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ORIGINAL ARTICLE

Biphasic prognostic significance of PD-L1 expression status in patients with early- and locally advanced-stage non-small cell lung cancer

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Abstract

Programmed cell death-ligand 1 (PD-L1) expression on tumor cells is induced by interferon-gamma, suggesting the induction of an anti-tumor immune response. In turn, binding of PD-L1 to programmed cell death 1 (PD-1) triggers an immune checkpoint pathway that contributes to tumor growth. Though it remains to be elucidated, the clinical significance of PD-L1 expression might vary with tumor progression in non–small-cell lung cancer (NSCLC). Immunohistochemical analysis of PD-L1 was done in tumor specimens from patients who underwent radical surgery for stage I–IIIA NSCLC ($n=228$). Tumor PD-L1 expression intensity was semi-quantitatively scored and its correlation with various clinicopathological features and postoperative relapse-free survival (RFS) was assessed relative to pathological stage. In stage I, postoperative RFS was significantly prolonged in patients with a high PD-L1 score compared with a low PD-L1 score, exhibiting 5-year relapse-free probabilities of 94.1% and 75.1%, respectively ($P=0.031$). A multivariate analysis revealed that a high PD-L1 score was a prognostic factor of longer postoperative RFS (hazard ratio: 0.111, $P=0.033$). Conversely, in stages II and IIIA, patients with a high PD-L1 score tended to suffer from postoperative tumor recurrence. In early-stage NSCLC, high tumor PD-L1 expression status represents a biomarker to predict good prognosis after radical surgery and may reflect the induction of an antitumor immune response. However, in locally advanced stage NSCLC, tumor PD-L1 expression status may reflect the execution of an immune checkpoint pathway and predicts the incidence of postoperative tumor recurrence.

Keywords Programmed cell death-ligand 1 · Non-small cell lung cancer · Prognostic biomarker · Relapse-free survival · Surgery

Abbreviations

NSCLC	Non–small-cell lung cancer
OS	Overall survival
PD-L1	Programmed cell death-ligand 1
RFS	Relapse-free survival
5-y RFP	5-Year relapse-free probability

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Introduction

Binding of programmed cell death-ligand 1 (PD-L1) expressed on tumor cells to the T cell surface molecule programmed cell death-1 (PD-1) is thought to induce exhaustion and apoptosis of activated T lymphocytes [1, 2]. This effect has been exploited therapeutically, because inhibitors of the PD-1/PD-L1 interaction disrupt T cell non-responsiveness to promote a cytotoxic antitumor immune response. Patients with non–small-cell lung cancer (NSCLC) have shown significant benefit from treatment with inhibitors that target the PD-1/PD-L1 immune checkpoint axis. In two phase 3 trials, patients with previously treated NSCLC experienced significantly longer overall survival (OS) after treatment with the anti-PD-1 monoclonal antibody nivolumab compared with docetaxel [3, 4]. Similarly, when employed as a first line anticancer therapy, pembrolizumab, another anti-PD-1 antibody, resulted in longer progression-free survival and OS compared with platinum-based chemotherapy in patients with advanced NSCLC, in which PD-L1 was expressed in more than 50% of tumor cells [5].

The clinical success of immune checkpoint inhibitors has prompted an exploration of the association between PD-L1 expression on tumor cells and clinicopathological features of patients with NSCLC. We previously examined PD-L1 expression in pulmonary adenocarcinomas by immunohistochemistry (IHC) and found that tumors cells expressing PD-L1 were heterogeneously distributed throughout the tumor tissue [6–8]. On the basis of this finding, we semi-quantitatively evaluated PD-L1 expression in whole tissue sections by microscopy, and found that postoperative relapse-free survival (RFS) was significantly shorter for patients with high PD-L1 expression score compared with low PD-L1 expression score for stage I–IIIB NSCLC tumors [6].

Subsequent studies have examined the prognostic value of PD-L1 expression status in NSCLC [reviewed in ref. [9]]. However, the data have generally been inconsistent, with some studies finding that high PD-L1 expression status was associated with a poor prognosis for NSCLC patients [10–15], while other studies concluded the opposite [16–18]. These discrepant results may be due to the heterogeneity of the patient population [16], because patients with all, early to advanced, stages of NSCLC were included in a single study. Patient prognosis may be strongly influenced by the clinical stage of disease and the corresponding therapeutic strategies. Therefore, in settings targeting patients with all stages of NSCLC, it may be difficult to accurately assess the prognostic value of PD-L1 expression.

PD-L1 expression status may have been evaluated only in the context of the PD-1/PD-L1 immune checkpoint mechanism. However, given that PD-L1 expression is regulated by interferon-γ that is released from activated T lymphocytes [19–21], PD-L1 expression on tumor cells may reflect the induction of an antitumor immune response. We previously reported that in pN0M0 NSCLC, postoperative relapse-free survival (RFS) was significantly longer in patients with PD-L1-positive cancer-associated fibroblasts (CAFs) compared with PD-L1-negative CAFs and PD-L1 expression on CAFs was an independent prognostic factor of higher 5-year relapse-free probability (5-y RFP) [22]. Based on these data, we also focused on another aspect of PD-L1 expression, the induction of an antitumor immune responses, and interpret the clinical significance of PD-L1 expression status in tumor cells from NSCLC patients.

In this study, we focused on both aspects of the immunological significance of PD-L1 expression on tumor cells, their role in the immune checkpoint mechanism and the induction of an antitumor immune response. In addition, we reduced the confounding factors in the patient population and investigated correlation of PD-L1 expression status of tumor cells with postoperative tumor recurrence for each pathological stage.

Here, we report a difference in the prognostic significance of PD-L1 expression status in patients with early- and locally advanced-stage NSCLC.

Materials and methods

Patients and NSCLC tumor samples

A total of 228 patients who received surgery for NSCLC at Shiga University of Medical Science Hospital between January 2008 and December 2014 were enrolled in this study. To reduce the influence of therapeutic strategies on patient prognosis, we included only the patients who had received radical surgery with complete resection of their tumor plus systematic dissection of regional lymph nodes. Patients undergoing segmentectomy or partial resection of the tumor were excluded. None of the included patients received neoadjuvant chemotherapy or any other antitumor therapy prior to surgery. The pathological subtypes of NSCLC included in this study were invasive pulmonary adenocarcinomas and squamous cell carcinomas of the lung but excluded non-invasive adenocarcinomas. Clinicopathological data were obtained from patient medical records. Tumor tissue samples for immunohistochemistry (IHC) were obtained from resected specimens and processed using standard formalin fixation/paraffin embedding protocols. The study design was approved by the Ethical Committee of Shiga University of Medical Science, and informed consent was obtained from all patients.

PD-L1 immunohistochemistry

Whole tumor sections (4-μm-thick) of formalin-fixed paraffin-embedded tissue were deparaffinized in xylene and rehydrated in ethanol and distilled water. Antigen retrieval was performed by microwaving the sections in 10 mM sodium citrate buffer (pH 6.0) for 10 min. Endogenous peroxidase activity was blocked by treatment with 3% H_2O_2 for 10 min, and non-specific binding was blocked by treatment with 5% normal goat serum in Tris-buffered saline containing 0.1% Tween 20 for 1 h at room temperature. The sections were then incubated overnight at 4 °C with anti-human PD-L1 monoclonal antibody (clone E1L3N, 1:200; Cell Signaling Technology, Danvers, MA, USA). The sections were then incubated with SignalStain boost IHC detection reagent and visualized using SignalStain DAB substrate (both Cell Signaling Technology). Finally, sections were counterstained with hematoxylin.

For negative control staining, the anti-PD-L1 primary antibody was replaced with a rabbit IgG monoclonal antibody (Cell Signaling Technology). PD-L1 staining intensity in alveolar macrophages was used as an internal positive control as described previously [6].

Evaluation of PD-L1 expression on NSCLC cells

Following IHC staining, to evaluate PD-L1 expression status of MSCLC cells, we used a semi-quantitative scoring method reflecting both the intensity and extent of PD-L1 expression of tumor cells and expressed as the H-score, as described previously [6]. Sections were independently examined by two researchers, including a pathologist. Briefly, PD-L1 staining on tumor cells was scored relative to that on alveolar macrophages in the same section, with score 0, 1, 2, and 3 corresponded to no staining, weak staining (tumor cell intensity lower than alveolar macrophages), moderate staining (tumor cell intensity similar to that of alveolar macrophages), and strong staining (tumor cell intensity stronger than that of alveolar macrophages), respectively. The total number of tumor cells was counted in three randomly selected fields under 200 × magnification, and the percentage of PD-L1-stained tumor cells was calculated. The final PD-L1 expression score (H-score) was calculated as: [(1 × % of cells scoring 1) + (2 × % of cells scoring 2) + (3 × % of cells scoring 3)]. The scale thus ranges from 0 to 300 and is weighted towards cells with high-intensity staining.

Statistical analysis

Correlations between variables were analyzed using the Fisher's exact test for categorical variables and the Mann–Whitney *U* test for continuous variables. Relapse-free survival after surgery was calculated using Kaplan–Meier analysis, and RFS in different groups was compared with the log-rank test. Multivariate analysis of postoperative RFS was performed using the Cox proportional hazards model. *P* values of less than 0.05 were considered statistically significant. All analyses were performed using SPSS Statistics 22.0 software (IBM, Armonk, NY, USA).

Results

Patient characteristics

In total, 228 patients were included in this study (Table 1), which consisted of 162 men (71.1%) and 66 women (28.9%) with a median age at surgery of 68.0 years (range 36–88 years). Of these, 168 (73.7%) were smokers. The pathological subtypes included invasive pulmonary adenocarcinomas ($n = 147$, 64.5%) and squamous cell carcinomas of the lung ($n = 81$, 35.5%). The median tumor diameter was 28 mm (range 4–127 mm). Eighty-four tumors were grade 1 (36.8%), 106 were grade 2 (46.5%), and 38 were grade 3 (16.7%). Postoperative pathological staging of the tumor was IA in 86 cases (37.7%), IB in 34 (14.9%), IIA in 8 (3.5%), IIB in 47 (20.6%) and IIIA in 53 (23.2%). Invasion of tumor cells to lymphatics was observed in 134 cases (58.8%) and

Table 1 Patient characteristics ($n = 228$)

Age (y.o.) (median, range)	68, 36–88
Gender, *n* (%)	
Male	162 (71.1)
Female	66 (28.9)
Smoking habits, *n* (%)	
Never	60 (26.3)
Current/former	168 (73.7)
Adjuvant chemotherapy, *n* (%)	92 (40.4)
Pathology, *n* (%)	
Adenocarcinoma	147 (64.5)
Squamous cell ca.	81 (35.5)
Tumor size (mm) (median, range)	28, 4–127
Cell grade, *n* (%)	
Grade 1	84 (36.8)
Grade 2	106 (46.5)
Grade 3	38 (16.7)
Pathological stage, *n* (%)	
1A	86 (37.7)
1B	34 (14.9)
2A	8 (3.5)
2B	47 (20.6)
3A	53 (23.2)
Invasion to lymphatics, *n* (%)	134 (58.8)
Invasion to microvessels, *n* (%)	158 (69.3)

to microvessels in 158 cases (69.3%). The median time of observation was 53 months (range 1–139 months).

Correlation between tumor PD-L1 expression intensity and clinicopathological factors in respective pathological stages

Using the H-score as a semi-quantitative evaluation of the intensity and extent of PD-L1 expression, the median PD-L1 score was 48.9, with a range of 0.0–294.2. The median PD-L1 scores for patients with stage I ($n=120$), stage II ($n=155$), and stage IIIA NSCLC ($n=53$) were 46.9 (range 0.0–283.5), 74.3 (range 0.0–292.7) and 72.2 (0.1–294.2), respectively (Fig. 1, Supplementary Table 1). The score tended to be lower in patients with stage I compared with stage II or IIIA NSCLC patients; however, significant differences were not observed between different the two groups (stage I vs. II, $P=0.103$; stage I vs. IIIA, $P=0.591$; stage II vs. IIIA, $P=0.381$).

In the cohort consisting of patients with stage I–IIIA NSCLC, tumor PD-L1 expression intensity was not associated with age ($P=0.975$), gender ($P=0.082$), smoking habits ($P=0.156$), pathological type ($P=0.118$) or tumor cell grade ($P=0.063$) (Fig. 2, Supplementary Tables 2 and 3). With respect to the invasiveness of tumor cells, tumor PD-L1 expression intensity was significantly associated with microvessel invasion of tumor cells, with the score being lower for tumors with microvessel invasion ($n=52$) compared with tumors without ($n=176$) [36.8 (0.1–276.5) and 62.2 (0.0–294.2), respectively; $P=0.029$]. The data indicate that the invasiveness of tumor cells to lymphatic and microvascular vessels is associated with PD-L1 expression in tumor cells. However, when the correlations were analyzed in patients with stage I, II or IIIA, it did not reach the level of statistical significance ($P=0.057$, $P=0.393$, $P=0.324$, respectively).

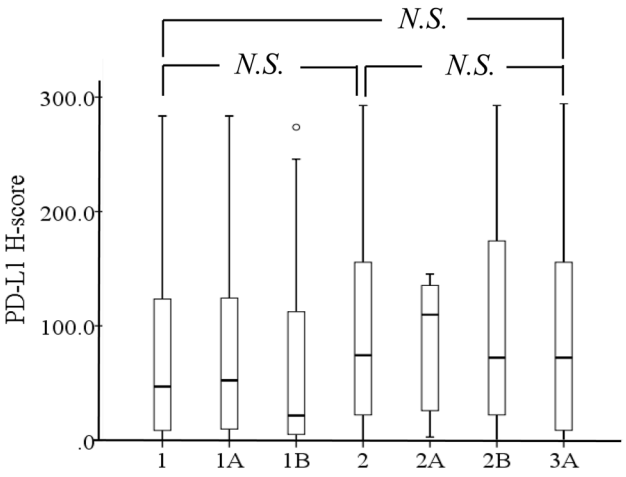

Fig. 1 Tumor PD-L1 expression intensity (PD-L1 H-score) in respective pathological stages

In patients with stage I NSCLC, tumor PD-L1 expression intensity was significantly lower in the tumors of female patients compared with those of male patients [26.3 (0.0–283.5) and 59.4 (0.0–277.4), respectively; $P=0.027$]. In the cohort, significant correlations were not observed in other clinicopathological factors. For the stage II nor IIIA NSCLC groups, PD-L1 expression intensity was not associated with any of the clinicopathological factors that we examined in the study. These data indicate that there are few specific correlations between PD-L1 expression on tumor cells and clinicopathological factors in early-stage as well as locally advanced stage NSCLC.

Correlation between tumor PD-L1 expression intensity and postoperative relapse-free survival

Patients were classified into two groups according to tumor PD-L1 expression score: ≥ 150 (high PD-L1 group) and < 150 (low PD-L1 group). When the PD-L1 expression score was above 150.0, the PD-L1 tumor proportion score was estimated to be more than 50%. Therefore, for a case in which a PD-L1 expression score was above 150.0, it was defined as having high PD-L1 expression intensity. In the cohort consisting of patients with stage I–IIIA NSCLC, Kaplan–Meier analysis revealed that postoperative RFS was not significantly different between the high PD-L1 group ($n=55$) and the low PD-L1 group ($n=173$) ($P=0.253$, Fig. 3a), which had 5-y RFP of 54.7% and 62.7%, respectively. In patients with stage I NSCLC, postoperative RFS for the high PD-L1 group ($n=25$) was significantly longer than postoperative RFS for the low PD-L1 group ($n=95$) ($P=0.031$, Fig. 3b), which had 5-y RFP of 94.1% and 75.1%, respectively. Conversely, in patients with stage II (Fig. 3c) or IIIA (Fig. 3d) NSCLC, postoperative RFS for the high PD-L1 group tended to be shorter than postoperative RFS for the low PD-L1 group ($P=0.105$, $P=0.139$, respectively). These data demonstrate that the prognostic value of tumor PD-L1 expression intensity is different between patients with early-stage and locally advanced-stage NSCLC.

Next, we analyzed the value of tumor PD-L1 expression status on postoperative tumor relapse according to pT-stage. In patients with pT1, Kaplan–Meier analysis revealed that postoperative RFS for the high PD-L1 group ($n=23$) tended to be longer than postoperative RFS for the low PD-L1 group ($n=89$) ($P=0.290$, Fig. 4a), which had 5-y RFP of 78.4% and 66.8%, respectively. However, in patients with pT2 (Fig. 4b), pT3 (Fig. 4c) or pT4 (Fig. 4d) NSCLC, postoperative RFS for the high PD-L1 group tended to be shorter than postoperative RFS for the low PD-L1 group ($P=0.726$, $P=0.051$, $P=0.407$, respectively).

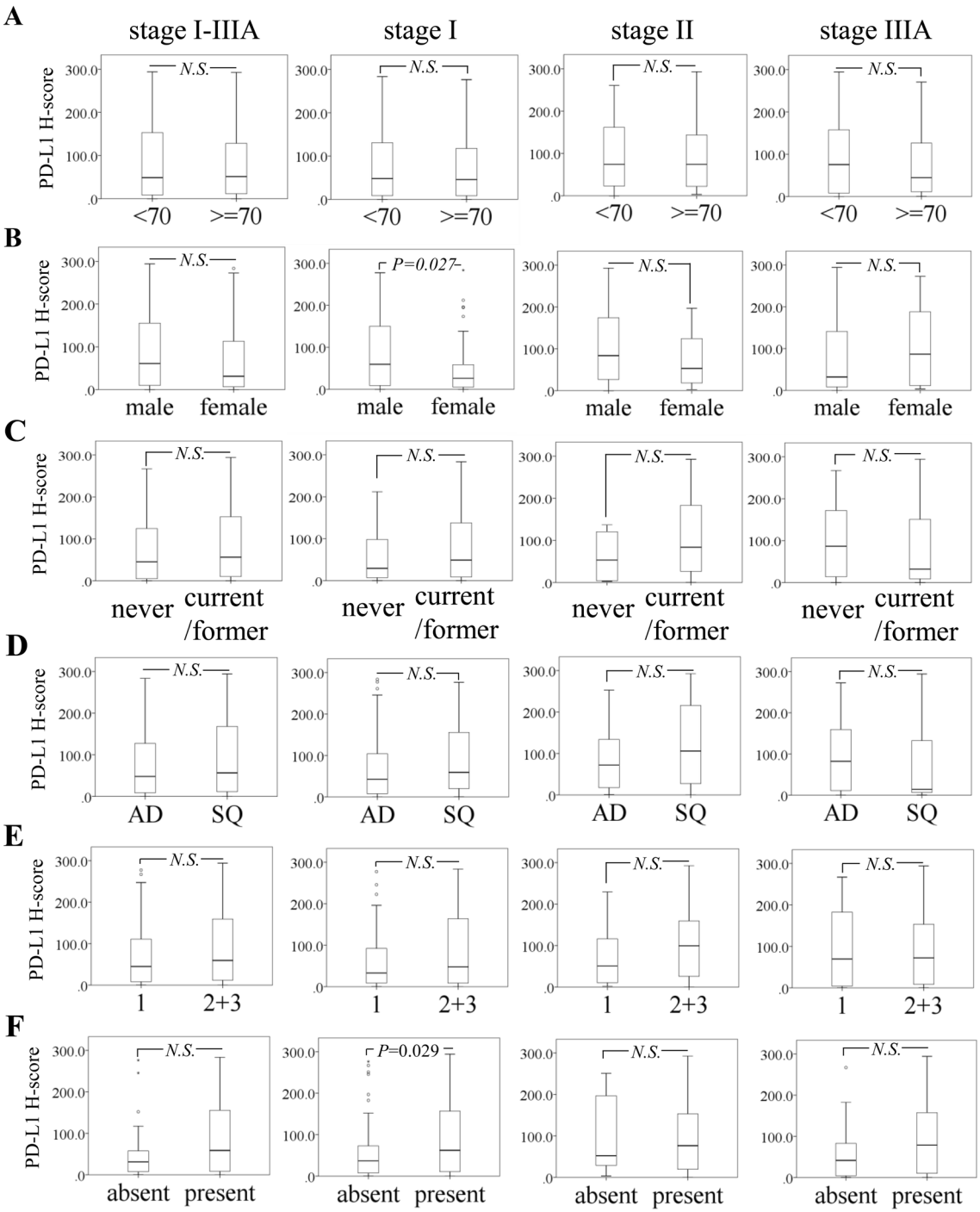

Fig. 2 Tumor PD-L1 expression intensity and clinicopathological factors relative to the pathological stages. The correlations between PD-L1 H-score and **a** age, **b** gender, **c** smoking habits, **d** pathological types, **e** tumor cell grade, and **f** microvessel invasion

In addition, we analyzed associations with respect to pN-stage. In patients with pN0 (Fig. 3a), postoperative prognosis tended to be better in the high PD-L1 group ($n = 33$) compared with the low PD-L1 group ($n = 129$) ($P = 0.731$) (Fig. 5a). Conversely, in the pN1 (Fig. 5b) and pN2 (Fig. 5c) cohorts, postoperative prognosis for the high PD-L1 group tended to be poorer compared with the prognosis for the low PD-L1 group ($P = 0.190$, $P = 0.051$, $P = 0.649$, respectively). These data demonstrate that the value of tumor PD-L1 expression status on postoperative tumor relapse may vary with tumor progression in NSCLC.

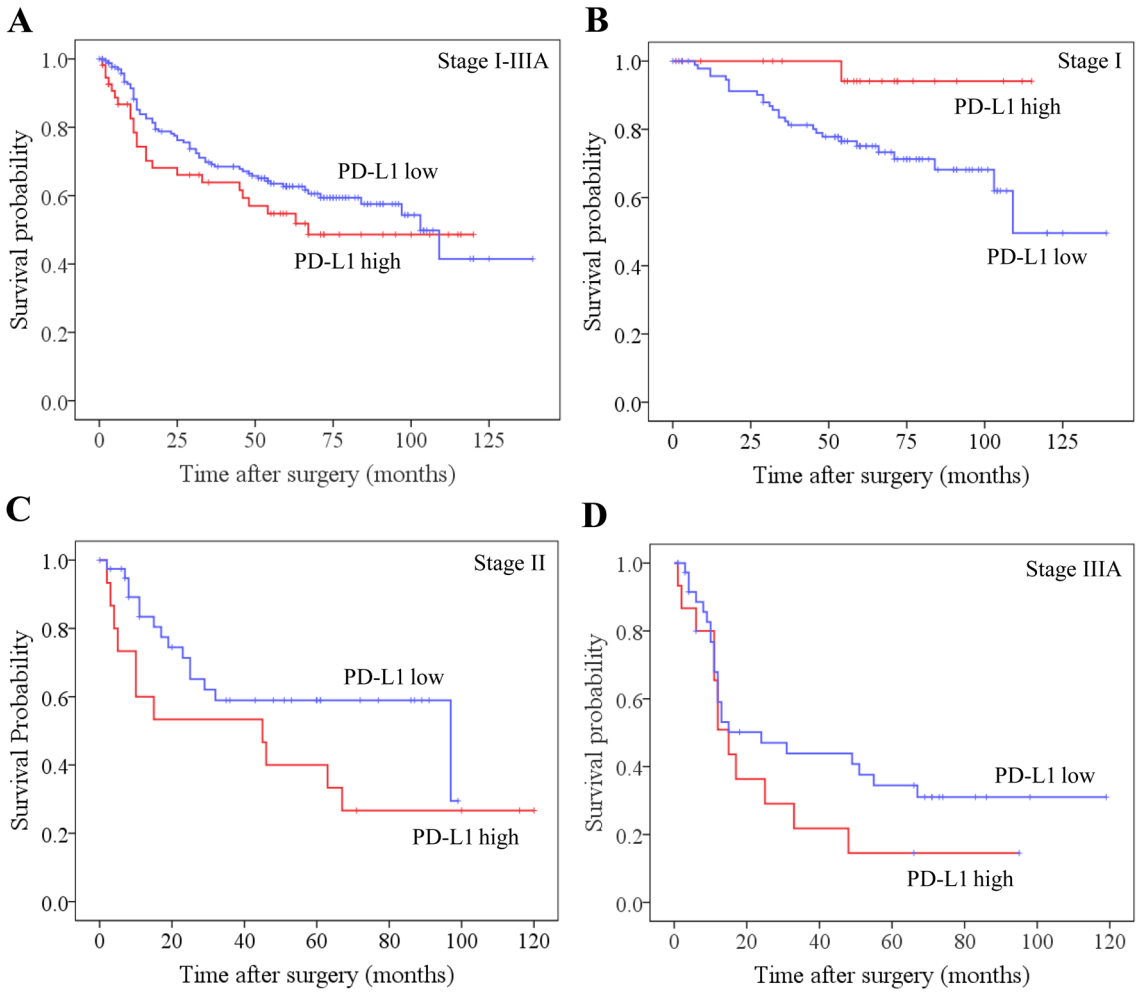

Fig. 3 Tumor PD-L1 expression intensity and relapse-free survival relative to the pathological stage. **a** Kaplan–Meier analysis on relapse-free survival in patients with stage I–IIIA. **b** Kaplan–Meier analysis on relapse-free survival in patients with stage I. **c** Kaplan–Meier analysis on relapse-free survival in patients with stage II. **d** Kaplan–Meier analysis on relapse-free survival in patients with stage IIIA

Tumor PD-L1 expression intensity to predict postoperative tumor relapse in NSCLC

Next, we examined whether tumor PD-L1 expression status was a predictive biomarker for tumor relapse after radical surgery in NSCLC. In the cohort consisting of patients with stage I–IIIA NSCLC, univariate analysis revealed that postoperative RFS was not correlated with several clinicopathological factors including age ($P=0.555$), pathological types ($P=0.475$), tumor cell grade ($P=0.121$) or tumor PD-L1 expression intensity ($P=0.253$) (Table 3). However, patients with smoking habits and those with microvessel invasion of tumor cells more frequently experienced postoperative tumor relapse compared with their counterparts ($P=0.009$ and $P=0.007$, respectively). In patients with stage I NSCLC, postoperative RFS was significantly correlated with clinicopathological factors such as smoking habits ($P=0.035$) or tumor PD-L1 expression status ($P=0.031$) (Table 2). In both stage II and IIIA NSCLC cohorts, postoperative RFS was not correlated with clinicopathological factors analyzed in the study (Table 2).

We further examined the prognostic value of tumor PD-L1 expression status by multivariate analysis. In the cohort consisting of patients with stage I–IIIA NSCLC, as well as both cohorts consisting of stage II and IIIA NSCLC, tumor PD-L1 expression status did not serve as a significant predictive factor for tumor relapse after radical surgery (Table 3). However, in patients with stage I NSCLC, high tumor PD-L1 expression status was revealed to be a positive prognostic factor of higher 5-y RFP [hazard ratio (HR): 0.111, 95% confidence interval (CI): 0.015–0.841, $P=0.033$]. In addition, microvessel invasion of tumor cells was also revealed to be a positive prognostic factor of lower 5-y RFP (HR: 3.370, 95% CI: 1.232–9.220, $P=0.018$) in

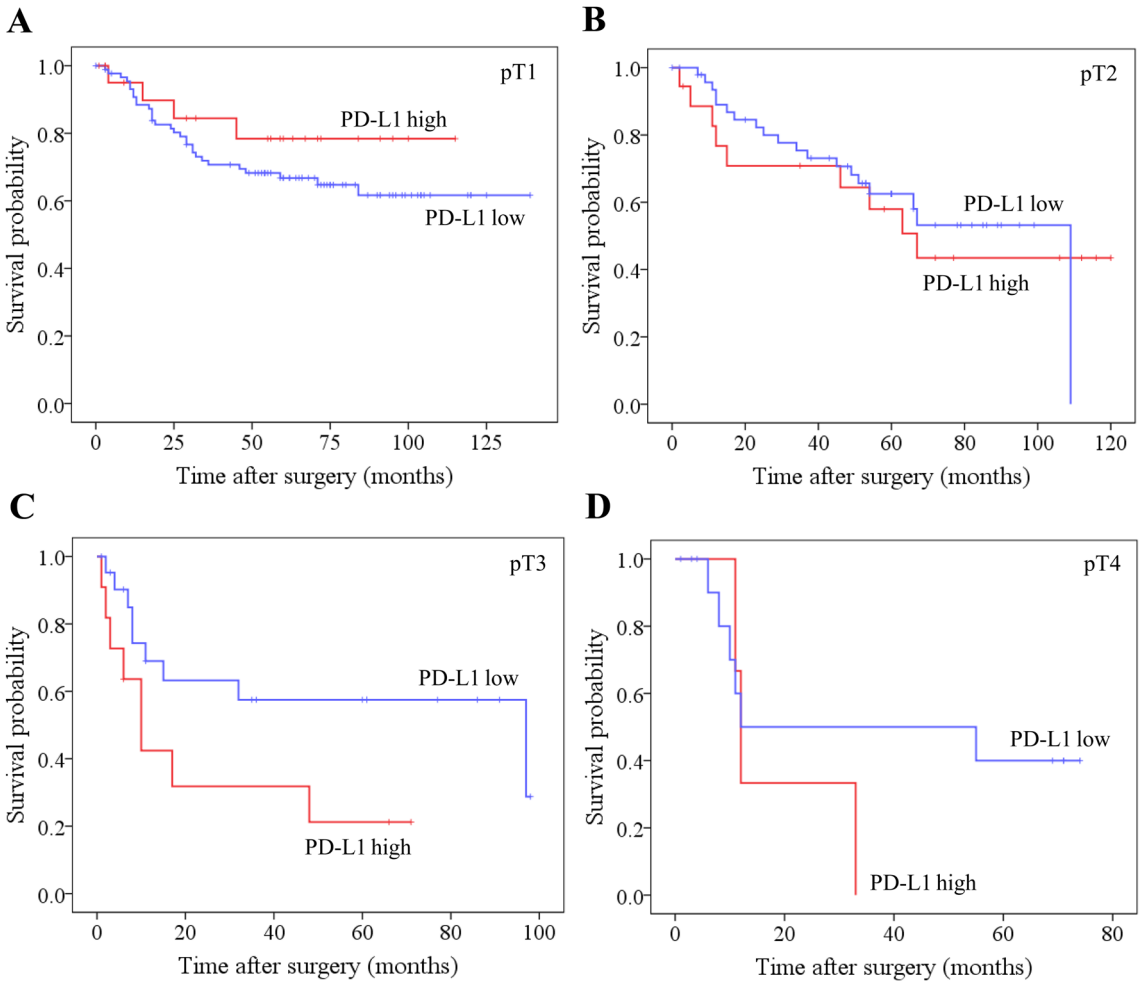

Fig. 4 Tumor PD-L1 expression intensity and relapse-free survival relative to the pT-stage. **a** Kaplan–Meier analysis on relapse-free survival in patients with pT0. **b** Kaplan–Meier analysis on relapse-free survival in patients with pT1. **c** Kaplan–Meier analysis on relapse-free survival in patients with pT3. **d** Kaplan–Meier analysis on relapse-free survival in patients with pT4

the cohort, and the variable was not associated with tumor PD-L1 expression intensity ($P = 0.057$, Table 1). These data demonstrate that tumor PD-L1 expressions status is an independent prognostic factor of tumor relapse after radical surgery in stage I NSCLC, but not in locally advanced stage NSCLC.

Discussion

In this study, we examined the prognostic value of PD-L1 expression status in NSCLC and found that tumor PD-L1 expression status has the biphasic prognostic significance in patients with early- and locally advanced-stage NSCLC. In early-stage NSCLC, high PD-L1 expression status in tumor cells was significantly associated with longer RFS after radical surgery, whereas in locally advanced-stage NSCLC, it was associated with tumor recurrence after radical surgery.

The roles of the PD-1/PD-L1 immune checkpoint mechanism in tumor progression has been the subject of intense research, since the discovery that anti-PD-1/PD-L1 inhibitors exhibit clinical efficacy in patients with NSCLC. Previous papers have described the prognostic significance of PD-L1 expression in tumor cell of NSCLC patients. In a meta-analysis, in which 50 studies containing 11,383 patients were analyzed, high PD-L1 expression status was associated with poor OS [9]. Given that PD-L1-positive tumor cells can suppress the antitumor immune response through exhaustion of PD-1-positive activated T lymphocytes [1, 2], the data regarding a correlations between PD-L1 expression and poor prognosis are plausible. However, it is unclear whether PD-L1 expression in tumor cells is associated with OS. The PD-L1 expression status of tumor cells may temporally and spatially change depending on the immunological and physiological properties of the tumor microenvironment [6, 19], and it is constantly changing as a

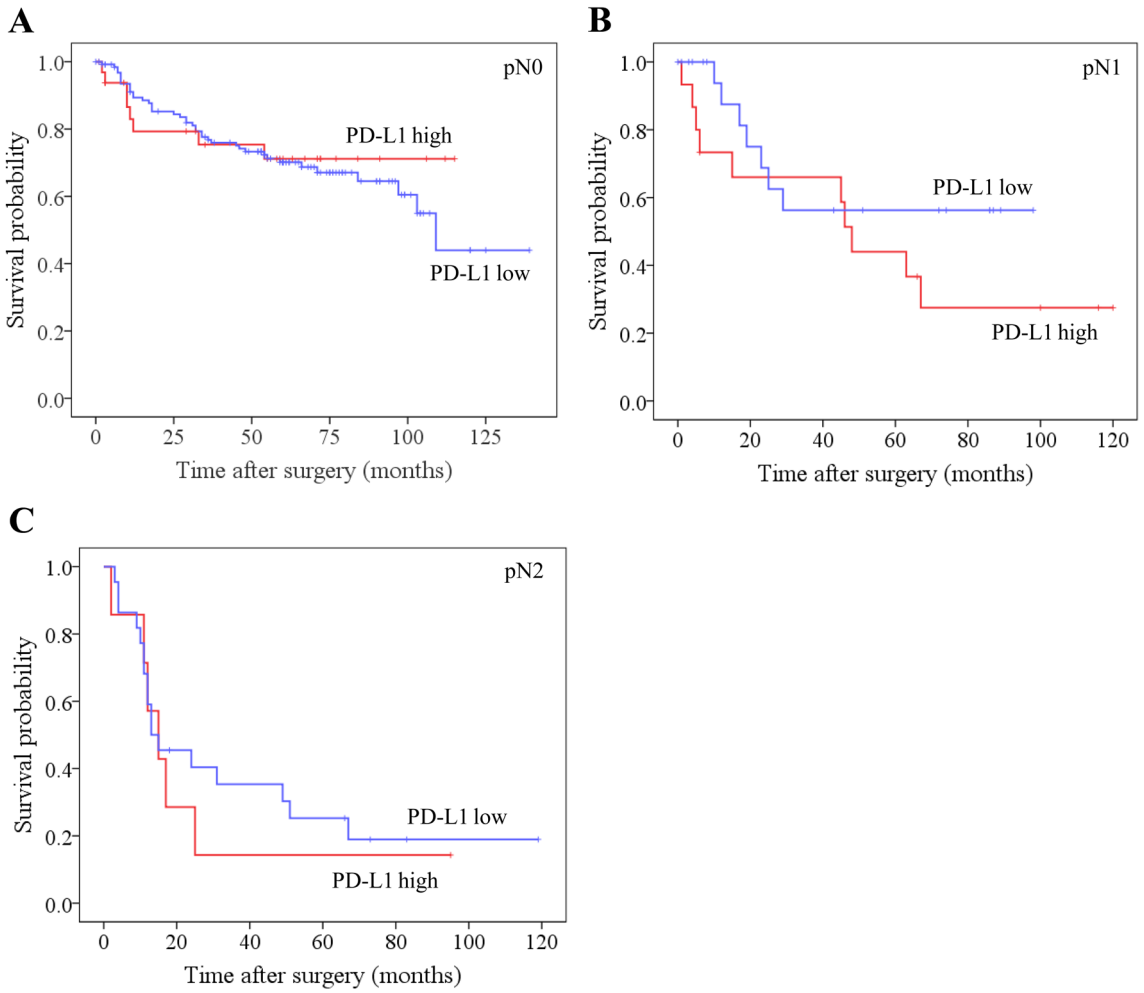

Fig. 5 Tumor PD-L1 expression intensity and relapse-free survival relative to the pN-stage. **a** Kaplan–Meier analysis on relapse-free survival in patients with pN0. **b** Kaplan–Meier analysis on relapse-free survival in patients with pN1. **c** Kaplan–Meier analysis on relapse-free survival in patients with pN2

result of the therapy that a patient receives. The PD-L1 status of tumor cells at the time of surgery is unlikely to persist throughout the clinical course of the disease. Therefore, it is difficult to predict the OS of NSCLC patients based solely on tumor cell PD-L1 expression at the time of surgery. In this context, we examined the significance of PD-L1 expression in tumor cell in association with tumor recurrence, and found that high PD-L1 expression intensity is associated with poor RFS after radical surgery. Similar results were reported by Takada and colleagues [14], suggesting that PD-L1 status of tumor cells at surgery is associated with tumor recurrence.

However, an additional concern has been raised about these studies. For example, as pointed out in another report [16], the patient populations in these studies were quite heterogeneous making it difficult to evaluate the prognostic value of PD-L1 expression in tumors. In most studies, patients with all stages (early to advanced) of NSCLC were included in a single study [23–30]. As such, the patient cohort consisted of patients who received different treatment regimens and had different prognoses according to their respective stage. To eliminate the heterogeneity of the patient population, we analyzed the data relative to pathological stage and showed that the prognostic value of tumor PD-L1 expression intensity varied with tumor progression. Thus, the prognostic value of PD-L1 in NSCLC can only be accurately determined when such potentially confounding factors are eliminated.

In the present study, high PD-L1 expression status of tumor cells was significantly associated with extended RFS after radical surgery in stage I NSCLC patients. This demonstrates that tumor PD-L1 is a useful biomarker for predicting a good postoperative prognosis. To address the reason why tumor PD-L1 expression is associated with a good prognosis in early-stage NSCLC, we must consider the role of interferon-γ. As we previously reported, PD-L1 expression

Table 2 Univariate analysis of correlations between patient prognosis and clinicopathological factors

	Stage I–IIIA ($n=228$)		Stage I ($n=120$)		Stage II ($n=55$)		Stage IIIA ($n=53$)	
Variables	5-y RFP (%)	*P*	5-y RFP (%)	*P*	5-y RFP (%)	*P*	5-y RFP (%)	*P*
Age, years								
< 70	59.7	0.555	77.5	0.661	60.3	0.530	29.5	0.652
≥ 70	62.1		79.2		45.0		26.4	
Gender								
Male	56.5	0.032	74.5	0.204	47.2	0.101	30.9	0.951
Female	70.8		84.9		77.8		23.2	
Smoking								
Never	73.5	0.009	86.8	0.035	87.5	0.110	18.2	0.703
Current/former	55.9		73.7		46.0		32.0	
Pathology								
Adenocarcinoma	63.9	0.475	79.6	0.790	62.9	0.658	24.5	0.592
Squamous cell ca	54.6		74.6		41.5		36.2	
Cell grade								
Grade 1	70.2	0.121	82.5	0.647	63.6	0.892	18.2	0.519
Grade 2–3	55.5		74.8		49.3		31.8	
Microvessel invasion								
Absent	77.9	0.007	90.6	0.082	50.0	0.119	45.0	0.119
Present	55.8		73.0		53.3		25.2	
PD-L1 status								
Low	62.7	0.253	75.1	0.031	59.0	0.105	34.5	0.139
High	54.7		94.1		40.0		14.5	

5-y RFP 5-year relapse-free probability

Table 3 Multivariate analysis on relapse-free survival after surgery

	Stage I–IIIA ($n=228$)		Stage I ($n=120$)		Stage II ($n=55$)		Stage IIIA ($n=53$)	
	HR	*P*	HR	*P*	HR	*P*	HR	*P*
Variables	(95% CI)		(95% CI)		(95% CI)		(95% CI)	
Age, years	0.868	0.523	1.759	0.18	0.644	0.351	0.753	0.484
< 70 vs. ≥ 70	(0.563–1.339)		(0.786–3.934)		(0.255–1.624)		(0.340–1.666)	
Gender	1.008	0.982	1.165	0.82	2.186	0.43	0.834	0.738
Male vs. female	(0.482–2.108)		(0.313–4.342)		(0.313–15.282)		(0.288–2.413)	
Smoking	1.905	0.111	2.903	0.147	1.703	0.592	0.901	0.853
Current/former vs. never	(0.862–4.207)		(0.688–12.234)		(0.243–11.933)		(0.299–2.714)	
Cell grade	1.064	0.794	1.126	0.771	1.413	0.528	0.829	0.647
Grade 2–3 vs. grade 1	(0.666–1.700)		(0.506–2.504)		(0.483–4.136)		(0.371–1.851)	
Microvessel invasion	2.14	0.017	3.37	0.018	1.057	0.973	2.269	0.135
Present vs. absent	(1.146–3.995)		(1.232–9.220)		(0.271–4.125)		(0.774–6.653)	
PD-L1 status	1.131	0.614	0.111	0.033	1.45	0.402	1.536	0.242
High vs. low	(0.701–1.826)		(0.015–0.841)		(0.608–3.458)		(0.749–3.151)	

HR hazard ratio, *95% CI* 95% confidence interval

in NSCLC cells is regulated by interferon-γ [19]. Given that this cytokine is primarily released from activated T lymphocytes in the tumor microenvironment [20, 21], the presence of PD-L1-positive tumor cells is indicative of an ongoing antitumor immune response. Thus, complete tumor resection may effectively reduce the tumor burden while leaving the antitumor immune response intact. This cytotoxic immune response could attack residual tumor cells after surgery

and prevent tumor recurrence, thus yielding a good clinical course after surgery in patients with stage I NSCLC.

Similar results have been reported for stage I pulmonary adenocarcinomas by Yang and colleagues [16] despite the use of a different method of PD-L1assessment. They defined a PD-L1-positive case as one in which more than 5% of tumor cells were PD-L1- positive for by immunohistochemical analysis. In contrast, we defined a PD-L1-positive case as having a PD-L1 H-score greater than 150. In these cases, all cases are estimated to be more than 50% in their tumor proportional score (TPS) of PD-L1. On the other hand, cases with a PD-L1 H-score less than 150 consist of cases with the TPS less than 50% and those with the TPS more than 50%, because the staining intensity is not considered in the TPS counting. Therefore, though the criteria for PD-L1-positive cases were comparatively more strict in our study, the clinical significance of its expression in stage I NSCLC patients was consistent, suggesting the presence of an antitumor immune response. As further evidence for the induction of an antitumor immune response, we examined the correlation between the frequency of tumor-infiltrating lymphocytes and PD-L1 expression. However, we found no correlations were found out (data not shown), which is consistent with a previously reported study [16]. Given that tumor-infiltrating lymphocytes are freely moving around in the tumor microenvironment, it may be difficult to capture the exact time of their interaction with tumor cells.

In locally advanced stage NSCLC (stage II–IIIA), high PD-L1 expression status of tumor cells was associated with poor RFS after radical surgery, which was in contrast to the results observed for early-stage disease. Therefore, the PD-L1 expression status of tumor cells reflects the induction of an antitumor immune response in early-stage NSLCL, whereas it reflects suppression of an antitumor immune response in later stages. Activated T lymphocytes can induce PD-L1 expression on tumor cells through interferon-γ [19], and their antitumor activity may be exhausted by the PD-1/PD-L1 immune checkpoint mechanism [1]. In locally advanced stage NSCLC with high PD-L1 expression, the antitumor immune response resulting from activated T lymphocytes could be suppressed by the PD-1/PD-L1 immune checkpoint mechanism. We hypothesize that the clinical significance of PD-L1 expression status of tumor cells may depend on the time that activated T lymphocytes are influenced by the PD-1/PD-L1 interaction.

With respect to the association between tumor PD-L1 expression status and prognosis of NSCLC patients, antitumor immunity in the tumor microenvironment, which is represented by PD-L1 expression status at the time of surgery, may influence tumor recurrence. In the study, however, we did not examine PD-L1 expression status in the tumor tissues of recurrent cases. Therefore, we cannot evaluate the effect of PD-L1 expression status at the time of surgery on tumor recurrence, and this is considered to be a limitation of our study.

In conclusion, the prognostic significance of tumor PD-L1 expression status is different between patients with early- and locally advanced-stage NSCLC. In early-stage NSCLC, high PD-L1 expression status of tumor cells reflects the induction of an antitumor immune response and have utility as a biomarker to predict a good prognosis after radical surgery. However, in locally advanced-stage NSCLC, tumor PD-L1 expression status may reflect the execution of an immune checkpoint pathway that predicts the incidence of postoperative tumor recurrence. On the basis of the data, evaluation of tumor PD-L1 expression status focusing on its biphasic prognostic significance is suggested to be of value for the postoperative disease management of NSCLC patients.

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Compliance with ethical standards

Conflict of interest The authors have no conflicts of interest.

Ethical approval The study design was approved by the Ethical Committee of Shiga University of Medical Science (approval number: R2016-115).

Informed consent Informed consent was obtained from all patients in the study. The patients agreed to the use of their specimens and clinical data for research and publication.

References

1. Dong H, Strome SE, Salomao DR, Tamura H, Hirano F, Flies DB, Roche PC, Zhu G, Tamada K, Lennon VA, Celis E, Chen L (2002) Tumor-associated B7–H1 promotes T-cell apoptosis: a potential mechanism of immune evasion. Nat Med 8(8):793–800
2. Hansen JD, Du Pasquier L, Lefranc MP, Lopez V, Benmansour A, Boudinot P (2009) The B7 family of immunoregulatory

receptors: a comparative and evolutionary perspective. Mol Immunol 46(3):457–472
3. Brahmer J, Reckamp KL, Baas P, Crinò L, Eberhardt WEE, Antonia S, Pluzanski A, Vokes EE, Holgado E, Waterhouse D, Ready N, Gainor J, Frontera OA, Havel L, Steins M, Garassino M, Aerts JG, Domine M, Paz-Ares L, Reck M, Baudelet C, Harbison C, Lestini B, Spigel D (2015) Nivolumab versus docetaxel in advanced squamous-cell non-small-cell lung cancer. N Engl J Med 373(2):123–135
4. Borghaei H, Paz-Ares L, Horn L, Spigel D, Steins M, Ready NE, Chow LQ, Voles EE, Felip E, Holgado E, Barlesi F, Kohlhäufl M, Arrieta O, Burgio MA, Fayette J, Lena H, Poddubskaya E, Gerber DE, Gettinger SN, Rudin CM, Rizvi N, Crinò L, Blumenschein GR, Antonia SJ, Dorange C, Harbison CT, Finckenstein FG (2015) Brahmer JR (2015) Nivulumab versus docetaxel nonsquamous non-small-cell lung cancer. N Engl J Med 373(17):1627–1639
5. Reck M, Rodriguez-Abreu D, Robinson AG, Hui R, Csőszi T, Fülöp A, Gottfried M, Peled N, Tafreshi A, Cuffe S, O'Brien M, Rao S, Hotta K, Leiby MA, Lubiniecki GM, Shentu Y, Rangwala R, Brahmer JR (2016) Pembrolizumab versus chemotherapy for PD-L1-positive non-small-cell lung cancer. N Engl J Med 375(19):1823–1833
6. Igarashi T, Teramoto K, Ishida M, Hanaoka J, Daigo Y (2016) Scoreing of PD-L1 expression intensity on pulmonary adenocarcinomas and the correlations with clinicopathological factors. ESMO Open 1(4):e000083
7. McLaughlin J, Han G, Schalper KA, Carvajal-Hausdorfet D, Pelekanou V, Rehman J, Velcheti V, Herbst R, LoRusso P, Rimm DL (2016) Quantitative assesment of the heterogeneity of PD-L1 expression in non-small-cell lung cancer. JAMA Oncol 2(1):46–54
8. Nakamura S, Hayashi K, Imaoka Y, Kitamura Y, Akazawa Y, Tabata K, Groen R, Tsuchiya T, Yamasaki N, Nagayasu T, Fukuoka J (2017) Intratumoral heterogeniety of programmed cell death ligand-1 expression is common in lung cancer. PLoS ONE 12(10):e0186192
9. Li H, Xu Y, Ban D, Song Y, Zhan P, Hu Y, Zhang Q, Zhang F, Liu H, Li T, Sugiura H, Cappuzzo F, lin D, Lv T, (2019) The clinicopathological and prognostic significance of PD-L1 expression assessed by immunohistochemistry in lung cancer: a meta-analysis of 50 studies with 11,383 patients. Transl Lung Cancer Res 8(4):429–449
10. Mu C-Y, Huang J-A, Chen Y, Chen C, Zhang X-G (2011) High expression of PD-L1 in lung cancer may contribute to poor prognosis and tumor cells immune escape through suppressing tumor infiltrating dendritic cells maturation. Med Oncol 28(3):682–688
11. Shimoji M, Shimizu S, Sato K, Suda K, Kobayashi Y, Tomizawa K, Takemoto T, Mitsudomi T (2016) Clinical and pathologic features of lung cancer expressing programmed cell death ligand (PD-L1). Lung Cancer 98:69–75
12. Sun J-M, Zhou W, Choi Y-L, Choi S-J, Kim SE, Wang Z, Dolled-Filhart M, Emancipator K, Wu D, Weiner R, Frisman D, Kim HK, Choi YS, Shim YM, Kim J (2016) Prognostic significance of PD-L1 in patients with non-small cell lung cancer: A large cohort study of surgically resected cases. J Thorac Oncol 11(7):1003–1011
13. Zhou C, Tang J, Sun H, Zheng X, Li Z, Sun T, Li J, Wang S, Zhou X, Sun H, Cheng Z, Zhang H, Ma H (2017) PD-L1 expression as poor prognostic factor in patients with non-squamous non-small cell lung cancer. Oncotarget 8(35):58457–58468
14. Takada K, Okamoto T, Toyokawa G, Kozuma Y, Matsubara T, Haratake N, Akamine T, Takamori S, Katsura M, Shoji F, Oda Y, Maehara Y (2017) The expression of PD-L1 protein as a prognostic factor in lung squamous cell carcinoma. Lung Cancer 104:7–15
15. Sepesi B, Cuentas EP, Canales JR, Behrens C, Correa AM, Vaporciyan A, Weissferdt A, Kalhor N, Maron C, Swisher S, Wistuba I (2017) Programmed death cell ligand 1 (PD-L1) is associated with survival in stage I non-small cell lung cancer. Semin Thorac Cardiovasc Surg 29(3):408–415
16. Yang C-Y, Lin M-W, Chang Y-L, Wu C-T, Yang P-C (2014) Programmedn cell death-ligand I expression in surgically resected stage I pulmonary adenocarcinoma and its correlation with driver mutations and clinical outcomes. Eur J Cancer 50(7):1361–1369
17. Cooper WA, Tran T, Vilain RE, Madore J, Selinger CI, Kohonen-Corish M, Yip PY, Yu B, O'Toole SA, McCaughan BC, Yearley JH, Horvath LG, Kao S, Boyer M, Scolyer RA (2015) PD-L1 expression is a favorable prognostic factor in early stage non-small cell carcinoma. Lung Cancer 89(2):181–188
18. Yang C-Y, Lin M-W, Chang Y-L, Wu C-T, Yang P-C (2016) 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 57:91–103
19. Igarashi T, Teramoto K, Ishida M, Hanaoka J, Daigo Y (2017) The mechanism of de novo expression of programmed cell death-ligand 1 in squamous cell carcinoma of the lung. Oncol Rep 38(4):2189–2196
20. Abiko K, Matsumura N, Hamanishi J, Horikawa N, Murakami R, Yamaguchi K, Yoshida Y, Baba T, Konishi I, Mandai M (2015) IFN-γ from lymphocytes induces PD-L1 expression and promotes progression of ovarian cancer. Br J Cancer 112(9):1501–1509
21. Spranger S, Spaapen RM, Zha Y, Williams J, Meng Y, Ha TT, Gajewski TF (2013) Up-regulation of PD-L1, IDO, and T (regs) in the melanoma tumor microenvironment is driven by CD8(+) T cells. Sci Transl Med 5(200):200ra116
22. Teramoto K, Igarashi T, Kataoka Y, Ishida M, Hanaoka J, Sumimoto H, Daigo Y (2019) Clinical significance of PD-L1-positive cancer-associated fibroblasts in pN0M0 non-small cell lung cancer. Lung Cancer 173:56–63
23. Chen Y-B, Mu C-Y, Huang J-A (2012) Clinical significance of programmed death-1 ligand-1 expression in patients with non-small cell lung cancer: a 5-year-follow-up study. Tumori 98(6):751–755
24. Azuma L, Ota K, Kawahara A, Hattori S, Ikawa E, Harada T, Matsumoto K, Takayama K, Takamori S, Kage M, Hoshimno T, Nakanishi Y, Okamoto I (2014) Association of PD-L1 overexpression with activating EGFR mutations in surgically resected non small-cell lung cancer. Ann Oncol 25(10):1935–1940
25. Mao Y, Li W, Chen K, Xie V, Liu Q, Duan W, Zhou X, Liang R, Tao M (2015) B7–H1 and B7–H3 are independent predictors of poor prognosis in patients with non-small cell lung cancer. Oncotarget 6(5):3452–3461
26. Ji M, Liu Y, Li Q, Li X, Ning Z, Zhao W, Shi H, Jiang J, Wu C (2016) PD-1/PD-L1 expression in non-small-cell lung cancer and its correlation with EGFR/KRAS mutations. Cancer Biol Ther 17(4):407–413
27. Okita R, Maeda A, Shimizu K, Nojima Y, Saisho S, Nakata M (2017) PD-L1 overexpression is partially regulated by EGFR/HER2 signaling and associated with poor prognosis in patients with non-small-cell lung cancer. Cancer Immunol Immunother 66(7):865–876
28. Zhang Y, Wang L, Li Y, Pan Y, Wang R, Hu H, Li H, Luo X, Ye T, Sun Y, Chen H (2014) Protein expression of programmed death 1 ligand 1 and ligand 2 independently predict poor prognosis in surgically resected lung adenocarcinoma. Onco Targets Ther 7:567–573
29. Cha YJ, Kim HR, Lee CY, Cho BC, Shim HS (2016) Clinicopathological and prognostic significance of programmed cell death ligand-1 expression in lung adenocarcinoma and its relationship with p53 status. Lung Cancer 297:73–80

30. Huynh TG, Morales-Oyarvide V, Campo MJ, Gainor JF, Bozkurlar E, Uruga H, Zhao L, Gomez-Caraballo M, Hata AN, Mark EJ, Lanuti M, Engelman JA, Mino-Kenudson M (2016) Programmed cell death ligand 1 expression in resected lung adenocarcinomas: association with immune microenvironment. J Thorac Oncol 11(11):1869–1918