Clin. Lab. 2020;66:XXX-XXX

ORIGINAL ARTICLE

PD-L1 Overexpression on Tumor-Infiltrating Lymphocytes Related to Better Prognosis of Colorectal Cancer

Ji-Lin Wang [1, #], TaChung Yu [1, #], Tian-Tian Sun [1], Yan Feng [2], Hua Xiong [1], Jing-Yuan Fang [1]

[#] *Ji-Lin Wang and TaChung Yu contributes equally to this work*
[1] *Division of Gastroenterology and Hepatology, Shanghai Institute of Digestive Disease; Key Laboratory of Gastroenterology and Hepatology, Ministry of Health; State Key Laboratory for Oncogenes and Related Genes; Renji Hospital, School of Medicine, Shanghai Jiao-Tong University, Shanghai, China*
[2] *The Fifth Department of Oncology, Longhua Hospital Shanghai University of Traditional Chinese Medicine, Shanghai, China*

SUMMARY

Background: **PD-L1 expression on tumor-infiltrating lymphocytes (TILs) has recently been reported as a biomarker for colorectal cancer (CRC). However, the prognostic and clinical significance of PD-L1 on TILs in CRC remains controversial. We performed this meta-analysis to evaluate the association between the PD-L1 expression on TILs and clinicopathological features and prognosis of CRC patients.**
Methods: **A comprehensive literature search for relevant studies published up to Feb 2020 was performed using Medline, Embase, and Web of Science. Odds ratio (OR) with 95% CI was selected to appraise the correlation between PD-L1 expression on TILs with prognostic and clinicopathological characteristics of CRC patients. Begg's and Egger's test were used to assess publication bias. The statistical analysis was conducted using Stata software.**
Results: **A total of 19 studies including 5,213 CRC cases were included in this meta-analysis. The pooled results showed that PD-L1 overexpression on TILs was relevant to longer OS (OR = 1.36, 95% CI = 1.19 - 1.55, p < 0.01) and longer DFS/RFS (OR = 1.22, 95% CI = 1.03 - 1.44, p = 0.02). Moreover, CRC patients with high expression of PD-L1 on TILS was associated with lower T stage (OR = 2.30, 95% CI = 1.85 - 2.87, p < 0.01), less lymph node invasion (OR = 1.48, 95% CI = 1.03 - 2.13, p = 0.03), less distant metastasis (OR = 2.56, 95% CI = 1.81 - 3.64, p < 0.01), earlier TNM stage (OR = 1.93, 95% CI = 1.34 - 2.66, p < 0.01), later tumor grade (OR = 0.38, 95% CI = 0.23 - 0.62, p < 0.01) and high MSI status (OR = 0.36, 95% CI = 0.25 - 0.52, p < 0.01). But it is not related to tumor size, tumor differentiation, MMR status, BRAF mutant, and KRAS mutant.**
Conclusions: **This meta-analysis revealed that PD-L1 expression on TILs can serve as a significant biomarker for positive prognosis and clinicopathological features of CRC. Our results may provide some useful information when using PD-L1 expression to predict the survival of CRC patients and to select the beneficial CRC patients from PD-1/PD-L1 antibody treatment.**
(Clin. Lab. 2020;66:xx-xx. DOI: 10.7754/Clin.Lab.2020.200325)

Correspondence:
Jing-Yuan Fang
Middle Shandong Road 145
200001 Shanghai
China
Phone: +86 13918386561
Email: jingyuanfang@sjtu.edu.cn

Hua Xiong
Middle Shandong Road 145
200001 Shanghai
China
Phone: +86 13636466690
Email: huaxong88@126.com
Email: wangjilin811123@163.com

Manuscript accepted April 29, 2020

KEY WORDS

PD-L1, tumor-infiltrating lymphocyte, prognosis, colorectal cancer

INTRODUCTION

Colorectal Cancer (CRC) was the third most diagnosed tumor and the second leading cause of tumor-related death worldwide in 2018 [1]. The incidence of CRC has increased rapidly in China in recent years [2]. Although the overall survival rate of CRC has been improved in recent years, the prognosis of metastatic CRC is still very poor [3]. Immune checkpoint blockers (ICBs), especially PD-1/PD-L1 inhibitors, have now become the standard treatment for many metastatic tumors. Unfortunately, most of metastatic CRCs are not sensitive to ICBs. Therefore, it is urgent to find a marker that can predict the efficacy of ICBs in CRC.

It has been reported that the expression status of PD-L1 could predict the efficacy of PD-1/PD-L1 mAbs in some types of tumors [4]. PD-L1 is the main ligand of PD-1. PD-1 is strongly expressed on activated lymphocytes, while PD-L1 is expressed not only on lymphocytes, but also on tumor cell surfaces. The binding of PD-1 on PD-L1 may suppress the immune response of T cells and results in immune tolerance and immune escape [5]. Many studies have shown that PD-L1 expression is elevated in many types of cancer tissues, and its increased expression is related to poor prognosis of cancer patients [6]. However, the expression of PD-L1 in tumor-infiltrating lymphocytes (TILs) may have different roles [7].

PD-L1 could also be expressed in both tumor cells and TILs in CRC; however, the role of PD-L1 in CRC has not been fully clarified. While many studies have confirmed that the expression of PD-L1 on CRC tumor cells correlated with a poor prognosis for CRC patients [8,9], the association between PD-L1 in TILs and the prognosis for CRC patients has been controversial. There have been many studies which explore the prognostic significance of PD-L1 expression in TILs for CRC patients in recent years [10-28]. Some of the studies reported better prognosis of PD-L1 TILs expression for CRC [10,13-15,20-24,26]; however, other studies reported reverse results or no significant association [11,12,16,17]. To address the controversial issues, this meta-analysis was conducted to explore the correlation between PD-L1 expression on TILs and several clinicopathological features and the prognosis of CRC.

MATERIALS AND METHODS

Search Strategy

Eligible studies were retrieved by searching the following databases: Medline, Embase, and Web of Science. The search strategy included the following keywords: "PD-L1", "CD724", "B7-H1", "Programmed Cell Death1 Ligand1", "colorectal cancer", "colon cancer", "rectal cancer", "Colorectal Tumor", "Colorectal Carcinoma". The reference list of each primary study and of previous reviews and systematic reviews were also manually searched to avoid missing studies.

Study selection criteria

All eligible studies which evaluated the association between PD-L1 expression on TILs and the prognosis of CRC were selected in this meta-analysis. Studies meeting the following inclusion criteria were included: 1) Immunohistochemistry (IHC) was used to detect the expression of PD-L1 on TILs in CRC; 2) The relationship between PD-L1 expression on TILs and the clinicopathological features or prognosis of CRC was reported; 3) studies provided sufficient data to estimate odds ratios (ORs) for overall survival (OS) or disease free survival (DFS) or relapse free survival (RFS). The following studies are excluded: 1) meta-analyses, reviews, comments, letters, or case reports; 2) deficient data to report the ORs; 3) not using IHC; 4) non-English. We did not assess the methodological quality of the included studies, given that quality scorings of observational studies in meta-analyses is controversial.

Data extraction

All data of the included studies were independently extracted by two investigators (JW and TY), and disagreements in data extraction were resolved by discussion. The following data were recorded from each included article: the name of first author, publication year, cutoff value, PD-L1 antibody, PD-L1 positivity on TILs, tissue section, CRC type, number of cases, T category, N category, distant metastasis, tumor size, histology, tumor grade, AJCC stage, MSI status, MMR status, BRAF mutant, KRAS mutant, and most importantly the 5-year overall survival (OS) rate, 5-year disease free survival (DFS) rate, and relapse free survival. For those studies which did not provide 5-year OS and DFS directly, Kaplan-Meier curves were read by GetData Graph Digitizer (http://getdatagraph-digitizer.com).

Statistical analysis

The software used for statistical analysis is STATA 11.0 (Stata Corporation, College Station, TX, USA). The odds ratio (OR) with 95% CI was utilized to assess the relationship between PD-L1 expression on TILs and different clinicopathological features and prognosis of CRC. The heterogeneity between included studies was examined by chi-squared (χ^2) test and I^2 statistics. $p < 0.1$ or $I^2 > 50\%$ was considered significant heterogeneity. Fixed-effects model (Mantel-Haenszel) was used when there was no between-study heterogeneity; otherwise, the random effect model (DerSimonian and Laird) was used. The potential publication bias was determined by Begg's and Egger's test. Influence analysis was conducted by omitting each study to find potential outliers. p-values less than 0.05 were considered statis-

Table 1. Characteristics of included studies for examination of PD-L1 expression in immune cells.

Author (year) [Ref]	PD-L1 Ab clone	cutoff value	Tissue section	No. of patients	CRC type	Positivity	Endpoints	Prognosis
Droeser 2013 [10]	27A2	22 cells/punch	TMA	424	all stage	2.50%	OS	better
Wang 2016 [11]	SP142	score > 1	TMA	262	stage II - III	21%	RFS	worse
Kollmanna 2017 [12]	E1L3N	> 5%	WS	53	stage IV	96.20%	OS and RFS	NS
Jabbour 2017 [13]	SP142	> 10%	WS	104	all stage	72%	NR	better
Koganemaru 2017 [14]	SP142	> 5%	WS	235	stage III	8.10%	DFS	better
Lee 2017 [15]	E1L3N	> 5%	TMA	339	all stage	30.70%	OS	better
Masugi 2017 [16]	MIH1	score > 1	TMA	823	all stage	5%	OS	NS
Berntsson 2018 [17]	E1L3N	> 10%	TMA	555	all stage	55.40%	OS	NS
Valentini 2018 [18]	E1L3N	> 5%	WS	63	all stage	78%	NR	/
Korehisa 2018 [19]	SP142	> 1%	WS	499	all stage	36.10%	NR	/
Lee 2018a [20]	27A2	> 5%	WS	89	stage I - III	68.60%	DFS	better
Lee 2018b [21]	MIH1	> 5%	TMA	336	all stage	45.92%	OS and RFS	better
Yomoda 2018 [22]	E1L3N	NR	WS	132	all stage	18.20%	OS and RFS	better
Calik 2019 [23]	CAL10	> 5%	WS	157	all stage	54.10%	OS	better
Ho 2019 [24]	22C3	> 10%	TMA	238	all stage	26.90%	OS	better
Ahtiainen 2019 [25]	E1L3N	> 5%	WS	242	all stage	51.24%	NR	/
Kong 2019 [26]	NR	> 5%	WS	337	all stage	53.30%	OS and RFS	better
Mona 2020 [27]	NR	> 5%	WS	60	all stage	38.30%	NR	/
Pyo 2020 [28]	SP263	> 10%	WS	265	all stage	17.70%	OS and RFS	NS

Note: NR - not reported, NS - not significant, tma - tissue microassay, WS - whole section, OS - overall survival, DFS - disease-free survival, RFS - relapse free survival.

tically significant in this study. Two authors performed the statistical analysis independently and obtained the same results.

RESULTS

Search results and study characteristics

Three hundred and sixty-five potential papers were identified initially using the search strategy above, 330 of which were excluded after reading the titles and abstracts. After reading full texts, we excluded another 16 studies, among which eight studies had no usable data, seven studies were not about PD-L1 expression on TILs, and one study was not in English. Finally, 19 studies published from 2013 to 2020 met the inclusion criteria and were included in this meta-analysis. The detailed literature selection procedure was described in Figure 1. The 19 studies included 5,213 cases, the number of patients in each study ranges from 53 to 823. CRC type in most of the studies covered all stages of CRC, but Wang's study [11] only included stage II - III patients, Kollmanna's study [12] only included stage IV patients, Koganemaru's study [14] only included stage III patients, and Lee's study [20] only included stage I - III patients. The detailed characteristics of the studies are shown in Table 1 and Table 2.

Correlation between PD-L1 expression on TILs and survival

We evaluated the correlation between PD-L1 expression on TILs and prognosis of CRC. A total of eleven studies reported OS. The pooled analysis revealed that PD-L1 overexpression in TILs was significantly associated with better 5-year OS rate in a fixed-effects model (OR = 1.36, 95% CI = 1.19 - 1.55, $p < 0.01$) (Figure 2A).

Table 2. Data extracted from the included studies.

Author [Ref]	Tumor size < 5cm/ ≥ 5cm	Tumor grade (G1 + G2/ G3)	Differen-tiation Well/ poor	T (T 1, 2/ T 3, 4)	n Negative/ positive)	Distance Metastasis (M 0/M 1)	TNM (I + II/ III + IV)	MSI status low/high	MMR status proficient/ deficient	BRAF mutation wild/ mutant	KRAS mutation wild/ mutant	5-year OS rate	5-year DFS/ RFS
Droeser 2013 [10]	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	H (9/11)	NR
												L (131/413)	
Wang 2016 [11]	H (32/22)	NR	H (43/11)	H (5/49)	H (27/27)	NR	H (28/26)	NR	NR	NR	NR	NR	H (31/54)
	L (141/67)		L (170/38)	L (10/198)	L (111/97)		L (110/98)						L (156/208)
Kollmanna 2017 [12]	NR	NR	NR	NR	NR	H (29/12)	H (12/26)	NR	NR	NR	NR	H (10/41)	H (2/41)
						L (6/4)	L (3/7)					L (0/10)	L (0/10)
Jabbour 2017 [13]	NR	NR	NR	H (25/50)	H (42/33)	NR	H (38/37)	NR	NR	NR	NR	NR	NR
				L (1/28)	L (15/14)		L (13/16)						
Koganemaru 2017 [14]	NR	NR	H (35/1)	H (11/25)	NR	NR	NR	NR	NR	NR	NR	NR	H (31/36)
			L (182/17)	L (42/157)									L (135/199)
Lee 2017 [15]	NR	NR	H (84/23)	H (95/12)	H (100/7)	H (106/1)	H (77/29)	NR	NR	H (10/16)	NR	H (91/107)	NR
			L (59/20)	L (66/13)	L (65/13)	L (71/8)	L (44/35)			L (69/91)		L (53/79)	
Masugi 2017 [16]	NR	NR	H (36/8)	H (16/27)	H (26/13)	H (39/4)	H (25/18)	H (31/12)	NR	H (33/10)	H (29/14)	H (23/44)	NR
			L (708/69)	L (208/507	L (424/266)	L (602/114)	L (395/321)	L (632/124)		L (650/110)	L (446/310)	L (342/771)	

Table 2. Data extracted from the included studies (continued).

Author [Ref]	Tumor size < 5cm/ ≥ 5cm	Tumor grade (G1 + G2/ G3)	Differen-tiation Well/ poor	T (T 1, 2/ T 3, 4)	n Negative/ positive)	Distance Metastasis (M 0/M 1)	TNM (I + II/ III + IV)	MSI status low/high	MMR status proficient/ deficient	BRAF mutation wild/ mutant	KRAS mutation wild/ mutant	5-year OS rate	5-year DFS/ RFS
Berntsson 2018 [17]	**NR**	**NR**	**H (63/230)**	**H (82/202)**	**H (173/89)**	**H (257/36)**		**H (227/58)**	**NR**	**H (229/44)**	**H (179/95)**	**H (209/ 299)**	**NR**
			L (54/179)	**L (29/201)**	**L (112/115)**	**L (183/53)**		**L (199/17)**		**L (200/31)**	**L (141/90)**	**L (119/239)**	
Valentini 2018 [18]	**NR**	**H (23/26)**	**NR**	**NR**	**H (37/12)**	**NR**	**NR**	**H (25/24)**	**NR**	**H (40/9)**	**H (32/17)**	**NR**	**NR**
		L (10/4)			**L (9/5)**			**L (12/2)**		**L (14/0)**	**L (9/5)**		
Korehisa 2018 [19]	**NR**	**H (14/12)**	**H (16/10)**	**NR**	**H (21/5)**	**NR**	**H (21/5)**	**NR**	**NR**			**NR**	**NR**
		L (9/1)	**L (8/2)**		**L (7/3)**		**L (4/6)**						
Lee 2018a [20]	**NR**	**NR**	**H (42/14)**	**NR**	**H (37/19)**	**NR**	**NR**	**NR**	**NR**	**NR**	**NR**	**NR**	**H (50/56)**
			L (29/4)		**L (20/13)**								**L (23/33)**
Lee 2018b [21]	**NR**	**NR**	**H (144/8)**	**H (37/115)**	**H (93/59)**	**H (145/7)**	**H (92/59)**	**H (15/13)**	**NR**	**NR**	**NR**	**H (164/180)**	**H (164/180)**
			L (170/9)	**L (26/156)**	**L (66/113**	**L (154/25)**	**L (66/113)**	**L (15/5)**				**L (114/155)**	**L (113/155)**
Yomoda 2018 [22]	**NR**	**NR**	**H (23/1)**	**H (13/11)**	**H (19/5)**	**NR**	**H (19/5)**	**NR**	**NR**	**NR**	**NR**	**H (24/24)**	**H (23/24)**
			L (97/11)	**L (26/82**	**L (56/52)**		**L (51/57)**					**L (82/108)**	**L (74/108)**
Calik 2019 [23]	**H (35/50)**	**NR**	**H (75/10)**	**H (56/29)**	**NR**	**NR**	**NR**	**NR**	**NR**	**NR**	**NR**	**H (83/85)**	**NR**
	L (25/47)		**L (59/13)**	**L (34/38)**								**L (38/72)**	

Table 2. Data extracted from the included studies (continued).

Author [Ref]	Tumor size < 5cm/ ≥ 5cm	Tumor grade (G1 + G2/ G3)	Differen-tiation Well/ poor	T (T 1, 2/ T 3, 4)	n Negative/ positive)	Distance Metastasis (M 0/M 1)	TNM (I + II/ III + IV)	MSI status low/high	MMR status proficient/ deficient	BRAF mutation wild/ mutant	KRAS mutation wild/ mutant	5-year OS rate	5-year DFS/RFS
Ho 2019 [24]	NR	NR	NR	NR	NR	NR	NR	H (87/12)	NR	NR	NR	H (31/45)	NR
								L (133/6				L (81/193)	
Ahtiainen 2019 [25]	NR	H (47/31)	NR	NR	NR	NR	H (60/19)	NR	H (23/56)	NR	NR	NR	NR
		L (108/27)					L (64/51)		L (77/38)				
Kong 2019 [26]	NR	NR	NR	NR	NR	NR	NR	NR	H (90/82)	NR	NR	H (161/172)	H (152/172)
				A					L (79/86)			L (134/165)	L (112/165)
Mona 2020 [27]	NR	H (18/5)	NR	H (3/20)	H (10/13)	H (22/1)	NR	NR	NR	NR	NR	NR	NR
		L (31/6)		L (2/35)	L (11/26)	L (35/2)							
Pyo 2020 [28]	H (18/29)	NR	H (39/8)	H (14/33)	H (18/29)	H (47/0)	H (29/18)	NR	NR	NR	NR	H (34/47)	H (31/47)
	L (88/130)		L (171/47)	L (27/191)	L (127/91)	L (189/29)	L (88/130)					L (115/218)	L (105/218)

Note: NR - not reported, H - PD-L1 positive or high expression, L - PD-L1 negative or low expression.

Table 3. PD-L1 with the clinicopathological features of CRC.

Features	OR (95% CI)	p-value	P_{het}
T category	2.30 (1.85 - 2.87)	< 0.01	0.51
(T 1 + 2/T 3 + 4)			
Lymph node	1.48 (1.03 - 2.13)	0.03	0.01
(N 0/N 1)			
Metastasis	2.56 (1.81 - 3.64)	< 0.01	0.44
(M 0/M 1)			
Tumor size	1.50 (0.47 - 4.75)	0.49	< 0.01
(Small/large)			
Differentiation	0.97 (0.76 - 1.23)	0.78	0.32
(Well/poor)			
Grade	0.38 (0.23 - 0.62)	< 0.01	0.63
(Grade 1 + 2/Grade 3)			
TNM stage	1.93 (1.34 - 2.66)	< 0.01	0.05
(I + II/III + IV)			
MSI status	0.36 (0.25 - 0.52)	< 0.01	0.76
(Low/high)			
MMR status	0.50 (0.09 - 2.84)	0.43	< 0.01
(Proficient/deficient)			
BRAF mutation	0.71 (0.49 - 1.02)	0.047	0.59
(Wild/mutant)			
KRAS mutation	1.26 (0.92 - 1.73)	0.16	0.64
(Wild/mutant)			
5y-OS	1.36 (1.19 - 1.55)	< 0.01	0.76
DFS/RFS	1.22 (1.03 - 1.44)	0.02	0.77

Eight studies reported DFS or RFS. The pooled analysis revealed that PD-L1 overexpression in TILs was significantly associated with better DFS/RFS in a fixed-effects model (OR = 1.22, 95% CI = 1.03 - 1.44, p = 0.02) (Figure 2B).

Correlation of PD-L1 expression on TILs with clinicopathological features of CRC

Moreover, we investigated the relationship between PD-L1 expression on TILs and clinicopathological features of CRC including tumor size, tumor differentiation, tumor grade, T stage, lymph node invasion, distant metastasis, TNM stage, MSI status, MMR status, BRAF mutant, and KRAS mutant. The merged results demonstrated that PD-L1 overexpression on TILs suggested in CRC patients with lower T stage (OR = 2.30, 95% CI = 1.85 - 2.87, p < 0.01), less lymph node invasion (OR = 1.48, 95% CI = 1.03 - 2.13, p = 0.03, Figure 2C), less distant metastasis (OR = 2.56, 95% CI = 1.81 - 3.64, p < 0.01, Figure 2D), and earlier TNM stage (OR = 1.93, 95% CI = 1.34 - 2.66, p < 0.01). However, the merged results also demonstrated that PD-L1 overexpression on TILs suggested in CRC patients with later tumor grade (OR = 0.38, 95% CI = 0.23 - 0.62, p < 0.01) and high MSI status (OR = 0.36, 95% CI = 0.25 - 0.52, p < 0.01). There was no association between PD-L1 expression on TILs and tumor size, tumor differentiation, MMR status, BRAF mutant, and KRAS mutant. The detailed results are illustrated in Table 3.

Sensitivity analysis

Influence analysis was performed to assess the influence of each individual study on the pooled results by sequential deletion of a single study. The analysis suggested that no individual trial could significantly affect the pooled results (Figure 3); therefore, the results from this meta-analysis are credible.

Publication Bias

Potential publication bias was examined by Begg's test and Egger's test. The shapes of the funnel plots from Begg's test was symmetric (Figure 4), suggesting no

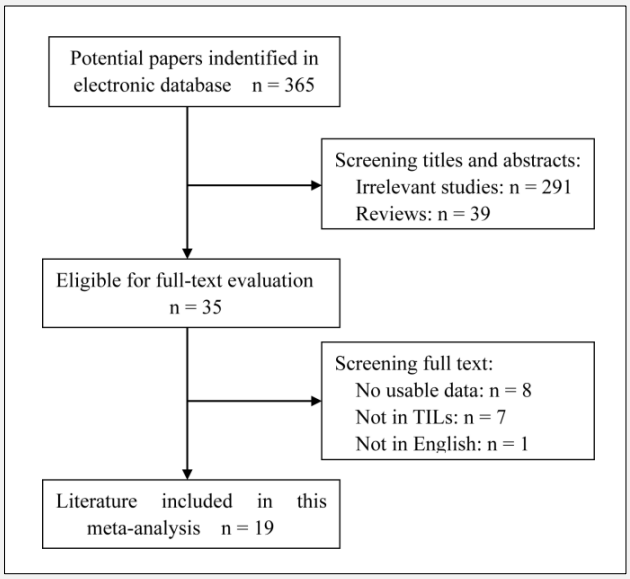

Figure 1. Flowchart of literature selection.

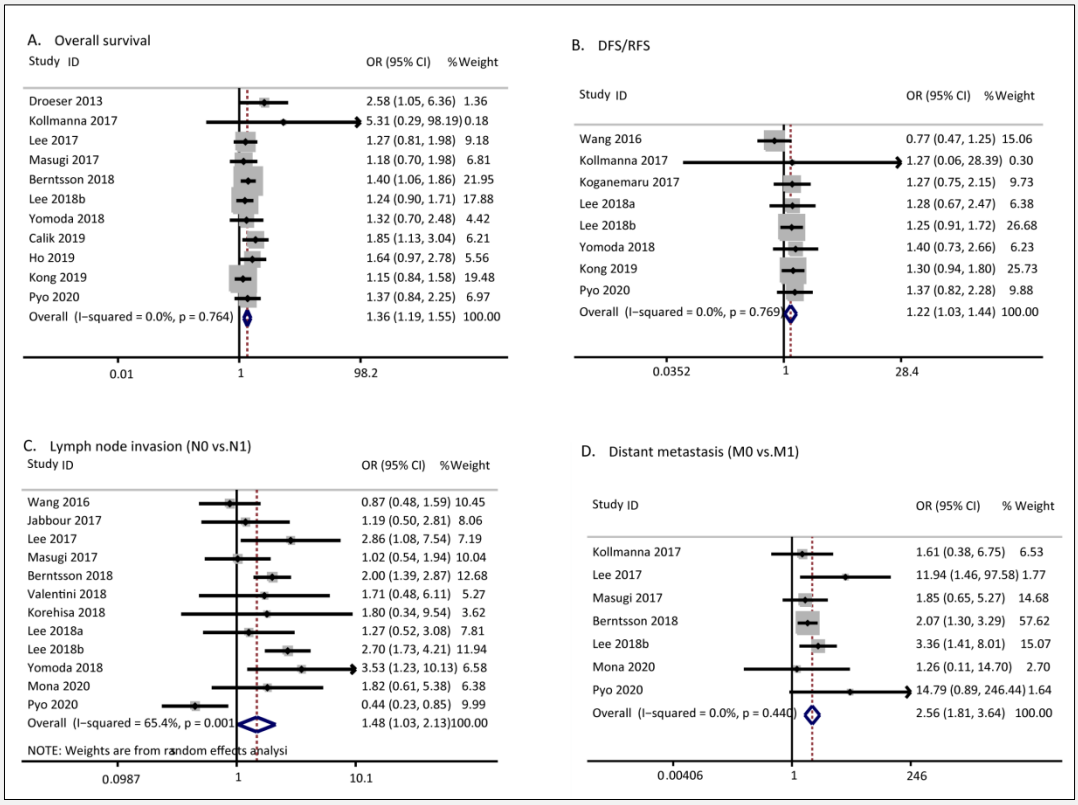

Figure 2. Forest plot about the association between PD-L1 expression on TILs and prognosis of CRC patients: A - OS, B - DFS/RFS, C - Lymph node invasion, D - Distant metastasis.

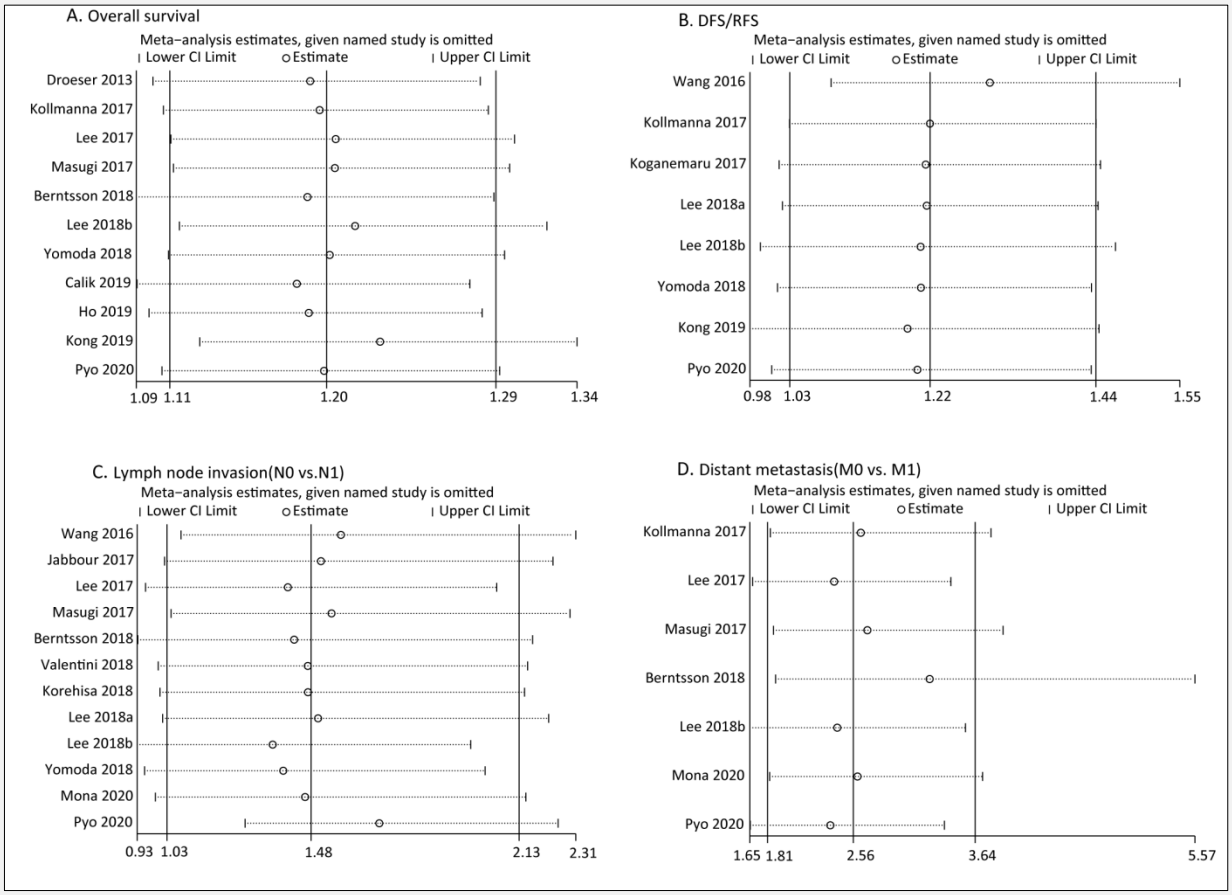

Figure 3. Influence analysis regarding A - OS, B - DFS/RFS, C - Lymph node invasion, D - Distant metastasis.

obvious publication bias. However, the p-value regarding 5-year-OS assessed by Egger's test was less than 0.05, indicating potential publication bias among these studies regarding the OR for 5-year OS rate.

DISCUSSION

PD-L1 has attracted more and more attention in the last decade. Previous studies mainly focused on the prognostic significance of PD-L1 expressed on tumor cells (TCs), and suggested expression of PD-L1 on TCs usually correlated with an obviously poor survival in various types of tumors [29-32]. Several meta-analyses have also confirmed that PD-L1 overexpression on CRC TCs was associated with worse survival [8,9,33]. In recent years, some studies suggested that not only tumor cells, but also tumor-infiltrating immune cells could express PD-L1, and recent studies indicated that the PD-L1 expression in TILs also played important roles in tumor immune escape and influenced tumor progression. A series of studies have also been conducted to explore the prognostic role of PD-L1 on TILs in CRC patients; however, the results were controversial. Therefore, we conducted this meta-analysis to clarify this important issue. There is a meta-analysis exploring the relationship between PD-L1 on TILs and cancers [34]. However, this meta-analysis is quite different from our study because it included all kinds of cancers and only one article is about PD-L1 and CRC.

Our meta-analysis included 19 studies with a total of 5,213 cases. Our data indicated that PD-L1 overexpression on TILs was associated with longer OS and longer DFS/RFS. Moreover, PD-L1 overexpression on TILs was also associated with lower T stage, less lymph node invasion, less distant metastasis and earlier TNM stage. The results from our meta-analysis are opposite from the results about PD-L1 expression on CRC TCs. However, our results are consistent from the studies about PD-L1 expression on TILs in other kinds of cancer. For

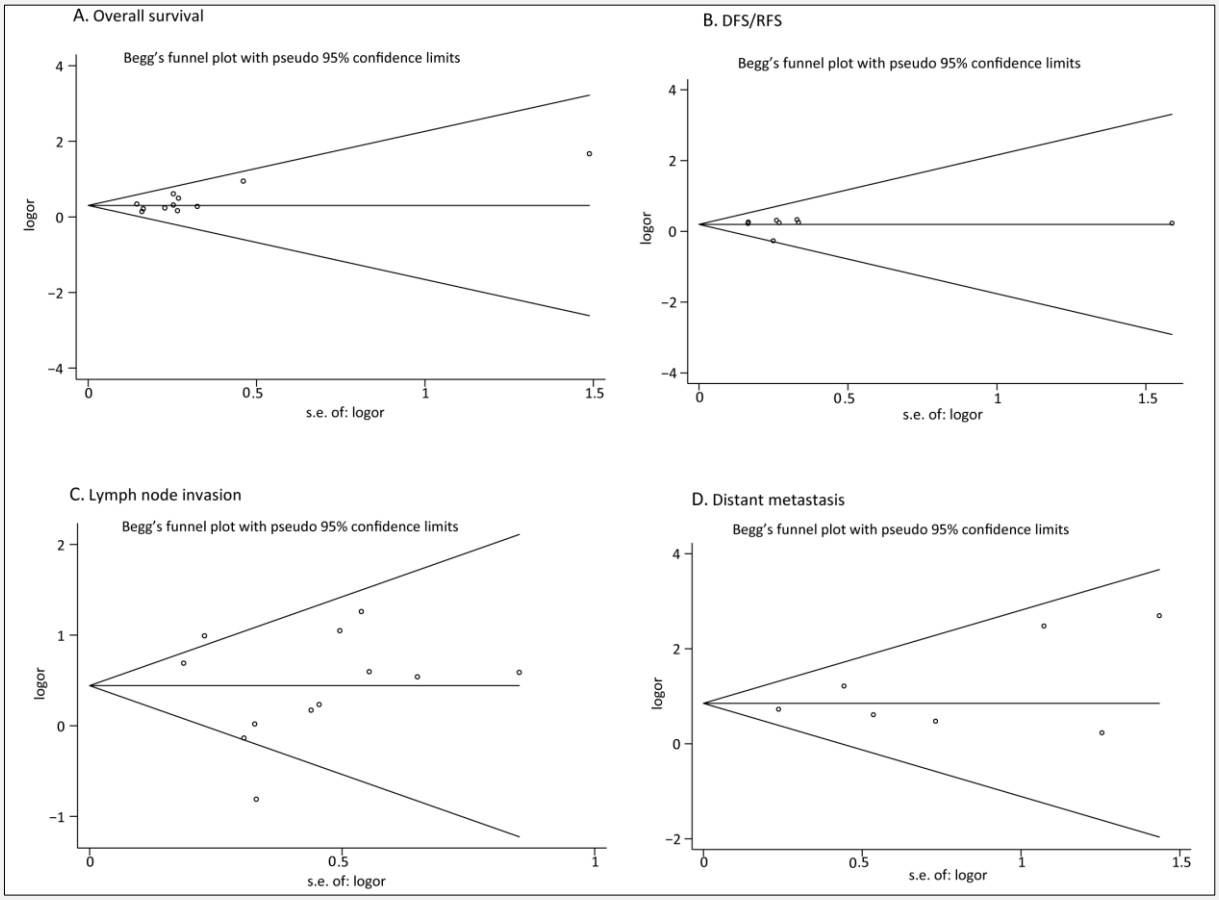

Figure 4. Begg's test for publication bias: A - OS, B - DFS/RFS, C - Lymph node invasion, D - Distant metastasis.

example, Huang's study found that PD-L1 expression on TILs related to better OS and DFS in primary breast cancer [31]. Chovanec's study suggested that testicular germ cell tumor patients with high infiltration of PD-L1 positive TILs had significantly better OS and PFS compared to patients with lower expression of PD-L1 [35]. Darb-Esfahani's study revealed that PD-L1 in TILs was a positive factor for OS and RFS of ovarian carcinoma [36].

The findings from our meta-analysis have further confirmed that PD-L1 expression on TILs has quite different roles compared to its expression on TCs. The results could be explained by the different mechanisms between PD-L1 expression in TCs and TILs. In general, when expressing on TCs, PD-L1 could induce anergy and apoptosis of PD-1 positive T cells by interfering with T cell receptor signal transduction, resulting in T cell immune tolerance and cancer tumor escape [37]. However, expression of PD-L1 on TILs may represent effective host immune responses in the presence of a favorable immune microenvironment abundant with CD4 and CD8 positive T cells, which may lead to restraining of tumor growth [38]. Furthermore, PD-L1 overexpression on TILs was positive correlated to the quantity of multiple TIL cells, such as CD4 and CD8 positive T lymphocytes. Since the high expression of these TILs was associated with better outcomes of cancer patients [39,40], the PD-L1 expression on TILs was possibly associated with better prognosis of cancer patients. However, the detailed function and mechanism of PD-L1 on TILs need to be further clarified. Tumor microenvironment could be classified into four types based on the status of PD-L1 and TILs, and type I is PD-L1 positive and TIL positive. This type of cancer patient is considered most likely to benefit from treatment with PD-1/PD-L1 immune checkpoint inhibitors [41]. Therefore, it is quite important to clarify the role and mechanism of PD-L1 expression on TILs.

Although this meta-analysis aimed to provide the best possible estimate of the correlation between the clinical

significance of PD-L1 on TILs in CRC, it may have several limitations. First, the sample size in each included study was relatively small, although we included 19 studies, the total sample size was only 5,213. Second, PD-L1 positivity was evaluated by using different antibody and cutoff values in each study; therefore, it may affect the sensitivity of IHC. Third, although most of the studies in this meta-analysis includes all stages of CRC patients, there are still some studies that only included CRC patients at a specific stage and only a few studies have considered the correlation of PD-L1 with MSI status, MMR status, KRAS mutation, and BRAF station, so the value of PD-L1 on TILs has not been fully explored. Forth, only studies published in English were included in this meta-analysis, publication bias may have occurred. In fact, the Egger's test has indicated potential publication bias regarding the results of OS.

CONCLUSION

In conclusion, our meta-analysis has demonstrated that the overexpression of PD-L1 on TILs indicates a better prognosis and clinicopathological features of CRC. Our results may provide some useful information when using PD-L1 expression to predict the survival of CRC patients and to select the beneficial CRC patients from PD-1/PD-L1 ICB treatment.

Acknowledgment:
This work was supported by the National Nature Science Foundation of China (81502015, 31970718, 81830081).

Declaration of Interest:
The authors report no competing financial interests.

References:

1. Bray F, Ferlay J, Soerjomataram I, Siegel RL, Torre LA, Jemal A. Global cancer statistics 2018: GLOBOCAN estimates of incidence and mortality worldwide for 36 cancers in 185 countries. CA Cancer J Clin 2018;68:394-424 (PMID: 30207593).

2. Feng RM, Zong YN, Cao SM, Xu RH. Current cancer situation in China: good or bad news from the 2018 Global Cancer Statistics? Cancer Commun (Lond) 2019;39:22 (PMID: 31030667).

3. Yu IS, Cheung WY. Metastatic Colorectal Cancer in the Era of Personalized Medicine: A More Tailored Approach to Systemic Therapy. Can J Gastroenterol Hepatol 2018;2018:9450754 (PMID: 30519549).

4. Patel SP, Kurzrock R. PD-L1 Expression as a Predictive Biomarker in Cancer Immunotherapy. Mol Cancer Ther 2015;14: 847-56 (PMID: 25695955).

5. Payandeh Z, Khalili S, Somi MH, et al. PD-1/PD-L1-dependent immune response in colorectal cancer. J Cell Physiol 2020 (PMID: 31960962).

6. Ju X, Zhang H, Zhou Z, Wang Q. Regulation of PD-L1 expression in cancer and clinical implications in immunotherapy. Am J Cancer Res 2020;10:1-11 (PMID: 32064150).

7. Zhu X, Zhang Q, Wang D, Liu C, Han B, Yang JM. Expression of PD-L1 Attenuates the Positive Impacts of High-level Tumor-infiltrating Lymphocytes on Prognosis of Triple-negative Breast Cancer. Cancer Biol Ther 2019;20:1105-12 (PMID: 30929569).

8. Yang L, Xue R, Pan C. Prognostic and clinicopathological value of PD-L1 in colorectal cancer: a systematic review and meta-analysis. Onco Targets Ther 2019;12:3671-82 (PMID: 31190 869).

9. Li Y, He M, Zhou Y, Yang C, et al. The Prognostic and Clinicopathological Roles of PD-L1 Expression in Colorectal Cancer: A Systematic Review and Meta-Analysis. Front Pharmacol 2019; 10:139 (PMID: 30873025).

10. Droeser RA, Hirt C, Viehl CT, et al. Clinical impact of programmed cell death ligand 1 expression in colorectal cancer. Eur J Cancer 2013;49:2233-42 (PMID: 23478000).

11. Wang L, Ren F, Wang Q, et al. Significance of Programmed Death Ligand 1 (PD-L1) Immunohistochemical Expression in Colorectal Cancer. Mol Diagn Ther 2016;20:175-81 (PMID: 268 91728).

12. Kollmann D, Schweiger T, Schwarz S, et al. PD1-positive tumor-infiltrating lymphocytes are associated with poor clinical outcome after pulmonary metastasectomy for colorectal cancer. Oncoimmunology 2017;6:e1331194 (PMID: 28932634).

13. El Jabbour T, Ross JS, Sheehan CE, et al. PD-L1 protein expression in tumour cells and immune cells in mismatch repair protein-deficient and -proficient colorectal cancer: the foundation study using the SP142 antibody and whole section immunohistochemistry. J Clin Pathol 2018;71:46-51 (PMID: 28667193).

14. Koganemaru S, Inoshita N, Miura Y, et al. Prognostic value of programmed death-ligand 1 expression in patients with stage III colorectal cancer. Cancer Sci 2017;108:853-8 (PMID: 28267 224).

15. Lee KS, Kwak Y, Ahn S, et al. Prognostic implication of CD274 (PD-L1) protein expression in tumor-infiltrating immune cells for microsatellite unstable and stable colorectal cancer. Cancer Immunol Immunother 2017;66:927-39 (PMID: 28405764).

16. Masugi Y, Nishihara R, Yang J,et al. Tumour CD274 (PD-L1) expression and T cells in colorectal cancer. Gut 2017;66:1463-73 (PMID: 27196573).

17. Berntsson J, Eberhard J, Nodin B, Leandersson K, Larsson AH, Jirstrom K. Expression of programmed cell death protein 1 (PD-1) and its ligand PD-L1 in colorectal cancer: Relationship with sidedness and prognosis. Oncoimmunology 2018;7:e1465165 (PMID: 30221062).

18. Valentini AM, Di Pinto F, Cariola F, et al. PD-L1 expression in colorectal cancer defines three subsets of tumor immune microenvironments. Oncotarget 2018;9:8584-96 (PMID: 29492219).

19. Korehisa S, Oki E, Iimori M, et al. Clinical significance of programmed cell death-ligand 1 expression and the immune microenvironment at the invasive front of colorectal cancers with high microsatellite instability. Int J Cancer 2018;142:822-32 (PMID: 29044503).

20. Lee SJ, Jun SY, Lee IH, et al. CD274, LAG3, and IDO1 expressions in tumor-infiltrating immune cells as prognostic biomarker for patients with MSI-high colon cancer. J Cancer Res Clin Oncol 2018;144:1005-14 (PMID: 29520442).

21. Lee KS, Kim BH, Oh HK, et al. Programmed cell death ligand-1 protein expression and CD274/PD-L1 gene amplification in colorectal cancer: Implications for prognosis. Cancer Sci 2018;109: 2957-69 (PMID: 29949671).

22. Yomoda T, Sudo T, Kawahara A, et al. The Immunoscore is a Superior Prognostic Tool in Stages II and III Colorectal Cancer and is Significantly Correlated with Programmed Death-Ligand 1 (PD-L1) Expression on Tumor-Infiltrating Mononuclear Cells. Ann Surg Oncol 2019;26:415-24 (PMID: 30569297).

23. Calik I, Calik M, Turken G, et al. Intratumoral Cytotoxic T-Lymphocyte Density and PD-L1 Expression Are Prognostic Biomarkers for Patients with Colorectal Cancer. Medicina (Kaunas) 2019; 55:723 (PMID: 31683723).

24. Ho HL, Chou TY, Yang SH, et al. PD-L1 is a double-edged sword in colorectal cancer: the prognostic value of PD-L1 depends on the cell type expressing PD-L1. J Cancer Res Clin Oncol 2019;145:1785-94 (PMID: 31129768).

25. Ahtiainen M, Wirta EV, Kuopio T, et al. Combined prognostic value of CD274 (PD-L1)/ PDCDI (PD-1) expression and immune cell infiltration in colorectal cancer as per mismatch repair status. Mod Pathol 2019;32:866-83 (PMID: 30723299).

26. Kong P, Wang J, Song Z, et al. Circulating Lymphocytes, PD-L1 Expression on Tumor-infiltrating Lymphocytes, and Survival of Colorectal Cancer Patients with Different Mismatch Repair Gene Status. J Cancer 2019;10:1745-54 (PMID: 31205530).

27. Elfishawy M, Abd ESA, Hegazy A, El-Yasergy DF. Immunohistochemical Expression of Programmed Death Ligand-1 (PDL-1) in Colorectal carcinoma and Its Correlation with Stromal Tumor Infiltrating Lymphocytes. Asian Pac J Cancer Prev 2020;21:225-32 (PMID: 31983188).

28. Pyo JS, Ko SH, Ko YS, Kim NY. Clinicopathological significance of PD-L1 expression in colorectal cancer: Impact of PD-L1 expression on pFOXO1 expression. Pathol Res Pract 2020;216: 152764 (PMID: 31836325).

29. Bastaki S, Irandoust M, Ahmadi A, et al. PD-L1/PD-1 axis as a potent therapeutic target in breast cancer. Life Sci 2020;247: 117437 (PMID: 32070710).

30. Zhu L, Sun J, Wang L, Li Z, Wang L, Li Z. Prognostic and Clinicopathological Significance of PD-L1 in Patients With Bladder Cancer: A Meta-Analysis. Front Pharmacol 2019;10:962 (PMID: 31616289).

31. Huang W, Ran R, Shao B, Li H. Prognostic and clinicopathological value of PD-L1 expression in primary breast cancer: a meta-analysis. Breast Cancer Res Treat 2019;178:17-33 (PMID: 31359 214).

32. Wang L. Prognostic effect of programmed death-ligand 1 (PD-L1) in ovarian cancer: a systematic review, meta-analysis and bioinformatics study. J Ovarian Res 2019;12:37 (PMID: 31039 792).

33. Shen Z, Gu L, Mao D, Chen M, Jin R. Clinicopathological and prognostic significance of PD-L1 expression in colorectal cancer: a systematic review and meta-analysis. World J Surg Oncol 2019; 17:4 (PMID: 30609938).

34. Kim Y, Wen X, Cho NY, Kang GH. Intratumoral immune cells expressing PD-1/PD-L1 and their prognostic implications in cancer: a meta-analysis. Int J Biol Markers 2018;1724600818770941 (PMID: 29779430).

35. Chovanec M, Cierna Z, Miskovska V, et al. Prognostic role of programmed-death ligand 1 (PD-L1) expressing tumor infiltrating lymphocytes in testicular germ cell tumors. Oncotarget 2017;8: 21794-805 (PMID: 28423520).

36. Darb-Esfahani S, Kunze CA, Kulbe H, et al. Prognostic impact of programmed cell death-1 (PD-1) and PD-ligand 1 (PD-L1) expression in cancer cells and tumor-infiltrating lymphocytes in ovarian high grade serous carcinoma. Oncotarget 2016;7:1486-99 (PMID: 26625204).

37. Kuol N, Stojanovska L, Nurgali K, Apostolopoulos V. PD-1/PD-L1 in disease. Immunotherapy 2018;10:149-60. (PMID: 29260 623).

38. Yang CY, Lin MW, Chang YL, Wu CT, Yang PC. Programmed cell death-ligand 1 expression is associated with a favourable immune microenvironment and better overall survival in stage I pulmonary squamous cell carcinoma. Eur J Cancer 2016;57:91-103 (PMID: 26901614).

39. Zeng DQ, Yu YF, Ou QY, et al. Prognostic and predictive value of tumor-infiltrating lymphocytes for clinical therapeutic research in patients with non-small cell lung cancer. Oncotarget 2016;7: 13765-81 (PMID: 26871598).

40. Hwang WT, Adams SF, Tahirovic E, Hagemann IS, Coukos G. Prognostic significance of tumor-infiltrating T cells in ovarian cancer: a meta-analysis. Gynecol Oncol 2012;124:192-8 (PMID: 22040834).

41. Wang Q, Wu X. Primary and acquired resistance to PD-1/PD-L1 blockade in cancer treatment. Int Immunopharmacol 2017;46: 210-9 (PMID: 28324831).