PD-L2 Expression as a Potential Predictive Biomarker for the Response to Anti-PD-1 Drugs in Patients with Non-small Cell Lung Cancer

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Abstract. *Background/Aim:* To investigate the role of programmed cell death-ligand 2 (PD-L2) expression as a predictive biomarker for response to anti-programmed cell death-1 (PD-1) drugs in patients with non-small cell lung cancer (NSCLC). *Patients and Methods:* Ten patients who had undergone curative lung resection and received the anti-PD-1 drugs for the recurrence were enrolled. The cut-off value for PD-L2 (antibody clone 176611) expression on tumor cells was set at 50%. Tumor response was evaluated according to immune-related response criteria. *Results:* Seven patients (70.0%) were positive for PD-L2. The response rates were 28.6% (2/7) and 33.3% (1/3) in patients with PD-L2-positive and PD-L2-negative NSCLC, respectively. Disease control was obtained in 2 patients despite the programmed cell death-ligand 1 (PD-L1)-negativity (antibody clone 22C3: 0%, antibody clone SP142: 0%), and these tumors expressed PD-L2 ($\geq 1\%$). *Conclusion:* PD-L2 expression may be a target of immunotherapy in patients with PD-L1-negative NSCLC.

Lung cancer is one of the most lethal malignancies in the world, and non-small cell lung cancer (NSCLC) accounts for 85% of cases (1, 2). There have been dramatic developments in pharmacotherapy for patients with NSCLC, and immunotherapy targeting programmed cell death 1 (PD-1) or programmed death-ligand 1 (PD-L1) has become one of the standard pharmacological therapies (3, 4). Since the anti-PD-1 drugs became a standard therapy for patients with NSCLC, biomarkers predicting the response to anti-PD-1 antibodies are being sought. According to previous reports, PD-L1 expression by tumor cells is one of the promising predictive biomarkers for the response to the immunotherapy (5-8). However, given the results of CheckMate-057 that the overall survival of anti-PD-1 treated patients who were negative for PD-L1 expression was equivalent to that of patients treated by conventional standard chemotherapy (3), other ligands to PD-1, including programmed death-ligand 2 (PD-L2), may be considered as therapy targets.

PD-L2 protein is expressed mainly by dendritic cells, macrophages, and tumor cells, and down-regulates the effector functions of T cells via PD-1/PD-L2 axis in the tumor microenvironment (9, 10). A recent retrospective study has suggested the relationship between PD-L2 expression and tumor response to anti-PD-1 drugs in patients with human solid tumors (11), but their association in patients with NSCLC is unknown. Therefore, in this translational study, the clinical impact of PD-L2 expression as a predictive biomarker to anti-PD-1 antibodies in patients with NSCLC was examined.

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Table I. Patient characteristics (n=10).

Case	Age	Gender	Histological type	Target lesion	PD-L1 (22C3)	PD-L1 (SP142)	PD-L2 (176611)	Anti-PD-1 drug	PFS (months)	Best Response	irRC
1	69	M	Sq	Liver metastasis	5	0	50	Pembrolizumab	10.5	PR	-78.7%
2	70	M	Ad	Lung metastasis, Mediastinal LN	5	0	50	Nivolumab	6.3	PR	-78.5%
3	86	M	Ad	Lung metastasis	50	10	0	Nivolumab	10.5	PR	-73.2%
4	60	M	Carcinosarcoma	Liver metastasis	0	0	50	Nivolumab	8.2	SD	-10.8%
5	73	M	Ad	Lung metastasis	0	0	1	Nivolumab	2.6*	SD	-6.3%
6	66	M	Ad	Mediastinal LN	5	0	50	Nivolumab	3.7	PD	+34.3%
7	60	M	Sq	Lung metastasis	0	0	10	Nivolumab	6.7	PD	+39.4%
8	69	M	Sq	Lung metastasis	0	0	50	Nivolumab	6.4	PD	+42.0%
9	83	M	Sq	Bone metastasis (Rib)	50	0	50	Nivolumab	2.1	PD	+69.2%
10	62	M	Sq	Lung metastasis, Mediastinal LN	1	1	50	Nivolumab	2.9	PD	+144.7%

PD-L1: Programmed cell death-ligand 1; PD-L2: programmed cell death-ligand 2; PD-1: programmed cell death-1; PFS: progression-free survival; irRC: immune-related response criteria; Sq: squamous cell carcinoma; Ad: adenocarcinoma; LN: lymph node; PR: partial response; SD: stable disease; PD: progression disease. *PD reason: vertebral body invasion.

Patients and Methods

Patients and samples. From January 2015 to December 2017, 11 patients underwent complete resection of primary lung cancer at the Department of Surgery and Science, Graduate School of Medical Sciences, Kyushu University (Fukuoka, Japan). Among them, one patient who did not have a target lesion was excluded. Thus, the data of the remaining 10 patients were included in this retrospective study. The age, sex, histological type, target lesion, and type of anti-PD-1 drug were investigated. Clinical information was obtained from medical records. Nivolumab and Pembrolizumab were administered intravenously at a dose of 3 mg/kg every 2 weeks and 200 mg every 3 weeks, respectively. Tumor response was evaluated by computed tomography every 6 to 8 weeks according to the immune-related response criteria (irRC) (12-15). The institutional review board approved this study (Kyushu University, IRB No. 29-261).

Immunohistochemical analysis of PD-L1 and PD-L2 expression. Formalin-fixed and paraffin-embedded tumor tissue sections were used to determine the expression of PD-L1 and PD-L2 in tumor tissue. The immunohistochemical analysis was conducted using antibodies against PD-L1 (clone 22C3, dilution 1:50; Agilent/Dako, Carpinteria, CA, USA), PD-L1 (clone SP142, dilution 1:100; Spring Bioscience, Ventana, Tucson, AZ, USA) and PD-L2 (clone 176611, dilution 1:200; R&D systems, Inc., Minneapolis, MN, USA). Immunohistochemistry (IHC) for PD-L1 was performed as described previously (16-18). Regarding IHC for PD-L2, 4 µm thick sections were mounted on glass slides with the use of the B Bond-III autostainer (Leica Microsystems, Newcastle, UK). Briefly, slices were treated with proteinase K (Agilent/Dako, Carpinteria, CA, USA) for 5 min and then incubated with anti-human PD-L2 antibody for 30 min. This automated system used a Refine polymer detection system (Leica Microsystems, Newcastle, UK) with horseradish peroxidase-polymer as secondary antibody and 3,3' diaminobenzidine (DAB) as the chromogen. The slides were visualized using DAB.

The proportion of carcinoma cells positive for PD-L1 or PD-L2 was estimated as the percentage of total tumor cells in whole sections. All

immunohistochemical images were evaluated independently by three investigators (S.T., K.T., and T.J.) who were unaware of the outcomes. In case the determinations were different among three observers, the slides were reviewed by all the three investigators together to reach consensus. We set the cut-off values of PD-L1 (22C3), PD-L1 (SP142), and PD-L2 positivity to 50% (4), 1% (6), and 50%, respectively.

Results

Patient characteristics. This study included 10 patients who underwent curative lung resection and received anti-PD-1 drugs for recurrence. The patient characteristics are described in Table I. The median patient age was 69 years (range=60-86 years), and all patients were men. Half of the patients were diagnosed as having squamous cell carcinoma. Target lesions included lung, liver, lymph node, and bone metastases. Immunohistochemistry for PD-L1, using two different primary antibodies, and PD-L2 was performed and patients were classified into positive or negative expression as described in Methods. Two patients (20.0%) were positive for PD-L1 expression as assayed using the 22C3 antibody, and two patients (20.0%) were positive for PD-L1 expression as assessed using the SP142 antibody. Seven patients (70.0%) were positive for PD-L2 expression. Nine patients (90.0%) received nivolumab. The median progression-free survival was 6.3 months (range=2.1-10.5 months). Tumor response to anti-PD-1 antibodies according to irRC included partial response (PR), stable disease (SD), and progressive disease (PD) in 3, 2, and 5 patients, respectively.

Association between PD-L2/PD-L1 expression and tumor response rate to anti-PD-1 drugs. The range of PD-L2 positive expression was 0%, 1-4%, 10-49%, and ≥50% in 1, 1, 1, and 7 patients, respectively. Figure 1 shows waterfall plots for the

Figure 1. Waterfall plot for best percentage change from baseline in tumor measurement according to the expression of (a) programmed cell death-ligand 2, (b) programmed cell death-ligand 1 (PD-L1; antibody clone 22C3), and (c) PD-L1 (antibody clone SP142).

best percentage change from baseline in tumor measurement according to the expression of PD-L2/PD-L1. In NSCLC patients positive for PD-L2 ($\geq 50\%$), 2 (28.6%), 1 (14.3%), and 4 (57.1%) of 7 tumors showed PR, SD, and PD, respectively. The tumor response rate to anti-PD-1 drugs was 28.6% (2/7) and 33.3% (1/3) in NSCLC patients positive for PD-L2 ($\geq 50\%$) and negative for PD-L2 ($< 50\%$), respectively. In evaluating PD-L1 expression using 22C3, the tumor response rate was 50.0% (1/2) and 25.0% (2/8) in NSCLC patients positive for PD-L1 ($\geq 50\%$) and negative for PD-L1-negative ($< 50\%$), respectively. Similarly, in evaluating PD-L1 positivity using SP142, the tumor response rate was 50.0% (1/2) and 25.0% (2/8) in NSCLC patients positive for PD-L1 ($\geq 1\%$) and negative for PD-L1 ($< 1\%$), respectively. These data are summarized in Table II. Of note, disease control (SD) was obtained in two patients despite PD-L1-negativity (22C3: 0%; SP142: 0%), and positivity for PD-L2 ($\geq 1\%$).

Discussion

In the present study, tumor response rate to anti-PD-1 drugs was found to be 28.6% in NSCLC patients positive for PD-L2 ($\geq 50\%$). To the best of our knowledge, this is the first study to

Table II. The relationship between PD-L2/PD-L1 expression and response rate to anti-PD-1 drugs in patients with non-small cell lung cancer.

PD-L2/PD-L1 expression	Response (+)	Response (-)	Number of patients
PD-L2 (176611) $\geq 50\%$	2 (28.6%)	5 (71.4%)	7
PD-L2 (176611) $< 50\%$	1 (33.3%)	2 (66.7%)	3
PD-L1 (22C3) $\geq 50\%$	1 (50.0%)	1 (50.0%)	2
PD-L1 (22C3) $< 50\%$	2 (25.0%)	6 (75.0%)	8
PD-L1 (SP142) $\geq 1\%$	1 (50.0%)	1 (50.0%)	2
PD-L1 (SP142) $< 1\%$	2 (25.0%)	6 (75.0%)	8

PD-L1: Programmed cell death-ligand 1; PD-L2: programmed cell death-ligand 2; PD-1: programmed cell death-1.

investigate the predictive role of PD-L2 expression to immunotherapy in patients with NSCLC, although the sample size was very small. Of note, disease control (SD) observed in 2 tumors with PD-L1-negativity and PD-L2-positivity suggesting that PD-L2 expression may have a clinical role as a target of immunotherapy (Figure 1). While the clinical significance of PD-L1 expression has been examined in many

previous studies (19-26), that of PD-L2 has not been well investigate in NSCLC patients.

Recently, few studies have suggested the relationship between PD-L2 expression and clinical response to immunotherapy (11, 27). Yearley *et al.* reported that in patients with several kinds of solid malignancies treated by PD-1 axis targeted therapy, PD-L2 positivity was statistically significant predictor of progression-free survival, while PD-L1 was not (11). Given the results of CheckMate-057 that overall survival of anti-PD-1 drug administered patients with PD-L1 negativity was equivalent to that of patients treated by chemotherapy, the potential role of other PD-1 ligands except of PD-L1 may exist (3). As shown in Figure 1, two tumors with PD-L1-negativity and PD-L2-positivity showed SD to anti-PD-1 drugs, which may at least partly explain a positive clinical response to anti-PD-1 antibodies in NSCLC patients negative for PD-L1. Because high PD-L2 positivity was observed in the current study (7/10), therapeutic strategies targeting PD-L2 would be of interest. The potential role of PD-L2 expression alone or in combination with PD-L1 expression as a predictive biomarker for the response to immunotherapy may need to be investigated in future prospective studies.

The present study had several limitations. First, this study did not analyze the influence of chemotherapy and radiotherapy on PD-L1 expression before administration of anti-PD-1 drugs due to the small sample size. Chemotherapy is reported to induce PD-L1 expression (28, 29), and our previous studies suggested that radiotherapy may contribute to the conversion of PD-L1-negative into PD-L1 positive NSCLC (30-32). Thus, further analyses including therapy-derived effects are necessary to draw definitive conclusions. Second, PD-L1 and PD-L2 expression was examined using only 22C3, SP142 and 176611 antibody clones. The positive rate of PD-L1 using SP142 was reported to be lower than that of other antibodies such as 28-8 and SP263 (33). However, according to a previous study of patients with small-cell lung cancer, SP142-positivity was higher than that of other antibodies (34). Therefore, both of PD-L1 and PD-L2 expression should be evaluated using additional antibodies.

In conclusion, our study demonstrated that PD-L2 expression may be a target of anti-PD-1 drugs in NSCLC patients negative for PD-L1. The potential role of PD-L2 expression as a predictive biomarker for the response to immunotherapy needs to be elucidated in future studies.

Conflicts of Interest

All the Authors declare no conflicts of interest.

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