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Original Article

Effect of the thyroid transcription factor 1 expression and treatment discontinuation due to adverse events on progression-free survival in patients with advanced non-squamous non-small cell lung cancer treated with pembrolizumab plus pemetrexed and platinum chemotherapy: a Japanese four-hospital, retrospective study

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Abstract: Although a significant improvement in progression-free survival (PFS) has been reported in the thyroid transcription factor 1 (TTF-1) positive patients under treatment for non-squamous non-small cell lung cancer (NS-NSCLC), including immune checkpoint inhibitor therapy, the association between TTF-1 expression and adverse event occurrence remains unclear. Therefore, this study investigated the impact of TTF-1 and its adverse events on PFS during pembrolizumab plus pemetrexed and platinum chemotherapy for NS-NSCLC. Patients who received the pembrolizumab plus pemetrexed and platinum chemotherapy from 1/1/2018 to 12/31/2022 and whose TTF-1 expression was measured were included in the study. This was a retrospective study conducted using electronic medical records. The mean age of the 79 patients was 67.5 ± 8.4 years, with 75.95% patients being male. Among them, 59.49% were TTF-1 positive. PFS comparison between TTF-1-positive and -negative patients showed a trend toward longer PFS for TTF-1 positive patients, though the results were statistically insignificant (P = 0.190). Proportional hazards analysis indicated significant PFS extension from treatment interruption, as adverse events related to cancer therapy stopped (hazard ratio [HR] = 0.32, P = 0.005) and the number of anticancer agents used (HR = 0.01, P < 0.001). Additionally, pembrolizumab plus pemetrexed and platinum chemotherapy for TTF-1-positive NS-NSCLC significantly extended PFS after treatment discontinuation as related adverse events stopped (827 vs. 210 days, P = 0.021). Measurement of TTF-1 may accordingly serve as a predictor of treatment response to the pembrolizumab plus pemetrexed and platinum chemotherapy. It may also be a predictor of patient prognosis when treatment is discontinued due to related adverse events.

Keywords: Thyroid transcription factor 1, non-squamous non-small cell lung cancer, adverse events, immune checkpoint inhibitor therapy

Introduction

Lung cancer is the leading cause of cancer-related death worldwide. However, the advent of immune checkpoint inhibitors (ICIs) has greatly improved survival prognosis. The improvement in prognosis is particularly marked in the treatment of advanced non-squamous non-small cell lung cancer (NS-NSCLC), which accounts for approximately 80% of all lung cancer cases. Currently, a combination regimen consisting of pembrolizumab, pemetrexed, and

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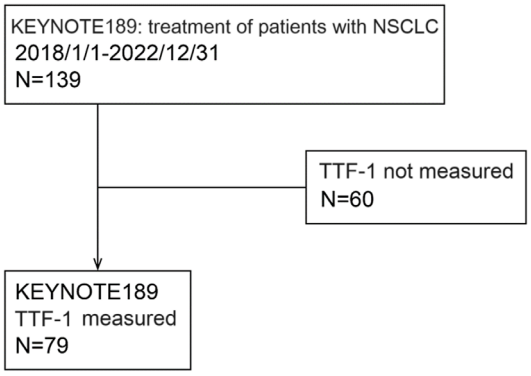

Figure 1. Flow chart of population cohort selection. NSCLC, non-small cell lung cancer.

platinum preparation chemotherapy is the first-line treatment for NS-NSCLC without mutations/translocations in driver genes such as *KRAS*, *EGFR*, *ALK*, *RET*, or *ROS1*.

A biomarker that may influence the efficacy of this pembrolizumab plus pemetrexed and platinum chemotherapy is the thyroid transcription factor 1 (TTF-1). Frost et al. investigated the efficacy of a regimen comprised a conventional platinum preparation and pemetrexed without an ICI and reported that TTF-1 positive patients had significantly improved progression-free survival (PFS) and overall survival (OS), while TTF-1 negative patients had shorter OS [1].

Moreover, Takeuchi et al. reported that in lung adenocarcinoma treatment with the addition of the molecularly targeted agent bevacizumab in addition to platinum and pemetrexed, TTF-1 positive patients exhibited significantly improved PFS and OS, while the addition of bevacizumab in TTF-1 negative patients did not result in significant improvement [2]. Further, Ibusuki et al. reported the first significant improvement in PFS in TTF-1-positive patients in the treatment of NS-NSCLC that included an ICI [3].

Recently, the expression of immune-related adverse events (irAEs) have reported to affect OS and PFS [4-6]. TTF-1 positive patients who underwent pembrolizumab plus pemetrexed and platinum chemotherapy for NS-NSCLC tended to have extended PFS compared to TTF-1 negative patients and may also be more prone to hematologic toxicities such as leukopenia and neutropenia [7]. However, this previous study was a single-center study, thus the insufficient number of cases and the unclear association between TTF-1 expression and the occurrence of adverse events. In the present study, we investigated the impact of TTF-1 and its adverse events on PFS with respect to pembrolizumab plus pemetrexed and platinum chemotherapy for NS-NSCLC.

Materials and methods

Target patients

We studied 139 patients who received the pembrolizumab plus pemetrexed and platinum chemotherapy at Kurashiki Central Hospital, Fukuyama Medical Center, Okayama Medical Center, and Tsuyama Central Hospital between 1/1/2018 and 12/31/2022. Of these patients, 79 in whom the presence or absence of TTF-1 expression was measured were included in the study (**Figure 1**). As this was a retrospective study, informed consent was waived.

Surveying method and survey items

This was a retrospective study conducted using electronic medical records. The survey method was retrospective, using electronic medical records. Survey items included age, gender, TTF-1 expression, body surface area, cancer stage, existence of metastasis, site of metastasis, history of treatment discontinuation due to side effect(s), names of side effect(s) leading to discontinuation, PFS, presence of progressive disease (PD), and date of blood sampling.

Ethical considerations

This study was conducted with the approval of the Ethics and Safety Committee for Education and Research of Shujitsu University and Shujitsu Junior College (Control Number: 260).

Statistical analysis

When comparing baseline characteristics between TTF-1 positive and negative patients, continuous variables were compared by Student's *t*-test, and categorical variables were compared by the χ^2 test. Fisher's exact test was used when the number of cases between comparator groups was less than 5. For survival analysis, the Kaplan-Meier method was used, and a Cox proportional hazards analysis was performed using eight explanatory variables

Table 1. Patient characteristics

	TTF-1 expression			
	Total n = 79	TTF-1 positive n = 47	TTF-1 negative n = 32	*p* value
Age (years)	67.5 ± 8.4	67.0 ± 7.9	68.2 ± 9.1	0.545
Sex				0.485
Male	60 (75.95%)	37 (78.72%)	23 (71.88%)	
Female	19 (24.05%)	10 (21.28%)	9 (28.13%)	
Body surface area (m^2)	1.61 ± 0.19	1.62 ± 0.20	1.59 ± 0.18	0.439
Smoking status				0.569
Non smoker	11 (13.92%)	6 (12.77%)	5 (15.63%)	
Former smoker	51 (64.56%)	29 (61.70%)	22 (68.75%)	
Current smoker	17 (21.52%)	12 (25.53%)	5 (15.63%)	
Clinical stage				0.100
Stage 1	1 (1.27%)	0 (0.00%)	1 (3.13%)	
Stage 2	1 (1.27%)	0 (0.00%)	1 (3.13%)	
Stage 3	9 (11.39%)	3 (6.38%)	6 (18.75%)	
Stage 4	68 (86.08%)	44 (93.62%)	24 (75.00%)	
PD-L1				0.027
< 1%	26 (32.91%)	10 (21.28%)	16 (50.00%)	
1-49%	18 (22.78%)	12 (25.53%)	6 (18.75%)	
≥ 50%	35 (44.30%)	25 (53.19%)	10 (31.25%)	
Metastatic site at primary diagnosis				
Pleura	10 (12.66%)	6 (12.77%)	4 (12.50%)	0.908
Bone	27 (34.18%)	17 (36.17%)	10 (31.25%)	0.859
Brain	25 (31.65%)	19 (40.43%)	6 (18.75%)	0.069
Adrenal gland	18 (22.78%)	12 (25.53%)	6 (18.75%)	0.651
Liver	13 (16.46%)	8 (17.02%)	5 (15.63%)	0.959

that could affect the length of PFS: age, sex, smoking history, PD-L1 expression rate, TTF-1 expression rate, presence of metastasis, treatment discontinuation as an adverse event, and number of anticancer agents administered. The log-rank test was used to compare PFS between patient groups. *P*-values were calculated by a two-tailed test, and the level of statistical significance was set at 5%. The JMP®Pro17 software (SAS Institute Inc., Cary, NC, USA) was used for the data analysis.

Results

The mean age of all 79 patients was 67.5 ± 8.4 years, with 75.95% (60/79) being male. The average body surface area was 1.61 ± 0.19 m^2. Among them, 86.08% (68/79) were smokers, including past smokers, and the same percentage had stage 4 disease. Additionally, 67.09% (53/79) exhibited PD-L1 expression of at least 1%. The most common metastatic sites, in descending order, were the bone, brain, and adrenal glands. Of the total 79 patients, 59.49% (47/79) were TTF-1 positive, and significantly more patients expressed PD-L1 compared to TTF-1 negative patients (P = 0.027, χ^2 test). No differences were observed between TTF-1-positive and TTF-1-negative patients with respect to other survey items (**Table 1**).

The PFS of TTF-1 positive and negative patients was compared (**Figure 2**). The results revealed a trend toward prolonged PFS in TTF-1-positive patients, although the results were not statistically significant (P = 0.190, log-rank test).

A proportional hazards analysis was performed to explore factors affecting PFS (**Table 2**). Factors that significantly extended PFS included patients who had treatment interruptions due to adverse events from anticancer therapy (HR = 0.32, P = 0.005) and more frequent administration of anticancer drugs (HR = 0.01,

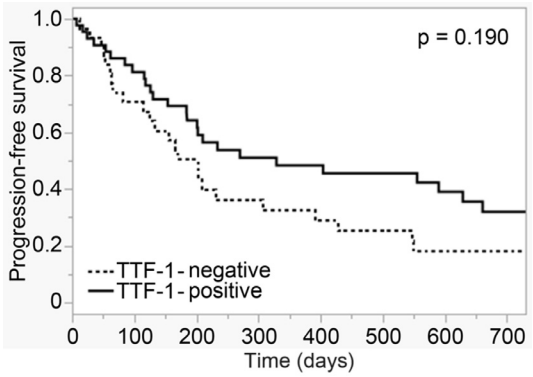

Figure 2. Progression-free survival with or without TTF-1 expression.

P < 0.001). Furthermore, age, sex, smoking history, PD-L1 expression, the presence of TTF-1 expression, and metastasis status did not significantly affect PFS extension.

The group of patients who had to discontinue treatment due to related adverse events, (PFS, median 590 days) had a statistically significantly longer PFS (P = 0.005, log-rank test) than did the group of patients who did not (201 days) (**Figure 3**).

We compared the effect of treatment discontinuation due to adverse events on PFS for each TTF-1-positive and TTF-1-negative patient (**Figure 4**). The results indicated no difference in PFS in TTF-1 negative patients (treatment discontinuation due to adverse events vs. no treatment discontinuation; median 208 days vs. 169 days) (**Figure 4B**), whereas, in TTF-1-positive patients only, PFS was significantly prolonged in those who discontinued anticancer therapy due to related adverse events (median 827 days vs. 210 days, P = 0.021, log-rank test) (**Figure 4A**).

Discussion

To the extent of our knowledge, the present study is the first to investigate the combined effect of factors affecting treatment response, such as TTF-1 expression and treatment discontinuation due to adverse events, in patients treated with pembrolizumab plus pemetrexed and platinum chemotherapy therapy for NS-NSCLC.

Ibusuki et al. reported that in NS-NSCLC cases, patients who were TTF-1 positive had significantly extended PFS compared to TTF-1 negative patients when an ICI was combined with cytotoxic anticancer agents [3]. We obtained similar results in the present study. However, Ibusuki et al.'s study included patients treated with not only the pembrolizumab plus pemetrexed and platinum chemotherapy but also with the IMpower132 regimen with atezolizumab as an ICI. In addition, this study included no investigation of adverse events in TTF-1-positive patients. We found that single regimen of pembrolizumab combined with pemetrexed and platinum chemotherapy significantly prolonged PFS in TTF-1-positive patients who discontinued treatment due to side effects. These adverse events included immune checkpoint inhibitor-related issues such as skin rash, thyroid dysfunction, myasthenia gravis, interstitial pneumonitis, and chemotherapy-induced hematologic toxicity. Therefore, the possible extension of OS and PFS due to IrAEs may be limited to TTF-1-positive patients. This finding suggests that measuring TTF-1 in patients on the pembrolizumab plus pemetrexed and platinum chemotherapy may be important in predicting prognosis following treatment discontinuation in these patients. TTF-1 measurement may also be useful in predicting prognosis when treating NS-NSCLC, including other ICIs.

PD-L1 expression should be confirmed when treating patients with ICI. Among the patients in this study, those with high PD-L1 expression were more likely to be TTF-1 positive, and in this regard, a positive correlation between TTF-1 and PD-L1 expression has been reported histologically [8]. Therefore, we believe that PD-L1 expression alone can predict therapeutic prognosis without measuring TTF-1. However, we performed a stratified analysis of the patients in this study to compare the effect of treatment discontinuation on PFS in four groups according to the expression of TTF-1 and PD-L1: a negative-negative group, a positive-negative group, a negative-positive group, and a positive-positive group. The results showed a significant extension of PFS only in the positive-positive group (median, 988 days vs. 202 days, P = 0.019, log-rank test) (data not shown). PD-L1 positivity did not significantly prolong PFS, but patients who tested positive for both TTF-1 and PD-L1 did. Therefore, we believe that the expression of TTF-1 and PD-L1 alone should be confirmed together.

Table 2. Multivariate analysis of clinicopathologic factors for PFS

Characteristics	HR (95% CI)	*p* value
Age (years)	2.31 (0.44-14.30)	0.341
Sex		
Male	1.08 (0.51-2.28)	0.843
Female	0.93 (0.44-1.96)	0.843
Smoking status		
Former smoker (vs. Non smoker)	0.81 (0.35-1.87)	0.629
Current smoker (vs. Non smoker)	1.10 (0.51-2.28)	0.879
PD-L1		
1-49% (vs. < 1%)	1.32 (0.59-2.71)	0.506
> 50% (vs. < 1%)	0.47 (0.20-1.13)	0.092
TTF-1		
Positive	0.78 (0.42-1.48)	0.457
Negative	1.27 (0.68-2.39)	0.457
Metastasis status		
Brain metastasis	1.13 (0.53-2.42)	0.747
Liver metastasis	1.81 (0.86-3.81)	0.117
Discontinuation of treatment due to adverse events		
Discontinued	0.32 (0.15-0.71)	0.005
Not discontinued	3.08 (1.41-6.74)	0.005
Number of anticancer therapy administrations	0.01 (0.00-0.08)	< 0.001

HR, hazard ratio; CI, confidence interval.

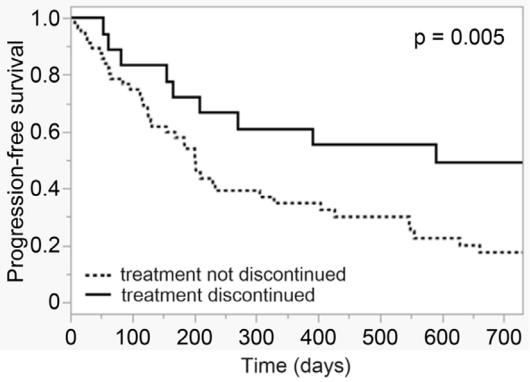

Figure 3. Progression-free survival with or without discontinuation of treatment due to adverse events.

Currently, the extension of PFS in TTF-1 and PD-L1 positive patients undergoing ICI treatment is explained by the following mechanisms. First, TTF-1 has been reported to induce PD-L1 expression through PI3K/Akt signaling [9, 10]. In various human NSCLC cell lines, such as A549, H2122, Calu-3, and H292 cells, forced expression of TTF-1 significantly increased PD-L1 expression at both the mRNA and protein levels. Furthermore, TTF-1 was observed to colocalize with PD-L1 in a significant proportion of human NSCLC cells [11]. Consequently, in TTF-1 positive patients, PD-L1 is induced, increasing the target molecules of ICIs, and thereby potentially extending PFS with ICI treatment. Second, TTF-1 suppresses the epithelial-to-mesenchymal transition (EMT), thereby suppressing the metastatic potential and invasiveness of cancer cells via TGF-beta [12]. Tumor-associated neutrophils suppress T cell activation and diminish the effectiveness of PD-1/PD-L1 inhibitors; conversely, TTF-1-negative cells induce the accumulation of these neutrophils within tumor cells by inducing tumor-associated CXCL5 [13]. Consequently, TTF-1-negative patients are associated with a poor prognosis for treatment [14]. These basic research findings support our results.

This study was subject to several limitations. First, this study was essentially a retrospective study, although it included pembrolizumab plus pemetrexed and platinum chemotherapy patients at four hospitals and employed a study design that minimized selection bias. Second, we examined the prognosis of TTF-1-positive patients who discontinued treatment due to pembrolizumab plus pemetrexed and platinum chemotherapy adverse events and confirmed that the adverse events that led to treatment discontinuation did not extend PFS in patients with interstitial pneumonia. However, the details of other adverse events are unknown because no information was collected (Supplementary Table 1). Third, this is a retrospective study conducted since 2018. Because of the measurement environments across the hospitals, comprehensive information such as TMB and MSI was not uniformly obtainable from all patients. Therefore, the influence of these factors on PFS could not be evaluated.

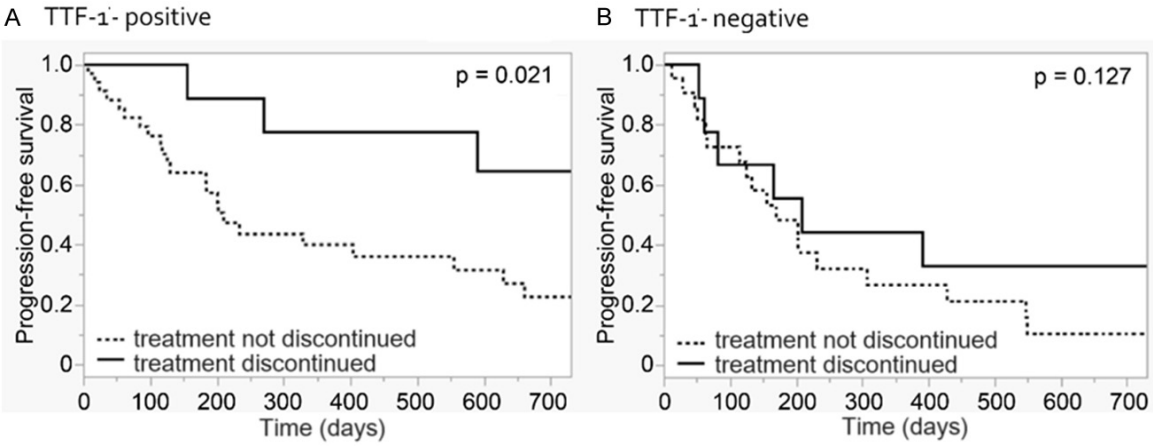

Figure 4. Progression-free survival with or without TTF-1 expression and discontinuation of treatment due to adverse events.

Conclusion

We found that the pembrolizumab plus pemetrexed and platinum chemotherapy for TTF-1-positive NS-NSCLC significantly prolonged PFS in patients whose treatment had previously been discontinued due to an adverse event. Measurement of TTF-1 may accordingly serve as a predictor of treatment response to the pembrolizumab plus pemetrexed and platinum chemotherapy, but it may also be a predictor of patient prognosis when there is adverse event-related treatment discontinuation.

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Disclosure of conflict of interest

None.

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Supplementary Table 1. Details of patients discontinuing treatment for side effects

ID	TTF-1 expression	Age	Sex	Adverse events	PFS	Number of administrations
1	Negative	81	M	Other	53	1
2	Negative	67	M	Myelosuppression	60	1
3	Negative	77	F	Interstitial pneumonia	390	3
4	Negative	74	M	Interstitial pneumonia	81	4
5	Negative	72	F	Other	208	4
6	Negative	74	F	Other	166	5
7	Negative	72	M	Interstitial pneumonia	1445	7
8	Negative	67	F	Other	1397	26
9	Negative	61	M	Other	1128	35
10	Positive	63	M	Interstitial pneumonia	590	2
11	Positive	74	M	Interstitial pneumonia	154	4
12	Positive	62	M	Other	-	4
13	Positive	75	M	Myelosuppression	270	5
14	Positive	77	F	Other	827	5
15	Positive	74	M	Other	988	5
16	Positive	61	M	Other	-	5
17	Positive	66	M	Other	499	8
18	Positive	58	M	Other	592	18
19	Positive	67	M	Myelosuppression	1424	34
20	Positive	61	M	Other	1345	43

PFS, progression-free survival (day); other, Adverse events other than skin rash, thyroid dysfunction, myasthenia gravis, interstitial pneumonia, and myelosuppression; -, censoring.