ORIGINAL ARTICLE

Comparing Three Different Anti–Programmed Death-Ligand 1 Antibodies in Immunohistochemical Evaluation of Combined Chemoimmunotherapy Response in Patients With NSCLC: A Prospective Study

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ABSTRACT

Introduction: Multiple programmed death-ligand 1 (PD-L1) immunohistochemistry assays performed using different antibodies including DAKO 22C3, DAKO 28-8, and Ventana SP142 PD-L1—predictive markers for response to various immune checkpoint inhibitors in NSCLC—have been approved in several countries. The differences in multiple PD-L1 immunohistochemistry assay results in predicting the therapeutic response to combined chemoimmunotherapy in patients with NSCLC remain unclear.

Methods: In this multicenter prospective observational study, we monitored 70 patients with advanced NSCLC treated with combined chemoimmunotherapy at 10 institutions in Japan. The expression of PD-L1 in pretreatment tumors was evaluated using the 22C3, 28-8, and SP142 assays in all patients.

Results: The PD-L1 level in tumor cells determined using the 22C3 assay was the highest among the three assays performed with different antibodies. According to the 22C3 assay results, the PD-L1 tumor proportion score greater than or equal to 50% group had a significantly longer progression-free survival period than the PD-L1 tumor proportion score less than 50% group. Nevertheless, the

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other assays did not reveal remarkable differences in the objective response rate or progression-free survival.

Conclusions: In our study, PD-L1 expression determined using the 22C3 assay was more correlated with the therapeutic response of patients with NSCLC treated with combined chemoimmunotherapy than that determined using the 28-8 and SP142 assays. Therefore, the 22C3 assay may be useful for clinical decision-making for patients with NSCLC treated with combined chemoimmunotherapy. Trial registration number: UMIN 000043958.

Keywords: Chemoimmunotherapy; PD-L1; Non–small cell lung cancer; Prospective analysis; Therapeutic response

Introduction

The recent expansion in the use of immune checkpoint inhibitors (ICIs), including programmed cell death protein 1 and programmed death-ligand 1 (PD-L1) inhibitors, has resulted in a paradigm shift in the management of patients with advanced NSCLC.[1–3] Combined chemotherapy is the standard treatment option for patients with treatment-naive advanced NSCLC.[4,5] Several biomarkers for predicting responses to ICIs in patients with NSCLC, such as PD-L1 expression, neutrophil-to-lymphocyte ratio, microbiome, and tumor mutation burden, have been reported.[6–8] Nevertheless, predictive factors for combined chemoimmunotherapy response in patients with NSCLC have not been extensively investigated.

PD-L1 expression is the most frequently used factor in clinical practice for predicting the response to immunotherapy in patients with NSCLC. Several immunohistochemistry (IHC) assays have been used to assess PD-L1 expression, including the 22C3, 28-8, and SP142 assays, because different reagents and evaluations for PD-L1 expression have been developed based on the findings of various clinical trials. The PD-L1 IHC Dako 28-8 pharmDx assay has been approved in several countries as an optional test for second-line nivolumab therapy response, based on the findings of clinical trials CheckMate 017 and CheckMate 057.[1,9] The PD-L1 IHC Dako 22C3 pharmDx assay has also been approved in several countries as a companion diagnostic tool for assessing pembrolizumab response, based on the findings of KEYNOTE-024 and KEYNOTE-042 studies.[6,10] In addition, the Ventana PD-L1 SP142 assay has been approved by the Food and Drug Administration as a companion diagnostic tool for identifying patients with metastatic NSCLC who may be suitable for treatment with atezolizumab based on the findings of the OAK, IMpower130, and IMpower150 studies.[3,11,12] Owing to complex clinical trial designs, the clinical application of PD-L1 IHC assays varies. Several studies have investigated the concordance of PD-L1 expression results of multiple PD-L1 IHC assays. The SP142 assay has lower sensitivity than other PD-L1 IHC assays, such as the 22C3 assay, and presents different staining patterns.[13,14] Nevertheless, the three PD-L1 IHC assays have not been compared in terms of their predictive efficacy for the clinical outcomes of combined chemoimmunotherapy in patients with NSCLC. Therefore, in this prospective study, we assessed the clinical effects of PD-L1 expression evaluated using three different PD-L1 antibodies (22C3, 28-8, and SP142) in patients with advanced NSCLC treated with combined chemoimmunotherapy.

Matrerials and Methods

Patients

We prospectively enrolled patients with advanced or recurrent NSCLC who provided written informed consent and were treated with combination chemotherapy at 10 institutions in Japan (University Hospital Kyoto Prefectural University of Medicine, Japanese Red Cross Kyoto Daini Hospital, Saiseikai Suita Hospital, Japanese Red Cross Kyoto Daiichi Hospital, Uji-Tokushukai Medical Center, Otsu City Hospital, Matsushita Memorial Hospital, Fukuchiyama City Hospital, Kyoto City Hospital, and Saiseikai Shiga Hospital) between November 2019 and March 2021. The study involved patients who met the following criteria: (1) histologically and cytologically confirmed unresectable advanced or recurrent NSCLC and (2) not previously treated with chemotherapy or immunotherapy. The exclusion criteria were patients for whom evaluation using residual specimens after a pathologic diagnosis was challenging or impossible. Combined chemoimmunotherapy was performed as routine care. All patients were followed up from the onset of treatment to November 2021. The study protocol was approved by the Ethics Committee of the University Hospital, Kyoto Prefectural University of Medicine (Kyoto, Japan; approval number: ERB-C-1545), and was conducted in accordance with the tenets of the Declaration of Helsinki. The protocol was registered at the University Medical Hospital Information Network Clinical Trials Registry (UMIN000043958).

Analysis of PD-L1 Expression

Formalin-fixed, paraffin-embedded tissue blocks were serially cut into 4-μm-thick sections and deparaffinized. For the 22C3 assay, PD-L1 IHC was performed using the 22C3 pharmDx assay in a commercial clinical

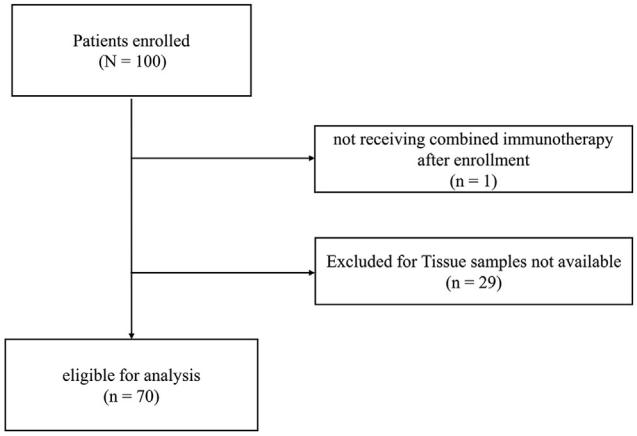

Figure 1. CONSORT diagram of the study.

laboratory (SRL, Inc., Tokyo, Japan). Pathologists affiliated to commercial vendors interpreted tumor PD-L1 expression according to the assay results. For the 28-8 assay, the sections were stained with anti–PD-L1 28-8 rabbit monoclonal primary antibodies on the Dako Autostainer Link48 system. For the SP142 assay, the sections were stained with anti–PD-L1 SP142 rabbit monoclonal primary antibodies using the Ventana Benchmark Ultra system. In addition to IHC staining, hematoxylin and eosin staining was performed to identify tumor cells (TCs). The percentages of PD-L1–positive TCs and tumor-infiltrating immune cells (ICs) were assessed by two experienced pathologists (A.M.H. and N.T.) who were blinded to the clinicopathologic characteristics of the patient samples. In the 22C3 and 28-8 assays, PD-L1 expression was determined using tumor proportion score (TPS), which is the percentage of viable TCs having partial or complete membrane staining. TPS greater than 1% indicated PD-L1 expression and TPS greater than or equal to 50% indicated high PD-L1 expression. In the SP142 assay, PD-L1 expression as a percentage of total TCs and proportion of tumor-infiltrating ICs expressing PD-L1 as a percentage of tumor area were scored. TC3, TC2, and TC1 indicate greater than or equal to 50%, greater than or equal to 5%, and greater than or equal to 1% TCs expressing PD-L1, respectively. IC3, IC2, and IC1 indicate tumor-infiltrating ICs expressing PD-L1 in greater than or equal to 10%, greater than or equal to 5%, and greater than or equal to 1% of the tumor area, respectively.

Statistical Analysis

All statistical tests were two sided, and statistical significance was set at p less than 0.05. Staining concordance was assessed to compare the dichotomized expression values among the assays using Cohen's kappa method with quadratic weighting. The clinical end points were progression-free survival (PFS), overall survival (OS), objective response rate (ORR), and disease control rate based on the Response Evaluation Criteria in Solid Tumors version 1.1 criteria. Patients with no events were censored on the date of their last follow-up. The data cutoff point was November 2021. PFS and OS were calculated using the Kaplan-Meier method, and differences were compared using the log-rank test. For univariate analysis, Cox proportional hazard models were used to estimate hazard ratio (HR) and 95% confidence interval (CI). OS and PFS were censored on the last date of survival confirmation for patients who had no documented disease progression and were alive. On the basis of previous reports, Eastern Cooperative Oncology Group performance status greater than or equal to 2, sex, age ($\geq$75 y), and smoking status were selected as the covariates.[4,15,16] Statistical analyses were performed using EZR statistical software version 1.40 (EZR Project; https://cran.r-project.org).[17]

Results

Patient Characteristics

From the 100 enrolled patients with advanced NSCLC who received combined chemoimmunotherapy during the study at the 10 institutions, 30 were excluded based on the following criteria: did not receive combined immunotherapy after enrollment (n = 1) and unavailability of tissue samples (n = 29). Therefore, 70 patients were eligible for analysis in this study (Fig. 1). The median age of the patients at enrollment was 69 (44–86) years, and 55 (78.6%) of the patients were men. Furthermore, 56 patients (80.0%) had a history of smoking, and most (94.3%) had an Eastern Cooperative Oncology Group performance status of 0 or 1. The histologic subtypes included adenocarcinoma (n = 44, 62.9%) and squamous cell carcinoma (n = 14, 20.0%). Ten patients (14.3%) had EGFR mutations and none had ALK fusion. Except three patients (two with exon20ins and one with no EGFR mutation at diagnosis), the remaining seven patients were treated with EGFR tyrosine kin ase inhibitors before combined chemoimmunotherapy. Pembrolizumab and atezolizumab were administered to 48 (68.6%) and 22 patients (31.4%), respectively. Furthermore, 13 patients (18.6%) were on a platinum plus paclitaxel or pemetrexed plus bevacizumab plus atezolizumab regimen (Table 1).

Comparison of PD-L1 Expression Results of the Three IHC Assays

The PD-L1 expression levels determined using the three IHC assays in the 70 patients with NSCLC are found in Figure 2*A*. The interobserver variability of the interpretation of PD-L1 for 28-8 and SP142 is found in

Table 1. Patient Characteristics

Characteristics	All patients (n = 70)
Age (y)	
Median (range)	69 (44-86)
Sex	
Male	55 (78.6)
Female	15 (21.4)
ECOG PS	
0	21 (30.0)
1	45 (64.3)
2	4 (5.7)
Stage	
III or IV	63 (90.0)
Recurrence	7 (10.0)
Oncogenic driver	
EGFR mutation positivity	10 (14.3)
ALK-rearranged positivity	0 (0.0)
Smoking status	
Current or former	56 (80.0)
Never	14 (20.0)
Histology	
Adeno	44 (62.9)
Squamous	14 (20.0)
Others	12 (17.1)
Regimen	
Platinum + pemetrexed + pembrolizumab	26 (37.1)
Platinum + paclitaxel or nab-paclitaxel + pembrolizumab	22 (29.3)
Platinum + paclitaxel or pemetrexed + bevacizumab + atezolizumab	13 (18.6)
Platinum + pemetrexed + atezolizumab	4 (5.7)
Platinum + paclitaxel or nab-paclitaxel + atezolizumab	5 (7.1)
Response assessment	
CR	1 (1.4)
PR	41 (58.6)
SD	15 (21.4)
PD	7 (10.0)
NE	6 (8.6)
Objective response rate (95% CI)	60.00% (47.6%-71.5%)
Disease control rate (95% CI)	81.40% (70.3%-89.7%)

Notes: All values are n (%) unless otherwise specified.
CI, confidence interval; CR, complete response; ECOG PS, Eastern Cooperative Oncology Group performance status; NE, non evaluable; PD, progressive disease; PR, partial response; SD, stable disease.

Supplementary Figure 1. According to the 22C3 assay, the number of patients with PD-L1 TPS less than 1%, 1% to 49%, and greater than or equal to 50% was 22 (31.4%), 27 (38.6%), and 21 (30.0%), respectively. According to the 28-8 assay, the number of patients with PD-L1 TPS less than 1%, 1% to 49%, and greater than or equal to 50% was 25 (35.7%), 30 (42.9%), and 15 (21.4%), respectively. The number of patients with PD-L1 TPS less than 1%, 1% to 49%, and greater than or equal to 50% according to the SP142 assay was 37 (52.9%), 27 (38.6%), and six (8.6%), respectively. In 33 (47.1%) of the tumor specimens, the same level of PD-L1 expression was observed using the three antibodies (either TPS $\geq$ 1% with the three assays or TPS $<$ 1% with the three assays); 11 (15.7%) of the tumor specimens were triple negative and 22 (31.4%) were triple positive. Furthermore, 59 (84.3%) of all tumor specimens were positive in at least one IHC assay (22C3, 28-8, or SP142); 48 (81.4%) were positive in the 22C3 assay; 45 (76.3%) were positive in the 28-8 assay; and 33 (55.9%) were positive in the SP142 assay (Fig. 2*B*). The average TPS values were 31.5%, 18.8%, and 8.9% in the 22C3, 28-8, and SP142 assays, with higher TC-positive staining in the 22C3 assay than in the 28-8 and SP142 assays (Fig. 2*C*, Fig. 2*D* and Supplementary Fig. 2). Moderate concordance was observed between the 22C3 and 28-8 assays (k = 0.60). There was a slight concordance between the 22C3 and SP142 assays and between the 28-8 and SP142 assays (k = 0.39, k = 0.45, respectively) (Supplementary Table 1).

Analysis of the Response Rate to Combined Chemoimmunotherapy Using Different PD-L1 IHC Assays

The ORR and disease control rate in patients receiving combined chemoimmunotherapy were analyzed according to two cutoff levels in the 22C3 (TPS $\geq$ 50% versus TPS $<$ 50% and TPS $\geq$ 1% versus TPS $<$ 1%), 28-8 (TPS $\geq$ 50% versus TPS $<$ 50% and TPS $\geq$ 1% versus TPS $<$ 1%), and SP142 (TC3 or IC3 versus TC0, 1, 2 and IC0, 1, 2 and TC1, 2, 3 or IC1, 2, 3 versus TC0 and IC0) assays. Using the 22C3 assay, the ORR in the PD-L1 TPS greater than or equal to 50% and PD-L1 TPS greater than or equal to 1% groups was determined to be significantly higher than that in the PD-L1 TPS less than 50% and PD-L1 TPS less than 1% groups (90.5% versus 46.9%, $p < 0.001$, 70.8% versus 36.4%, $p = 0.008$, respectively). There was no significant difference in ORR evaluated based on PD-L1 expression using the 28-8 and SP142 assays (Table 2).

PFS and OS Evaluated Based on PD-L1 Expression Levels Measured Using Different PD-L1 Assays

The median follow-up time was 12.2 months. Among the 70 patients with NSCLC, 54 had disease progression and 22 had died by the cutoff date.

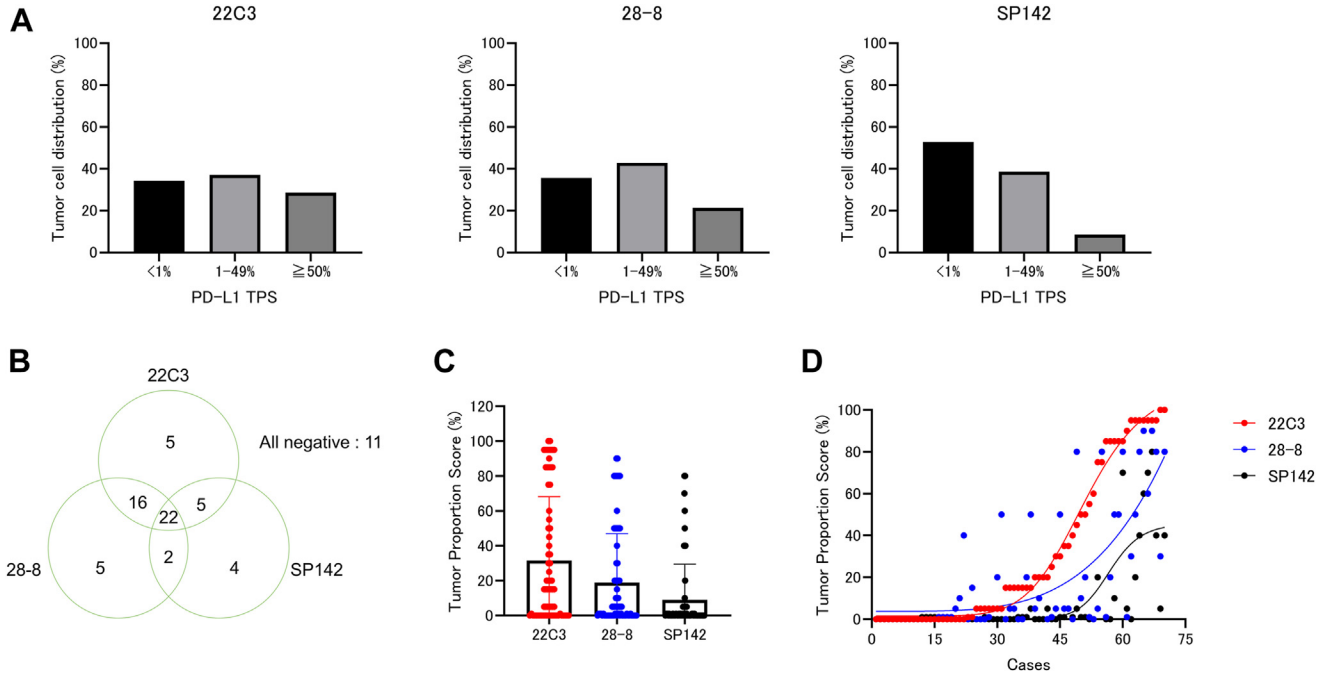

Figure 2. PD-L1 expression in tumor cells determined using three immunohistochemistry assays in 70 patients with NSCLC. (*A*) The number of cases with TPS less than 1%, 1% to 49%, and greater than or equal to 50% in the 22C3, 28-8, and SP142 assays. (*B*) The median TPS in the 22C3, 28-8, and SP142 assays. (*C*) PD-L1 TPS in the 22C3, 28-8, and SP142 assays in all cases. (*D*) Comparability of PD-L1 staining on tumor cells among the 22C3, 28-8, and SP142 assays. PD-L1, programmed death-ligand 1; TPS, tumor proportion score.

Using the 22C3 assay, the PD-L1 TPS greater than or equal to 50% group was found to have had a significantly longer PFS period than the PD-L1 TPS less than 50% group (12.0 versus 5.8 mo, $p = 0.004$) (Fig 3*A*). The PD-L1 TPS greater than or equal to 1% and PD-L1 TPS less than 1% groups had comparable PFS periods (8.5 versus 5.5 mo, $p = 0.24$) (Fig 3*B*). Using the 28-8 assay, we noted that the PFS of the PD-L1 TPS greater than or equal to 50% and PD-L1 TPS greater than or equal to 1% groups was comparable with that of the PD-L1 TPS less than 50% and PD-L1 TPS less than 1% groups (11.3 versus 7.9 mo, $p = 0.84$, 8.1 versus 5.6 mo, $p = 0.52$, respectively) (Fig. 3*C* and *D*). Using the SP142 assay, we found that the PFS of the TC3 or IC3 group and TC1, 2, 3 or IC1, 2, 3 group was comparable with that of the TC0, 1, 2 and IC0, 1, 2 group and TC0 and IC0 group (11.3 versus 7.9 mo, $p = 0.60$, and 11.3 versus 7.6 mo, $p = 0.29$, respectively) (Fig 3*E* and *F*). Using the 22C3 assay, the OS of the PD-L1 TPS greater than or equal to 50% and PD-L1 TPS greater than or equal to 1% groups was observed to be comparable with that of the PD-L1 TPS less than 50% and PD-L1 TPS less than 1% groups ($p = 0.33$, $p = 0.59$, respectively) (Supplementary Fig. 3*A* and *B*). Using the 28-8 assay, the OS of the PD-L1 TPS greater than or equal to 50% and PD-L1 TPS greater than or equal to 1% groups was observed to be comparable with that of the PD-L1 TPS less than 50% and PD-L1 TPS less than 1% groups ($p = 0.92$, $p = 0.53$, respectively) (Supplementary Fig. 3*C* and *D*). Using the SP142 assay, the OS of the TC3 or IC3 group and TC1, 2, 3 or IC1, 2, 3 was comparable with that of the TC0, 1, 2 and IC0, 1, 2 group and TC0 and IC0 group ($p = 0.49$, $p = 0.79$, respectively) (Supplementary Fig. 3*E* and *F*). In the univariate analysis, PD-L1 TPS greater than or equal to 50% determined using the 22C3 assay was significantly correlated with prolonged PFS (HR = 0.41, 95% CI: 0.38–0.74, $p < 0.01$). This result was confirmed using the multivariate analysis (HR = 0.38, 95% CI: 0.19–0.74) (Table 3).

Discussion

To the best of our knowledge, this is the first prospective real-world study on the relationship between three different PD-L1 IHC assay results and therapeutic responses in patients with NSCLC receiving combined chemoimmunotherapy. In this study, the PD-L1 level in TCs determined using the 22C3 assay was the highest and that determined using the SP142 assay the lowest. These findings are consistent with those of the Blueprint phase 1 and phase 2 studies, which have reported relatively high sensitivity of the 22C3 and 28-8 assays and consistently low TC staining in the SP142 assay.[11,12] Although these previous studies concluded that the 22C3 and 28-8 assays were comparable, a recent large cohort study reported

Table 2. Analysis of the Response Rate to Combined Chemoimmunotherapy Using Each PD-L1 IHC Assay

PD-L1 status	ORR (%) (95% CI)	*p*	DCR (%) (95% CI)
Total	60.0 (47.6-71.5)		81.4 (70.3-89.7)
22C3			
TPS $\geq$ 50%	90.5 (69.6-98.8)	<0.001	95.2 (76.2-99.9)
TPS < 50%	46.9 (32.5-61.7)		75.5 (61.1-86.7)
TPS $\geq$ 1%	70.8 (55.9-83.0)	0.008	85.4 (72.2-93.9)
TPS < 1%	36.4 (17.2-59.3)		72.7 (49.8-89.3)
28-8			
TPS $\geq$ 50%	73.3 (44.9-92.2)	0.37	80.0 (51.9-95.7)
TPS < 50%	56.4 (42.3-69.7)		81.8 (69.1-90.9)
TPS $\geq$ 1%	68.9 (53.4-81.8)	0.07	86.7 (73.2-94.9)
TPS < 1%	44.0 (24.4-65.1)		72.0 (50.6-87.9)
SP142			
TC3 or IC3	83.3 (35.9-99.6)	0.39	83.3 (35.9-99.6)
TC0, 1, 2 and IC0, 1, 2	57.8 (44.8-70.1)		81.2 (69.5-89.9)
TC1, 2, 3 or IC1, 2, 3	72.7 (54.5-86.7)	0.05	81.8 (64.5-93.0)
TC0 and IC0	48.6 (31.9-65.6)		81.1 (64.8-92.0)

CI, confidence interval; DCR, disease control rate; IC, immune cell; IHC, immunohistochemistry; ORR, objective response rate; PD-L1, programmed death-ligand 1; TC, tumor cell; TPS, tumor proportion score.

that the 22C3 assay had higher PD-L1 expression in TCs than the 28-8 assay.[18] In this study, 81.4%, 76.3%, and 55.9% of the specimens in the 22C3, 28-8, and SP142 assays tested positive in at least one IHC assay, respectively, consistent with the findings of previous studies.[18,19] Differences in specific epitopes recognized by various PD-L1 antibodies (22C3, 28-8, and SP142) might be one of the major factors influencing variation in the staining characteristics of the PD-L1 IHC assays. The epitope recognized by 22C3 spans 31 amino acids and is located predominantly in extracellular residues 166 to 190, whereas the main epitopes recognized by 28-8 are within extracellular residues 86 to 93, 125 to 145, and 205 to 223, and SP142 antibody clones reportedly bind to amino acid residues 284 to 290 in the cytoplasmic tail of PD-L1.[20] Therefore, the differences in the detection results of the PD-L1 IHC assays assessed in this study may be explained by the differences in the epitopes of the extracellular domain of PD-L1 to which each PD-L1 antibody binds. Differences in PD-L1 staining are attributed to

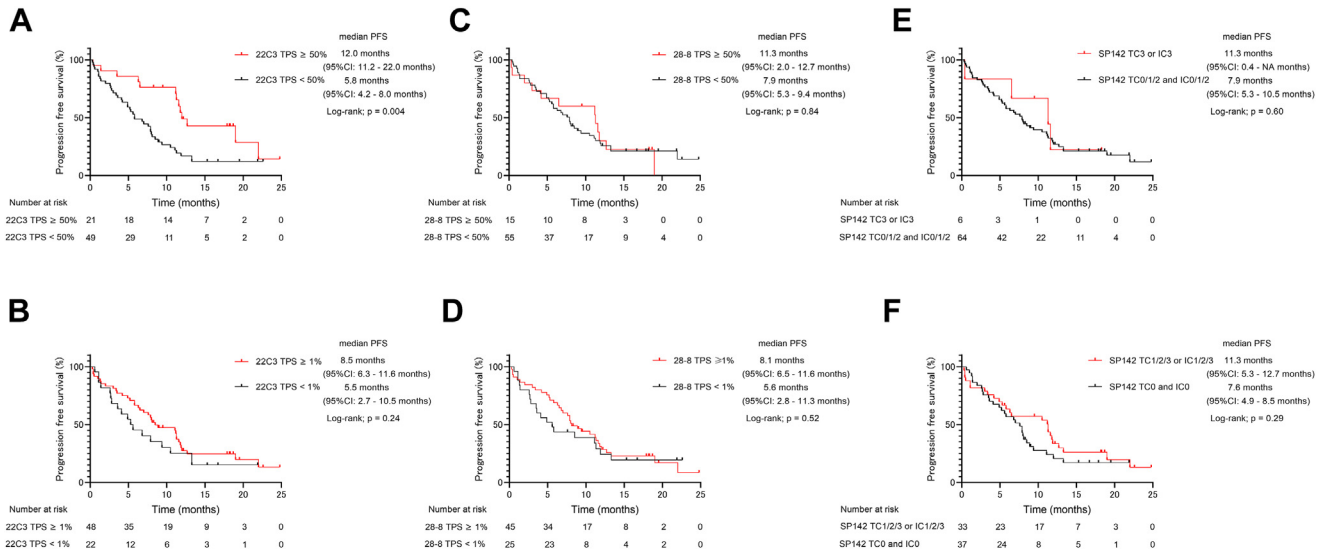

Figure 3. PFS based on the expression of PD-L1 determined using different PD-L1 IHC assays. PFS analyses were performed by stratifying the 22C3, 28-8, and SP142 assay results. A comparison of Kaplan-Meier curves of PFS between patients with (*A*) 22C3 TPS greater than or equal to 50% and TPS less than 50%, (*B*) 22C3 TPS greater than or equal to 1% and TPS less than 1%, (*C*) 28-8 TPS greater than or equal to 50% and TPS less than 50%, (*D*) 28-8 TPS greater than or equal to 1% and TPS less than 1%, (*E*) SP142 TC3 or IC3 and TC0, 1, 2 and IC0, 1, 2, and (*F*) SP142 TC1, 2, 3 or IC1, 2, 3 and TC0 and IC0. IC, immune cell; IHC, immunohistochemistry; PD-L1, programmed death-ligand 1; PFS, progression-free survival; TC, tumor cell; TPS, tumor proportion score.

Table 3. Univariate and Multivariate Analyses of Progression-Free Survival in Patients With NSCLC Treated With Combined Chemoimmunotherapy

Parameter	Progression-Free Survival	
	Univariate HR (95% CI);	Multivariate HR (95% CI);
(Comparator)	*p*	*p*
PD-L1 22C3 TPS ≥ 50%	0.41 (0.22-0.77);	0.38 (0.19-0.74);
(vs. <50%)	<0.01	<0.01
PD-L1 22C3 TPS ≥ 1%	0.71 (0.40-1.26);	
(vs. <1%)	0.24	
PD-L1 28-8 TPS ≥ 50%	0.93 (0.49-1.78);	
(vs. <50%)	0.84	
PD-L1 28-8 TPS ≥ 1%	0.83 (0.48-1.46);	
(vs. <1%)	0.52	
PD-L1 SP142 TC3 or IC3	0.76 (0.27-2.12);	
(vs. others)	0.60	
PD-L1 SP142 TC0 and IC0	0.74 (0.43-1.28);	
(vs. others)	0.29	
Age ≥ 75 y	0.89 (0.50-1.61);	1.18 (0.63-2.21);
(vs. <75 y)	0.70	0.61
Male sex	1.23 (0.65-2.32);	1.97 (0.87-4.50);
(vs. female sex)	0.53	0.10
Smoker	0.80 (0.43-1.49);	0.57 (0.26-1.25);
(vs. never smoker)	0.49	0.16
ECOG PS ≥ 2	4.01 (1.42-11.3);	3.27 (1.15-9.34);
(vs. 0, 1)	<0.01	0.03
Stage III or IV	1.32 (0.53-3.33)	
(vs. recurrence)	0.55	
EGFR mutation positive	0.95 (0.45-2.00);	
(vs. all others)	0.89	
Pembrolizumab regimen	0.76 (0.44-1.34);	
(vs. atezolizumab regimen)	0.35	

CI, confidence interval; ECOG PS, Eastern Cooperative Oncology Group performance status; HR, hazard ratio; PD-L1, programmed death-ligand 1; TPS, tumor proportion score.

intratumor heterogeneity and assay- or platform-specific variables.[21] Notably, our results revealed that PD-L1 staining with 22C3 was strongly correlated with the efficacy of combined chemoimmunotherapy. A previous study revealed that high PD-L1 expression determined using the SP142 assays corresponded to very high PD-L1 expression in other assays and strongly correlated with the efficacy of ICI monotherapy.[8] Another study revealed that the 22C3 and SP142 assays can predict the efficacy of ICI monotherapy to the same extent.[22,23] Nevertheless, high PD-L1 expression determined using the SP142 assay was weakly correlated with the efficacy of combined chemoimmunotherapy in this study. This finding could be attributed to the small number of specimens and the limited number of patients with high PD-L1 expression determined using the SP142 test or the different clinical roles of ICI monotherapy and combined chemoimmunotherapy. On the basis of our findings, PD-L1 expression determined using the 22C3 assay might be a stronger predictive marker than that determined using the 28-8 and SP142 assays in patients with NSCLC treated with combined chemoimmunotherapy; however, further studies are required to determine useful PD-L1 IHC assay(s).

Our study has certain limitations. First, the sample size was small. In particular, PD-L1 expression determined using the SP142 assay may have revealed a weak correlation with treatment response owing to the limited number of specimens. Second, different pathologists evaluated the 22C3, 28-8, and SP142 assay results. TC PD-L1 expression has been reported to have a high interassay concordance rate. Although the staining concordance rate among the PD-L1 IHCs in this study was similar to that in a previous study, the results of this study should be carefully interpreted, and further large cohort studies are needed. Third, the expertise of each pathologist for different PD-L1 assays is a limitation. The pathologists evaluate PD-L1 expression using the SP142

assay in routine practice; however, the assessment of PD-L1 in the routine care of NSCLC is carried out on a commercial basis using the 22C3 assay, suggesting that their evaluation of the SP142 assay results might be more accurate than that of other assay results. Moreover, discordance was observed between the pathologists who assessed the immunostaining for anti–PD-L1 antibodies. Fourth, the follow-up period was too short for OS evaluation, although the PFS period was relatively adequate. OS could not be evaluated sufficiently owing to the limited follow-up period and small number of patients. Finally, PD-L1 expression was determined using the 22C3 assay in routine care in Japan before enrollment, suggesting that the inclusion of patients with high PD-L1 expression receiving ICI monotherapy might introduce bias in patient selection.

In conclusion, our prospective observations revealed that PD-L1 expression determined using the 22C3 assay was correlated with combined chemoimmunotherapy effects in patients with NSCLC. These results could serve as an additional resource for clinicians to select the most appropriate PD-L1 IHC assay for patients with advanced NSCLC on combined chemoimmunotherapy.

CRediT Authorship Contribution Statement

Yuki Katayama: Software, Validation, Investigation, Visualization, Writing—original draft.

Tadaaki Yamada: Conceptualization, Resources, Writing—original draft, Project administration, Methodology, Funding acquisition.

Kenji Morimoto: Data curation.

Hiroyuki Fujii: Resources.

Satomi Morita: Investigation.

Keiko Tanimura: Resources.

Takayuki Takeda: Resources.

Asuka Okada: Resources.

Shinsuke Shiotsu: Resources.

Yusuke Chihara: Resources.

Osamu Hiranuma: Resources.

Takahiro Yamada: Resources.

Takahiro Ota: Resources.

Taishi Harada: Resources.

Isao Hasegawa: Resources.

Masahiro Iwasaku: Validation.

Shinsaku Tokuda: Validation.

Noriyuki Tanaka: Investigation, Resources.

Aya Miyagawa-Hayashino: Investigation, Resources.

Koichi Takayama: Supervision.

Disclosure

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Data Statement

The data supporting the study findings are available on request from the corresponding author.

Supplementary Data

Note: To access the supplementary material accompanying this article, visit the online version of the *JTO Clinical and Research Reports* at www.jtocrr.org and at https://doi.org/10.1016/j.jtocrr.2024.100644.

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