



ORIGINAL ARTICLE

PD-L1 expression and its effect on clinical outcomes of EGFR-mutant NSCLC patients treated with EGFR-TKIs

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ABSTRACT

Objective: Epidermal growth factor receptor (EGFR) activation was reported to upregulate programmed death-ligand 1 (PD-L1) expression in lung cancer cells and subsequently contribute to immune escape, indicating its critical role in EGFR-driven lung tumors. This study characterized PD-L1 expression in patients with surgically resected EGFR-mutant non-small cell lung cancer (NSCLC). The effect of PD-L1 expression on clinical outcomes was also investigated in advanced EGFR-mutant NSCLC treated with EGFR-tyrosine kinase inhibitors (TKIs).

Methods: In total, 73 patients with surgically resected NSCLC and EGFR mutations were identified. PD-L1 expression and CD8+ tumor-infiltrating lymphocyte (TIL) density were assessed by immunohistochemistry. A literature review of publications that assessed the predictive and prognostic value of PD-L1 expression in advanced EGFR-mutant NSCLC patients treated with EGFR-TKIs was performed.

Results: Nineteen (26.0%) patients were positive for PD-L1 expression, which was significantly associated with concomitant KRAS mutation ($P = 0.020$) and marginally associated with higher CD8+ TILs density ($P = 0.056$). Positive PD-L1 expression was associated with markedly inferior overall survival (OS) in multivariate analysis ($P = 0.032$). The combination of PD-L1 and CD8+ TILs expression could be used to stratify the population into three groups with distinct prognoses. A meta-analysis of six publications showed that positive PD-L1 expression was not associated with OS [hazard ratio (HR) = 0.90; 95% confidence interval (CI), 0.42–1.38] or progression-free survival (HR = 1.03; 95 CI, 0.73–1.33) in advanced EGFR-mutant NSCLC patients receiving EGFR-TKIs.

Conclusions: PD-L1 expression tended to correlate with CD8+ TIL expression, concomitant KRAS mutation, and poor survival in surgically resected EGFR-mutant NSCLC. PD-L1 expression was neither the predictive nor the prognostic factor in advanced EGFR-mutant NSCLC patients treated with EGFR-TKIs.

KEYWORDS

Non-small cell lung cancer; EGFR mutation; PD-L1; CD8; survival

Introduction

Targeted therapy on epidermal growth factor receptor (EGFR)-activating mutations has revolutionized the treatment landscape of patients with advanced EGFR-mutant non-small cell lung cancer (NSCLC)¹. Several large-scale phase 3 trials have demonstrated the superior efficacy of first-

and second-generation EGFR tyrosine kinase inhibitors (TKIs) to standard platinum-based chemotherapy in the first-line setting²⁻⁴. However, almost all responders acquired drug resistance after 9–12 months (acquired resistance), and 30% of the initial cohorts showed no response to EGFR-TKIs (primary resistance)^{1,5-7}. A series of resistance mechanisms have been associated with primary and/or acquired resistance, including Bcl-2 family member (BIM) deletion polymorphism, second site mutations, downstream or bypass signaling pathway activation, and histological transformation; however, 25%–30% of acquired resistance and half of primary resistance mechanisms remain undetermined¹⁻⁷. Therefore, drug resistance is the major

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challenge for the application of EGFR-TKI in EGFR-mutant NSCLC. There is an urgent need to identify more effective biomarkers to better predict the efficacy of EGFR-TKIs.

Immune checkpoints are orchestrated by a set of co-stimulatory and co-inhibitory molecules to regulate the activation and effector functions of tumor-infiltrating lymphocytes (TILs)⁸. Blockade of inhibitory immune checkpoints by antibodies may release the immune inhibition of effector T cells, especially CD8+ T cells⁹. To date, therapeutic antibodies targeting programmed cell death 1 (PD-1) and its ligand (PD-L1) have been associated with remarkable response rates in various cancers and are another significant breakthrough in the treatment of patients with advanced NSCLC. Three PD-1/PD-L1 antibodies, including nivolumab, pembrolizumab, and atezolizumab, have been approved for second-line or subsequent treatment of advanced NSCLC for their promising anti-tumor effect compared to that of standard chemotherapy¹⁰⁻¹³. Furthermore, pembrolizumab was approved in the first-line setting for patients with PD-L1 expression $\geq 50\%$ ^{14,15}. However, in clinical practice, Gainor et al.¹⁶ reported a limited effect of PD-1/PD-L1 inhibitors in patients with EGFR-mutant NSCLC. A recent phase 2 clinical study reported a 12.2% objective response rate (ORR) to an anti-PD-L1 antibody among EGFR-mutant NSCLC patients, with at least 25% of tumor cells expressing PD-L1, compared to an ORR of 3.6% in those with PD-L1 expression $< 25\%$ ¹⁷, suggesting the clinical value of PD-L1 expression in EGFR-mutant NSCLC patients treated with anti-PD-L1 antibody. However, there are limited data to characterize the PD-L1 expression in EGFR-mutant NSCLC patients. A recent preclinical study demonstrated that the expression of mutant EGFR in bronchial epithelial cells could significantly induce PD-L1 expression and that EGFR-TKIs could reduce PD-L1 expression in NSCLC cell lines with activated EGFR¹⁸, indicating a potential relationship between PD-L1 expression and EGFR mutations in NSCLC. Several groups, therefore, investigated the predictive and prognostic value of PD-L1 expression on EGFR-mutant NSCLC patients treated with EGFR-TKIs. However, their findings were paradoxical. Lin et al.¹⁹ reported that PD-L1 expression was associated with a better response to EGFR-TKI and longer survival, while Tang et al.²⁰ suggested that PD-L1 expression was neither the predictive nor the prognostic factor in EGFR-mutant NSCLC patients treated with EGFR-TKIs.

Therefore, the current study aimed to characterize PD-L1 expression in patients with surgically resected EGFR-mutant NSCLC and to investigate the effect of PD-L1 expression on clinical outcomes in patients with advanced EGFR-mutant

NSCLC treated with EGFR-TKIs. Because the antitumor effect of anti-PD-1/PD-L1 antibodies is mainly dependent on the proliferation and activation of CD8+ TILs^{8,9}, we further evaluated the prognostic value of the combination of PD-L1 expression and CD8+ TIL density in surgically resected NSCLC with EGFR mutations.

Patients and methods

Patient cohort

We retrospectively identified patients who underwent surgical resection of the lung (lobectomy or pneumonectomy) due to the histologically-confirmed NSCLC, between 2011 and 2015, at Shanghai Pulmonary Hospital and our cooperative medical centers. The major patient baseline characteristics including age, sex, smoking history, histology [World Health Organization (WHO) classification]²¹, and stage of NSCLC at initial diagnosis were collected. Never smoker was defined as a person who has smoked fewer than 100 cigarettes during their lifetime. This study was conducted in accordance with the provisions of the Declaration of Helsinki and was approved by the ethics committee of Shanghai Pulmonary Hospital. The exclusion criteria included histologically-confirmed SCLC, stage IIIB-IV before surgery, and chemotherapy and/or radiotherapy administered before surgery. Patients who could not provide adequate samples for EGFR detection and PD-L1/CD8 staining or who disagreed with the study protocols were ineligible.

PD-L1 expression analysis

PD-L1 expression was evaluated by immunohistochemistry (IHC) as described previously²². In brief, tumor sections of formalin-fixed and paraffin-embedded specimens were treated with 3% H₂O₂ for 10 min, blocked with 5% goat serum, and incubated with a primary anti-human PD-L1 antibody (#13684, clone E1L3N, Cell Signaling Technology, Danvers, MA, diluted 1:200). Next, a peroxidase-labeled secondary antibody (REAL EnVision Detection Reagent Peroxidase Rabbit/Mouse, DAKO, Denmark) was applied to the sections for 30 min at room temperature. PD-L1 positive/negative expression was defined using a cut-off value of 5%²³⁻²⁶.

CD8+ TIL analysis

A mouse anti-CD8 monoclonal antibody (M7103, clone

C8144B, DAKO, Denmark) was utilized for CD8+ TIL density assessment as described previously^{27,28}. TILs were enumerated on immunostained CD8 preparations and scored using a four-tier scale (details in supplemental file). The cut-off point for high/low CD8+ TIL expression was 5%.

EGFR and KRAS mutation detection

EGFR and KRAS mutation analyses were conducted at Shanghai Pulmonary Hospital & Thoracic Cancer Institute, Tongji University School of Medicine (Shanghai, China). The samples were tested using an Amplification Refractory Mutation System (ARMS) as previously described (Amoy Diagnostics Co., Ltd., Xiamen, China)^{22,29,30}.

Meta-analysis

We performed a publication search of the PubMed/Medline, EMBASE, Google Scholar, Cochrane Library, and Web of Science databases through December 31, 2017, using “lung cancer” and “PD-L1” and “EGFR-TKI” and their related words. Data on the relationship between PD-L1 expression and overall survival (OS), progression-free survival (PFS), and clinicopathological characteristics in EGFR-mutant NSCLC patients treated with EGFR-TKIs were collected from publications meeting the eligibility criteria (**Supplementary Table S1**). The details of our methodology are described in the supplemental file.

Statistical analysis

Categorical variables were analyzed using the Chi-square or Fisher's exact tests. Continuous variables were analyzed by analysis of variance and Tukey's multiple comparison tests. Kaplan-Meier curves were used to assess patient survival, and log-rank tests were used to evaluate the significance of differences between two or four groups. Cox proportional hazards models were used for uni- and multivariate survival analyses to calculate the hazard ratios (HRs) and corresponding 95% confidence intervals (CIs). The OS was calculated from the date of lung cancer diagnosis to death from any cause or was censored at the last follow-up date. *P*-values less than 0.05 (two-sided) were considered to be significant. Meta-analysis was conducted using STATA version 12.0 (Stata Corporation, TX). All statistical analyses were performed using IBM SPSS Statistics for Windows, version 22.0 (IBM Corp., Armonk, NY, USA).

Results

Characterization of PD-L1 expression in EGFR-mutant NSCLC

In total, 201 patients with surgically resected NSCLC were initially enrolled. After EGFR detection, 73 patients with surgically resected EGFR-mutant NSCLC were finally identified. Most (63.0%) were < 65 years old at the time of diagnosis. Forty-two (57.5%) of the patients were female and 44 (60.3%) were never-smokers. The most predominant histological type was adenocarcinoma (71 patients). Three patients (4.1%) had concomitant KRAS mutations. Nineteen patients (26.0%) had positive PD-L1 expression and 22 (30.1%) had high CD8+ TILs density. Representative images of IHC for PD-L1 expression and CD8+ TILs are shown in **Supplementary Figure S1**. The clinical features of the 73 patients are summarized in **Table 1**.

There was a significant correlation between PD-L1 expression and concomitant KRAS mutations ($P = 0.020$). Patients with high CD8+ TIL density were more likely to have positive PD-L1 expression, but the relationship did not reach statistical significance ($P = 0.056$). There were no significant differences in PD-L1 expression in terms of age ($P = 0.570$), gender ($P = 0.297$), smoking status ($P = 0.429$), pathologic types (adenocarcinoma vs. non-adenocarcinoma) ($P = 0.973$), pathologic stages (I/IIIA vs. IIIB/IV) ($P = 0.591$), and lymph node metastasis ($P = 0.661$).

Prognostic value of PD-L1 expression in surgically resected NSCLC with EGFR mutation

The median follow-up time was 25.7 months (range, 5.6–54.9 months). Univariate analysis revealed that patients with positive PD-L1 expression showed a marginally shorter OS than that in patients with negative expression (HR = 2.339, 95% CI: 0.992–9.835; $P = 0.057$) (**Table 2** and **Figure 1A**). High CD8+ TIL density tended to be associated with longer OS but it did not reach the statistical significance (HR = 0.409, 95% CI: 0.185–1.246; $P = 0.138$) (**Table 2** and **Figure 1B**). Other factors including gender, age, smoking status, lymph node metastasis, and EGFR mutant types were not associated with OS (**Table 2**). Multivariate analyses suggested that positive PD-L1 expression was significantly associated with a shorter OS (HR = 2.995, 95% CI: 1.097–8.178; $P = 0.032$) (**Table 2**). While patients with high CD8+ TIL expression showed a longer OS than that in those with low CD8+ TIL expression, the difference was not statistically

Table 1 Baseline characteristics of the study population

Variables	All patients	PD-L1 positive	PD-L1 negative	<i>P</i>
Total	73	19	54	
Age at diagnosis, years				0.570
< 65	46	13	33	
≥ 65	27	6	21	
Gender				0.297
Male	31	10	21	
Female	42	9	33	
Smoking history				0.429
Never-smoker	44	10	34	
Former/current smoker	29	9	20	
Pathological classification				0.973
Adenocarcinoma	71	18	53	
Non-adenocarcinoma	2	1	1	
pTNM stage				0.591
pI-III A	69	17	52	
pIII B-IV	4	2	2	
Lymph node metastasis				0.661
Yes	30	7	23	
No	43	12	31	
KRAS mutation				0.020
KRAS mutation	3	3	0	
KRAS wild type	70	16	54	
CD8+ TILs expression				0.056
Yes	22	9	13	
No	51	10	41	

pTNM, pathological TNM; TILs, tumor infiltrating lymphocytes.

significant (HR = 0.296, 95% CI: 0.080–1.103; *P* = 0.070) (Table 2).

We further assigned the population into four groups according to PD-L1 and CD8+ TIL expression. The median OS was not reached in the PD-L1+/CD8-high and PD-L1-/CD8-high expression groups but was 29.0 months in the PD-L1+/CD8-low group and 45.0 months in the PD-L1-/CD8-low group (*P* = 0.056, Figure 1C). Patients with PD-L1-/high CD8+ TIL density expression had the longest OS (HR = 0.196, *P* = 0.072), while the PD-L1+/CD8-low group had the shortest OS (HR = 3.087, *P* = 0.020) (Supplementary Figure S2).

Features of the studies included in the meta-analysis

A total of 192 potentially relevant studies were screened. Most of the excluded publications were reviews, comments, duplications, or studies with incomplete data. The current study assessed 648 cases from six publications^{19,20,31-34} to investigate both the predictive and prognostic value of PD-L1 expression in advanced EGFR-mutant NSCLC patients treated with EGFR-TKIs. The main features of the eligible studies are shown in Table 3 and Supplementary Table S2. In addition, prognostic and predictive data on OS and PFS were obtained from the included studies. Figure 2 depicts a flowchart of publication inclusion.

Predictive and prognostic value of high PD-L1 expression in EGFR-mutant NSCLC treated with EGFR-TKIs

A meta-analysis was performed to evaluate the overall effect of positive PD-L1 expression in the studies containing predictive and prognostic data. The pooled results showed that positive PD-L1 expression was not correlated with OS (HR = 0.90, 95% CI: 0.42–1.38; *P* > 0.05; Figure 3A). Similarly, the pooled results indicated that positive PD-L1 expression was not associated with PFS (HR = 1.03, 95% CI: 0.73–1.33; *P* > 0.05; Figure 3B). Both results showed high heterogeneity (*I*² = 80.3%, *P* < 0.001; *I*² = 60.8%; *P* = 0.013; respectively).

Publication bias

Sensitivity analysis was conducted by deleting one study at one time to assess its effect on the pooled HRs. Deletion of the study by Meniawy et al.³¹ slightly decreased the heterogeneity in the analysis of pooled HRs of PFS and OS. No other studies influenced the pooled results. Begg's funnel plots and Egger's tests were used to evaluate the publication bias. The Begg's funnel plot was symmetric, and Egger's tests suggested no evidence of publication bias (Supplementary Figure S3).

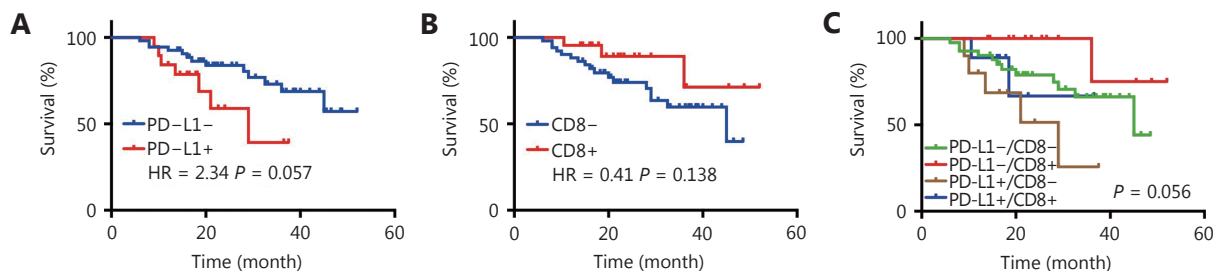
Discussion

Emerging evidence suggested that blocking PD-1/PD-L1 showed a limited efficacy in patients with EGFR-mutant NSCLC. A preclinical study reported that EGFR activation could regulate PD-L1 expression in lung cancer, indicating a sophisticated interaction between EGFR mutations and

Table 2 Univariate and multivariate analyses of clinical parameters on OS in patients with EGFR mutations.

Factor	Univariate analysis			Multivariate analysis		
	HR (log rank)	95% CI	P	HR (log rank)	95% CI	P
Gender (male/female)	0.990	0.404–2.413	0.982	1.904	0.541–6.695	0.316
Age, years (≥ 65 / <65)	1.221	0.494–3.096	0.656	1.098	0.431–2.795	0.845
Smoking (smoking/never)	1.339	0.523–3.425	0.544	1.988	0.557–7.095	0.290
Lymph node metastases (yes/no)	1.591	0.679–4.113	0.279	1.544	0.587–4.060	0.579
PD-L1 expression (yes/no)	2.339	0.992–9.385	0.057	2.995	1.097–8.178	0.032
CD8 expression (yes/no)	0.409	0.185–1.246	0.138	0.296	0.080–1.103	0.070
EGFR (rare/sensitizing)*	1.279	0.343–5.025	0.693	1.451	0.389–5.408	0.579
KRAS (mutation/wild type)	1.089	0.135–8.839	0.934	1.357	0.132–13.978	0.798

HR: hazard ratio; CI: confidence interval; *: Sensitizing mutations include EGFR exon 19 deletion and L858R; rare mutations include EGFR mutations except exon 19 deletion and L858R.

**Figure 1** Prognostic value of PD-L1 expression (A) CD8 TILs expression (B) and combination of PD-L1 and CD8 TILs expression (C) in surgically resected NSCLC with EGFR mutation.

PD-L1 expression. Improved understanding of PD-L1 expression in EGFR-mutant NSCLC would contribute to the development of more effective immunotherapy. Additionally, clarifying the predictive and prognostic value of PD-L1 expression in EGFR-mutant NSCLC patients treated with EGFR-TKIs would be helpful to precisely determine the populations who could most benefit from EGFR-TKIs therapy. To achieve these goals, the current study systematically investigated the clinicopathological features of PD-L1 expression in surgically resected EGFR-mutant NSCLC and the effect of this expression on clinical outcome patients with advanced EGFR-mutant NSCLC treated with EGFR-TKIs. Our results showed that positive PD-L1 expression was significantly correlated with concomitant KRAS mutations and seemed to be associated with high CD8+ TILs density without statistical significance, mainly due to the small sample size. Positive PD-L1 expression was associated with a significantly shorter OS, and the combination of PD-L1 and CD8+ expression could further stratify the population into three groups with distinct prognoses. Furthermore, a meta-analysis of six publications

indicated that positive PD-L1 expression was neither a predictive nor a prognostic factor in advanced EGFR-mutant NSCLC patients treated with EGFR-TKIs.

Several studies have reported a positive association between EGFR mutations and positive PD-L1 expression in completely resected NSCLC^{20,35-37}. However, few studies have characterized PD-L1 expression in patients with EGFR-mutant NSCLC. In our study, we observed that positive PD-L1 expression was significantly correlated with concomitant KRAS mutations in patients with EGFR-mutant NSCLC. PD-L1 is frequently highly expressed in NSCLC with driver mutations, which subsequently activates key oncogenic pathways such as PI3K-AKT and RAS-MAPK. Hence, it is anticipated that more cases with high PD-L1 expression had concomitant EGFR and KRAS mutations. Notably, only three patients in the present study had concomitant EGFR and KRAS mutations. We should interpret this result with caution. In addition, positive PD-L1 expression was marginally significantly associated with high CD8+ TIL density in this population, suggesting high PD-L1 expression of CD8+ TILs in EGFR-mutant NSCLC. Further investigation

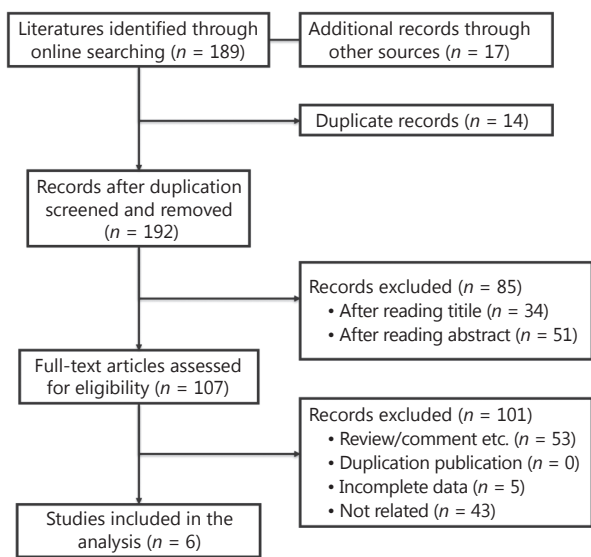


Figure 2 The flowchart of publication selection.

of the potential role of PD-1/PD-L1 pathway in inhibiting CD8+ TIL function in EGFR-mutant NSCLC is required.

We further evaluated the prognostic value of PD-L1 expression in surgically resected NSCLC with EGFR mutations. As expected, positive PD-L1 expression was significantly associated with inferior OS and CD8+ TIL density was marginally correlated with a longer OS. When we combined PD-L1 expression and CD8+ TIL density, the total populations could be divided into three groups with distinct prognoses. Similarly, a large number of studies consistently reported positive PD-L1 expression to be an independent negative prognostic factor in NSCLC. In 2014, Azuma et al.³⁵ assessed the prognostic value of PD-L1 expression in 164 surgically resected NSCLC, reporting high PD-L1 expression to be significantly associated with a shorter OS independently of other factors. Two recent meta-analyses also demonstrated the prognostic value of PD-L1 expression in NSCLC.

However, another meta-analysis reported no statistically significant difference between PD-L1 expression and prognosis in patients with NSCLC^{38,39}. Of note, the driver gene mutations of the populations in these studies were unknown. Thus, high-quality studies with larger sample sizes are needed to determine the prognostic value of PD-L1 expression in NSCLC with specific driver mutations. Our finding of the prognostic value of PD-L1 expression combined with CD8+ TIL density was consistent with those of other studies²⁸. For example, Tokito et al.²⁸ observed that a combination of a lack of PD-L1 expression and CD8+ TIL density was significantly associated with favorable survival in patients with locally advanced NSCLC receiving concurrent chemoradiotherapy, indicating that PD-L1 expression in combination with CD8+ TIL density was a useful predictive biomarker in patients with stage III NSCLC. These results suggested that the combination of PD-L1 expression and CD8+ TIL density may be useful to predict the prognosis and efficacy of immunotherapy in NSCLC.

To clarify the predictive and prognostic value of PD-L1 expression in EGFR-mutant NSCLC patients treated with EGFR-TKIs, we conducted a meta-analysis of six eligible publications. Although preclinical studies reported that EGFR activation could induce PD-L1 expression to facilitate immune escape and that EGFR-TKI could significantly down-regulate PD-L1 expression in EGFR-mutant NSCLC cells^{18,35}, the pooled results indicated that positive PD-L1 expression was not correlated with OS or PFS in this population. Whether PD-L1 expression is a reliable biomarker for EGFR-TKI treatment in advanced EGFR-mutant NSCLC patients requires further investigation.

The current study had several limitations. First, the number of publications included in the meta-analysis was relatively small and all were retrospective studies. Second, publication bias is inevitable in this area of research. We identified several abstracts published at meetings without

Table 3 Baseline characteristics of included studies

Author	Year	Treatment record	No. of cases	No. < 65 years	No. of female	No. of ECOG = 0-1	No. of never smoker	No. of adenocarcinoma	Outcome assessment
Lin et al.	2015	Yes	56	40	35	39	38	56	OS, PFS
Tang et al.	2015	Yes	170	85	77	NA	113	145	OS, PFS
Meniawy et al.	2016	Yes	33	12	17	32	NA	23	OS, PFS
Sorensen et al.	2016	Yes	38	13	23	31	9	NA	OS, PFS
Cho et al.	2017	Yes	319	NA	194	318	114	319	OS, PFS
Kobayashi et al.	2018	Yes	32	13	20	NA	27	NA	OS, PFS

NA: not available; OS: overall survival; PFS: progression-free survival; ORR, overall response rate; DCR, disease control rate.

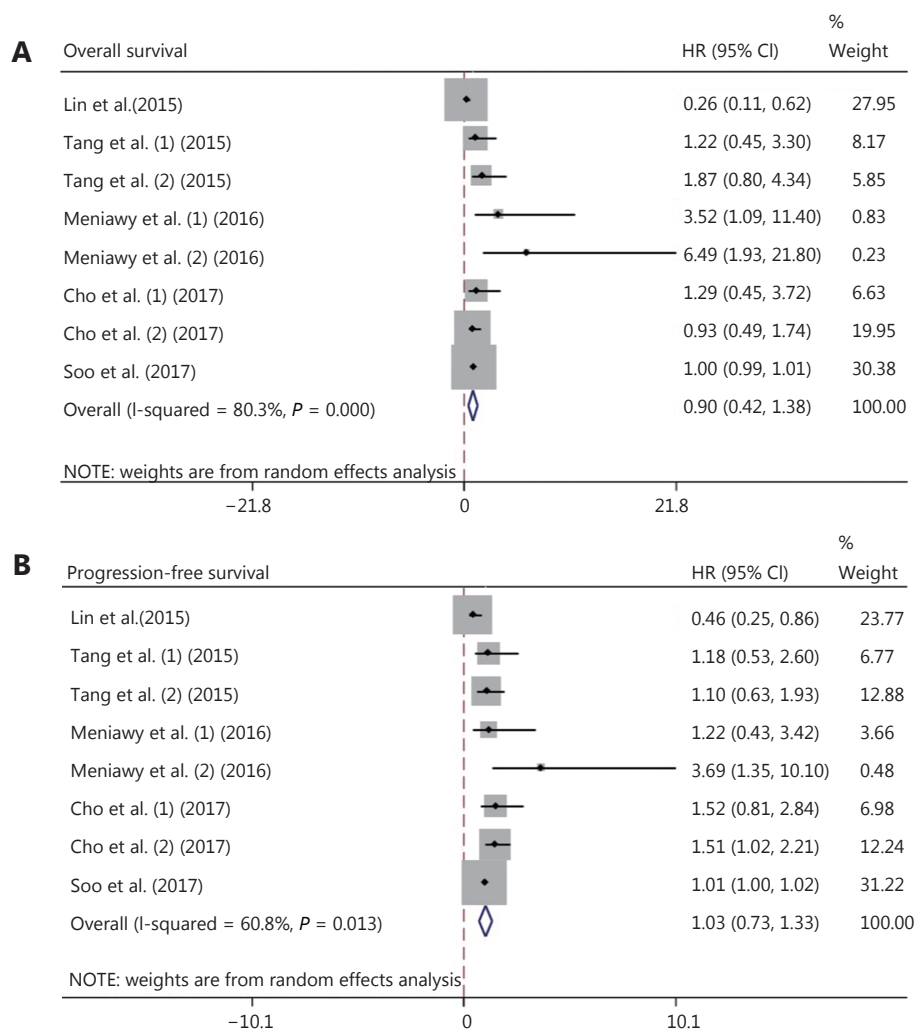


Figure 3 Meta-analysis to evaluate the predictive (A) and prognostic (B) value of high PD-L1 expression in EGFR-mutant NSCLC treated with EGFR-TKIs.

further detailed publications and excluded these abstracts from the meta-analysis. Third, the quality of data was heterogeneous due to a multitude of confounding factors (laboratory conditions, PD-L1 antibody, cutoff values of PD-L1 expression, etc.) that made direct comparisons difficult.

In conclusion, the present study showed that PD-L1 expression had a particular clinicopathological feature and was correlated with a shorter OS in patients with EGFR-mutant NSCLC. The combination of PD-L1 expression and CD8+ TIL density more precisely differentiated subpopulations with distinct prognoses after surgery. Positive PD-L1 expression was neither the predictive nor the prognostic factor in EGFR-mutant NSCLC patients treated with EGFR-TKIs. These results suggested that a meaningful

graded prognostic assessment for patients with surgical EGFR-mutant NSCLC should incorporate PD-L1 and CD8+ TILs and that PD-L1 expression should not be evaluated as a biomarker of EGFR-TKIs.

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Conflict of interest statement

No potential conflicts of interest are disclosed.

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Supplementary materials

CD8+ TIL expression analysis

Immunohistochemical staining for CD8+ TILs (Leica Microsystems, Newcastle-upon-Tyne, UK) was conducted on the same fully automated Bond-III system (Leica Microsystems) using onboard heat-induced antigen retrieval with epitope retrieval solution 2 for 10 min at 99 °C, and incubated with the antibody for 30 min at room temperature. This automated system utilized a Refine polymer detection kit with horseradish peroxidase-polymer as a secondary antibody and DAB, and incubation with a secondary antibody was performed for 30 min at room temperature.

Methodology of meta-analysis

Publication online search

We performed a publication search through PubMed/Medline, EMBASE, Google Scholar, Cochrane Library and Web of Science until December 31, 2017 by using “lung cancer” and “PD-L1” and “EGFR-TKI” or their related words. Titles and abstracts were firstly reviewed to determine publications. We then collected the data on the relationship of PD-L1 expression with overall survival (OS), progression-free survival (PFS) and clinicopathological characteristics in EGFR-mutant NSCLC patients. This analysis was conducted in line with Preferred Reporting Items for Systematic Reviews and Meta-Analyses: the PRISMA Statement.

Publication selection

Publications met the following criteria were eligible: (1) evaluated positive PD-L1 expression in NSCLC with EGFR mutations; (2) PD-L1 expression was determined on tumor specimens, instead of the peripheral blood or cell lines or any other types of tissue; (3) reported data could analyze the rate of positive PD-L1 expression and/or high risk (HR) on clinical outcomes including PFS (EGFR-TKI treatment) and OS. Studies were ineligible if they were: (1) comment, reviews, case-only studies, editorial, or familial studies; (2) insufficient data for analysis of rate and/or HR with 95% confidence intervals (CIs); and (3) repeat of previous publications or replicated samples. The study eligibility was independently evaluated by two reviewers. Disagreements were resolved by discussion.

Data extraction and quality assessment

We extracted the following information from the included studies: name of first author, publication year, treatment record, study population, cut-off value of positive PD-L1 expression, and rate of positive PD-L1 expression with 95% CIs, HR for PFS, and/or OS with related 95% CIs. To avoid the selection bias, we did not utilize the data from the Kaplan-Meier curves. When univariate and multivariate analysis were simultaneously reported, the results of multivariate analysis were selected. Two reviewers (Y.B and T.J.) independently extracted the data by using a predefined Excel form. Disagreements were solved by consensus. As we previously mentioned^{1,2}, two reviewers assessed the study quality independently by using the listed factors.

Statistical analysis

95% CIs were determined per estimate and presented in forest plots. For time-to-event data, the HRs with related 95% CIs were directly extracted from the eligible publications or calculated using previous methods proposed by Tierney et al. Cochran's Q test and I² statistic were used to determine the heterogeneity of different studies. Low-level heterogeneity was defined as $P < 0.1$ for the χ^2 test and $I^2 < 25\%$. If the heterogeneity was non-significant, a pooled effect was calculated with a fixed-effects model. A random-effects model was used when the heterogeneity was statistically significant. Publication bias was assessed by using funnel plots, Begg's and Egger's tests. Statistical analysis was conducted by Review Manager 5.0 software and STATA v12.0 (Stata Corporation, TX). *P* values were two-sided and considered significant if < 0.05 except for the Q-test.

Table S1 Search strategies included: PubMed and EMBASE (date was from the inception through June 2017)

1) PubMed search strategy

1. "Lung neoplasms" [Mesh]
2. Lung cancer [Title/Abstract]
3. Lung tumor [Title/Abstract]
4. Lung tumour [Title/Abstract]
5. Lung carcinoma* [Title/Abstract]
6. Lung neoplas* [Title/Abstract]
7. 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. "EGFR gene" [Mesh]
15. "erbB1 gene" [Mesh]
16. EGFR [Title/Abstract]
17. TKI [Title/Abstract]
18. EGFR Tyrosine kinase inhibitor [title/Abstract]
19. (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) AND (14 OR 15 OR 16 OR 17 OR 18)

2) EMBASE search strategy

1. 'lung neoplasm'/exp
2. lung cancer:ab,ti
3. lung tumor:ab,ti
4. lung tumour:ab,ti
5. lung 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. 'EGFR'/exp
16. 'TKI'/exp
17. 'EGFR-TKI'/exp
18. EGFR:ab,ti
19. TKI:ab,ti
20. EGFR tyrosine kinase inhibitor:ab,ti
21. (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) AND (15 OR 16 OR 17 OR 18 OR 19 OR 20)

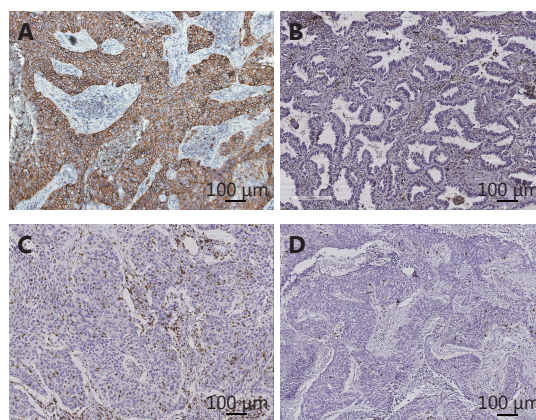


Figure S1 Positive (A) and negative staining (B) pattern for programmed cell death ligand-1 (PD-L1); positive (C) and negative staining (D) pattern for CD8+ tumor infiltrating lymphocytes (TILs).

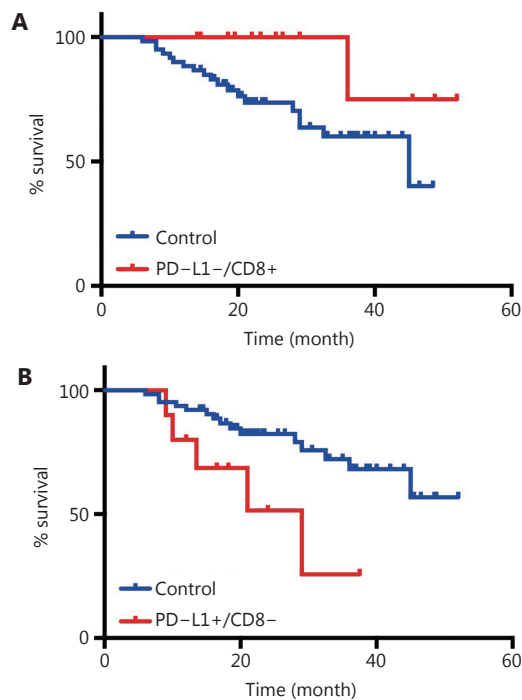


Figure S2 Patients with PD-L1-/CD8+ TILs expression had the longest OS (A) and PD-L1+/CD8- low group had the shortest OS (B).

Table S2 Methodological characteristics of included studies 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	Lin et al.	2015	0	1	1	1	2	2	2
2	Tang et al.	2015	0	1	1	1	2	1	2
3	Meniawy et al.	2016	0	1	1	1	1	1	2
4	Sorensen et al.	2016	0	1	1	1	1	1	2
5	Cho et al.	2017	0	1	1	1	2	2	2
6	Kobayashi et al.	2018	0	1	1	1	2	1	2

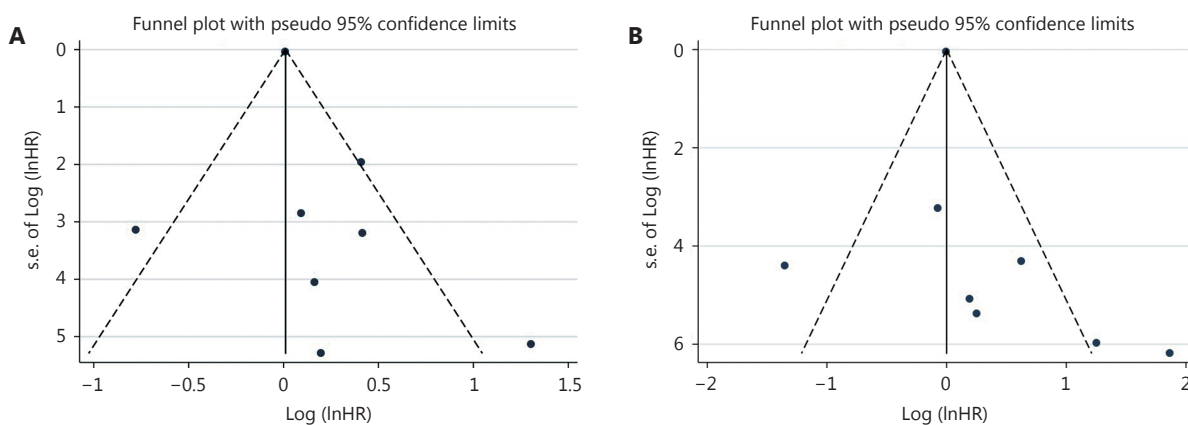


Figure S3 Funnel plots for progression-free survival (A) and overall survival (B) from included studies.

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