Significance of programmed cell death-ligand 1 expression and its association with survival in patients with small cell lung cancer

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Abstract

Background: Programmed cell death 1 (PD-1) receptor-ligand interaction is a major pathway often hijacked by tumors in order to suppress immune control. The aim of this retrospective study was to investigate the prevalence and prognostic roles of PD-ligand 1 (PD-L1) expression in small cell lung cancer (SCLC).

Methods: The expression of PD-L1 was evaluated by immunohistochemical analysis in 102 specimens of SCLC. Tumors with staining in over 5% of tumor cells were scored as positive for PD-L1 expression. Survival analysis was performed using the Kaplan-Meier method.

Results: Expression of PD-L1 in tumor cells was observed in 71.6% (73/102) of SCLCs, and was significantly correlated with a limited disease (LD) stage. SCLC patients with PD-L1-positive tumors showed significantly longer overall survival (OS) than those with PD-L1-negative (median OS, 16.3 versus 7.3 months; $p<0.001$, respectively). Multivariate analyses demonstrated that a good performance status, LD stage and expression of PD-L1 were significantly predictive of better OS, independently of other factors. We found no relevance between PD-L1 expression and progression-free survival for first-line treatment in LD- and extensive disease-SCLC patients.

Conclusions: In patients with SCLC, expression of PD-L1 is positively correlated with a LD stage, and is independently predictive of a favorable outcome.

Key words: small cell lung cancer, PD-L1, immunohistochemistry, prognostic factor

Introduction

Lung cancer is the leading cause of cancer death worldwide.[1] Small cell lung cancer (SCLC) is a histological subtype that accounts for approximately 15% of all lung cancers cases.[2] Systemic chemotherapy is the standard type of care for SCLC. Although SCLC shows a good response to initial treatment, most patients suffer disease recurrence and become refractory to chemotherapy. Despite intensive research, the prognosis of SCLC remains poor, and therefore new strategies to improve outcome are urgently needed.

Blockade of immune checkpoints with monoclonal antibodies has also recently emerged as a new therapeutic tool in oncology.[3, 4] Programmed cell death 1 (PD1), which belongs to the CD28 family of proteins, is a receptor expressed on the surface of T cells that regulates their activation and proliferation.[3, 4] Its ligand, programmed cell death–ligand 1 (PD-L1), is frequently overexpressed in many types of human cancer.[5] Recent clinical trials have indicated that inhibition of this pathway with anti-PD-1/PD-L1 antibodies exerts a promising antitumor effect against several human malignancies, including non-small cell lung cancer (NSCLC), melanoma, and renal cell cancer.[6, 7] Preliminary analysis of these trials suggests that tumor expression of PD-L1 predicts response to PD-1/PD-L1 directed therapy. However, the clinical relevance of PD-L1 expression in SCLC has remained unclear. We therefore examined PD-L1 expression in SCLC and analyzed its associated clinicopathologic characteristics and prognostic relevance.

Patients and Methods

Patients

We retrospectively screened consecutive 178 patients who were diagnosed as having SCLC at Kurume University Hospital between 2002 and 2013. Among these patients, 108 were histologically diagnosed on the basis of tissue samples obtained by biopsy or surgery, and 70 patients were diagnosed by cytological specimens. Adequate histological specimens containing abundant tumor cells were available for 102 of these patients, who were enrolled in this retrospective study. The tumor specimens were derived from primary lung lesion in 83 (81.4%) patients, liver metastasis in four (3.9%), brain metastasis in three (2.9%), bone metastasis in two (2.0%), skin metastasis in three (2.9%) and lymph node metastasis in seven (6.9%). The clinical characteristics of the patients, including age, sex, Eastern Cooperative Oncology Group (ECOG) performance status (PS), limited disease (LD)-extensive disease (ED) stage, serum lactate dehydrogenase (LDH) level, serum pro-gastrin-releasing peptide (Pro-GRP) level, and serum neuron-specific enolase (NSE) level, were recorded. LD stage was defined as location of disease within an anatomic region that could be safely encompassed within a tolerable radiation field, whereas ED stage was defined as extending beyond locoregional boundaries, possibly including malignant pleural or pericardial effusion, and hematogenous metastases. The present study was conducted in accordance with the provisions of the Declaration of Helsinki and was approved by the Institutional Review Board of Kurume University Hospital.

Immunohistochemical analysis of PD-L1 proteins

We used 4-μm-thick sections of formalin-fixated, paraffin-embedded tissues. The sections were mounted on glass slides and then incubated with anti-rabbit monoclonal antibody against PD-L1 (abcam, Cambridge, UK) for immunohistochemical analysis with the use of BenchMark XT (Ventana Automated Systems, Inc., Tucson, AZ, USA).

Briefly, each slide was heat-treated using Ventana's CC1 retrieval solution for 30 min, and incubated with the PD-L1 antibody for 30 min. This automated system used the ultraVIEW DAB detection kit with 3, 3' diaminobenzidine (DAB) as the chromogen (Ventana Automated Systems). All immunohistochemical analysis was evaluated by two experienced observers (A.K. and M.K.) who were unaware of the patients' conditions. Spots for which the pathologists disagreed regarding the staining category were reviewed jointly and a single consensus category was established. Cases with less than 5% tumor staining were considered negative as previous studies.[8, 9]

Statistical analyses

Correlations between PD-L1 expression and patient characteristics were analyzed using the chi-squared or Fisher's exact test for categorical variables. We evaluated whether parameters including PD-L1 expression were associated with the survival of SCLC patients. Overall survival (OS) was measured from administration of treatment or initial diagnosis until the date of death or last follow-up. PFS was defined as the period from the date of initiation of first-line treatment to the date of disease progression or death due to any cause. The Kaplan-Meier method was used to assess the patients' survival curves and the log-rank test was used to evaluate the significance of differences between two groups. Multivariate regression was performed using the Cox proportional hazards model. All variables that had p values of <0.05 were included in the Cox model. All tests were two-sided, and differences were considered statistically significant at $P<0.05$. All of the statistical analyses were conducted using JMP version 10 (SAS Institute Inc., Cary, NC).

Results

Patient characteristics

The clinical characteristics of the 102 patients are shown in Table 1. The median age of the patients at diagnosis was 70 years (range, 36-85 years). Eighty-nine (87.3%) of the patients were male, and 87 (85.3%) patients had a good PS (0-1). Forty-one patients (40.2%) had LD and 61 (59.8%) had ED at the time of diagnosis. The median LDH level was 245 U/L (range, 138–3476 IU/L). The LDH level was found to be lower than or equal to the upper normal limit of 229 IU/L in 37 patients (36.3%), and was higher than 229 IU/L in 65 patients (63.7%). The Pro-GRP and NSE serum levels were available in 101 (99.0%) and 79 (77.5%) patients, respectively, and the median values were 294 pg/mL (range, 12.6-33300 pg/mL) and 22.4 ng/mL (range, 5.2-581 ng/mL), respectively. ED-SCLC patients were treated with platinum-based chemotherapy as a first line treatment. Among 41 LD-SCLC patients, 31 (75.6%) patients were treated by concurrent chemoradiotherapy, three (7.3%) were treated by platinum-based chemotherapy, and seven (17.1%) were treated by surgical resection followed by platinum-based chemotherapy.

PD-L1 protein expression

Immunostaining for PD-L1 was observed in the membrane and/or cytoplasm of the tumor cells and stromal lymphocytes. Representative PD-L1 staining patterns in the

tumor specimens are shown in Figure 1. Seventy-three (71.6%) SCLC patients had positive tumor PD-L1 staining.

Correlation between PD-L1 expression and patient characteristics

The relationship between PD-L1 expression and patient demographics is shown in Table 1. Expression of PD-L1 was significantly higher in SCLC patients with LD than in those with ED (p=0.011). No significant correlation was observed between PD-L1 expression and age (p=0.272), sex (p=0.186), PS (p=0.758), serum LDH level (p=0.108), serum Pro-GRP level (p=0.609), and serum NSE level (p=0.666).

Survival analysis

At the time of analysis, the median duration of follow-up was 11.4 months (range, 0.7-134.6 months). The PD-L1-positive group showed significantly longer OS than the PD-L1-negative group (median 16.3 months versus 7.3 months, p<0.001; Figure 2). Univariate analysis revealed that a LD stage (p<0.001), a good PS (p<0.001), a low serum NSE level (p<0.001), a normal serum LDH level (p=0.039), and expression of PD-L1 (p<0.001) were significantly associated with a favorable OS, whereas none of the other factors examined was significantly associated with OS (Table 2). We performed multivariate analysis to examine which factors were associated with expression of PD-L1. Multivariate analyses demonstrated that a good PS, a LD stage and expression of PD-L1 were independent and significant predictive factors for OS (Table 3). Sub-analysis of ED-SCLC patients showed that the PD-L1-positive group had a longer OS than PD-L1-negative group (median 9.2 months versus 5.4 months, p=0.037; Figure 3A), whereas there was no significant difference in PFS between the positive and negative groups (median 5.2 months in the positive group versus 4.6 months in the negative group, p=0.747; Figure 3B). In LD-SCLC patients, there was no

significant difference in OS (median 25.5 months in the positive group versus 21.8 months in the negative group, p=0.146; Figure 3C) and PFS (median 10.6 months in the positive group versus 7.9 months in the negative group, p=0.083; Figure 3D) between the positive and negative groups.

Discussion

Although blockade of immune checkpoints with monoclonal antibodies has also recently emerged as a new therapeutic strategy in several malignancies,[3, 4] the clinicopathologic characteristics associated with PD-L1 expression in SCLC have remained largely unknown. Here, using immunohistochemistry, we examined PD-L1 expression in 102 specimens of SCLC, and found that expression of PD-L1 in SCLC (71.6%) was relatively higher than those of NSCLC [10-12] , and was correlated with a LD stage, which also corresponded to the subsets of patients who were more likely to have a good outcome.

Several studies have reported the association between clinicopathologic factors and PD-L1 expression in lung cancer.[10-12] Some have shown that expression of PD-L1 is more correlated with a higher grade of differentiation, while others have found no significant correlations.[10, 11] Additional work will be needed to clarify why these factors are associated with PD-L1 expression.

We found that patients with expression of PD-L1 had significantly better survival than those with negative expression. Multivariate analysis revealed that expression of PD-L1, a good PS and a LD stage were significantly associated with better prognosis independently of the other factors examined. Given that a LD stage has been reported as prognostic factor in SCLC patients, expression of PD-L1 might be

related to a good prognosis. As far as we are aware, a significant association between PD-L1 expression and better prognosis has not previously been demonstrated for individuals with SCLC. These results are in line with previous studies showing that expression of PD-L1 is associated with better prognosis in patients with NSCLC, colorectal cancer, breast cancer, and malignant melanoma.[10, 12, 14-17] In contrast to our study, several previous studies have reported that expression of PD-L1 protein was associated with poor prognosis in patients with NSCLC, esophageal carcinoma, gastric carcinoma, hepatocellular carcinoma, pancreatic carcinoma, renal cell carcinoma, and ovarian carcinoma.[11, 18-26] These conflicting results may be due to a number of reasons. One explanation is that determination of PD-L1 expression in tumor samples has generally been performed by immunohistochemistry using various antibodies in different malignancies. Second, the threshold for positivity has not been clearly defined, and reproducibility has not been formally assessed. For future clinical applications, further efforts to standardize a quantitative assay for PD-L1 expression are warranted.

We further analyzed whether PD-L1 expression in tumor cells is correlated with PFS in LD- and ED-SCLC. We found no relevance between PD-L1 expression and PFS in SCLC, suggesting that PD-L1 expression may be a prognostic factor rather than a factor predictive of the response to chemotherapy. Previous studies have shown that PD-L1 expression is driven by various oncogenic signaling pathways.[27-30] Accordingly, we speculate that effective chemotherapy may weaken anti-tumor immunity by regulating the expression of PD-L1. Additional work will be needed to clarify these issues.

Our study had a number of limitations. One major weakness was that the number of patients studied was relatively small. Secondly, the information was collected retrospectively, and thirdly, PD-L1 expression was evaluated from formalin-fixed

paraffin-embedded specimens obtained by trans-bronchial lung biopsy (TBLB) in most cases. Although most SCLC patients are diagnosed at a late disease stage, surgically resected samples containing an adequate number of tumor cells are not obtained in clinical practice.

In conclusion, we have demonstrated that expression of PD-L1 was present in over 70% of SCLC patients and was associated with a better prognosis. Further studies are warranted to clarify the role of PD-L1 expression, and the therapeutic effect of PD-1/PD-L1 blockade, in SCLC models and clinical trials. These data provide a basis for implementation of cancer immunotherapy in patients with SCLC.

Conflict of interest statement

The authors declare no conflict of interest.

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Figure legends

Figure 1. Positive programmed cell death-ligand 1 (PD-L1) immunohistochemical

staining pattern (A) and negative immunohistochemical staining pattern (B).

Figure 2. Kaplan-Meier overall survival curves for SCLC patients with positive and negative expression of PD-L1.

Figure 3. Kaplan-Meier curves for overall survival (OS) (A) and progression-free survival (PFS) (B) of ED-SCLC patients with high or low expression of PD-L1. Kaplan-Meier curves for overall survival (OS) (C) and progression-free survival (PFS) (D) of LD-SCLC patients with high or low expression of PD-L1

Figure 1

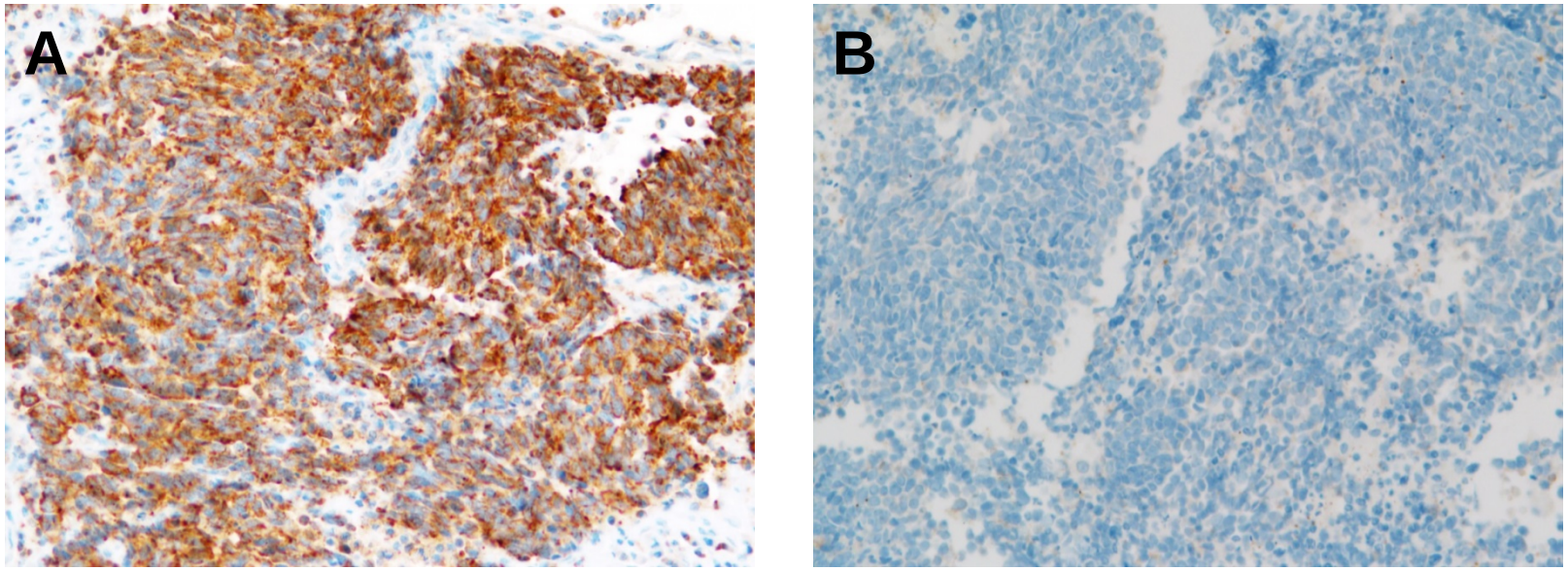

Figure 2

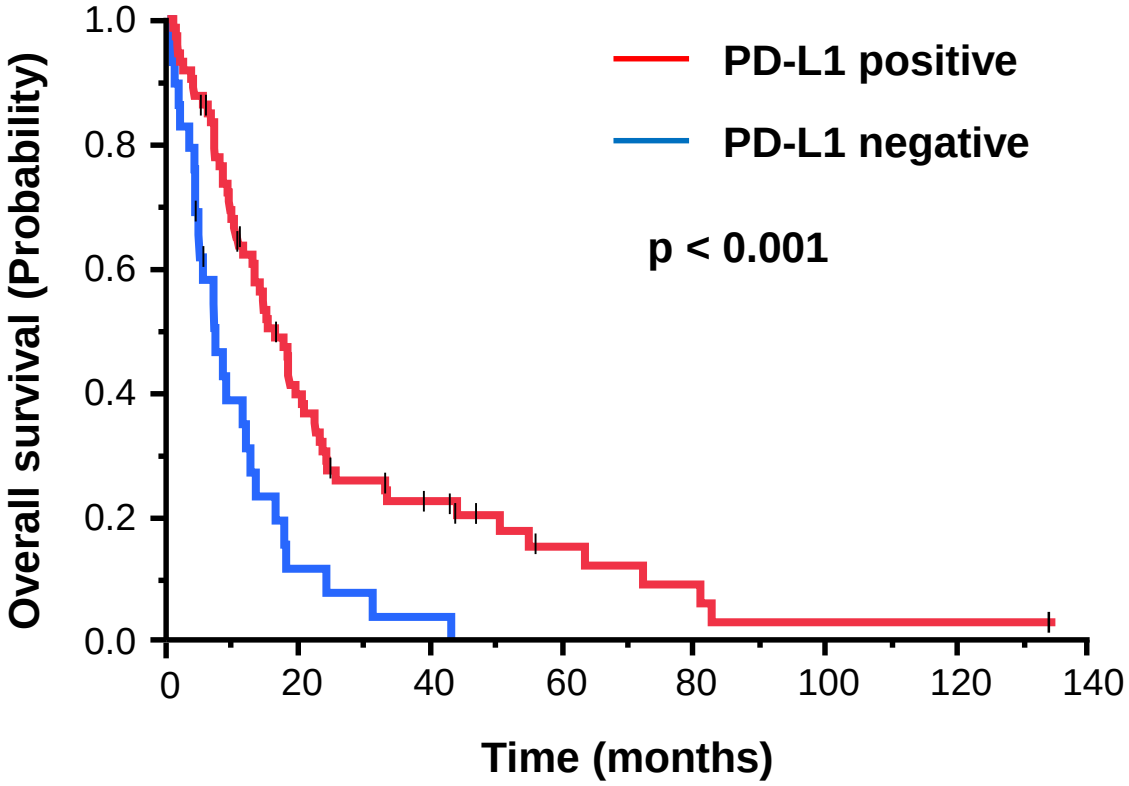
Overall survival (Probability)
1.0
0.8
0.6
0.4
0.2
0.0
0
20
40
60
80
100
120
140
Time (months)
PD-L1 positive
PD-L1 negative
p < 0.001

Figure 3

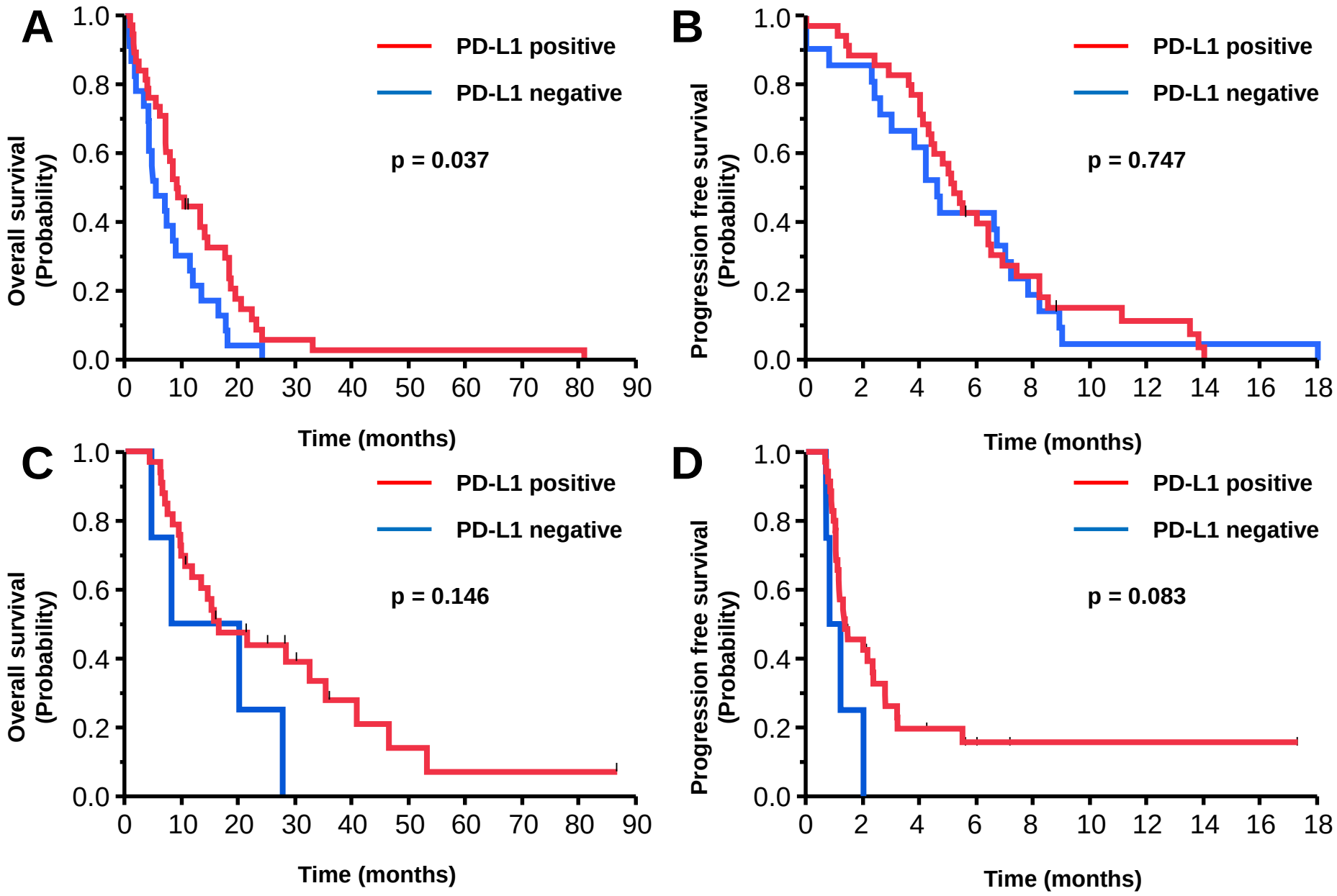
A
Overall survival (Probability)
1.0
0.8
0.6
0.4
0.2
0.0
0
10
20
30
40
50
60
70
80
90
Time (months)
PD-L1 positive
PD-L1 negative
p = 0.037
B
Progression free survival (Probability)
1.0
0.8
0.6
0.4
0.2
0.0
0
2
4
6
8
10
12
14
16
18
Time (months)
PD-L1 positive
PD-L1 negative
p = 0.747
C
Overall survival (Probability)
1.0
0.8
0.6
0.4
0.2
0.0
0
10
20
30
40
50
60
70
80
90
Time (months)
PD-L1 positive
PD-L1 negative
p = 0.146
D
Progression free survival (Probability)
1.0
0.8
0.6
0.4
0.2
0.0
0
2
4
6
8
10
12
14
16
18
Time (months)
PD-L1 positive
PD-L1 negative
p = 0.083

Table 1. Patient characteristics and their association with PD-L1 expression.

Variables	No. of patients	PD-L1 expression		*p*-value
		Positive	Negative	
Age				
<70	51	39	12	0.272
≥70	51	34	17	
Sex				
Male	89	66	23	0.186
Female	13	7	6	
Performance status				
0-1	87	63	24	0.758
2-3	15	10	5	
Stage				
LD	41	35	6	0.011
ED	61	38	23	
Serum LDH level				
Normal	37	30	7	0.108
Abnormal	65	43	22	
Serum Pro-GRP level				
Median	294	304	265	0.609
Range	12.6-33300	13.8-31400	12.6-33300	
Serum NSE level				
Median	22.4	19.6	26.7	0.666
Range	5.2-581	5.2-581	5.6-309	

Abbreviations: PD-L1, programmed cell death-ligand 1; LDH, lactate dehydrogenase;

Pro-GRP, pro-gastrin-releasing peptide; NSE, neuron-specific enolase.

Table 2. Univariate analysis of prognostic factors for overall survival.

Factor	Number	Median OS (mo)	HR (95% CI)	*p*-value
Age				
<70	51	14.6	1.161 (0.755-1.778)	0.492
≥70	51	11.9		
Sex				
Male	89	13.2	0.908 (0.512-1.761)	0.756
Female	13	17.6		
Performance status				
0-1	87	15.2	0.227 (0.131-0.419)	<0.001
2-3	15	1.9		
Stage				
Limited disease	41	25.5	0.254 (0.154-0.408)	<0.001
Extensive disease	61	8.4		
Serum LDH level				
Normal	37	18.3	0.628 (0.398-0.974)	0.039
Abnormal	65	9.7		
Serum Pro-GRP level				
Low (< median)	50	14.5	0.682 (0.440-1.054)	0.104
High (> median)	51	12.9		
Serum NSE level				
Low (< median)	39	18.0	0.433 (0.259-0.718)	<0.001
High (> median)	40	9.5		
PD-L1 expression				
Positive	73	16.3	0.408 (0.257-0.664)	<0.001
Negative	29	7.3		

Abbreviations: OS, overall survival; HR, hazard ratio; CI, confidence interval; LDH, lactate dehydrogenase; Pro-GRP, pro-gastrin-releasing peptide; NSE, neuron-specific enolase; PD-L1, programmed cell death-ligand 1.

Table 3. Multivariate analysis of factors for overall survival.

Factor	Hazard ratio	95% CI	*p*-value
PS (0-1/2-3)	0.390	0.192-0.841	0.018
Stage (LD/ED)	0.403	0.199-0.804	0.010
NSE level (Low/High)	0.671	0.358-1.225	0.196
LDH level (Normal/Abnormal)	1.130	0.628-1.995	0.679
PD-L1 expression (Positive/Negative)	0.435	0.241-0.803	0.008

Abbreviations: CI, confidence interval; PS, performance status; LD, limited disease; ED, extensive disease; LDH, lactate dehydrogenase; NSE, neuron-specific enolase; PD-L1, programmed cell death-ligand 1.