

High programmed death ligand 1 expression in carcinomatous components predicts a poor prognosis in pulmonary pleomorphic carcinoma

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Abstract

Purpose: This study reviewed the clinicopathological characteristics and programmed death ligand 1 (PD-L1) expression of 46 patients with pulmonary pleomorphic carcinoma to better understand its clinical behavior and factors affecting the survival.

Methods: Data of patients with pulmonary pleomorphic carcinomas resected in our institution were retrospectively reviewed. The tumors were classified as carcinomatous or sarcomatous according to the tissue components. Pathological characteristics were evaluated on hematoxylin and eosin-stained sections. The percentages of tumor cells with membrane staining for PD-L1 in carcinomatous and sarcomatous components were determined.

Results: We reviewed data of 46 patients (41 males, 5 females; median age 70.5 years old, range 36–83 years old). Most patients with pulmonary pleomorphic carcinoma expressed PD-L1 (80.4%), and the proportion of PD-L1 expression in tumor cells was significantly higher in sarcomatous components than in carcinomatous components. In univariable analyses, high p-stage (III), necrosis on pathological findings, and high PD-L1 expression in carcinomatous components ($\geq 50\%$) were poor prognostic factors for the overall survival. In multivariable analyses, high PD-L1 expression in carcinomatous components was significantly associated with a poor prognosis after surgery.

Conclusions: High PD-L1 expression in carcinomatous components was significantly associated with a poor prognosis after surgery.

Keywords: carcinomatous components, programmed death ligand 1, pulmonary pleomorphic carcinoma

Introduction

Pulmonary pleomorphic carcinoma (PC) is a rare disease that accounts for <1% of all lung cancers [1]. It is a histological type of lung cancer characterized by poorly differentiated non-small-cell lung carcinoma (NSCLC), particularly squamous cell carcinoma, adenocarcinoma, or undifferentiated non-small-cell carcinoma, that contains spindle and/or giant cells, accounting for at least 10% of tumor cells [2]. Pulmonary PC has a more aggressive tumor phenotype [3, 4] and is more resistant to chemoradiotherapy than other NSCLC subtypes. Owing to the resistance of pulmonary PC to chemoradiotherapy, there is a need for new therapeutic strategies, including immunotherapy targeting the programmed death-1 (PD-1)/PD ligand 1 (PD-L1) pathway. However, data concerning clinicopathological characteristics and the prognosis of pulmonary PC remain limited and elusive because of the rarity of the disease.

With regard to immune checkpoint inhibitors, it has been reported that PD-L1 expression in lung cancer is associated with a poor prognosis but good response to immunotherapy [5, 6].

Thus, the present study reviewed the clinicopathological characteristics and PD-L1 expression of 46 patients with pulmonary PC resected at our institution to better understand its clinical behavior and factors affecting the survival.

Methods

Patients and methods

This retrospective study included patients with pulmonary PC who consecutively underwent lung resection at Kyoto University Hospital between 2002 and 2017. This study included 46 patients. The pathologic (p-) stage was determined according to the tumor, node, metastases (TNM) classification (UICC TNM staging system, 8th edition) [7]. Patient characteristics and postoperative outcomes were assessed.

This study was approved by the Institutional Ethics Review Board of Kyoto University.

Immunohistochemical staining

Immunohistochemical analyses of the main lesions of the primary tumors of all patients were performed. Tissue samples were collected from formalin-fixed paraffin-embedded (FFPE) tissues of patients. Serial 4- μ m-thick sections were cut from FFPE primary tumor specimens and stained with hematoxylin-eosin (HE). Carcinomatous and sarcomatous components were directly abutted against each other, and bending was noted between them in some areas. Immunohistochemical analyses were performed to detect specific molecules (thyroid transcription factor 1 [TTF-1], p40, and vimentin) to distinguish between carcinomatous and sarcomatous components. Tissue sections were incubated with antibodies against TTF-1, p40, vimentin, and PD-L1 (Supplemental Table 1). Anti-human PD-L1 was a rabbit monoclonal IgG antibody (Abgent Cat# ab205921, RRID:AB_2687878, clone 28-8 from Abcam, Cambridge, MA, USA) that was used for immunostaining with a Ventana Benchmark Ultra autostainer (Ventana Medical Systems, Tucson, AZ, USA). Each slide was evaluated by three investigators (M.N., N.N., and A.Y.) who did not have access to clinical data.

The tumors were classified according to the current World Health Organization histologic classification criteria [8], and the tissue components of pulmonary PC were classified

as adenocarcinoma, squamous cell carcinoma, large-cell carcinoma, spindle cell carcinoma, or giant-cell carcinoma. Lymphatic invasion, tumor necrosis of cancer cells, and pulmonary metastasis were evaluated on HE-stained sections. Elastica van Gieson, Elastica-Masson, or Victoria Blue staining was performed to confirm vascular and pleural invasion. However, in cases wherein differentiating between carcinomatous and sarcomatous components was difficult, the cases were classified as sarcomatous when vimentin staining was positive by $\geq 50\%$. The percentages of tumor cells with membrane staining for PD-L1 were determined. In addition, the percentages of tumor cells with membrane staining for PD-L1 in carcinomatous and sarcomatous components were also determined.

Stained lesions containing both carcinomatous and sarcomatous components in the same slide were selected to compare these two components. However, carcinomatous components could not be found in six patients in whom the PD-L1 expression had been evaluated.

Statistical analyses

Various clinical and pathological data were retrospectively reviewed to analyze prognostic factors for the disease-free survival (DFS) and overall survival (OS) after surgery. Data were analyzed using the JMP[®] Pro 12.2.0 software program (JMP, Cary, NC, USA). The postoperative survival rate was analyzed using the Kaplan-Meier method. Prognostic risks of variables for the DFS and OS were analyzed using the log-rank test for univariable analyses. For multivariable analyses, Cox hazards models were used. P-values < 0.05 were considered statistically significant.

Results

Patients' characteristics

Clinical data of the patients are summarized in Table 1. There were 41 male and 5 female patients. The age of patients at the time of the diagnosis ranged from 36 to 83 years old (median age: 70.5 years old). Among 40 (87.0%) smokers, 28 were moderate smokers (20–60 pack-years), and 12 were heavy smokers (>60 pack-years). The locations of the tumors were as follows: 21 cases in the right upper lobe, 11 in the right lower lobe, 11 in the left upper lobe, 2 in the left lower lobe, and 1 in the right middle lobe. There were 21 (45.7%), 16 (34.8%), and 9 (19.6%) patients with stage I, II, and III disease, respectively. KL-6 at the time of the diagnosis ranged from 131 to 4140 U/ml (median: 332 U/ml), and high KL-6 (≥ 500 U/ml) levels were observed in 11 (26.8%) patients.

Operative procedures

Three (6.5%) of 46 patients underwent wedge resection of the lung, and 4 (8.7%) underwent segmentectomy. Thirty-seven (80.4%) patients underwent lobectomy, and 2 (4.3%) underwent pneumonectomy. Twenty patients required extended resections, including 10 chest wall resections (21.3%), 4 parietal pleura resections (8.5%), 2 bronchoplasties (4.3%), 2 pulmonary artery resections (4.3%), 2 great vessel resections (4.3%), and 1 pericardial resection (2.1%). Some patients required multiple extended resection procedures. Forty-five patients underwent macroscopically complete resection; however, in three patients, pathological examinations of the resected specimens showed microscopic tumor residues at the resection margins (Supplemental Table 2).

PD-L1 expression in patients with pulmonary PC

When the 1% cut-off value of tumor cells with PD-L1 staining was adopted, PD-L1 expression

was positive in 37 (80.4% of all pulmonary PC) patients. The number of patients with PD-L1 expression values of 0%, 1%–49%, and $\geq 50\%$ in a section was 21 (52.5%), 14 (35.0%), and 5 (12.5%), respectively, in carcinomatous components and 10 (22.2%), 15 (33.3%), and 20 (44.4%), respectively, in sarcomatous components. Statistical analyses showed that the PD-L1 expression proportion was significantly higher in sarcomatous components than in carcinomatous components (Figs. 1 and 2).

Pathological findings

The maximum diameter of the tumors ranged from 1.4 to 11.3 cm (median: 4.0 cm). A spindle cell carcinoma component was observed in 13 patients (28.3%), and a giant-cell carcinoma component was observed in 9 patients (19.6%). Both components were observed in 23 patients (50.0%), and a chondrosarcoma was observed in 1 patient (2.2%). An adenocarcinoma component was found in 32 (69.6%) patients, and a squamous cell carcinoma component was found in 10 patients (21.7%). Other carcinoma components were found in 7 patients (15.2%) (there was a duplication due to a case of adenosquamous carcinoma). Thirteen (28.3%), 16 (34.8%), and 17 (37.0%) patients had stage I, II, and III disease, respectively.

Vascular invasion was observed in 31 patients (67.4%). Lymphatic invasion was observed in 4 patients (8.7%). Lymph node metastases were present in 14 patients (30.4%). Metastases were noted in the sarcomatous and carcinomatous components in 4 (28.6%) and 5 (35.7%) patients, respectively, and metastases were found in both sarcomatous and carcinomatous components in 5 patients (35.7%). Tumor necrosis was observed in 33 patients (71.7%) (Supplemental Table 3).

The survival

The median period of follow-up examinations was 38.5 months (range, 3.0–158.2 months).

The median DFS was 16.5 months, and the median OS was 37.0 months. The 5-year OS and DFS rates were 53.4% and 42.9%, respectively (Fig. 3). In univariable analyses, high p-stage (III) and necrosis on pathological findings were poor prognostic factors for the DFS (Supplemental Table 4). High p-stage (III), necrosis on pathological findings, and high PD-L1 expression in carcinomatous components ($\geq 50\%$) were poor prognostic factors for the OS (Table 2, Supplemental Figure 1-3). In multivariable analyses, there was no significant difference in the DFS (Supplemental Table 5), and a high PD-L1 expression in carcinomatous components ($\geq 50\%$) was significantly associated with a poor OS (Table 3).

Discussion

The current study showed that PD-L1 was expressed in most patients with pulmonary PC, and that the proportion of PD-L1 expression in tumor cells was significantly higher in sarcomatous components than in carcinomatous components. A high PD-L1 expression in carcinomatous components was significantly associated with a poor prognosis after surgery.

Pulmonary PC is an uncommon morphologic variant of lung carcinoma, and there are few studies on it. Pulmonary PC has a more aggressive clinical course and poorer outcome than NSCLC [9] and is generally resistant to chemotherapy and radiotherapy. Previous studies have reported that the PD-L1 expression in patients with pulmonary PC ranged from 53% to 91% (median: 76.6%) (considering a 1% or 5% cut-off) [10-15]. The PD-L1 expression was also higher in patients with pulmonary PC than in those with NSCLC (38%) [16]. Some studies have reported that PD-L1 positivity was high in sarcomas (5.87%–64.7%). These studies included osteosarcomas, soft tissue sarcomas, and chondrosarcomas [17, 18]. A study on pulmonary PC reported that the proportion of PD-L1-positive tumor cells was higher in the sarcomatous component than in the carcinomatous component [13]. However, another study reported that the proportion of PD-L1-positive tumor cells was similar between sarcomatous and carcinomatous components [14].

Because the optimal cut-off value for tumor proportion scores remains unestablished, the cut-off values used in clinical settings were adopted in the present study. Similar to previous studies, the current study noted a high PD-L1 expression in patients with pulmonary PC (80.4%). Furthermore, the PD-L1 expression was higher in the sarcomatous component (77.7%) than in the carcinomatous component (47.5%), supporting the results of a previous study [13]. These results suggested that the ability of pulmonary PC to evade immune response was stronger than that of other NSCLCs, possibly because of the relatively poor prognosis of pulmonary PC.

193 In the current study, univariable and multivariable analyses revealed that the OS was
194 worse in patients with a high PD-L1 expression in carcinomatous components than in patients
195 with a low PD-L1 expression in carcinomatous components. Initially, sarcomatous components
196 were associated with a poor prognosis; however, in the current study, our findings of high PD-
197 L1 expression in carcinomatous components being associated with a poor prognosis may imply
198 that, in addition to the malignancy of sarcomatous components, acquiring immune escape with
199 carcinomatous components, i.e. an increasing grade of malignancy, may be associated with a
200 worse prognosis in pulmonary pleomorphic carcinoma. In short, this finding suggested that
201 PD-L1 expression in carcinomatous components contributed to worse prognosis. This has not
202 been reported in previous studies on pulmonary PC, so a further evaluation of the individual
203 PD-L1 expression of the carcinomatous and sarcomatous components is warranted. Since the
204 PD-L1 expression in pulmonary PC is high, adjuvant anti-PD-L1 antibody treatment may be
205 considered to develop optimal treatment strategies for resected cases of pulmonary PC.

206 According to univariable analyses, necrosis on pathological findings was significantly
207 associated with a poor prognosis. Necrosis, a common feature of tumors, is caused by chronic
208 ischemic injury. The degree of tumor necrosis reflects the level of tumor hypoxia. Increased
209 cellular hypoxia was correlated with resistance to radiotherapy and chemotherapy, increased
210 metastatic potential, and a poor prognosis in patients with solid tumors [19].

211 Several limitations associated with the present study warrant mention. First, the results
212 were based on a small number of patients at a single institution. The retrospective design was
213 the most practical means of addressing our question owing to the rarity of pulmonary PC;
214 however, the results should be interpreted with caution. This was the biggest limitation of our
215 study, and therefore, a prospective large-scale study at multiple institutions is needed to confirm
216 the current results. Second, immunohistochemical staining of pulmonary PC cases performed
217 more than three years ago may have carried a high possibility of false negatives. Third, because

there were some cases wherein distinguishing the components was difficult, classification was made based on the positive rate of vimentin expression. This was different from the diagnostic criteria of sarcomatous components.

Conclusions

PD-L1 expression was significantly higher in sarcomatous components than in carcinomatous components. Furthermore, a high PD-L1 expression in carcinomatous components suggested a poor OS. Immunotherapy may thus be a potential therapeutic candidate for this aggressive tumor.

Acknowledgements

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Conflict of interest statement

Misa Noguchi and other co-authors have no conflicts of interest.

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Figure legends

Figure 1. PD-L1 expression of a tumor that included both carcinomatous and sarcomatous components. This image shows Hematoxylin-Eosin and immunohistochemical staining (anti-human PD-L1) of a tumor that included both carcinomatous and sarcomatous components. In this case, the PD-L1 expression was negative in the carcinomatous component but was $\geq 50\%$ in the sarcomatous component.

Figure 2. Proportions of PD-L1 expression. The proportion of PD-L1 expression is significantly higher in the sarcomatous components than in the carcinomatous components.

Figure 3. The overall and disease-free survival curves of patients after pulmonary resection. The 5-year overall survival rate was 53.4%, and the 5-year disease-free survival rate was 42.9% (n=46).

Supplemental Figure 1. The overall survival curves divided into high and low PD-L1 expression in all patients

Supplemental Figure 2. The overall survival curves of patients with high and low PD-L1 expression in carcinomatous components

Supplemental Figure 3. The overall survival curves of patients with high and low PD-L1 expression in sarcomatous components

323 **Table 1.** Preoperative patient characteristics

Variable		All patients (N=46)
Age (years)		70.5 (range: 36–83)
Sex	Male	41 (89.1%)
	Female	5 (10.9%)
Smoking status	Never	6 (13.0%)
	Former or current	40 (87.0%)
Tumor location	RUL	21 (45.7%)
	RML	1 (2.2%)
	RLL	11 (23.9%)
	LUL	11 (23.9%)
	LLL	2 (4.3%)
Clinical stage	I	21 (45.7%)
	II	16 (34.8%)
	III	9 (19.6%)
KL-6		332 (range: 131–4140)

325 **Table 2.** Results of a univariable analysis of the overall survival

Variable		Number of patients	P-value
Age	≥75 years	16	0.369
	<75 years	30	
Sex	Male	41	0.851
	Female	5	
Smoking habit	Yes	40	0.533
	No	6	
KL-6 levels	High (≥500 U/ml)	11	0.279
	Low (<500 U/ml)	30	
Pathological stage	I, II	29	0.024
	III	17	
Pleural invasion	Present	32	0.987
	Absent	14	
Vascular invasion	Present	31	0.827
	Absent	15	
Lymphatic invasion	Present	4	0.097
	Absent	42	
Necrosis	Present	33	0.043
	Absent	13	
PD-L1 expression	<50%	31	0.592
	≥50%	15	
PD-L1 expression in carcinomatous	<50%	35	0.009
	≥50%	5	
PD-L1 expression in sarcomatous	<50%	38	0.568
	≥50%	8	

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Table 3. Results of a multivariable analysis of prognostic factors for the overall survival

Variable		HR and 95% CI		P-value
Pathological stage	I, II/III	0.556	0.205–1.481	0.237
Necrosis	Yes/No	2.670	0.785–12.220	0.121
PD-L1 expression in carcinomatous components	≥50% vs. <50%	3.740	1.158–10.583	0.029

CI, confidence interval

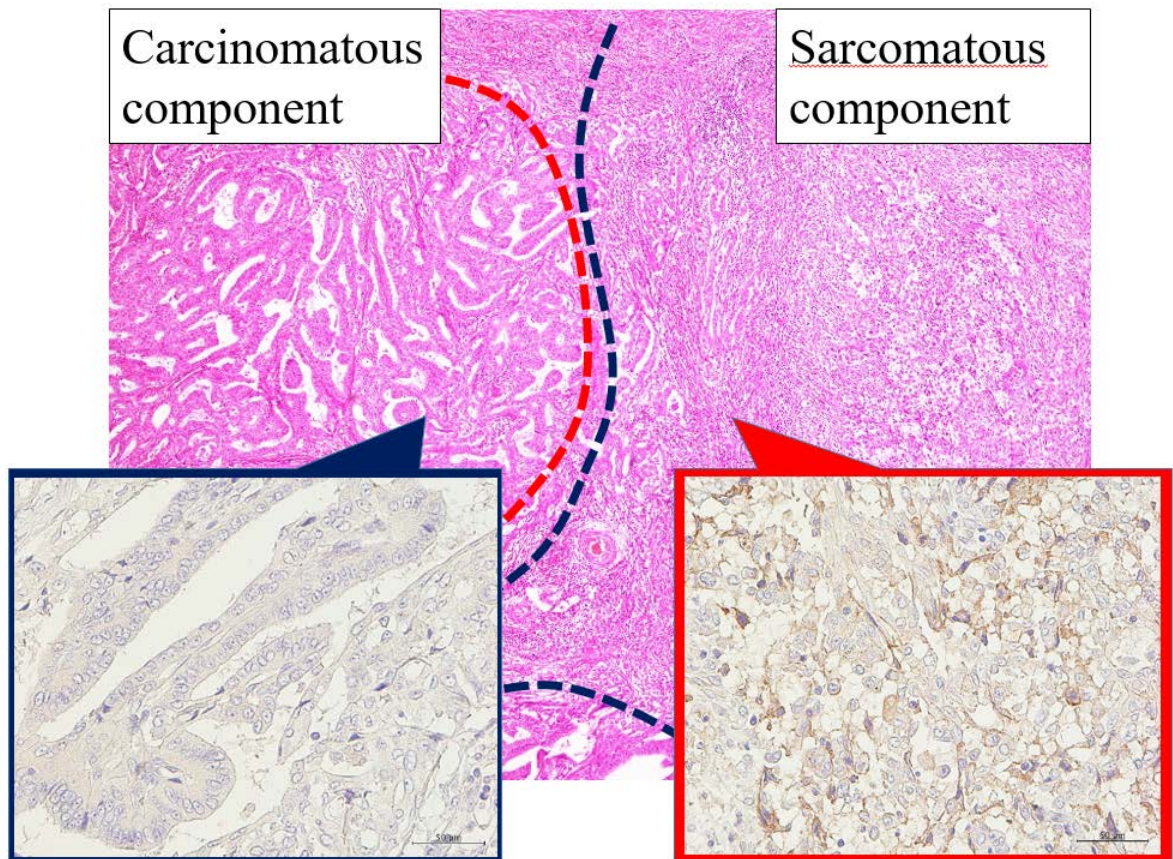


Figure 1. PD-L1 expression of a tumor that included both carcinomatous and sarcomatous components. This image shows Hematoxylin-Eosin and immunohistochemical staining (anti-human PD-L1) of a tumor that included both carcinomatous and sarcomatous components. In this case, the PD-L1 expression was negative in the carcinomatous component but was $\geq 50\%$ in the sarcomatous component.

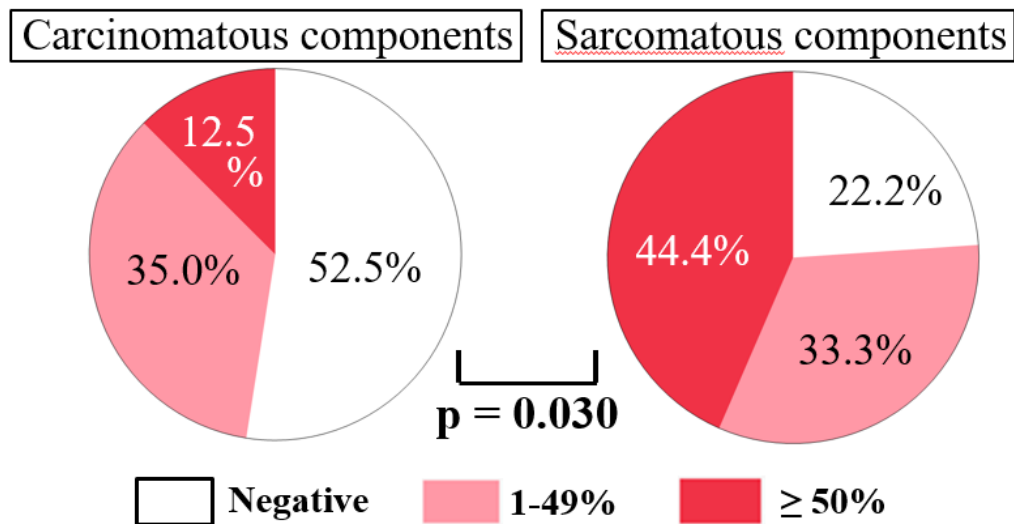


Figure 2. Proportions of PD-L1 expression. The proportion of PD-L1 expression is significantly higher in the sarcomatous components than in the carcinomatous components.

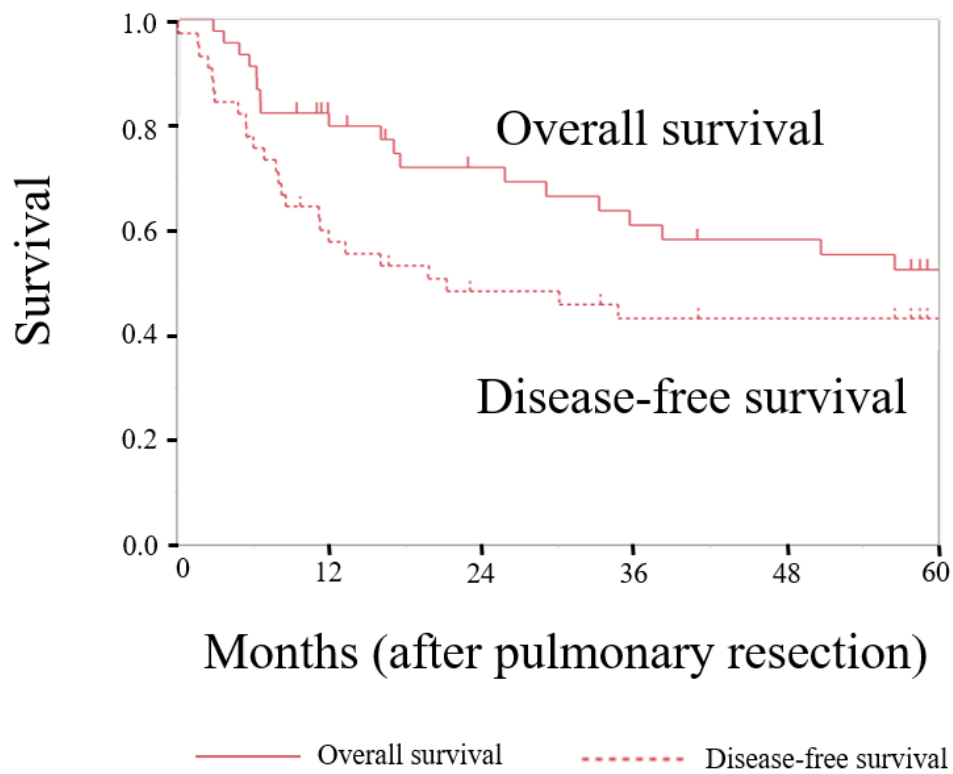


Figure 3. The overall and disease-free survival curves of patients after pulmonary resection. The 5-year overall survival rate was 53.4%, and the 5-year disease-free survival rate was 42.9% (n=46).

397 **Supplemental Table 1.** Antibodies used in immunohistochemical analyses

Antibody	Clone	Vendor	Dilution	Incubation time	Antigen retrieval		Detection kit
PD-L1	28-8	Abcam	1:200	16 min	Benchmark ULTRA CC1 (EDTA base, pH=8.5)	32 min	OptiView DAB IHC Detection Kit
TTF-1	8G7G3/1	Dako	1:200	32 min		64 min	iVIEW DAB Universal Kit
p40	BC28	Roche	RTU	16 min		32 min	OptiView DAB IHC Detection Kit
Vimentin	V9	Roche	RTU	32 min		36 min	iVIEW DAB Universal Kit

398 Automated staining system: Ventana Medical Systems/Roche, Tucson, AZ

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417 **Supplemental Table 2.** Operative procedures

Variable	All patients (N=46)
Operation	
Pneumonectomy	2 (4.3%)
Lobectomy	37 (80.4%)
Segmentectomy	4 (8.7%)
Wedge resection	3 (6.5%)
Combined modality	
No	26 (56.5%)
Yes	20 (43.5%)
Chest wall resection	9 (19.6%)
Parietal pleura resection	4 (8.7%)
Bronchoplasty	2 (4.3%)
Pulmonary artery plasty	2 (4.3%)
Great vessel resection	2 (4.3%)
Pericardium resection	1 (2.2%)
Complete resection	
Yes	43 (93.5%)
No	3 (6.5%)

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431 **Supplemental Table 3.** Pathological findings

Variable		All patients (N=46)
Pathological stage	I	13 (28.3%)
	II	16 (34.8%)
	III	17 (37.0%)
Tumor size (cm)		4.0 (range: 1.4–11.3)
Pathologic T-factor	T1	10 (21.7%)
	T2	13 (28.3%)
	T3	13 (28.3%)
	T4	10 (21.7%)
Pathologic N-factor	N0	32 (69.6%)
	N1	9 (19.6%)
	N2	5 (10.9%)
Necrosis	Present	33 (71.7%)
	Absent	13 (28.3%)
Pleural invasion	Present	32 (69.6%)
	Absent	14 (30.4%)
Vascular invasion	Present	31 (67.4%)
	Absent	15 (32.6%)
Lymphatic invasion	Present	4 (8.7%)
	Absent	42 (91.3%)
PD-L1 expression	Negative	9 (19.6%)
	1%–49%	22 (47.8%)
	50%–	15 (32.6%)

Supplemental Table 4. Results of a univariable analysis of the disease-free survival

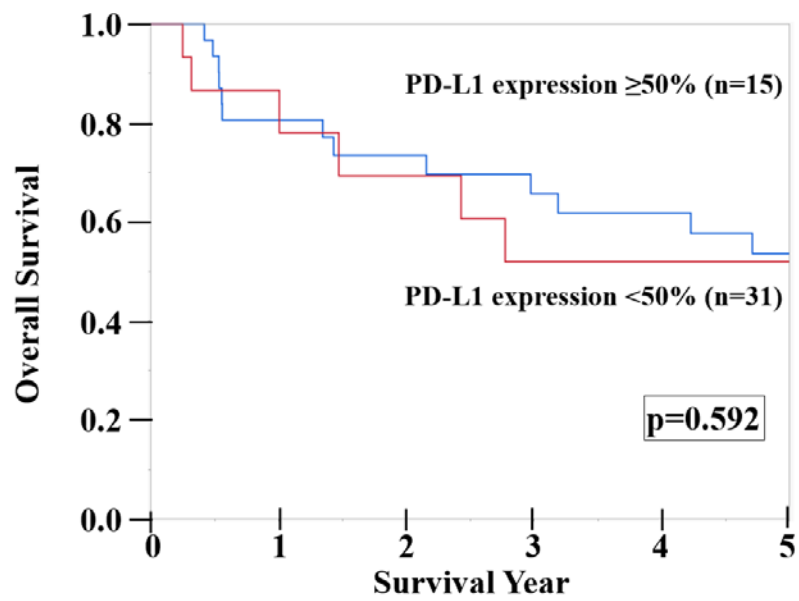
Variable		Number of patients	P-value
Age	≥75 years	16	0.431
	<75 years	30	
Sex	Male	41	0.961
	Female	5	
Smoking habit	Yes	40	0.578
	No	6	
KL-6 levels	High (≥500 U/ml)	11	0.832
	Low (<500 U/ml)	30	
Pathological stage	I, II	29	0.040
	III	17	
Pleural invasion	Present	32	0.494
	Absent	14	
Vascular invasion	Present	31	0.877
	Absent	15	
Lymphatic invasion	Present	4	0.326
	Absent	42	
Necrosis	Present	33	0.025
	Absent	13	
PD-L1 expression	<50%	31	0.292
	≥50%	15	
PD-L1 expression in carcinomatous	<50%	35	0.544
	≥50%	5	
PD-L1 expression in sarcomatous	<50%	38	0.897
	≥50%	8	

Supplemental Table 5. Results of a multivariable analysis of prognostic factors for the disease-free survival

Variable		HR and 95% CI		P-value
Pathological stage	I, II/III	0.588	0.259–1.320	0.196
Necrosis	Yes/No	2.640	0.952–9.338	0.063

CI, confidence interval

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478 **Supplemental Figure 1.** The overall survival curves divided into high and low PD-L1
479 expression in all patients

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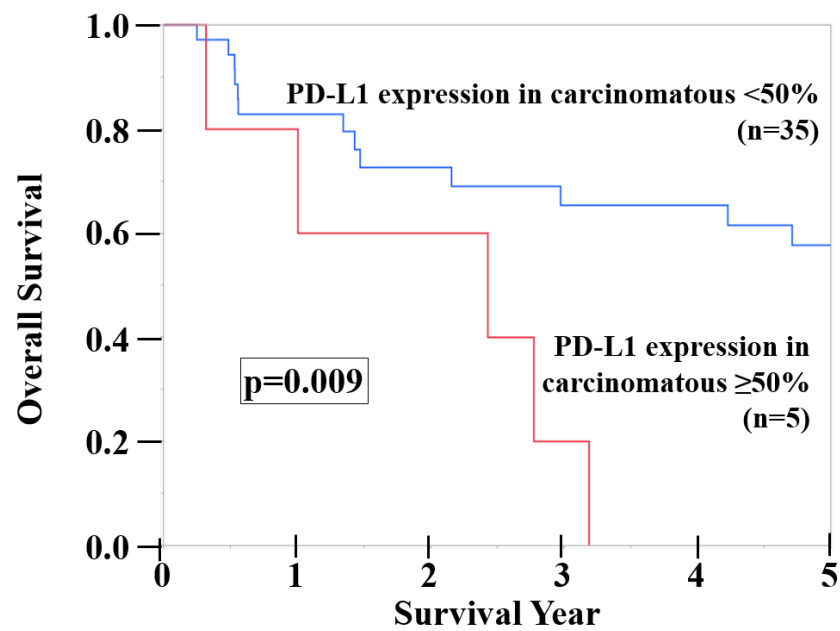
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496 **Supplemental Figure 2.** The overall survival curves of patients with high and low PD-L1
497 expression in carcinomatous components

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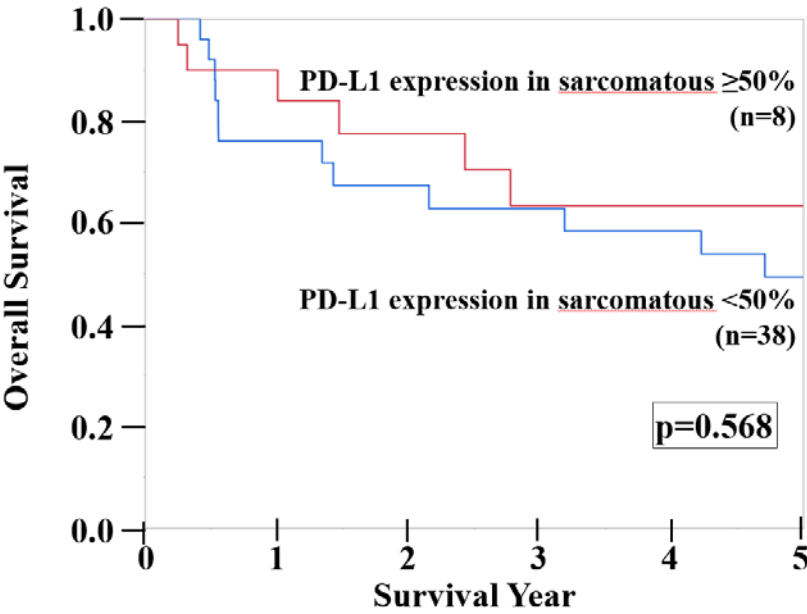
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513 **Supplemental Figure 3.** The overall survival curves of patients with high and low PD-L1

514 expression in sarcomatous components