



The clinicopathological and prognostic value of PD-L1 in urothelial carcinoma: a meta-analysis

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Abstract

The prognostic value of programmed death-ligand 1 (PD-L1) in urothelial carcinoma (UC) has been assessed in previous studies, while the results remain controversial and heterogeneous. Therefore, we performed this meta-analysis to explore the prognostic effect of PD-L1 in patients with UC. PubMed, Embase and Web of Science were searched to identify the studies. Hazard ratios (HR) with 95% confidence interval (95% CI) and clinicopathological factors were extracted from included studies. A total of 1819 patients with UC from 11 published studies were incorporated. The results of meta-analysis showed that positive PD-L1 expression was significantly associated with poorer overall survival (OS) (HR 1.59, 95% CI 1.05–2.40) and disease-free survival (DFS) (HR 1.83, 95% CI 1.03–3.25), but not recurrence-free survival. Moreover, in the subgroup analysis, significant associations between PD-L1 expression and OS or DFS were found in bladder UC, the cutoff value of positive expression of PD-L1 $\geq 5\%$ and the expression of PD-L1 on the tumor cell membrane. Interestingly, positive PD-L1 expression was correlated with poorer pathological T stage (OR 2.03, 95% CI 1.46–2.82). Our meta-analysis implies that PD-L1 might be a valuable biomarker of poor prognosis for UC, especially bladder UC, although further large-scale and well-designed studies are warranted to verify the prognostic value of PD-L1 for UC.

Keywords PD-L1 · Urothelial carcinoma · Prognosis · Meta-analysis · Biomarker

Introduction

Urothelial carcinoma (UC) is the most common aggressive tumor involving the urinary tract [1]. UC can arise from anywhere along the urinary tract, including the renal pelvis and ureter in the upper tract as well as bladder and urethra in the lower tract. Among them, the majority are located in the bladder and only 5% are located in the upper urinary tract [2]. In the USA alone, it was estimated that 79,030 cases were diagnosed and 16,870 cases died from bladder UC in 2017 [1]. One of the main reasons for the high mortality rate of UC is lacking effective detection methods for prognosis monitoring. Hence, there is an urgent need to identify novel molecular targets to predict the prognosis of UC.

Immunotherapy with immune checkpoint inhibitors, particularly against the programmed death-ligand 1/programmed death-1 (PD-L1/PD-1) axis, has shown robust antitumor activity and improved treatment outcomes in several tumor types [3, 4], including UC [5]. PD-L1 is a surface glycoprotein that belongs to B7/CD28 costimulatory factor superfamily, and expressed on sorts of immune cells and tumor cells [6, 7]. The expression of PD-L1 by tumor cells can impede T cell activation, and induce apoptosis or inhibition of tumor-specific T cells, resulting in tumor growth [7, 8]. Growing evidence has shown that PD-L1 overexpression is associated with worse prognosis and resistance to immune therapies in several human cancers [9–13]. However, the prognosis role of PD-L1 in UC is still controversial. Some studies showed that elevated PD-L1 expression in UC tissue was correlated with a worse survival rate [14–16], but others found that PD-L1 expression has no correlation with prognosis in UC [17–19]. Krabbe et al. [19] even found that prognostic value of PD-L1 in UC seems to follow an opposite trend. So there is an urgent need to analyze the data of PD-L1 in UC to reach a reasonable conclusion, and detection

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of PD-L1 expression in UC may be a great help to verify a high-risk population of UC patients with poor prognosis.

So far, there was no systematic study on the prognostic value of PD-L1 in UC. Therefore, in this study, we performed a meta-analysis of the available evidence to explore the expression of PD-L1 in association with clinicopathological factors and prognosis of UC patients in order to determine the prognostic value of PD-L1 in patients with UC.

Materials and methods

A prospective protocol of objectives, literature-search strategies, inclusion and exclusion criteria, outcome measurements and methods of statistical analysis was prepared a priori according to the Preferred Reporting Items for Systematic Reviews and Meta-analysis (PRISMA) guidelines [20]. Our study was based on data from previously published studies. Therefore, patient's consent or ethical approval was not necessary.

Literature search

Comprehensive literature retrieval was conducted independently by two investigators in PubMed, Web of Science and Embase with a cutoff date of April 1, 2018. The following MeSH terms were used in search strategy: (“PD-L1” OR “programmed death-ligand 1” OR “B7-H1” OR “B7 homolog 1” OR “CD274”) AND (“Transitional Cell Carcinoma” OR “Transitional Cell Carcinomas” OR “urothelial carcinoma” OR “urothelial carcinomas”). In addition, the references of other relevant articles were also manually reviewed for additional eligible studies.

Inclusion and exclusion criteria

Inclusion criteria included: (1) studies that explored the association between PD-L1 expression and prognostic parameters of UC; (2) the expression of PD-L1 was tested using immunohistochemistry (IHC) staining on tumor cells in primary cancer tissues; (3) the association between PD-L1 expression and prognosis and/or clinicopathological features; (4) hazard ratio (HR) and 95% confidence interval (95% CI) was reported or could be calculated from the data provided; (5) the articles were written only in English. Exclusion criteria included: (1) duplicate publications; (2) reviews, letters, case reports and expert opinions; (3) studies without available or usable data.

Study selection and data extraction

We screened the studies according to inclusion and exclusion criteria. Two investigators independently extracted and

reviewed the data from the eligible studies. Any disagreements were consulted with a third investigator to resolve the dispute. Through a same standardized information collection form, the following data were extracted from all eligible studies: first author's name, publication year, country of origin, primary tumor site of UC, number of patients, cutoff value used to define PD-L1 positive and PD-L1 expression location in tumor cells and HR with corresponding 95% CI. In addition, the data of clinicopathological parameters (patient gender, lymph node metastasis and lymphovascular invasion, pathological T stage) were also extracted from all eligible studies.

In order to minimize bias, the HR and 95% CI were extracted preferentially from multivariable analyses. If not available, HR and 95% CI from Kaplan–Meier curves were retrieved using Engauge Digitizer version 4.1 (<http://digitizer.sourceforge.net/>) and spreadsheet designed, as previously described [21, 22].

Assessment of study quality and statistical analysis

The Newcastle–Ottawa Scale (NOS) was used to evaluate the methodological quality of all included studies. The study with a NOS score ≥ 6 was indicated to be of high quality. Discrepancies in the score were resolved through discussion between the authors. $I^2 < 50\%$ or $p > 0.05$ were considered as no heterogeneity, and a fixed-effects model was performed. Otherwise, random-effects model was applied. Subgroup analysis was performed to further assess the prognostic value of PD-L1 [23]. The heterogeneity of pooled data was assessed using the Cochran Q -test and I -squared test. Sensitivity analysis was applied to evaluate the stability of the results. Begg's funnel plot and Egger's test were applied to assess the potential publication bias. All analyses were performed using STATA 12.0 software (STATA Corporation, College Station, TX, USA). And statistical significance was defined as p value < 0.05 .

Results

Literature retrieval and analysis

A flowchart of the literature retrieval process is shown in Fig. 1. Initially, a total of 254 potential studies were found utilizing the electronic databases' search. Based on the above-mentioned inclusion and exclusion criteria, a total of 11 studies comprising 1819 patients were included in the meta-analysis at last [15–19, 24–28]. A summary of the main characteristics of the included studies is shown in Table 1. The year of publication of all eligible studies ranged from 2014 to 2017, but one in 2008 [14]. Immunohistochemical techniques used in the eligible

Fig. 1 Flow diagram of the literature search and selection

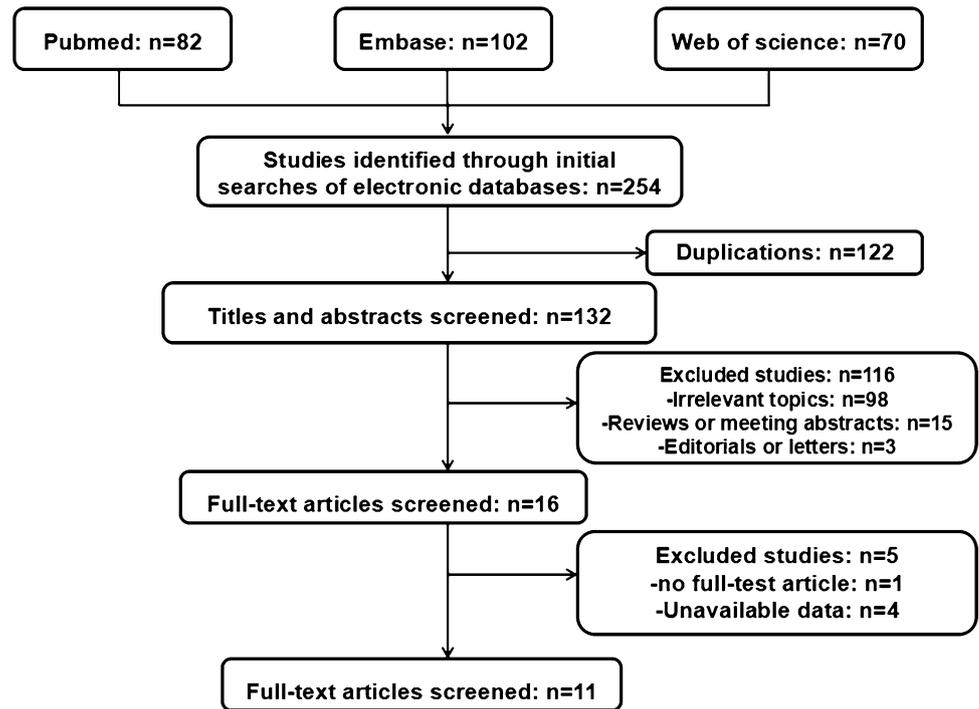


Table 1 Main characteristic of the eligible studies

First author, year	Patient source	Samples	Primary tumor site	Expression location	Staging	Outcome	Analysis method	Median (months) Follow-up
Bellmunt, 2015	USA	100	Bladder	TCM	M1	OS	Multivariate analysis	25
Boorjian, 2008	USA	167	Bladder	TCM, cytoplasmic	Ta–T2/N0/M0	OS	Multivariate analysis	164.4
Erlmeier, 2016	Germany	42	Bladder, upper tract	TCM	T1–T4/N1–N3	OS	K–M	63
Krabbe, 2017	USA, Europe	423	Upper tract	TCM, cytoplasmic	Ta–T4/Nx–N3	OS, RFS, DFS	Univariate analyses	37
Necchi, 2017	Italy	87	Bladder, upper tract	N/A	T2–T4/N0–N3/M0–M1	OS	K–M	N/A
Pichler, 2017	Austria	50	Bladder	N/A	T1–T4/N1–N3/M0	OS, RFS, DFS	K–M	25.5
Sharma, 2017	USA, Asian, Europe	265	Bladder, upper tract	TCM	M1	OS	K–M	7
Skala, 2016	USA	149	Upper tract	TCM	Ta–T4/Nx–N2/M0–M1	DFS	Multivariate analysis	N/A
Wu, 2016	Taiwan	72	Bladder	N/A	T2–T4/N0–N+/M0–M1	OS, RFS, DFS	Univariate analyses	45.6
Xylinas, 2014	USA	302	Bladder	TCM	Ta–T4/N0–N+/M0–M1	OS, RFS, DFS	Multivariate analysis	120
Zhang, 2017	China	162	Upper tract	TCM	Ta–T4/N0–N+	DFS	Multivariate analysis	79

studies are presented in Table 2. In all 11 eligible studies, PD-L1 expression levels were measured in tumor cells via immunohistochemistry (IHC) staining. Each article had a distinct cutoff value used to define positive and negative

PD-L1 expressions. Overall survival (OS), recurrence-free survival (RFS) and disease-free survival (DFS) were used as the end points to evaluate the prognosis.

Table 2 Immunohistochemical technique used in the eligible studies

First author, year	Primary antibody					Cutoff expression (%)
	Antibody	Clone	Specie	Dilution	Source	
Bellmunt, 2015	Anti-PD-L1	405.9A11	Mouse, monoclonal	NA	Dr. Gordon Freeman's Laboratory, USA	5
Boorjian, 2008	Anti-B7-H1	5H1	NA	1:100	NA	5
Erlmeier, 2016	Anti-PD-L1	NA	Rabbit, monoclonal	1:200	Cell Signaling Technology, USA	10
Krabbe, 2017	Anti-PD-L1	E1L3N(R)	Rabbit monoclonal	1:250	Cell Signaling Technology, USA	1
Necchi, 2017	The Ventana SP142 IHC assay					1
Pichler, 2017	Anti-PD-L1	CLA-10	Monoclonal	NA	Biocare, UK	1
Sharma, 2017	Dako PD-L1 immunohistochemical 28-8 pharmDx kit					1
Skala, 2016	Anti-PD-L1	5H1	NA	1:500	NA	5
Wu, 2016	NA	NA	NA	NA	NA	> 10
Xylinas, 2014	Anti-B7-H1	5H1	NA	1:100	NA	5
Zhang, 2017	Anti-PD-L1	E1L3 N	Rabbit monoclonal	1:200	Cell Signaling Technology, USA	5

Relationship between PD-L1 expression and survival

Among 11 eligible studies, nine studies reported results of OS toward PD-L1 expression with a total of 1508 UC patients. Because heterogeneity among studies was statistically significant ($p < 0.001$, $I^2 = 72.8\%$), the random-effects model was applied. The meta-analysis of those studies showed that positive expression of PD-L1 was significantly associated with poor OS of UC patients (HR 1.59, 95% CI 1.05–2.40, $p = 0.026$, Fig. 2).

There were six studies comprising 847 patients concerning the association between DFS and PD-L1 expression. Owing to the significant heterogeneity ($p = 0.005$, $I^2 = 70.5\%$) among these studies, a random-effects model was applied. Our results suggested that positive PD-L1

expression was significantly associated with DFS of UC patients (HR 1.83, 95% CI 1.03–3.25, $p = 0.04$, Fig. 3).

As shown in Fig. 4, there were three studies including 775 patients which provided results regarding RFS and PD-L1 expression. The pooled data demonstrated that PD-L1 expression was not associated with RFS of UC patients (HR 0.99, 95% CI 0.69–1.40, $p = 0.941$).

Subgroup analyses and sensitivity analyses in OS

To solve the heterogeneity, the subgroup meta-analysis was conducted, which was stratified by the patient source, primary tumor site, cutoff value used to define positive expression of PD-L1 and location of PD-L1 expression in tumor cells (Table 3). In the stratified analysis by the patient source, positive expression of PD-L1 was significantly associated

Fig. 2 Forest plot for the relationship between PD-L1 expression and OS

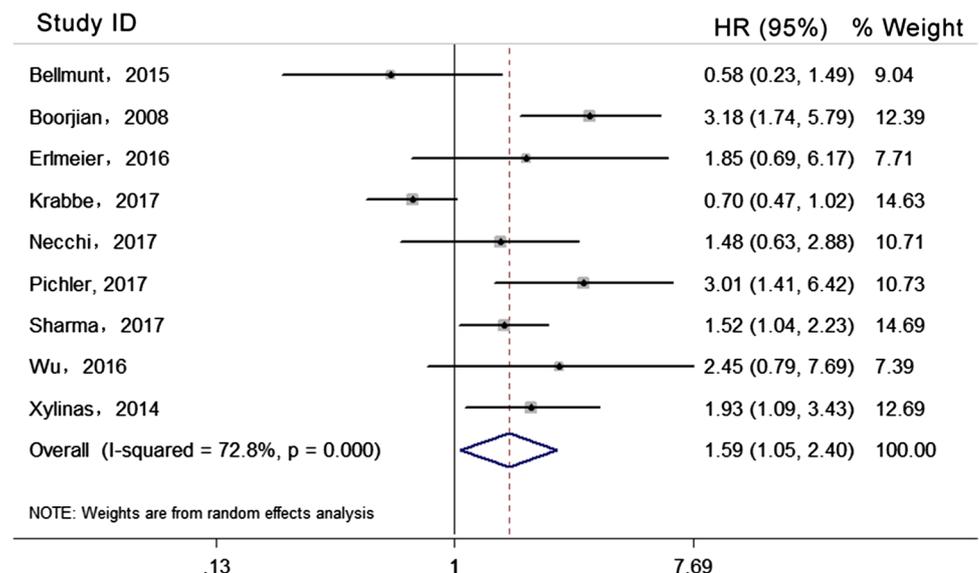


Fig. 3 Forest plot for the relationship between PD-L1 expression and DFS

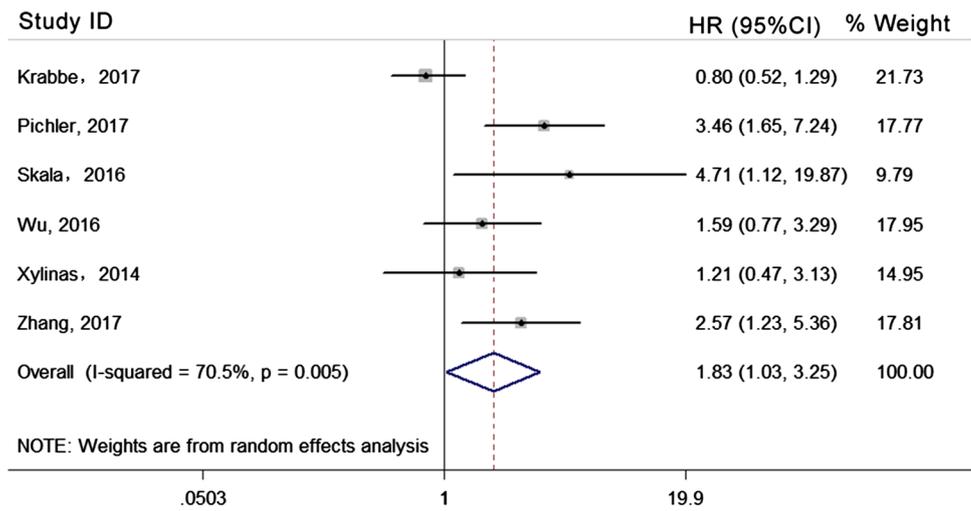


Fig. 4 Forest plot for the relationship between PD-L1 expression and RFS

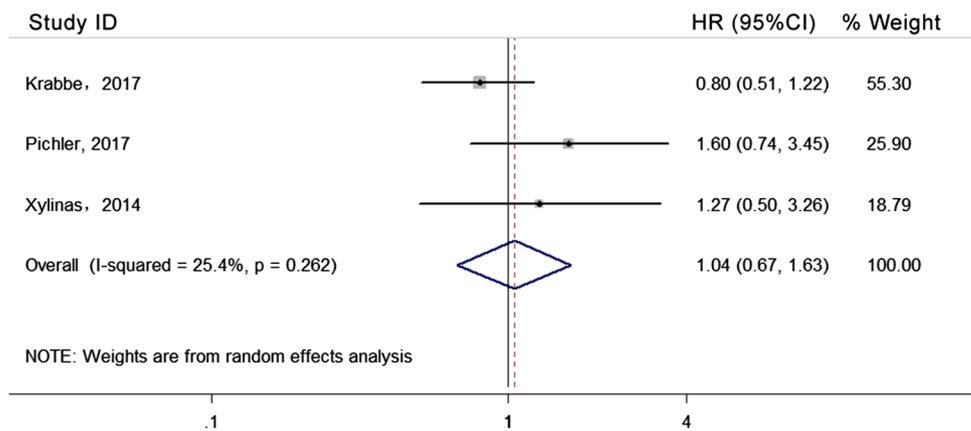


Table 3 Results of subgroup analyses in OS

Stratified analysis	No. of studies	No. of patients	Pooled HR (95% CI)	p value	Heterogeneity		
					I ² (%)	p value	Model
<i>Patients source</i>							
Only USA	3	569	1.64 (0.71–3.80)	0.247	77.8	0.011	Random
Only Europe	3	179	2.06 (1.27–3.33)	0.003*	0	0.422	Fixed
Others	3	760	1.23 (0.62–2.42)	0.554	79.8	0.007	Random
<i>Primary site</i>							
Bladder	5	691	2.01 (1.17–3.45)	0.012*	60.5	0.038	Random
Others	4	817	1.44 (0.86–2.43)	0.169	74.1	0.004	Random
<i>Cutoff</i>							
<5%	4	825	1.40 (0.78–2.52)	0.263	79.8	0.002	Random
≥5%	5	683	1.96 (1.40–2.77)	<0.001*	56.4	0.057	Fixed
<i>Location</i>							
Only TCM	4	407	1.49 (1.12–2.00)	0.007*	38.3	0.182	Fixed
Others	5	1101	1.82 (0.87–3.80)	0.110	83.5	<0.001	Random

with worse OS in patients only from Europe (HR 2.06, 95% CI 1.27–3.33, $p=0.003$) but not other patient sources. The subgroup analysis by primary tumor site suggested that

positive expression of PD-L1 was significantly associated with a reduced OS in urothelial carcinoma of the bladder (HR 2.01, 95% CI 1.17–3.45, $p=0.012$) but not others.

Further, subgroup analysis based on cutoff value was performed, providing high-quality evidence that PD-L1 positive was significantly associated with a reduced OS when a cutoff value $\geq 5\%$ was used (HR 1.96, 95% CI 1.40–2.77, $p < 0.001$). However, elevated PD-L1 expression was not associated with a reduced OS when a cutoff value $< 5\%$ was used. For PD-L1 expression location in tumor cells, subgroup analysis showed that PD-L1 positive was significantly associated with worse OS when PD-L1 expression was only located on the tumor cell membrane (TCM) (HR 1.49, 95% CI 1.12–2.00, $p = 0.007$). Meanwhile, it is worth mentioning that there is no heterogeneity ($p > 0.05$ or $I^2 = 0$) in the subgroups where PD-L1 positive was significantly associated with worse OS.

The result of sensitivity analysis showed that the removal of Krabbe et al. [19] eliminates statistical heterogeneity and significantly changes the pooled HR; the 95% CI of HR was

still greater than 1, which meant that the removal of Krabbe et al. [19] does not affect our conclusion. A graphical representation of the effect size is shown in Fig. 5 by using Stata 12.0.

Subgroup analyses and sensitivity analyses in DFS

We also performed subgroup analysis according to the patient source, primary tumor site, cutoff value used to define positive expression of PD-L1 and location of PD-L1 expression in tumor cells among studies focused on DFS (Table 4). The subgroup analysis by primary tumor site suggested that PD-L1 positive was associated with a reduced DFS in urothelial carcinoma of the bladder (HR 2.00, 95% CI 1.27–3.16, $p = 0.003$) but not upper tract. In addition, PD-L1 overexpression was found to be associated with worse DFS when a cutoff value $\geq 5\%$

Fig. 5 Sensitivity analysis for the meta-analysis among those studies reporting OS

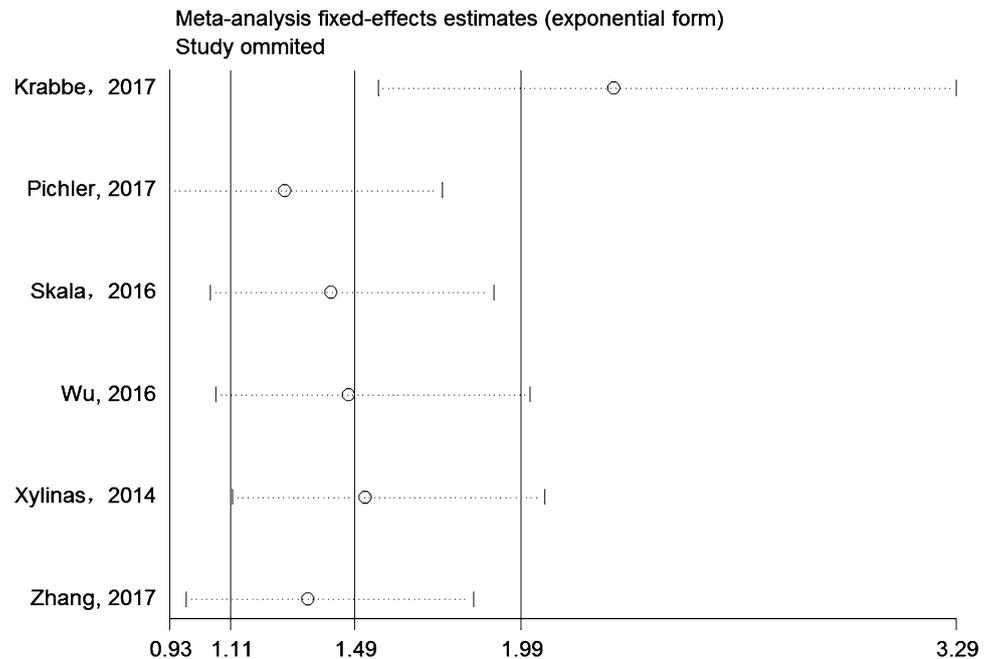


Table 4 Results of subgroup analyses in DFS

Stratified analysis	No. of studies	No. of patients	Pooled HR (95% CI)	P value	Heterogeneity		
					I^2 (%)	P value	Model
<i>Primary site</i>							
Bladder	3	424	2.00 (1.27–3.16)	0.003*	44.0	0.167	Fixed
Upper tract	3	734	1.86 (0.65–5.32)	0.248	81.2	0.005	Random
<i>Cutoff</i>							
<5%	2	473	1.61 (0.38–6.77)	0.513	90.9	0.001	Random
$\geq 5\%$	4	685	1.96 (1.27–3.02)	0.002*	8.0	0.353	Fixed
<i>Location</i>							
Only TCM	3	613	2.19 (1.28–3.76)	0.004*	27.9	0.250	Fixed
Others	3	545	1.58 (0.66–3.78)	0.110	82.5	0.003	Random

was used (HR 1.96, 95% CI 1.27–3.02, $p = 0.002$). For PD-L1 expression location in tumor cells, subgroup analysis showed that PD-L1 positive was associated with worse DFS when PD-L1 expression was only located in TCM (HR 2.19, 95% CI 1.28–3.76, $p = 0.004$). Sensitivity analysis of DFS has a similar result to OS, which is shown in Fig. 6.

Association between PD-L1 expression and clinicopathological features

Meta-analysis (Table 5) showed that PD-L1 positive was not associated with patient gender, lymph node metastasis and lymphovascular invasion, but was statistically associated with poorer pathological T stage (OR 2.03, 95% CI 1.46–2.82, $p < 0.001$). We failed to analyze the association between the overexpression of PD-L1 and other clinicopathological features because of the insufficient data.

Publication bias

For the meta-analysis of the relationship between PD-L1 expression level and prognosis, Begg’s funnel plots and Egger’s test were used to assess the publication bias. Begg’s funnel plot (Fig. 7a) shows that there was no significant publication bias observed among those studies reporting OS ($Pr > |z| = 0.917$, $z = 0.10$). In addition, a similar result was obtained among studies from Egger’s test ($t = 1.01$, $p > |t| = 0.348$). Moreover, no publication bias was found among studies reporting DFS by Begg’s funnel plot (Figure, $Pr > |z| = 0.260$, $z = 1.13$, Fig. 7b) and Egger’s test ($t = 2.08$, $p > |t| = 0.106$).

Discussion

A substantial amount of researches have demonstrated that malignant tumor cells can express immunosuppressive molecules to inhibit antitumor lymphocytes, thus evading host immune responses [29–31]. Recently, a novel immune

Fig. 6 Sensitivity analysis for the meta-analysis among those studies reporting DFS

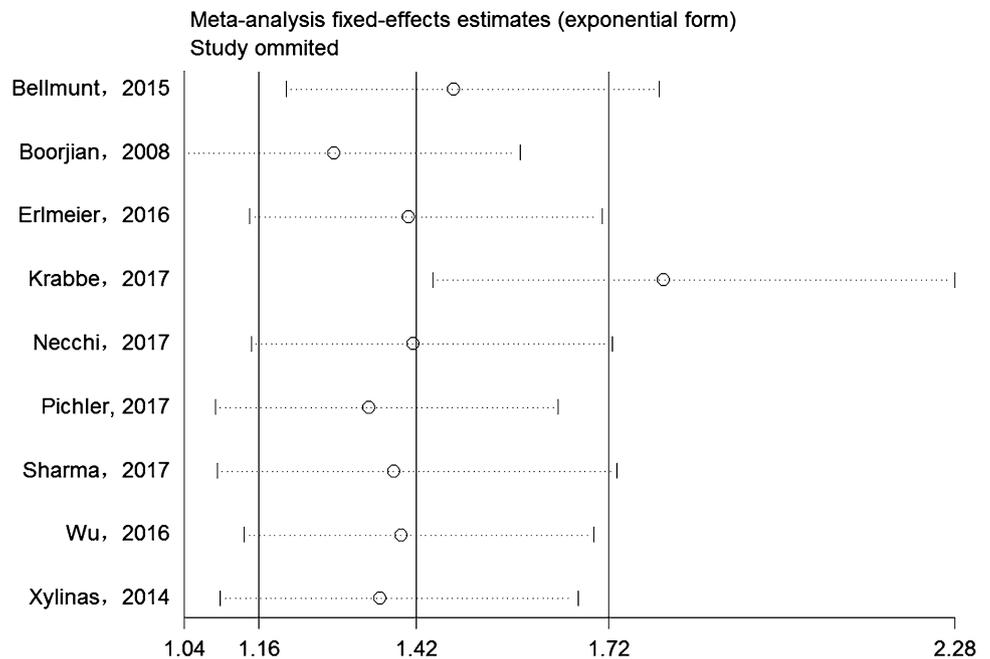
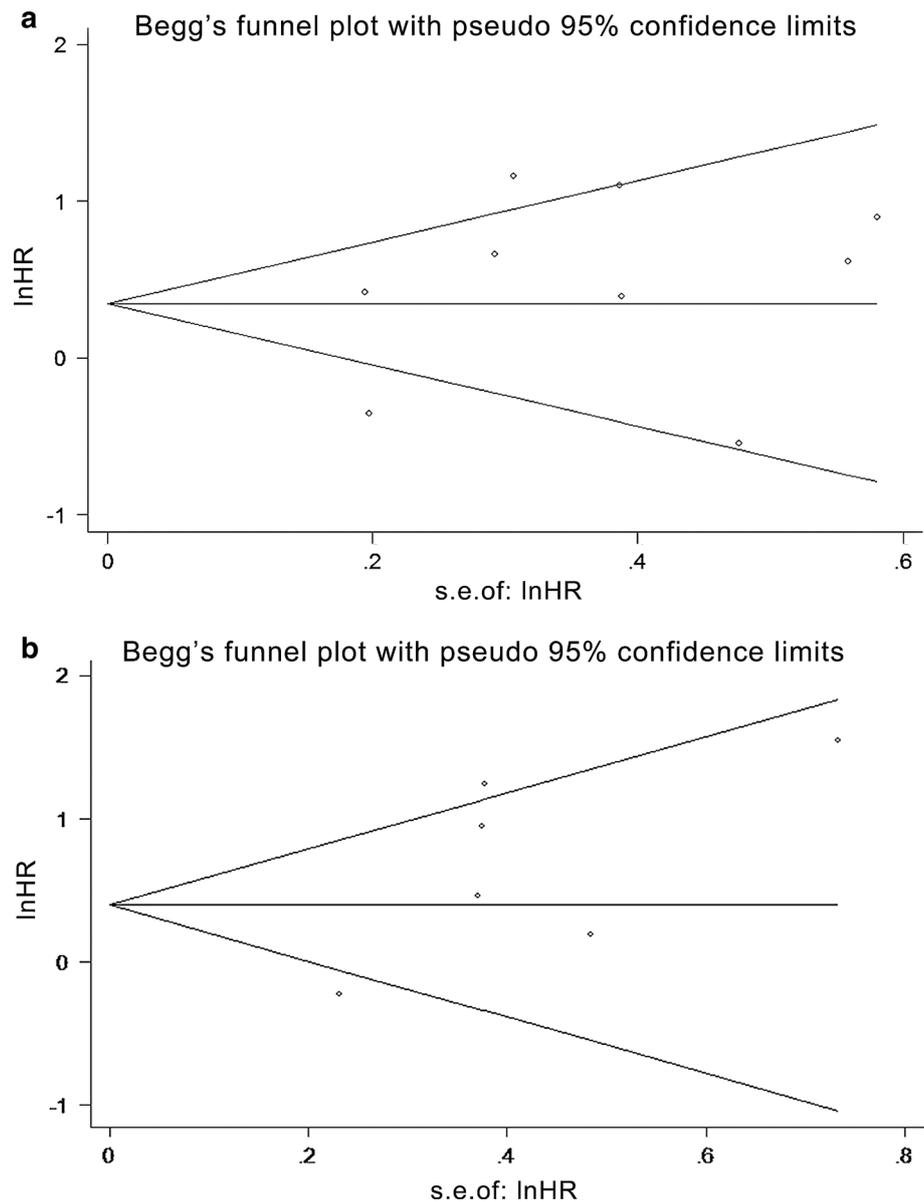


Table 5 Meta-analysis results of the associations of positive PD-L1 expression with clinicopathological features

Clinicopathological parameters	No. of studies	No. of patients	OR (95% CI)	p value	Heterogeneity		
					I^2 (%)	p value	Model
Gender (male vs. female)	6	1471	0.87 (0.66–1.15)	0.336	0.0	0.529	Fixed
T stage ($\geq T2$ vs. $\leq T1$)	4	1127	2.03 (1.46–2.82)	<0.001	56.6	0.075	Fixed
Lymph node metastasis (yes vs. no)	6	898	0.91 (0.65–1.29)	0.611	35.8	0.168	Fixed
Lymphovascular (yes vs. no)	4	685	0.81 (0.20–3.31)	0.770	87.8	<0.001	Random

Fig. 7 **a** Funnel plot analysis of potential publication bias among those studies reporting OS. **b** Funnel plot analysis of potential publication bias among those studies reporting DFS



checkpoint receptor named as PD-L1 that tumor cells express to evade host immune response has been a research hot spot. At first, PD-1/PD-L1 was regarded as an important co-inhibitory signaling pathway aiming to protect healthy cells from autoimmune responses or excessive inflammatory [32, 33]. However, accumulating evidence shows that this signaling pathway was also involved in the escape of cancer immunoediting, thus promoting the survival and growth of tumor cells [34, 35]. Further researches showed that PD-1/PD-L1 pathway blockade promised results for increasing survival rates and long-term remission in both solid tumors and hematologic malignancies [35–37]. Multiple studies have found that UC cells are also able to express PD-L1, and anti-PD-L1 therapy has been proved to exert clinical activity in UC [38–40]. An increasing number of researchers

have performed studies aimed at investigating the prognostic value of PD-L1 in UC, but the relationship between PD-L1 expression and the prognosis of UC patients remains inconclusive. Therefore, a meta-analysis of the data available regarding the prognostic significance of PD-L1 would be useful and is urgently needed.

To our knowledge, our meta-analysis is the first to systematically estimate the correlation between PD-L1 expression and survival of UC patients. In this meta-analysis, we investigated the survival data of 1819 UC patients from 11 cohort studies. Our results provided strong evidence that overexpression of PD-L1 was statistically significantly associated with poor OS and DFS in UC patients, while it had no significant impact on RFS. The possible reason was the insufficiently large sample size included when performing

the analysis between PD-L1 expression and RFS. Because of the presence of heterogeneity, subgroup analyses were applied to identify the sources of any observed heterogeneity.

The subgroup analysis suggested that positive expression of PD-L1 was associated with poor OS in UC patients only from Europe countries/regions. Because of the inconsistency in cutoff values for assessing PD-L1 expression, we performed the subgroup analysis based on cutoff value and a significant correlation between positive PD-L1 expression and a worse prognosis was observed, suggesting that a cutoff value of 5% used to define PD-L1 positive can be used to estimate OS and DFS. When performing a subgroup analysis by subtype of the primary tumor site, we found that positive expression of PD-L1 correlated with poor OS and DFS in patients with bladder UC. Moreover, our meta-analysis also showed that elevated level of PD-L1 expression was significantly associated with poorer pathological T stage. Taken together, PD-L1 might be a potential predictive biomarker for clinical outcomes.

We made an effort to conduct a comprehensive analysis, but several limitations still exist in our meta-analysis. First, the sample sizes and the number of studies included in this meta-analysis were relatively small. However, the results of the sensitivity analysis remained stable after the sequential exclusion of each individual study. Second, some studies included only provided Kaplan–Meier curves, without HR and 95% CIs, which might influence the accuracy of our results. Third, certain studies with positive results might be published more easily than negative results, which may account for publication bias. Based on these observations, we believe that additional studies are needed.

In conclusion, our data suggest that positive expression of PD-L1 was statistically associated with worse OS and DFS in the patients with UC, especially with bladder UC. However, no significant association between PD-L1 expression and PFS was observed for patients with UC. Moreover, positive PD-L1 expression was correlated with poorer pathological T stage of UC. These results indicate that PD-L1 may play an important role in a valuable biomarker of poor prognosis for UC, although further large-scale and well-designed studies are warranted to verify the prognostic value of PD-L1 for UC.

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Compliance with ethical standards

Conflict of interest All authors declare that they have no competing interests.

Ethical approval This article does not contain any studies with human participants or animals performed by any of the authors. All the data involved in this study were extracted from published articles.

Informed consent Informed consent was obtained from all individual participants included in the study.

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