


Programmed Death-ligand 1 Expression With Clone 22C3 in Non-small Cell Lung Cancer: A Single Institution Experience

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ABSTRACT:

BACKGROUND: In recent years, the anti-programmed cell death 1 (PD-1) drug pembrolizumab (Keytruda) was approved for treatment of unresectable advanced non-small cell lung cancer (NSCLC) as first- or second-line therapy depending on the clone 22C3-programmed death-ligand 1 (PD-L1) immunohistochemical expression score by the companion diagnostic assay. We herein evaluated 22C3-PD-L1 expression of NSCLC in a single institution experience and compared it with clinicopathologic features.

MATERIALS AND METHODS: We assessed 22C3-PD-L1 expressions of 411 patients with NSCLC from our institution, including in past specimens. Programmed death-ligand 1 immunohistochemistry (IHC) testing was performed using the PD-L1 clone 22C3 pharmDx kit (Agilent Technologies/Dako, Carpinteria, CA, USA). Patients were separated into 3 groups with <1% (no expression), 1% to 49% (low expression), or ≥50% (high expression) positive tumor cells.

RESULTS: In all, 137 patients (33%) did not express PD-L1, 155 (38%) showed low expression, and 119 (29%) demonstrated high expression. Archival samples showed lower PD-L1 expression than that of recent samples, and the ratios of no expression case significantly increased by using paraffin blocks embedded particularly in more than 4 years ago. Programmed death-ligand 1 positivity was significantly associated with male sex, smoking, higher tumor grade, squamous cell carcinoma in histologic type, wild-type *EGFR*, and *ALK* rearrangement positive.

CONCLUSIONS: The rate of 22C3-PD-L1 expression of NSCLC detected in this study was similar to the frequencies of the previous reports, although the ratio of expression case decreased when using old paraffin blocks.

KEYWORDS: Non-small lung cancer, PD-L1 expression testing, 22C3, pembrolizumab, EGFR

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Introduction

Recently, nivolumab and pembrolizumab were approved for immune-checkpoint therapy in unresectable and recurrent non-small cell lung cancer (NSCLC) cases.¹⁻³ Atezolizumab is also approved by Food and Drug Administration (FDA) for the treatment of patients with metastatic NSCLC. For pembrolizumab, a companion diagnostic assay was developed by Dako in NSCLC clinical trials to examine programmed death-ligand 1 (PD-L1) expression by immunohistochemistry (IHC). The Dako PD-L1 IHC 22C3 pharmDx assay is the companion diagnostic (CDx) assay for PD-L1 with approval in the United States and Japan. Many studies about PD-L1 IHC in

NSCLC have been performed, and the association between PD-L1 expression of tumor cells and therapeutic response to the anti-programmed cell death 1 (PD-1)/PD-L1 immunotherapy has been indicated.^{1,4} It was reported in some studies that PD-L1 expression was a prognostic factor and associated with sex, smoking history, driver oncogene mutation including *EGFR* status, and pathologic differentiation of the tumor.^{2,5-8} Nevertheless there are still few reports on 22C3-PD-L1 expression in NSCLC in Japan.

We herein evaluated the 22C3-PD-L1 expression of NSCLC, including in past specimens, and compared it with clinicopathologic features.



Materials and Methods

Tumor and data collection

Patients who underwent biopsy and surgical resection for NSCLC between December 2004 and December 2017 at the National Hospital Organization Kinki-Chuo Chest Medical Center, Osaka, Japan, were consecutively selected. Clinical factors, including age, sex, and smoking history were examined from clinical records. For evaluation of the pathologic tumor stage, only surgical materials were estimated in this study. Tumor PD-L1 analysis was carried out from February 2017 to December 2017. The archived samples were newly sliced from formalin-fixed paraffin blocks and used for examination. The histopathologic diagnosis and pathologic tumor stage was confirmed by 2 pathologists (MT, TK) according to the current 2015 World Health Organization Classification.⁹ We analyzed 411 patients (139 resection specimens, 272 biopsies) in this study. Patients who had received neoadjuvant chemotherapy were excluded, because previous therapies may affect the expression of PD-L1. Cytology specimens and cell block samples were not included in this study. In all, 325 patients were analyzed using the *EGFR* mutation test by the testing laboratories: PCR-Invader assay by BML Inc. (Tokyo, Japan). *ALK* status was tested by IHC with the ALK Detection Kit (Histofine ALK iAEP kit, clone 5A4, Nichirei Bioscience, Tokyo, Japan) and 2 different fluorescence in situ hybridization (FISH) methods, Vysis FISH and SureFISH.¹⁰ Patients with *ROS1* and *BRAF V600E* positive patients were excluded in this study, because *ROS1* or *BRAF V600E* related lung cancer had only 1 case of each in our institution.

This work was conducted according to the Declaration of Helsinki (2000) of the World Medical Association and was approved by our institutional review board (Approval number: 634).

Tumor PD-L1 analysis

All viable cancer cells on the entire pathologic tissue section were evaluated and included in the PD-L1 scoring analysis.

Programmed death-ligand 1 IHC testing was performed using the PD-L1 clone 22C3 pharmDx kit and Dako Automated Link 48 platform (Agilent Technologies/Dako, Carpinteria, CA, USA). The PD-L1 tumor proportion score (TPS) was calculated as the percentage of the at least 100 viable cancer cells with complete or partial membrane staining. Necrotic areas were excluded from scoring. Each patient sample was separated into 3 groups with <1% (no expression), 1% to 49% (low expression), or \geq 50% (high expression) positive tumor cells. Programmed death-ligand 1 expression positivity is defined as low and high expression on the basis of the clinical trial assay that may maximally predict the clinical response of patients with NSCLC treated with pembrolizumab.¹

Statistical methods

Fisher's exact test was used to compare categorical variables. All *P* values reported are 2 sided, and tests were performed at the .05 significance level.

Results

The 411 NSCLC tumor samples were evaluated for PD-L1 expression of tumor cells (Table 1). The average age of patients examined was 70 years old. A total of 137 (33%) specimens did not express PD-L1 (no expression), 155 (38%) expressed PD-L1 in 1% to 49% of cells (low expression), and 119 (29%) expressed PD-L1 with a TPS of >50% (high expression). This prevalence is similar with KEYNOTE-001 study (Figure 1).² With the old specimen obtained from 2004 to 2012, 21 of 35 (60%) had no expression, 9 of 35 (26%) showed low expression, and 5 of 35 (14%) displayed high expression. With the archived samples taken from 2013 to 2015, 22 of 51 (43%) patients had no expression, 20 of 51 (39%) showed low expression, and 9 of 51 (18%) displayed high expression. In the samples from the last 2 years, 94 of 325 (29%) patients had no expression, 126 of 325 (39%) showed low expression, and 105 of 325 (32%) displayed high expression. In the archived samples, there were significantly more cases of low and no PD-L1 expression than in the recent samples (Figure 1, *P* = .001). The association between PD-L1 positivity and sampling method (biopsy or surgical operation) was not confirmed (data not shown).

Programmed death-ligand 1 protein expression with clone 22C3 was higher in men than in women and in smokers than in non-smokers. In addition, PD-L1 expression was more common in squamous cell carcinoma than in adenocarcinoma. High-expression was more frequent in higher histologic grades. The PD-L1 expression group had a significantly higher proportion of *EGFR* mutation-negative carcinoma and *ALK*-rearranged lung carcinoma. Programmed death-ligand 1 expression was not significantly associated with patient age or pathologic stage.

Discussion

The rapid progress of immunotherapy for lung cancer may bring a revolution for cancer therapy. Recently, the anti-PD-L1 drug pembrolizumab (Keytruda) was approved for treatment of locally advanced and metastasizing NSCLC as first- or second-line therapy depending on the PD-L1 expression score. The best predictive factor for effect of immunotherapy is tumor PD-L1 expression analyzed by IHC on the pathologic tissue sections.

The companion diagnostic assay for this drug, the PD-L1 IHC 22C3 pharmDx kit (Dako Agilent, Denmark), is based on the primary monoclonal antibody 22C3 and is carried out on the semiautomatic Dako Autostainer Link 48 platform.

Association between clinicopathologic features and PD-L1 expression in NSCLC reported in some studies remains controversial.^{2,5-8} There are still only a few reports about

Table 1. PD-L1 expression status by subgroup.

SUBGROUP	N	PD-L1 EXPRESSION STATUS			P VALUE
		TPS ≥ 50% HIGH EXPRESSION	TPS 1% TO 49% LOW EXPRESSION	TPS < 1% NO EXPRESSION	
		N (%)	N (%)	N (%)	
Overall	411	119 (29)	155 (38)	137 (33)	
Sex					
Women	141	27 (19)	51 (36)	63 (45)	.0004
Men	270	92 (34)	104 (39)	74 (27)	
Age					
<70 years	198	52 (26)	72 (36)	74 (37)	.221
≥70 years	213	67 (31)	83 (39)	63 (30)	
Smoking status					
Never	114	18 (16)	46 (40)	50 (44)	.0005
Smoker	297	101 (34)	109 (37)	87 (29)	
Histology					
ADC	238	55 (23)	81 (34)	102 (43)	<.0001
SqCC	97	34 (35)	48 (49)	15 (15)	
Other	76	30 (39)	26 (34)	20 (26)	
Histologic grade					
1, 2	253	48 (19)	97 (38)	108 (43)	<.0001
3, 4	158	71 (45)	58 (37)	29 (18)	
Pathologic stage					
I, II	103	23 (22)	40 (39)	40 (39)	.424
III, IV	36	12 (33)	12 (33)	12 (33)	
Genomic aberrations					
<i>EGFR</i> mutation	105	16 (15)	34 (32)	55 (52)	<.0001
<i>ALK</i> FISH-positive	19	6 (32)	10 (53)	3 (16)	
<i>EGFR/ALK</i> -negative	201	72 (36)	70 (35)	59 (29)	

All *P* values calculated by using Fisher's exact test. Abbreviations: ADC, adenocarcinoma; *ALK*, anaplastic lymphoma receptor tyrosine kinase gene; FISH, fluorescence in situ hybridization; PD-L1, programmed death-ligand 1; SqCC, squamous cell carcinoma; TPS, tumor proportion score.

22C3-PD-L1 expression in NSCLC in Japan. Yoneshima et al¹¹ reported that a subset of patients with *EGFR* mutations or *ALK* arrangement showed 22C3-PD-L1 high expression. Kato et al¹² reported that nodal metastasis, sample preservation time and carcinoembryonic antigen (CEA) levels were associated with 22C3-PD-L1 expression.

We retrospectively evaluated PD-L1 expression and analyzed its correlation with clinicopathologic characteristics. In this study, there was no association between PD-L1 expression and age or pathologic stage, although more men and smokers

were found in the PD-L1 expression group than women and non-smokers. There are several reports about the association between smoking and PD-L1 expression. Calles et al⁷ suggested that the expression of PD-L1 was induced by smoking with *KRAS*-mutant NSCLC. Deng et al¹³ reported that current/former smokers showed significantly higher expression of PD-L1 compared with never smokers. These results are consistent with ours. On the contrary, the association between smoking and PD-L1 expression was not noted in some reports.¹⁴

According to previous studies, PD-L1 expression in NSCLC was significantly more common in squamous cell carcinoma than in non-squamous NSCLC of the lung, as in our study.^{15,16} The correlation between classic driver oncogene mutations in *EGFR* or *ALK* and PD-L1 expression in lung carcinoma has been studied, and the results obtained thus far are controversial. *EGFR* mutation status was reported to not be associated with PD-L1 expression in some reports.² Other reports suggested that the presence of *EGFR* gene mutation and *ALK* rearrangement are associated with the lack of PD-L1 expression on tumor cells.¹⁷ Takada et al¹⁸ recently found that PD-L1 positivity was significantly associated with wild-type *EGFR*. In our study, wild-type *EGFR* or *ALK*-rearranged carcinoma was associated with PD-L1 expression.

Although we did not examine the association between PD-L1 expression and prognosis, there are many studies using

numerous modalities indicating high PD-L1 expression to be a poor prognostic factor in NSCLC.^{2,19,20}

The proportion of positive PD-L1 expression was less in the specimens newly sliced from older paraffin blocks in this study. Grillo et al²¹ reported that membrane and nuclear antigens presented reduced IHC staining intensity in older blocks. The cause of degradation of antigen preservation is uncertain, but archival conditions and oxidation processes may be involved. The report showing similar results to ours about PD-L1 expression in archival specimens was published recently.¹² Furthermore, Herbst et al²² reported that TPS for PD-L1 was superior in fresh samples compared with archival samples, although patient survival was not related to the time of sample collection. Therefore, cautious judgment is necessary when using older paraffin blocks. But this study has some limitations that should be examined in future. Sample type (biopsy vs resection), site (primary vs metastasis), tumor stage, and any other clinicopathologic feature has been reported to be significantly associated with PD-L1 expression in NSCLC.²³⁻²⁵ Any association with other clinicopathologic features should be excluded, in considering the effect of archived samples.

Although the fact that PD-L1 expression remains an independent predictive marker of response to therapy has been emphasized, it may be controversial. Most recently, combination chemotherapy plus immunotherapy has been reported to be the new standard of care in metastatic treatment naive NSCLC now based on KEYNOTE-189 and KEYNOTE-407.^{26,27} In addition, Tumor Mutation Burden (TMB) has been reported to be another predictive marker in the CHECKMATE-227 study, and so the studies on a larger series of patients with NSCLC about correlation of PD-L1 expression with TMB and chemotherapy will be examined in the future.²⁸

In conclusion, the rate of 22C3-PD-L1 expression of NSCLC (Figure 2) detected in this study was similar to the

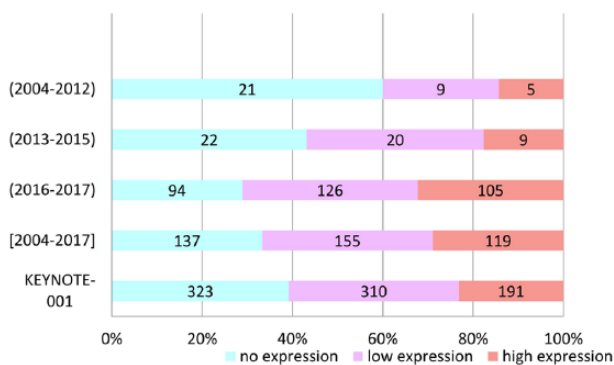


Figure 1. Distribution of 22C3-PD-L1 scoring among different years in which samples were taken compared with the KEYNOTE-001 study.¹ Archival samples showed lower PD-L1 expression than that of recent samples ($P=.001$). The figures on the graph represent actual numbers. PD-L1 indicates programmed death-ligand 1.

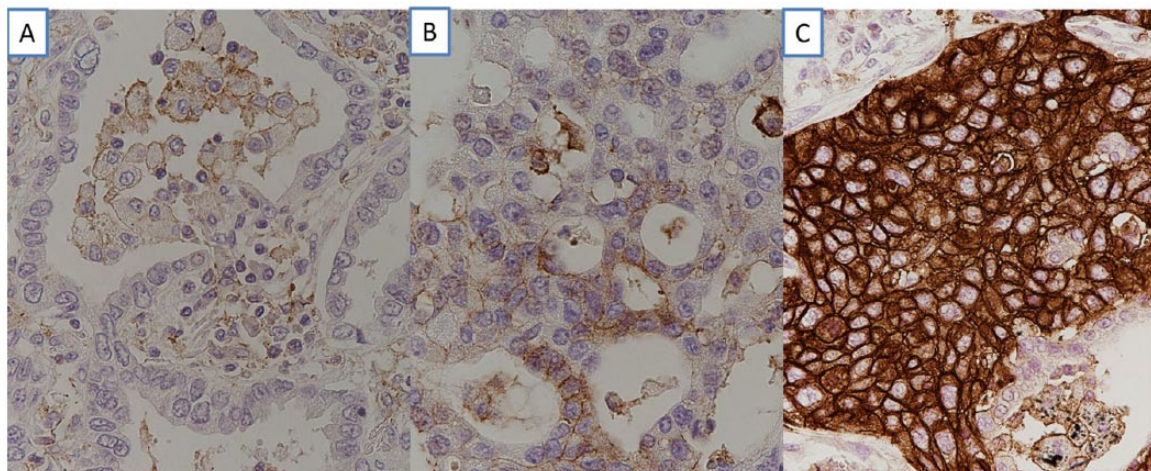


Figure 2. Representative images of PD-L1 IHC 22C3 in NSCLC. (A) Papillary adenocarcinoma demonstrating no expression (TPS=0%), but was associated with macrophages exhibiting membranous staining. (B) Acinar adenocarcinoma exhibiting low expression (TPS=35%). (C) Squamous cell carcinoma demonstrating high expression (TPS=100%). IHC indicates immunohistochemistry; NSCLC, non-small cell lung cancer; PD-L1, programmed death-ligand 1; TPS, tumor proportion score.

frequencies of the previous reports, although the ratio of expression case decreased when using old paraffin blocks. The rate of 22C3-PD-L1 positivity in IHC was significantly associated with male sex, smoking, higher tumor grade, squamous cell carcinoma in histologic type, wild-type *EGFR*, and *ALK* rearrangement positive.

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Author Contributions

MT contributed to corresponding author, study design, data collection, review of pathology samples, data analysis and manuscript write-up. TK contributed to study design, review of pathology samples, data analysis and manuscript editing. MN, AT, YT, NS, YN, KO, SS, KK, AN, TS, TU, H-EY, AM and SA contributed to data collection and interpretation. All authors read and approved the final manuscript.

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