Clinical Significance of PD-L1 Protein Expression in Surgically Resected Primary Lung Adenocarcinoma

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ORIGINAL ARTICLE

Clinical Significance of PD-L1 Protein Expression in Surgically Resected Primary Lung Adenocarcinoma

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ABSTRACT

Introduction: The clinicopathological features of carcinomas expressing programmed death ligand 1 (PD-L1) and their associations with common driver mutations, such as mutations in the *EGFR* gene, in lung adenocarcinoma are not clearly understood. Here, we examined PD-L1 protein expression in surgically resected primary lung adenocarcinoma and the association of PD-L1 protein expression with clinicopathological features, *EGFR* mutation status, and patient outcomes.

Methods: The expression of PD-L1 protein in 417 surgically resected primary lung adenocarcinomas was evaluated by immunohistochemical analysis. The cutoff value for defining PD-L1 positivity was determined according to the histogram of proportions of PD-L1–positive cancer cells.

Results: Samples from 85 patients (20.4%) and 144 patients (34.5%) were positive for PD-L1 protein expression according to 5% and 1% PD-L1 cutoff values, respectively. Fisher's exact tests showed that PD-L1 positivity was significantly associated with male sex, smoking, higher tumor grade, advanced T status, advanced N status, advanced stage, the presence of pleural and vessel invasions, micropapillary or solid predominant histological subtypes, and wild-type *EGFR*. Univariate and multivariate survival analyses revealed that patients with PD-L1 positivity had poorer prognoses than those without PD-L1 protein expression at the 1% cutoff value (disease-free survival $p < 0.0001$, overall survival $p < 0.0001$).

Conclusions: PD-L1 protein expression was significantly higher in smoking-associated adenocarcinoma and in *EGFR* mutation–negative adenocarcinoma. PD-L1 protein expression was associated with poor survival in patients with lung adenocarcinoma. The PD-L1/programmed cell death 1 pathway may contribute to the progression of smoking-associated tumors in lung adenocarcinoma.

Keywords: Programmed death ligand 1; Immunohistochemistry; EGFR; Lung adenocarcinoma

Introduction

Lung cancer is the leading cause of cancer-related death worldwide.[1] Recently, molecular targeted therapy has greatly improved the clinical course for patients with NSCLC having common driver mutations, such as mutations in the *EGFR* and anaplastic lymphoma receptor tyrosine kinase gene (*ALK*).[2] Despite advances in therapies, the prognosis of patients with NSCLC who do not have driver oncogene mutations remains poor.[3] Immune checkpoint inhibitors have recently been shown to improve prognoses in multiple types of cancers.[4,5] The interaction of programmed death ligand 1 (PD-L1)

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and programmed cell death 1 (PD-1) acts as an immune checkpoint signal, suppressing the effector functions of activated T cells. PD-1 is a receptor expressed on the surface of T cells that regulates the activation of T cells; PD-L1, the ligand of PD-1, is expressed in many cancers, including NSCLC, and is believed to promote evasion of the antitumor immune response at the tumor site.[4,6,7] Recently, antibodies that target PD-L1 or PD-1 have been developed for anticancer therapy in various types of cancers[8,9] and have been shown to be efficacious in the treatment of NSCLC.[10–12] However, whether PD-L1 protein expression in tumor cells predicts the efficacy of anti–PD-L1/PD-1 therapy is unclear.[7] Moreover, the clinicopathological features and prognostic impact of PD-L1 protein expression have not been elucidated. Some recent studies have demonstrated that high expression of PD-L1 protein is associated with the presence of *EGFR* mutations and that mutated *EGFR* up-regulates PD-L1 by activating downstream signaling pathways in NSCLC.[13–15] However, some other studies have shown opposite results; thus, the association between *EGFR* mutation status and PD-L1 protein expression is still controversial.[16–18] Accordingly, elucidation of the clinical significance of PD-L1 protein expression in lung cancer may provide insights into effective strategies for PD-1/PD-L1–inhibitory treatment.

Therefore, in this study, we examined PD-L1 protein expression in surgically resected primary lung adenocarcinoma and investigated the associations of PD-L1 protein expression with clinicopathological features, common *EGFR* mutations, and patient outcomes. Because the PD-L1 cutoff level for immunohistochemical (IHC) analysis has not been established, we constructed a histogram based on the proportions of PD-L1–positive cells and conducted further analyses using both 5% and 1% cutoff values.

Materials and Methods

Patients and Samples

We retrospectively examined patients who underwent surgical resection of their primary lung adenocarcinoma between January 2003 and December 2012 at the Department of Surgery and Science, Graduate School of Medical Sciences, Kyushu University. Thirteen patients who had received neoadjuvant therapy and five patients with stage IV disease were excluded because of a previous report showing inconsistencies in PD-L1 expression in tumor cells before and after neoadjuvant chemotherapy.[19] Finally, 417 paraffin-embedded specimens were available and retrieved from the registry of the Department of Anatomic Pathology, Graduate School of Medical Sciences, Kyushu University. Clinicopathological features, including the following, were examined: age at operation; sex; smoking history; tumor differentiation; pathological tumor, node, and metastasis stage (seventh edition of the American Joint Committee on Cancer lung cancer staging system); pleural or lymphovascular invasion; histological subtype (2015 WHO classification); surgical procedure; and *EGFR* mutation status. *EGFR* status had been determined in tumor tissue using the peptide nucleic acid–locked nucleic acid polymerase chain reaction clamp method (Mitsubishi Chemical Medience, Tokyo, Japan) in 235 specimens.[20] Briefly, systemic dissection of hilar and mediastinal lymph nodes was performed at the same time as pulmonary lobectomy. Selected lymph node sampling was performed during sublobar resection. Perioperative therapy, which was selected by the physician, was performed within the clinical practice guidelines for lung cancer in Japan. An EGFR tyrosine kinase inhibitor was used after recurrence in patients with tumors harboring *EGFR*-sensitive mutations. After surgical resection, routine examinations, including a physical examination, blood tests (including serum tumor markers), and chest radiography, were performed at 3-month intervals for the first 3 years and at 6-month intervals thereafter. Computed tomography was performed twice a year for the first 3 years and then at least annually thereafter. Adjuvant chemotherapy was administered in some patients when the treatment was required. The eligibility criteria for patients receiving adjuvant chemotherapy were as follows: (1) had pathological stage (p-stage) IB to IIIA disease, (2) was less than 76 years of age, (3) had a performance status of 0 or 1, and (4) provided written informed consent. The regimen for p-stage IB disease was uracil-tegafur, and that for p-stage IIA to IIIA disease was a platinum-based combined regimen in principle. Clinical information and follow-up data were obtained from medical records. This study was approved by our institutional review board (Kyushu University, IRB No. 27-435).

IHC Analysis

IHC analysis was performed in 417 surgically resected primary lung adenocarcinomas using formalin-fixed tissue sections. Sections (4-μm-thick) were cut, dewaxed with xylene, and rehydrated through a graded series of ethanol. After inhibition of endogenous peroxidase activity for 30 minutes with 3% hydrogen peroxide in methanol, the sections were pretreated with Target Retrieval Solution (Dako, Glostrup, Denmark) in a decloaking chamber at 110°C for 15 minutes and then incubated with monoclonal antibodies at 4°C overnight. The immune complex was detected with a DAKO EnVision Detection System (Dako). The sections were finally reacted in 3,3′-diaminobenzidine, counterstained with hematoxylin, and mounted.

The primary antibody was an antihuman PD-L1 rabbit monoclonal antibody (clone SP142, dilution 1:100 [Spring Bioscience, Ventana, Tucson, AZ). Carcinoma cells showing membranous staining for PD-L1 were evaluated as positive cells. The proportion of PD-L1–positive cells was estimated as the percentage of total carcinoma cells in whole sections independently by three investigators (K.T., M.K., and G.T.). If the independent assessments did not agree, the slides were reviewed by all three investigators together to achieve consensus. The consensus judgments were adopted as the final results. The percentages of PD-L1–positive carcinoma cells in all cases are shown in Figure 1*F*, and we first set the cutoff values at both 5% and 1%. Sections from human placentas for PD-L1 were used as positive controls (Fig. 1*E*).

Statistical Analysis

Statistical analyses for categorical factors were performed using Fisher's exact tests, and univariate and multivariate analyses of the relationships between PD-L1 protein expression and other patient characteristics were performed by logistic regression analysis with the backward elimination method. Disease-free survival (DFS) was considered to be the period between surgery and the date of the recurrence, and overall survival (OS) was considered to be the period between surgery and the date of the last follow-up or death. These rates were estimated using the Kaplan-Meier method with the log-rank test. Cox proportional hazards regression analysis was performed to estimate the hazard ratios for positive risk factors with the backward elimination method. All statistical analyses were performed using JMP Statistical Discovery Software, version 11.0 (SAS Institute, Cary, NC). All results were considered statistically significant if p was less than 0.05.

Results

Association between PD-L1 Protein Expression and Clinicopathological Characteristics in Patients with Primary Lung Adenocarcinoma

A total of 417 patients with primary lung adenocarcinoma who underwent surgical resection were included in the present study (Supplementary Table 1); 205 patients (49.2%) were male, and 218 (52.3%) had never smoked. The median age of all patients was 69 years (range 29–85 years). *EGFR* status was available for 235 patients; of these, 123 (52.3%) had wild-type *EGFR* and 112 (47.7%) had mutant *EGFR*.

IHC staining for PD-L1 was detected at the membrane of carcinoma cells (Fig. 1*B–D*). The associations between PD-L1 protein expression and patients' clinicopathological characteristics are described in Table 1. Eighty-five patients (20.4%) were positive for PD-L1 when the cutoff value was set at 5%, whereas 144 patients (34.5%) were positive for PD-L1 at the 1% cutoff value. PD-L1 protein expression was higher in men than in women, in smokers than in never-smokers, and in patients without *EGFR* mutations than in those with *EGFR* mutations at both cutoff values. Additionally, PD-L1 protein expression was higher in patients with more advanced-stage (including tumor and node stage) cancer, in tumors with higher pathological grades (micropapillary or solid predominant), and in tumors with pleural or vessel invasion. These associations were also observed at both the 5% and 1% cutoff values. We further examined the association between PD-L1 protein expression and *EGFR* mutation site; however, no significant association was found (Supplementary Table 2). Multivariate analysis was performed in two ways: (1) adjustment for all factors and (2) adjustment by all factors except *EGFR* status because the number of available cases was small. This analysis revealed that smoking, higher pathological grade (micropapillary or solid predominant), and wild-type *EGFR* were independent predictors of PD-L1 positivity (Supplementary Table 3).

Univariate Survival Analysis for Surgically Resected Primary Lung Adenocarcinoma according to PD-L1 Protein Expression

Next, we assessed the associations between PD-L1 protein expression and patient postoperative survival at both PD-L1 cutoff values. Survival analyses by the Kaplan-Meier method showed that patients with PD-L1 positivity had significantly shorter DFS and shorter OS after surgery than did patients without PD-L1 protein expression at both cutoff values (log-rank test values of $p = 0.0010$ and $p = 0.0049$, respectively, at the 5% cutoff value [Fig. 2*A* and *B*] and $p < 0.0001$ and $p < 0.0001$, respectively, at the 1% cutoff value [Fig. 2*C* and *D*]).

To determine the preferable PD-L1 cutoff level for prognostic analyses, we then conducted forest plot analyses for both cutoff levels, assessing the DFS and OS of each subgroup (Fig. 3*A* and *B*). These forest plot analyses revealed that the 1% cutoff value provided a more sensitive value for prediction of postoperative prognosis in terms of both PFS and OS in each subgroup.

Multivariate Survival Analysis for Surgically Resected Primary Lung Adenocarcinoma according to PD-L1 Protein Expression

On the basis of the aforementioned results, we adopted the 1% cutoff value for further survival

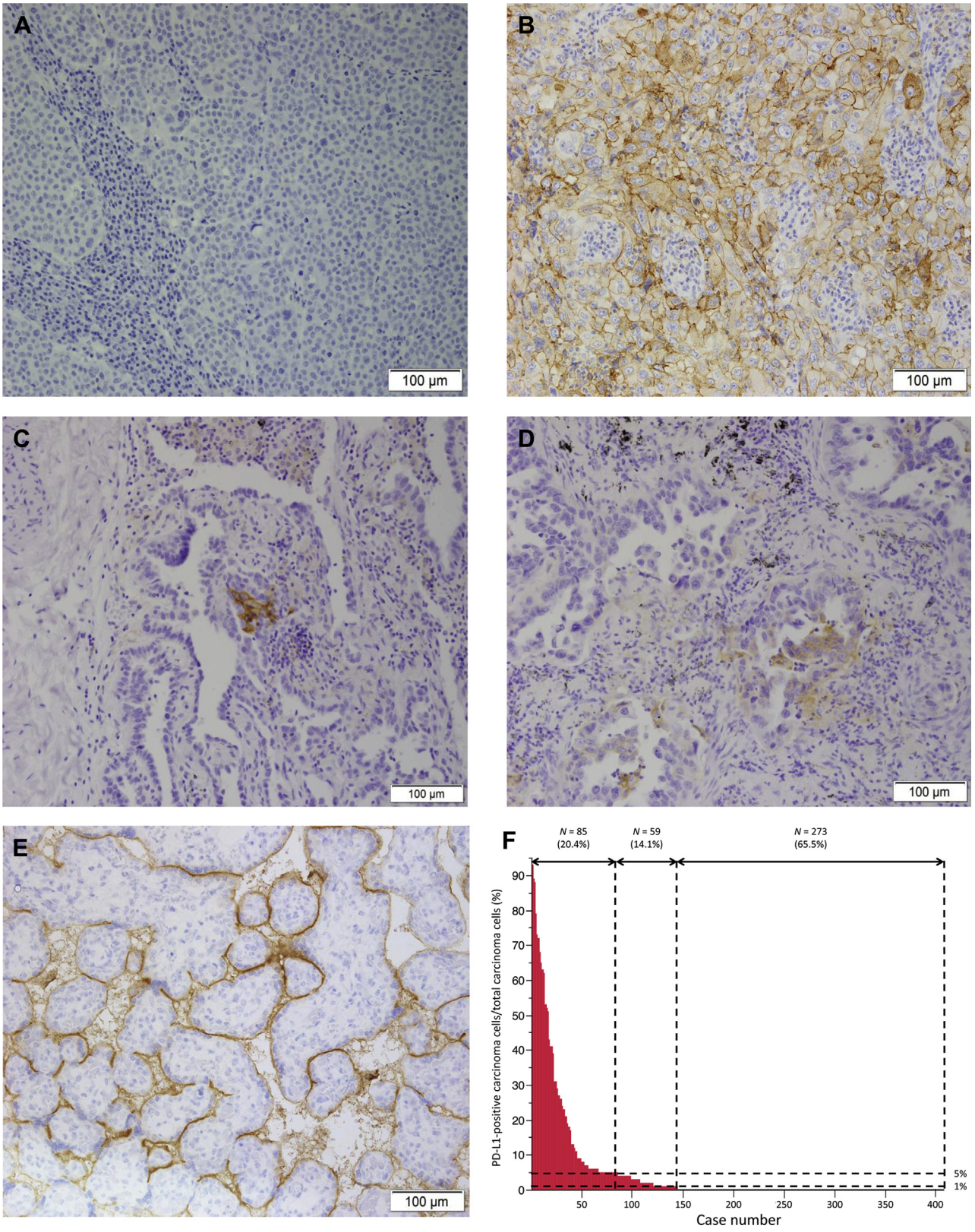

Figure 1. Immunohistochemical staining of programmed death ligand 1 (PD-L1) in patients with primary lung adenocarcinoma. (*A*) Negative staining for PD-L1. (*B*) Positive membrane staining for PD-L1. (*C*) Representative image of a PD-L1–positive case with the proportion closer to the 1% cutoff. (*D*) Representative image of a PD-L1–positive case with the proportion closer to the 5% cutoff. (*E*) Positive control of human placenta tissue. (*F*) Histogram of the percentages of PD-L1–positive carcinoma cells in all cases.

Table 1. Association between PD-L1 Protein Expression and Clinicopathological Factors in All Patients

		5% Cutoff			1% Cutoff		
		PD-L1, n (%)			PD-L1, n (%)		
Factors	n	Negative	Positive	*p* Value	Negative	Positive	*p* Value
Age, y							
<70	222	177 (53.3)	45 (52.9)	1	143 (52.4)	79 (54.9)	0.68
≥70	195	155 (46.7)	40 (47.1)		130 (47.6)	65 (45.1)	
Sex							
Male	205	148 (44.6)	57 (67.1)	0.0002	121 (44.3)	84 (58.3)	0.0074
Female	212	184 (55.4)	28 (32.9)		152 (55.7)	60 (41.7)	
Smoking status							
Never-smoker	218	196 (59.0)	22 (25.9)	<0.0001	160 (58.6)	58 (40.3)	0.0004
Smoker	199	136 (41.0)	63 (74.1)		113 (41.4)	86 (59.7)	
Grade							
G1	202	193 (58.1)	9 (10.6)	<0.0001	170 (62.3)	32 (22.2)	<0.0001
≥G2	215	139 (41.9)	76 (89.4)		103 (37.7)	112 (77.8)	
T							
T1	247	207 (62.3)	40 (47.1)	0.0132	177 (64.8)	70 (48.6)	0.0016
≥T2	170	125 (37.7)	45 (52.9)		96 (35.2)	74 (51.4)	
N							
N0	337	278 (83.7)	59 (69.4)	0.005	234 (85.7)	103 (71.5)	0.0006
≥N1	80	54 (16.3)	26 (30.6)		39 (14.3)	41 (28.5)	
Stage							
I	305	255 (76.8)	50 (58.8)	0.0015	216 (79.1)	89 (61.8)	0.0002
≥II (II/III)	112 (63/49)	77 (23.2)	35 (41.2)		57 (20.9)	55 (38.2)	
pl							
Absent	323	271 (81.6)	52 (61.2)	0.0001	224 (82.1)	99 (68.8)	0.003
Present	94	61 (18.4)	33 (38.8)		49 (17.9)	45 (31.2)	
ly							
Absent	357	283 (85.2)	74 (87.1)	0.7324	233 (85.3)	124 (86.1)	0.8842
Present	60	49 (14.8)	11 (12.9)		40 (14.7)	20 (13.9)	
v							
Absent	300	261 (78.6)	39 (45.9)	<0.0001	217 (79.5)	83 (57.6)	<0.0001
Present	117	71 (21.4)	46 (54.1)		56 (20.5)	61 (42.4)	
Histological subtype							
Micropapillary/solid	27	6 (1.8)	21 (24.7)	<0.0001	4 (1.5)	23 (16.0)	<0.0001
Others	390	326 (98.2)	64 (75.3)		269 (98.5)	121 (84.0)	
Surgical procedure							
≥Lobectomy	317	251 (75.6)	66 (77.6)	0.7765	205 (75.1)	112 (77.8)	0.6296
Sublobar resection	100	81 (24.4)	19 (22.4)		68 (24.9)	32 (22.2)	
EGFR[a]							
Wild-type	123	91 (46.7)	32 (80.0)	<0.0001	79 (46.2)	44 (68.8)	0.0021
Mutant	112	104 (53.3)	8 (20.0)		92 (53.8)	20 (31.2)	

[a]Cases for which data were available.
PD-L1, programmed death-ligand 1; pl, pleural invasion; ly, lymphatic invasion; v, vascular invasion.

analyses. Cox proportional hazards regression models showed that PD-L1 positivity, age 70 years or older, male sex, and smoking were related to shorter DFS and OS (PD-L1–positive versus PD-L1–negative: hazard ratio [HR] = 1.99, $p < 0.0001$, and HR = 2.51, $p < 0.0001$, respectively; Table 2). Advanced stage and histological invasiveness were also associated with poor prognoses (p-stage II–III versus I DFS: HR = 5.28, $p < 0.0001$, and OS: HR = 4.23, $p < 0.0001$). With regard to driver oncogenes of lung adenocarcinoma, tumors with *EGFR* mutations showed better DFS and OS. In the multivariate analysis, age, stage, and lymphatic invasion remained predictors of both DFS and OS, and PD-L1 positivity remained a predictor of OS but not DFS (see Table 2).

Discussion

In the present article, we demonstrated that PD-L1 protein was elevated in 34.5% of patients with lung adenocarcinoma who underwent surgical resection. PD-L1 protein expression was significantly higher in

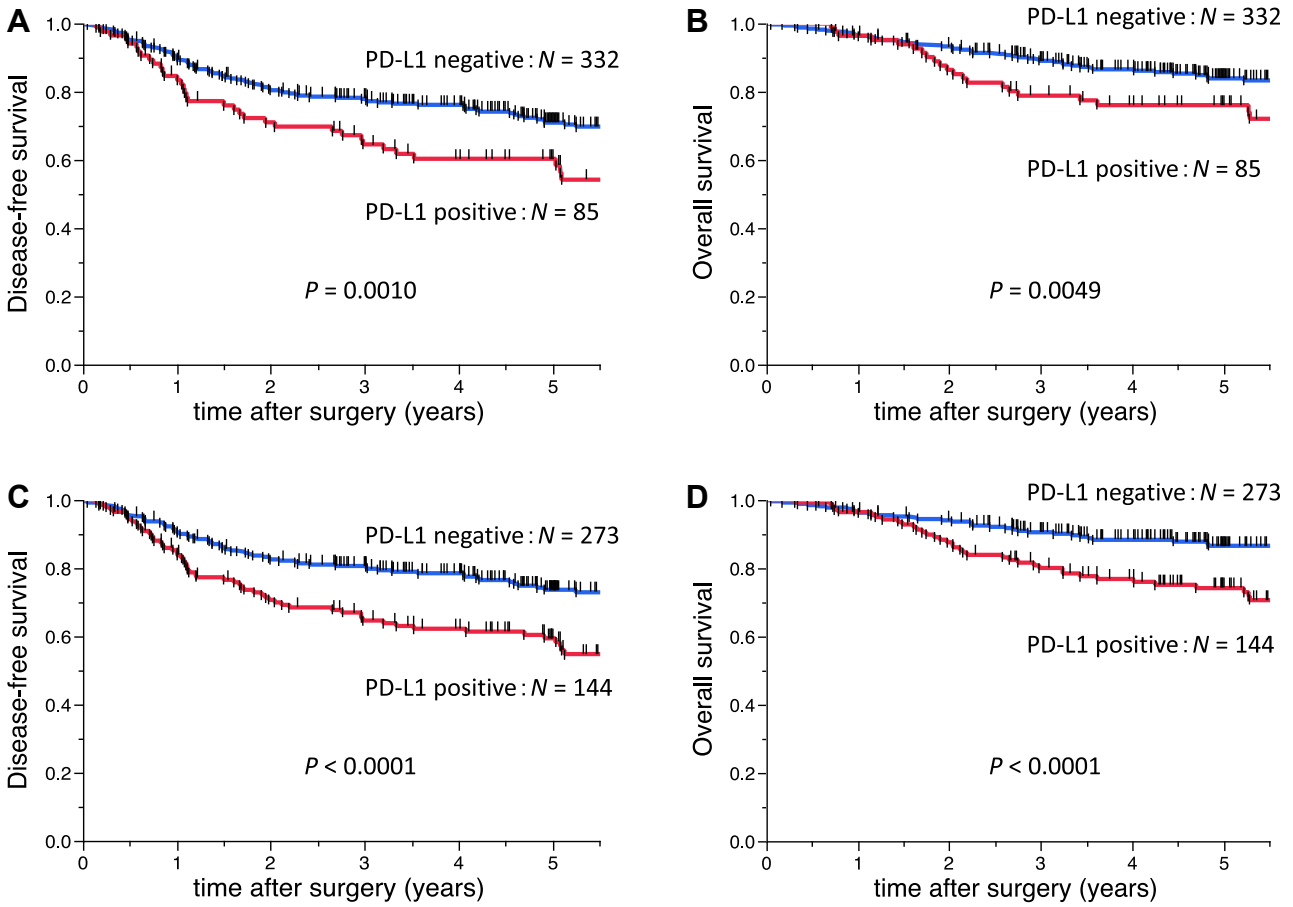

Figure 2. Kaplan-Meier curves showing survival of patients with primary lung adenocarcinoma according to programmed death ligand 1 (PD-L1) expression. (*A*) Disease-free survival and (*B*) overall survival in all patients according to PD-L1 protein expression status determined by the 5% cutoff value. (*C*) Disease-free survival and (*D*) overall survival in all patients according to PD-L1 protein expression status, as determined by the 1% cutoff value.

male patients, smokers, and patients without *EGFR* mutations. PD-L1 protein expression was also related to more advanced stage and higher pathological grade. Multivariate analysis revealed that higher pathological grade (micropapillary or solid predominant) and wild-type *EGFR* were independent predictors of PD-L1 positivity. On the other hand, smoking was not shown to be an independent predictor of PD-L1 positivity in this analysis, possibly because the number of available cases in the statistical model was small; the number of cases available for both *EGFR* status and smoking status was 40 in the analysis with a 5% cutoff value and 64 in the analysis with a 1% cutoff value. Multivariate analysis adjusted by all factors except *EGFR* status revealed that smoking was an independent predictor of PD-L1 positivity. Recently, some studies have shown that high PD-L1 protein expression is associated with certain clinicopathological features in NSCLC, including *EGFR* mutations.[15–18,21–26] Azuma et al. lately reported that PD-L1 protein expression is higher in women than in men, in never-smokers than in smokers, and in patients with *EGFR* mutations than in patients without *EGFR* mutations,[15] and D'Incecco et al. recently showed that PD-L1 positivity was significantly associated with the presence of *EGFR* mutations[26]; these data conflicted with the results of our study. Other studies have found no significant correlations between PD-L1 protein expression and *EGFR* mutation status.[16–18] One possible reason for this discordance may be the heterogeneity of NSCLC histological cell types. The studies by Azuma and D'Incecco included squamous cell carcinomas (30.5% and 18.4% of the patient cohorts, respectively), whereas the present study consisted of adenocarcinomas only. In a subset analysis of a phase III trial assessing nivolumab treatment for nonsquamous cell cancers, patients with a smoking habit and patients without *EGFR* mutation showed better OS in the nivolumab group than in the docetaxel group.[27] Additionally, patients with high PD-L1 protein expression experienced better sensitivity to anti–PD-1 treatment.[11,27,28] These findings suggested that smoking-associated cancers without *EGFR* mutations tend to exhibit higher PD-L1 protein expression

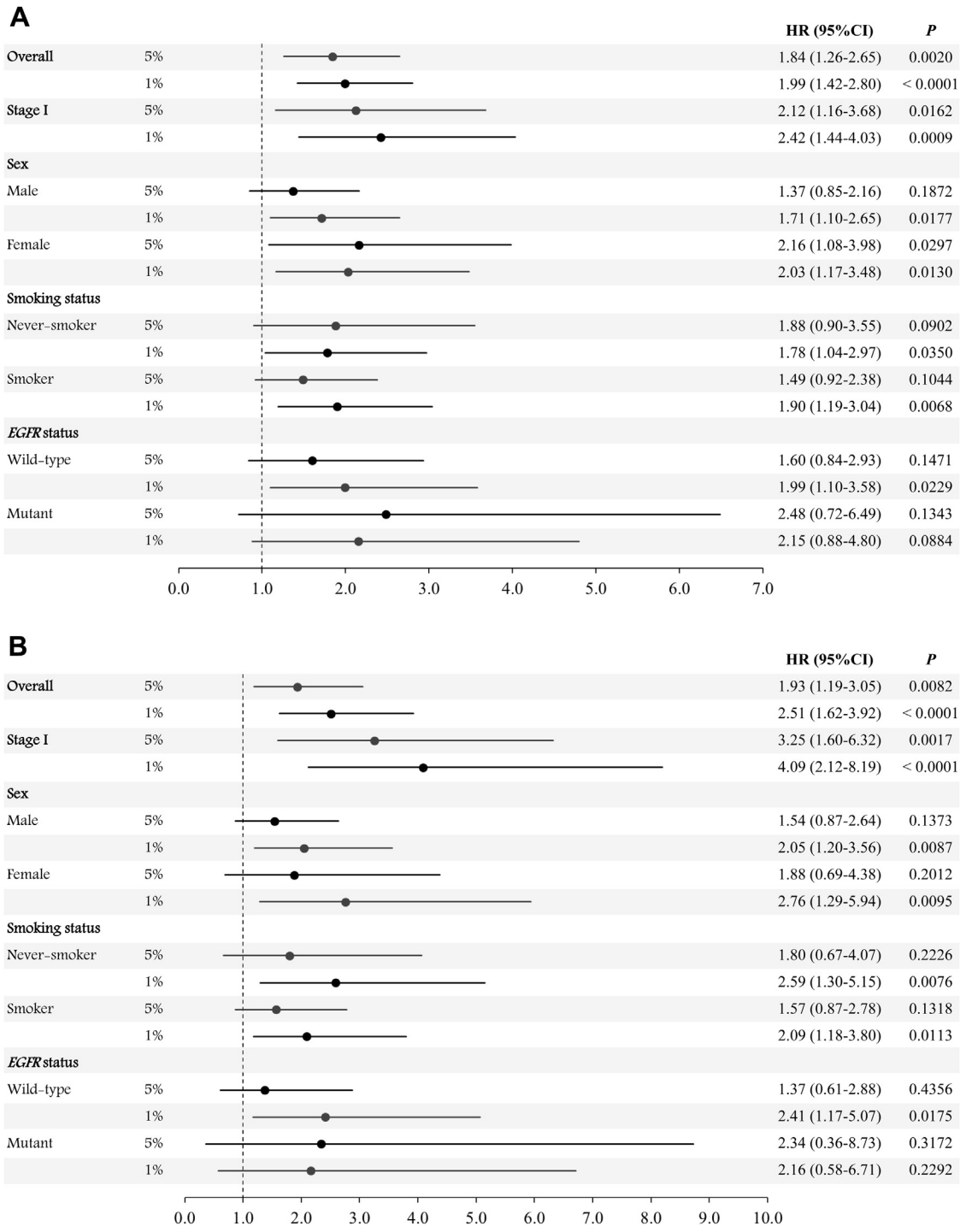

Figure 3. Summary of hazard ratios (programmed death ligand 1 [PD-L1]-positive and PD-L1-negative) according to subgroups. (*A*) Disease-free survival. (*B*) Overall survival. These forest plot analyses revealed that the 1% cutoff value provided a more sensitive value for prediction of postoperative prognosis.

than do cancers from nonsmokers having *EGFR* mutations; thus, these cancers may show greater sensitivity to anti–PD-1 treatment.

Recent genetic analysis investigating predictive factors for immune checkpoint inhibitors suggested that tumors with a greater number of somatic mutations were more sensitive to treatment.[29] Studies based on The Cancer Genome Atlas project demonstrated that melanoma and NSCLC, which showed significant sensitivity to anti–PD-1 antibody therapy, had the greatest mutational burden per cell compared with other solid tumors.[30,31] These data implied that tumors with a

Table 2. Univariate and Multivariate Analyses of DFS and OS in All Patients Using the 1% Cutoff Value

	DFS		OS	
	Univariate Analysis	Multivariate Analysis	Univariate Analysis	Multivariate Analysis
	HR (95% CI)	HR (95% CI)	HR (95% CI)	HR (95% CI)
Factors	*p* Value	*p* Value	*p* Value	*p* Value
Age, y				
≥70	1.46 (1.04-2.05)	1.67 (1.19-2.36)	2.71 (1.72-4.37)	3.43 (2.16-5.58)
<70	0.0288	0.003	<0.0001	<0.0001
Sex				
Male	1.91 (1.36-2.72)		2.42 (1.54-3.90)	2.23 (1.41-3.61)
Female	0.0002		0.0001	0.0005
Smoking status				
Smoker	1.52 (1.08-2.15)		1.78 (1.15-2.80)	
Never-smoker	0.0159		0.0097	
Grade				
≥G2	4.03 (2.73-6.13)	2.31 (1.51-3.63)	3.80 (2.30-6.60)	
G1	<0.0001	<0.0001	<0.0001	
Stage				
≥II	5.28 (3.75-7.47)	3.30 (2.27-4.81)	4.23 (2.73-6.59)	2.88 (1.82-4.60)
I	<0.0001	<0.0001	<0.0001	<0.0001
pl				
Present	3.47 (2.44-4.90)		3.65 (2.34-5.65)	
Absent	<0.0001		<0.0001	
ly				
Present	4.95 (3.42-7.06)	2.60 (1.75-3.82)	4.28 (2.68-6.70)	3.94 (2.38-6.41)
Absent	<0.0001	<0.0001	<0.0001	<0.0001
v				
Present	3.08 (2.19-4.33)		3.58 (2.31-5.56)	
Absent	<0.0001		<0.0001	
Histological subtype				
Micropapillary/solid	2.06 (1.15-3.40)		1.14 (0.44-2.42)	
Others	0.0166		0.7572	
Surgical procedure				
≥Lobectomy	1.60 (1.04-2.56)		1.66 (0.95-3.14)	
Sublobar resection	0.0322		0.0787	
EGFR[a]				
Wild-type	1.76 (1.10-2.89)		2.21 (1.18-4.40)	
Mutant	0.0185		0.0128	
PD-L1				
Positive	1.99 (1.42-2.80)		2.51 (1.62-3.92)	2.30 (1.46-3.65)
Negative	<0.0001		<0.0001	0.0004

[a]Cases for which data were available.
DFS, disease-free survival; OS, overall survival; pl, pleural invasion; ly, lymphatic invasion; v, vascular invasion; PD-L1, programmed death-ligand 1; HR, hazard ratio; CI, confidence interval.

greater number of somatic mutations produce more immunogenic neoantigens, which would be more frequently recognized and attacked by T lymphocytes.[31] Therefore, tumors developed in such an environment may have enhanced PD-L1/PD-1 signaling to evade immune attack. Interestingly, some studies have shown that adenocarcinomas from nonsmokers and adenocarcinomas with *EGFR* mutations had smaller somatic mutation burdens than did smoking-associated adenocarcinomas.[30,32] To the best of our knowledge, there have been no direct data demonstrating the correlation between the mutation burden of the tumors and PD-L1 protein expression in tumors. The present study is the first to demonstrate the correlation between PD-L1 protein expression and smoking-associated cancers in lung adenocarcinoma. Our findings supported the predictive role of tumor mutational burden for anti–PD-L1/PD-1 therapy against solid tumors.

In addition, we examined the association between histological subtype of adenocarcinoma and PD-L1

protein expression in this study. Our results showed that PD-L1 protein expression was significantly higher in micropapillary or solid predominant tumors than in other subtypes. Micropapillary and solid predominant subtypes are associated with a poorer prognosis compared with other subtypes.[33–35] Thus, the histological subtype of adenocarcinoma may be associated with differences in PD-L1 protein expression in lung adenocarcinomas.

Many recent studies, including the present study, have evaluated the prognostic impact of PD-L1 protein expression in NSCLC (summarized in Table 3).[15–18,21–26,36] However, the results of these studies have varied greatly; many studies have shown that patients with high PD-L1 protein expression have poorer prognoses, similar to our results,[15,18,21,22] whereas others showed a favorable prognosis for these patients.[16,17,23] As already described, one of the possible reasons for this discrepancy may be the heterogeneity of cancer cells. Eight out of 13 cohorts in these studies consisted of NSCLCs, including adenocarcinoma, squamous cell carcinoma, and others (see Table 3). The proportions of the cell types also differed among these eight studies. Among these recent studies, that of Cooper et al.[16] investigated the largest patient cohort of NSCLCs (including adenocarcinoma, squamous cell carcinoma, and others [276, 271, and 131 patients, respectively]). Although they reported that PD-L1–positive patients had better prognoses in the analysis of all patients, in their subset analysis, the prognostic correlation was only found in patients with squamous cell carcinoma, not in patients with adenocarcinoma.[16] Similarly, another study also found that patients with squamous cell carcinoma had better survival rates when the tumor showed high PD-L1 protein expression, whereas patients with nonsquamous cell carcinoma did not.[24] In a study of 143 patients with adenocarcinoma, PD-L1 positivity was associated with poor pronosis,[18] similar to our results; however, another study reported opposite results.[17]

Two recent large phase III studies that compared the efficacy of an anti–PD-1 antibody with cytotoxic agents showed different efficacies between squamous cell carcinoma and nonsquamous cell carcinoma,[10,27] suggesting that PD-L1 signaling status differs among different NSCLC cell types. Here, we focused on PD-L1 protein expression in adenocarcinoma only and evaluated the largest number of patients with adenocarcinoma among recent studies investigating PD-L1 protein expression in NSCLC. Patients with high PD-L1 protein expression showed significantly poor OS in univariate and multivariate survival analyses. Our data indicated that adenocarcinomas with more malignant behaviors may have developed a greater ability to harness PD-L1/PD-1 signaling in tumor progression, evading the tumor immune response. Some studies have shown that patients with adenocarcinomas harboring *EGFR* mutations had better prognoses than did those with wild-type *EGFR*[37,38]; thus, the poor prognoses in patients with high PD-L1 protein expression may be attributed to the larger proportion of patients with wild-type *EGFR* among patients with high PD-L1 protein expression in tumors.

Data discrepancy may also be related to the use of different anti–PD-L1 antibodies. Additionally, IHC evaluation methods have varied greatly among studies. Velcheti et al. conducted a validation for four antibodies comparing the IHC staining results of their tissue microarray system with the results of in situ PD-L1 mRNA expression[23] and showed that only one antibody, the noncommercial mouse monoclonal antibody 5H1, could be validated. In correlative studies of clinical trials investigating anti–PD-1 and anti–PD-L1 antibody in NSCLC, independent monoclonal antibodies were used (clone 28–8 from Epitomics [Burlingame, CA] in the nivolumab studies, clone 22C3 from Merck [Kenilworth, NJ] in the pembrolizumab study, and clone SP142 from Ventana Medical Systems [Tucson, AZ] in the MPDL3280A study). Here, we used clone SP142, and set the cutoff point of positive IHC staining result at 1% of cells with membrane staining. Some other studies have used scoring systems in which the intensity and extent of staining are multiplied, and some studies have used a cutoff value of 5% (see Table 3). In our study, if we set the cutoff value to 5%, no significance was observed in multivariate analysis (data not shown). With regard to subgroup analyses, the 1% cutoff value may be a better predictive marker for DFS and OS than the 5% cutoff value (see Fig. 3 and Table 2). However, appropriate antibodies and cutoff values for PD-L1 protein expression have not yet been established. Thus, standardized methods to evaluate the predictive role of PD-L1 IHC analysis are needed to develop a robust marker for anti–PD-1/anti–PD-L1 antibodies.

Although our study was based on one of the largest cohorts among recent studies that investigated resected lung cancer specimens, it was retrospective and was not a trial-based correlative study; thus, the possibility of bias could not be excluded. A large prospective study for clinicopathological, genetic, and prognostic significance of tumors with PD-L1 overexpression is needed to verify this exploratory result. In conclusion, we demonstrated that PD-L1 protein expression was significantly higher in smoking-associated lung adenocarcinoma without *EGFR* mutations. PD-L1 protein expression was higher in tumors with more aggressive disease and was associated with poor survival in patients with lung adenocarcinoma.

Table 3. Summary of Recent Studies Investigating PD-L1 Protein Expression in Surgically Resected NSCLCs

Cell Types	Author	Year	n	Ad/Sq	I/II/III/IV	PD-L1 Distribution	IHC Evaluation	Antibody	Tumors with Positive PD-L1 Expression, %	Prognosis of Patients with PD-L1–Positive Tumors
NSCLC (Ad + Sq)	Mu[21]	2011	109	46/63 (42%/58%)	36/35/38/0	Membrane cytoplasm	Intensity × extent	Unclear (monoclonal)	53.2	Poor
	Azuma[15]	2014	164	114/50 (70%/30%)	67/46/51/0	Membrane cytoplasm	Intensity × extent	Lifespan Biosciences (polyclonal)	50	Poor
	Chen[22]	2012	120	50/50 (42%/42%)	11/33/76/0	Membrane cytoplasm	Intensity × extent	Abcam 236A/E7 (polyclonal)	57.5	Poor
	Velcheti (Greek cohort)[23]	2014	303	124/152 (41%/50%)	95/80/94/33	AQUA	Unclear	5H1 (monoclonal)	24.8	Better
	Velcheti (Yale cohort)[23]	2014	155	102/30 (66%/19%)	74/22/35/11	AQUA	Unclear	5H1 (monoclonal)	36.1	Better
	Cooper[16]	2015	678	276/271 (41%/40%)	I/II-III 339/339	Membrane	Intensity, any ≥50%	Merck 22C3 (monoclonal)	32.8	Better (better in Sq, not in Ad)
	Schmidt[24]	2015	321	125/149 (39%/46%)	187/83/51/0	Cytoplasm	Intensity, moderate ≥5%	Cell signaling E1L3N (monoclonal)	24	No correlation (better in Sq)
	D'Incecco[26]	2015	125	83/23 (66%/18%)	Not available	Not available	Intensity, moderate ≥5%	Abcam ab58810 (polyclonal)	55.3	Not available
Sq only	Boland[36]	2013	214	0/214	104/67/40/3	Membrane	Intensity, any ≥5%	5H1 (monoclonal)	19.6	No correlation
	Kim[25]	2015	331	0/331	131/118/79/0	Membrane cytoplasm	Intensity moderate ≥5%	Cell signaling E1L3N (monoclonal)	26.9	No correlation
Ad only	Zhang[18]	2014	143	143/0	I/II-III 66/77	Membrane cytoplasm	Intensity × extent	Sigma-Aldrich SAB2900365 (polyclonal)	49	Poor
	Yang[17]	2014	163	163/0	all I	Membrane	Intensity, any ≥5%	Proteintech	39.9	RFS better
	Present study	2016	417	417/0	305/63/49/0	Membrane	Intensity, any ≥1%	Ventana SP142 (monoclonal)	34.5	Poor

PD-L1, programmed death-ligand 1; Ad, adenocarcinoma; Sq, squamous cell carcinoma; IHC, immunohistochemistry; RFS, recurrence-free survival.

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Supplementary Data

Note: To access the supplementary material accompanying this article, visit the online version of the *Journal of Thoracic Oncology* at www.jto.org and at http://dx.doi.org/10.1016/j.jtho.2016.06.006.

References

1. Siegel R, Naishadham D, Jemal A. Cancer statistics, 2012. *CA: Cancer J Clin*. 2012;62:10–29.
2. Morgensztern D, Campo MJ, Dahlberg SE, et al. Molecularly targeted therapies in non-small-cell lung cancer annual update 2014. *J Thorac Oncol*. 2015;10:S1–S63.
3. Oxnard GR, Binder A, Janne PA. New targetable oncogenes in non-small-cell lung cancer. *J Clin Oncol*. 2013;31:1097–1104.
4. Pardoll DM. The blockade of immune checkpoints in cancer immunotherapy. *Nat Rev Cancer*. 2012;12:252–264.
5. Postow MA, Callahan MK, Wolchok JD. Immune checkpoint blockade in cancer therapy. *J Clin Oncol*. 2015;33:1974–1982.
6. Gatalica Z, Snyder C, Maney T, et al. Programmed cell death 1 (PD-1) and its ligand (PD-L1) in common cancers and their correlation with molecular cancer type. *Cancer Epidemiol Biomarkers Prev*. 2014;23:2965–2970.
7. Patel SP, Kurzrock R. PD-L1 Expression as a predictive biomarker in cancer immunotherapy. *Mol Cancer Ther*. 2015;14:847–856.
8. Topalian SL, Hodi FS, Brahmer JR, et al. Safety, activity, and immune correlates of anti-PD-1 antibody in cancer. *N Engl J Med*. 2012;366:2443–2454.
9. Brahmer JR, Tykodi SS, Chow LQ, et al. Safety and activity of anti-PD-L1 antibody in patients with advanced cancer. *N Engl J Med*. 2012;366:2455–2465.
10. Brahmer J, Reckamp KL, Baas P, et al. Nivolumab versus docetaxel in advanced squamous-cell non-small-cell lung cancer. *N Engl J Med*. 2015;373:123–135.
11. Garon EB, Rizvi NA, Hui R, et al. Pembrolizumab for the treatment of non-small-cell lung cancer. *N Engl J Med*. 2015;372:2018–2028.
12. Herbst RS, Soria JC, Kowanetz M, et al. Predictive correlates of response to the anti-PD-L1 antibody MPDL3280A in cancer patients. *Nature*. 2014;515:563–567.
13. Akbay EA, Koyama S, Carretero J, et al. Activation of the PD-1 pathway contributes to immune escape in EGFR-driven lung tumors. *Cancer Discov*. 2013;3:1355–1363.
14. Chen N, Fang W, Zhan J, et al. Upregulation of PD-L1 by EGFR activation mediates the immune escape in EGFR-driven NSCLC: implication for optional immune targeted therapy for NSCLC patients with EGFR mutation. *J Thorac Oncol*. 2015;10:910–923.
15. Azuma K, Ota K, Kawahara A, et al. Association of PD-L1 overexpression with activating EGFR mutations in surgically resected nonsmall-cell lung cancer. *Ann Oncol*. 2014;25:1935–1940.
16. 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*. 2015;89:181–188.
17. Yang CY, Lin MW, Chang YL, et al. 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*. 2014;50:1361–1369.
18. Zhang Y, Wang L, Li Y, et al. Protein expression of programmed death 1 ligand 1 and ligand 2 independently predict poor prognosis in surgically resected lung adenocarcinoma. *Onco Targets Ther*. 2014;7:567–573.
19. Sheng J, Fang W, Yu J, et al. Expression of programmed death ligand-1 on tumor cells varies pre and post chemotherapy in non-small cell lung cancer. *Sci Rep*. 2016;6:20090.
20. Kohno M, Okamoto T, Suda K, et al. Prognostic and therapeutic implications of aromatase expression in lung adenocarcinomas with EGFR mutations. *Clin Cancer Res*. 2014;20:3613–3622.
21. Mu CY, Huang JA, Chen Y, et al. High expression of PD-L1 in lung cancer may contribute to poor prognosis and tumor cells immune escape through suppressing tumor infiltrating dendritic cells maturation. *Med Oncol*. 2011;28:682–688.
22. Chen YB, Mu CY, Huang JA. Clinical significance of programmed death-1 ligand-1 expression in patients with non-small cell lung cancer: a 5-year-follow-up study. *Tumori*. 2012;98:751–755.
23. Velcheti V, Schalper KA, Carvajal DE, et al. Programmed death ligand-1 expression in non-small cell lung cancer. *Lab Invest*. 2014;94:107–116.
24. Schmidt LH, Kummel A, Gorlich D, et al. PD-1 and PD-L1 Expression in NSCLC indicate a favorable prognosis in defined subgroups. *PloS One*. 2015;10:e0136023.
25. Kim MY, Koh J, Kim S, et al. Clinicopathological analysis of PD-L1 and PD-L2 expression in pulmonary squamous cell carcinoma: comparison with tumor-infiltrating T cells and the status of oncogenic drivers. *Lung Cancer*. 2015;88:24–33.
26. D'Incecco A, Andreozzi M, Ludovini V, et al. PD-1 and PD-L1 expression in molecularly selected non-small-cell lung cancer patients. *Br J Cancer*. 2015;112:95–102.
27. Borghaei H, Paz-Ares L, Horn L, et al. Nivolumab versus docetaxel in advanced nonsquamous non-small-cell lung cancer. *N Engl J Med*. 2015;373:1627–1639.
28. Carbognin L, Pilotto S, Milella M, et al. Differential activity of nivolumab, pembrolizumab and MPDL3280A according to the tumor expression of programmed death-ligand-1 (PD-L1): sensitivity analysis of trials in melanoma, lung and genitourinary cancers. *PloS One*. 2015;10:e0130142.
29. Rizvi NA, Hellmann MD, Snyder A, et al. Cancer immunology. Mutational landscape determines sensitivity to PD-1 blockade in non-small cell lung cancer. *Science*. 2015;348:124–128.
30. Alexandrov LB, Nik-Zainal S, Wedge DC, et al. Signatures of mutational processes in human cancer. *Nature*. 2013;500:415–421.

31. Champiat S, Ferte C, Lebel-Binay S, et al. Exomics and immunogenics: bridging mutational load and immune checkpoints efficacy. *Oncoimmunology*. 2014;3:e27817.
32. Vogelstein B, Papadopoulos N, Velculescu VE, et al. Cancer genome landscapes. *Science*. 2013;339:1546–1558.
33. Yoshizawa A, Motoi N, Riely GJ, et al. Impact of proposed IASLC/ATS/ERS classification of lung adenocarcinoma: prognostic subgroups and implications for further revision of staging based on analysis of 514 stage I cases. *Mod Pathol*. 2011;24:653–664.
34. Miyoshi T, Satoh Y, Okumura S, et al. Early-stage lung adenocarcinomas with a micropapillary pattern, a distinct pathologic marker for a significantly poor prognosis. *Am J Surg Pathol*. 2003;27:101–109.
35. Amin MB, Tamboli P, Merchant SH, et al. Micropapillary component in lung adenocarcinoma: a distinctive histologic feature with possible prognostic significance. *Am J Surg Pathol*. 2002;26:358–364.
36. Boland JM, Kwon ED, Harrington SM, et al. Tumor B7-H1 and B7-H3 expression in squamous cell carcinoma of the lung. *Clin Lung Cancer*. 2013;14:157–163.
37. Izar B, Sequist L, Lee M, et al. The impact of EGFR mutation status on outcomes in patients with resected stage I non-small cell lung cancers. *Ann Thorac Surg*. 2013;96:962–968.
38. Lee YJ, Park IK, Park MS, et al. Activating mutations within the EGFR kinase domain: a molecular predictor of disease-free survival in resected pulmonary adenocarcinoma. *J Cancer Res Clin Oncol*. 2009;135:1647–1654.