

Radiological Features of Programmed Cell Death-Ligand 2-positive Lung Adenocarcinoma: A Single-institution Retrospective Study

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Abstract. Aim: Programmed cell death-ligand 1 and 2 (PD-L1 and PD-L2) are ligands of the programmed cell death-1 (PD1) receptor. PD1/PD-L1 inhibitors have shown clinical efficacy in non-small cell lung cancer (NSCLC). However, relatively little is known about the expression of PD-L2, or its association with the clinicopathological features of NSCLC. Here, the radiological features of PD-L2-positive lung adenocarcinoma were evaluated. Materials and Methods: PD-L1 and PD-L2 expression were evaluated by immunohistochemical staining of surgically-resected specimens from 393 patients with primary lung adenocarcinoma who underwent preoperative thin-section computed tomography (CT), 222 of whom also underwent ¹⁸F-fluorodeoxyglucose positron-emission tomography/CT (¹⁸F-FDG-PET/CT). Results: Among the 393 specimens, 132 (33.6%) and 266 (67.7%) were positive for PD-L1 and PD-L2 expression, respectively. Multivariate analysis showed that the absence of surrounding ground glass opacity and the presence of air bronchogram were significantly associated with PD-L2 expression; however, there was no significant association between PD-L2 expression and the consolidation/tumor ratio. In 222 ¹⁸F-FDG-PET/CT, the maximum standardized uptake value was significantly higher in patients with PD-L2-positive

compared to those with PD-L2-negative tumors. Conclusion: PD-L2-positive lung adenocarcinomas are less radiologically malignant and invasive than their PD-L1-positive counterparts.

Immunotherapeutic targeting of the T-cell-inhibitory protein programmed cell death-1 (PD1) and of its ligand programmed cell death-ligand 1 (PD-L1) has garnered a great deal of attention in recent years as a novel treatment option for non-small cell lung cancer (NSCLC). Indeed, anti-PD1/PD-L1 immunotherapy has been shown to significantly prolong the survival of patients with NSCLC compared to conventional standard chemotherapy in many clinical trials (1-6). To date, immunohistochemical (IHC) detection of tumor PD-L1 expression has proven to be the most useful predictive biomarker of response to PD1/PD-L1 immunotherapy. However, some patients with PD-L1-negative tumors do respond to such therapy, suggesting that additional biomarkers will be necessary to accurately predict the response to therapy targeting the PD1 checkpoint (7, 8).

Programmed cell death-ligand 2 (PD-L2) is the second identified ligand for PD-1 (9). Although PD-L1 appears to be the main ligand, its binding affinity for PD1 is 2- to 6-fold lower than that of PD-L2 (10). However, there have been relatively few reports about the clinical significance of PD-L2 expression in NSCLC, and its precise roles in the human tumor microenvironment and its utility as a prognostic or predictive marker have not yet been established (11-15). We recently investigated the clinical utility of PD-L2 expression in NSCLC and identified a significant association between its expression in primary lung adenocarcinoma and patient survival (unpublished observation). Here, we extended this study to investigate the clinical features of PD-L2-positive lung adenocarcinoma in more detail.

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Recently, we identified several associations between PD-L1 expression and clinical features in 394 patients with primary lung adenocarcinomas; these included radiological characteristics on computed tomography (CT) (16) and metabolic characteristics by ^{18}F -fluorodeoxyglucose positron-emission tomography/CT (^{18}F -FDG PET/CT) (17, 18). In the present study, we analyzed the same features for the same patient cohort in the context of tumor PD-L2 expression. The results allow for comparison and clarification of the clinical significance of PD-L2 and PD-L1 expression in patients with primary lung adenocarcinoma.

Materials and Methods

Patients and samples. This study retrospectively examined patients who underwent surgical resection of primary lung adenocarcinoma between January 2003 and December 2012 at the Department of Surgery and Science, Graduate School of Medical Sciences, Kyushu University. We previously analyzed the relationship between PD-L1 expression and clinical features in 394 patients who had undergone preoperative thin-section CT at our Institution (16). Among those, 393 patients whose formalin-fixed and paraffin-embedded tumor tissue sections were available for IHC of PD-L2 were enrolled in this study. The clinicopathological features, including age at surgery, sex, smoking history, pathologic tumor-node-metastasis stage (seventh edition of the American Joint Committee on Cancer lung cancer staging system) (19), pleural and lymphovascular invasion, histological subtype (World Health Organization Classification 2015) (20), and epidermal growth factor receptor (*EGFR*) mutation status were examined. *EGFR* status had been determined in 230 specimens of tumor tissue using the peptide nucleic acid–locked nucleic acid polymerase chain reaction clamp method (Mitsubishi Chemical Medience, Tokyo, Japan) (21). Clinical information was obtained from medical records. This study was approved by our Institutional Review Board (Kyushu University, IRB No. 29-261).

Chest CT. Chest CT was performed in the supine position during inspiratory breath-hold using various multi-detector row scanners: Aquilion 4, Aquilion 64, Aquilion ONE, Aquilion ONE Vision (all Toshiba), SOMATOM Plus4 Volume Zoom (Siemens Medical Solutions, Erlangen, Germany), Brilliance CT, and Brilliance iCT (both Philips Healthcare, Amsterdam, the Netherlands). The imaging parameters for thin-section CT were as follows: tube voltage 120 kVp, tube current 100-500 mA, scan field of view 320-360 mm, and slice thickness 2 mm. Real exposure control (Toshiba, Tokyo, Japan) or automatic exposure control (Siemens and Phillips) was included in each study. All of the CT data sets were transferred to a Picture Archiving and Communication System, which was accessible by the workstations (Volume Analyzer Synapse-Vincent; Fujifilm, Tokyo, Japan) using a specialized application for lung CTs. The diameter of consolidation in each tumor (C) and the diameter of the whole tumor (T), including ground glass opacity (GGO), were measured manually with axial 2-dimensional CT data on 2-mm slice sections and the C/T ratio was calculated. Three thoracic oncologists (KT, GT, and ST) evaluated all of the CT images, and disagreements were resolved by consensus.

^{18}F -FDG PET/CT. ^{18}F -FDG PET/CT scanning was performed using SECAT EXACT HR+, Biograph mCT (both Siemens), and

Discovery STElite16 (GE) scanners. The maximum standardized uptake value (SUVmax) of the tumor was calculated.

Immunohistochemical analysis. PD-L1 and PD-L2 expression in formalin-fixed, paraffin-embedded tumor sections was performed by IHC using primary antibodies against PD-L1 (1:100 dilution, rabbit monoclonal, clone SP142; Spring Bioscience, Ventana, Tucson, AZ, USA) and PD-L2 (1:200 dilution, mouse monoclonal, clone 176611; R&D Systems, Minneapolis, MN, USA). For PD-L1, staining was performed as previously described (16-18, 22-31). For PD-L2, 4- μm -thick sections were mounted on glass slides and stained using a B Bond-III autostainer (Leica Microsystems, Bannockburn, IL, USA). Briefly, sections were treated with proteinase K (Agilent/Dako, Carpinteria, CA, USA) for 5 min, and incubated with the antibody for 30 min. The automated staining set-up used a Bond Polymer Refine Detection system (Leica Microsystems) with a horseradish peroxidase-coupled polymer secondary antibody and 3,3'-diaminobenzidine (DAB) as the chromogen. The slides were visualized using DAB.

Carcinoma cells showing membranous staining for PD-L1 or PD-L2 were classified as positive, and the percentage of positive cells was calculated from the whole stained section. Sections with less than 1% tumor membrane staining were considered negative in this study. IHC images were evaluated independently by three investigators (KT, ST, and TJ) who were blinded to the patient clinical data. Disagreements were resolved by consensus.

Statistical analysis. Univariate and multivariate analyses of the relationship of PD-L1 and PD-L2 expression with CT features (convergence, surrounding GGO, air bronchogram, notching, pleural indentation, spiculation, and cavitation) were performed by logistic regression analysis with a backward elimination method. Associations between PD-L1/PD-L2 expression, the C/T ratio and clinicopathological factors were examined by Fisher's exact test. Associations between PD-L1/PD-L2 expression and SUVmax in preoperative ^{18}F -FDG PET/CT were evaluated using Student's *t*-test. All statistical analyses were performed using JMP Statistical Discovery software (version 11.0; SAS Institute, Cary, NC, USA). A value of $p < 0.05$ was considered statistically significant.

Results

Patient characteristics. Patient characteristics are shown in Table I. The median age of the 393 patients was 69 years (range=29-85 years); 196 (49.9%) were male and 201 (51.1%) had never smoked. The *EGFR* mutation status was available for 230 patients. Of these, 119 (51.7%) and 111 (48.3%) expressed wild-type and mutant *EGFR*, respectively.

PD-L1 and PD-L2 positivity was defined as positive membranous staining of $\geq 1\%$ of cancer cells on IHC staining of lung adenocarcinoma sections (Figure 1). Of the 393 patients, 132 (33.6%) and 266 (67.7%) were positive for PD-L1 and PD-L2 expression, respectively. PD-L1 and PD-L2 were co-expressed by 98 (24.9%) patients. Figure 2 shows representative images of the CT features investigated: convergence, surrounding GGO, air bronchogram, notching, pleural indentation, spiculation, and cavitation. These features were observed in 264

Table I. Patient clinicopathological characteristics (N=393).

Factors	Value
Age, years	
Median (range)	69 (29-85)
Gender, n	
Male	196
Female	197
Smoking status, n	
Never-smoker	201
Smoker	192
Radiological tumor diameter, cm	
Median (range)	2.1 (0.3-10.7)
Pathological stage, n	
IA	213
IB	73
IIA	33
IIB	28
IIIA	36
IIIB	5
IV	5
Pleural invasion, n ^a	
No	305
Yes	87
Lymphatic invasion, n	
No	337
Yes	56
Vascular invasion, n	
No	279
Yes	114
Histological subtype, n	
AAH/AIS/MIA	39
Lepidic-predominant	24
Papillary-predominant	290
Acinar-predominant	6
Micropapillary-predominant	1
Solid-predominant	26
Variant	7
EGFR, n ^a	
Wild-type	119
Mutant	111
PD-L1, n	
Negative	261
Positive	132
PD-L2, n	
Negative	127
Positive	266
PD-L1/PD-L2, n	
Co-expression	98
Other ^b	295

EGFR: Epidermal growth factor receptor, PD-L1: programmed cell death-ligand 1, PD-L2: programmed cell death-ligand 2, AAH: atypical adenomatous hyperplasia, AIS: adenocarcinoma *in situ*, MIA: minimally invasive adenocarcinoma. ^aCases for which data were available. ^bNo expression or single protein expression.

(67.2%), 189 (48.1%), 312 (79.4%), 142 (36.1%), 311 (79.1%), 178 (45.3%), and 73 (18.6%), respectively, of the 393 patients (Tables II and III).

Association between tumor PD-L2 expression and CT features. Table II shows the relationship between PD-L2 expression and CT features. Univariate analysis identified significant associations between PD-L2 expression and the presence of convergence, air bronchogram, and spiculation, and the absence of surrounding GGO. In multivariate analysis, the presence of air bronchogram and the absence of surrounding GGO were significantly associated with PD-L2 expression (Table II).

Table IV shows the PD-L2 expression status stratified by the C/T ratio. Positive PD-L2 expression was observed in 8.3%, 6.0%, 12.0%, and 73.7% of tumors with C/T ratios of 0, 0.1-0.25, 0.26-0.5, and ≥ 0.51 , respectively ($p=0.3188$).

Association between tumor PD-L2 expression and metabolic activity on ¹⁸F-FDG PET/CT. the relationship between tumor PD-L2 expression and metabolic characteristics of primary lung adenocarcinoma was evaluated in the 222 patients for whom ¹⁸F-FDG PET/CT data were available. The average SUV_{max} of the patients with positive PD-L2 expression was significantly higher than that of PD-L2-negative patients [6.33 (range=0-30.4) and 3.92 (range=0-14.9), respectively; $p=0.0009$] (Figure 3A).

Association between tumor PD-L1/PD-L2 co-expression and features on CT and ¹⁸F-FDG PET/CT. We next evaluated the relationship between radiological and metabolic features and PD-L1/PD-L2 co-expression in primary lung adenocarcinoma. Univariate analysis revealed that PD-L1/PD-L2 co-expression was significantly associated with the presence of convergence, notching, spiculation, and cavitation and the absence of surrounding GGO. In multivariate analysis, the presence of convergence and cavitation and the absence of surrounding GGO remained significantly associated with PD-L1/PD-L2 co-expression (Table III). Positive PD-L1/PD-L2 co-expression was observed in 3.1%, 3.1%, 6.1%, and 87.7% of tumors with C/T ratios of 0, 0.1-0.25, 0.26-0.5, and ≥ 0.51 , respectively ($p=0.0006$, Table V). With regard to ¹⁸F-FDG PET/CT, the SUVmax was significantly higher in patients with PD-L1/PD-L2 co-expression than all other patients ($p<0.0001$, Figure 3B).

Association between tumor PD-L1/PD-L2 expression and patient clinicopathological features. Finally, we examined the association between PD-L1 and/or PD-L2 expression and clinicopathological factors in our patient cohort (Table VI). PD-L2 expression was significantly higher in non-smokers than in smokers. Unlike PD-L1, however, PD-L2 expression was not significantly associated with EGFR status. Moreover, PD-L1 expression tended to be more significantly associated than PD-L2 expression with pathologically invasive features such as pleural invasion, vascular invasion, and histological subtype.

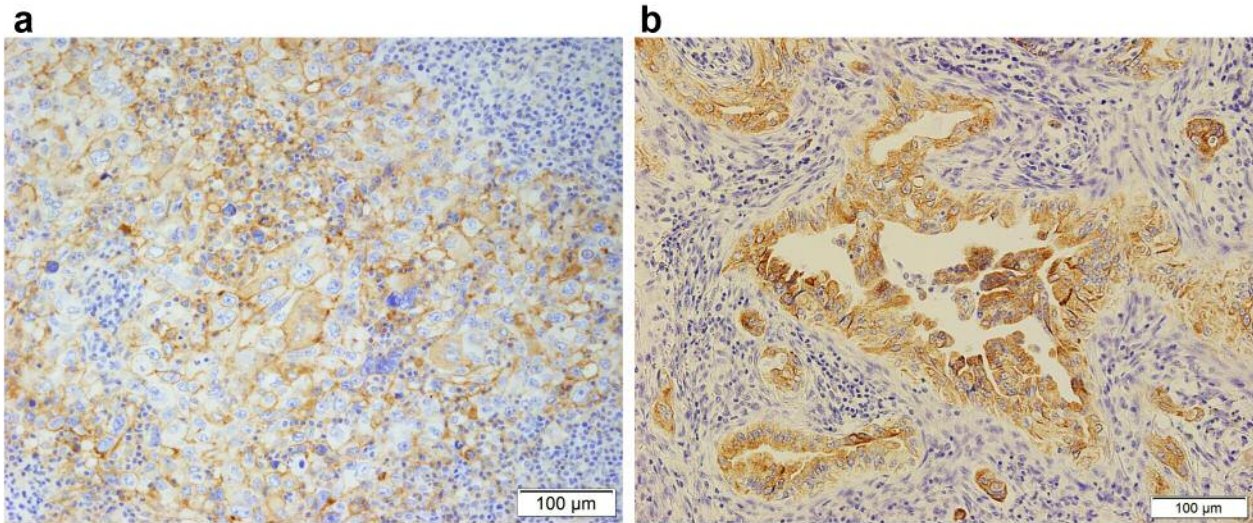


Figure 1. Representative images of positive membranous immunohistochemical staining for programmed cell death-ligand 1 (PD-L1) (A) and programmed cell death-ligand 2 (PD-L2) (B) in surgically resected specimens from patients with primary lung adenocarcinoma. Scale bar: 100 μ m.

Discussion

In this study, we examined the relationship between PD-L2 expression and radiological features of primary lung adenocarcinoma. We previously reported that PD-L1 expression was significantly associated with the absence of surrounding GGO and air bronchogram and the presence of convergence and cavitation in lung adenocarcinoma (16). In the present study, however, we found that the absence of surrounding GGO and the presence of air bronchogram were significantly associated with PD-L2 expression in the same patient cohort and tumor samples. Convergence is considered to reflect tumor fibrosis, whereas cavitation is thought to be associated with tumor necrosis, which is observed when rapid tumor growth exceeds the available blood supply (16). Therefore, convergence and cavitation, which were significantly associated with tumor expression of PD-L1 but not PD-L2, are thought to be associated with tumor malignancy in NSCLC. Air bronchogram is a radiological finding in which an air-filled bronchus is surrounded by fluid-filled airspaces; notably, it is not found in invasive adenocarcinoma where alveolar septa, bronchi, and vasculature are destroyed (16).

Air bronchogram on CT scans of lung cancer patients has been the subject of several reports and was shown to be a significant predictor of pathological N0 status (32-35). Moreover, in studies examining the associations between air bronchogram, PD-L1, and driver oncogenes in patients with lung cancer, PD-L1 expression was significantly associated with the absence of air bronchogram (16), whereas *EGFR* mutation was significantly associated with the presence of

air bronchogram (34, 35), and anaplastic lymphoma kinase rearrangement was unrelated to air bronchogram on CT (34). These findings suggest that the absence of air bronchogram is associated with malignancy and invasive features in lung cancer. Although a mechanistic link between air bronchogram and PD-L2 expression remains to be determined, our finding of a significant association between the presence of air bronchogram and PD-L2 expression suggests that PD-L2-positive lung adenocarcinoma may be less malignant and invasive than PD-L1-positive tumor. Moreover, we found a significant association between the C/T ratio and PD-L1 expression, as previously reported (23), but not PD-L2 expression. The predominance of GGO suggests noninvasive of the tumor, as the Japan Clinical Oncology Group 0201 study defined the tumor with a C/T ratio ≤ 0.25 on thin-section CT as radiologically noninvasive lung adenocarcinoma, since such tumors correspond well to pathologically noninvasive adenocarcinomas (36). Actually, in this study, PD-L1 expression tended to be more associated with pathologically invasive features than PD-L2 expression. From these findings, PD-L2 expression may not be as closely associated with tumor invasiveness as PD-L1 expression in primary lung adenocarcinoma.

We evaluated the metabolic characteristics of primary lung adenocarcinoma in 222 patients for whom preoperative ^{18}F -FDG PET/CT data were available, and found that SUVmax was significantly higher in patients with PD-L2-positive compared with those with PD-L2-negative tumors. We previously showed a similar association for metabolic activity and PD-L1-positive lung adenocarcinoma (17, 18). A

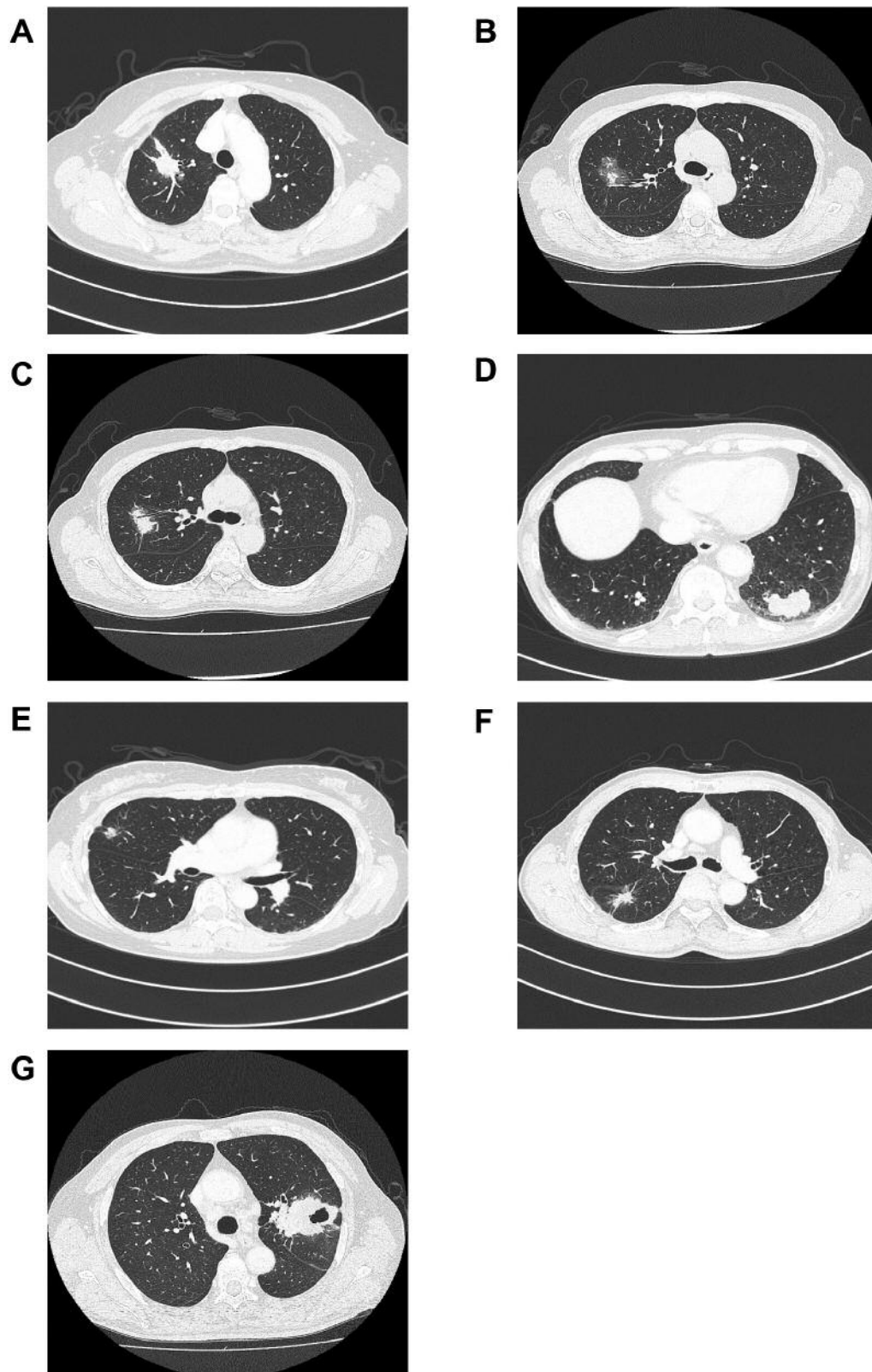


Figure 2. Representative computed tomographic images of lung adenocarcinoma patients showing typical features of Convergence (A), surrounding ground glass opacity (B), air bronchogram (C), notching (D), pleural indentation (E), spiculation (F), and cavitation (G).

Table II. Univariate and multivariate analysis of the associations between programmed cell death-ligand 2 (PD-L2) expression and computed tomographic (CT) features.

CT feature		N (%)	PD-L2, N (%)		Univariate analysis			Multivariate analysis		
			Negative	Positive	OR	95% CI	p-Value	OR	95% CI	p-Value
Convergence	–	129 (32.8)	54 (42.5)	75 (28.2)	1.00					
	+	264 (67.2)	73 (57.5)	191 (71.8)	1.88	1.21-2.93	0.0051			
Surrounding GGO	–	204 (51.9)	54 (42.5)	150 (56.4)	1.00			1.00		
	+	189 (48.1)	73 (57.5)	116 (43.6)	0.57	0.37-0.88	0.0100	0.52	0.33-0.80	0.0032
Air bronchogram	–	81 (20.6)	34 (26.8)	47 (17.7)	1.00			1.00		
	+	312 (79.4)	93 (73.2)	219 (82.3)	1.70	1.03-2.81	0.0400	1.95	1.16-3.29	0.0121
Notching	–	251 (63.9)	86 (67.7)	165 (62.0)	1.00					
	+	142 (36.1)	41 (32.3)	101 (38.0)	1.28	0.82-2.02	0.2702			
Pleural indentation	–	82 (20.9)	32 (25.2)	50 (18.8)	1.00					
	+	311 (79.1)	95 (74.8)	216 (81.2)	1.46	0.87-2.40	0.1489			
Spiculation	–	215 (54.7)	82 (64.6)	133 (50.0)	1.00					
	+	178 (45.3)	45 (35.4)	133 (50.0)	1.82	1.18-2.83	0.0063			
Cavitation	–	320 (81.4)	110 (86.6)	210 (79.0)	1.00					
	+	73 (18.6)	17 (13.4)	56 (21.0)	1.73	0.97-3.19	0.0615			

GGO: Ground glass opacity, OR: odds ratio, CI: confidence interval.

Table III. Univariate and multivariate analysis of the relationship between programmed cell death-ligand 1 (PD-L1)/PD-L2 co-expression and computed tomographic features.

CT feature		N (%)	PD-L1/PD-L2, N (%)		Univariate analysis			Multivariate analysis		
			Other ^a	Co-expression	OR	95% CI	p-Value	OR	95% CI	p-Value
Convergence	–	129 (32.8)	117 (39.7)	12 (12.2)	1.00			1.00		
	+	264 (67.2)	178 (60.3)	86 (87.8)	4.71	2.55-9.42	<0.0001	2.39	1.19-5.07	0.0136
Surrounding GGO	–	204 (51.9)	128 (43.4)	76 (77.6)	1.00			1.00		
	+	189 (48.1)	167 (56.6)	22 (22.4)	0.22	0.13-0.37	<0.0001	0.34	0.19-0.59	0.0001
Air bronchogram	–	81 (20.6)	59 (20.0)	22 (22.5)	1.00					
	+	312 (79.4)	236 (80.0)	76 (77.6)	0.86	0.50-1.53	0.6061			
Notching	–	251 (63.9)	202 (68.5)	49 (50.0)	1.00					
	+	142 (36.1)	93 (31.5)	49 (50.0)	2.17	1.36-3.47	0.0011			
Pleural indentation	–	82 (20.9)	68 (23.1)	14 (14.3)	1.00					
	+	311 (79.1)	227 (76.9)	84 (85.7)	1.80	0.98-3.48	0.0563			
Spiculation	–	215 (54.7)	178 (60.3)	37 (37.8)	1.00					
	+	178 (45.3)	117 (39.7)	61 (62.2)	2.51	1.57-4.04	<0.0001			
Cavitation	–	320 (81.4)	254 (86.1)	66 (67.4)	1.00			1.00		
	+	73 (18.6)	41 (13.9)	32 (32.6)	3.00	1.75-5.13	<0.0001	2.04	1.15-3.60	0.0145

GGO: Ground glass opacity, OR: odds ratio, CI: confidence interval. ^aNo expression or single protein expression.

previous study identified significant differences in FDG uptake across histological subtypes and differentiation groups of NSCLC, which paralleled differences in the Ki-67 index (37). Therefore, PD-L2-positive lung adenocarcinoma may harbor more proliferative traits than PD-L2-negative tumors. We recently found that PD-L1 positivity was an independent predictor of shorter disease-free survival, and that both PD-L1 and PD-L2 were predictors of shorter overall survival in 433

patients with primary lung adenocarcinoma (unpublished observations). Thus, PD-L2-positive lung adenocarcinoma was not as pathologically or radiologically invasive as PD-L1-positive lung adenocarcinoma, but PD-L2 expression appears to be related to tumor malignancy in lung adenocarcinoma.

Some recent studies have suggested that PD-L2 expression may be related to the response to PD-1-targeted immunotherapy. For example, Yearley *et al.* showed that PD-L2

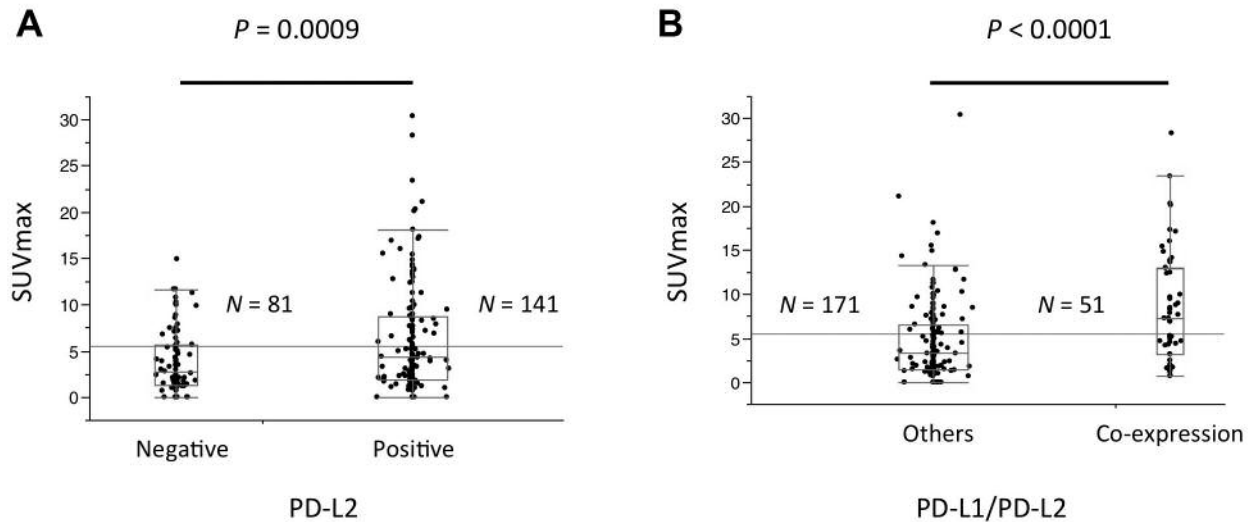


Figure 3. Maximum standardized uptake value (SUV_{max}) stratified by programmed cell death-ligand 1 (PD-L1) and PD-L2 expression. A: SUV_{max} was significantly higher in patients with PD-L2-positive compared with PD-L2-negative tumors ($p=0.0009$). B: SUV_{max} was significantly higher in patients with tumors co-expressing PD-L1 and PD-L2 compared to those expressing no or single protein ($p<0.0001$).

expression was a significant predictor of progression-free and overall survival in patients with squamous cell carcinoma of the head and neck treated with the PD-1 inhibitor pembrolizumab (38). Although a similar relationship has not yet been established for PD-L2 expression, our data are likely to help clarify the clinical significance of PD-L2 expression in NSCLC.

There are several limitations to this study. Firstly, it was a single institutional retrospective study and not a trial-based correlative study; thus, the possibility of bias cannot be excluded. Validation cohort studies should be conducted to confirm our results. However, to our knowledge this is the first report to demonstrate the relationship between PD-L2 expression and features of imaging modalities in resected lung adenocarcinomas. Secondly, we conducted PD-L1 IHC using only one antibody. Several recent studies have shown that positive PD-L1 expression is detected at a lower rate with the SP142 antibody used here than with other antibodies, such as 28-8, 22C3, and SP263 (39-42). We should conduct the same analysis using these and other available PD-L1-specific antibodies. Thirdly, there are no definitive guidelines for antibody use or quantification of PD-L2 expression in NSCLC, and no comparative data are available for different PD-L2 antibodies. We used clone 176611 and set the cut-off value for positivity as membranous staining 1% of tumor cells in this study. However, this antibody has not been evaluated in a clinical setting. Therefore, PD-L2 expression should be further evaluated using other antibodies and cut-off values. The fourth limitation is the lack of analysis of only patients with advanced cancer such as stage IV, which was due to our focus being on resected lung adenocarcinoma. Further

Table IV. Frequency of programmed cell death-ligand 2 (PD-L2) expression according to the consolidation/tumor (C/T) ratio.

C/T ratio	PD-L2, N (%)		p-Value
	Negative	Positive	
0 (pure GGO)	17 (13.4)	22 (8.3)	0.3188
0.10-0.25	5 (3.9)	16 (6.0)	
0.26-0.50	18 (14.2)	32 (12.0)	
≥ 0.51	87 (68.5)	196 (73.7)	

GGO: Ground glass opacity.

Table V. Frequency of programmed cell death-ligand 1 (PD-L1)/PD-L2 co-expression according to the consolidation/tumor (C/T) ratio.

C/T ratio	PD-L1/PD-L2, N (%)		p-Value
	Other ^a	Co-expression	
0 (pure GGO)	36 (12.2)	3 (3.1)	0.0006
0.10-0.25	18 (6.1)	3 (3.1)	
0.26-0.50	44 (14.9)	6 (6.1)	
≥ 0.51	197 (66.8)	86 (87.7)	

GGO: Ground glass opacity. ^aNo expression or single protein expression.

studies should include analysis of PD-L2 expression only in advanced lung adenocarcinoma specimens to validate our results here with resected tumors.

Table VI. Association of programmed cell death-ligand 1 (PD-L1) and PD-L2 expression with clinicopathological factors.

Factor	PD-L1, N (%)				PD-L2, N (%)			PD-L1/PD-L2, N (%)		
	N (%)	Negative	Positive	<i>p</i> -Value	Negative	Positive	<i>p</i> -Value	Co-expression	Other ^b	<i>p</i> -Value
Age										
<70 Years	207 (52.7)	136 (52.1)	71 (53.8)	0.8307	65 (51.2)	142 (53.4)	0.7460	51 (52.0)	156 (52.9)	0.9074
≥70 Years	186 (47.3)	125 (47.9)	61 (46.2)		62 (48.8)	124 (46.6)		47 (48.0)	139 (47.1)	
Gender										
Male	196 (49.9)	117 (44.8)	79 (59.9)	0.0055	74 (58.3)	122 (45.9)	0.0237	51 (52.0)	145 (49.1)	0.6423
Female	197 (50.1)	144 (55.2)	53 (40.1)		53 (41.7)	144 (54.1)		47 (48.0)	150 (50.9)	
Smoking status										
Never-smoker	201 (51.2)	149 (57.1)	52 (39.4)	0.0010	52 (40.9)	149 (56.0)	0.0069	48 (49.0)	153 (51.9)	0.6422
Smoker	192 (48.8)	112 (42.9)	80 (60.6)		75 (59.1)	117 (44.0)		50 (51.0)	142 (48.1)	
Radiologic tumor diameter										
≤2.0 cm	180 (45.8)	127 (48.7)	53 (40.2)	0.1333	79 (62.2)	101 (38.0)	<0.0001	33 (33.7)	147 (49.8)	0.0069
>2.0 cm	213 (54.2)	134 (51.3)	79 (59.8)		48 (37.8)	165 (62.0)		65 (66.3)	148 (50.2)	
Pathological stage										
I	286 (72.8)	204 (78.2)	82 (62.1)	0.0011	103 (81.1)	183 (68.8)	0.0109	58 (59.2)	228 (77.3)	0.0010
≥II	107 (27.2)	57 (21.8)	50 (37.9)		24 (18.9)	83 (31.2)		40 (40.8)	67 (22.7)	
Pleural invasion ^a										
No	305 (77.8)	215 (82.7)	90 (68.2)	0.0019	104 (82.5)	201 (75.6)	0.1520	67 (68.4)	238 (81.0)	0.0116
Yes	87 (22.2)	45 (17.3)	42 (31.8)		22 (17.5)	65 (24.4)		31 (31.6)	56 (19.0)	
Lymphatic invasion										
No	337 (85.8)	223 (85.4)	114 (86.4)	0.8792	116 (91.3)	221 (83.1)	0.0308	81 (82.7)	256 (86.8)	0.3195
Yes	56 (14.2)	38 (14.6)	18 (13.6)		11 (8.7)	45 (16.9)		17 (17.3)	39 (13.2)	
Vascular invasion										
No	279 (71.0)	206 (78.9)	73 (55.3)	<0.0001	96 (75.6)	183 (68.8)	0.1914	55 (56.1)	224 (75.9)	0.0003
Yes	114 (29.0)	55 (21.1)	59 (44.7)		31 (24.4)	83 (31.2)		43 (43.9)	71 (24.1)	
Histological subtype										
AAH/AIS/MIA/lepidic	63 (16.0)	57 (21.8)	6 (4.6)	<0.0001	32 (25.2)	31 (11.7)	0.0011	5 (5.1)	58 (19.7)	0.0004
Other	330 (84.0)	204 (78.2)	126 (95.4)		95 (74.8)	235 (88.3)		93 (94.9)	237 (80.3)	
<i>EGFR</i> ^a										
Wild-type	119 (51.7)	79 (46.5)	40 (66.7)	0.0103	46 (54.8)	73 (50.0)	0.4969	29 (65.9)	90 (48.4)	0.0441
Mutant	111 (48.3)	91 (53.5)	20 (33.3)		38 (45.2)	73 (50.0)		15 (34.1)	96 (51.6)	

EGFR: Epidermal growth factor receptor, AAH: atypical adenomatous hyperplasia, AIS: adenocarcinoma *in situ*, MIA: minimally-invasive adenocarcinoma. ^aCases for which data were available. ^bNo expression or single protein expression.

To the best of our knowledge, this is the first report of a relationship between PD-L2 expression and radiological and metabolic imaging features in lung adenocarcinoma. PD-L2-positive lung adenocarcinoma was less radiologically malignant and invasive compared to PD-L1-positive tumors. Further studies are warranted to provide more detailed information about the clinical features of PD-L2-positive NSCLC, including squamous cell carcinoma.

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Conflicts of Interest

The Authors have declared no conflicts of interest in regard to this study.

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