



# Characterization of PD-L1 protein expression and CD8<sup>+</sup> tumor-infiltrating lymphocyte density, and their associations with clinical outcome in small-cell lung cancer

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**Background:** This study aimed to characterize programmed death ligand-1 (PD-L1) expression and CD8<sup>+</sup> tumor-infiltrating lymphocytes (TILs) density, and their impact on survival in patients with surgically resected small-cell lung cancer (SCLC).

**Methods:** Fifty-six patients with surgically resected SCLC were included. PD-L1 protein expression and CD8<sup>+</sup> TILs were tested by immunohistochemistry. A meta-analysis of 15 articles with 1,505 patients that investigated the prevalence and prognostic significance of PD-L1 expression in SCLC was conducted.

**Results:** Twenty-two (39.3%) patients had positive PD-L1 protein expression and 42 (75.0%) had high CD8<sup>+</sup> TILs density. PD-L1 expression level was not associated with CD8<sup>+</sup> TILs density ( $P=0.528$ ). No any association between clinicopathological features and PD-L1 expression level or CD8<sup>+</sup> TILs density was observed. Positive PD-L1 expression [hazard ratio (HR) =0.374,  $P=0.002$ ] and high CD8<sup>+</sup> TILs density (HR =0.429,  $P=0.008$ ) were independently associated with significantly longer overall survival (OS), which remain the statistical significance in multivariate analyses ( $P=0.007$ ,  $P=0.002$ ; respectively). Meta-analysis showed that the prevalence of positive PD-L1 expression was 0.35 [95% confidence interval (CI), 0.22–0.48] and positive PD-L1 expression was correlated with markedly longer OS (HR =0.61; 95% CI, 0.31–0.91) in patients with SCLC.

**Conclusions:** The prevalence of PD-L1 expression in surgically resected SCLC is lower than that published for NSCLC. There was no association between PD-L1 expression or CD8<sup>+</sup> TILs density and clinicopathological parameters. PD-L1 expression and CD8<sup>+</sup> TILs density was independently correlated with better outcome in patients with SCLC.

**Keywords:** Small-cell lung cancer (SCLC); programmed death ligand-1 (PD-L1); CD8; survival

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## Introduction

Small-cell lung cancer (SCLC) accounts for approximately 15% of all lung cancers (1,2). The high aggressiveness and early widespread metastasis of SCLC result in the majority of patients being diagnosed with extensive-stage disease (ES-SCLC) (1-3). Therapeutic strategies have not substantially changed in more than 40 years. The median overall survival (OS) for early stage (I-III) SCLC is 15–20 months and for ES-SCLC is 9–11 months; the 5-year survival rate is 20–25% for early-stage SCLC and only about 2–6% for ES-SCLC (4-7). In spite of a high response rate with initial platinum-based chemotherapy, almost all patients with ES-SCLC will subsequently relapse after a short period of response (1-4).

Immune checkpoint inhibitors such as cytotoxic T lymphocyte antigen-4 (CTLA-4), programmed death-1 (PD-1) and its ligand (PD-L1) could significantly enhance the antitumor immunity (8-11). A number of clinical trials demonstrated that anti-PD-1/PD-L1 antibodies monotherapy could only show a response rate of nearly 20% in non-small cell lung cancer (NSCLC) (12-16), but this strategy only showed very limited efficacy in pre-treated SCLC (17). The combination of anti-CTLA-4 and anti-PD-1 antibody showed promising results with the 2-year OS rate of ~30% (18,19). Moreover, a recent randomized phase III trial (IMpower133) demonstrated a markedly prolonged progression-free survival (PFS) and OS with atezolizumab plus etoposide and carboplatin than with placebo plus etoposide and carboplatin (20), which has become a new standard of care in the first-line setting of ES-SCLC. Nevertheless, immune checkpoints inhibitors alone or combinations is still challenging due to their modest antitumor activities in SCLC.

Emerging evidence suggest that PD-L1 expression and pre-existing anti-tumor immunity [such as CD8<sup>+</sup> tumor-infiltrating lymphocytes (TILs)] play a significant role in the clinical activity of anti-PD-1/PD-L1 immunotherapy (11,21,22). Although a large number of studies characterize PD-L1 expression and CD8<sup>+</sup> TILs in NSCLC, the reported data on SCLC is limited. Furthermore, published results are inconsistent on the prevalence and prognostic significance of PD-L1 expression and CD8<sup>+</sup> TILs in SCLC. The investigation of PD-L1 expression and CD8<sup>+</sup> TILs characterization in relation to clinicopathological features and clinical outcomes in SCLC may guide the development of new treatment strategies, by providing novel stratification parameters for therapeutic selection and the future design

of clinical trials with immune checkpoints inhibitors. Therefore, we conducted this study with 56 surgically resected SCLC and a meta-analysis of 15 publications with 1,505 patients to systematically characterize PD-L1 expression and CD8<sup>+</sup> TIL, and their impacts on clinical outcome in patients with SCLC.

## Methods

### *Patients' selection*

We retrospectively screened patients who underwent surgical resection, palliative operation or open biopsy due to the histologically-confirmed SCLC, between 2012 and 2015, at three hospitals. Patient clinicopathological parameters including age, gender, smoking history, tumor location, pathological stage, pathological lymph nodal factors, pleural invasion, lymphatic invasion, and vascular invasion were recorded. Pathological staging was performed using the 7<sup>th</sup> edition of the TNM Classification of Malignant Tumors (23,24). A person who has smoked fewer than 100 cigarettes during their lifetime was defined as never smoker. For postoperative chemotherapy (POCT), cisplatin/carboplatin plus etoposide was administrated (4–6 cycles). Postoperative thoracic irradiation (PORT) with a total dose of 50–60 Gy with 1.8–2.0 Gy per fraction for 5 days per week was administered. For patients without brain metastasis identified by brain magnetic resonance imaging (MRI) prior to prophylactic cranial irradiation (PCI), a total dose of 25 Gy with 2.5 Gy per fraction, or a total dose of 30 Gy with 3.0 Gy per fraction was administrated. The exclusion criteria included histologically-confirmed mixed SCLC, patients with inadequate samples for PD-L1 and CD8 staining or who disagreed with the research protocols. This study was approved by the ethics committee of Shanghai Pulmonary Hospital (FK-17-0113) and conducted in line with the provisions of the Declaration of Helsinki.

### *PD-L1 protein expression analysis*

PD-L1 protein expression was evaluated in patients with SCLC by immunohistochemistry (IHC) as described in our previous studies (25,26). Briefly, tumor sections of formalin-fixed and paraffin-embedded (FFPE) samples were cut at widths of 4–5 μm, dewaxed with xylene, and rehydrated through a graded series of ethanol. Next, the sections were incubated with 3% H<sub>2</sub>O<sub>2</sub> (10 minutes), blocked with 5% goat serum, and incubated with an anti-human

PD-L1 antibody (diluted 1:100; #13684, clone E1L3N, Cell Signaling Technology). Then, a peroxidase-labeled secondary antibody was applied to the sections (30 minutes) at room temperature. All immunohistochemical images were assessed by two pathologists (Z Dong and L Hou). The cut-off point for PD-L1 positive/negative expression was 5% (25-28).

### **CD8<sup>+</sup> TIL density assessment**

CD8<sup>+</sup> TIL density assessment was performed according to the previous reports (25,26,29,30). IHC for CD8<sup>+</sup> TIL density was conducted on the fully automated Bond-III system (Leica Microsystems, Newcastle-upon-Tyne, UK) by using onboard heat-induced antigen retrieval with epitope retrieval solution 2 for 10 minutes at 99 °C, and then, incubated with a mouse anti-CD8 monoclonal antibody (M7103, clone C8144B, DAKO, Denmark) for 30 minutes at room temperature. This automated system utilized a Refine polymer detection kit with horseradish peroxidase (HRP)-polymer as a secondary antibody and DAB. All immunohistochemical images were also evaluated by two senior pathologists (Z Dong and L Hou). The cut-off value for high/low CD8<sup>+</sup> TIL density was 5%.

### **Systematic review with meta-analysis**

We then performed a literature review of publication search via the online databases including PubMed/Medline, Cochrane Library, EMBASE, Web of Science, and Google Scholar through June 2019, using “lung cancer” and “PD-L1”, and their corresponding keywords. Data on the association between PD-L1 expression and clinical outcomes, and clinicopathological features in patients with SCLC were identified from published articles meeting the inclusion criteria (Figure S1). The details of methodology are summarized in the Supplementary file 1.

### **Statistical analyses**

Chi-square or Fisher's exact tests were used to assess the associations between PD-L1 expression or CD8<sup>+</sup> TILs density and clinicopathological characteristics. Continuous variables were analyzed by analysis of variance and Tukey's multiple comparison tests. Kaplan-Meier curves were utilized to evaluate patients' outcomes, and the log-rank tests were used to assess the significance of differences

among groups. Cox proportional hazards models were leveraged for uni- and multivariate analyses to calculate the hazard ratios (HRs) and corresponding 95% confidence intervals (CIs). The OS was calculated from the date of SCLC diagnosis to death from any cause or was censored at the last follow-up date.  $P < 0.05$  (two-sided) were considered statistically significant. Meta-analysis was performed using Stata version 14.0 (Stata Corporation, TX, USA). All statistical analyses were conducted using IBM SPSS Statistics v22.0 (IBM Corp., Armonk, NY, USA).

## **Results**

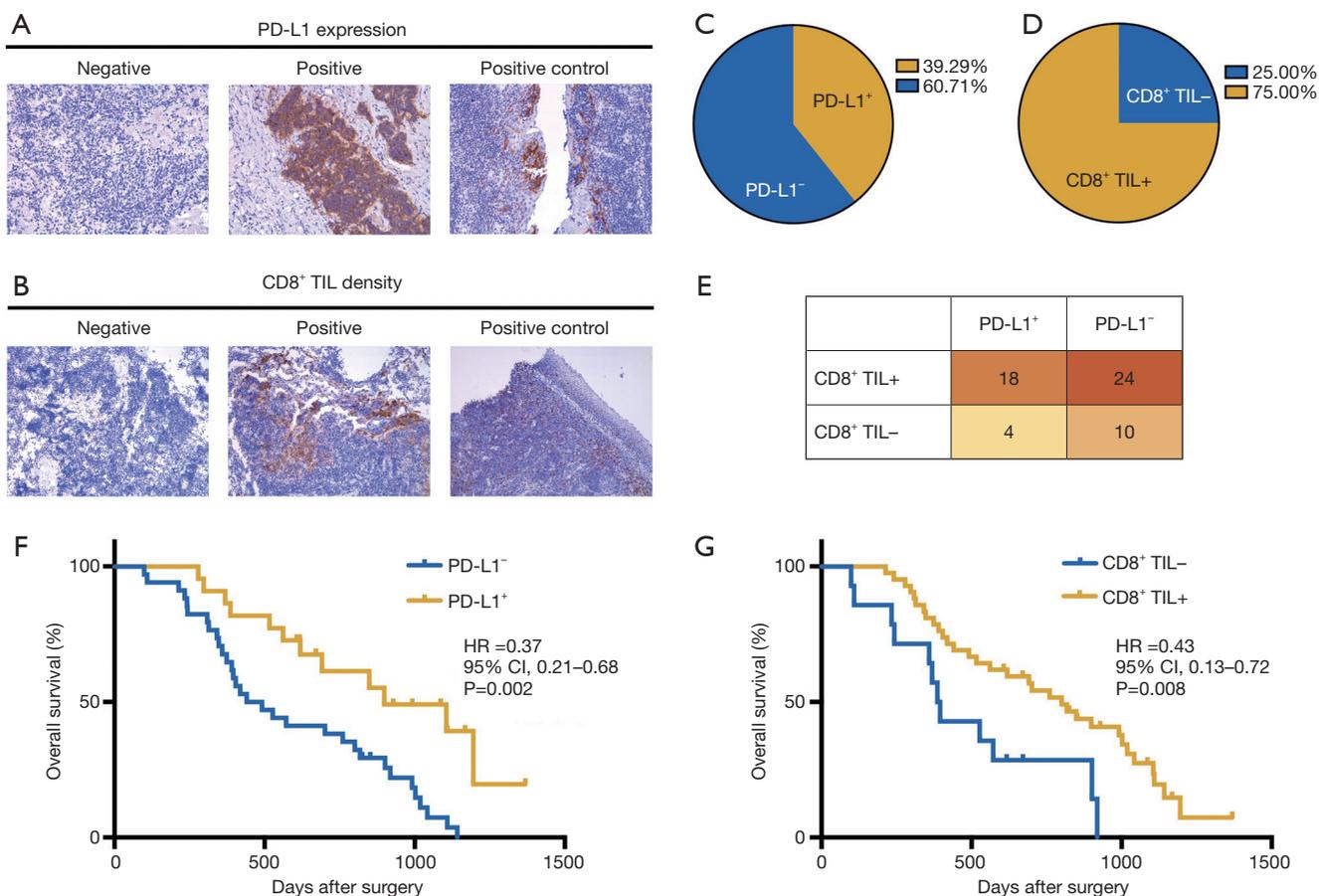
### **Characterization of PD-L1 expression and CD8<sup>+</sup> TIL density**

Fifty-six patients were finally analyzed. Most of them (75.0%) were <65 years old at initial diagnosis. Seven (12.5%) of them were female and 5 (8.9%) were never-smokers. Thirty-one (55.4%) patients were diagnosed with pathological stage I–II. Most (85.7%) of them had central tumor location. Seven (12.5%) of the patients had pleural invasion and none had vascular invasion. Twenty-eight (50.0%) patients received POCT, and 6 (10.7%) received PORT.

Representative images of PD-L1 protein expression and CD8<sup>+</sup> TILs are listed in Figure 1A and Figure 1B. Twenty-two (39.3%) patients had positive PD-L1 expression (Figure 1C) and 42 (75.0%) had high CD8<sup>+</sup> TIL density (Figure 1D). The clinicopathological features of all included patients are listed in Table 1. PD-L1 expression level was not associated with CD8<sup>+</sup> TILs density ( $P = 0.528$ ; Figure 1E). No significant differences in PD-L1 expression including age ( $P = 0.114$ ), sex ( $P = 0.535$ ), smoking history ( $P = 0.656$ ), pathologic stages (I vs. II/III,  $P = 0.586$ ), lymph node metastasis ( $P = 0.153$ ), tumor location (peripheral vs. central,  $P = 0.780$ ), pleural invasion ( $P = 0.535$ ), POCT ( $P = 0.101$ ) and PORT ( $P = 0.312$ ) were observed. Of note, patients received PCI had higher proportion of positive PD-L1 expression than those without PCI ( $P = 0.041$ ). There were no significant differences in CD8<sup>+</sup> TIL density in terms of all listed clinicopathological features (Table 1).

### **Prognostic value of PD-L1 protein expression and CD8<sup>+</sup> TILs density**

The median follow-up time was 618 days (range, 99–1,369 days). Kaplan-Meier curves indicated that positive



**Figure 1** Characterization of PD-L1 protein expression and CD8<sup>+</sup> TILs density, and their correlations with prognosis. (A) Representative images of immunohistochemistry for PD-L1 expression (×200); (B) representative images of immunohistochemistry for CD8<sup>+</sup> TILs (×200); (C) distribution of PD-L1 expression; (D) distribution of CD8<sup>+</sup> TILs; (E) correlation between PD-L1 expression and CD8<sup>+</sup> TILs density; (F) prognostic value of PD-L1 expression; (G) prognostic value of CD8<sup>+</sup> TILs density. PD-L1, programmed death ligand-1; TILs, tumor-infiltrating lymphocytes; HR, hazard ratio; CI, confidence interval.

PD-L1 expression was associated with a significantly longer OS (HR = 0.37, 95% CI: 0.21–0.68; P = 0.002; *Figure 1F*). High CD8<sup>+</sup> TIL density was correlated with longer OS (HR = 0.43, 95% CI: 0.13–0.72; P = 0.008) (*Figure 1G*). Univariate analysis found age (HR = 0.416, P = 0.031), TNM stage (HR = 5.105, P < 0.001), T stage (HR = 2.182, P = 0.014), lymph node metastasis (HR = 1.926, P = 0.045) were also associated with prolonged OS (*Table 2*). Multivariate analyses showed that positive PD-L1 expression (HR = 0.352, 95% CI: 0.166–0.748; P = 0.007) and high CD8<sup>+</sup> TILs density (HR = 0.261, 95% CI: 0.114–0.601; P = 0.002) were independently associated with significantly longer OS (*Table 2*). We further divided the population into four groups according to PD-L1 expression and CD8<sup>+</sup> TIL density. Patients with negative

PD-L1 expression and low CD8<sup>+</sup> TILs density had the shortest OS (HR = 0.36, P = 0.003), while the positive PD-L1 expression and high CD8<sup>+</sup> TIL density group had the longest OS (HR = 0.34, P = 0.001) (*Figure S2*).

### Features of included studies in the meta-analysis

The detailed methodology of meta-analysis is summarized in Supplementary file 1 and *Figure S1*. Totally, 103 relevant publications were screened. The majority of the excluded publications were reviews, comments, duplications, or studies with incomplete data. A flowchart of publication selection was shown in *Figure S3*. The present study included 1,505 cases from 15 articles to investigate the

**Table 1** Baseline characteristics of included patients

Factor	Category	Total, n	PD-L1					CD8 <sup>+</sup> TIL				
			PD-L1 <sup>+</sup>		PD-L1 <sup>-</sup>		P value	High		Low		P value
			N	%	N	%		N	%	N	%	
Age	<65	42	14	33.3	28	66.7	0.114	32	76.2	10	23.8	0.999
	≥65	14	8	57.1	6	42.9		10	71.4	4	28.6	
Sex	Male	49	18	36.7	31	63.3	0.535	35	71.4	14	28.6	0.243
	Female	7	4	57.1	3	42.9		7	100.0	0	0.0	
Smoking	Never	5	2	40.0	3	60.0	0.656	5	100.0	0	0.0	0.417
	Current/former	51	20	39.2	31	60.8		37	72.5	14	27.5	
p-Stage	I	18	8	44.4	10	55.6	0.586	16	88.9	2	11.1	0.186
	II	13	4	30.8	9	69.2		11	84.6	2	15.4	
	III	25	10	40.0	15	60.0		15	60.0	10	40.0	
pT status	T1	6	4	66.7	2	33.3	0.312	6	100.0	0	0.0	0.318
	T2	26	10	38.5	16	61.5		22	84.6	4	15.4	
	T3	16	6	37.5	10	62.5		12	75.0	4	25.0	
	T4	8	2	25.0	6	75.0		2	25.0	6	75.0	
pN status	N0	29	14	48.3	15	51.7	0.153	21	72.4	8	27.6	0.643
	N1	6	0	0.0	6	100.0		4	66.7	2	33.3	
	N2	21	8	38.1	13	61.9		17	81.0	4	19.0	
Tumor location	Peripheral	8	4	50.0	4	50.0	0.780	8	100.0	0	0.0	0.186
	Central	48	18	37.5	30	62.5		34	70.8	14	29.2	
Pleural invasion	Yes	7	4	57.1	3	42.9	0.535	5	71.4	2	28.6	0.816
	No	49	18	36.7	31	63.3		37	75.5	12	24.5	
Vascular invasion	Yes	0	0	0.0	0	0.0	-	0	0.0	0	0.0	-
	No	56	22	39.3	34	60.7		42	75.0	14	25.0	
POCT	Yes	28	14	50.0	14	50.0	0.101	24	85.7	4	14.3	0.123
	No	28	8	28.6	20	71.4		18	64.3	10	35.7	
PORT	Yes	6	4	66.7	2	33.3	0.312	6	100.0	0	0.0	0.318
	No	50	18	36.0	32	64.0		36	72.0	14	28.0	
PCI	Yes	4	4	100.0	0	0.0	0.041	2	50.0	2	50.0	0.549
	No	52	18	34.6	34	65.4		40	76.9	12	23.1	

p, pathological; T, tumor; N, lymph node; PD-L1, programmed death ligand-1; TIL, tumor-infiltrating lymphocyte; POCT, postoperative chemotherapy; PORT, postoperative radiotherapy; PCI, prophylactic cranial irradiation.

**Table 2** Univariate and multivariate analyses of clinicopathological parameters on overall survival

Factor	Univariate analysis			Multivariate analysis		
	HR (log rank)	95% CI	P value	HR (log rank)	95% CI	P value
Sex (male/female)	1.454	0.570–3.710	0.433	–	–	–
Age (<65/≥65)	0.416	0.187–0.922	0.031	0.790	0.330–1.889	0.596
Smoking (yes/no)	1.144	0.448–2.920	0.779	–	–	–
TNM (II–III/I)	5.105	2.240–11.634	<0.001	18.125	4.099–80.146	<0.001
T stage (3–4/1–2)	2.182	1.168–4.076	0.014	3.953	1.447–10.753	0.007
N stage (1–2/0)	1.926	1.014–3.661	0.045	1.957	0.813–4.717	0.134
Pleural invasion (yes/no)	1.679	0.639–4.412	0.293	–	–	–
Location (central/peripheral)	2.189	0.775–6.182	0.139	–	–	–
POCT (no/yes)	1.205	0.662–2.197	0.542	–	–	–
PORT (no/yes)	1.955	0.821–4.651	0.130	–	–	–
PD-L1 (positive/negative)	0.374	0.211–0.683	0.002	0.352	0.166–0.748	0.007
CD8 <sup>+</sup> TIL (positive/negative)	0.429	0.134–0.722	0.008	0.261	0.114–0.601	0.002

HR, hazard ratio; CI, confidence interval; PD-L1, programmed death ligand-1; TIL, tumor-infiltrating lymphocyte; POCT, postoperative chemotherapy; PORT, postoperative radiotherapy.

prevalence of positive PD-L1 expression and its prognostic value in patients with SCLC (31–45). The main features of each study are summarized in *Table 3* and *Table S1*.

#### ***Prevalence of positive PD-L1 expression and its prognostic value***

Meta-analysis showed that the prevalence of positive PD-L1 expression was 0.35 (95% CI: 0.22–0.48; *Figure 2*) and positive PD-L1 expression was correlated with significantly better OS (HR = 0.61, 95% CI: 0.31–0.91;  $P < 0.05$ ; *Figure 3*). But both results exhibited high heterogeneity ( $I^2 = 97.7\%$ ,  $P < 0.001$ ;  $I^2 = 93.8\%$ ;  $P < 0.001$ ; respectively).

#### ***Publication bias***

Sensitivity analysis was performed by deleting one study at one time to evaluate its effect on the pooled HRs. Deletion of the publication by Xu *et al.* or Zhao *et al.* slightly decreased the heterogeneity in the analysis of pooled HRs of PFS and OS (43,44). No other studies influenced the pooled results. Begg's funnel plots and Egger's tests were utilized to assess the publication bias. The Begg's funnel plot was symmetric, and Egger's tests suggested no evidence of publication bias (*Figure S4*).

## **Discussion**

Accumulating evidence indicated that blockade of PD-1/PD-L1 interaction yielded a narrow antitumor effect in patients with ES-SCLC when compared with NSCLC. As the most important factors of antitumor immune response, PD-L1 expression and CD8<sup>+</sup> TIL often determines whether anti-PD-1/PD-L1 antibodies works or not in various solid tumors (46,47). Understanding of PD-L1 expression and CD8<sup>+</sup> TIL in SCLC could contribute to the research and development of more effective immune checkpoints blockade therapy. Furthermore, clarifying the prognostic value of PD-L1 expression and CD8<sup>+</sup> TILs density in patients with SCLC would be helpful to precisely choose the sub-populations who could most benefit from anti-PD-1/PD-L1 antibodies therapy. In order to achieve these aims, the present study investigated the clinicopathological parameters of PD-L1 expression and CD8<sup>+</sup> TIL density, and their correlations with clinical outcome in patients with surgically resected SCLC. The current results showed that the prevalence of PD-L1 expression in surgically resected SCLC is lower than that published for NSCLC. There was no any association between PD-L1 expression or CD8<sup>+</sup> TIL density and clinicopathological parameters in SCLC. Positive PD-L1 expression and high CD8<sup>+</sup> TIL density was

**Table 3** Baseline characteristics of included studies

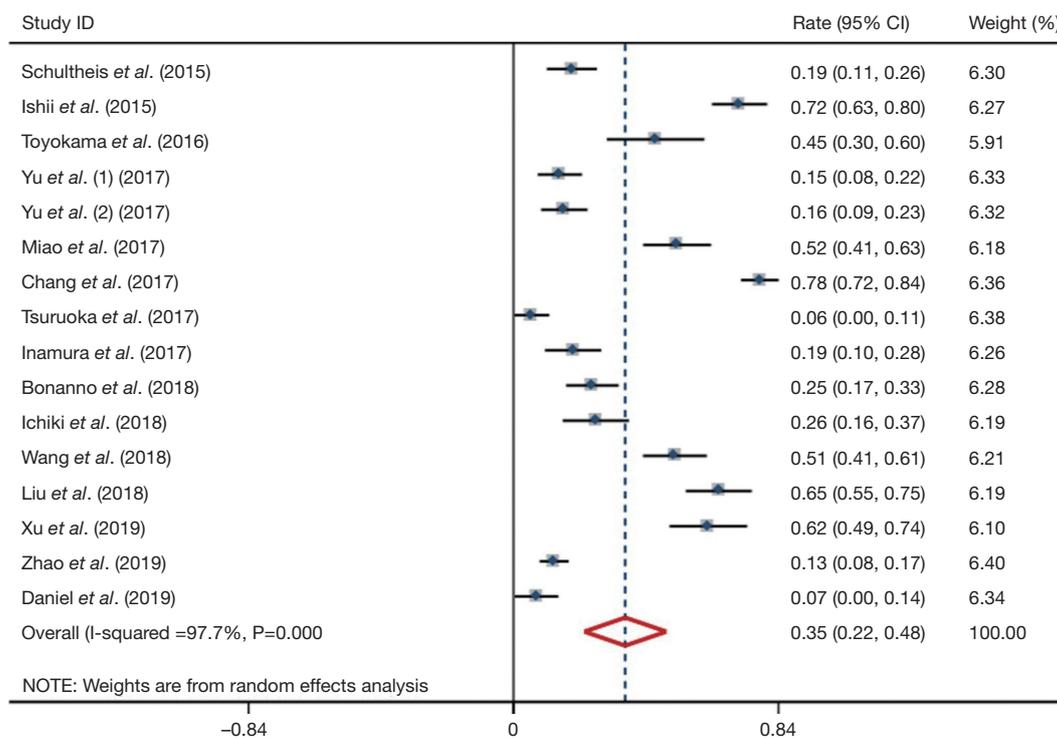
Author	Year	No. of cases	No. of >60 years	No. of male	No. of smoker	No. of ES-SCLC	Positive rate	PD-L1 IHC assay	Cutoff value	Study endpoints
Schultheis <i>et al.</i>	2015	92	–	–	–	–	0.185	5H1 and E1L3N	Staining intensity H-score $\geq 1$	None
Ishii <i>et al.</i>	2015	102	51	89	–	61	0.716	Abcam	$\geq 5\%$ of PD-L1 expression	OS
Toyokama <i>et al.</i>	2016	40	21	34	36	5	0.450	Clone SP142	$\geq 5\%$ of TCs or ICs stained for PD-L1	OS and DFS
Yu <i>et al.</i> [1]	2017	98	–	57	–	0	0.147	Clone SP142 and SP28-8	Tumor proportion score $\geq 1\%$	OS and PFS
Yu <i>et al.</i> [2]	2017	96	–	46	92	96	0.161	Clone SP142 and SP28-8	Tumor proportion score $\geq 1\%$	OS
Miao <i>et al.</i>	2017	83	8	72	–	36	0.518	Clone SP142	$\geq 5\%$ of TCs or ICs stained for PD-L1	OS
Chang <i>et al.</i>	2017	186	49	167	173	112	0.780	Cat. no. 66248-1-Ig	$\geq 5\%$ of PD-L1 expression	OS
Tsuruoka <i>et al.</i>	2017	69	–	–	–	–	0.058	E1L3N	Staining intensity H-score $\geq 1$	OS and DFS
Inamura <i>et al.</i>	2017	74	–	–	–	–	0.189	E1L3N	Staining intensity H-score $\geq 1$	OS and DFS
Bonanno <i>et al.</i>	2018	104	–	72	99	38	0.250	Clone 22C3	$\geq 1\%$ of TCs for PD-L1	OS
Ichiki <i>et al.</i>	2018	67	30	55	–	34	0.261	E1L3N	Staining intensity H-score $\geq 1$	OS
Wang <i>et al.</i>	2018	94	–	69	–	52	0.511	Clone SP142	$\geq 5\%$ of TCs or $\geq 1\%$ of ICs stained for PD-L1	OS and PFS
Liu <i>et al.</i>	2018	80	–	45	–	0	0.650	Clone SP142	$\geq 5\%$ of TCs or ICs stained for PD-L1	OS
Xu <i>et al.</i>	2019	60	–	43	–	40	0.617	Cat. no. 66248-1-Ig	$\geq 5\%$ of PD-L1 expression	OS
Zhao <i>et al.</i>	2019	205	101	164	129	103	0.129	Clone 22C3	$\geq 50\%$ of TCs for PD-L1	OS
Daniel <i>et al.</i>	2019	55	37	32	–	32	0.073	E1L3N	Staining intensity H-score $\geq 1$	None

No., number; ES-SCLC, extensive-stage small cell lung cancer; PD-L1, programmed death ligand-1; IHC, immunohistochemistry; OS, overall survival; PFS, progression-free survival; DFS, disease-free survival; TCs, tumor cells; ICs, immune cells.

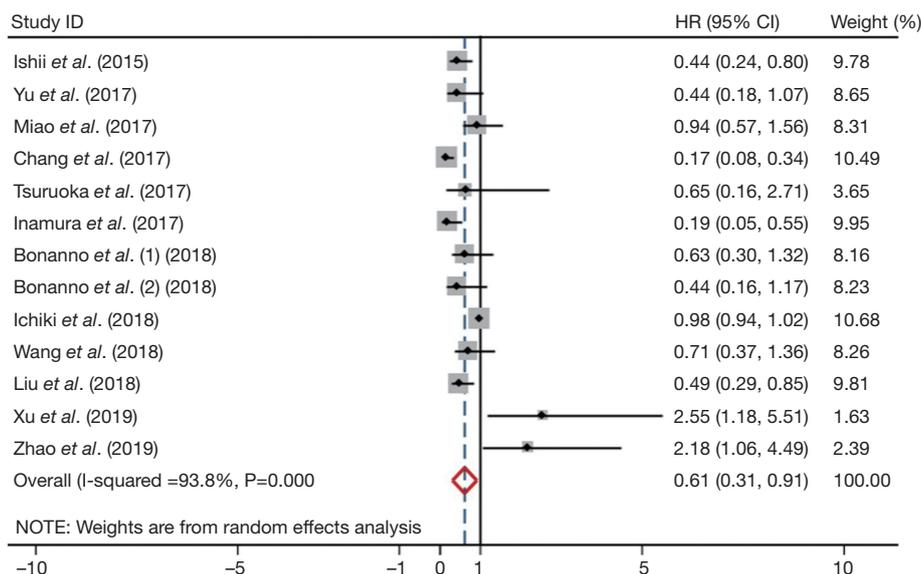
independently correlated with better prognosis in patients with SCLC, and PD-L1 expression plus CD8<sup>+</sup> TIL density could more precisely differentiate sub-populations with discrepant OS after surgical resection. Moreover, a meta-analysis of 15 published articles with 1,505 cases confirmed the lower prevalence and prognostic value of PD-L1

expression in patients with SCLC.

The prevalence of positive PD-L1 expression was 39.3% and 35.0% in the pooled analysis, which was lower than that reported in NSCLC (25,48-51). Although the detection of PD-L1 expression was influenced by a multitude of factors including laboratory conditions, testing platform and



**Figure 2** Meta-analysis of the prevalence of PD-L1 expression from all included publications. PD-L1, programmed death ligand-1; CI, confidence interval.



**Figure 3** Meta-analysis of the prognostic value of positive PD-L1 expression from all included studies. PD-L1, programmed death ligand-1; CI, confidence interval.

process, PD-L1 antibody assay and so on, most of published studies consistently reported the relatively low rate of PD-L1 expression in SCLC. For example, Yu *et al.* reported that the overall prevalence of PD-L1 expression in tumors was 16.5% with a tumor proportion score (TPS) cutoff  $\geq 1\%$  by using two approved anti-PD-1/PD-L1 antibodies (SP142 and clone 28-8) in 249 SCLC patients (34). Similarly, Zhao *et al.* reported that only 12.9% of 205 patients with surgically resected SCLC had positive PD-L1 expression by using clone 22C3 with a cutoff value of 1% (44). Interestingly, these two studies included patients from different ethnicities, indicating that low rate of PD-L1 expression is common in patients with SCLC. However, Chang *et al.* observed that the frequency of PD-L1 overexpression in tumors was 78.0% in 186 patients with SCLC (36), which was comparable to the expression rate in NSCLC. Of note, most of the included cases in Chang's study was diagnosed with stage IV SCLC (60.2%). As they mentioned in the study, high PD-L1 expression was significantly associated with stage IV disease ( $P=0.048$ ) (36), which could be partially explain the high prevalence of PD-L1 overexpression in their cohort. Taken together, our results together with other findings suggested that overall frequency of PD-L1 expression in SCLC is low and not influenced by the ethnicity. Whether disease stage of SCLC had impact on the prevalence of PD-L1 expression need further study.

To better select the targeted population who had the tendency to express PD-L1 and CD8<sup>+</sup> TIL, we did the analysis of association between clinicopathological parameters and PD-L1 expression or CD8<sup>+</sup> TIL density. We observed no any association between PD-L1 expression or CD8<sup>+</sup> TIL density and clinicopathological features in current cohort, mainly due to the small sample size. Although the previous studies suggested that PD-L1 expression or CD8<sup>+</sup> TIL density was significantly correlated with patient age, the absence of nodal metastasis, the presence of vascular invasion, disease stage, primary tumor size, normal levels of serum neuron-specific enolase (NSE) and lactate dehydrogenase (LDH) (33,35,36,42-44), all of these studies are retrospective design together with small sample size. It still warrants the future studies with large sample size to investigate the correlations between clinicopathological features and PD-L1 expression or CD8<sup>+</sup> TIL density.

In our study, we observed that positive PD-L1 expression and high CD8<sup>+</sup> TIL density was independently correlated with prolonged OS in surgically resected SCLC.

Specifically, the meta-analysis of 15 publications with 1,505 cases further validated the prognostic significance of positive PD-L1 expression in SCLC. Similar to our findings, a number of studies reported the correlation between PD-L1 expression and better prognosis in SCLC (32,36,38,42). High CD8<sup>+</sup> TIL density was also previously reported to be associated with improved OS (41,45). However, several studies indicated that PD-L1 expression was not the independent prognostic factor for OS. Even some of them revealed that positive PD-L1 expression was correlated with worse OS (43,44). The potential reasons for this inconsistent result should consider the different populations, testing process and technical difference for PD-L1 expression, as well as the heterogeneity of PD-L1 expression in tumor or immune cells. Nevertheless, when we utilized PD-L1 expression plus CD8<sup>+</sup> TIL density to predict the clinical outcome, we observed that it could more precisely stratify the total population into two groups with different prognoses after surgical resection, indicating the incorporation of these factors into multivariable prognostic models worth further exploration.

Several limitations of this study should be acknowledged. Firstly, the number of eligible patients and identified publications in meta-analysis were relatively small and all of them were retrospective studies, which suggested that the findings should be interpreted with caution and large-scale studies are still warranted. Secondly, PD-L1 antibody assay used in this study is clone E1L3N. Whether it could result in the low detection rate of PD-L1 expression in SCLC remained undetermined. Thirdly, publication bias is inevitable since we identified several meeting abstracts without detailed publications and not included these them for the meta-analysis. Fourthly, the data quality of each included article in meta-analysis was heterogeneous due to a series of confounding factors (PD-L1 antibody assay, laboratory conditions, testing process, cutoffs of positive PD-L1 expression, etc.) that made direct comparisons difficult.

In conclusion, this study reported that PD-L1 expression and CD8<sup>+</sup> TIL density had a lower expression level and particular clinicopathological feature in patients with SCLC when compared with NSCLC. Both positive PD-L1 expression and high CD8<sup>+</sup> TIL density was independently correlated with longer OS, and combination of PD-L1 expression and CD8<sup>+</sup> TIL density could further stratify the total population into two groups with discrepant prognosis, suggesting that a meaningful graded prognostic evaluation for patients with surgically resected SCLC should

incorporate PD-L1 expression and CD8<sup>+</sup> TILs.

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## Footnote

*Conflicts of Interest:* The authors have no conflicts of interest to declare.

*Ethical Statement:* The authors are accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved. This study was approved by the ethics committee of Shanghai Pulmonary Hospital (FK-17-0113) and conducted in line with the provisions of the Declaration of Helsinki.

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## Supplementary file 1 Methodology of meta-analysis

### Publication search

We conducted a literature review of publication search via the online databases including PubMed/Medline, Cochrane Library, EMBASE, Web of Science, and Google Scholar through May 2019, using “lung cancer” and “PD-L1”, and their corresponding words. Titles and abstracts were firstly reviewed to determine publications. We collected the data on the association of PD-L1 expression with prognosis, and clinicopathological characteristics in patients with small-cell lung cancer (SCLC). This analysis was performed in accordance with Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) Statement.

### Publication selection, data extraction and quality assessment

Studies met the following criteria were identified: (I) evaluated positive PD-L1 expression in patients with SCLC; (II) PD-L1 expression was tested on tumor samples, instead

of the peripheral blood or cell lines or any other types of tissue; (III) published data could assess the rate of positive PD-L1 expression and/or high risk on overall survival (OS). Publications were excluded if they were: (I) reviews, case-only studies, editorial, comment, or familial studies; (II) inadequate data for analysis of rate and/or high risk with 95% confidence intervals (CIs); and (III) repeat of previous studies or replicated samples. Two reviewers independently evaluated the study eligibility.

The following information from the eligible studies: name of first author, publication year, study population, number of age >60 years old, number of male, number of smoker, number of extensive-stage SCLC (ES-SCLC), rate of positive PD-L1 expression with 95% CIs, cut-off value of positive PD-L1 expression, anti-PD-L1 antibody assay, and hazard ratio (HR) for OS with related 95% CIs, were extracted. We only chose the results of multivariate analysis when univariate and multivariate analysis were simultaneously reported. Two reviewers independently extracted the data. Disagreements were solved by discussion. As we previously mentioned (25,52,53), two reviewers independently assessed the study quality via using the listed factors.

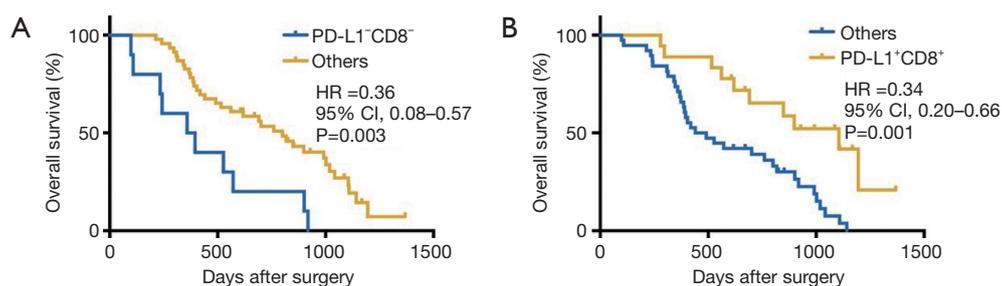
#### 1) PubMed search strategy

1. "small cell lung cancer"[Mesh]
2. small cell lung cancer [Title/Abstract]
3. small cell lung tumor [Title/Abstract]
4. small cell lung tumour [Title/Abstract]
5. small cell lung carcinoma* [Title/Abstract]
6. small cell lung neoplas* [Title/Abstract]
7. small cell lung malignan*[Title/Abstract]
8. "B7-H1 antigen" [Mesh]
9. "CD274 protein" [Mesh]
10. PD-L1 [Title/Abstract]
11. PDL1 [Title/Abstract]
12. B7-H1 [Title/Abstract]
13. CD274 [Title/Abstract]
14. (1 OR 2 OR 3 OR 4 OR 5 OR 6 OR 7) AND (8 OR 9 OR 10 OR 11 OR 12 OR 13)

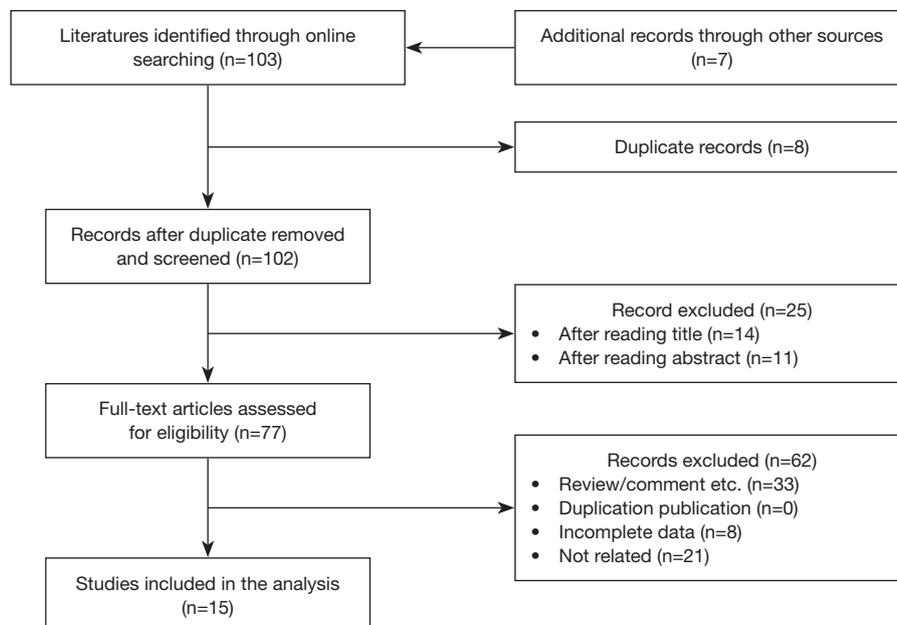
#### 2) EMBASE search strategy

1. 'lung neoplasm'/exp
2. lung cancer:ab,ti
3. lung tumor:ab,ti
4. lung tumour:ab,ti
5. ung carcinoma:ab,ti
6. lung neoplas*:ab,ti
7. lung malignan*:ab,ti
8. lung adenoma*:ab,ti
9. 'B7-H1 antigen'/exp
10. B7-H1
11. 'CD274 protein'/exp
12. CD274
13. 'PD-L1 expression'/exp
14. PDL1 expression:ab,ti
15. (1 OR 2 OR 3 OR 4 OR 5 OR 6 OR 7 OR 8) AND (((9 OR 10) OR (11 OR 12)) OR 13 OR 14)

**Figure S1** Search strategies. Search included: PubMed and EMBASE; date was from the inception through May 2019.



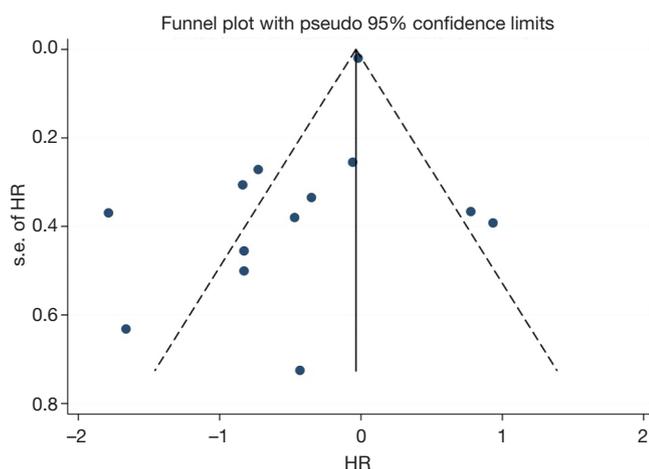
**Figure S2** Subgroup analysis of OS based on the PD-L1 expression and CD8<sup>+</sup> TILs density. (A) Patients with negative PD-L1 expression and low CD8<sup>+</sup> TILs density had the shortest OS; (B) patients with positive PD-L1 expression and high CD8<sup>+</sup> TILs density had the longest OS. OS, overall survival; PD-L1, programmed death ligand-1; TILs, tumor-infiltrating lymphocytes; HR, hazard ratio; CI, confidence interval.



**Figure S3** The flowchart of publication selection.

**Table S1** Methodological features of included publications and quality score

No.	Authors	Year	Representativeness of population	Non exposed cohort	Ascertainment of exposure	Outcome not present at start of study	Appropriate confounding measurement and account	Sufficient measurement of outcomes	Completeness of follow-up
1	Schultheis <i>et al.</i>	2015	0	1	1	1	2	1	1
2	Ishii <i>et al.</i>	2015	0	1	1	1	2	1	2
3	Toyokama <i>et al.</i>	2016	0	1	1	1	1	1	1
4	Yu <i>et al.</i>	2017	0	1	1	1	1	2	2
5	Miao <i>et al.</i>	2017	0	1	1	1	2	1	2
6	Chang <i>et al.</i>	2017	0	1	0	1	1	2	2
7	Tsuruoka <i>et al.</i>	2017	0	1	1	1	2	2	2
8	Inamura <i>et al.</i>	2017	0	1	1	1	2	2	2
9	Bonanno <i>et al.</i>	2018	0	1	0	1	2	2	2
10	Ichiki <i>et al.</i>	2018	0	1	1	1	1	2	2
11	Wang <i>et al.</i>	2018	0	1	1	1	2	2	2
12	Liu <i>et al.</i>	2018	0	1	1	1	2	2	2
13	Xu <i>et al.</i>	2019	0	1	1	1	2	2	2
14	Zhao <i>et al.</i>	2019	0	1	1	1	2	2	2
15	Daniel <i>et al.</i>	2019	0	1	1	1	1	1	1



**Figure S4** Funnel plots for hazard ratio of overall survival from included studies. HR, hazard ratio.

## References

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