on the genomic profile of urothelial tumors, potentially opening new avenues for prognosis and therapy [3], its clinical application is still premature.

Non-muscle invasive UC (NMIUC) has been historically recognized as an immunogenic tumor [4]. Tumor-infiltrating mononuclear cells (TIMCs) appear to be involved in the local anti-tumor responses [5] when immunotherapy with Bacillus Calmette–Guerin (BCG) is used, preventing local recurrences and tumor progression in High-grade/ Carcinoma *in situ* (CIS) non-invasive disease [6].

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Recently, blocking immune checkpoint molecules with monoclonal antibodies has emerged as a promising strategy in advanced urothelial cancer treatment [7]. The interaction of programmed cell death-1 (PD-1) on T-cells with its ligand PD-L1 (B7-H1) on tumor cells and immune cells limits T-cell-mediated immune responses [8]. Therefore, it is hypothesized that the PD-1/PD-L1 signaling pathway plays an important role in immune system escape by the tumor [9].

PD-L1 has been shown to be expressed in several malignancies, including UC [10–12]. In addition, it has been suggested that higher PD-L1 expression in tumor cell membrane or tumor-infiltrating immune cells is associated with different clinico-pathological features and clinical outcome in multiple different tumor types [13]. However, the prognostic impact of this biomarker has not been established across different tumor types.

In this study, we sought to characterize PD-L1 expression and its correlation with clinico-pathological features as well as OS in a large series of patients with UC as well as OS including patients who developed metastatic disease and were subsequently treated with platinum-based chemotherapy (M1).

methods

patients and samples

A total of 160 patients with UC from two institutions, Dana-Farber Cancer Institute, Boston, USA, and Hospital del Mar, Barcelona, Spain, were identified. Formalin-fixed paraffin-embedded (FFPE) blocks from radical cystectomy or transurethral resection of bladder tumors (TURB) were retrieved from the Department of Pathology. Baseline clinico-pathological characteristics including smoking history, prior BCG treatment, TNM stage at diagnosis, copy number variation (CNV) at chromosome 9, prognostic factors in patients with metastatic disease, and clinical follow-up data were retrospectively collected from our database. Institutional Review Board approval was obtained at both institutions before data acquisition and tumor staining.

immunohistochemistry

PD-L1 expression was evaluated in a tissue micro array (TMA) by immunohistochemistry using a mouse monoclonal anti-PD-L1 antibody (405.9A11) developed in Dr Gordon Freeman's laboratory (Figure 1). This antibody attaches to the PD-L1 ligand in the cytoplasmic domain, providing a clearer stain on the membrane of cells. The immunohistochemical assay is described in supplementary material S1, available at *Annals of Oncology* online.

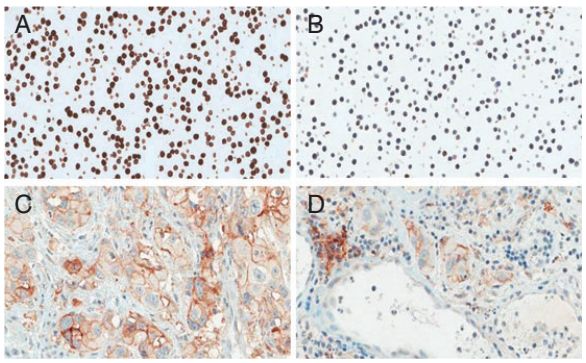

Figure 1. PD-L1 expression in FFPE samples stained with anti-PD-L1 antibody (405.9A11). Positive and negative controls for PD-L1 expression are presented in (A) and (B), respectively. Positive membranous staining is present in tumor cells in (C). In (D), tumor cells are negative, and tumor-infiltrating mononuclear cells are positive for PD-L1.

scoring of PD-L1 expression

For each sample, the TIMCs infiltrate and the membranous expression of PD-L1 in tumor cells or TIMCs were determined by two independent pathologists (SS and MC) blinded to clinical data.

PD-L1 tumor positivity was defined by the presence of ≥5% of tumor cells with membrane staining.

The extent of TIMCs was assessed in hematoxylin and eosin-stained slides and recorded as absent (0), focal (1), mild (2), moderate (3), and severe (4) with score 0 or 1 considered negative. The extent of PD-L1-positive TIMCs was also assessed using the same scoring scale (0–4) and samples with a score of 2–4 were considered PD-L1-positive. Seventeen samples were non-evaluable for TIMCs extent or PD-L1 staining in TIMCs.

recurrent copy number alterations

Array comparative genomic hybridization was carried out on 71 samples as previously described (supplementary material S2, available at *Annals of Oncology* online) [14].

statistical analysis

The primary objective of this study was to correlate the levels of PD-L1 expression with OS in patients with metastatic disease and who received first-line platinum-based chemotherapy. We also carried out an exploratory analysis to correlate PD-L1 expression and clinico-pathological features. Patient clinico-pathological characteristics were summarized descriptively. OS was defined as the time period between the date of the first chemotherapy application and the date of death, or censored on the date of last follow-up. Smoking history was only available for 74 patients from one institution. The time point for current smokers was at the time of cystectomy. Current and former smokers were combined into the smokers' category for analysis. Fisher's exact tests were used to assess the associations of smoking status, use of BCG with PD-L1 positivity in tumor cells, and TIMCs. The Cox regression model was used to assess the association of PD-L1 positivity and TIMCs with OS in both univariate and multivariate analysis adjusting for Eastern Cooperative Oncology Group (ECOG) performance status and whether patients had visceral disease or not. Hazard ratio and 95% confidence interval were also listed.

All statistical computations were carried out using SAS v.9.2 (SAS Institute Inc., Cary, NC) and a *P*-value (two-sided) of <0.05 was considered statistically significant.

results

Patient and tumor characteristics are described in Table 1. One hundred and sixty patients had tumor samples and adequate clinical data to be evaluated for PD-L1 expression in tumor cells. Among them, 143 had TIMCs in tumor samples and were evaluable for PD-L1 expression in TIMCs. Out of the 160 patients, 100 patients developed metastatic disease and received treatment (M1) (Figure 2). Of the 100 M1 patients, 89 had TIMCs in their tumor sample and were evaluable for PD-L1 expression in TIMCs ($M1^{+TIMCs}$) (supplementary Figure S3, available at *Annals of Oncology* online). Of note, patients were not treated with immunotherapy during the course of metastatic disease.

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Table 1. Patient characteristics [patient characteristics at the time of initial diagnosis (Stage) and at the time of starting treatment for metastatic disease]

Clinico-pathological features	All cohort ($n = 160$), n (%)	Patients with metastatic disease ($n = 100$), n (%)
Staging		
Non-invasive tumors	23 (14.4)	
T2	60 (37.5)	
T3	57 (35.7)	
T4	16 (10)	
Not available	4 (2.5)	
Visceral disease		
Yes		47 (47%)
No		53 (53%)
ECOG PS		
0		35 (35%)
1		58 (58%)
2 or 3		7 (7%)
PD-L1 expression on tumor cell membrane		
Negative (<5%)	128 (80)	86 (86%)
Positive (≥5%)	32 (20)	14 (14%)
Extent of TIMCs		
Absent	3 (1.9)	2 (2%)
Focal	43 (26.9)	32 (32%)
Mild	50 (31.2)	28 (28%)
Moderate	34 (21.2)	21 (21%)
Severe	13 (8.1)	6 (6%)
Not available	17 (10.6)	11 (11%)
PD-L1 expression in TIMC[a]		
Absent	34 (23.8)	26 (29.2%)
Focal	51 (35.6)	30 (33.7%)
Mild	42 (29.4)	23 (25.8%)
Moderate	13 (9.1)	8 (9%)
Severe	3 (2.1)	2 (2.3%)

[a]Patients with absent TIMCs were not stained for PD-L1 in TIMCs ($n = 143$ and $n = 89$ in the metastatic cohort).

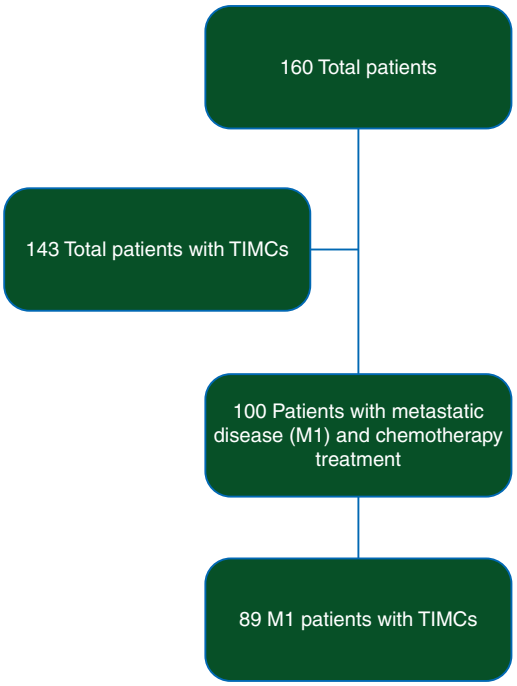

Figure 2. Study design.

PD-L1 expression on tumor cell membrane or TIMCs

Overall, PD-L1 expression in tumor cells was negative in 128 patients (80%) and positive in 32 patients (20%). In the M1 subset ($n = 100$), PD-L1 expression was negative in 86 (86%) and positive in 14 patients (14%) (Table 1).

Seventeen patients (10.6%) were not evaluable for TIMCs and were not included in the PD-L1 expression analysis. Out of the 143 patients with TIMCs present, PD-L1 expression in TIMCs was scored as absent (0) in 34 patients (23.8%), focal (1) in 51 patients (35.6%), mild (2) in 42 patients (29.3%), moderate (3) in 13 patients (9.1%), and severe (4) in 3 patients (2.1%). PD-L1 expression in TIMCs was considered negative (0 or 1) in 85 of 143 patients (63%) and positive (2–4) in 58 patients (37%).

Among the $M1^{+TIMCs}$ subset ($n = 89$), PD-L1 expression in TIMCs were scored as absent (0) in 25 patients (28.1%), focal (1) in 30 patients (33.7%), mild (2) in 23 patients (25.8%), moderate (3) in 8 patients (9.0%), and severe (4) in 2 patients (2.2%). PD-L1 in TIMCs expression was considered negative (0–1) in 56 of 89 patients (63%) and positive (2–4) in 33 of 89 patients (37.1%) (Table 1).

association of PD-L1 expression and OS in patients with metastatic disease

The median time from cystectomy to the development of metastatic disease in these patients was 20 months. All received first-line treatment with platinum-based chemotherapy. The median follow-up was 25 months for M1 patients. In the $M1^{+TIMCs}$ subset, the presence (score of 2–4) versus the absence (score of 0–1) of TIMCs infiltrate was associated with longer OS (11 versus 18 months, $P = 0.02$). Positive PD-L1 expression (score of 2–4) in TIMCs was significantly associated with longer OS (12 versus 23 months) in both univariate ($P = 0.04$) and multivariable analysis ($P = 0.0007$) (adjusting for ECOG status and visceral disease) (Table 2; Figure 3). PD-L1 expression in tumor cell membrane was not associated with survival ($P = 0.45$).

association of PD-L1 expression and staging

Overall, 23 patients had NMIUC (T0 and T1) and 133 patients had high-grade muscle invasive UC (≥T2). Staging was not available in four patients. For muscle-invasive UC, TNM stages II, III, and IV at diagnosis were found in 60, 57, and 16 patients, respectively. There were no statistically significant differences in PD-L1 expression on TIMCs or on tumor cells between non-invasive or invasive bladder cancer (41.8% versus 30%; $P = 0.53$; 8.7% versus 21.8%; $P = 0.25$) (Table 3).

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Table 2. Association of PD-L1 expression and OS in patients who develop metastatic disease

	n	Deaths	Median OS and 95% CI	HR and 95% CI (univariate)	*P*-value	HR and 95% CI (multivariable)	*P*-value
PD-L1 expression in TIMCs							
Absent, focal	56	37	12 (9, 16)	1.87 (1.02, 3.47)	0.04	3.19 (1.64, 6.22)	0.0007
Mild, moderate, severe	33	14	23 (12, not reached)	1 (reference)		1 (reference)	
PD-L1 expression in tumor cell membrane							
<5%	86	52	14 (11, 18)	1.42 (0.57, 3.55)	0.45	1.72 (0.67, 4.40)	0.26
≥5%	14	5	Not reached	1 (reference)		1 (reference)	

The extent of PD-L1-positive TIMCs was also assessed using a scale (0–4): absent (0), focal (1), mild (2), moderate (3), and severe (4) with score 0 or 1 considered negative. Samples with a score of 2–4 were considered PD-L1-positive. PD-L1 tumor positivity was defined by the presence of ≥5% of tumor cells (membrane staining).

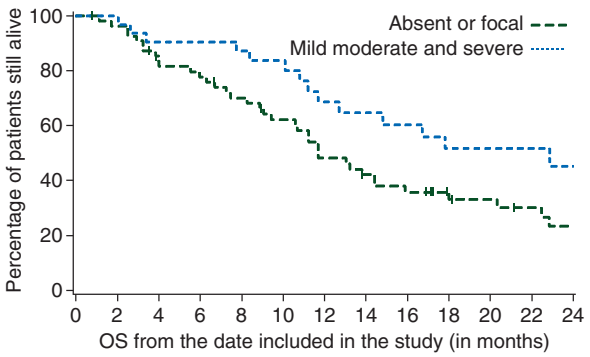

Figure 3. PD-L1 expression in TIMC and OS ($n = 89$). Correlation of PD-L1 expression (Absent or Focal vs. Mild, Moderate or Severe) and OS (months) in patients with metastatic disease.

association of PD-L1 expression and BCG treatment

Information regarding the prior use of BCG was available in a subset of 69 out of the total 160 patients (43.1%). Out of the 69 patients, 17 patients (23%) were treated with at least one BCG instillation and 52 (70%) did not receive any BCG therapy (supplementary Table S4, available at *Annals of Oncology* online). All patients who underwent BCG treatment had their last treatment within 1 year of cystectomy. There was no correlation with prior adjuvant BCG exposure and PD-L1 expression in tumor cell membrane or TIMCs ($P = 0.12$ and 0.99, respectively) (Table 3).

association of PD-L1 expression and smoking status

In a subset of 73 patients, smoking history was available: 9 (12%) were active smokers, 46 (62%) were former smokers, and 18 (24%) had never smoked. Smoking history was not associated with PD-L1 expression in tumor cell membrane or TIMCs ($P = 0.86$ and 0.99, respectively) (Table 3).

association of PD-L1 expression and CNV at chromosome 9

CNV data were available for 71 of the 100 M1 patients. CNV at the PD-L1 locus (9p24) was not significant in terms of standard GISTIC parameters. We also looked for the correlation of loss of all of chromosome 9 defined as having a loss in all four loci (9p11.2, 9p21.3, 9q34.3, and 9p23) that were shown to be significant based on GISTIC cut-offs. Chromosome 9 loss was identified in five patients. In our analysis, loss of chromosome 9 did not correlate with PD-L1 expression in tumor cell membrane nor TIMCs ($P > 0.99$).

discussion

Higher PD-L1 expression in tumor cells has been correlated with both favorable and unfavorable outcome in several malignancies [15–17]. In UC, PD-L1 expression on tumor cells has been associated with high grade, stage, and worse outcome in some reports. However, the overall impact of PD-L1 expression on prognosis remains controversial in UC [18]. To our knowledge, this is the first study to demonstrate that PD-L1 expression in TIMCs is correlated with improved OS in patients with UC who developed metastatic disease and were homogeneously treated with platinum-based chemotherapy. PD-L1 expression can occur on the tumor cell or on TIMCs. When T-cells recognize antigen and become activated, they express cytokines such as interferon-γ which in turn can induce PD-L1 expression on surrounding immune and tumor cells. The expression of PD-L1 on TIMCs is consistent with the idea that these intratumoral lymphocytes are tumor antigen-specific and responding to the tumor.

The correlation between PD-L1 expression in tumors cells and worse clinical outcome (higher risk of recurrence and shorter OS) in 65 patients with UC was first reported by Nakanishi et al. [19]. In addition, levels of PD-L1 expression were found to be high in inflammatory cells in 13 randomly selected patients.

Recently, Boorjian et al. [20] reported that higher PD-L1 expression in tumor cells was associated with the presence of advanced disease in patients with UC and also correlated with shorter OS in patients with organ-confined UC after radical cystectomy. In another series, which evaluated 302 UC patients, PD-L1 expression in tumor cell membrane was not correlated with recurrence, cancer-specific, or OS. However, in patients with organ-confined UC, higher PD-L1 expression was associated with an increased risk of death ($P = 0.02$) [21].

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Table 3. Association of PD-L1 expression and clinico-pathological features

Staging	PD-L1 expression in tumor cell membrane (*n*)		*P*-value	PD-L1 expression TIMCs (*n*)		*P*-value
	Negative	Positive		Negative	Positive	
Association of PD-L1 expression with staging at the time of radical cystectomy						
Non-invasive tumors	21	2	0.25	7	3	0.53
Muscle-invasive tumors	104	29		75	54	
Clinical features	PD-L1 expression in tumor cell (*n*)		*P*-value	D-L1 expression in TIMCs (n)		*P*-value
	<5%	≥5%		Positive	Negative	
Association of PD-L1 expression with BCG use or smoking history						
Prior BCG						
No	35	17	0.12	21	27	0.99
Yes	15	2		7	8	
Smoking history						
Active smokers	7	2	0.86	4	5	0.99
Former smokers	32	14		19	23	
Never smoked	14	4		7	9	

In the first part, the association between PD-L1 expression and staging (non-invasive tumors versus muscle invasive tumors) at the time of radical cystectomy is presented. The association between PD-L1 expression and BCG use or smoking history is presented in the second part.

Based on the potential predictive role recently described for PD-L1 expression on immune cells in patients receiving checkpoint inhibitors in UC, attention has now switched toward the analysis of PD-L1 expression in immune cells instead of tumors cells. In our study, no association between tumor cell PD-L1 expression and clinical outcome was found. However, in addition to seeing a correlation with the presence of TIMCs and survival (supplementary Table S5, available at *Annals of Oncology* online) as previously reported [22], higher PD-L1 expression in TIMCs was statistically correlated with longer OS in the multivariate analysis in patients who developed metastatic disease and subsequently received chemotherapy. Moreover, we found that patients with TIMCs were more likely to have higher PD-L1 expression on tumor cell membrane (supplementary Table S5, available at *Annals of Oncology* online). It is still an open question how these exploratory correlations will impact patient's prognosis. Therefore, further prospective studies are warranted.

Recently, preliminary results from a phase I study to evaluate the efficacy of MPDL3280A, an anti-PD-L1 mAb, in patients with advanced UC were presented. The overall response rate in those patients who express PD-L1 (score 2/3) in immune cells was 52% versus 14% in those who were considered PD-L1 negative (score 0/1) [23]. Interestingly, most of the responses were ongoing at the cut-off time of analysis. These results support the rationale of PD-L1 expression in immune cells as a potential predictive biomarker for immunotherapies in UC. The fact that PD-L1-negative patients had response to anti-PD-L1 therapy highlights the need for better biomarkers for response to agents targeting this pathway.

The success of BCG in NMIUC has highlighted UC as an immune sensitive disease and the role of immune checkpoints like PD-1/PD-L1 in patients failing BCG is under evaluation [24]. Inman et al. evaluated PD-L1 expression in tumor cells in 280 UC of the bladder. In this study, they reported that PD-L1 expression was associated with high-grade tumors and tumor infiltration by mononuclear cells ($P = 0.009$ and 0.004, respectively). Interestingly, higher PD-L1 expression was seen in 11 out of 12 patients who had BCG-induced pathological inflammatory changes and failed BCG treatment suggesting that tumor cells might be protected from attack by immune cells through immune checkpoints, like PD-L1 [12]. Notably, in our analysis, PD-L1 expression was not correlated with prior use of BCG.

Smoking history is the most important risk factor for bladder cancer and highly complex mutational profiles have been described for both, smokers and non-smokers [25]. Responses to agents targeting PD-1/PD-L1 pathway have been described to be more robust in smokers than non-smokers in patients with lung cancer [26]. In our study, no correlation between PD-L1 expression and smoking history was observed in an exploratory analysis.

PD-L1 gene is located on chromosome 9p24. Green et al. [27] demonstrated that *PD-L1* amplification was associated with significantly higher PD-L1 expression Hodgkin's lymphomas cells. UC is associated with multiple somatic CNVs, including frequent chromosome 9 loss [28]. Therefore, we speculated that CNV on chromosome 9 may correlate with PD-L1 expression in UC. No correlation was found between copy number changes and PD-L1 expression.

Our study has limitations. First, although we have analyzed a large cohort, this is a retrospective analysis and there is a potential for selection bias. Secondly, clinical data were limited for some of our patients, resulting in lack of power for the analyses of smoking status and BCG due to the small sample size. Thirdly, whether the presence of TIMCs correlates with PD-L1 expression, as shown in our study, should be a focus of further prospective investigations. In addition, it is difficult to compare the results from our study with previous studies due to different methodologies used to evaluate PD-L1 expression in tumor cell membrane and TIMCs. Finally, even though we tried to minimize tumor

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heterogeneity using three different cores for each patient in the TMAs, heterogeneity of PD-L1 expression within the tumor may limit the ability for an adequate assessment.

In conclusion, PD-L1 is widely expressed in tumor cell membrane and TIMCs in UC. No significant correlation was found with prior BCG treatment, smoking history, staging, or chromosome 9 copy number changes. However, PD-L1 positivity in TIMCs and not in tumor cells was significantly associated with better OS in those patients who subsequently developed metastatic disease and received platinum-based chemotherapy. Further prospective studies should be carried out in order to define the role of PD-L1 expression in immune cells as a predictive and prognostic biomarker in UC.

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disclosure

APF, MC, LW, SAM, JJL, and SS declare no conflict of interest for this study. GJF: significant financial interest from DFCI administered patent royalties from BMS, Merck, Roche/Genentech, EMD-Serono, Amplimmune, Boehringer-Mannheim, CoStim. Scientific founder and scientific board member of CoStim. TKC: consultancy: Pfizer, Novartis; advisory board: Pfizer, Novartis, Aveo, GlaxoSmithKline, Exelixis; research: Pfizer; No Speakers bureau. FSH: advisory board: Genentech. JB: advisory board: Merck, Genentech, OncoGenex.

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Immunohistochemical Staining

The immunohistochemical assay was validated using FFPE cell line controls known to be positive or negative for PD-L1 expression by flow cytometry. A tissue microarray (TMA) was constructed with all UC samples. Each tumor sample in the TMA had three cores punched per tumor sample to represent tumor heterogeneity. The TMA also included normal urothelium tissue cores. The TMA was stained with the anti-PD-L1 antibody 405.9A11 (final concentration of 3.25 ug/ml) on a Benchmark XT autostainer (Ventana Medical System, Tucson, AZ) using standard antigen retrieval (CC1 buffer, pH8.0, #950-124, Ventana). UltraView Universal DAB Detection kit (#760-500, Ventana) was used according to the manufacturer's instruction. Counterstaining was performed as part of the automated staining protocol using hematoxylin (#760-2021, Ventana). After staining, slides were washed in soapy water and distilled water, dehydrated in graded alcohol and xylene, mounted and cover slipped.

Recurrent copy number alterations

Array comparative genomic hybridization was performed on 71 samples. Normalized copy number data was segmented using GLAD with default parameters available in GenePattern version 3.3.3. Genomic Identification of Significant Targets in Cancer (GISTIC) software[20] (v2.0.12) was then used to identify regions of the genome that were significantly gained or deleted across a set of samples. The software estimated false discovery rates (q-values), as well as potential targets (drivers) of the aberrations. Copy numbers of significantly gained or deleted regions (q-value < 0.25) were dichotomized based on the standard GISTIC cutoffs for amplifications or deletion (log base 2 ratio > 0.9 or < -1.3, respectively). For this analysis, only GISTIC regions on chromosome 9 were analyzed.

Association of PD-L1 tumor expression and TIMCs for the entire cohort (N=153)

	PD-L1 tumor expression		P-value
TIMCs	<5%	≥5%	0.02
Absent (0,1)	45	5	
Present (2,3,4)	75	28	

Association of PD-L1 tumor expression cell and TIMCs for patients with metastatic disease (N=89)

	PD-L1 tumor expression		P-value
TIMCs	<5%	≥5%	0.07
Absent (0,1)	32	2	
Present (2,3,4)	43	12	

Association between presence of TIMCs and OS

TIMCs presence	N	Deaths	Median OS	P-value
Absent (0,1)	34	23	11	0.02
Present (2,3,4)	55	28	18	

Table 2: Smoking history and use of BCG

Clinico-pathological feature		Total 74 UC *n*(%)
Prior BCG use	Yes	17 (23)
	No	52 (70)
	Unknown	5 (7)
Smoking History*	Active smokers	9 (12)
	Former Smokers	46 (62)
	Never Smoked	18 (24)
	Unknown	1 (1)

*Current and former smokers were combined into the smokers' category for analysis.