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【 ORIGINAL ARTICLE 】

A High PD-L1 Expression in Pulmonary Pleomorphic Carcinoma Correlates with Parietal-pleural Invasion and Might Predict a Poor Prognosis

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Abstract:

Objective Pleomorphic carcinoma (PC) is a rare pulmonary epithelial malignant tumor with a poor prognosis. The objective of the present study was to investigate the programmed death-ligand 1 (PD-L1) expression in PC and its correlation between the clinicopathological factors and prognosis.

Methods Clinical and pathological data of 35 patients with surgically resected PC encountered from 2002 to 2016 at our institution were collected. The PD-L1 expression on tumor cells was evaluated via immunohistochemistry (clone 22C3). We examined the correlation between the PD-L1 expression and patients' clinicopathological factors and their prognosis.

Results A high PD-L1 expression (≥50%) was seen in 21 (60%) patients, and parietal-pleural invasion was significantly correlated with a high PD-L1 expression (p=0.012). The 5-year overall survival and relapse-free survival were 68.2% and 43.2%, respectively. Tumor size ≥50 mm (p=0.021), lymph node metastasis (p=0.023), and a high PD-L1 expression (p=0.047) were correlated with a short relapse-free survival. Since lymph node metastasis was an independent risk factor of a poor overall survival (p=0.012), patients with a high PD-L1 expression also tended to have a worse overall survival than those with low levels (p=0.081).

Conclusion A high PD-L1 expression is frequently seen in PC. The PD-L1 expression is associated with parietal-pleural invasion and might indicate a poor prognosis.

Key words: parietal-pleural invasion, pleomorphic carcinoma, programmed death-ligand 1, prognosis

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Introduction

Pulmonary pleomorphic carcinoma (PC) is a rare pulmonary epithelial malignant tumor that accounts for approximately 0.4% of all cases of primary lung cancer (1, 2). According to the 2015 World Health Organization (WHO) classification, PC is defined as poorly differentiated adenocarcinoma, squamous cell carcinoma, or large cell carcinoma, containing a component of spindle cells or giant cells with a sarcomatoid tumor component of at least 10% (3). The clinical course of PC is more aggressive and its prognosis significantly poorer than that of non-small cell carcinoma (NSCLC) (1, 4). Although the best treatment modality is complete surgical resection, PC tends to recur even after complete resection in early-stage disease (5). Furthermore, resistance to chemotherapy and radiotherapy makes it difficult to manage PC after relapse (1, 6).

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In some reports, PC showed a high programmed cell death-ligand 1 (PD-L1) expression (7, 8). However, due to its rarity, the relationship between the PD-L1 expression and the clinicopathological characteristics of PC and the implications of the PD-L1 expression in PC are poorly understood. We reviewed the clinicopathological data of patients with PC who underwent pulmonary resection at our institution to better understand the correlation between the PD-L1 expression and the clinical behavior in this histotype of pulmonary carcinoma.

Materials and Methods

Patients

We included consecutive patients with pulmonary PC diagnosed by surgical resection from January 1, 2002, to December 31, 2016, at Kinki-chuo Chest Medical Center (KCMC). The histopathological diagnosis was confirmed by two pathologists (M.T and T.K) according to the current 2015 WHO classification of lung tumors (3). The clinical characteristics, surgical staging, tumor-node-metastasis (TNM) classification according to the seventh edition (9), treatments, and outcomes were collected from the medical records. This study was approved by the Institutional Review Board of KCMC (Approval number: 602).

Pathological analyses and PD-L1 immunohistochemistry

For each tumor sample, the histological subtype of carcinomatous components (adenocarcinoma, squamous cell carcinoma, and large-cell carcinoma) and sarcomatoid components were collected.

To measure the PD-L1 expression, we used the PD-L1 clone 22C3 pharmDx kit and Dako Automated Link 48 platform (Dako, Carpenteria, USA), which is clinically used for the evaluation of the PD-L1 expression in NSCLC at our institution. The PD-L1 expression was calculated as the percentage of complete or partial membrane staining in a section that included at least 100 viable tumor cells. Necrotic areas were excluded from scoring. A high expression of PD-L1 was defined as ≥50% staining of tumor cell membrane, and PD-L1 negative was defined as <1% staining.

Patient evaluation

The relapse-free survival (RFS) was defined as the interval from the date of resection to the date of diagnosed recurrence or metastases or to the date of death. The overall survival (OS) was calculated from the date of surgical resection until the date of death from any cause or the last follow-up date. After relapse, the progression-free survival (PFS) was defined as the time from the first day of chemotherapy until disease progression or the date of death. Responses to chemotherapy and/or radiotherapy were assessed using the Response Evaluation Criteria in Solid Tumors (RECIST) ver. 1.1. guidelines (10).

Statistical analyses

Fisher's exact test was used to compare the clinicopathologic characteristics of PD-L1 expression positive and negative PCs. The OS and RFS were assessed using the Kaplan-Meier method and log-rank tests. Cox's model was used for univariate and multivariate analyses. Independent variables with a p value <0.2 in univariate analyses were included in the multivariate analysis. A two-sided p value < 0.05 was considered statistically significant. All statistical tests were performed using the JMP 11.0 software program (SAS Institute Japan, Tokyo, Japan).

Results

Patient characteristics

In total, 35 consecutive cases of surgically treated pulmonary PC were identified. The clinical characteristics of the study population are shown in Table 1. The median age was 62 years (range, 34-80). Most patients were men (71%) and smokers (83%), and approximately 51% were asymptomatic. The levels of serum carcinoembryonic antigen (CEA, normal: 0.0-5.0 ng/mL) and cytokeratin fragment 19 (CYFRA 21-1, normal: 0.0-2.8 ng/mL) were high in 9 patients (29%) each; however, the level of pro-gastrin-releasing peptide (Pro-GRP, normal: 0.0-80.0 pg/mL) was not increased in any patients. Chest computed tomography (CT) was performed before surgery in all cases, and in 20 cases (57%), the primary tumor was located in the upper lobe. Fluorodeoxyglucose-positron emission tomography (FDG-PET) was performed in 14 patients, and the median standardized uptake value (SUV) max of the primary lesions was 8.0 (range, 2.2-22.8).

Pathological findings

Before surgical resection, 32 patients underwent a transbronchial biopsy (TBB). The results were negative in 12 patients, adenocarcinoma in 8 patients, squamous cell carcinoma in 6 patients, NSCLC in 5 patients, and necrosis in 1 patient.

Regarding the histological subtypes and pathological stage of the study population (Table 1), 69% of the patients had early-stage disease (stage I and II). The median maximal diameter of the resected tumor was 48 mm (range, 20-110). Blood vessel invasion was observed in 86% of cases, and visceral and parietal pleural extension was found in 74% and 23% of cases, respectively.

The PD-L1 expression was negative (<1% positively stained cells) in only 3 cases (8.6%), and a high PD-L1 expression (≥50% positively stained cells) was observed in 21 of the 35 PC cases (60%). The PD-L1 expression according to patient characteristics and tumor pathological features is presented in Table 2. Parietal-pleural invasion was significantly associated with a positive PD-L1 expression (p= 0.012). Adenocarcinoma components (p=0.112) and pleo-

Table 1. Patients Characteristics and Pathological Features (n=35).

Characteristics	n (%) or median (range)
Age, years (range)	65 (34–80)
Sex	
Male	25 (71%)
Female	10 (29%)
Smoking status	
Never-smoker	6 (17%)
Current/former smoker	29 (83%)
Symptoms	
None	17 (49%)
Cough	13 (37%)
Bloody sputum	3 (9%)
Chest pain	1 (3%)
Fever	1 (3%)
Tumor marker	
CEA, ng/mL	3.4 (0.6-16.4)
CYFRA, ng/mL	1.9 (0.6-6.0)
proGRP, pg/mL	41.6 (16.9–67.8)
Tumor size (CT), mm	45.5 (18–130)
Tumor location	
RUL	11 (31%)
RML	1 (3%)
RLL	8 (23%)
LUL	8 (23%)
LLL	7 (20%)
Epithelial components	
Adenocarcinoma	26 (74%)
Squamous cell carcinoma	9 (26%)
Large cell carcinoma	0 (0%)
PD-L1 staining	
≥50%	21 (60%)
50%<	14 (40%)
Pathological Stage	
I	10 (29%)
II	14 (40%)
III	10 (29%)
IV	1 (3%)
Pathological T	
T1	2 (6%)
T2	19 (54%)
T3	12 (34%)
T4	2 (6%)
Pathological N	
N0	24 (69%)
N≥1	11 (31%)
Visceral-pleural invasion	
Negative	9 (26%)
Positive	26 (74%)
Parietal-pleural invasion	
Negative	27 (77%)
Positive	8 (23%)
Blood-vessel invation	
Negative	5 (14%)
Positive	30 (86%)

morphic components ≥50% (p=0.176) tended to show a high PD-L1 expression. However, other clinical factors, including the age, sex, smoking status, and TNM classification, were not associated with the PD-L1 expression.

Treatment

Surgical procedures included lobectomy (82%), pneumonectomy (14%), and segmentectomy (3%). Combination surgery of wedge resection (14%), thoracic wall resection (20%), and pleural resection (9%) was performed in some cases. Adjuvant chemotherapy was administered to 13 patients (37%).

Postoperative recurrence was seen in 17 patients (49%), and post-recurrence treatment was administered to 13 patients. The details of the treatment are shown in Supplementary material. Anti PD-1 therapy was administered to 2 patients. For the patient with 1% PD-L1 positive staining, nivolumab resulted in only a short progression-free duration of 52 days. By contrast, for the patient with 100% PD-L1 positive staining, pembrolizumab showed a partial response that continued after 13 cycles, and the treatment is still ongoing.

Survival analyses

The median follow-up was 24 months (range, 2.3-129). The 1- and 5-year OS were 75.2% and 68.2%, respectively, whereas the RFS was 61.8% at 1 year and 43.2% at 5 years (Fig. 1A, 2A). The median period until recurrence was 449 days (range, 38-3,881), and the median survival time after initial relapse was 236 days (range, 13-2,700).

A univariate analysis showed that lymph node metastasis (p=0.013) and tumor size ≥50 mm (p=0.016) were associated with short a RFS (Fig. 1B and C, Table 3). A high PD-L1 expression also tended to result in a poor RFS, but this was not significant (p=0.078) (Fig. 1D, Table 3). In a multivariate analysis, lymph node metastasis (p=0.023), tumor size ≥50 mm (p=0.021), and a high PD-L1 expression (p=0.047) were significantly associated with a poor RFS (Table 3). Regarding the OS, only lymph node metastasis was significantly associated with a poor OS (p=0.007) (Fig. 2B, Table 4), since a high PD-L1 expression (p=0.084) and tumor size ≥50 mm (p=0.129) tended to result in a short OS according to the univariate analysis (Fig. 2C and D, Table 4). The same result was found in the multivariate analysis of the OS; lymph node metastasis (p=0.012) was significantly associated with a poor OS, since tumor size ≥50 mm and a high PD-L1 expression tended to result in a short OS (Table 4).

Discussion

We analyzed 35 patients with surgically resected PC. The PD-L1 expression was measured by 22C3 antibody. A total of 60% of the patients were found to have a high PD-L1 expression. Previous studies have reported a high PD-L1 expression in PC. Kim et al. reported a high PD-L1 expression

Table 2. Clinical and Pathological Data Related to PD-L1 Expression (n=35).

	PD-L1<50%	PD-L1≥50%	p value
Sex			0.704
Male	11	14	
Female	3	7	
Smoking status			1.000
Never smoker	2	4	
Current/former smoker	12	17	
Epithelial components			0.112
Adenocarcinoma	8	18	
Squamous cell carcinoma	6	3	
Pleomorphic components			0.176
<50%	9	8	
≥50%	5	13	
Parietal-pleural invasion			0.012*
yes	0	8	
no	14	13	

p<0.05*.

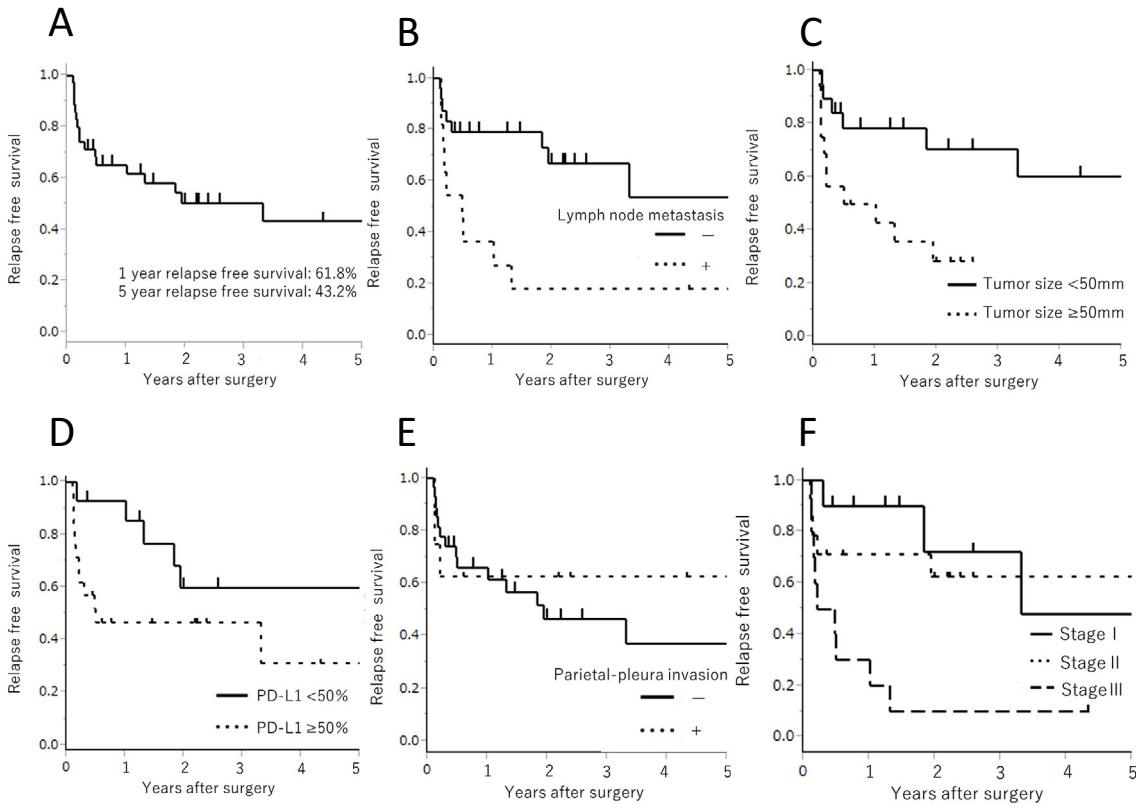

Figure 1. Relapse-free survival curves in all patients for each clinicopathological factors. (A) All pulmonary pleomorphic carcinoma patients (n=35); (B) according to lymph node metastasis; (C) according to tumor size; (D) according to the PD-L1 expression; (E) according to parietal-pleural invasion; (F) according to tumor stage.

in 90.2% of patients with PCs in their study (11), and Yvorel et al. also reported a high PD-L1 expression in 75% of cases of pleomorphic, spindle cell, and giant cell carcinoma (8). Both studies used rabbit anti-PD-L1 (E1L3N) monoclonal antibody. However, using murine anti-B7-H1 monoclonal antibody (clone 5H1), Vieira et al. reported that 53% of cases of sarcomatoid lung carcinoma (including 79% of PC) showed high levels of PD-L1 (12). As observed on staining with other antibodies, PC also showed a high PD-L1 expression with 22C3 antibody staining, indicating

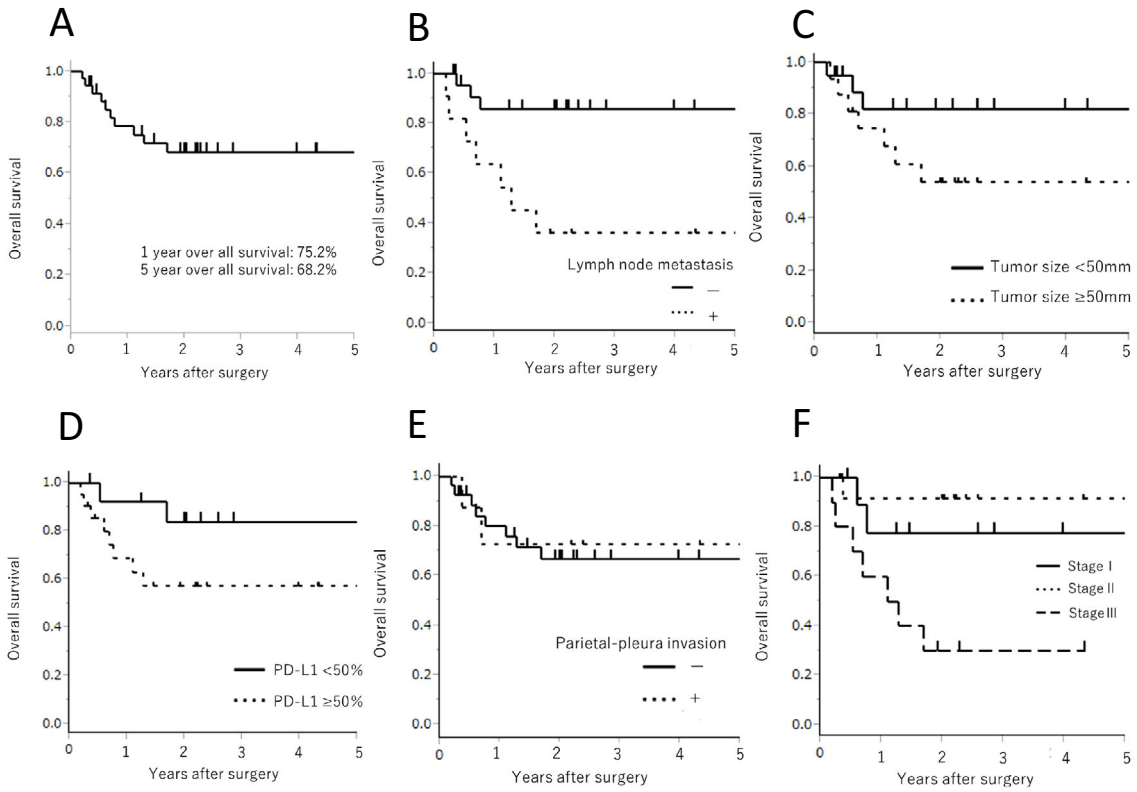

Figure 2. **Overall survival curves of all patients for each clinicopathological factors. (A) All pulmonary pleomorphic carcinoma patients (n=35); (B) according to lymph node metastasis; (C) according to tumor size; (D) according to the PD-L1 expression; (E) according to parietal-pleural invasion; (F) according to tumor stage.**

Table 3. **Univariate and Multivariate Analyses of Prognostic Factors for Relapse Free Survival.**

Factor	Univariate analysis		Multivariate analysis	
	HR (95%CI)	p value	HR(95%CI)	p value
Age (≥65 years vs. <65 years)	1.174 (0.446–3.148)	0.742		
Gender (male vs. female)	0.814 (0.300–2.567)	0.704		
Smoking history (yes vs. no)	0.844 (0.275–3.666)	0.794		
Tumor size (≥50 mm vs. <50 mm)	3.451 (1.252–10.97)	0.016*	3.358 (1.194–10.84)	0.021*
Lymphnode metastasis (yes vs. no)	3.451 (1.304–9.306)	0.013*	3.204 (1.181–8.903)	0.023*
Parietal-pleural invasion (yes vs. no)	0.731 (0.168–2.249)	0.613		
PD-L1 expression (≥50% vs. <50%)	2.469 (0.907–7.813)	0.078	2.795 (1.012–8.975)	0.047*

*p<0.05.

that PC can also be stained with this widely used antibody for NSCLC. Furthermore, only 8.6% of cases were PD-L1 negative in our research, which is consistent with the findings of a previous report citing PD-L1 negativity in 9.8% cases (11). PC frequently shows a high expression of PD-L 1, and few cases are PD-L1 negative.

We found that a high PD-L1 expression in PC was associated with parietal-pleural invasion and correlated with a poor RFS. Parietal-pleural invasion is reported to be a factor influencing a poor prognosis in sarcomatoid carcinoma of the lung (13). However, other studies have reported that blood-vessel invasion, which is known to be associated with a poor OS, was also associated with the PD-L1 expression in sarcomatoid lung cancer (8, 12). A similar phenomenon is seen in NSCLC. Poor tumor differentiation and a high tumor grade were associated with a high PD-L1 expression (14, 15), and a high PD-L1 expression was significantly correlated with a poor prognosis in NSCLC (16, 17). Taken together, these findings indicate that PC with a highly invasive character might show a high PD-L1 expression, which indicates a poor prognosis. A high PD-L1 expression is more frequently seen in PC than in NSCLC (12). This may be consistent with the highly disseminated nature of the disease, the increased rate of relapse after surgical resection, and the poor prognosis of PC when compared with NSCLC.

Since PC is resistant to chemotherapy and radiotherapy,

Table 4. Univariate and Multivariate Analyses of Prognostic Factors for over All Survival.

Factor	Univariate analysis		Multivariate analysis	
	HR (95%CI)	p value	HR(95%CI)	p value
Age (≥65years vs. <65years)	1.741 (0.496–6.820)	0.386		
Gender (male vs. female)	0.894 (0.247–4.159)	0.872		
Smoking history (yes vs. no)	0.923 (0.231–6.118)	0.920		
Tumor size (≥50mm vs. <50mm)	2.716 (0.755–12.60)	0.129	2.249 (0.610–10.63)	0.229
Lymphnode metastasis (yes vs. no)	5.740 (1.594–26.66)	0.007*	5.208 (1.426–24.42)	0.012*
Parietal-pleural invasion (yes vs. no)	0.823 (0.124–3.289)	0.802		
PD-L1 expression (≥50% vs. <50%)	3.437 (0.857–22.83)	0.084	3.526 (0.866–23.70)	0.081

*p<0.05.

the potential efficacy of immune checkpoint inhibitors (ICIs) for this malignancy is gaining increasing attention. PD-1 is an immunoinhibitory receptor expressed on the surface of T cells that normally negatively regulates immune responses (18, 19). Binding of PD-1 to its ligands (PD-L1 and PD-L2) on tumor cells suppresses T cells and leads to evasion of the immune response (20, 21). The Keynote-024 study, an open label phase III trial, reported that, in patients with advanced NSCLC with PD-L1 expression in at least 50% of tumor cells, the PD-1 inhibitor pembrolizumab was associated with a significantly longer PFS and OS than platinum-based chemotherapy (22). In our study, an ICI was administered after relapse, and the outcomes suggest that the PD-L1 expression might be useful for predicting the effectiveness of immune therapy not only in NSCLC but also in PC. Nevertheless, we should note that a high PD-L1 expression does not always guarantee treatment response to ICIs, as cases of PC with a poor outcome following treatment with a PD-1 antibody despite a high PD-L1 expression have been reported (23).

This study has several limitations. First, this was a retrospective study conducted at a single institution. Second, the number of PD-L1 negative cases was too small to statistically evaluate its influence. The relationship of this protein with the OS may not have been statistically significantly indicated because of the small sample size, which was unavoidable due to the rarity of PC. In addition, we did not investigate the tumor microenvironment, including the presence of tumor-infiltrating lymphocytes, which is known to be a predictor of the efficacy of ICIs. Finally, the number of patients treated with ICIs was small. Although PC is rare and difficult to diagnose preoperatively by TBB samples, further large-scale studies with an evaluation of the tumor microenvironment are needed to obtain more information on the clinical significance of PD-L1 in PC.

In conclusion, a high PD-L1 expression was frequently seen in surgically resected PC with parietal-pleural invasion. A high PD-L1 expression might indicate highly invasive behavior and a poor prognosis in PC.

The authors state that they have no Conflict of Interest (COI).

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References

1. Fishback NF, Travis WD, Moran CA, Guinee DG Jr, McCarthy WF, Koss MN. Pleomorphic (spindle/giant cell) carcinoma of the lung. A clinicopathologic correlation of 78 cases. Cancer **73**: 2936-2945, 1994.

2. Travis WD. Pathology of lung cancer. Clin Chest Med **23**: 65-81, 2002.

3. Travis WD, Brambilla E, Burke AP, Marx A, Nicholson AG. WHO Classification of Tumours of the Lung, Pleura, Thymus and Heart. International Agency for Research on Cancer, Lyon, 2015.

4. Chang YL, Lee YC, Shih JY, Wu CT. Pulmonary pleomorphic (spindle) cell carcinoma: peculiar clinicopathologic manifestations different from ordinary non-small cell carcinoma. Lung Cancer **34**: 91-97, 2001.

5. Raveglia F, Mezzetti M, Panigalli T, et al. Personal experience in surgical management of pulmonary pleomorphic carcinoma. Ann Thorac Surg **78**: 1742-1747, 2004.

6. Bae HM, Min HS, Lee SH, et al. Palliative chemotherapy for pulmonary pleomorphic carcinoma. Lung Cancer **58**: 112-115, 2007.

7. Chang YL, Yang CY, Lin MW, Wu CT, Yang PC. High co-expression of PD-L1 and HIF-1alpha correlates with tumour necrosis in pulmonary pleomorphic carcinoma. Eur J Cancer **60**: 125-135, 2016.

8. Yvorel V, Patoir A, Casteillo F, et al. PD-L1 expression in pleomorphic, spindle cell and giant cell carcinoma of the lung is related to TTF-1, p40 expression and might indicate a worse prognosis. PLoS One **12**: e0180346, 2017.

9. American Joint Committee on Cancer. AJCC Cancer Staging Manual. 7th ed. Springer, New York, 2010.

10. Eisenhauer EA, Therasse P, Bogaerts J, et al. New response evaluation criteria in solid tumours: revised RECIST guideline (version 1.1). Eur J Cancer **45**: 228-247, 2009.

11. Kim S, Kim MY, Koh J, et al. Programmed death-1 ligand 1 and 2 are highly expressed in pleomorphic carcinomas of the lung: comparison of sarcomatous and carcinomatous areas. Eur J Cancer **51**: 2698-2707, 2015.

12. Vieira T, Antoine M, Hamard C, et al. Sarcomatoid lung carcinomas show high levels of programmed death ligand-1 (PD-L1) and strong immune-cell infiltration by TCD3 cells and macrophages. Lung Cancer **98**: 51-58, 2-16.

13. Vieira T, Antoine M, Ruppert AM, et al. Blood vessel invasion is a major feature and a factor of poor prognosis in sarcomatoid carcinoma of the lung. Lung Cancer **85**: 276-281, 2014.

14. Pan ZK, Ye F, Wu X, An HX, Wu JX. Clinicopathological and prognostic significance of programmed cell death ligand1 (PD-L1) expression in patients with non-small cell lung cancer: a meta-analysis. J Thorac Dis **7**: 462-470, 2015.

15. Cooper WA, Tran T, Vilain RE, et al. PD-L1 expression is a favorable prognostic factor in early stage non-small cell carcinoma. Lung Cancer **89**: 181-188, 2015.
16. Shimoji M, Shimizu S, Sato K, et al. Clinical and pathologic features of lung cancer expressing programmed cell death ligand 1 (PD-L1). Lung Cancer **98**: 9-75, 2016.
17. Zhang M, Wang D, Sun Q, et al. Prognostic significance of PD-L1 expression and ^{18}F-FDG PET/CT in surgical pulmonary squamous cell carcinoma. Oncotarget **8**: 51630-51640, 2017.
18. Francisco LM, Sage PT, Sharpe AH. The PD-1 pathway in tolerance and autoimmunity. Immunol Rev **236**: 219-242, 2010.
19. Pardoll DM. The blockade of immune checkpoints in cancer immunotherapy. Nat Rev Cancer **12**: 252-264, 2012.
20. Iwai Y, Ishida M, Tanaka Y, Okazaki T, Honjo T, Minato N. Involvement of PD-L1 on tumor cells in the escape from host immune system and tumor immunotherapy by PD-L1 blockade. Proc Natl Acad Sci USA **99**: 12293-12297, 2002.
21. Freeman GJ, Long AJ, Iwai Y, et al. Engagement of the PD-1 immunoinhibitory receptor by a novel B7 family member leads to negative regulation of lymphocyte activation. J Exp Med **192**: 1027-1034, 2000.
22. Reck M, Rodríguez-Abreu D, Robinson AG, et al. KEYNOTE-024 investigators: pembrolizumab versus chemotherapy for PD-L1-positive non-small-cell lung cancer. N Engl J Med **375**: 1823-1833, 2016.
23. Kanazu M, Uenami T, Yano Y, et al. Case series of pleomorphic carcinomas of the lung treated with nivolumab. Thorac Cancer **8**: 724-728, 2017.