ORIGINAL ARTICLE

Predictive Impact of Diffuse Positivity for TTF-1 Expression in Patients Treated With Platinum-Doublet Chemotherapy Plus Immune Checkpoint Inhibitors for Advanced Nonsquamous NSCLC

Yuto Terashima, MD,[a] Masaru Matsumoto, MD, PhD,[a,*] Hiroki Iida, MD,[a] Sae Takashima, MD,[a] Aya Fukuizumi, MD, PhD,[a] Susumu Takeuchi, MD, PhD,[a] Akihiko Miyanaga, MD, PhD,[a] Yasuhiro Terasaki, MD, PhD,[b] Kazuo Kasahara, MD, PhD,[a] Masahiro Seike, MD, PhD[a]

[a]*Department of Pulmonary Medicine and Oncology, Graduate School of Medicine, Nippon Medical School, Tokyo, Japan*
[b]*Department of Analytic Human Pathology, Graduate School of Medicine, Nippon Medical School, Tokyo, Japan*

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ABSTRACT

Introduction: Pervious studies reported the association of TTF-1 expression with the efficacy of platinum-doublet chemotherapy in combination with immune checkpoint inhibitors in advanced nonsquamous NSCLC. Nevertheless, the predictive value of extent of TTF-1 expression (diffuse or focal TTF-1 positivity) remains unclear.

Methods: The present study retrospectively reviewed 74 patients with TTF-1–positive recurrent or advanced nonsquamous NSCLC receiving first-line chemoimmunotherapy in a single institution in Japan. TTF-1 expression score in pretreatment tumor specimens was evaluated using immunohistochemistry, and the impact of chemoimmunotherapy response was analyzed.

Results: In the total cohort, ≥50% of the tumor cells were TTF-1 positive (i.e., diffusely TTF-1 positive) in specimens of 61 patients (82.4%), whereas 10% to 49% of the tumor cells were TTF-1 positive (i.e., focally TTF-1 positive) in specimens of the remaining 13 patients (17.6%). In multivariate analysis, the median progression-free survival and overall survival (OS) were significantly longer in patients with diffusely TTF-1–positive tumors than in those with focally TTF-1–positive tumors (14.2 versus 9.2 mo, $p = 0.01$ and 30.2 versus 17.3 mo, $p = 0.01$, respectively). Moreover, the median OS was significantly longer in patients receiving chemoimmunotherapy including pemetrexed than in those receiving chemoimmunotherapy not including pemetrexed among the patients with diffusely TTF-1–positive tumors (not attained versus 23.2 mo, $p < 0.01$).

Conclusions: The positive extent of diffuse TTF-1 expression associated with patient outcome was an independent predictive factor for better progression-free survival and OS in patients with advanced nonsquamous NSCLC receiving chemoimmunotherapy.

***Corresponding author.**

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Address for correspondence: Masaru Matsumoto, MD, PhD, Department of Pulmonary Medicine and Oncology, Graduate School of Medicine, Nippon Medical School, 1-1-5 Sendagi, Bunkyo-ku, Tokyo 113-8603, Japan. E-mail: s7062@nms.ac.jp

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Introduction

Lung cancer, including the histologic subtype of NSCLC, remains the leading cause of cancer-related mortality worldwide.[1,2] The standard therapeutic options for unresectable advanced or recurrent NSCLC have recently expanded by the development of immune checkpoint inhibitors (ICIs), including anti–programmed cell death protein-1, anti–programmed death-ligand 1 (anti–PD-L1), and anti–CTLA-4 antibodies. Several important clinical trials have revealed the clinical benefit and remarkably long-lasting responses in patients who received combination therapy with platinum-doublet chemotherapy plus ICIs (i.e., chemoimmunotherapy) compared with those who received chemotherapy alone.[3–7] Thus, currently, chemoimmunotherapy is a standard treatment regimen for advanced NSCLC.

TTF-1, which is expressed in 60% to 80% of nonsquamous NSCLCs,[8] is one of the most frequently used markers to diagnose the histologic type of lung cancer and to differentiate primary lung adenocarcinoma from other metastatic adenocarcinomas.[9–12] Several retrospective studies suggested that TTF-1 positivity was associated with chemoimmunotherapy efficacy in patients with nonsquamous NSCLC, particularly in those receiving regimens including pemetrexed.[13,14] Nevertheless, the association of chemoimmunotherapy efficacy with the extent of TTF-1 expression (diffuse or focal TTF-1 positivity), that is not on the presence or absence of TTF-1 expression, remains unclear.

In this retrospective study, we aimed to evaluate the predictive impact of extent of TTF-1 expression on chemoimmunotherapy response in patients with advanced nonsquamous NSCLC.

Materials and Methods

Patients

Between January 1, 2019, and December 31, 2022, 223 patients were diagnosed with recurrent or advanced nonsquamous NSCLC, 107 patients underwent first-line chemoimmunotherapy, and 104 patients underwent immunohistochemistry testing for TTF-1 in Nippon Medical School Hospital in Tokyo, Japan. Of the 104 patients, 74 patients with TTF-1–positive recurrent or advanced nonsquamous NSCLC were administrated first-line chemoimmunotherapy. The medical records of these patients were retrospectively reviewed to collect data on age, sex, histologic subtype, smoking history, Eastern Cooperative Oncology Group performance status score at chemoimmunotherapy initiation, driver mutation status, PD-L1 expression, and clinical stage. All study procedures were performed in accordance with the tenets of the Declaration of Helsinki (as revised in 2021). The study was approved by the Institutional Review Board of the Graduate School of Medicine Nippon Medical School (approval number B-2023-666). Informed consent was waived off because of the retrospective nature of this study, and an opt-out option was included.

Analysis of TTF-1 Expression

TTF-1 expression was determined using immunohistochemistry in 4- to 5-μm–thick paraffin-embedded tissue sections prepared from lung cancer tissue samples obtained during surgery, bronchoscopy, or computed tomography-guided biopsy at the time of diagnosis. Antigen retrieval was performed by autoclaving the sections at 95°C for 36 minutes in a citrate buffer (pH 8.5). The sections were incubated with a rabbit monoclonal anti–TTF-1 antibody (clone SP141; Roche, Basel, Switzerland) for 16 minutes at room temperature. Primary antibody binding was detected using the VENTANA ultra View DAB universal kit (Roche), staining was visualized using diaminobenzidine chromogen, and all sections were counterstained with hematoxylin. TTF-1 expression was evaluated on the basis of the nuclear staining of neoplastic cells and scored in the categories of $\geq$50%, 10% to 49%, and less than 10% by an experienced pathologist who was blinded to the clinical information of the patients. In previous studies, 10% TTF-1–positive tumor cells were identified as the best cutoff to distinguish nonsquamous lung cancer from squamous cell carcinoma using TTF-1 immunostaining with the SP141 clone.[9] Thus, in the present study, we also used 10% as the cutoff value for positive staining for TTF-1. Patients with less than 10% TTF-1–positive tumor cells, that is, those with TTF-1–negative tumors, were excluded from the study analyses. In addition, high PD-L1 expression was defined as a PD-L1 tumor proportion score (TPS) of $\geq$50%.[3–7] The study patients were categorized into the following two groups on the basis of the extent and pattern of TTF-1 expression as follows: diffusely TTF-1 positive, $\geq$50% TTF-1 positive (Fig. 1*A* and *B*); focally TTF-1 positive, 10% to 49% TTF-1 positive (Fig. 1*C* and *D*).

Statistical Analysis

Data on baseline patient characteristics were presented as numbers and percentages. Univariate analysis

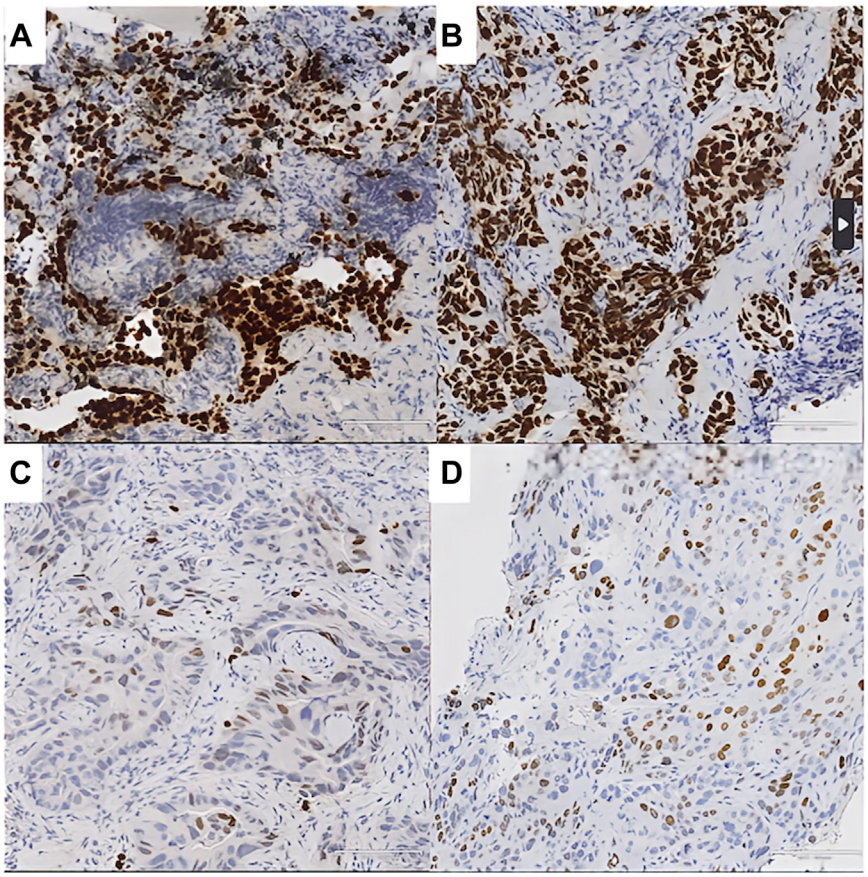

Figure 1. Immunohistochemical staining for TTF-1 with the SP141 clone in tumor biopsy specimens of patients with TTF-1–positive primary lung adenocarcinoma. (*A*, *B*) Lung adenocarcinoma with diffuse TTF-1 positivity, defined as ≥50% tumor cells positive for TTF-1. (*C*, *D*) Lung adenocarcinoma with focal TTF-1 positivity, defined as 10% to 49% tumor cells positive for TTF-1. Scale bars: 100 μm (*A*-*D*).

was performed using Fisher's exact test for categorical data. Differences in baseline patient characteristics and clinical response to chemoimmunotherapy were compared between the patients with diffusely and focally TTF-1–positive tumors. Progression-free survival (PFS) was defined as the time from chemoimmunotherapy initiation to the first documented disease progression or death. Overall survival (OS) was defined as the time from chemoimmunotherapy initiation to death regardless of the cause of death. Patients without disease progression or those who died at the time of analysis were censored at the date of last contact. PFS and OS curves were estimated using the Kaplan–Meier method, and differences between the groups were compared using the log-rank test and Cox proportional hazards regression analysis. Overall response rate (ORR) was defined as the proportion of patients with complete response or partial response as best overall response according to the Response Evaluation Criteria in Solid Tumors version 1.1.[15] The data of patients' outcome were presented as hazard ratios (HRs) with 95% confidence intervals (CIs), and a two-tailed *p* value of less than 0.05 was considered to indicate statistical significance. All statistical analyses were performed using EZR (Saitama Medical Center, Jichi Medical University, Saitama, Japan).[16]

Results

Patient Characteristics

The study cohort consisted of 74 patients with TTF-1–positive recurrent or advanced nonsquamous NSCLC receiving first-line chemoimmunotherapy. Table 1 reveals the pretreatment characteristics of all patients. The median patient age was 69.5 (range: 38–81) years, and 53 patients (71.6%) were male. Most of the patients (78.4%) had a smoking index ([number of cigarettes smoked per d] × [years spent smoking]/20) of ≥20, and 67 patients (90.5%) had an Eastern Cooperative Oncology Group performance status score of 0 or 1. Furthermore, 68 patients (91.9%) had adenocarcinoma, and six patients (8.1%) had nonadenocarcinoma such as large cell neuroendocrine carcinoma and not otherwise specified

Table 1. Baseline Patient Characteristics

Characteristics	Total (n = 74)	Diffuse TTF-1 Positivity (n = 61)	Focal TTF-1 Positivity (n = 13)	*p*
Median age (range), y	69.5 (38-81)	70 (38-81)	67 (47-79)	
Age group, y, n (%)				
<75	60 (81.1)	50 (82.0)	10 (76.9)	0.70
≥75	14 (18.9)	11 (18.0)	3 (23.1)	
Sex, n (%)				
Male	53 (71.6)	44 (72.1)	9 (69.2)	1.00
Female	21 (28.4)	17 (27.9)	4 (30.8)	
Smoking status, n (%) smoking index ≥ 20	58 (78.4)	48 (78.7)	10 (76.9)	1.00
ECOG PS score, n (%)				
0 or 1	67 (90.5)	55 (90.2)	12 (92.3)	1.00
≥2	7 (9.5)	6 (9.8)	1 (7.7)	
Histologic subtypes, n (%)				
Adenocarcinoma	68 (91.9)	58 (95.1)	10 (76.9)	0.063
Nonadenocarcinoma	6 (8.1)	3 (4.9)	3 (23.1)	
Stage, n (%)				
IV	63 (85.1)	53 (86.9)	10 (76.9)	0.53
III	4 (5.4)	3 (4.9)	1 (7.7)	
Recurrent	7 (9.5)	5 (8.2)	2 (15.4)	
PD-L1 expression, n (%)				
≥50%	18 (24.3)	17 (27.9)	1 (7.7)	0.28
1%-49%	18 (24.3)	15 (24.6)	3 (23.1)	
<1%	29 (39.2)	23 (37.7)	6 (46.1)	
Unknown	9 (12.2)	6 (9.8)	3 (23.1)	
EGFR or *ALK* mutation status, n (%)				
Positive	7 (9.5)	7 (11.5)	0 (0)	0.34
Negative or unknown	67 (90.5)	54 (88.5)	13 (100)	
Chemotherapy regimen, n (%)				
Platinum + pemetrexed + pembrolizumab	42 (56.7)	36 (59.0)	6 (46.1)	0.72
Carboplatin + pemetrexed + atezolizumab	6 (8.1)	5 (8.1)	1 (7.7)	
Platinum + pemetrexed + nivolumab + ipilimumab	6 (8.1)	4 (6.6)	2 (15.4)	
Carboplatin + nab-paclitaxel + atezolizumab	5 (6.8)	4 (6.6)	1 (7.7)	
Carboplatin + paclitaxel + bevacizumab + atezolizumab	15 (20.3)	12 (19.7)	3 (23.1)	
Response assessment				
CR	1 (1.4)	1 (1.6)	0 (0.0)	
PR	30 (40.5)	24 (39.4)	6 (46.2)	
SD	34 (45.9)	27 (44.3)	7 (53.8)	
PD	3 (4.1)	3 (4.9)	0 (0.0)	
NA	6 (8.1)	6 (9.8)	0 (0.0)	

CR, complete response; ECOG PS, Eastern Cooperative Oncology Group performance status; NA, not available; PD, progressive disease; PD-L1, programmed death-ligand 1; PR, partial response; SD, stable disease.

carcinoma. On the basis of the eight edition of the TNM classification for lung cancer, 63 (85.1%), four (5.4%), and seven patients (9.5%) had stage IV, stage III, and recurrent cancer, respectively. The PD-L1 expression status was known in 65 patients (87.8%), and 36 (48.6%) of these patients had a PD-L1 TPS of ≥1%. Seven patients (9.5%) of 74 were positive for EGFR or ALK mutation, and 63 patients (89.2%) were negative for EGFR and ALK mutation, but the result was unknown for one patient (1.3%). Regarding chemotherapy, 54 (73.0%), 20 (27.0%), and 15 patients (20.3%) received regimens containing pemetrexed, paclitaxel or nab-paclitaxel, and bevacizumab, respectively. Regarding ICIs, 42 (56.8%), 26 (35.1%), and six patients (8.1%) received regimens containing pembrolizumab, atezolizumab, and nivolumab plus ipilimumab, respectively.

Association of the Extent of TTF-1 Expression With Chemoimmunotherapy Efficacy

The patients were categorized as those with diffusely and focally TTF-1–positive tumors (n = 61 [82.4%] and n = 13 [17.6%], respectively). The median PFS was significantly better in patients with diffusely TTF-1–

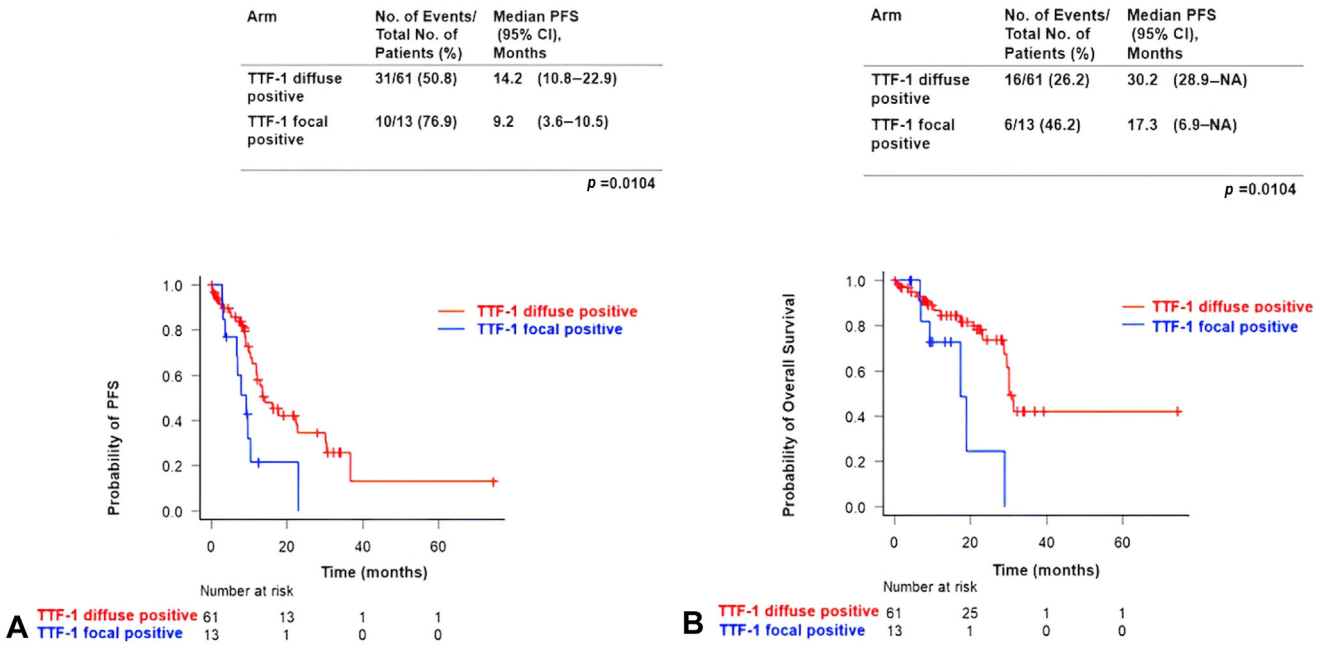

Figure 2. Estimated Kaplan-Meier survival curves for (*A*) PFS and (*B*) overall survival comparing patients with diffusely TTF-1-positive tumors (n = 61) and those with focally TTF-1-positive tumors (n = 13). CI, confidence interval; NA, not available; PFS, progression-free survival.

positive tumors than in those with focally TTF-1–positive tumors (14.2 [95% CI: 10.8–22.9] mo versus 9.2 [95% CI: 3.6–10.5] mo; $p = 0.01$; Fig. 2*A*). Similarly, the median OS was significantly better in patients with diffusely TTF-1–positive tumors than in those with focally TTF-1–positive tumors (30.2 [95% CI: 28.9–not attained (NA)] mo versus 17.3 [95% CI: 6.9–NA] mo; $p = 0.01$; Fig. 2*B*).

The univariate analysis revealed that PFS was significantly associated with the extent of TTF-1–positive expression (diffuse versus focal) (HR = 0.39, 95% CI: 0.19–0.82, $p = 0.014$; Table 2). Similarly, the multivariate analysis revealed a significant association between PFS and the extent of TTF-1–positive expression (diffuse versus focal) (HR = 0.42, 95% CI: 0.18–0.99, $p = 0.047$; Table 2). The univariate analysis revealed that OS was significantly associated with the extent of TTF-1–positive expression (diffuse versus focal) (HR = 0.29, 95% CI: 0.11–0.79, $p = 0.016$; Table 3). The multivariate analysis also revealed a significant association between OS and the extent of TTF-1–positive expression (diffuse versus focal) (HR = 0.27, 95% CI: 0.087–0.86, $p = 0.026$; Table 3).

Table 2. Univariate and Multivariate Analysis of Progression-Free Survival

	Univariate Analysis			Multivariable Analysis		
Factor	HR	95% CI	*p*	HR	95% CI	*P*
TTF-1 expression (diffuse vs. focal)	0.39	0.19-0.82	0.014	0.42	0.18-0.99	0.047
Age (<75 vs. ≥75 y)	0.94	0.50-1.78	0.86	1.13	0.53-2.44	0.75
Smoking status (smoking index, <20 vs. ≥20)	0.93	0.52-1.67	0.81	1.05	0.49-2.23	0.90
ECOG PS score (<2 vs. ≥2)	0.66	0.31-1.40	0.28	0.47	0.16-1.41	0.18
Stage (IV vs. III or relapse)	0.75	0.36-1.58	0.45	1.13	0.43-2.94	0.81
PD-L1 TPS (≥50% vs. other)	0.54	0.25-1.20	0.13	0.59	0.21-1.68	0.32
EGFR or *ALK* mutation status (positive vs. negative or unknown)	0.67	0.21-2.15	0.50	1.02	0.29-3.57	0.98

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

Table 3. Univariate and Multivariate Analysis of Overall Survival

	Univariate Analysis			Multivariable Analysis		
Factor	HR	95% CI	*p*	HR	95% CI	*p*
TTF-1 expression score (diffuse vs. focal)	0.29	0.11-0.79	0.016	0.27	0.087-0.86	0.026
Age (<75 vs. ≥75 y)	0.67	0.26-1.74	0.41	0.46	0.13-1.63	0.23
Smoking status (smoking index, <20 vs. ≥20)	0.82	0.37-1.84	0.64	1.10	0.37-3.26	0.87
ECOG PS score (<2 vs. ≥2)	0.94	0.33-2.72	0.92	0.73	0.15-3.54	0.70
Stage (IV vs. III or relapse)	0.86	0.30-2.46	0.78	0.85	0.20-3.56	0.82
PD-L1 TPS (≥50% vs. other)	0.63	0.19-2.07	0.44	0.83	0.17-4.14	0.82
EGFR or *ALK* mutation status (positive vs. negative or unknown)	0.46	0.06-3.40	0.45	0.61	0.073-5.09	0.65

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

Finally, the median ORR was not significantly different between the patients with diffusely TTF-1–positive tumors and those with focally TTF-1–positive tumors (41.0% [95% CI: 28.6%–54.3%] versus 46.2% [95% CI: 19.2%–74.9%], $p = 0.89$; Supplementary Fig. 1).

Association of the TTF-1 Expression Score With the Efficacy of Pemetrexed-Containing Regimens

In patients with diffusely TTF-1–positive tumors (n = 61), 45 patients (73.7%) were treated with chemoimmunotherapy including pemetrexed and 16 patients (26.3%) were treated with chemoimmunotherapy including paclitaxel or nab-paclitaxel, whereas in patients with focally TTF-1–positive tumors (n = 13), nine patients (69.2%) were treated with chemoimmunotherapy including pemetrexed and four patients (30.8%) were treated with chemoimmunotherapy including paclitaxel or nab-paclitaxel.

Among the patients with diffusely TTF-1–positive tumors, the PFS did not significantly differ between those treated with chemoimmunotherapy including pemetrexed and those treated with chemoimmunotherapy including paclitaxel or nab-paclitaxel (median, 16.2 versus 12.1 mo, $p = 0.22$; Fig. 3*A*).

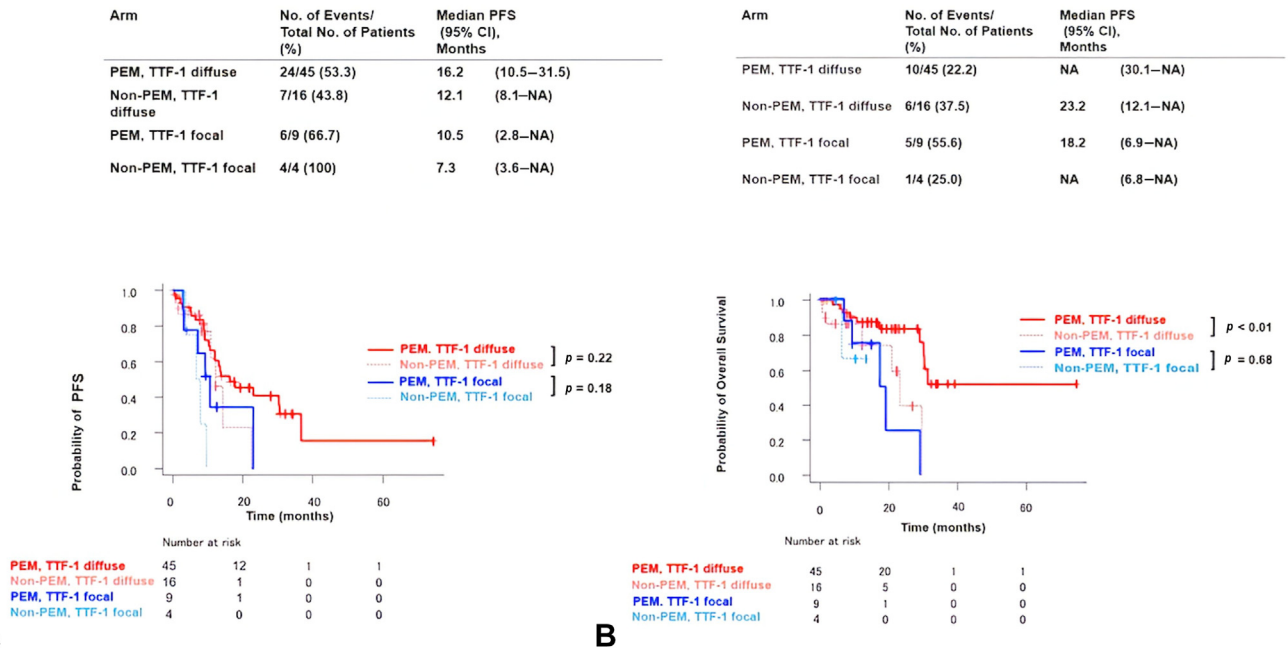

Figure 3. Estimated Kaplan-Meier survival curves for (*A*) PFS and (*B*) overall survival comparing patients with diffusely TTF-1-positive tumors receiving chemoimmunotherapy containing pemetrexed (PEM) (n = 45), those with diffusely TTF-1-positive tumors receiving chemoimmunotherapy including paclitaxel or nab-paclitaxel (non-PEM) (n = 16), those with focally TTF-1-positive tumors receiving PEM (n = 9), and those with focally TTF-1-positive tumors receiving non-PEM (n = 4). CI, confidence interval; NA, not available; PFS, progression-free survival.

Nevertheless, within the same group of patients with diffusely TTF-1–positive tumors, the OS was significantly longer in patients treated with chemoimmunotherapy including pemetrexed than in those treated with chemoimmunotherapy including paclitaxel or nab-paclitaxel (median, NA versus 23.2 mo, $p < 0.01$; Fig. 3*B*). The ORR was 46.7% (95% CI: 31.7%–62.1%) in patients with diffusely TTF-1–positive tumors treated with chemoimmunotherapy including pemetrexed and 25.0% (95% CI: 7.3%–52.4%) in those with diffusely TTF-1–positive tumors treated with chemoimmunotherapy including paclitaxel or nab-paclitaxel; the difference in ORR was not statistically significant ($p = 0.15$; Supplementary Fig. 2).

Among the patients with focally TTF-1–positive tumors, the PFS and the OS did not significantly differ between those treated with chemoimmunotherapy including pemetrexed and those treated with chemoimmunotherapy including paclitaxel or nab-paclitaxel (PFS: median = 10.5 versus 7.3 mo, $p = 0.18$; Fig. 3*A*; OS: median = 18.2 mo versus NA, $p = 0.68$; Fig. 3*B*). Finally, the ORR was 33.3% (95% CI: 7.5%–70.1%) in patients with focally TTF-1–positive tumors treated with chemoimmunotherapy including pemetrexed and 75.0% (95% CI: 19.4%–99.4%) in those with focally TTF-1–positive tumors treated with chemoimmunotherapy including paclitaxel or nab-paclitaxel, with no statistically significant difference found between the two groups ($p = 0.27$; Supplementary Fig. 2).

Discussion

In this retrospective study of 74 patients with nonsquamous NSCLC, the multivariate analysis revealed that the PFS and OS were significantly longer in patients with diffusely TTF-1–positive tumors, defined as tumors with ≥50% TTF-1–positive cells, than in those with focally TTF-1–positive tumors, defined as tumors with 10% to 49% TTF-1–positive cells, receiving chemoimmunotherapy. Moreover, the OS was significantly longer in patients with diffusely TTF-1–positive tumors receiving chemoimmunotherapy including pemetrexed than in those receiving chemoimmunotherapy including paclitaxel or nab-paclitaxel. To the best of our knowledge, this is the first study to identify the extent of TTF-1 expression as a predictive factor in patients with advanced nonsquamous NSCLC treated with chemoimmunotherapy. In previous studies, the PFS and OS were significantly longer in patients with TTF-1–positive tumors than in those with TTF-1–negative tumors among those receiving chemoimmunotherapy,[13,14] owing to different mechanisms of carcinogenesis. Lung adenocarcinomas can be divided into terminal respiratory unit (TRU) and non-TRU carcinoma types. TRU-type carcinomas are well differentiated and exhibit TTF-1 positivity; these tumors arise from type II pneumocytes and Clara cells. In contrast, non–TRU-type carcinomas are negative for TTF-1 and sometimes coexist with squamous cell carcinomas arising from centrally located dysplastic mucous columnar cells.[17] Thus, TTF-1–negative tumors may behave like squamous cell carcinoma and are associated with worse prognosis. In addition, survival response to chemoimmunotherapy including pemetrexed was significantly better in patients with TTF-1–positive tumors than those with TTF-1–negative tumors.[13,18–24] It has been reported that KEAP1 mutations, interleukin 35, and A2A adenosine receptor, which are associated with the efficacy of immunotherapy, were more expressive in TTF-1–positive tumors than TTF-1–negative tumors.[25–28] In light of these previous studies, although these factors were not assessed in this study, the present study findings indicate that the behavior of tumors with focal TTF-1 positivity might be more similar to the behavior of TTF-1–negative tumors than that of tumors with diffuse TTF-1 positivity, and chemoimmunotherapy was less effective in patients with focally TTF-1–positive tumors. Moreover, the administration of pemetrexed can augment the release of more damage-associated molecular patterns from tumor cells with the subsequent induction of immunogenic cell death compared with other cytotoxic agents.[29,30] This mechanism might explain the improved OS achieved with chemoimmunotherapy combining pemetrexed with ICIs in patients with NSCLC. Previous reports have suggested that pemetrexed is less effective in patients with smoking history and TTF-1 negativity.[20–23] Thus, considering the possibility of similarities between TTF-1–negative and focally TTF-1–positive tumors, which have been seemed to be less effective toward pemetrexed, the results of our study indicated that survival response to chemoimmunotherapy including pemetrexed was significantly better in patients with diffusely TTF-1–positive tumors.

The extent of TTF-1 expression might be considered as a predictive factor for chemoimmunotherapy efficacy. Previous studies reported that the PD-L1 TPS was a predictive factor for chemoimmunotherapy efficacy in patients with stage IV NSCLC. Specifically, the PFS and OS were better in patients with high PD-L1 TPS (≥50%) than in those with low (1%–49%) or negative (<1%) PD-L1 TPS.[3,5,7,31] Although current efforts are intensely exploring potential predictive factors for chemoimmunotherapy, no definitive marker other than the PD-L1 TPS has been validated as predictors of chemoimmunotherapy response in advanced NSCLC without driver mutations. Several studies exploring TTF-1 expression status as a treatment response marker reported that the PFS and OS were significantly better in patients with TTF-1–positive tumors than in those with

TTF-1–negative tumors.[13,14,18–20,32–34] Low thymidylate synthase, which is the main target of pemetrexed, was also associated with significantly better clinical response in patients with advanced nonsquamous NSCLC receiving pemetrexed-based chemotherapy.[18,35] Nevertheless, some patients with TTF-1–positive tumors exhibited minimal response to chemoimmunotherapy. In the present study, the PFS and OS were significantly longer in patients with diffusely TTF-1–positive tumors than in those with focally TTF-1–positive tumors, indicating that the extent of TTF-1 expression should be considered as a potential predictive factor for chemoimmunotherapy efficacy in advanced nonsquamous NSCLC.

In the present study, TTF-1 immunostaining was evaluated with the SP141 clone. Immunohistochemical staining for TTF-1 is often used to distinguish primary lung adenocarcinoma from squamous cell carcinoma and metastatic adenocarcinoma.[9–12] Among the commercially available TTF-1 clones for immunohistochemical staining, such as mouse (e.g., 8G7G3/1, SPT24, BGX-397A, SMP150, and 5S143) and rabbit (e.g., SP141, EP15844, C12-I, and G21-G) monoclonal antibodies,[36] the 8G7G3/1, SPT24, and the newly introduced SP141 clones are routinely used in clinical practice. Although 8G7G3/1 is the most frequently used clone in studies investigating the relationship between lung carcinoma and TTF-1 expression,[13,14,18,20,33] the SP141 clone was used to assess TTF-1 expression because of new technology in the present study. The SP141 clone is more sensitive but less specific than the 8G7G3/1 and SPT24 clones.[9,36] Thus, it remains possible that the focally TTF-1–positive tumors on the basis of immunohistochemical staining with the SP141 clone might be negative for TTF-1 on the basis of immunohistochemical staining with the 8G7G3/1 clone, thereby explaining the worse prognosis observed in patients with focally TTF-1–positive tumors than in those with diffusely TTF-1–positive tumors. Future studies are warranted to delineate the relationship between lung carcinoma and TTF-1 expression evaluated using the SP141 clone.

In this study, 66 patients (89.2%) had negative results for *EGFR* or *ALK* mutation, because patients with *EGFR* or *ALK* mutation positivity were administered TKIs as first-line therapy. All patients with *EGFR* or *ALK* positivity were included in diffuse TTF-1–positive group. Generally, patients with *EGFR* positivity are reported to have a good prognosis.[37] Nevertheless, the *EGFR*-positive patients in this study had little effect on EGFR TKI, such as EGFR exon20.[38] Therefore, we concluded that *EGFR* had only positive or little effect on the results of this study.

The present study has a number of limitations that should be acknowledged. First, one experienced pathologist in a single institution evaluated the extent of TTF-1 expression. Second, this was a retrospective and nonrandomized study. Thus, selection bias could not be ruled out. Third, the sample size was small and validation studies were not performed. Finally, the follow-up duration was not sufficient to adequately evaluate long-term survival outcomes. Nevertheless, the multivariate analyses were performed to minimize the confounding bias. Further prospective trials are necessary to validate the present study findings.

In patients with advanced nonsquamous NSCLC, the PFS and OS were significantly better in patients with diffusely TTF-1–positive tumors, defined as TTF-1 expression in $\geq$50% of the tumor cells, than in those with focally TTF-1–positive tumors, defined as TTF-1 expression in 10% to 49% of the tumor cells, indicating that the extent of TTF-1 expression might be a predictive factor for chemoimmunotherapy efficacy in advanced nonsquamous NSCLC.

CRediT Authorship Contribution Statement

Yuto Terashima: Conceptualization, Data curation, Formal analysis, Investigation, Validation, Visualization, Writing—original draft, Writing—review and editing.

Masaru Matsumoto: Conceptualization, Data curation, Formal analysis, Supervision, Writing—review and editing.

Hiroki Iida: Conceptualization, Investigation.

Sae Takashima: Conceptualization, Data curation, Formal analysis, Investigation.

Aya Fukuizumi: Conceptualization, Supervision.

Susumu Takeuchi: Conceptualization, Supervision.

Akihiko Miyanaga: Conceptualization, Supervision.

Yasuhiro Terasaki: Conceptualization, Investigation.

Kazuo Kasahara: Conceptualization, Supervision.

Masahiro Seike: Conceptualization, Supervision, Writing—review and editing.

Ethics Approval and Consent for Publication

The study protocol was approved by the Institutional Review Board of the Graduate School of Medicine at Nippon Medical School (approval no. B-2023-666). The study was conducted in accordance with the tenets of the Declaration of Helsinki.

Data Availability

The data sets generated and analyzed during the study are available from the corresponding author on reasonable request.

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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.2023.100578.

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