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Fluorodeoxyglucose uptake is associated with low tumor-infiltrating lymphocyte levels in patients with small cell lung cancer

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ABSTRACT

Objectives: Positron emission tomography (PET) using 2-deoxy-2-[^{18}F] fluoro-D-glucose (^{18}F-FDG) is a clinically useful modality for cancer evaluation. The mechanism of ^{18}F-FDG uptake within cancer cells involves the glucose transporter 1 (GLUT1) and hypoxia-inducible factor-1 α (HIF-1α). Although recent research has shown its clinical efficacy in small-cell lung cancer (SCLC), no suitable biomarker has been identified. We conducted a clinicopathological study to examine the relationship between tumor immunity and ^{18}F-FDG uptake in patients with SCLC.

Materials and methods: Tumor sections were stained by immunohistochemistry for GLUT1, HIF-1α, PD-L1, CD4, CD8, and Foxp3. The relationship between clinicopathological features and ^{18}F-FDG uptake was analyzed. Student's *t*-test, $\chi 2$ test, non-parametric Spearman's rank test, and Kaplan–Meier method were used to evaluate associations between the variables.

Results: A total of 98 patients 78 men and 20 women who underwent ^{18}F-FDG PET, were enrolled in this study. PD-L1 was expressed in 36.7% (36/98) of all patients; this was significantly associated with GLUT1 expression ($p = 0.04$). The accumulation of ^{18}F-FDG was significantly higher in patients with low CD8 and CD4 TILs than in those with high TILs ($p = 0.03$ and $p = 0.01$, respectively). The uptake of ^{18}F-FDG was not significantly associated with the expression of either Foxp3 or PD-L1. Multivariate analysis demonstrated that advanced stage, poor ECOG-PS, and high SUV_{max} were independent predictors of poor OS. Among patients with limited-stage disease, multivariate analysis confirmed high PD-L1 expression and a high SUV_{max} to be independent predictors of poor OS. However, only ECOG-PS was found to be an independent predictor of poor OS among patients with extensive-stage tumors.

Conclusion: High SUV_{max} on ^{18}F-FDG-PET is correlated with low expression of CD8(+) and CD4(+) TILs, but is an independent prognostic factor for OS, particularly in those with limited disease. Further studies are warranted to validate our findings.

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1. Introduction

Positron emission tomography (PET) using 2-deoxy-2-[^{18}F] fluoro-D-glucose (^{18}F-FDG) is a clinically useful modality for evaluating cancer invasion and metastases and for differentiating between malignant and benign lesions [1]. ^{18}F-FDG PET can be used to monitor chemotherapeutic efficacy and predict the survival of patients following treatment [2,3]. The mechanism for ^{18}F-FDG uptake within cancer cells involves the presence of glucose transporter 1 (GLUT1) and hypoxia-inducible factor-1 α (HIF-1α). The uptake level of ^{18}F-FDG is closely linked to the expression of these markers [4,5].

Recent research has revealed that immune checkpoint inhibitors, such as antibodies to programmed cell death-1 (PD-1) and programmed cell death ligand-1 (PD-L1), have high clinical efficacy against several cancer types. In particular, anti-PD-1/PD-L1 antibodies such as nivolumab, pembrolizumab, and atezolizumab are commonly used to treat patients with non-small cell lung cancer (NSCLC) [6–9]. In the case of small cell lung cancer (SCLC), the CheckMate 032 trial found that nivolumab monotherapy resulted in an objective response rate of 10%, while the median duration of response was not reached after a median follow-up time of 6.6 months [10]. In the KEYNOTE-028 trial that evaluated pembrolizumab monotherapy in patients with PD-L1-positive extensive-stage SCLC [11], the objective response rate was 33.3% while the median duration of response was 19.4 months. These studies showed a potential for long-term disease control in a subset of SCLC patients.

Although established biomarkers that can accurately predict the efficacy of PD-1/PD-L1 immune checkpoint inhibitors are much needed, no suitable predictor has been identified for the efficacy of PD-1 immune checkpoint inhibitors. Nowadays, PD-L1 expression is regarded as a biomarker for anti-PD-1/PD-L1 antibodies in NSCLC [6–9]. However, tumor responses may occur irrespective of PD-L1 expression levels [10], indicating that this ligand might not be a useful biomarker in patients with SCLC. Additionally, the reported expression levels of PD-L1 in patients with SCLC were relatively low [12–16]. Since ^{18}F-FDG PET is a radiological modality for determining the status of tumor activity, it may also be suitable for monitoring the efficacy of PD-1/PD-L1 immune checkpoint inhibitors. Takada et al. reported no correlation between PD-L1 expression and the ^{18}F-FDG maximum standardized uptake value (SUV_{max}) in patients with neuroendocrine tumors [17]. However, their study was limited by its small sample size (16 and 15 patients with small cell carcinoma and large cell neuroendocrine carcinoma, respectively), which may have led to bias. We conducted a clinicopathological study based on the above information, to assess tumor immunity including the evaluation of PD-L1 expression and tumor infiltrating lymphocytes (TILs), in patients with SCLC who underwent ^{18}F-FDG PET.

2. Materials and methods

2.1. Patients

This retrospective study evaluated consecutive patients with histologically or cytologically diagnosed SCLC who underwent ^{18}F-FDG PET for systemic evaluation at Gunma University Hospital between April 2000 and December 2017. The study protocol was approved by the institutional review board of Gunma University Hospital (2018-144) and followed the guidelines of the Helsinki Declaration. The stages of pathological tumor-node-metastasis (TNM) were established using the International System for Staging Lung Cancer adopted by the American Joint Committee on Cancer and the Union Internationale Centre le Cancer [18]. The follow-up duration for censored patients ranged from 0 to 152.2 months (median: 20.0 months).

2.2. Immunochemical staining

Immunohistochemical staining for PD-L1 using a rabbit monoclonal antibody (E1L3N; 1:200 dilution; Cell Signaling Technology, Danvers, Massachusetts, United States and 28-8; 1:400 dilution; Abcam, Tokyo, Japan) was performed according to the procedures described in previous studies [19–21]. The expression of PD-L1 was considered positive when membrane staining was observed. A semi-quantitative scoring method was used for PD-L1: 1 = < 1% of cells were stained, 2 = 1–5%, 3 = 6–10%, 4 = 11–25%, 5 = 26–50%, and 6 = > 50%. Tumors with a score of ≥2 were defined as PD-L1-positive.

Immunohistochemical staining was also performed for CD4 (1:200 dilution; Dako, Tokyo, Japan), CD8 (1:1000 dilution; Abcam, Tokyo, Japan), and Foxp3 (1:200 dilution; Abcam, Tokyo, Japan) in the tumor specimens. After evaluating the specimens, the numbers of CD4(+), CD8(+), and Foxp3(+) cells were counted in a selected 0.26 mm^2 field area hotspot (400× magnification). We determined the cutoff points for CD4-, CD8-, and Foxp3-positivity using receiver operating characteristic (ROC) analysis. The expression levels of GLUT1 (1:200 dilution; Abcam, Tokyo, Japan) and HIF-1α (1:100 dilution; Abcam, Tokyo, Japan) were scored according to the stained tumor area as follows: 1 = ≤10% of the cells were stained, 2 = 11–25%, 3 = 26–50%, and 4 = ≥51%. Tumors scored as 1–2 and 3–4 were defined as low-expression and high-expression, respectively. The tissue sections were examined in a blinded fashion by at least two investigators using a light microscope. In case of any discrepancies, both investigators evaluated the slides simultaneously until they reached a final assessment consensus. Both investigators were blinded to the patients' outcomes.

2.3. PET imaging and data analysis

All patients fasted for at least 6 h before undergoing PET, which was performed using a PET/CT scanner (Discovery STE; GE Healthcare, USA) with a 700 mm field of view at Gunma University Hospital. Three-dimensional data acquisition was initiated 50 min after the injection of 5 MBq/kg of ^{18}F-FDG. We acquired between four and 10 bed positions (3 min acquisition per bed position) according to the range of the imaging. Attenuation-corrected transverse images obtained with ^{18}F-FDG were reconstructed with the ordered subsets expectation maximization algorithm into 128 × 128 matrices with a slice thickness of 3.27 mm.

All ^{18}F-FDG images were interpreted by two experienced nuclear physicians who were unaware of the patients' clinical history and data. Tracer uptake in the primary tumor was defined as positive if the uptake was higher than that in the normal mediastinum. Discrepant results were resolved by a consensus review. For semi-quantitative analysis, functional images of the SUV were produced using the attenuation-corrected transaxial images, injected doses of ^{18}F-FDG, patient's body weight, and cross-calibration factor between PET and the dose calibrator. The SUV was defined using the following equation: SUV = radioactive concentration in the region of interest (ROI) (MBq/g)/injected dose (MBq)/patient's body weight (g). The ROI was manually drawn over the primary tumor on the SUV images. When the tumor was > 1 cm in diameter or the shape of the tumor was irregular or multifocal, an ROI of approximately 1 cm in diameter was drawn over the area corresponding to the maximal tracer uptake. ROI analysis was conducted by a nuclear physician with the aid of corresponding computed tomography (CT) scans. The SUV_{max} in the ROI was used as a representative value for the assessment of ^{18}F-FDG uptake in the lesion. CT scanning for initial staging was performed with intravenous contrast medium; the images were interpreted by board-certified radiologists.

2.4. Statistical analysis

Continuous variables were analyzed using Student's *t*-test and categorical variables were analyzed using the χ^2 test. The relationships

Table 1
Patient's characteristics.

Variables		All patients	FDG uptake		
		Total (n = 98)	High (n = 85)	Low (n = 13)	p-value
Age	< 65/65≦	25/73	19/66	6/7	0.06
Sex	Male /Female	78/20	68/17	10/3	0.79
Stage	I + II/III + IV	20/78	16/69	4/9	0.31
Smoking	Yes/No	88/10	76/9	12/1	0.74
ECOG-PS	0–1/2–4	70/28	60/25	10/3	0.63
T factor	1 + 2/3 + 4	76/22	65/20	11/2	0.51
N factor	0/1–3	20/78	16/69	4/9	0.31
limited	LD/ED	40/58	32/53	8/5	0.1

Abbreviations: FDG, Fluorodeoxyglucose; PS, performance status; LD, limited disease; ED, extensive disease.

between different variables or matched pairs were analyzed using the non-parametric Spearman rank test or Wilcoxon singed-rank test, as appropriate. The Kaplan-Meier method and log-rank test were used to compare differences in survival.

Overall survival (OS) was defined as the interval from diagnosis with cancer to death from any cause.

Univariate and multivariate analyses were performed using Cox's proportional hazard models for survival. Differences were considered statistically significant at p-values of < 0.05, and all analyses were performed using the JMP Pro software (version 12.0; SAS Institute Inc., Cary, NC, USA). ROC curve analyses were used to discriminate evented and censored patients during OS analysis.

3. Results

3.1. Patient demographics

The patients' demographics are shown in Table 1. Ninety-eight patients (78 men and 20 women) were enrolled in this study. The median age was 70 years (range: 49–88 years), and 88 patients were smokers. The Eastern Cooperative Oncology Group performance status (ECOG PS) scores were 0 or 1 in 70 patients, 2 in 19 patients, 3 in 8 patients and 4 in 1one patient. The TNM stages were as follows: stage I in 14 patients, stage II in 6, stage III in 30, and stage IV in 48. The tumors were of limited-stage were 40 patients, and extensive-stage were 58 patients.

Among the 98 patients, the SUV_{max} of ^{18}F-FDG uptake ranged from 0 to 18.4, with a median of 8.3. The cutoff SUVmax was determined as 5.1; a high SUV_{max} was identified in 85 of 98 patients (87%). There was no correlation between ^{18}F-FDG uptake and clinical characteristics.

3.2. Immunohistochemical findings

Immunohistochemical analysis was performed on the 98 primary SCLC tumor specimens; representative immunostained images are shown in Fig. 1. PD-L1 immunostaining was localized predominantly in the plasma membrane of SCLC cells. The positive rates of PD-L1 expression with E1L3N clone and 28-8 clone were 36.7% (36/98) and 41.8% (41/98), respectively. The percentages of patients with PD-L1 (E1L3N) and PD-L1 (28-8) expression scores of 1, 2, 3, 4, 5, and 6 were 63.3% (62/98), 7.1% (7/98), 4.0% (4/98), 8.2% (8/98), 9.2% (9/98), and 8.2% (8/98), respectively, and 58.2% (57/98), 17.3% (17/98), 2.0% (2/98), 12.2% (12/98), 8.2% (8/98), and 2.0% (2/98), respectively. High expression levels of GLUT1, HIF-1α, CD8, CD4, and Foxp3 were identified in 15% (15/98), 17% (17/98), 27% (26/98), 22% (22/98), and 15% (15/98) of the lesions, respectively. PD-L1 (E1L3N) expression was significantly associated with GLUT1 ($p = 0.04$, Table 2); moreover, low CD8 low was significantly associated with low CD4 ($p < 0.01$, Table 2). In patients with low CD8 expression, GLUT1 expression was closely associated with those of PD-L1 (E1L3N) and Foxp3 ($p = 0.02$ and $p = 0.01$, respectively), whereas GLUT1 expression was significantly linked to Foxp3 in patients with low CD4 expression, ($p = 0.02$, Supplemental Table 1, online only).

3.3. Correlation between ^{18}F-FDG uptake and different variables

The accumulation of ^{18}F-FDG was significantly higher in patients with low CD8 and CD4 TILs than in those with high TILs (Fig. 2A and B). The uptake of ^{18}F-FDG was not significantly associated with the expression of Foxp3 or of PD-L1 (E1L3N) (Fig. 2C and D).

3.4. Survival analysis

Among all patients, the median OS was 16.3 months (95% confidence interval [CI]: 11.9–21.9 months), and 66 patients eventually died. Univariate analyses revealed significant differences in OS according to stage, ECOG-PS, SUV_{max}, and PD-L1 (E1L3N) expression (Table 3). However, multivariate analyses found that advanced stage, worse ECOG-PS scores, and high SUV_{max} were independent predictors of worse OS (hazard ratio [HR]: 0.29, $p < 0.01$; HR: 0.45, $p < 0.01$; and HR: 3.55, $p < 0.01$, respectively). The Kaplan-Meier curves for OS according to SUV_{max} and PD-L1 (E1L3N) are shown in Fig. 3.

In patients with limited-stage SCLC, univariate analysis showed a significant difference in survival according to SUV_{max} and PD-L1 (E1L3N) expression. Multivariate analysis revealed that high PD-L1 expression and a high SUV_{max} were independent predictors of poor OS (HR: 4.37, $p = 0.01$ and HR: 3.03, $p = 0.02$, respectively; Supplementary Table 2 online only). In patients with extensive-stage disease, univariate analysis showed that survival was significantly influenced by sex, ECOG-PS, and FoxP3 expression; on multivariate analysis, only ECOG-PS was found to be an independent predictor of worse OS (HR: 0.50, $p = 0.04$; Supplementary Table 3 online only).

4. Discussion

To the best of our knowledge, ours is the first study to examine the relationship between the immune microenvironment and ^{18}F-FDG PET results in patients with SCLC. The low expressions of CD8 and CD4 correlated with a high ^{18}F-FDG uptake. Our study also indicated that the SUV_{max} was an independent prognostic factor in patients with limited disease-SCLC but not in those with extensive disease-SCLC.

The concept of "hot" and "cold" tumors, wherein tumors are immunologically classified into "hot" or "cold" phenotypes based on the demonstration of either high or low immune-cell infiltration, respectively, is a relatively new idea [22,23]. Immunologically "cold" tumors are biologically prone to possess a stem-like phenotype [22], and recent studies found a correlation between the SUV_{max} and expression of PD-L1 in NSCLC [20,21,24,25]. Lopci et al. reported a correlation between the SUV_{max} and CD8-TIL expression [24]. However, other studies showed no correlations between the SUV_{max} and TILs in patients with NSCLC [20,21]. The results of our study demonstrated that the low expressions of CD8 and CD4 were closely correlated with high ^{18}F-FDG uptake, although ^{18}F-FDG uptake was not significantly associated with the expression of PD-L1 and GLUT1. Likewise, GLUT1 and Foxp3 expression levels were correlated in patients with low CD8 and CD4 levels. These data suggest that a high SUV_{max} might reflect a "cold" tumor microenvironment in SCLC. ^{18}F-FDG PET might be useful in distinguishing responders from non-responders during treatment with immunocheckpoint inhibitors for SCLC.

The PD-L1 positivity rates in patients with SCLC ranged between 0% and 71.6% in previous studies [12–16,26–29]; in our study, this rate was 36.7% with the E1L3N clone and 41.8% with the 28-8 clone. PD-L1 is reported to be closely correlated with the expression of GLUT1 in patients with renal-cell carcinoma, Hodgkin's lymphoma, squamous cell lung carcinoma, and lung adenocarcinoma [20,21,30,31]. A

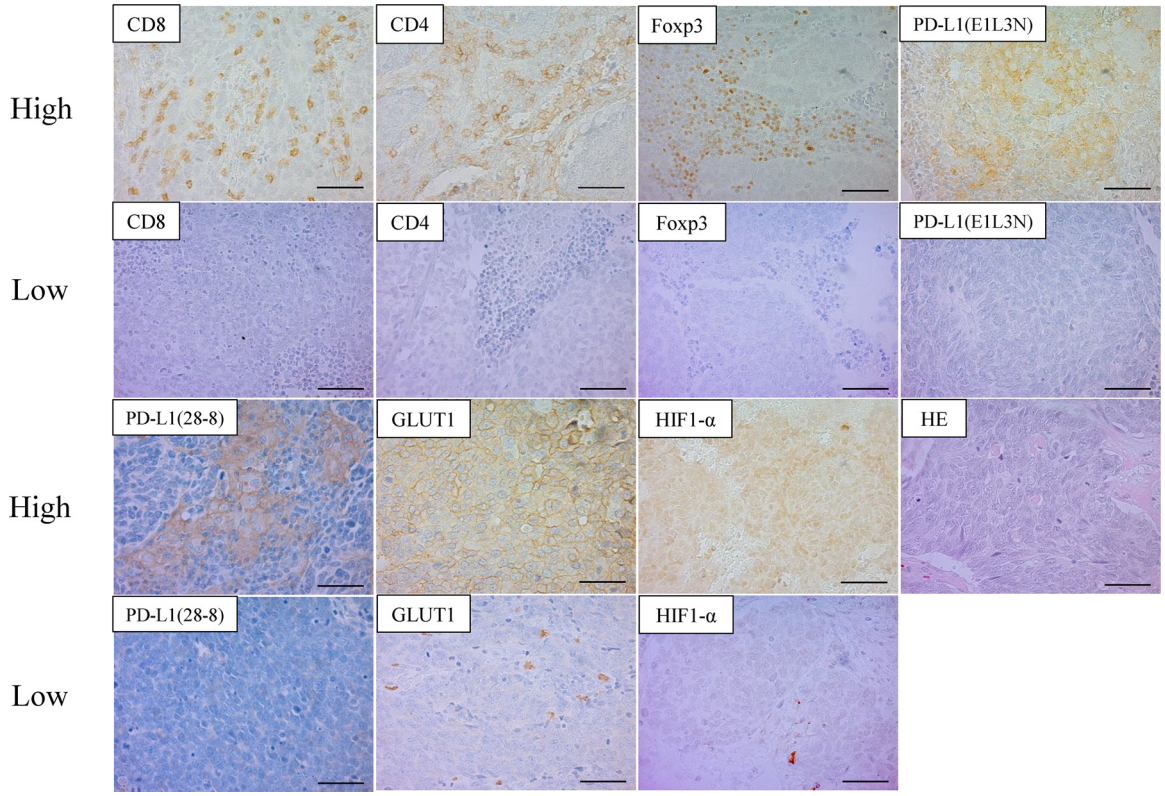

Fig. 1. Representative immunostained images of CD8, CD4, Foxp3, PD-L1, GLUT1, HIF-1α, and HE. A semi-quantitative scoring method was used for PD-L1 (1 = < 1%, 2 = 1–5%, 3 = 6–10%, 4 = 11–25%, 5 = 26–50%, and 6 = > 50% of positive cells). Tumors with a score of ≥1 were graded as positive. The receiver operating characteristic was used as the cut-off point for CD4(+), CD8(+), and Foxp3(+) TIL density. The expression of GLUT1 and HIF-1α was scored according to the stained tumor area as follows: 1 = ≤10% stained, 2 = 11–25% stained, 3 = 26–50% stained, and 4 = ≥51% stained. Tumors scored as 1 or 2 and 3 or 4 were defined as low expression and high expression tumors, respectively (scale bar = 200 μm). Upper and lower columns represent high and low expression, respectively.

significant relationship has also been observed between PD-L1 and HIF-1α expression in patients with pulmonary pleomorphic carcinoma, oral squamous carcinoma, squamous cell lung carcinoma, and lung adenocarcinoma [20,21,32,33]. To our knowledge, our study is the first to demonstrate an association between PD-L1 and GLUT1 in patients with SCLC (p = 0.04). Additionally, a high expression of PD-L1 was correlated with worse prognosis. The role of PD-L1 expression as a prognostic marker is controversial; some previous studies demonstrated that high PD-L1-positivity is associated with better prognosis [26,29,34–38], while others found it to be associated with poor prognosis [27,39–46]. This inconsistency may stem from the timing of obtaining the tumor sample, the diversity of anti-PD-L1 antibodies used, and the inconsistent cut-off values for PD-L1 expression. In our study, the PD-L1 and SUV_{max} were independent prognostic factors for patients with limited disease-SCLC but not in those with extensive disease-SCLC. Since previous studies have discovered a correlation between limited stage disease and PD-L1 expression [26,29], earlier stage may be associated with a greater invasion of lymphocytes.

This study has some limitations. First, the number of patients was relatively small; second, the study population was heterogeneous and included both those with limited and extensive disease. These limitations made it difficult to calculate disease-free or progression-free survival. Third, most specimens were obtained via transbronchial lung biopsy. However, this is a general limitation in clinical practice, as most SCLC patients are diagnosed at a later stage. Fourth, the lack of an independent cohort to validate the results of our study also limits the interpretation of our study results. The FDG-PET device used in our institution may be different from that of other institutions. The SUV_{max} assessment may differ according to type of FDG-PET machine, introducing a possible variability in SUV_{max} results. Future large-sample prospective studies are warranted to overcome these limitations and validate our results.

In conclusion, in our cohort, the uptake of ^{18}F-FDG on PET significantly correlated with low expression of CD8(+) and CD4(+) TILs. High ^{18}F-FDG uptake appears to be predictive of a shorter survival in SCLC patients, particularly those with limited disease. Further studies

Table 2
Correlation with immunostained variables.

Variables		CD8			CD4			PD-L1		
		High (n = 26)	Low (n = 72)	P-value	High (n = 22)	Low (n = 76)	P-value	Positive (n = 36)	Negative (n = 62)	P-value
PD-L1	High/low	8/18	28/44	0.46	9/13	27/49	0.64			
HIF-1	High/low	2/24	15/57	0.12	7/18	13/63	0.90	8/28	9/53	0.33
Glut1	High/low	7/19	8/64	0.05	3/19	12/64	0.80	9/27	6/56	0.04
CD4	High/low	12/14	10/62	< 0.01				9/27	13/49	0.64
Foxp3	High/low	7/19	8/64	0.05	5/17	10/66	0.27	6/30	9/53	0.77

Abbreviations: PD-L1, programmed death ligand-1; HIF-1α, hypoxia-inducible factor-1α; GLUT1, glucose transporter type 1.

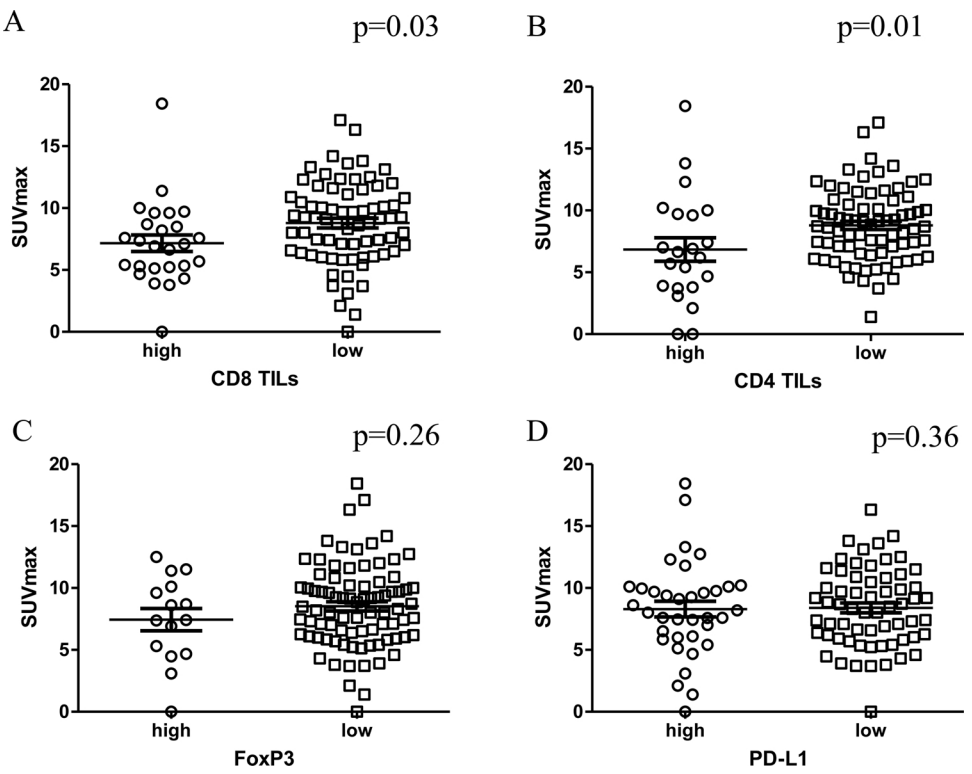

Fig. 2. (A, B): Comparison of ^{18}F-FDG uptake according to PD-L1 expression and TILs (CD4, CD8 and Foxp3): The accumulation of ^{18}F-FDG was significantly higher in patients with low CD4 and CD8 TILs than in those with high TILs. (C, D): The uptake of 18 F-FDG was not significantly associated with the expression of Foxp3 (p = 0.26) or of PD-L1 (p = 0.36).

Table 3
Univariate and multivariate survival analysis for overall survival.

Variables		Univariate analysis			Multivariate analysis		
		HR	p-value	95%CI	HR	p-value	95%CI
Age	< 65/65≦	0.88	0.66	0.50–1.50			
Sex	Male / Female	1.63	0.13	0.86–3.40			
stage	I + II / III + IV	0.22	< 0.01	0.09–0.45	0.29	< 0.01	0.12-0.63
ECOG-PS	0-1/2-4	0.38	< 0.01	0.23–0.65	0.45	< 0.01	0.26-0.77
SUV_{max}	High / low	2.84	< 0.01	1.25–8.18	3.55	< 0.01	1.49-10.56
PD-L1	High / low	1.84	0.01	1.11–3.03	1.60	0.07	0.95-2.66
Glut1	High / low	1.09	0.79	0.52–2.05			
HIF-1	High / low	1.33	0.4	0.65–2.46			
CD8	High / low	0.56	0.05	0.30–1.00			
CD4	High / low	0.68	0.21	0.34–1.23			
FoxP3	High / low	1.07	0.81	0.54–1.95			

Abbreviations: PS, performance status, PD-L1, programmed death ligand-1; GLUT1, glucose transporter type 1; HIF-1α, hypoxia-inducible factor-1α.

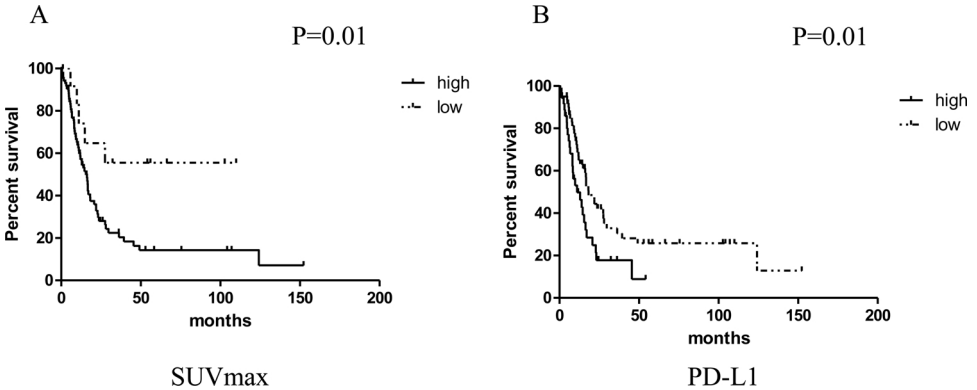

Fig. 3. Kaplan–Meier curves of overall survival (OS) according to SUV_{max} and PD-L1. (A) The OS of patients with a high SUV_{max} was shorter than that of patients with a low SUV_{max} (p = 0.01). (B) The OS of patients with high PD-L1 expression was shorter than that of patients with low PD-L1 expression (p = 0.01).

are warranted to validate our findings.

Declaration of competing interest

KK has received research grants and a speaker honorarium from Boehringer Ingelheim Company, Ono Pharmaceutical Company, and Bristol-Myers Company and Chugai Pharmaceutical Company. All remaining authors have declared no conflicts of interest.

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Appendix A. Supplementary data

Supplementary material related to this article can be found, in the online version, at doi:https://doi.org/10.1016/j.lungcan.2019.06.009.

References

[1] R.M. Pieterman, J.W. van Putten, J.J. Meuzelaar, E.L. Mooyaart, W. Vaalburg, G.H. Koëter, V. Fidler, J. Pruim, H.J. Groen, Preoperative staging of non-small-cell lung cancer with positron-emission tomography, N. Engl. J. Med. 343 (2000) 254–261, https://doi.org/10.1056/NEJM200007273430404.

[2] S.H. Moon, S.H. Cho, L.C. Park, J.H. Ji, J.M. Sun, J.S. Ahn, K. Park, J.Y. Choi, M.J. Ahn, Metabolic response evaluated by 18F-FDG PET/CT as a potential screening tool in identifying a subgroup of patients with advanced non-small cell lung cancer for immediate maintenance therapy after first-line chemotherapy, Eur. J. Nucl. Med. Mol. Imaging 40 (2013) 1005–1013, https://doi.org/10.1007/s00259-013-2400-4.

[3] W.A. Weber, V. Petersen, B. Schmidt, L. Tyndale-Hines, T. Link, C. Peschel, M. Schwaiger, Positron emission tomography in non-small-cell lung cancer: prediction of response to chemotherapy by quantitative assessment of glucose use, J. Clin. Oncol. 21 (2003) 2651–2657, https://doi.org/10.1200/JCO.2003.12.004.

[4] K. Kaira, M. Endo, M. Abe, Y. Ohde, T. Okumura, T. Takahashi, H. Murakami, A. Tsuya, Y. Nakamura, T. Naito, I. Hayashi, M. Serizawa, Y. Koh, H. Hanaoka, H. Tominaga, N. Kondo, T. Nakajima, N. Yamamoto, Biologic correlation of 2-[18F]-fluoro-2-deoxy-D-glucose uptake on positron emission tomography in thymic epithelial tumors, J. Clin. Oncol. 28 (2010) 3746–3753, https://doi.org/10.1200/JCO.2009.27.4662.

[5] K. Kaira, M. Serizawa, Y. Koh, T. Takahashi, A. Yamaguchi, H. Hanaoka, N. Oriuchi, M. Endo, Y. Ohde, T. Nakajima, N. Yamamoto, Biological significance of 18F-FDG uptake on PET in patients with non-small-cell lung cancer, Lung Cancer 83 (2014) 197–204, https://doi.org/10.1016/j.lungcan.2013.11.025.

[6] J. Brahmer, K.L. Reckamp, P. Baas, L. Crinò, W.E. Eberhardt, E. Poddubskaya, S. Antonia, A. Pluzanski, E.E. Vokes, E. Holgado, D. Waterhouse, N. Ready, J. Gainor, O. Arén Frontera, L. Havel, M. Steins, M.C. Garassino, J.G. Aerts, M. Domine, L. Paz-Ares, M. Reck, C. Baudelet, C.T. Harbison, B. Lestini, D.R. Spigel, Nivolumab versus docetaxel in advanced squamous-cell non-small-cell lung cancer, N. Engl. J. Med. 373 (2015) 123–135, https://doi.org/10.1056/NEJMoa1504627.

[7] H. Borghaei, L. Paz-Ares, L. Horn, D.R. Spigel, M. Steins, N.E. Ready, L.Q. Chow, E.E. Vokes, E. Felip, E. Holgado, F. Barlesi, M. Kohlhäufl, O. Arrieta, M.A. Burgio, J. Fayette, H. Lena, E. Poddubskaya, D.E. Gerber, S.N. Gettinger, C.M. Rudin, N. Rizvi, L. Crinò, G.R. Blumenschein Jr., S.J. Antonia, C. Dorange, C.T. Harbison, F. Graf Finckenstein, J.R. Brahmer, Nivolumab versus docetaxel in advanced non-squamous non-small-cell lung cancer, N. Engl. J. Med. 373 (2015) 1627–1639, https://doi.org/10.1056/NEJMoa1507643.

[8] Reck M, D. Rodríguez-Abreu, A.G. Robinson, R. Hui, T. Csőszi, A. Fülöp, M. Gottfried, N. Peled, A. Tafreshi, S. Cuffe, M. O'Brien, S. Rao, K. Hotta, M.A. Leiby, G.M. Lubiniecki, Y. Shentu, R. Rangwala, J.R. Brahmer, KEYNOTE-024 Investigators, Pembrolizumab versus chemotherapy for pd-l1-positive non-small-cell lung cancer, N. Engl. J. Med. 375 (2016) 1823–1833, https://doi.org/10.1056/NEJMoa1606774.

[9] R.S. Herbst, P. Baas, D.W. Kim, E. Felip, J.L. Pérez-Gracia, J.Y. Han, J. Molina, J.H. Kim, C.D. Arvis, M.J. Ahn, M. Majem, M.J. Fidler, G. de Castro Jr., M. Garrido, G.M. Lubiniecki, Y. Shentu, E. Im, M. Dolled-Filhart, E.B. Garon, Pembrolizumab versus docetaxel for previously treated, PD-L1-positive, advanced non-small-cell lung cancer (KEYNOTE-010): a randomised controlled trial, Lancet 387 (2016) 1540–1550, https://doi.org/10.1016/S0140-6736(15)01281-7.

[10] S.J. Antonia, J.A. López-Martin, J. Bendell, P.A. Ott, M. Taylor, J.P. Eder, J.äger D, M.C. Pietanza, D.T. Le, F. de Braud, M.A. Morse, P.A. Ascierto, L. Horn, A. Amin, R.N. Pillai, J. Evans, I. Chau, P. Bono, A. Atmaca, P. Sharma, C.T. Harbison, C.S. Lin, O. Christensen, E. Calvo, Nivolumab alone and nivolumab plus ipilimumab in recurrent small-cell lung cancer (CheckMate 032): a multicentre, open-label, phase 1/2 trial, Lancet Oncol. 17 (2016) 883–895, https://doi.org/10.1016/S1470-2045(16)30098-5.

[11] P.A. Ott, E. Elez, S. Hiret, D.W. Kim, A. Morosky, S. Saraf, B. Piperdi, J.M. Mehnert, Pembrolizumab in patients with extensive-stage small-cell lung cancer: results from the phase Ib KEYNOTE-028 study, J. Clin. Oncol. 35 (2017) 3823–3829, https://doi.org/10.1200/JCO.2017.72.5069.

[12] A.M. Schultheis, A.H. Scheel, L. Ozretić, J. George, R.K. Thomas, T. Hagemann, T. Zander, J. Wolf, R. Buettner, PD-L1 expression in small cell neuroendocrine carcinomas, Eur. J. Cancer 51 (2015) 421–426, https://doi.org/10.1016/j.ejca.2014.12.006.

[13] K. Tsuruoka, H. Horinouchi, Y. Goto, S. Kanda, Y. Fujiwara, H. Nokihara, N. Yamamoto, K. Asakura, K. Nakagawa, H. Sakurai, S.I. Watanabe, K. Tsuta, Y. Ohe, PD-L1 expression in neuroendocrine tumors of the lung, Lung Cancer 108 (2017) 115–120, https://doi.org/10.1016/j.lungcan.2017.03.006.

[14] A. Kasajima, Y. Ishikawa, A. Iwata, K. Steiger, N. Oka, H. Ishida, A. Sakurada, H. Suzuki, T. Kameya, B. Konukiewitz, G. Klöppel, Y. Okada, H. Sasano, W. Weichert, Inflammation and PD-L1 expression in pulmonary neuroendocrine tumors, Endocr. Relat. Cancer 25 (2018) 339–350, https://doi.org/10.1530/ERC-17-0427.

[15] H.S. Kim, J.H. Lee, S.J. Nam, C.Y. Ock, J.W. Moon, C.W. Yoo, G.K. Lee, J.Y. Han, Association of PD-L1 expression with tumor-infiltrating immune cells and mutation burden in high-grade neuroendocrine carcinoma of the lung, J. Thorac. Oncol. 13 (2018) 636–648, https://doi.org/10.1016/j.jtho.2018.01.008.

[16] G. Toyokawa, K. Takada, N. Haratake, S. Takamori, T. Akamine, M. Katsura, T. Fujishita, F. Shoji, T. Okamoto, Y. Oda, Y. Maehara, Favorable disease-free survival associated with programmed death ligand 1 expression in patients with surgically resected small-cell lung cancer, Anticancer Res. 36 (2016) 4329–4336.

[17] K. Takada, G. Toyokawa, T. Okamoto, S. Baba, Y. Kozuma, T. Matsubara, N. Haratake, T. Akamine, S. Takamori, M. Katsura, F. Shoji, H. Honda, Y. Oda, Y. Maehara, Metabolic characteristics of programmed cell death-ligand 1-expressing lung cancer on (18) F-fluorodeoxyglucose positron emission tomography/computed tomography, Cancer Med. 6 (2017) 2552–2561, https://doi.org/10.1002/cam4.1215.

[18] C.F. Mountain, Revisions in the international system for staging lung cancer, Chest 111 (1997) 1710–1717.

[19] K. Kaira, T. Higuchi, I. Naruse, Y. Arisaka, A. Tokue, B. Altan, S. Suda, A. Mogi, K. Shimizu, N. Sunaga, T. Hisada, S. Kitano, H. Obinata, T. Yokobori, K. Mori, M. Nishiyama, Y. Tsushima, T. Asao, Metabolic activity by (18)F-FDG-PET/CT is predictive of early response after nivolumab in previously treated NSCLC, Eur. J. Nucl. Med. Mol. Imaging 45 (2018) 56–66, https://doi.org/10.1007/s00259-017-3806-1.

[20] N. Kasahara, K. Kaira, P. Bao, T. Higuchi, Y. Arisaka, B. Erkhem-Ochir, N. Sunaga, Y. Ohtaki, T. Yajima, T. Kosaka, T. Oyama, T. Yokobori, T. Asao, M. Nishiyama, Y. Tsushima, H. Kuwano, K. Shimizu, A. Mogi, Correlation of tumor-related immunity with 18F-FDG-PET in pulmonary squamous-cell carcinoma, Lung Cancer 119 (2018) 71–77, https://doi.org/10.1016/j.lungcan.2018.03.001.

[21] K. Kaira, K. Shimizu, S. Kitahara, T. Yajima, J. Atsumi, T. Kosaka, Y. Ohtaki, T. Higuchi, T. Oyama, T. Asao, A. Mogi, 2-Deoxy-2-[fluorine-18] fluoro-d-glucose uptake on positron emission tomography is associated with programmed death ligand-1 expression in patients with pulmonary adenocarcinoma, Eur. J. Cancer 101 (2018) 181–190, https://doi.org/10.1016/j.ejca.2018.06.022.

[22] N. Nagarsheth, M.S. Wicha, W. Zou, Chemokines in the cancer microenvironment and their relevance in cancer immunotherapy, Nat. Rev. Immunol. 17 (2017) 559–572, https://doi.org/10.1038/nri.2017.49.

[23] J.B.A.G. Haanen, Converting cold into hot tumors by combining immunotherapies, Cell 170 (2017) 1055–1056, https://doi.org/10.1016/j.cell.2017.08.031.

[24] E. Lopci, L. Toschi, F. Grizzi, D. Rahal, L. Olivari, G.F. Castino, S. Marchetti, N. Cortese, D. Qehajaj, D. Pistillo, M. Alloisio, M. Roncalli, P. Allavena, A. Santoro, F. Marchesi, A. Chiti, Correlation of metabolic information on FDG-PET with tissue expression of immune markers in patients with non-small cell lung cancer (NSCLC) who are candidates for upfront surgery, Eur. J. Nucl. Med. Mol. Imaging 43 (2016) 1954–1961, https://doi.org/10.1007/s00259-016-3425-2.

[25] M. Zhang, D. Wang, Q. Sun, H. Pu, Y. Wang, S. Zhao, Y. Wang, Q. Zhang, Prognostic significance of PD-L1 expression and 18F-FDG PET/CT in surgical pulmonary squamous cell carcinoma, Oncotarget 8 (2017) 51630–51640, https://doi.org/10.18632/oncotarget.18257.

[26] H. Ishii, K. Azuma, A. Kawahara, K. Yamada, Y. Imamura, T. Tokito, T. Kinoshita, M. Kage, T. Hoshino, Significance of programmed cell death-ligand 1 expression and its association with survival in patients with small cell lung cancer, J. Thorac. Oncol. 10 (2015) 426–430, https://doi.org/10.1097/JTO.0000000000000414.

[27] Y. Fan, K. Ma, C. Wang, J. Ning, Y. Hu, D. Dong, X. Dong, Q. Geng, E. Li, Y. Wu, Prognostic value of PD-L1 and PD-1 expression in pulmonary neuroendocrine tumors, Onco. Ther. 9 (2016) 6075–6082, https://doi.org/10.2147/OTT.S115054.

[28] A.S. Berghoff, G. Ricken, D. Wilhelm, O. Rajky, G. Widhalm, K. Dieckmann, P. Birner, R. Bartsch, M. Preusser, Tumor infiltrating lymphocytes and PD-L1 expression in brain metastases of small cell lung cancer (SCLC), J. Neurooncol. 130 (2016) 19–29, https://doi.org/10.1007/s11060-016-2216-8.

[29] L. Miao, Y. Lu, Y. Xu, G. Zhang, Z. Huang, L. Gong, Y. Fan, PD-L1 and c-MET expression and survival in patients with small cell lung cancer, Oncotarget 8 (2017) 53978–53988, https://doi.org/10.18632/oncotarget.9765.

[30] M. Ruf, H. Moch, P. Schraml, PD-L1 expression is regulated by hypoxia inducible factor in clear cell renal cell carcinoma, Int. J. Cancer 139 (2016) 396–403, https://doi.org/10.1002/ijc.30077.

[31] Y.W. Koh, J.H. Han, S.Y. Park, D.H. Yoon, C. Suh, J. Huh, GLUT1 as a prognostic factor for classical Hodgkin's lymphoma: correlation with PD-L1 and PD-L2 expression, J. Pathol. Transl. Med. 51 (2017) 152–158, https://doi.org/10.4132/

jptm.2016.11.03.
[32] T.C. Chen, C.T. Wu, C.P. Wang, W.L. Hsu, T.L. Yang, P.J. Lou, J.Y. Ko, Y.L. Chang, Associations among pretreatment tumor necrosis and the expression of HIF-1alpha and PD-L1 in advanced oral squamous cell carcinoma and the prognostic impact thereof, Oral Oncol. 51 (2015) 1004–1010, https://doi.org/10.1016/j.oraloncology.2015.08.011.
[33] Y.L. Chang, C.Y. Yang, M.W. Lin, C.T. Wu, P.C. Yang, High co-expression of PD-L1 and HIF-1alpha correlates with tumour necrosis in pulmonary pleomorphic carcinoma, Eur. J. Cancer 60 (2016) 125–135, https://doi.org/10.1016/j.ejca.2016.03.012.
[34] E.J. Lipson, J.G. Vincent, M. Loyo, L.T. Kagohara, B.S. Luber, H. Wang, H. Xu, S.K. Nayar, T.S. Wang, D. Sidransky, R.A. Anders, S.L. Topalian, J.M. Taube, PD-L1 expression in the Merkel cell carcinoma microenvironment: association with inflammation, Merkel cell polyomavirus and overall survival, Cancer Immunol. Res. 1 (2013) 54–63, https://doi.org/10.1158/2326-6066.CIR-13-0034.
[35] R.A. Droeser, C. Hirt, C.T. Viehl, D.M. Frey, C. Nebiker, X. Huber, I. Zlobec, S. Eppenberger-Castori, A. Tzankov, R. Rosso, M. Zuber, M.G. Muraro, F. Amicarella, E. Cremonesi, M. Heberer, G. Iezzi, A. Lugli, L. Terracciano, G. Sconocchia, D. Oertli, G.C. Spagnoli, L. Tornillo, Clinical impact of programmed cell death ligand 1 expression in colorectal cancer, Eur. J. Cancer 49 (2013) 2233–2242, https://doi.org/10.1016/j.ejca.2013.02.015.
[36] C.Y. Yang, M.W. Lin, Y.L. Chang, C.T. Wu, P.C. Yang, Programmed cell death-ligand 1 expression in surgically resected stage I pulmonary adenocarcinoma and its correlation with driver mutations and clinical outcomes, Eur. J. Cancer 50 (2014) 1361–1369, https://doi.org/10.1016/j.ejca.2014.01.018.
[37] V. Velcheti, K.A. Schalper, D.E. Carvajal, V.K. Anagnostou, K.N. Syrigos, M. Sznol, R.S. Herbst, S.N. Gettinger, L. Chen, D.L. Rimm, Programmed death ligand-1 expression in non-small cell lung cancer, Lab. Invest. 94 (2014) 107–116, https://doi.org/10.1038/labinvest.2013.130.
[38] K.A. Schalper, V. Velcheti, D. Carvajal, H. Wimberly, J. Brown, L. Pusztai, D.L. Rimm, In situ tumor PD-L1 mRNA expression is associated with increased TILs and better outcome in breast carcinomas, Clin. Cancer Res. 20 (2014) 2773–2782, https://doi.org/10.1158/1078-0432.CCR-13-2702.
[39] Y. Ohigashi, M. Sho, Y. Yamada, Y. Tsurui, K. Hamada, N. Ikeda, T. Mizuno, R. Yoriki, H. Kashizuka, K. Yane, F. Tsushima, N. Otsuki, H. Yagita, M. Azuma, Y. Nakajima, Clinical significance of programmed death-1 ligand-1 and programmed death-1 ligand-2 expression in human esophageal cancer, Clin. Cancer Res. 11 (2005) 2947–2953, https://doi.org/10.1158/1078-0432.CCR-04-1469.
[40] C. Wu, Y. Zhu, J. Jiang, J. Zhao, X.G. Zhang, N. Xu, Immunohistochemical localization of programmed death-1 ligand-1 (PD-L1) in gastric carcinoma and its clinical significance, Acta Histochem. 108 (2006) 19–24, https://doi.org/10.1016/j.acthis.2006.01.003.
[41] R.H. Thompson, S.M. Kuntz, B.C. Leibovich, H. Dong, C.M. Lohse, W.S. Webster, S. Sengupta, I. Frank, A.S. Parker, H. Zincke, M.L. Blute, T.J. Sebo, J.C. Cheville, E.D. Kwon, Tumor B7-H1 is associated with poor prognosis in renal cell carcinoma patients with long-term follow-up, Cancer Res. 66 (2006) 3381–3385, https://doi.org/10.1158/0008-5472.CAN-05-4303.
[42] R.H. Thompson, H. Dong, E.D. Kwon, Implications of B7-H1 expression in clear cell carcinoma of the kidney for prognostication and therapy, Clin. Cancer Res. 13 (2 Pt 2) (2007) 709s–715s, https://doi.org/10.1158/1078-0432.CCR-06-1868.
[43] T. Nomi, M. Sho, T. Akahori, K. Hamada, A. Kubo, H. Kanehiro, S. Nakamura, K. Enomoto, H. Yagita, M. Azuma, Y. Nakajima, Clinical significance and therapeutic potential of the programmed death-1 ligand/programmed death-1 pathway in human pancreatic cancer, Clin. Cancer Res. 13 (2007) 2151–2157, https://doi.org/10.1158/1078-0432.CCR-06-2746.
[44] R. Hino, K. Kabashima, Y. Kato, H. Yagi, M. Nakamura, T. Honjo, T. Okazaki, Y. Tokura, Tumor cell expression of programmed cell death-1 ligand 1 is a prognostic factor for malignant melanoma, Cancer 116 (2010) 1757–1766, https://doi.org/10.1002/cncr.24899.
[45] J.M. Taube, R.A. Anders, G.D. Young, H. Xu, R. Shrma, T.L. McMiller, S. Chen, A.P. Klein, D.M. Pardoll, S.L. Topalian, L. Chen, Colocalization of inflammatory response with B7-h1 expression in human melanocytic lesions supports an adaptive resistance mechanism of immune escape, Sci. Transl. Med. 4 (2012) 127ra37, , https://doi.org/10.1126/scitranslmed.3003689.
[46] S.K. Padda, J.W. Riess, E.J. Schwartz, L. Tian, H.E. Kohrt, J.W. Neal, R.B. West, H.A. Wakelee, Diffuse high intensity PD-L1 staining in thymic epithelial tumors, J. Thorac. Oncol. 10 (2015) 500–518, https://doi.org/10.1097/JTO.0000000000000429.