Detection of programmed cell death-ligand 1 using 22C3 antibody in patients with unresectable stage III non-small cell lung cancer receiving chemoradiotherapy

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ORIGINAL ARTICLE

Detection of programmed cell death-ligand 1 using 22C3 antibody in patients with unresectable stage III non-small cell lung cancer receiving chemoradiotherapy

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Abstract

Background The expression of programmed cell death-ligand 1 (PD-L1) is a biomarker for administering immune check point inhibitors in patients with advanced stage non-small cell lung cancer. Although the consolidation therapy of durvalumab after definitive chemoradiotherapy has become the new standard of care for patients with unresectable stage III non-small cell lung cancer, the prevalence and prognostic role of PD-L1 expression in this population remain unclear.

Methods We retrospectively reviewed data from patients with unresectable stage III non-small cell lung cancer who received definitive chemoradiotherapy at our institution between 2012 and 2017. Levels of PD-L1 were assessed using 22C3 antibody, and associations of progression-free and overall survival rates with PD-L1 statuses at a tumor proportion score cutoff of 1% were analyzed.

Results Among the 104 patients enrolled, PD-L1 statuses were as follows: tumor proportion score < 1%, 73 (70.2%); 1–49%, 21 (20.2%); and ≥ 50%, 10 (9.6%). The number of patients with stage III non-small cell lung cancer with pretreatment PD-L1 tumor proportion score ≥ 1% was less than the number with advanced stage disease. There was no association between patient characteristics and PD-L1 status, and no significant differences were observed in progression-free and overall survival rates relative to PD-L1 status.

Conclusion Expression of PD-L1 in patients with stage III non-small cell cancer before chemoradiotherapy should be assessed because of the low prevalence of tumors with tumor proportion scores ≥ 1%. Further studies are needed to clarify whether durvalumab improves survival after definitive chemoradiotherapy, irrespective of tumor PD-L1 expression.

Keywords Chemoradiotherapy · Immunohistochemistry · PD-L1 expression · Unresectable stage III non-small cell lung cancer

Introduction

Non-small cell lung cancer (NSCLC) is the most common cause of cancer-related deaths, and half of the diagnosed patients have locally advanced disease at the time of diagnosis [1]. Among locally advanced stage III NSCLC, 30–50% of patients have been reported to have inoperable disease [2], and definitive chemoradiotherapy (CRT) with platinum-based doublet regimens remains the standard of care for these patients [3–5]. Recently, the phase 3 of the PACIFIC trial revealed that consolidation therapy with the anti-programmed cell death-ligand 1 (PD-L1) antibody, durvalumab, prolonged progression-free survival (PFS) and

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overall survival (OS) in patients with unresectable stage III NSCLC who did not experience disease progression after 2 or more cycles of platinum-based CRT [6, 7]. Based on these data, the consolidation therapy of durvalumab after definitive CRT has become the new standard of care for patients with unresectable stage III NSCLC.

PD-L1 status has emerged as a predictive biomarker for administering immune checkpoint inhibitors (ICIs), such as those targeting programmed cell death-1 (PD-1) or PD-L1, to patients with stage IV NSCLC [8–10]. However, the pretreatment expression status of PD-L1 was not an inclusion criterion in the PACIFIC trial [6]. Nonetheless, post hoc analyses of the PACIFIC trial reported that in patients with PD-L1 expression less than 1%, durvalumab consolidation therapy after definitive CRT did not improve OS [11]. Therefore, it is still inconclusive whether pretreatment tumor PD-L1 expression can be used as a biomarker for the administration of consolidation therapy with durvalumab in patients with unresectable stage III NSCLC. Furthermore, to date, the expression of PD-L1 in these patients has not been fully investigated.

Based on the above, we conducted this study to investigate the prevalence and prognostic role of pretreatment PD-L1 protein expression using the 22C3 antibody in patients with unresectable stage III NSCLC who underwent definitive CRT without durvalumab as consolidation therapy.

Patients and methods

Study design

We retrospectively reviewed records from patients with histologically confirmed unresectable stage III NSCLC who received definitive CRT without durvalumab as consolidation therapy at Shizuoka Cancer Center between April 2012 and March 2017. Unresectable stage III NSCLC was defined according to the Union International for Cancer Control (UICC)-TNM 8th edition [12]. Tumor PD-L1 expression was measured in formalin-fixed paraffin-embedded (FFPE) tumor tissue samples collected for initial diagnosis. We prepared tumor tissue sections for PD-L1 immunostaining in August 2017. To ensure PD-L1 stainability, we investigated PD-L1 expression status in patients whose tumor tissue had been collected and formalin-fixed after April 2012. Patients whose tumor tissue samples were not stored at the institution were excluded, as were the patients who did not have adequate tissue specimens for evaluating a minimum of 100 cancer cells on the tissue sections. The present study was conducted in accordance with the guidelines of the Declaration of Helsinki (as revised in Fortaleza, Brazil, October 2013), and was approved by the Institutional Review Board of Shizuoka Cancer Center.

Immunohistochemical analysis

Tumor tissue sections (4 μm) were mounted on glass slides and PD-L1 expression was assessed by Dako's companion diagnostic test (PD-L1 IHC 22C3 pharmDx; Dako North America, Carpinteria, CA) on the Dako Autostainer Link 48 platform, which is commercially used for diagnostic tests. All stained slides were evaluated for PD-L1 membrane staining by the commercial vendor's pathologists (SRL, Inc.). PD-L1 protein expression was determined using tumor proportion score (TPS), the percentage of viable tumor cells that showed partial or complete membrane staining for PD-L1. Levels of PD-L1 expression were divided into three categories: negative (TPS < 1%), weakly positive (TPS 1–49%), and strongly positive (TPS ≥ 50%).

Patient characteristics

Baseline patient characteristics data, including sex; age at diagnosis; Eastern Cooperative Oncology Group-performance status (ECOG-PS); smoking history; sampling methods; histology; driver mutation status; clinical stage according to the UICC-TNM 8th edition; chemotherapy regimen; best response to treatment; and blood tests were retrospectively obtained from electronic medical records. Smoking history was assessed as the cumulative amount of smoking using the Brinkman Index (the number of cigarettes smoked per day multiplied by the number of years of smoking) [13]. Treatment response was defined according to the 'Response Evaluation Criteria in Solid Tumors (RECIST)' version 1.1 as assessed by computed tomography [14].

Statistical analyses

We examined the prevalence of PD-L1 protein expression and compared clinical baseline characteristics according to PD-L1 status using the Pearson chi-square test or Fisher's exact test for categorical variables and the Wilcoxon rank-sum test for continuous variables. PFS was defined as the interval between the date of the initial CRT and the date of detection of disease progression, death, or the last follow-up visit. OS was defined as the interval between the date of the initial CRT and the date of death or the last follow-up visit. Patients who remained alive were censored. We compared PFS and OS according to PD-L1 protein expression status at TPS cutoff of 1%. PFS and OS curves were constructed using the Kaplan–Meier method, and the groups were compared according to their PD-L1 statuses using log-rank tests. A multivariate logistic regression and Cox proportional hazards model were used to assess potential associations between survival benefit and patient characteristics,

including PD-L1 status. All *p* values are two-sided, and a value of < 0.05 was considered statistically significant. All statistical analyses were performed using the statistical software JMP® 13 (SAS Institute Inc., Cary, NC, USA).

Results

Patient characteristics

Figure 1 shows the flowchart for patient enrollment in this retrospective study. Among all 147 screened patients with stage III NSCLC who were scheduled to receive definitive radiation therapy with or without chemotherapy, 43 were excluded due to not having any preserved tissues at Shizuoka Cancer Center ($n = 27$); undergoing definitive radiotherapy without chemotherapy because of old age at initial treatment ($n = 5$), poor performance status ($n = 3$), or complicating comorbidities ($n = 1$); and inadequate tissue samples for PD-L1 immunohistochemical staining ($n = 7$). Finally, 104 patients were eligible and enrolled. All patients were treated with at least two cycles of platinum-based chemotherapies without durvalumab as a consolidation therapy. Thoracic radiotherapy was delivered at 60 Gy in 30 daily fractions, and was performed concurrently for all patients.

The baseline characteristics of the 104 unresectable stage III NSCLC patients are summarized in Table 1. The study population comprised 84 male and 20 female patients. The median age of patients at diagnosis was 65 years (range 40–82 years). Fifty-eight percent of patients had ECOG-PS 0, 21% had ECOG-PS 1, and 1% had ECOG-PS 2. 96 (92%) patients had a history of smoking, and 86 (83%) patients were heavy smokers (Brinkman index ≥ 400). Patients were diagnosed with stage IIIA (38%), IIIB (48%), and IIIC (14%) according to the UICC-TNM 8th edition. Overall, 82 (79%) samples were obtained transbronchially and 21 (20%) percutaneously. The biopsy sites of these tissue samples were the primary site in 83 (80%) cases and lymph nodes in 21(20%) cases. The median storage time for the archived FFPE tumor tissue samples was 42.0 months (range 5.4–68.4 months). The lung cancer histological types included 61 adenocarcinomas, 34 squamous cell carcinomas, and 9 diagnosed as other types. Seventy-two patients (69%) were examined for driver mutations, which were positive in 7 (6 had *EGFR* mutations and 1 showed *ALK* gene rearrangement).

Expression status of PD-L1

Representative PD-L1 staining patterns in the tumor specimens according to TPS are shown in Fig. 2. Figure 3 shows the prevalence of PD-L1 protein expression status in all patients (a) and according to histology (b, c). Of the 104 lung tumor specimens analyzed, 73 (70%), 21 (20%), and 10 (10%) patients showed PD-L1 expression status at TPS < 1%,

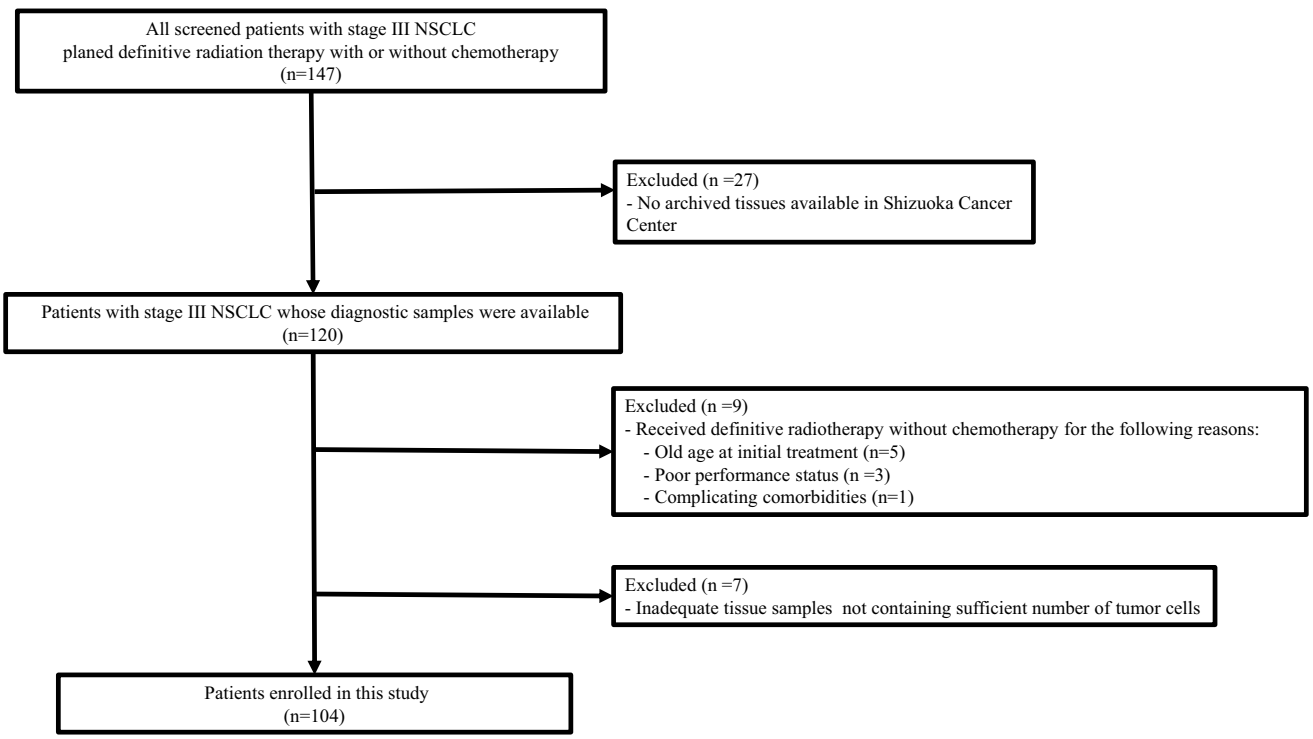

Fig. 1 Flow diagram of all screened patients with stage III NSCLC who received definitive radiotherapy. *NSCLC* non-small cell lung cancer

Table 1 Patient characteristics according to PD-L1 status at TPS cutoff of 1%

	All patients, $n=104$	TPS < 1%, $n=73$	TPS ≥ 1%, $n=31$	p value
Age, years				0.71
Median (range)	65 (40–82)	65 (46–81)	66 (40–82)	
Gender				0.60
Male	84 (81%)	58	26	
Female	20 (19%)	15	5	
Performance status				0.62
0	61 (59%)	41	20	
1	42 (40%)	31	11	
2	1 (1%)	1	0	
Smoking history				0.18
Smoker	96 (92%)	58	28	
BI ≥ 400	86 (83%)	15	3	
BI < 400*	18 (17%)			
Stage				0.18
IIIA	39 (38%)	24	15	
IIIB	50 (48%)	36	14	
IIIC	15 (14%)	13	2	
Histology				0.37
Ad	61 (59%)	46	15	
Sq	34 (33%)	21	13	
Other	9 (9%)	6	3	
Sampling method				0.76
Transbronchial biopsy	82 (79%)	58	24	
Forceps	65	45	20	
Needle aspiration	17	13	4	
Percutaneous methods	21 (20%)	14	7	
CT guided	14	10	4	
Sonography-guided	4	3	1	
Surgical resection	3	1	2	
Other	1 (1%)	1	0	
Biopsy site				0.89
Primary	83 (80%)	58	25	
Lymph node	21 (20%)	15	6	
Storage period, months				0.77
Median (range)	42.0 (5.4–68.4)	42.1 (5.4–66.1)	41.2 (5.5–68.4)	
The driver mutation status				0.10
Positive	7 (7%)	6	1	
EGFR mutation	6	5	1	
ALK rearrangement	1	1	0	
Negative	65 (63%)	49	16	
Unknown	32 (31%)	18	14	
Chemotherapy regimens				0.19
CDDP based	71 (68%)	47	71	
CBDCA based	33 (32%)	26	33	
Pretreatment WBC, /μL				0.30
Median (IQR)	7435 (6262–8982)	7290 (6095–8840)	7580 (6610–9330)	
Pretreatment Neut, /μL				0.27
Median (IQR)	4988 (3928–6196)	4704 (3873–6308)	5223 (4609–6009)	
Pretreatment Alb, g/dL				0.16
Median (IQR)	3.9 (3.6–4.2)	3.9 (3.6–4.2)	4.0 (3.7–4.4)	

Table 1 (continued)

	All patients, $n=104$	TPS < 1%, $n=73$	TPS ≥ 1%, $n=31$	p value
Pretreatment LDH, U/L				0.86
Median (IQR)	189 (164–226)	192 (164–227)	183 (161–219)	
Pretreatment CRP, mg/dL				0.94
Median (IQR)	0.8 (0.1–2.9)	0.7 (0.2–2.6)	0.9 (0.1–4.3)	
Best response to treatment				0.29
CR	7	6	1	
PR	66	42	24	
SD	26	21	5	
PD	5	4	1	

PD-L1 programmed cell death-ligand 1, *TPS* tumor proportion score, *BI* Brinkman index, *CT* computed tomography, *EGFR* epidermal growth factor receptor, *ALK* anaplastic lymphoma kinase, *CDDP* cisplatin, *CBDCA* carboplatin, *WBC* white blood cell, *IQR* interquartile range, *Neut* neutrophils, *Alb* albumin, *LDH* lactate dehydrogenase, *CRP* C-reactive protein, *CR* complete response, *PR* partial response, *SD* stable disease, *PD* progressive disease

*Eight never-smokers were included

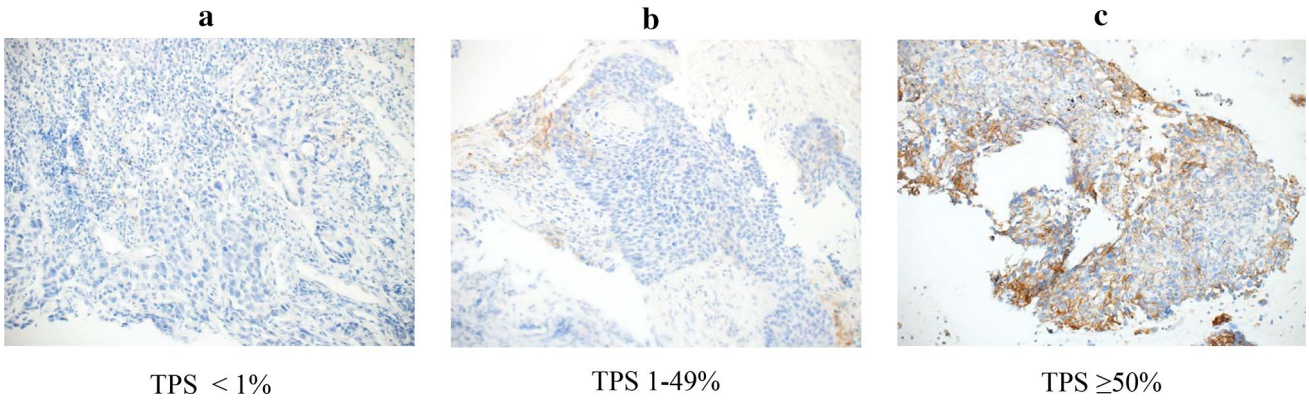

Fig. 2 Representative PD-L1-staining patterns in a tumor specimen. *PD-L1* programmed cell death-ligand 1, *TPS* tumor proportion score

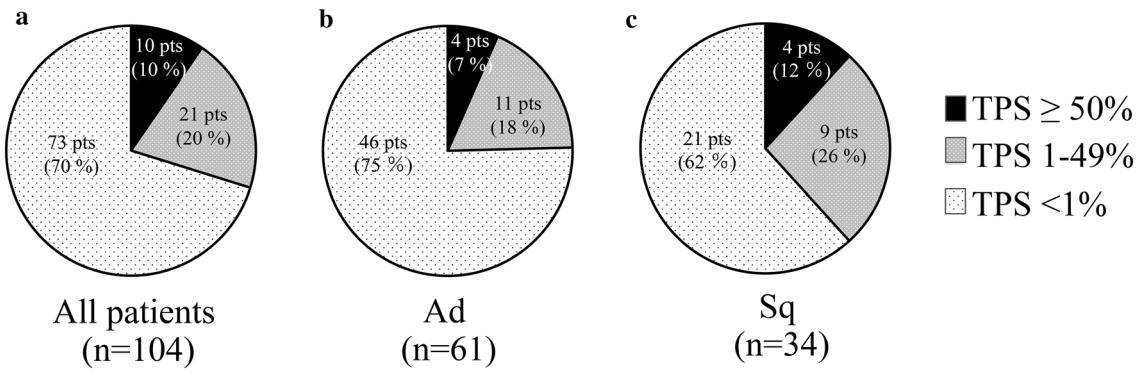

Fig. 3 The prevalence of PD-L1 protein expression status in all patients (**a**) and according to histology (**b**, **c**). *PD-L1* programmed cell death-ligand 1, *pts* patients, *TPS* tumor proportion score, *Ad* adenocarcinoma, *Sq* squamous cell carcinoma

1–49%, and ≥ 50%, respectively (Fig. 3a). When categorized by histological type, strongly positive PD-L1 expression (TPS ≥ 50%) was observed in 4 (7%) of 61 adenocarcinomas and 4 (12%) of 34 squamous cell carcinomas (Fig. 3b, c). Table 1 shows patients' characteristic in relation to PD-L1 expression at TPS cutoff of 1%. No statistically significant

differences were found between the TPS < 1% group and the TPS ≥ 1% group in any of the patient characteristics listed. The median storage times for the archived FFPE tissue samples did not result in any statistically significant differences between the TPS < 1% group and the TPS ≥ 1% group (42.1 vs 41.2 months, $p = 0.77$).

Treatment efficacy

Among the 104 patients, 63 (61%) had died by the study cutoff date (December 27, 2019). The median follow-up period for censored cases was 41.6 (range, 15.6–87.0) months. The objective response rates were 64.9% and 80.6% in patients with PD-L1 TPS < 1% and ≥ 1%, respectively. The Kaplan–Meier survival curves for PFS and OS are shown in Fig. 4. The median PFS was 11.4 months (95% confidence interval [CI], 8.9–15.4) in the TPS < 1% group and 10.9 months (95% CI, 8.3–17.3) in the TPS ≥ 1% group. No significant differences in PFS were observed relative to PD-L1 expression at the TPS cutoff of 1% (hazard ratio [HR] 1.17, 95% CI 0.74–1.81, log-rank $p = 0.49$) (Fig. 4a). Furthermore, the median OS was 36.8 (95% CI 31.0–50.7) months in the TPS < 1% group and 32.8 (95% CI 20.0–50.2) months in the TPS ≥ 1% group, respectively, which was not significantly different between the 2 groups (HR 1.26, 95% CI 0.73–2.09, log-rank $p = 0.40$) (Fig. 4b). Univariate and multivariate Cox hazard model analyses for PFS and OS are shown in Table 2. In the multivariate Cox hazard model analysis for PFS, the HR for smoking status (Brinkman index ≥ 400) was 0.53 (0.30–0.98, $p = 0.04$), and only smoking history, among patient characteristics, was associated with PFS. The hazard ratio for PD-L1 expression (TPS ≥ 1%) was 1.22 (95% CI 0.75–1.93, $p = 0.41$). In the multivariate Cox hazard model analysis for OS, there was no apparent predictor for OS among patient characteristics. The hazard ratio for the PD-L1 expression (TPS ≥ 1%) was 1.07 (95%CI 0.60–1.87, $p = 0.81$).

Treatment after relapse

Eighty-one (78%) patients experienced disease progression at the cutoff date (December 27, 2019). Of the 73 patients in the TPS < 1% group, 55 patients (75%) had tumor recurrence, whereas 26 of the 31 patients (84%) in the TPS ≥ 1% group experienced tumor recurrence. Among the 81 patients with relapse, the first recurrence site presented as distant metastasis in 45, locoregional relapse in 30, both distant and locoregional involvement in 6 patients. In the TPS < 1% group, distant relapse was observed in 36 patients (49%), whereas 15 patients (48%) demonstrated distant relapse in the TPS ≥ 1% group.

Fifty-seven (70%) of 81 patients with relapse received second-line chemotherapy. Four (67%) of 6 patients with *EGFR* mutation, who experienced relapse, received *EGFR* inhibitors, and 1 patient with *ALK* rearrangement received *ALK* inhibitors. Twenty (25%) of 81 relapsed cases received ICIs at any subsequent lines. Of the 73 patients in the TPS < 1% group, 12 patients (16%) were treated with ICIs, whereas 8 of the 31 patients (26%) in the TPS ≥ 1% group were treated with ICIs.

Discussion

In this retrospective study, we evaluated the prevalence of pretreatment PD-L1 expression in tumors from patients with unresectable stage III NSCLC who underwent definitive CRT without durvalumab as consolidation therapy, and observed a high frequency of the tumors in these patients had a TPS of < 1% compared to the levels found in patients

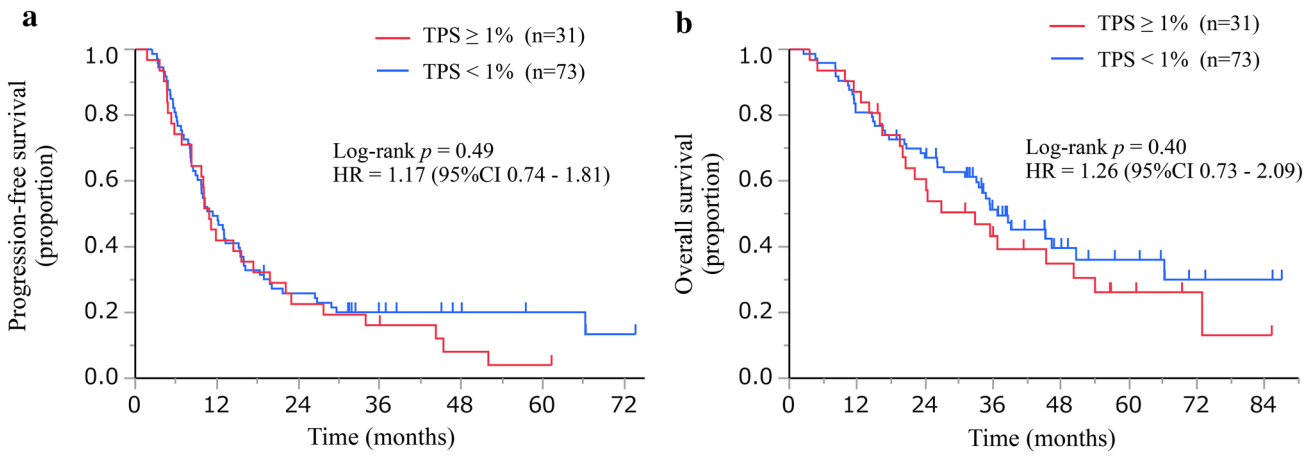

Fig. 4 Kaplan–Meier curves showing progression-free survival (**a**) and overall survival (**b**) according to PD-L1 protein expression status at the TPS cutoff of 1%. *PFS* progression-free survival, *PD-L1* programmed cell death-ligand 1, *TPS* tumor proportion score, *HR* hazard ratio

Table 2 Univariate and multivariate analysis for PFS and OS according to characteristics

Characteristics	PFS				OS			
	Univariate analysis		Multivariate analysis		Univariate analysis		Multivariate analysis	
	HR (95% CI)	*p* value	HR (95% CI)	*p* value	HR (95% CI)	*p* value	HR (95% CI)	*p* value
Age at diagnosis								
< 70	1.00 (Reference)	0.75	1.00 (Reference)	0.98	1.00 (Reference)	0.72	1.00 (Reference)	0.54
≥ 70	1.08 (0.68–1.67)		0.99 (0.61–1.58)		1.10 (0.64–1.86)		1.19 (0.67–2.04)	
Gender								
Male	1.00 (Reference)	0.97			1.00 (Reference)	0.56		
Female	0.98 (0.56–1.64)				1.20 (0.63–2.15)			
Performance status								
0	1.00 (Reference)	0.46	1.00 (Reference)	0.32	1.00 (Reference)	0.93	1.00 (Reference)	0.85
1–2	0.85 (0.55–1.30)		0.79 (0.49–1.25)		0.98 (0.59–1.61)		1.05 (0.60–1.80)	
Smoking history								
BI < 400	1.00 (Reference)	0.046	1.00 (Reference)	0.04	1.00 (Reference)	0.54	1.00 (Reference)	0.35
BI ≥ 400	0.56 (0.34–0.99)		0.53 (0.30–0.98)		0.81 (0.44–1.64)		0.71 (0.37–1.49)	
Histology								
Non-squamous	1.00 (Reference)	0.44	1.00 (Reference)	0.34	1.00 (Reference)	0.04	1.00 (Reference)	0.06
Squamous	1.19 (0.76–1.85)		1.27 (0.77–2.08)		1.72 (1.02–2.88)		1.75 (0.98–3.08)	
Stage								
IIIA	1.00 (Reference)	0.33	1.00 (Reference)	0.37	1.00 (Reference)	0.47	1.00 (Reference)	0.58
IIIB	1.41 (0.89–2.26)		1.35 (0.84–2.20)		1.21 (0.71–2.07)		1.15 (0.66–2.00)	
IIIC	1.30 (0.67–2.39)		1.49 (0.72–2.96)		0.74 (0.29–1.63)		0.73 (0.27–1.77)	
PD-L1 status								
< 1%	1.00 (Reference)	0.49	1.00 (Reference)	0.41	1.00 (Reference)	0.40	1.00 (Reference)	0.81
≥ 1%	1.17 (0.74–1.81)		1.22 (0.75–1.93)		1.26 (0.73–2.09)		1.07 (0.60–1.87)	
WBC								
< 7470	1.00 (Reference)	0.98			1.00 (Reference)	0.69		
≥ 7470	1.00 (0.65–1.52)				1.11 (0.67–1.82)			
LDH								
< 189	1.00 (Reference)	0.46			1.00 (Reference)	0.93		
≥ 189	0.85 (0.56–1.30)				1.02 (0.62–1.69)			
Alb								
< 3.9	1.00 (Reference)	0.50			1.00 (Reference)	0.37		
≥ 3.9	0.86 (0.57–1.32)				0.80 (0.48–1.31)			

PFS progression-free survival, *OS* overall survival, *HR* hazard ratio, *CI* confidence interval, *BI* Brinkman index, *PD-L1* programmed cell death-ligand 1, *WBC* white blood cell, *LDH* lactate dehydrogenase

with advanced stage disease. Furthermore, we also demonstrated that pretreatment PD-L1 expression status had no prognostic role in these patients.

The pivotal studies, KEYNOTE-189 [15] and 407 [16], in patients with previously untreated metastatic NSCLC demonstrated that 30.6–35.2% of patients had a PD-L1 TPS of < 1%. In contrast, 70% of patients with unresectable stage III NSCLC had tumors with TPS < 1% in this study. Several studies have reported the PD-L1 expression in early-stage NSCLC, including stage III (Table 3) [17–26]. As listed in Table 3, PD-L1 expression in tumors tended to be less frequent in early-stage NSCLC including stage III than in metastatic stage IV disease. A large retrospective cohort study investigating PD-L1 expression in surgically resected, stage I–III NSCLC showed PD-L1 positivity was more frequent in higher stages [24]. Most data of PD-L1 expression in Table 3 were obtained from surgically resected specimens, which are reportedly reliable in spite of tumor heterogeneity [27–29]. Moreover, there are two large studies that use the 22C3 antibody to evaluate tumor PD-L1 status in patients with early-stage operable NSCLC who have undergone surgical resection [25, 26]. The results of these two studies were similar to our study in the proportion of tumors with TPS > 1% [25, 26]. While, in the PACIFIC trial, the PD-L1 negative rate assessed by SP263 antibody was reported to be 33% of

Table 3 Previous studies on tumor PD-L1 protein expression in early-stage NSCLC, including stage III

Author	Year	Histology	Stage	Sample resource	Sample size All (stage III)	PD-L1 positive, *N* (%)	PD-L1 Antibody	IHC evaluation
Tokito [17]	2015	NSCLC	III	Biopsy	74 (74)	55 (74.3)	EPR1161 (Abcam)	≥ 5%
Schmidt [18]	2015	NSCLC	I–III	Surgical resection	321 (51)	77 (24.0)	E1L3N (CST)	≥ 5%
Tsao [19]	2017	NSCLC	I–IV	Surgical resection	982 (170)	314 (32.0) 204 (20.8) 141 (14.3)	E1L3N (CST)	≥ 1% ≥ 25% ≥ 50%
Huynh [20]	2016	Ad	I–IV	Surgical resection	261 (22)	129 (49.4) 95 (36.5) 62 (23.8)	E1L3N (CST)	≥ 1% ≥ 5% ≥ 50%
Inamura [21]	2016	Ad	I–IV	Surgical resection	268 (78)	43 (16.0)	E1L3N (CST)	≥ 5%
Takada [22]	2017	NSCLC	I–III	Surgical resection	499 (63)	189 (37.9) 119 (23.8) 71 (14.2) 39 (7.8)	SP142 (Ventana)	≥ 1% ≥ 5% ≥ 10% ≥ 50%
Wu [23]	2017	Ad	I–IV	Surgical resection	133 (42)	18 (13.5)	SP263 (Ventana)	≥ 25%
Keith [24]	2020	NSCLC	I–III	Surgical resection	2008 (447)	871 (43.4) 454 (22.6) 334 (16.6)	28–8 (Abcam)	≥ 1% ≥ 25% ≥ 50%
Cooper [25]	2015	NSCLC	I–III	Surgical resection	678 (NA)	191 (28.2) 50 (7.4)	22C3 (Merck)	≥ 1% ≥ 50%
Sun [26]	2016	NSCLC	I–IV	Surgical resection	1070 (201)	478 (44.7) 64 (6)	22C3 (Merck)	≥ 1% ≥ 50%
The present study	2020	NSCLC	III	Biopsy	104 (104)	31 (29.8) 10 (9.6)	22C3 (Merck)	≥ 1% ≥ 50%

NSCLC non-small cell lung cancer, *Sq* squamous cell carcinoma, *Ad* adenocarcinoma, *PD-L1* programmed cell death-ligand 1, *IHC* immunohistochemistry, *CST* cell signaling technology, *NA* not available

the evaluable population, which was different from our results [11]. These discrepancy in the prevalence of PD-L1 expression between studies, which may be due to differences in anti-PD-L1 antibodies, assays, platforms, and cutoff points. However, the Blueprint PD-L1 IHC Assay Comparison Project found that the extent and intensity of tumors stained for PD-L1 using 22C3, 28-8, and SP263 IHC assays were comparable [30]. So, we consider that the difference of PD-L1 antibodies may not be a main reason for the discrepancy in the prevalence of PD-L1 expression between the PACIFIC trial and our study. Notably, the time-related deterioration of PD-L1 staining is a major concern in our study [31], which may have been the cause of the differences between studies. To avoid this, only patients who had received CRT within 5 years prior to the time of examining the specimens for this study were included. Reanalyzing the data to include 35 patients whose specimens had been taken within three years of the study, did not change the proportion of patients with PD-L1 TPS ≥ 50% and TPS 1–49% tumor, both of which were still low (9% and 31%, respectively). Furthermore, a reanalysis of data containing 19 patients whose samples were taken within 1 year showed the same trend (TPS ≥ 50%, $n = 2$, 11% and TPS 1–49%, $n = 5$, 26%). Thus, we also consider that the difference in the prevalence of PD-L1 expression between the PACIFIC trial and our study may not be due to the time-related deterioration of PD-L1 staining.

We also showed that there was no association between PD-L1 expression status and therapeutic efficacy in patients with unresectable stage III NSCLC who underwent definitive CRT without durvalumab as consolidation therapy. Notably, PFS was significantly better for heavy smokers, but OS was not significantly different in our study. Contrary to our findings, smoking habits were reported to be an independent prognostic factor in patients with lung cancer [32]. It is unclear why our study results were favorable for PFS in heavy smokers. On the other hand, it has been reported that smoking status in NSCLC patients is not a prognostic factor for OS in Asian races, which might explain our results [33].

Regarding whether PD-L1 expression is a prognostic factor in patients with inoperable stage III NSCLC who receive concurrent CRT, only a couple of studies have been published. In one report, PD-L1 expression assessed by SP142 at the cutoff point of ≥ 5% was a negative prognostic factor for PFS and OS [34]. On the other hand, another study reported that PD-L1 expression assessed by anti-rabbit monoclonal antibody against PD-L1 (clone EPR1161) is not a prognostic factor [17], and this result supports our findings.

Recently, the PACIFIC trial demonstrates that durvalumab has a superior PFS advantage over placebo after CRT in patients with unresectable stage III NSCLC, regardless of their pretreatment PD-L1 expression levels [6]. The reason of this efficacy was explained by the hypothesis that radiation therapy could induce immunomodulatory changes in the tumor microenvironment which could affect the efficacy of immunotherapy [35–37]. Currently, the update results for the second primary endpoint of OS in PACIFIC trial were available [7]. Update analysis shows that the PFS benefit has translated to a significant OS prolongation. Moreover, the OS benefit of durvalumab was also observed in all the prespecified subgroups including pretreatment PD-L1 status assessed by SP263 at the cutoff point of 25%. However, exploratory post hoc subgroup analysis suggested that patients with tumor PD-L1 expression levels less than 1% would not benefit from OS due to durvalumab [7, 11]. However, no conclusion can be drawn, because the result was an exploratory post hoc analysis that excluded 36.7% of patients with unknown tumor PD-L1 expression. In addition, there is a lack of discussion about whether pretreatment PD-L1 expression status is a prognostic factor for definitive CRT without durvalumab as consolidation therapy.

Therefore, we believe our findings provide clues to the interpretation of the PACIFIC trial results as follows. In our study, the proportion of patients with PD-L1 TPS ≥ 1% tumors was low (30%), and there was no association between PD-L1 expression status at the cutoff of TPS 1% and patient characteristics of stage III NSCLC patients undergoing definitive CRT. In addition, there were no significant differences in PFS and OS according to PD-L1 expression status at the cutoff of TPS 1%. In other words, the baseline therapeutic effect of definitive CRT without durvalumab does not change significantly by pretreatment PD-L1 expression levels. Therefore, considering the results of the PACIFIC trial and our study, we should routinely evaluate PD-L1 expression in stage III NSCLC patients before CRT and accumulate clinical data whether durvalumab adds survival benefits after definitive CRT irrespective of tumor PD-L1 expression.

In this study, we focused on the potential importance of PD-L1 expression in pre-CRT as a biomarker for predicting the efficacy of durvalumab in patients receiving the current standard of care, durvalumab consolidation therapy. This is because data from the PACIFIC trial post hoc analysis showed that durvalumab did not affect OS prolongation in patients with PD-L1 TPS < 1%. A recent study has demonstrated that PD-L1 expression on tumor cells was significantly increased after CRT and there was no significant correlation between pre-CRT and post-CRT tumoral PD-L1 expression [38]. Because PD-L1 expression status is as a predictive biomarker for administering ICIs, further studies are warranted to evaluate whether pre- and post-CRT PD-L1 expression may be a biomarker for durvalumab consolidation therapy.

Our study had several limitations. First, it was a single-institution retrospective study. However, as shown in Table 3, we had a relatively large study population, despite focusing exclusively on patients with stage III NSCLC. Second, the tumor samples were small biopsy specimens and not surgically resected specimens, which can result in PD-L1 status mismatch among the samples [27, 29]. However, several prospective studies have shown that PD-L1 expression assessed by the 22C3 antibody is a useful predictive biomarker, even when assessed in small tissue biopsy specimens [8, 9, 39]. In addition, surgically resected samples are usually unavailable in this population during the diagnostic process, and so our study procedures are based on real-world settings. In spite of all the limitations, to the best of our knowledge, our study is the first to investigate the prognostic value of PD-L1 using 22C3 antibody in patients with inoperable stage III NSCLC who are receiving concurrent CRT.

In conclusion, the proportion of pretreatment PD-L1 TPS ≥ 1% in our samples was low, and we observed that pretreatment PD-L1 TPS ≥ 1%, as assessed by 22C3 antibody, is not a prognostic factor in patients with locally advanced unresectable stage III NSCLC receiving definitive CRT without durvalumab as consolidation therapy. PD-L1 expression in stage III NSCLC patients should be assessed prior to CRT, because it still remains inconclusive whether pretreatment expression of PD-L1 in tumor is a biomarker for durvalumab consolidation therapy. Further studies are needed to clarify whether or not durvalumab adds survival benefits after definitive CRT, irrespective of tumor PD-L1 expression in clinical settings.

Compliance with ethical standards

Conflict of interest This research did not receive any specific grant from funding agencies in the public, commercial, or not-for-profit sectors. NM reports grants and personal fees from Boehringer Ingelheim, personal fees from Boehringer Ingelheim, AstraZeneca KK, MSD Co., Ltd., Chugai Pharmaceutical Co.. HK reports personal fees from Eli Lilly K.K, and Taiho Pharmaceutical Co., Ltd.. SO reports personal fees from Chugai Pharmaceutical Co., Ltd., Ono Pharmaceutical Co., Ltd., Taiho Pharmaceutical Co., Ltd., MSD Co., Ltd., and Daiichi Sankyo Co., Ltd.. KW reports grants and personal fees from AstraZeneca KK, Chugai Pharmaceutical Co., Ltd., personal fees from Taiho Pharmaceutical, Boehringer Ingelheim, Eli Lilly K.K., Ono Pharmaceutical, and MSD Co., Ltd., grants from Novartis, and Abbvie. AO reports grants from Taiho Pharmaceutical, Ono Pharmaceutical, Chugai Pharmaceutical Co., Ltd., and Novartis Pharma K.K.. HK reports grants and personal fees from AstraZeneca KK, Pfizer Japan, Inc., Novartis Pharma K.K., and Chugai Pharmaceutical Co., Ltd., personal fees from Boehringer Ingelheim, Kyowa Hakko Kirin Co., Ltd., Pfizer K.K., Eli Lilly K.K, Bristol-Myers Squibb, Ono Pharmaceutical Co, Ltd., and MSD K.K., grants from Daiichi-Sankyo Co., Ltd.. TN reports grants from Ono Pharmaceutical Co., Ltd., Pfizer US, Inc., and Mochida Pharmaceutical Co. Ltd.. HM reports grants and personal fees from

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【主論文の和文要約】

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（化学放射線療法を受ける切除不能 III 期非小細胞肺がん患者における PD-L1 発現の検討）

【背景】

非小細胞肺がん（non-small cell lung cancer: NSCLC）は、がん関連死亡の最も大きな原因であり、診断された患者の半数は、診断時に局所進行した病変を有している[1]。局所進行の III 期 NSCLC のうち、30～50%の患者は手術不能であると報告されており[2]、これらの患者に対しては、白金製剤併用化学療法を用いた根治的化学放射線療法後に病勢進行を認めない切除不能の III 期 NSCLC 患者において、デュルバルマブによる地固め療法を行うことで、無増悪生存期間（progression-free survival: PFS）および全生存期間（Overall survival: OS）が延長することが示された[3, 4]。これらのデータに基づき、根治的化学放射線療法後のデュルバルマブによる地固め療法は、切除不能の III 期の NSCLC 患者に対する標準治療となった。IV 期 NSCLC 患者では、免疫チェックポイント阻害剤の効果予測バイオマーカーとして、programmed cell death-ligand 1（PD-L1）の発現状況が知られている[5-7]。しかし、PACIFIC 試験では、治療前の PD-L1 の発現状況は組み入れ基準とはなっていなかった[3]。それにもかかわらず、PACIFIC 試験の事後解析では、PD-L1 発現が 1%未満の患者において、根治的化学放射線療法後のデュルバルマブ併用療法は OS を改善しなかったと報告されている[8]。しかしながら、切除不能 III 期 NSCLC 患者において、治療前の腫瘍の PD-L1 発現をデュルバルマブによる地固め療法を行うためのバイオマーカーとして使用できるかどうかは、 まだ結論が出ていない。また、これまで切除不能 III 期 NSCLC 患者における PD-L1 の発現状況については十分に検討されていない。以上より、本研究では、デュルバルマブの地固め療法を併用しない根治的化学放射線療法を受けた切除不能 III 期 NSCLC 患者において、22C3 抗体を用いた治療前の PD-L1 発現の分布と予後との相関を検討した。

【対象患者と方法】

<u>研究デザイン</u>

2012 年 4 月から 2017 年 3 月までに静岡県立静岡がんセンターで、デュルバルマブの地固め療法を併用しない根治的化学放射線療法を受けた切除不能 III 期 NSCLC 患者を後方視的に検討した。初回診断時に採取したホルマリン固定パラフィン包埋（formalin-fixed paraffin-embedded: FFPE）腫瘍組織サンプルで腫瘍の PD-L1 発現を測定し評価した。腫瘍組織検体が研究所保管されていない患者や、組織切片上のがん細胞 100 個以上を評価するのに十分な組織検体を保有していない患者は除外した。本研究は、ヘルシンキ宣言のガイドラインに従って実施され，静岡がんセンターの倫理審査委員会の承認を得て実施した（IRB 承認番号：T29-16-29-1-5)。

免疫組織化学染色

腫瘍組織切片（4μm）をスライドガラスに載せ、診断検査用に市販されている Dako Autostainer Link 48 プラットフォームを用いたコンパニオン診断検査（PD-L1 IHC 22C3 pharmDx; Dako North America, Carpinteria, CA）を用いて腫瘍組織の PD-L1 発現状況を評価した。PD-L1 発現の評価は、商業用のコンパニオン診断検査会社（SRL, Inc.）によって実施したものを用いた。PD-L1 の発現は、PD-L1 の部分的または完全な膜染色を示した生存腫瘍細胞の割合である腫瘍割合スコア（Tumor proportion score: TPS）を使用して決定した。PD-L1 の発現レベルは、陰性（TPS ＜1%）、弱陽性（TPS 1-49%）、強陽性（TPS ≧50%）の 3 つのカテゴリーに分類した。

患者の特徴

性別、診断時年齢、Eastern Cooperative Oncology Group-performance status（ECOG-PS）、喫煙歴、組織のサンプリング方法、組織型、ドライバー変異の有無、Union International for Cancer Control（UICC）-TNM 第 8 版に基づく臨床病期、化学療法レジメン、治療に対する最良効果、血液検査などのベースライン患者の特性については、電子カルテ情報から取得した。

統計解析

研究対象内での PD-L1 の発現の分布を調べ、PD-L1 の発現状況に応じた臨床的ベースライン特性を、カテゴリー変数についてはピアソン・カイ二乗検定 またはフィッシャーの正確検定を用いて、連続変数についてはウィルコクソン・ランク和検定を用いて比較した。PFS は、根治的化学放射線治療開始日から疾患進行の確認日、死亡日、または最終受診日までの期間と定義した。OS は、根治的化学放射線治療開始日から死亡または最終受診日までの期間と定義した。カットオフ日の時点で生存していた患者は打ち切りとした。TPS カットオフ値 1%として PD-L1 発現別に患者集団を分け、PFS と OS を比較した。PFS と OS の曲線は Kaplan-Meier 法で解析し、log-rank 検定で PD-L1 発現別に群間比較を行った。多変量ロジスティック回帰と Cox 比例ハザードモデルを用いて、生存利益と患者特性との潜在的な関連性を評価した。

【結果】

対象患者

Figure 1（原本参照）に本研究の患者登録のフローチャートを示す。放射線治療を予定した III 期 N SCLC 患者 147 例のうち、静岡がんセンターに保存組織検体がない患者 27 例、放射線治療を単独で受けた患者（高齢 5 例、PS 不良 3 例、合併症 1 例のため）、および、残存した組織検体が PD-L1 免疫組織化学染色用として不十分であった患者 7 例の計 43 例が除外された。最終的に、104 例の患者が本研究の対象となった。対象患者は、少なくとも 2 サイクルの白金製剤併用化学療法を用いた根治的化学放射線療法を受け、地固め療法としてデュルバルマブを投与されなかった患者である。胸部放射線治療は、1 日 30 回分割で計 60Gy の放射線治療が計画され、全患者に対して化学療法と同時に照射された。切除不能 III 期 NSCLC 患者 104 例のベースラインの患者特性を Table1（原本参照）に示す。男性 84 名、女性 20 名で構成されており、診断時の年齢中央値は 65 歳（範囲 40-82 歳）、ECOG-PS 0 が 58%、ECOG-PS 1 が 21%、ECOG-PS 2 が 1%で、喫煙歴があったのは 86 例（83%）であった、UICC-TNM 第 8

版による病期分類では、IIIA 期（38%）、IIIB 期（48%）、IIIC 期（14%）であった。全体として、82 例（79%）の検体が経気管支的に、21 例（20%）の検体が経皮的に採取された。これらの組織検体の生検部位は、83（80%）例が原発部位、21（20%）例がリンパ節からの採取であった。保存された FFPE 腫瘍組織検体の保存期間の中央値は 42.0 ヶ月（範囲 5.4-68.4 ヶ月）であった。肺癌の組織型は、腺癌 61 例、扁平上皮癌 34 例、その他の組織型は 9 例であった。72 例（69%）でドライバー遺伝子変異の検索が行われており、7 例が陽性であった（6 例が EGFR 遺伝子変異、1 例が ALK 遺伝子再構成を示した)。 PD-L1 の発現状況別の代表的な PD-L1 染色パターンを Figure 2（原本参照）に示す。Figure 3（原本参照）は、全患者における PD-L1 発現状況の分布（Figure 3a）および組織型別の分布 （Figure 3b、3c）である。解析した 104 の肺腫瘍組織標本において、 PD-L1 発現状況：TPS＜1%、1～49%、50%以上は、73 例（70%）、21 例（20%）、10 例（10%）であった（Figure 3a)。また、組織型別では、PDL1 発現が強陽性（TPS≧50%）であったのは、腺癌 61 例のうち 4 例（7%）であり、扁平上皮癌では 34 例中 4 例（12%）であった（Figure 3b、c)。表 1 に、TPS のカットオフ値 1%として PDL1 発現別に患者の特徴を示した（TPS＜1%群と TPS≧1%群)。TPS＜1%群と TPS≧1%群との間には、いずれの患者特性においても統計学的な有意な差は認めなかった。保存された FFPE 組織サンプルの保存期間の中央値は、TPS＜1%群と TPS≧1%群の間で統計学的な有意な差は見られなかった（42.1 カ月対 41.2 カ月、p＝0.77)。

治療効果

全体 104 例のうち、63 例（61%）が研究のカットオフ日（2019 年 12 月 27 日）までに死亡していた。打ち切り症例の追跡期間中央値は 41.6 ヶ月（範囲、15.6-87.0）であった。PD-L1 TPS＜1%および≧1%の患者における客観的奏効割合は、それぞれ 64.9%および 80.6%であった。PFS と OS の生存曲線は Figure 4 に示す通りである。PFS 中央値は、TPS<1%群では 11.4 カ月（95%信頼区間[CI]、8.9-15.4)、TPS≧1%群では 10.9 カ月（95%CI、8.3-17.3）であった。TPS のカットオフ値 1%とした 2 群間では、PFS の統計学的な有意差は認められなかった（ハザード比[Hazard ratio: HR] 1.17, 95% CI 0.74-1.81, log-rank p = 0.49）(Figure 4a: 原本参照)。さらに、OS の中央値は、TPS＜1%群で 36.8 ヶ月（95%CI、31.0-50.7)、TPS≧1%群では 32.8 カ月（95%CI 20.0-50.2）であり、2 群間で有意差は認められなかった（HR 1.26、95%CI 0.73-2.09、log-rank p＝0.40）(図 4b)。PFS と OS の一変量および多変量 Cox 比例ハザードモデル解析結果を表 2 に示す。PFS の多変量 Cox 比例ハザードモデル解析では、喫煙状況（Brinkman index ≧400）の HR は 0.53（0.30-0.98、p = 0.04）で、患者特性のうち喫煙歴のみが PFS に関連していた。PDL1 発現（TPS≧1%）の HR は 1.22（95%CI 0.75-1.93、p＝0.41）であった。OS に関する多変量 Cox 比例ハザードモデル解析では、患者特性の中で OS の明らかな予測因子はなかった。PD-L1 発現（TPS≧1%）に関する HR は 1.07（95%CI 0.60- 1.87、p=0.81）であった。

再発後の治療

カットオフ日（2019 年 12 月 27 日）の時点で、81 例（78%）の患者が病勢進行を認めていた。TPS＜1%群の 73 例のうち、55 例（75%）が腫瘍の再発を認めていたのに対し 、TPS≧1%群の 31 例のうち 26 例 （84%）が腫瘍の再発を認めていた。再発した 81 例のうち、最初の再発部位は、遠隔転移が 45 例、局所再発が 30 例、遠隔転移と局所転移の両方が 6 例であった。TPS＜1%群では 36 例（49%）に

遠隔転移が認められ、TPS≧1%群 では 15 例（48%）に遠隔転移が認められた。 再発した 81 例中 57 例（70%）が二次化学療法を受けた。再発を経験した EGFR（Epidermal growth factor receptor）遺伝子変異を有する患者 6 例のうち 4 例（67%）が、EGFR 阻害剤を投与され、ALK（Anaplastic lymphoma kinase）遺伝子再構成を有する患者 1 例が ALK 阻害剤を投与された。再発した 81 例中 20 例（25%）が、その後のいずれかの治療ラインで免疫チェックポイント阻害薬を投与された。TPS＜1%群 73 例のうち、12 例（16%）が免疫チェックポイント阻害薬による治療を受け、TPS≧1%群 31 例のうち 8 例（26%）が免疫チェックポイント阻害薬による治療を受けていた。

【考察】

本研究では、切除不能 III 期 NSCLC において、デュルバルマブを併用しない根治的化学放射線療法を受けた患者の腫瘍における治療前の PD-L1 発現状況を評価し、TPS が 1%未満である患者の割合が、進行期 NSCLC 患者における分布よりも、より高い頻度であることが分かった。さらに、治療前の腫瘍における PD-L1 発現状況は、これらの患者の根治的化学放射線治療の予後を予測するものではないことが示された。前治療歴のない転移性 NSCLC 患者を対象とした重要な試験である KEYNOTE189 試験[9]と 407 試験[10]では、30.6～35.2%の患者が PD-L1 TPS が 1%未満であることが示されていた。一方 、本研究では、切除不能 III 期 NSCLC 患者においては、70%の患者が TPS＜1%を示した。いくつかの研究で、III 期を含む早期 NSCLC における PD-L1 発現状況について報告されている（表 3：原本参照）[11-20（原本では 17-26）]。表 3 に示すように、遠隔転移を有する IV 期 NSCLC よりも III 期を含む早期 NSCLC 患者においては、腫瘍における PD-L1 発現頻度は、低い傾向がある。外科的に切除された I-III 期の NSCLC における PD-L1 発現を調査した後方視的コホート研究では、PD-L1 の発現は、病期が進行する程、発現割合が上昇することが示された[18] 。表 3 の PD-L1 発現のデータのほとんどは外科的切除標本から得られたものであり、腫瘍の不均一性の観点から信頼性が高い報告である[21-23]。さらに、外科的切除を受けた手術可能な早期 NSCLC 患者において、腫瘍の PD-L1 の発現状況を評価するため、22C3 抗体を使用した 2 つの大規模研究があるが、TPS＞1%の腫瘍の割合において、我々の研究と同様であった[19, 20]。一方、PACIFIC 試験では、SP263 抗体で評価した PD-L1 陰性の割合は 33%と報告されており、我々の結果とは異なっていた[8]。これらの研究間の PD-L1 発現の分布の不一致は、染色に用いる PD-L1 抗体、アッセイ、プラットフォーム、カットオフポイントの違いに起因している可能性がある。しかし、Blueprint PD-L1 IHC Assay Comparison Project では、22C3、28-8、SP263 IHC アッセイを用いた PD-L1 による腫瘍の染色範囲と強度は同程度であることが示されている[24]。したがって、PD-L1 抗体の違いは、PACIFIC 試験と我々の研究の間の PD-L1 発現の分布の相違に大きな関与がないと考える。注目すべきは、PD-L1 の染色性に関して時間的な劣化であり[25]、これが研究間の差の原因になっている可能性がある。これを避けるために、本研究では、5 年以内に化学放射線療法を受けた患者のみを対象とした。さらに、検体を採取した時期が 3 年以内の患者 35 例のみを追加解析を行ったところ、PD-L1 TPS≧50%および TPS 1-49%の患者の割合は、やはりいずれも低かった（それぞれ 9%、31%）。さらに、1 年以内に採取された 19 例の患者の追加解析でも、同じ傾向が見られた（TPS≧50 %、n＝2、11%、TPS1-49%、n＝5、26%）。 したがって、PACIFIC 試験と我々の研究の間の PD-L1 発現の分布の違いについての原因として、時間経過による検体の染色性の悪化によるものではない可能性がある。また、本研究では切除不能 III 期 NSCLC 患者において、デュルバルマブを併用しない根治的化学放射線治療の治療効果と PD-L1 の発現状況に関連性がないことも明らかにした。手術不能 III 期

NSCLC で、同時に根治的化学放射線療法を受けた患者において、PD-L1 発現が予後因子であるかどうかについては、少数の研究で発表されている。ある報告では、 SP142 抗体で評価した PD-L1 発現を、カットオフ値 5%以上として、PFS と OS の予後を評価した有意差を示した[26]。一方、別の研究では、PD-L1 に対する抗ウサギモノクローナル抗体（クローン EPR1161）で評価した PD-L1 発現を検討しているが、予後因子ではないと報告しており [11]、 この結果は我々の知見を支持するものであった。

最近、PACIFIC 試験において、治療前の PD-L1 発現状況にかかわらず、切除不能 III 期 NSCLC 患者でデュルバルマブ地固め療法が根治的化学放射線療法後にプラセボに対して優れた PFS の延長を示すことが証明された[3]。この有効性の理由は、放射線療法が腫瘍の微小環境において免疫調節性の変化を誘発し、それが免疫療法の効果に影響を与える可能性があるという仮説によって説明されている[27-29]。現在では、PACIFIC 試験の第二の主要評価項目である OS のアップデート結果が示され、PFS の有効性および OS の有意な延長も示された[4]。さらに、デュルバルマブの OS 延長効果は、25%をカットオフ値として SP263 で評価した治療前の PD-L1 発現状況別を含む事前に規定されたすべてのサブグループ解析で観察された。しかし、探索的な事後解析では、腫瘍の PD-L1 発現状況が 1%未満の患者は、デュルバルマブによる OS の延長効果が認めないことが示唆された[4, 8]。しかし、この結果は、腫瘍の PD-L1 発現が不明な患者 36.7%を除外した探索的な事後分析であるため、結論を導くことはできない。また、治療前の PD-L1 発現状況が、デュルバルマブを併用しない根治的化学放射線療法の予後因子となるかどうかについては、これまでデータが不足していた。 したがって、今回の我々の研究結果では、PACIFIC 試験結果の解釈を補填するための手がかりを与えてくれるものと考える。我々の研究では、PD-L1 TPS 1%以上の患者割合は 30%と低く、TPS 1%のカットオフ値における PD-L1 発現状況と根治的化学放射線治療を受けた III 期 NSCLC 患者の患者特性の間には関連性は認められなかった。また、TPS 1%のカットオフ値における PD-L1 発現状況により、PFS および OS に有意差は認められなかった。つまり、デュルバルマブの地固め療法を行わない根治的化学放射線療法の治療効果は、治療前の PD-L1 発現状況により大きく変わることはないことを示した。したがって、III 期 NSCLC 患者において根治的化学放射線療法前の腫瘍の PD-L1 発現に関係なくデュルバルマブが根治的化学放射線療法の生存利益を追加するかどうかは、PACIFIC 試験の結果と我々の研究を参考に、臨床データのさらなる蓄積が必要があると思われる。最近の研究では、腫瘍細胞上の PD-L1 発現が化学放射線療法後に有意に増加することが示された[38]。PD-L1 の発現状況は免疫チェックポイント阻害薬投与の予測バイオマーカーとなる可能性があり、化学放射線療法前後の PD-L1 発現の違いを評価するためのさらなる研究も必要である。

本研究にはいくつかの限界があった。第一に、単一施設の後方視的研究であったことである。しかし、表 3 に示すように、III 期 NSCLC 患者のみに焦点を当てた研究としては、比較的大きな研究集団を得た。さらに、腫瘍標本は外科的切除標本ではなく微小な生検検体であったために腫瘍内の PD-L1 発現状態の不均一性を反映できていない可能性がある[21, 23]。一方で、いくつかの前向き研究により、22C3 抗体で評価した PD-L1 発現は、たとえ小さな腫瘍組織生検検体で評価した場合でも、有用な予測バイオマーカーであることが示されている[5, 6, 31]。さらに、この集団では、診断の過程で外科的切除検体は通常入手できないため、我々の研究手順は実際の臨床診断手順に基づくものである。これらの限界はあるが、我々の研究は、同時放射線治療を受けた手術不能 III 期 NSCLC 患者において、22C3 抗体を用いた PD-L1 発現と予後を調べた最初の研究である。

【結論】

我々の研究では、22C3 抗体で評価した治療前の PD-L1 TPS≧1%の割合は低く、治療前の PD-L1 TPS≧1%が、局所進行切除不能 III 期 NSCLC 患者における根治的化学放射線療法の予後因子ではなかった。治療前の腫瘍における PD-L1 発現がデュルバルマブ地固め療法のバイオマーカーとなるかどうかはまだ結論が出ていないが、III 期 NSCLC 患者における PD-L1 発現は、化学放射線療法前に評価されるべきである。実地臨床において、腫瘍の PD-L1 発現に関係なく、デュルバルマブが根治的化学放射線療法後の生存利益を追加するかどうかを明らかにするためには、さらなる研究が必要である。

【略語】

NSCLC：Non-small cell lung cancer

PFS：Progression-free survival

OS：Overall survival

PD-L1：Programmed cell death-ligand 1

FFPE：Formalin-fixed paraffin-embedded

TPS：Tumor proportion score

ECOG-PS：Eastern Cooperative Oncology Group-performance status

UICC：Union International for Cancer Control

EGFR：Epidermal growth factor receptor,

ALK：Anaplastic lymphoma kinase

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