Thyroid transcription factor-1 (TTF-1) expression and the efficacy of combination therapy with immune checkpoint inhibitors and cytotoxic chemotherapy in non-squamous non-small cell lung cancer

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Background: Thyroid transcription factor-1 (TTF-1) is expressed in approximately 70% of lung adenocarcinomas and is one of the most reliable makers to distinguish primary lung adenocarcinoma from metastatic disease. TTF-1-negative status is a poor prognostic factor, and TTF-1-negative lung adenocarcinoma is associated with poor efficacy of immune checkpoint inhibitor (ICI) monotherapy. However, the relationship between TTF-1 expression and the efficacy of ICI plus chemotherapy is still unclear.

Methods: We performed a retrospective analysis of 129 consecutive patients with advanced non-squamous non-small cell lung cancer (NS-NSCLC) treated with ICI monotherapy or ICI plus chemotherapy between January 2016 and December 2021. The expression of programmed death ligand-1 (PD-L1) and TTF-1 was also determined in cases for which no previous data were available. We then evaluated the association between TTF-1 expression status and treatment efficacy.

Results: Of the 129 cases, 33 were TTF-1-negative and 96 were positive. In the ICI monotherapy group (N=70), progression-free survival (PFS) was not significantly different between TTF-1-positive and negative patients (median 3.6 *vs.* 3.8 months, P=0.27); however, in patients with wild-type epidermal growth factor receptor (*EGFR*) and anaplastic lymphoma kinase (*ALK*), a trend for worse PFS was observed in TTF-1-negative cases compared with those that were TTF-1-positive (median 3.8 *vs.* 4.5 months, P=0.088). Moreover, long-term efficacy of ICI monotherapy (>2 years) was not observed in the TTF-1-negative group. TTF-1-negative patients tended to have worse overall survival (OS) than TTF-1-positive patients (median 15.6 *vs.* 19.5 months, P=0.13). In the ICI plus chemotherapy group (N=59), TTF-1-negative patients tended to have better PFS and similar OS compared with TTF-1-positive patients (median 9.9 *vs.* 9.6 months, P=0.14; median 32.3 *vs.* 18.9 months, P=0.78). Long-term efficacy was generally observed in TTF-1-negative patients treated with atezolizumab plus bevacizumab plus carboplatin plus paclitaxel (ABCP) (median PFS 22.5 months, median OS not reached).

Conclusions: ICI monotherapy is generally less efficacious in TTF-1-negative NS-NSCLC patients, and clinicians should consider ICI plus chemotherapy in these cases. Our study suggests that ABCP is an optimal regimen for TTF-1-negative NS-NSCLC.

Introduction

Immune checkpoint inhibitor (ICI) monotherapy or a combination of cytotoxic chemotherapy and ICI is the standard therapy for advanced non-squamous non-small cell lung cancer (NS-NSCLC) (1). Expression of programmed death ligand-1 (PD-L1) in tumor cells is the most widely used therapeutic indicator for ICIs, although the association between the efficacy of ICIs and PD-L1 expression has remained controversial (2). Furthermore, long-term survival is not achieved in all patients with high PD-L1 expression. Therefore, there is a need for biomarkers beyond PD-L1 that can be implemented in clinical practice to determine the optimal ICI and chemotherapy regimen.

Thyroid transcription factor-1 (TTF-1) is mainly expressed in type 2 alveolar epithelial cells; it has essential roles in morphogenesis and differentiation of the lung, thyroid, and forebrain (3,4). TTF-1 is expressed in approximately 70% of lung adenocarcinomas (5). Due to its restricted tissue expression, TTF-1 is one of the most reliable markers for distinguishing primary lung adenocarcinoma from metastatic lung tumors in clinical practice (6). The role of TTF-1 in lung cancer is poorly understood and, in some cases paradoxical. While it reportedly promotes carcinogenesis in lung cancer, some studies indicate it is a tumor suppressor in other tumor types (7-9). Before ICIs were widely used, TTF-1-negative status was reported as a poor prognosis factor in both the early and advanced stages of NS-NSCLC (10). TTF-1-negative status in lung adenocarcinoma is associated with poor efficacy of cytotoxic chemotherapies such as docetaxel and pemetrexed plus platinum (11,12). Even treatment with ICI monotherapy, one of the standard-of-care treatments in NSCLC, is poorly efficacious in TTF-1-negative cases (13,14). Due to these reports, TTF-1-negative status is considered a predictive factor of low therapeutic efficacy. However, the relationship between TTF-1 expression and the efficacy of ICI plus chemotherapy is still unclear. We conducted a retrospective study to elucidate the relationship between TTF-1 expression status and the efficacy of ICI-containing chemotherapy. We present this article in accordance with the STROBE reporting checklist (available at https://tlcr.amegroups.com/article/view/10.21037/tlcr-23-331/rc).

Highlight box

Key findings

- Thyroid transcription factor-1 (TTF-1)-negative non-squamous non-small cell lung cancer (NS-NSCLC) patients treated with immune checkpoint inhibitor (ICI) plus chemotherapy tended to have better PFS and similar overall survival (OS) compared with TTF-1-positive patients. In particular, atezolizumab plus bevacizumab plus carboplatin plus paclitaxel (ABCP) showed long-term efficacy compared with other regimens.

What is known and what is new?

- TTF-1-negative NS-NSCLC is associated with poor efficacy of ICI monotherapy and pemetrexed plus platinum cytotoxic chemotherapy.
- TTF-1-negative NS-NSCLC patients treated with ICI plus chemotherapy tended to have better PFS and similar OS compared with TTF-1-positive patients.

What is the implication, and what should change now?

- ICI monotherapy was generally not efficacious for patients with TTF-1-negative NS-NSCLC, thus clinicians should consider ICI plus chemotherapy. ABCP would be an optimal regimen for TTF-1-negative NS-NSCLC.

Methods

Patients and methods

We included patients who were administered ICI monotherapy or a combination of cytotoxic chemotherapy and ICI for advanced or recurrent NS-NSCLC at Nippon Medical School Tamanagayama Hospital between January 2016 and December 2021. We grouped patients into two treatment groups: ICI monotherapy or a combination of cytotoxic chemotherapy and ICI, and examined the association between the efficacy of each treatment and tumor TTF-1 expression. We retrieved

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Table 1 Patient characteristics of the entire population

Characteristics	Subtypes	All patients (N=129)
Age, median [range], year		72 [43–86]
Sex, n (%)	Male	91 (70.5)
	Female	38 (29.5)
Smoking, n (%)	Ever	96 (74.4)
	Never	33 (25.6)
PS, n (%)	0–1	98 (76.0)
	≥2	31 (24.0)
Stage, n (%)	III	3 (2.3)
	IV	95 (73.6)
	Recurrent	31 (24.0)
Pathology, n (%)	Adenocarcinoma	113 (87.6)
	Other	16 (12.4)
TTF-1, n (%)	Positive	96 (74.4)
	Negative	33 (25.6)
PD-L1, n (%)	<1%	46 (35.7)
	1–49%	39 (30.2)
	≥50%	44 (34.1)
EGFR, n (%)	Positive	20 (15.5)
	Negative	109 (84.5)
ALK, n (%)	Positive	3 (2.3)
	Negative	126 (97.7)
Regimen, n (%)	ICI	70 (54.3)
	ICI + chemo	59 (45.7)

PS, performance status; TTF-1, thyroid transcription factor-1; PD-L1, programmed death ligand-1; EGFR, epidermal growth factor receptor; ALK, anaplastic lymphoma kinase; chemo, chemotherapy; ICI, immune checkpoint inhibitor.

clinical data including pathological diagnosis, TTF-1 expression, and tumor proportion score (TPS) of PD-L1 as evaluated w the 22C3 assay. In cases with where TTF-1 expression or PD-L1 TPS was unknown, we re-examined the archived pathological specimens by performing immunohistochemistry (IHC) staining for TTF-1 and PD-L1. The PD-L1 TPS was evaluated by IHC using a Dako PD-L1 22C3 pharmDx kit (Dako, Carpinteria, CA, USA). TTF-1 expression was evaluated using TTF-1 monoclonal antibody SPT24 (Nichirei, Tokyo, Japan). Oncogenic drivers including epidermal growth factor receptor (EGFR)

and anaplastic lymphoma kinase (ALK) were examined using the Cobas® EGFR mutation test v2 (Roche, Basel, Switzerland) and FISH (Abbott Molecular, Des Plaines, IL, USA) or IHC (Roche, Basel, Switzerland) for ALK, and/or next-generation sequencing via the Oncomine™ DX target test (Thermo Fisher, Waltham, MA, USA). The decision of which test to use was made at the physician's discretion. Response and progression of the tumors were evaluated based on the Response Evaluation Criteria in Solid Tumors (RECIST) version 1.1 (15). This study was approved by the Ethics Committee of Nippon Medical School Tamanagayama Hospital (No. F-2022-010), and conducted according to the Declaration of Helsinki (as revised in 2013). Individual consent for this retrospective analysis was waived.

Statistical analysis

The categorical variables of each treatment were analyzed with Fisher's exact test and Student's *t*-test. We analyzed objective response rate (ORR), disease control rate (DCR), progression-free survival (PFS), and overall survival (OS) in each treatment group. PFS was defined as the period from the date of treatment initiation to the date of disease progression or last follow-up. OS was defined as the period from the date of treatment initiation to the date of death from any cause or last follow-up. Both OS and PFS were estimated with the Kaplan-Meier method and patient group differences were compared with the log-rank test. Hazard ratio (HR) and corresponding 95% confidence intervals (CIs) were calculated from a Cox proportional hazard model. We analyzed data with IBM SPSS Statistics program (version 28.0.1.0, IBM, Armonk, NY, USA).

Results

Patient characteristics

A total of 130 patients had been treated with regimens that included ICI. One patient was excluded because they suffered from a double cancer (lung adenocarcinoma and squamous cell carcinoma). We re-examined the pathological tissue samples from 17 of 129 patients to determine PD-L1 and TTF-1 expression status. The last follow-up date was March 31, 2023. The median follow-up time was 17.9 months (range, 0.73–67.2 months).

The characteristics of the entire patient population are shown in *Table 1*, and characteristics of each treatment group are shown in *Tables 2,3*. Thirty-three out of 129 patients

Table 2 Patient characteristics in the immune checkpoint inhibitor group

| Characteristics	Subtypes	TTF-1-negative (N=19)	TTF-1-positive (N=51)	P
Age, median [range], year		76 [50–86]	73 [43–85]	0.61
Sex, n (%)	Male	12 (63.2)	35 (68.6)	0.78
	Female	7 (36.8)	16 (31.4)	
Smoking, n (%)	Ever	14 (73.7)	35 (68.6)	0.78
	Never	5 (26.3)	16 (31.4)	
PS, n (%)	0–1	10 (52.6)	38 (74.5)	0.08
	≥2	9 (47.4)	13 (25.5)	
Stage, n (%)	III	1 (5.3)	0	0.13
	IV	14 (73.7)	32 (62.7)	
	Recurrent	4 (21.0)	19 (37.3)	
EGFR, n (%)	Positive	0	9 (17.6)	0.10
	Negative	19 (100.0)	42 (82.4)	
ALK, n (%)	Positive	1 (5.3)	2 (3.9)	1.00
	Negative	18 (94.7)	49 (96.1)	
Pathology, n (%)	Adenocarcinoma	14 (73.7)	47 (92.2)	0.05
	Other	5 (26.3)	4 (7.8)	
PD-L1, n (%)	<1%	6 (31.6)	12 (23.5)	0.17
	1–49%	8 (42.1)	13 (25.5)	
	≥50%	5 (26.3)	26 (51.0)	
Regimen, n (%)	Pembrolizumab	10 (52.6)	32 (62.7)	0.58
	Atezolizumab	5 (26.3)	8 (15.7)	
	Nivolumab	4 (21.1)	11 (21.6)	

TTF-1, thyroid transcription factor-1; PS, performance status; *EGFR*, epidermal growth factor receptor; *ALK*, anaplastic lymphoma kinase; PD-L1, programmed death ligand-1.

(25.6%) were TTF-1-negative (*Table 1*). In the ICI plus chemotherapy group, the proportion of females was slightly higher in TTF-1-positive compared to TTF-1-negative cases (*Table 3*). The proportion of worse performance status (PS) (≥2) was higher in TTF-1-negative than in TTF-1-positive patients treated with ICI monotherapy (47.4% *vs.* 25.5%, P=0.08, *Table 2*). *EGFR* mutations tended to be more frequent in the TTF-1-positive group (*Tables 2,3*). The proportion of adenocarcinomas was higher in TTF-1-positive patients in both treatment groups (*Tables 2,3*). There was no significant correlation between PD-L1 and TTF-1 expression, but a higher proportion of the TTF-1-negative cases were PD-L1 negative when compared with TTF-1-positive cases (*Tables 2,3*). Other baseline characteristics did not statistically differ between the treatment groups (*Tables 2,3*).

Treatment regimen

Of the 129 patients, 70 were treated with ICI monotherapy and 59 were treated with ICI plus chemotherapy. We separated the ICI monotherapy group across three main regimens: pembrolizumab (200 mg/body every 3 weeks), nivolumab (240 mg/body every 2 weeks or 480 mg/body every 4 weeks), and atezolizumab (1,200 mg/body every 3 weeks); the frequency at which these regimens were used was similar between the TTF-1-negative and -positive groups (*Table 2*). The ICI plus chemotherapy group was also

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Table 3 Patient characteristics in the immune checkpoint inhibitor plus chemotherapy group

Characteristics	Subtypes	TTF-1-negative (N=14)	TTF-1-positive (N=45)	P
Age, median [range], year		68.5 [55–76]	70.0 [45–82]	0.74
Sex, n (%)	Male	13 (92.9)	31 (68.9)	0.09
	Female	1 (7.1)	14 (31.1)	
Smoking, n (%)	Ever	12 (85.7)	35 (77.8)	0.71
	Never	2 (14.3)	10 (22.2)	
PS, n (%)	0–1	11 (78.6)	39 (86.7)	0.43
	≥2	3 (21.4)	6 (13.3)	
Stage, n (%)	III	1 (7.1)	1 (2.2)	0.51
	IV	12 (85.8)	37 (82.2)	
	Recurrent	1 (7.1)	7 (15.6)	
EGFR, n (%)	Positive	0	11 (24.4)	0.051
	Negative	14 (100.0)	34 (75.6)	
ALK, n (%)	Positive	0	0	1.00
	Negative	14 (100.0)	45 (100.0)	
Pathology, n (%)	Adenocarcinoma	9 (64.3)	43 (95.6)	0.006
	Other	5 (35.7)	2 (4.4)	
PD-L1, n (%)	<1%	9 (64.3)	19 (42.2)	0.26
	1–49%	2 (14.3)	16 (35.6)	
	≥50%	3 (21.4)	10 (22.2)	
Regimen, n (%)	CBDCA + PTX + BEV + ATZ	6 (42.9)	18 (40.0)	0.22
	CBDCA + nab-PTX + ATZ/Pembro	4 (28.6)	4 (8.9)	
	Platinum + PEM + Pembro	4 (28.6)	22 (48.9)	
	CBDCA + PEM + Nivo+Ipi	0	1 (2.2)	

TTF-1, thyroid transcription factor-1; PS, performance status; *EGFR*, epidermal growth factor receptor; *ALK*, anaplastic lymphoma kinase; PD-L1, programmed death ligand-1; CBDCA, carboplatin; PTX, paclitaxel; BEV, bevacizumab; ATZ, atezolizumab; nab-PTX, nanoparticle albumin-bound paclitaxel; Pembro, pembrolizumab; PEM, pemetrexed; Nivo, nivolumab; Ipi, ipilimumab.

separated according to three main regimens: atezolizumab (1,200 mg/body) plus bevacizumab (15 mg/kg) plus carboplatin (CBDCA) [area under the curve (AUC) 5] plus paclitaxel (175 mg/m^2) (ABCP), atezolizumab (1,200 mg/body) or pembrolizumab (200 mg/body) plus CBDCA (AUC 6) plus nab-paclitaxel (100 mg/m^2), and pembrolizumab (200 mg/body) plus platinum [cis-diamminedichloroplatinum (CDDP) (75 mg/m^2) or CBDCA (AUC5)] plus pemetrexed (500 mg/m^2) every 3–4 weeks up to 4 cycles. After four cycles, the following maintenance therapies were administered. The attending physician made appropriate decisions regarding the dose reduction of chemotherapy according to the patient's condition. The frequency with which these regimens was chosen did not differ between groups (*Table 3*).

Efficacy of ICI monotherapy and the relationship with TTF-1 expression

In Table S1, ORR and DCR were not statistically different between the TTF-1-positive and -negative groups (23.5% *vs*. 15.8%, P=0.74; 52.9% *vs*. 42.1%, P=0.59). In the ICI monotherapy group (N=70), PFS was not significantly different between TTF-1-negative and -positive patients (median 3.8 *vs*. 3.6 months, P=0.27; *Figure 1A,1B*).
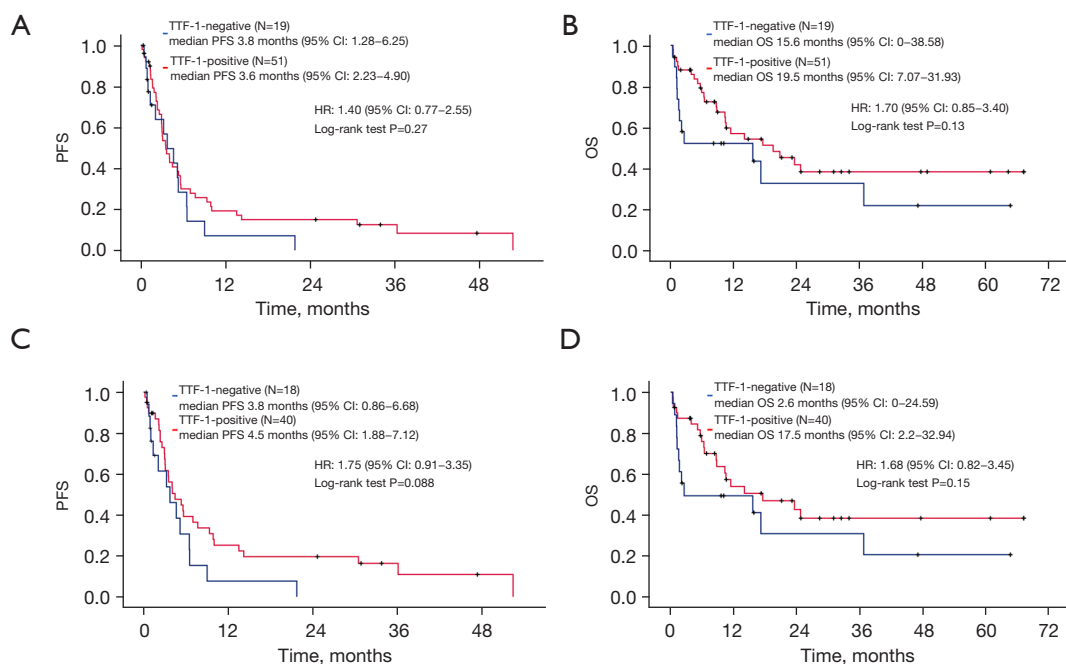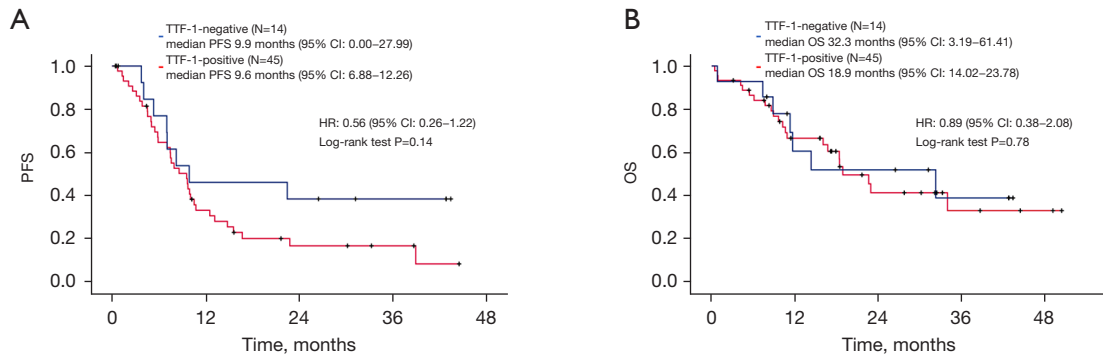
Figure 1 Kaplan-Meier curves in patients treated with ICI monotherapy according to TTF-1 expression status. (A) PFS in all patients who were treated with ICI monotherapy; (B) OS in all patients who were treated with ICI monotherapy; (C) PFS in patients with wild-type *EGFR* and *ALK*; (D) OS in patients with wild-type *EGFR* and *ALK*. TTF-1, thyroid transcription factor-1; PFS, progression-free survival; CI, confidence interval; HR, hazard ratio; OS, overall survival; ICI, immune checkpoint inhibitor; *EGFR*, epidermal growth factor receptor; *ALK*, anaplastic lymphoma kinase.

However, the long-term efficacy of ICI monotherapy (>2 years) was not observed in TTF-1-negative patients. TTF-1-negative patients tended to have worse OS than TTF-1-positive patients (median 15.6 *vs.* 19.5 months, P=0.13). *EGFR* mutations and *ALK* translocations are poor predictive factors for ICI monotherapy (16). Since such patients were included in our initial analysis and could confound our findings, we decided to perform a sub-analysis where we examined the impact of TTF-1 status in patients who had only wild-type *EGFR* and *ALK*. These patients had poorer PFS and OS when they had TTF-1-negative tumors (*Figure 1C,1D*). A trend for worse PFS was observed in TTF-1-negative cases compared with those that were TTF-1-positive (median PFS: 3.8 *vs.* 4.5 months, P=0.088; HR: 1.75, 95% CI: 0.91–3.35); this trend was therefore more obvious than the differences we observed in our initial analysis of all patients, including those with *EGFR* mutations and *ALK* translocations (*Figure 1A,1B*). Indeed, the shape of the Kaplan-Meier curve for OS was not significantly different between wild-type *EGFR* and *ALK* cases, and the corresponding curve based on all cases.

### Efficacy of ICI and chemotherapy, and the relationship with TTF-1 expression

Table S2 shows that ORR was higher in the TTF-1-negative group compared with TTF-1-positive patients (78.6% *vs.* 46.7%, P=0.063), whereas DCR was similar in the two groups (92.9% *vs.* 82.2%, P=0.67). As shown in *Figure 2A,2B*, there were no significant differences in PFS and OS; however, TTF-1 negative patients tended to have better PFS and a similar OS when compared with TTF-1-positive patients (median PFS: 9.9 *vs.* 9.6 months, P=0.14; median OS: 32.3 *vs.* 18.9 months, P=0.78). To elucidate which regimens contributed to prolonged PFS and OS in TTF-1-negative patients, we plotted Kaplan-Meier curves by each regimen (*Figure 3A,3B*). ABCP was the only regimen to elicit a long-term response. The efficacies of each regimen in TTF-1-positive patients are shown in Figure S1A,S1B. In the TTF-1 positive population, OS and PFS were similar for all regimens. The efficacy of ABCP and the relationship with TTF-1 expression is shown in *Figure 4A,4B*. The TTF-1-negative group tended to have longer PFS and OS compared with the TTF-1-positive
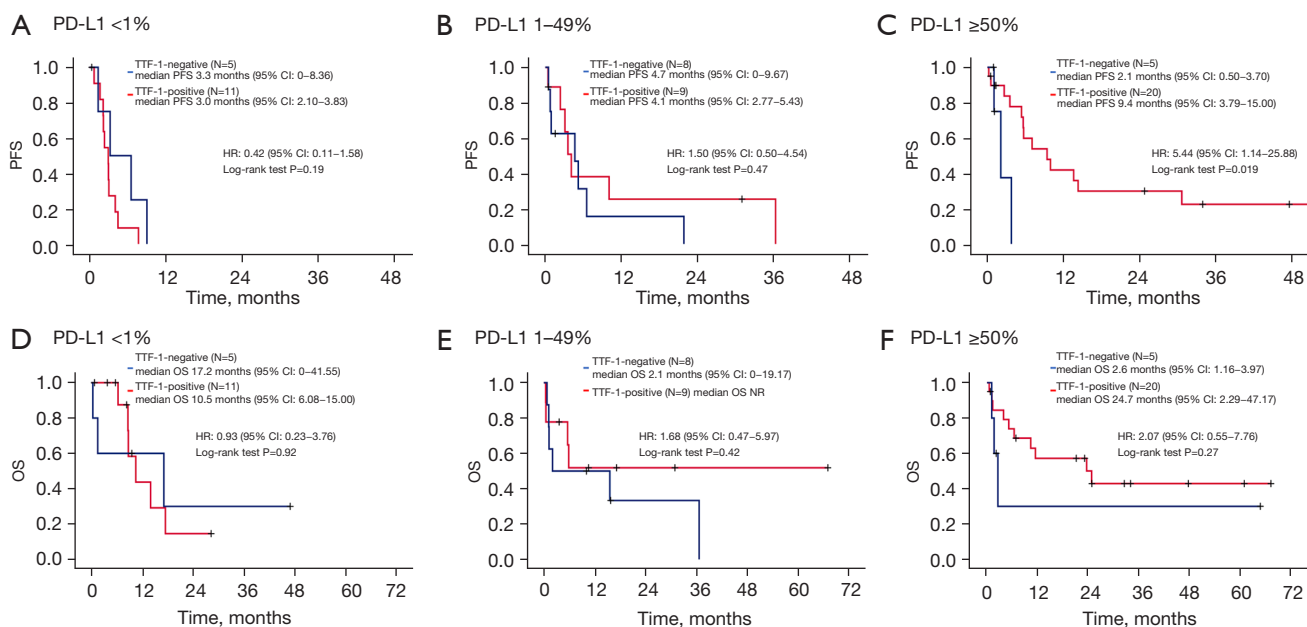
Figure 2 Kaplan-Meier curves in patients treated with ICI + chemotherapy according to TTF-1 expression status. (A) PFS; (B) OS. TTF-1, thyroid transcription factor-1; PFS, progression-free survival; CI, confidence interval; HR, hazard ratio; OS, overall survival; ICI, immune checkpoint inhibitor.
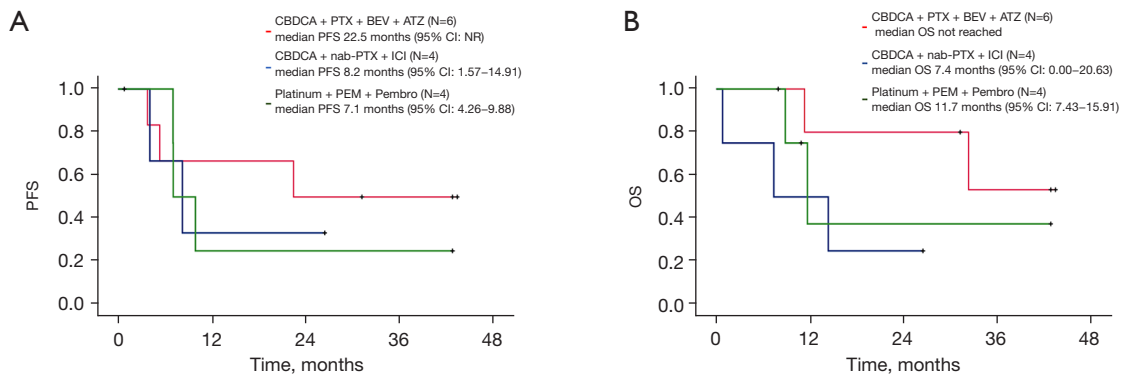
Figure 3 Kaplan-Meier curves in TTF-1-negative patients according to ICI + chemotherapy regimens. (A) PFS; (B) OS. CBDCA, carboplatin; PTX, paclitaxel; BEV, bevacizumab; ATZ, atezolizumab; PFS, progression-free survival; CI, confidence interval; NR, not reached; nab-PTX, nanoparticle albumin-bound paclitaxel; ICI, immune checkpoint inhibitor; PEM, pemetrexed; Pembro, pembrolizumab; OS, overall survival; TTF-1, thyroid transcription factor-1.
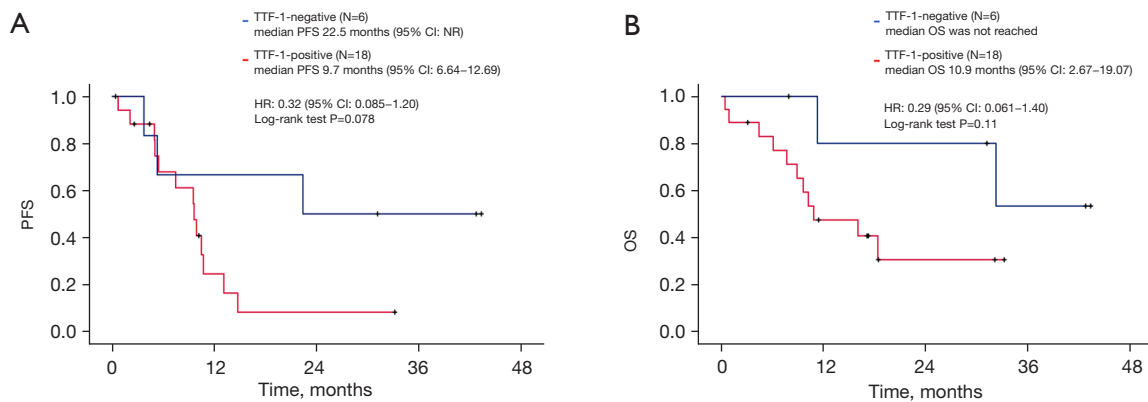
Figure 4 Kaplan-Meier curves in patients treated with ABCP according to TTF-1 expression status. (A) PFS; (B) OS. TTF-1, thyroid transcription factor-1; PFS, progression-free survival; CI, confidence interval; HR, hazard ratio; NR, not reached; OS, overall survival; ABCP, atezolizumab + bevacizumab + carboplatin + paclitaxel.

Figure 5 Kaplan-Meier curves in *EGFR* and *ALK* wild type patients treated with ICI monotherapy according to TTF-1 and PD-L1 expression status. (A) PFS in PD-L1 <1%; (B) PFS in PD-L1 1–49%; (C) PFS in PD-L1 ≥50%; (D) OS in PD-L1 <1%; (E) OS in PD-L1 1–49%; (F) OS in PD-L1 ≥50%. PD-L1, programmed death-ligand 1; TTF-1, thyroid transcription factor-1; PFS, progression-free survival; CI, confidence interval; HR, hazard ratio; OS, overall survival; NR, not reached; *EGFR*, epidermal growth factor receptor; *ALK*, anaplastic lymphoma kinase; ICI, immune checkpoint inhibitor.

group (median PFS: 22.5 *vs.* 9.7 months, P=0.078; median OS: not reached *vs.* 10.9 months, P=0.11). For all the other treatment regimens, PFS and OS in the TTF-1-negative group were not superior to those of TTF-1-positive patients (Figure S2A,S2B). The relationships between TTF-1 expression and the efficacy of all regimens except for ABCP are shown in Figures S3,S4.

Treatment efficacy by TTF-1 expression according to PD-L1 expression status

The relationship between TTF-1 expression status and treatment efficacy according to PD-L1 expression status is shown in *Figures 5,6*. In the ICI monotherapy group excluding *EGFR* and *ALK* mutations/translocations with PD-L1 ≥50% expression, TTF-1-negative patients had statistically worse PFS compared with TTF-1-positive patients (median PFS 2.1 *vs.* 9.4 months, P=0.019). There were no significant differences in OS (median OS 2.6 *vs.* 24.7 months, P=0.27), but mortality in the early stage of initiating treatment tended to be higher in the TTF-1-negative group. In the ICI + chemotherapy group, TTF-

1-negative patients tended to have better PFS and OS than TTF-1-positive patients in the PD-L1 <1% expression group (median PFS 22.5 *vs.* 8.0 months, P=0.056, median OS 32.3 *vs.* 16.1 months, P=0.15).

Discussion

In our study, TTF-1-negative patients treated with ICI monotherapy tended to have worse PFS and OS than TTF-1-positive patients. Additionally, long-term efficacy (>2 years) and therapeutic efficacy for high PD-L1 expression of ICI monotherapy were not observed in TTF-1-negative patients. On the other hand, TTF-1-negative patients treated with ICI plus chemotherapy tended to have better PFS and a similar OS when compared with TTF-1-positive patients. As a side note that ICI plus chemotherapy showed better PFS and OS compared with ICI monotherapy in TTF-1-negative patients (Figure S5). Thus, clinicians should consider ICI plus chemotherapy in TTF-1-negative patients. To our knowledge, this is the first report of a favorable therapeutic efficacy in TTF-1-negative NS-NSCLC. TTF-1-negative status has been recognized as
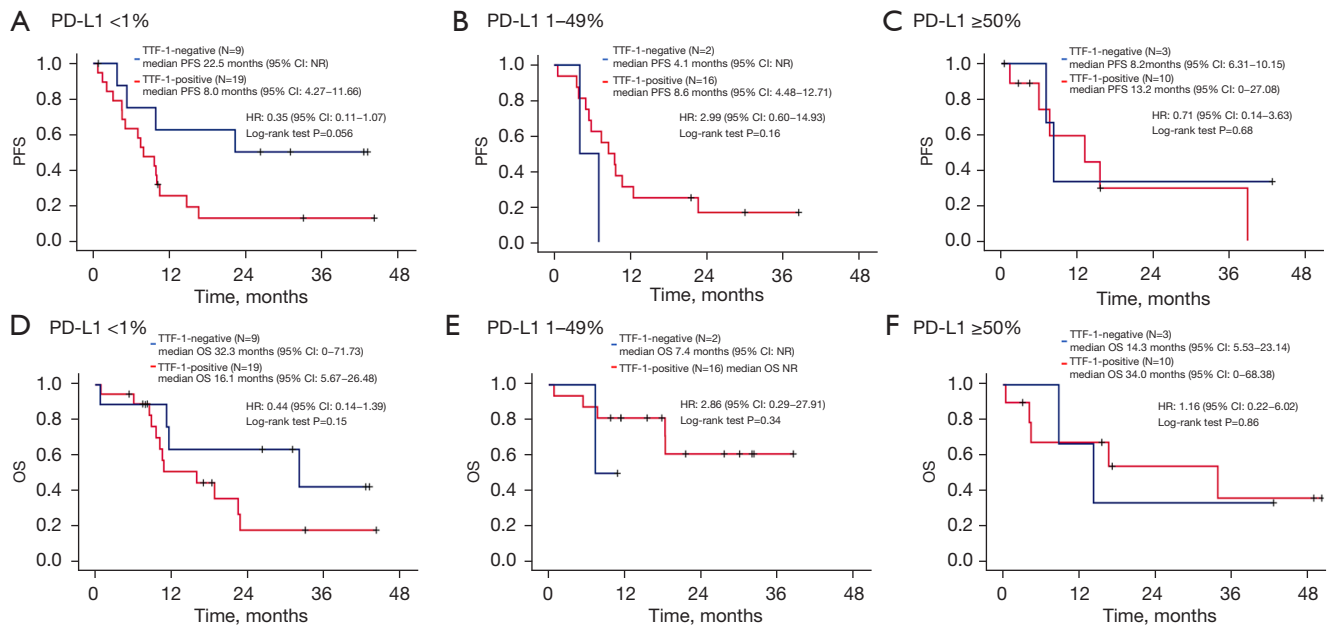
Figure 6 Kaplan-Meier curves in patients treated with ICI plus chemotherapy according to TTF-1 and PD-L1 expression status. (A) PFS in PD-L1 <1%; (B) PFS in PD-L1 1–49%; (C) PFS in PD-L1 ≥50%; (D) OS in PD-L1 <1%; (E) OS in PD-L1 1–49%; (F) OS in PD-L1 ≥50%. PD-L1, programmed death-ligand 1; TTF-1, thyroid transcription factor-1; PFS, progression-free survival; CI, confidence interval; NR, not reached; HR, hazard ratio; OS, overall survival; ICI, immune checkpoint inhibitor.

a poor predictive factor in various chemotherapy regimens, including platinum plus pemetrexed, docetaxel and EGFR-tyrosine kinase inhibitors (TKIs) (11,12,17). Our study showed that ICI/chemotherapy regimens (and especially ABCP) can have long-term efficacy in TTF-1-negative lung cancer patients.

In the ICI monotherapy group TTF-1-negative status tended to be associated with worse PFS and OS compared with TTF-1-positive status, and that PFS tended to be worse in the TTF-1-negative group once *EGFR* and *ALK* mutations/translocations were removed from the analysis. This finding is consistent with those of previous studies (13,14). Neither *EGFR* nor *ALK* status had a significant impact on OS. We suggest this is because our study included patients who had had a long-term response to EGFR-TKIs after progression on ICI monotherapy. Especially in the PD-L1 ≥50% group, TTF-1 negative patients had significantly worse PFS than positive patients. PD-L1 high expression state is generally considered to have a favorable therapeutic efficacy of ICI. Even with high PD-L1 expression, TTF-1-negative patients may not respond to ICI monotherapy as well as TTF-1-positive patients. We also note the fraction of patients with poor

PS was higher in TTF-1-negative compared to TTF-1-positive individuals. TTF-1-negative status is related to a higher frequency of serine-threonine kinase 11 (*STK11*) and Kelch-like epichlorohydrin-associated protein 1 (*KEAP1*) mutations (18,19). These mutations are associated with a tumor microenvironment that has a lower density of tumor-infiltrating leukocytes and cytotoxic CD8+ T lymphocytes, and lower PD-L1 expression (20-24). In our study, the TTF-1-negative group also had a higher proportion of PD-L1 negative tumors. TTF-1-negative status is therefore a poor predictive factor for ICI monotherapy, as it is associated with an 'immune cold' phenotype.

In the ICI plus chemotherapy group, there was no significant difference in PFS and OS between TTF-1-negative and -positive patients, although the Kaplan-Meier curves in both cases for TTF-1-negative patients were slightly above those of TTF-1-positive patients (*Figure 2*). To elucidate which regimens contributed to prolonged PFS and OS in TTF-1-negative cases, we analyzed Kaplan-Meier according to the individual treatment regimens. There was a notably improved trend with the ABCP regimen (*Figure 3A,3B*). Median PFS with ABCP was longer in the TTF-1-negative group than in the TTF-1-positive

group (22.5 *vs.* 9.7 months, respectively, *Figure 4A*). In the IMpower 150 trial, median PFS in the ABCP group was 8.3 months, suggesting that this treatment is by no means less effective in TTF-1-positive patients (25). We consider these differences in therapeutic efficacy are related to the specificity of the TTF-1-negative tumor microenvironment. TTF-1-negative status is associated with serglycin (SRGN) overexpression, and leads to secretion of chemokines and cytokines including interleukin (IL)-6, IL-8, C-X-C motif chemokine 1. These agents reprogram the tumor microenvironment to a more state that is more conducive to malignancy; the tumor cells induce angiogenesis and suppress immune recognition by interacting with fibroblasts and endothelial cells (26). In addition, chronic inflammation in the tumor microenvironment stimulates nuclear factor kappa B (NF-κB) activity. High NF-κB activity induces angiogenesis, suppresses the immune response, and stimulates cancer stem cell formation; together these processes engender a microenvironment that facilitates tumor cell survival and proliferation (27). ABCP is efficacious in NS-NSCLC with *STK-11* or *KEAP1* mutations which are associated with TTF-1-negative status and have a poor prognosis (28). Inhibiting the VEGF pathway improves vascular permeability in tumor tissue, suppresses regulatory T cells, decreases the recruitment of myeloid derived suppressor cells, and reduces the expression of programmed cell death protein 1 (PD-1) on CD8-positive cytotoxic T lymphocytes and regulatory T cells (29-32). We suggest that bevacizumab converts the immunosuppressive tumor microenvironment in TTF-1-negative patients to an immune stimulatory environment via inhibition of VEGF, thereby enhancing the efficacy of atezolizumab. This hypothesis is supported by our observation that atezolizumab plus bevacizumab exhibited long-term efficacy in our study, whereas bevacizumab plus chemotherapy is not efficacious compared with chemotherapy (33). On the other hand, the regimens of ICI plus CBDCA plus nab-paclitaxel and pembrolizumab plus platinum plus pemetrexed were not more effective in TTF-1-negative compared to TTF-1-positive patients (Figures S3,S4). A recent study demonstrated that PFS was significantly longer in TTF-1-positive than TTF-1-negative patients treated with ICI plus platinum plus pemetrexed (34). Our institution used ABCP after EGFR or ALK-TKI failure, in patients with massive pleural effusions, multiple liver metastases, and/or rapidly progressing tumors. ABCP therapy may also be promising for TTF-1-negative NS-NSCLC patients.

Our retrospective study contained several major limitations. First, it was a single center study and the sample size was relatively small, especially once we had subdivided the patients treated with ICI plus chemotherapy into each regimen. This limitation weakened our power to detect meaningful differences in efficacy between regimens. However, we did not exclude patients due to uncertain PD-L1 or TTF-1 status, as we re-examined such cases to confirm the relative status. To the best of our knowledge, previous studies evaluating therapeutic efficacy and TTF-1 expression in NSCLC only included cases where TTF-1 expression status was known, thus leading to selection bias. In contrast, we suggest that our study provides less biased and more clinically practical data. A second limitation of our study was our inability to determine SRGN expression or the mutational status of *KEAP1/STK11* and other druggable mutations (with the exception of *EGFR* and *ALK*). However, neither SRGN expression nor *KEAP1/STK11* mutation routinely screened for in clinical practice, and the status of these mutations is difficult to obtain in all cases. In addition, the status of druggable mutations is not available in all cases, as it is highly dependent on the volume of tumor specimens and the clinical course. The third limitation relates to the examination for TTF-1. Our institution has been using SPT24 antibody for examining tumor TTF-1 status. However, false positive signals with the SPT24 antibody have been reported in squamous cell carcinoma, and the 8G7G3 antibody has better specificity (35). Although we did not diagnose squamous cell carcinoma solely based on TTF-1 expression status, the use of different TTF-1 antibodies could impact the interpretation of our results.

Our study supports previous findings that ICI monotherapy for TTF-1-negative NS-NSCLC has poor efficacy compared with the same treatment in TTF-1-positive cases, and suggests the combination of ICI plus chemotherapy can be efficacious regardless of TTF-1 status. This appears to be the case especially for the ABCP regimen. For NS-NSCLC with poor prognostic factors including TTF-1-negative status, addition of a VEGF inhibitor enhances the efficacy of ICI, and this combination might be an optimal treatment option. Further prospective research with larger cohorts will be required to address this clinical hypothesis.

Conclusions

ICI monotherapy tended to have poor efficacy for TTF-1-negative NS-NSCLC; thus, clinicians should consider ICI plus chemotherapy. Based on our study, we suggest ABCP is an optimal regimen for TTF-1-negative NS-NSCLC.

Acknowledgments

Footnote

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