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Correlation between PD-L1 expression of the tumour cells and lymphocytes infiltration in the invasive front of urothelial carcinoma

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ABSTRACT

Purpose Programmed cell death-ligand 1 (PD-L1) as a cell surface glycoprotein can inhibit T cell function when binding to its receptor, PD-1. The newly developed therapy of targeting PD-1/PD-L1 signal pathway has shown great promise for the treatment of non-small cell lung cancer as well as melanoma. Approved by Food and Drug Administration, atezolizumab has become the first new drug to treat advanced bladder cancer. The aim of this study is to evaluate whether PD-L1 is associated with the lymphocytes infiltration in the tumour microenvironment and to assess the prognostic value of PD-L1 expression.

Materials and methods Among 96 invasive bladder urothelial carcinomas, some were used to construct tissue-microarrays, and some cases with shallow infiltration or large heterogeneity were performed, respectively, for the following work. By means of immunohistochemistry and HE, PD-L1 expression and immune cell infiltration in the invasive front of urothelial carcinoma were analysed.

Results We find that PD-L1 expression in tumour cells and lymphocytes are significantly associated with more tumour infiltrating lymphocytes (TILs) and more T cells. The integrated TILs, T-PD-L1 and I-PD-L1 are not significantly correlated with the overall survival (OS) of patients. However, the combination of T-PD-L1 and TILs, T-PD-L1 and I-PD-L1 is significantly correlated with the OS of patients. The T-PD-L1 (–)/TIL (–) group show the best prognosis and the T-PD-L1 (+)/I-PD-L1 (–) group show the worst prognosis. Furthermore, a multivariate analysis reveal that PD-L1 expression of lymphocytes is an independent prognostic factor for OS of patients.

Conclusions Our study reveal that PD-L1 of tumour cells are associated with the corresponding T cells infiltration and that the combination of T-PD-L1 and I-PD-L1, T-PD-L1 and TILs could be a relevant marker for the determination of the prognostic role of patients with the urothelial carcinoma.

WHAT IS ALREADY KNOWN ON THIS TOPIC

⇒ The newly developed therapy of targeting PD-1/PD-L1 signal pathway has shown great promise for the treatment of many cancers. However, there are not appropriate biomarkers for PD-1/PD-L1 inhibitor therapy and positive cut-off values for PD-L1 are still not standardised. So, we tested different biomarkers and combinations, cut-off values in this study.

WHAT THIS STUDY ADDS

⇒ Our study reveal that PD-L1 of tumour cells are associated with the corresponding T cells infiltration and that the combination of T-PD-L1 and I-PD-L1, T-PD-L1 and tumour infiltrating lymphocytes (TILs) could be a relevant marker for the determination of the prognostic role of patients with the urothelial carcinoma.

HOW THIS STUDY MIGHT AFFECT RESEARCH, PRACTICE OR POLICY

⇒ TILs, the combination of T-PD-L1 and I-PD-L1, T-PD-L1 and TILs may be appropriate biomarkers for PD-1/PD-L1 inhibitor therapy and 5% may be a good positive cut-off values for PD-L1.

INTRODUCTION

Urinary bladder cancer is the second most common genitourinary tumour.[1] Approximately 75% of patients with urothelial carcinoma (UC) were non-invasive and had a favourable prognosis following transurethral bladder tumer resection and intravesical chemotherapy or immunotherapy. However, the remaining 25% diagnosed with invasive UCs often have a poor prognosis despite systemic therapy.[2] A new therapy targeting of PD-1/PD-L1 signal pathway has been paid more attention in recent years.

Programmed cell death-ligand 1 (PD-L1) as a member of the B7 family is considered a coinhibitory molecule expressed on activated T cells, B cells, dendritic cells, and tumour cells as well.[3] The expression of PD-L1 on tumour cells is closely related to tumour development and poor prognosis. Therapy targeting PD-L1 has been a new approach compared with conventional treatment such as chemotherapy and radiotherapy.[4] PD-L1/PD-1 blockade therapy has shown unprecedented success in a wide variety of cancers, including melanoma,[5] non-small-cell lung cancer[6] and so on. Although PD-L1 immunohistochemistry is approved by the Food and Drug Administration (FDA) for selecting patients treated by Keytruda (pembrolizumab), the expression of PD-L1 is influenced by many factors such as immunotherapy, chemotherapy, and epidermal growth factor receptor-tyrosine kinase inhibitor (EGFR-TKI). Furthermore, the scoring cut-off has no standardisation.[7] So, it is urgent to find an effective predictor for response to PD-1/PD-L1 blockades and to decide an appropriate cut-off. Since the BCG vaccine was officially approved

by FDA for the bladder cancer in 1990, immunotherapy for bladder cancer had a long history.[8] Approved by FDA in May 2016, atezolizumab has become the first new drug for the treatment of advanced bladder cancer.

Many studies have indicated that tumour-infiltrating lymphocytes (TILs) have a prognostic value and a higher 'immunoscore' is correlated with longer disease-free survival and overall survival (OS) among various cancers.[9] Among the different cancers, higher infiltration of CD3+, CD8+, CD20+ is linked to a favourable prognosis[10 11]; however, a few studies have implicated that higher CD4+T cell density is associated with poor prognosis in non-muscle-invasive bladder cancer contrary to the non-small cell lung cancers.[12 13] Treatment by natural killer (NK) cells is a useful method, especially regarding to haematological malignancies. Yet until now, there are few studies about the infiltration of CD56+NK cells in the UC of the bladder.[14]

PD-L1 expression in tumour cells is significantly associated with local immune cell infiltration. But the correlation between the PD-L1 expression and the different subgroups of infiltrating immune cells in the UCs is unclear. Herein, we used 96 cases of patients with invasive UCs to analyse PD-L1 expression in the UCs and its association with clinical-pathological characteristics and the different kinds of lymphocytes.

MATERIAL AND METHOD

Patients, specimens and tissue-microarray

In this retrospective analysis, formalin-fixed and parrffin embedded tissue specimens of patients with invasive UCs from the First Affiliated Hospital of Wenzhou Medical University from 2010 to 2018 were represented in tissue-microarrays (TMA) format in this study. Each TMA spot included at least 50% of tumour cells. The infiltrating front of UC were selceted into TMA. At least three cores were taken to represent a case. In some cases with shallow infiltration or large heterogeneity, the following work was carried out respectively. The following information was collected: patients' personal information (ie, age, sex), histological characteristics (ie, invasion depth), and the dates of death. OS is defined as the date of UC surgery to the date of death or the last clinical follow-up before 29 March 2018.

Immunohistochemistry

The method of immunohistochemistry used in the current study and its validation has been previously described elsewhere.[15] In brief, the 3.5 µm thick, formalin-fixed, paraffin-embedded tissue-sections were dewaxed in xylene and rehydrated using graded alcohol and then blocked for endogenous peroxidase by incubating 20 min in 0.3% H_2O_2. Slides were then washed with 0.1% TBS three times, incubated with 1% diluted normal goat serum in TBST for 20 min, and incubated overnight at 4°C with the appropriate primary antibodies. And then slides were washed four times in TBS, incubated with biotinylated secondary antibody for 30 min at 37°C. Lastly, the result was revealed by incubating the slides with a DAB+H_2O_2 prepared in distilled water. The primary antibodies were from the following: anti-PD-L1(28-8) (ab-205921, Abcam, UK), anti-CD3 (ab-16669, Abcam, UK), anti-CD4 (ab-133616, Abcam, UK), anti-CD8 (ab-4055, Abcam, UK), anti-CD20 (ab-78237, Abcam, UK) and anti-CD56 (ab-28384, Abcam, UK).

Immunohistochemical analysis: PD-L1 expression

Two pathologists independently evaluated all stained slides for PD-L1 membrane staining. Here, we defined PD-L1 expression of 1%, 5%, 10%, 50% tumour cells as T-PD-L1 positive cut-off values and PD-L1 expression of 1% lymphocytes as I-PD-L1 positive cut-off value. PD-L1 staining on tumour-associated immune cells were conducted only in the infiltrating front of UC (also the area selected to TMA) without the external part of tumour and normal part. Then, the percentage of PD-L1 expression of lymphocytes in this region was calculated (the density of positive lymphocytes/the density of total lymphocytes in this region).

Evaluation of TILs

The TILs were performed in H&E-stained TMA preparations independently by two pathologists with five independent, 0.031 mm^2 areas in the tumour invasive front. Here, we defined 10% as the cut-off value of presence of TILs.

Immunohistochemical analysis: different kinds of TILs

For each specimen, five independent, 0.031 mm^2 areas in the tumour invasive front were selected and digitally imaged with an OLYMPUS camera. Immune cells were counted manually from the digital images displayed on a monitor. All counts were repeated three times by the same investigator, and the average of the repeat counts was used for statistical analyses. We determined the average value of five 0.031 mm^2 areas examined for each slide as the number of that slide. The observers were not informed of the results of the patients.

Statistical analysis

Patients' clinicopathological characteristics were compared using a χ^2 test for categorical variables. Using the Mann-Whitney U test, we compared the number of immune cells in the PD-L1 (+) and the PD-L1 (-) groups. OS were compared using Kaplan-Meier estimates and statistical significance was determined using the log-rank test. A multivariate Cox proportional hazards model was built. A value of $p<0.05$ was statistically significant. Statistical analyses were performed using SPSS software (V.22.0; SPSS).

RESULT

Patient clinicopathological characteristics

A total of 96 patients with invasive UCs (pT1-pT4) was enrolled (table 1), with 87 patients (90.6%) being male and 9 (9.4%) being female. Patients' mean age was 75 years old. 49 patients (51.1%) were over 75 years old and 47 (49.0%) were less than or equal to 75. Only 12 patients (12.5%) had the UC with divergent differentiation and 84 patients (87.5%) had pure UC. There were 15 patients (15.63%) with UCs in pT1, 65 patients (67.71%) in pT2, and 16 patients (16.7%) in pT3-pT4. Most patients (86.46%) did not have lymphatic metastasis (pN0), and only 13 patients (13.54%) had (pNx). According to standards, 71 patients (74.0%) had stage I–II, and 25 patients (26.0%) had stage III–IV disease, respectively.

Prognostic significance of T-PD-L1 and I-PD-L1 expression in UCs

PD-L1 was commonly expressed at the membrane of cancer cells and immune cells, and in the cytoplasm only in selective cases. Kaplan-Meier survival analysis was used for 96 invasive UCs with 1%, 5%, 10%, 50% cut-off values according to the expression of T-PD-L1 (PD-L1 on the tumour cells). At any cut-off value, the difference in survival was not significant between patients with T-PD-L1 (+) and T-PD-L1 (-). As shown in figure 1 and figure 2, at the 1% cut-off value,

Table 1 Associations between PD-L1 expression and clinicopathological characteristics

		T-PD-L1 (5% cut-off value)			I-PD-L1 (1% cut-off value)		
Subgroup	N	Negative	Positive	P value	Negative	Positive	P value
Overall	96	56	40		50	46	
Gender							
Female		6 (5.3)	3 (3.8)	0.859	3 (4.7)	6 (4.3)	0.405
Male		50 (50.8)	37 (36.3)		47 (45.3)	40 (41.7)	
Age (years)							
≤75		26 (27.4)	21 (19.6)	0.557	23 (24.5)	24 (22.5)	0.545
> 75		30 (28.6)	19 (20.4)		27 (25.5)	22 (23.5)	
Tumour differentiation							
Single differentiation		51 (49.0)	33 (35.0)	0.211	41 (43.8)	43 (40.3)	0.165
With other components		5 (7.0)	7 (5.0)		9 (6.3)	3 (5.8)	
Tumour depth							
T1		10 (8.8)	5 (6.3)	0.169	7 (7.8)	8 (7.2)	0.865
T2		40 (37.9)	25 (27.1)		34 (33.9)	31 (31.1)	
T3/4		6 (9.3)	10 (6.7)		9 (8.3)	7 (7.7)	
LN metastasis							
Absent		50 (48.4)	33 (34.6)	0.338	42 (43.2)	41 (39.8)	0.463
Present		6 (7.6)	7 (5.4)		8 (6.8)	5 (6.2)	
TNM stage							
I–II		46 (41.4)	25 (29.6)	0.031	36 (37.0)	35 (34.0)	0.649
III–IV		10 (14.6)	15 (10.4)		14 (13.0)	11 (12.0)	
TILs							
Absent		31 (22.8)	8 (16.3)	0.001	33 (20.3)	6 (18.7)	< 0.001
Present		25 (33.3)	32 (23.8)		17 (29.7)	40 (27.3)	
T-PD-L1 (5% cut-off value)							
Negative					34 (29.2)	22 (26.8)	0.045
Positive					16 (20.8)	24 (19.2)	
I-PD-L1 (1% cut-off value)							
Negative		34 (29.2)	16 (20.8)	0.045			
Positive		22 (26.8)	24 (19.2)				

TILs, tumour infiltrating lymphocytes.

the difference in survival was not significant between patients with I-PD-L1 (+) and the I-PD-L1 (−). However, with the 5% and 50% cutoff values, the T-PD-L1 (+) group showed a trend of disadvantage in survival over the T-PD-L1 (−) group (p=0.12 and p=0.11). Next, we defined the 5% cut-off value as the boundary of T-PD-L1 (+) and T-PD-L1 (-). In survival analysis for the combined prognostic effect of T-PD-L1 and I-PD-L1, patients with T-PD-L1 (+)/I-PD-L1 (-) showed the worst clinical outcomes respectively (p=0.0276).

Prognostic significance of TILs and T-PD-L1 expression in UCs

With the 5% cut-off value as the boundary of T-PD-L1 (+) and T-PD-L1 (-), patients with T-PD-L1 (-)/TIL (-) showed the best clinical outcomes respectively (p=0.041).

A multivariate analysis with a Cox proportional hazard regression model that included gender, age, tumour differentiation, tumour depth, LN metastasis, TNM stage, TILs, T-PD-L1 and I-PD-L1 expression was performed in the table 2. I-PD-L1 expression in UCs was significantly associated with lower risk (HR, 0.336; 95% CI 0.129 to 0.876; p=0.026; table 2) and this association was independent of T-PD-L1 expression. TNM stage and TILs were significantly associated with higher risk (HR, 2.713; 95% CI, 1.124 to 6.547; p=0.041 and HR, 3.699; 95% CI, 1.125 to 12.159; p=0.031; table 2).

The relationship between the PD-L1 expression and clinicopathological features

For the 5% cut-off value, PD-L1 was detected positive in 40 (41.7%) of 96 invasive UCs. For the 1% cut-off value, PD-L1 was detected positive in 46 (50%) of 96 invasive UCs. Double positivity in tumour cells and immune cells was observed in 24 cases (25%). The T-PD-L1 (+) phenotype was significantly associated with the existence of TILs (p<0.001) and the expression of I-PD-L1 (p=0.045) compared with T-PD-L1 (−) phenotype. The I-PD-L1 (+) phenotype was closely associated with the existence of TILs (p<0.001) and the expression of T-PD-L1 (p=0.045) compared with I-PD-L1 (−) phenotype in table 1.

Comparison of lymphocytes in the T-PD-L1 (+) and the T-PD-L1 (-) at the 5% cut-off value

Median, mean and IQR values of the density of each TIL type were listed in table 3. As shown in table 4, the density of CD3+, CD4+, CD8+T cells in the T-PD-L1 (+) group was more than T-PD-L1 (-) group (p=0.002; p=0.006; p=0.003). The density of CD20+, CD56+ cells was not significantly different between the cases with T-PD-L1 (+) and the T-PD-L1 (−).

DISCUSSION

The main histologic type of bladder cancer is UC. About 3/4 of the tumours are non-muscle invasive UC, and 1/4 of the cases are muscle invasive UC.[16] The overall 5-year survival rate

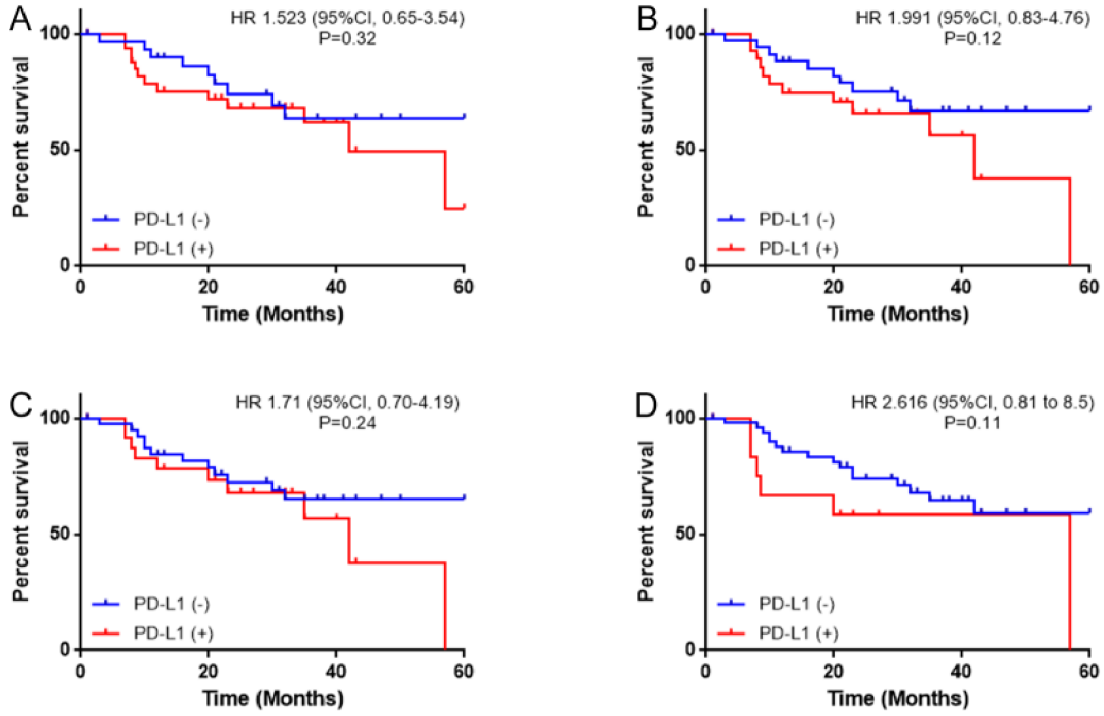

Figure 1 Kaplan-Meier survival analysis with log-rank test of the T-PD-L1 in different cut-off values. (A) Survival curves for OS according to the T-PD-L1 in 1% cut-off value. (B) Survival curves for OS according to the T-PD-L1 in 5% cut-off value. (C) Survival curves for OS according to the T-PD-L1 in 10% cut-off value. (D) Survival curves for OS according to the T-PD-L1 in 50% cut-off value. OS, overall survival.

for invasive bladder cancer is lower compared with others in the urinary system, with limited response to available treatments such as chemotherapy. This largely attributes to prone to develop regional and distant metastasis.[17] Immune checkpoint inhibitors have attracted significant attention because of their good effect in antitumour therapy. Ishida *et al* discovered and named programmed cell death-1 (PD-1).[18] Two studies of PD-1/PD-L1, published in the Journal of the *New England Journal of*

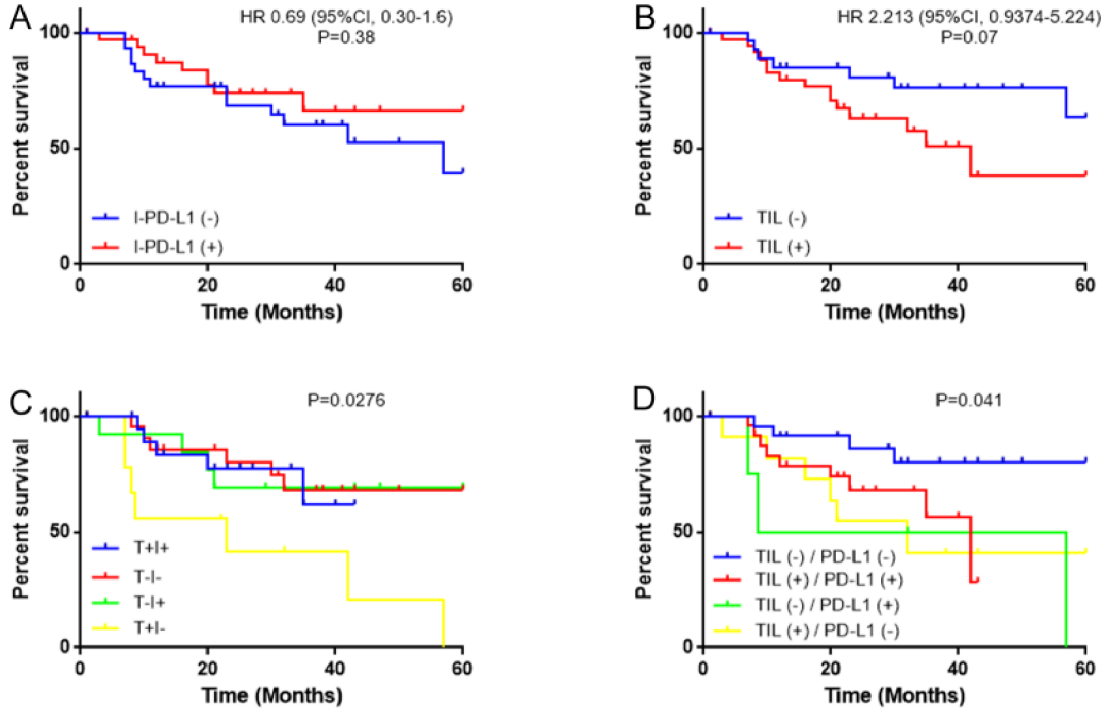

Figure 2 Kaplan-Meier survival analysis with log-rank test of the other indicators and the different combinations. (A) Survival curves for OS according to the I-PD-L1 in 1% cut-off value. (B) Survival curves for OS according to the TILs in the front of the cancer. (C) Survival curves for OS according to the combination of T-PD-L1 and I-PD-L1. (D) Survival curves for OS according to the combination of T-PD-L1 and TILs. OS, overall survival; TILs, tumour infiltrating lymphocytes.

Table 2 Univariate and multivariate analyses of OS in all patients

Variable	Univariate analyses		Multivariate analyses	
	HR (95% CI)	P value	HR (95% CI)	P value
Gender				
Female	1.000	0.400		
Male	2.368 (0.318 to 17.644)			
Age (years)				
≤75	1.000	0.102		
> 75	2.069 (0.865 to 4.949)			
Tumour differentiation				
Single differentiation	1.000	0.829		
With other components	1.177 (0.27 to 5.126)			
Tumour depth				
T1	1.000	0.269		
T2	3.904 (0.518 to 29.419)	0.186		
T3/4	6.629 (0.670 to 65.556)	0.106		
LN metastasis				
Absent	1.000	0.083		
Present	2.427 (0.891 to 6.613)			
TNM stage				
I–II	1.000	0.026	1.000	0.041
III–IV	2.713 (1.124 to 6.547)		2.570 (1.041 to 6.346)	
TILs				
Absent	1.000	0.079	1.000	0.031
Present	2.291 (0.91 to 5.768)		3.699 (1.125 to 12.159)	
T-PD-L1 (5% cut-off value)				
Negative	1.000	0.130	1.000	0.682
Positive	1.932 (0.823 to 4.533)		1.240 (0.443 to 3.467)	
I-PD-L1 (1% cut-off value)				
Negative	1.000	0.382	1.000	0.026
Positive	0.683 (0.291 to 1.605)		0.336 (0.129 to 0.876)	

OS, overall survival; TILs, tumour infiltrating lymphocytes.

Medicine in 2012, led to a flurry of research on the PD-1/PD-L1 pathway. In the immune system, the second signal can have positive or negative effects, and the PD-1/PD-L1 is the key pathway of negative regulation. Under normal circumstances, the activation of PD-1/PD-L1 pathway can prevent T cells from overproliferation and overactivation, thus avoiding the occurrence of autoimmune diseases and autoimmune injury, and maintaining the homeostasis of the immune system.[19 20] Now a wide range of researching evidences generally support that overexpression of PD-L1 on tumour cell surface combined with PD-1 on T cell surface can produce negative costimulatory signals, leading to loss of function and apoptosis of tumour antigen-specific T cells. The PD-1/PD-L1 pathway is the main mechanism of tumour escaping from immune surveillance.[21]

PD-L1 expression is detected in tumour cells from different sources, including oral squamous cell carcinoma,[22]

Table 3 Median, mean and IQR values of the density of CD3+, CD4+, CD8+, CD20+, CD56+ TILs in invasive front of the tumo

	Median (cells/mm^2)	Mean (cells/mm^2)	IQR (cells/mm^2)
CD3+	661	1027	308–1420
CD4+	374	574	167–647
CD8+	258	437	108–626
CD20+	48	204	2–168
CD56+	12	48	0–63

Table 4 Mean and IQR values of the density of CD3+, CD4+, CD8+, CD20+ and CD56+ TILs in invasive front of the tumour in two groups (PDL1+ and PDL1-)

	PD-L1- (cells/mm^2) (n=56)		PD-L1+ (cells/mm^2) (n=40)		
	Mean	SD	Mean	SD	P value
CD3+cells	741	732	1427	1163	0.002
CD4+cells	408	449	804	798	0.006
CD8+cells	306	359	621	565	0.003
CD20+cells	146	306	286	535	0.142
CD56+cells	37	76	62	89	0.157

TILs, tumour infiltrating lymphocytes.

non-small-cell lung carcinoma,[23] pancreatic carcinoma,[24] non-Hodgkin's lymphoma,[25] metastatic renal clear cell carcinoma,[26] oesophageal squamous cell carcinoma,[27] breast cancer[28] and so on. However, PD-L1 expression in lymphocytes remains unknown. In the study, we found expression of PD-L1 on the lymphocytes, and we defined the 1% cut-off value as the boundary of I-PD-L1 (+) and I-PD-L1 (−). The expression of PD-L1 was detected by immunohistochemistry in 96 cases of invasive UCs. PD-L1 expression was observed in tumour cells (n=40, 41.7% for 5% cut-off value) as well as in infiltrating immune cells (n=46, 47.9% for 1% cut-off value), but only was connected with TILs ($p<0.001$). Positive cut-off values for PD-L1 are still not standardised.[29] Our study compared 1%, 5%, 10% and 50% cutoff values and was evaluated for its role as a prognostic biomarker at each cut-off value. The T-PD-L1 (+) group showed a trend of disadvantage in survival over the T-PD-L1 (−) group with the 5% cut-off value ($p=0.11$), so the 5% cut-off value was applied. Many studies have shown that the PD-1/PD-L1 pathway plays an important role in the process of immune escaping from immune surveillance.[30–32] PD-L1 is an important independent factor of poor prognosis. In the study of pancreatic cancer, Masayo found that the level of PD-L1 expression was closely related to the prognosis of 235 patients with pancreatic cancer, tumour necrosis factor (TNF) can increase the expression of PD-L1 through NF-κb signalling pathway. The expression of PD-L1 in tumour cells is positively correlated with the infiltration of macrophages in tumour stroma. In another study, only low levels of PD-L1 were detected in normal pancreatic tissue, but high levels of PD-L1 were found in the membranes of pancreatic cancer cells. The prognosis of the patients was closely related to the expression of PD-L1 protein.[33] In our study, no significant survival difference was observed between the I-PD-L1 (+) and I-PD-L1 (−) groups, but a combined survival analysis revealed patients in the T-PD-L1 (+)/I-PD-L1 (-) groups had the worst OS compared with the other groups ($p=0.0276$) and in the multivariate Cox regression analysis, the I-PD-L1 was significantly associated with lower risk (HR=0.336; 95% CI, 0.129 to 0.876; $p=0.026$).

Tumourgenesis is not only the proliferation of tumour cells but also the involvement of stromal cells, inflammatory cells, blood vessels and other factors, which are combined and defined as the tumour microenvironment. Among them, TILs and other immune cells constitute the 'seventh characteristics' of tumour.[34] In addition to the expression of PD-L1, TILs may also be an important indicator for the use of checkpoint inhibitors in anti-tumour therapy. However, only under some certain conditions, TIL was used as an objective indicator. For example, intratumoural, rather than stromal, CD8+T cells

could be a potential negative prognostic marker in invasive in patients with breast cancer.[35] In this study, we only selected the invasive front of tumour. This explained why the presence of TILs showed a trend of disadvantage in survival and the TILs were significantly associated with higher risk (HR 0.86; 95% CI 1.125 to 12.159; p=0.031) in the multivariate Cox regression analysis. According to the TILs and the expression of PD-L1, the tumour microenvironment can be divided into four types: type I, (PD-L1 (+)/TILs (+)), type II (PD-L1 (−)/TILs (−)), type III, (PD-L1 (+)/TILs (−)), type IV, (PD-L1 (−)/TILs (+)). So, the most appropriate immunotherapeutic regimen should be chosen according to different conditions.[36] Studies had shown that these four subtypes can be used to screen for potential optimal therapeutic benefits, such as gastrointestinal cancer,[37] non-small-cell lung carcinoma[38] and so on. Here, patients with T-PD-L1 (−)/TILs (−) showed the best clinical outcomes (p=0.041), so type II was associated with a good prognosis in the patients with UCs.

In our study, the density of T cells in the T-PD-L1 positive group was significantly higher than those in the T-PD-L1 negative group. The expression of PD-L1 was related to the high density of T cells. This is consistent with previous studies of malignant melanoma.[39] The expression of PD-L1 in tumour cells is mainly regulated by internal and external regulation, which is mainly reflected in gene mutation and protein changes, such as in glioblastoma multiforme, loss of PTEN protein can cause PD-L1 protein to rise.[40] The activation of the PI3K-mTOR pathway and EGFR kinase pathway is an important pathway of the upregulation of PD-L1 in non-small-cell lung carcinoma and breast cancer.[41 42] Compared with the internal regulation, the external regulation of PD-L1 expression is mainly reflected in the effects of various inflammatory cytokines, among which interferon is the best inducer.[43–45] Interferon induces PD-L1 expression on the surface of endothelium, normal epithelial cells, myeloid cells, and immature T cells.[46] Other inflammatory mediators associated with PD-L1 expression include vascular endothelial growth factor, colony-stimulating factor, interleukin 4 (IL-4), and interleukin 10 (IL-10).[47–49] However, the mechanism of other inducers in tumour immune escape remains to be elucidated.[50 51] Our findings may reflect that PD-L1 expression in tumour cells is mainly controlled by an extrinsic pathway rather than an intrinsic mechanism in UCs.

In conclusion, our study reveals that the PD-L1 expression is associated with TILs and that the TILs can be a relevant marker in the determination of the prognostic role of PD-L1 expression in UCs. Our findings support that PD-L1 in the immune cells is a protective factor. The combination of T-PD-L1 expression and TILs, or T-PD-L1 and I-PD-L1 expression could be a significant prognostic factor for the UCs.

Take home messages

- ⇒ The 5% and 50% cut-off values are appropriate for a positive reading of PD-L1 expression on the tumour cells.
- ⇒ Perhaps the expression of PD-L1 on the tumour cells or the lymphocytes is not a good biomarker of the patients with the urothelial carcinoma, but the combination of expression of PD-L1 on the tumour cells and the lymphocytes plays a certain indicative role and the combination of PD-L1 expression of the tumour cells and lymphocytes infiltration in the invasive front of urothelial carcinoma plays a certain indicative role.
- ⇒ Lymphocyte infiltration in the invasive front of urothelial carcinoma affects the expression of PD-L1.

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