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ORIGINAL RESEARCH

Diffuse and Strong TTF-1 Expression Predicts Response to Pemetrexed-Based Immunochemotherapy in Advanced Lung Adenocarcinoma

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Purpose: Thyroid transcription factor-1 (TTF-1) is a good prognostic factor for non-small cell lung carcinoma (NSCLC). It is unclear how much TTF-1 staining is sufficient to predict therapeutic response in immunochemotherapy. We evaluated the cut-off of TTF-1 considering the percentage of positive cells and staining intensity as a predictive factor.

Patients and Methods: We conducted a retrospective multicenter study of patients with advanced lung adenocarcinoma or NSCLC favor adenocarcinoma treated with immunochemotherapy. One pathologist centrally examined the immunohistochemical staining of TTF-1 using 8G7G3/1 and provided scores of 0–5 based on the staining intensity and ratio.

Results: We analyzed 95 patients. As TTF-1 has previously been shown to be a predictive factor for pemetrexed, there were significant differences in PFS of patients treated with pemetrexed-based immunochemotherapy between TTF-1 scores of 5 (diffuse and strong staining) and ≤4, but not between 0 (no staining) and 2–4 (partial or weak staining). We defined a TTF-1 score of ≥5 as positive for the predictive factor and the positivity ratio was 61.1%. Patients who tested negative for TTF-1 had a significantly higher proportion of programmed death ligand 1 (PD-L1) tumor proportion score (TPS) <1%. Excluding mutation-positive patients, PFS in TTF-1 positivity was significantly longer than in negativity (8.0 and 5.9, hazard ratio (HR): 0.58 (0.34–0.98), p = 0.04), while TTF-1 negativity was not inferior to positivity in PFS with taxane-based immunochemotherapy. Patients treated with pemetrexed-based immunochemotherapy who tested positive for TTF-1 had significantly longer PFS than those who tested negative (HR: 0.51 (0.27-–0.99), p = 0.045) in the multivariate analysis incorporating age, PD-L1, PS, and TTF-1.

Conclusion: Diffuse and strong TTF-1 positivity may be useful for the predictive factor for pemetrexed-based immunochemotherapy. TTF-1 staining may be desirable to develop a more optimal immunochemotherapy for lung adenocarcinoma.

Keywords: TTF-1, advanced lung adenocarcinoma, immunochemotherapy, immunohistochemical staining

Introduction

Lung cancer is the leading cause of cancer-related deaths worldwide. Histological diagnosis is important as advanced non-small cell lung carcinoma (NSCLC), requires different treatment strategies for each histological type. Thyroid transcription factor-1 (TTF-1) is a transcription factor expressed in type II alveolar epithelial cells of the lung.[1–3] TTF-1 is associated with epithelial cell morphogenesis and differentiation[4,5] and lung tumorigenesis.[6] TTF-1 is expressed in almost 60–80% of lung adenocarcinoma[7–10] and is used as an immunohistochemical (IHC) marker for the diagnosis of lung adenocarcinoma.[11] Some

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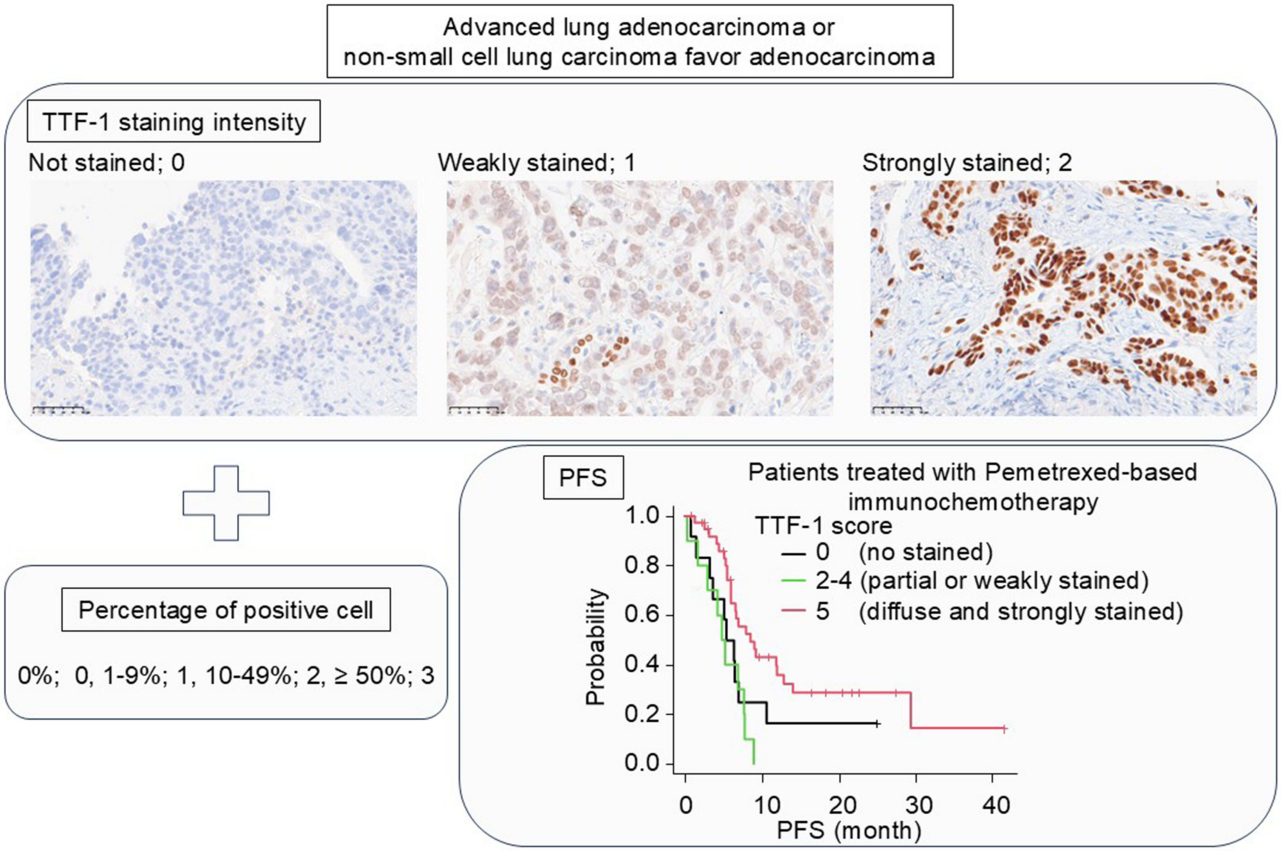
Graphical Abstract



studies have reported that TTF-1 is a good prognostic factor for NSCLC;[12–15] particularly in patients treated with pemetrexed-based chemotherapy.[16,17] Previous studies have investigated the association between TTF-1 and the outcomes of immune checkpoint inhibitor (ICI) monotherapy,[18,19] or ICI in combination with chemotherapy (immunochemotherapy).[20,21] However, in these studies, TTF-1 positivity was determined by each pathologist; no clear criteria were present for TTF-1 positivity. In the assessment of TTF-1, sensitivity and specificity vary depending on the IHC antibody clone. 8G7G3/1 was reported to have a higher specificity (99%), while 6% and 8% were reported to be positive with SPT24 and SP141, respectively in lung squamous cell carcinomas (SCC).[22] Therefore, we used the same IHC antibody clone (8G7G3/1) in this study. Partial positivity of TTF-1 is significant for diagnosis,[23] but was reported to be insufficient as a predictive factor for immunochemotherapy.[24] It is unclear how much TTF-1 staining is sufficient to predict therapeutic response in immunochemotherapy. In this study, we examined the cutoff of TTF-1 for a predictive factor of immunochemotherapy considering not only the percentage of positive cells but also the staining intensity, and aimed to evaluate the association between TTF-1 and the outcome of immunochemotherapy for advanced lung adenocarcinoma.

Materials and Methods

Study Design

We conducted a multicenter retrospective study on patients with advanced lung adenocarcinoma or NSCLC favor adenocarcinoma who began immunochemotherapy between January 2019 and May 2023 at six institutions in Japan. The primary endpoint was progression-free survival (PFS) with TTF-1 positivity or negativity. The secondary endpoints were the objective response rate (ORR), PFS, and overall survival (OS) associated with TTF-1, and programmed death

ligand 1 (PD-L1) expression. The eligibility criteria were as follows: patients aged 20 years or older, histologically diagnosed with advanced lung adenocarcinoma or NSCLC favor adenocarcinoma at each institution, and treated with immunochemotherapy for any treatment line. Informed consent was obtained using an opt-out method, where patients could refuse to participate after checking the study content through the hospital website. This study was approved by the Institutional Ethics Committee of Kobe University Hospital on March 23, 2022 (B210309) and by the Institutional Ethics Committee of each institution and registered with the University Hospital Medical Information Network (UMIN) Clinical Trials Registry (identification number UMIN000046901). The study conformed to the principles of the Declaration of Helsinki.

Data Collection

The following data were retrospectively collected from clinical records at each institution: age, sex, Eastern Cooperative Oncology Group (ECOG)-performance status (PS), staging based on the Union for International Cancer Control (UICC) 8th edition of tumor, nodes, and metastases (TNM) classification, tumor response assessment by each investigator based on the Response Evaluation Criteria in Solid Tumors (RECIST) version 1.1, PFS measured from the date of immunochemotherapy initiation to the date of disease progression or death, OS measured from the date of immunochemotherapy initiation to the date of death, regimen, treatment line, PD-L1 expression, and driver oncogene mutation status such as anaplastic lymphoma kinase (ALK), epidermal growth factor receptor (EGFR), and c-ros oncogene 1 (ROS-1). We collected tumor slides or tissue samples of the participating patients from each institution, and one pathologist (NJ) centrally re-diagnosed the tumors with hematoxylin and eosin (HE) staining according to the 5th edition of the World Health Organization (WHO) classification and re-examined the IHC staining at Kobe University Hospital. In this study, we defined NSCLC favor adenocarcinoma if 1% or more of tumor cells stained for TTF-1, although the tumors did not have morphological findings characteristic of adenocarcinoma or squamous cell carcinoma that would allow diagnosis on HE staining. We excluded patients who could not be diagnosed with lung adenocarcinoma or NSCLC favor adenocarcinoma.

Immunohistochemical Staining

In IHC staining, four-μm thick formalin-fixed, paraffin-embedded (FFPE) sections were used for the IHC staining of the anti TTF-1 antibody (Clone 8G7G3/1; 1:100 dilution) using an autostainer (BOND-III, Leica, Deer Park, US). Antigen retrieval was conducted using CC1 (high pH) for 60 min, and the sections were incubated with the primary antibody for 16 min. All sections were quantified using the staining intensity [negative, 0 (Figure 1A); weak, 1 (Figure 1B); strong, 2 (Figure 1C)] and the percentage of positive cells (0%, 0; 1–9%, 1; 10–49%, 2; ≥50%, 3) of TTF-1 by one pathologist (NJ). IHC scoring (0–5) was conducted based on the previous report[25] by adding up the staining intensity score and percentage of positive cells score.

Sample Size

We did not set a sample size because of the retrospective study. We retrospectively reviewed 27 patients with advanced lung adenocarcinoma or NSCLC favor adenocarcinoma who began immunochemotherapy between January 2019 and June 2021 at Kobe University Hospital. Nineteen patients showed TTF-1 scores of ≥2, and eight patients showed TTF-1 scores of 0. The nine-month PFS rate of the patients with TTF-1 scores of ≥2 was 49.1% and the nine-month PFS rate of

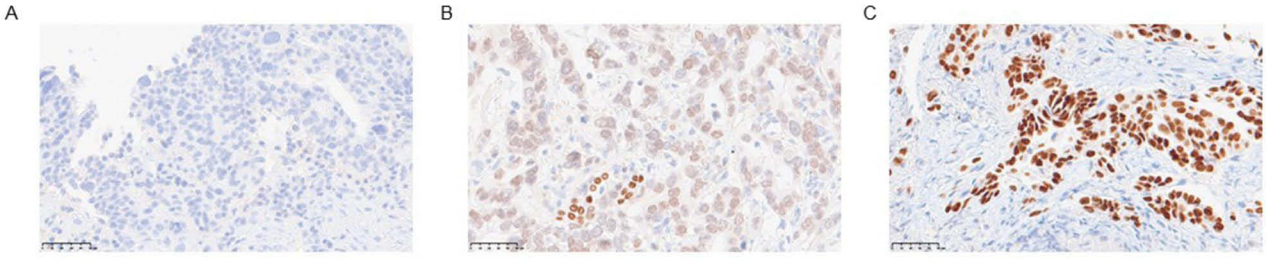


Figure 1 IHC staining of TTF-1. Examples of TTF-1 staining (clone 8G7G3/1) intensities of (**A**) negative, (**B**) weak, and (**C**) strong. Scale bars: 50 μm.

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the patients with TTF-1 scores of 0 was 25.0%. A one-sided type I error rate of 0.05, and a target power of 80% led to sample sizes of 68 patients.

Statistical Analyses

Statistical analyses were conducted with EZR software, version 1.51 (Saitama Medical Center, Jichi Medical University, Saitama, Japan).[26] We described age as the median and range and summarized the demographic variables as frequencies and percentages. We described PFS and OS as the medians and estimated 95% confidence intervals (CIs). Receiver operating characteristic (ROC) curve analysis was used to assess the cut-off for TTF-1 positivity. The Mann–Whitney *U*-test was used to assess the association between age and TTF-1. Fisher's exact test was used to assess the association between demographic variables and TTF-1 levels and used to compare the ORR. We used the Kaplan–Meier method to estimate PFS and OS, and the differences were assessed with the Log rank test. The hazard ratios (HRs) for PFS and OS with 95% CI were estimated for univariate and multivariate analyses using the Cox proportional hazards model. In multivariate analysis, we included the following factors: TTF-1, age, ECOG-PS, and PD-L1. The odds ratio (OR) for ORR with 95% CI was estimated for univariate analysis using the logistic regression model. We set statistical significance at $p < 0.05$.

Results

Patient Characteristics

Overall, 101 patients were treated with immunochemotherapy for advanced lung adenocarcinoma or NSCLC favor adenocarcinoma between January 2019 and May 2023 at six institutions. Six patients were excluded as they were not diagnosed with lung adenocarcinoma or NSCLC favor adenocarcinoma. We analyzed 95 patients in this study (Figure 2). Patient characteristics are shown in Table 1. The median age in this study was 71 (44–81) years; 70 (73.7%) males were included, and the number of patients with ECOG-PS less than 2 was 90 (94.7%). The number of patients with adenocarcinoma was 74 (77.9%). Twenty (21.1%) patients had PD-L1 tumor proportion score (TPS) of ≥ 50%. We included 16 (16.8%) mutation-positive patients, and all 14 (14.7%) patients received immunochemotherapy as the second or third-line treatment were previously treated with EGFR tyrosine kinase inhibitor (TKI). A total of 61 patients (64.2%) received pemetrexed-based regimens, while 34 (35.8%) received taxane-based regimens. All of the patients treated with pembrolizumab or nivolumab + ipilimumab were treated with pemetrexed, and all of the patients treated with atezolizumab were treated with taxane.

Efficacy of Immunochemotherapy

With the median follow-up of 14.5 (range: 0.6–45.1) months, the median PFS and median OS were 6.9 (95% CI: 5.9–8.0) months and 20.1 (95% CI: 16.2-NA) months, respectively. The ORR and disease control rate (DCR) were 45.3% and 88.4%, respectively, with 43 (45.3%) partial response (PR), 40 (42.1%) stable disease (SD), one (1.1%) non-PR/non-progressive disease (PD), five (5.3%) PD, and six (6.3%) not evaluable (NE). In the univariable analysis of patient

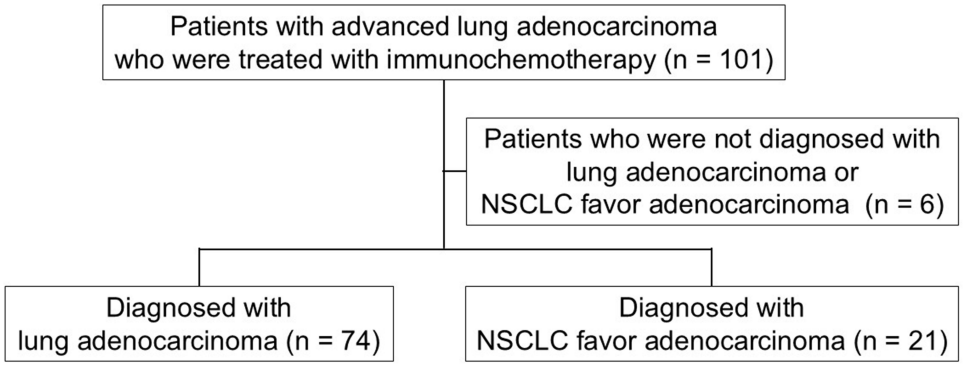

Figure 2 Consort diagram of this study.
Abbreviation: NSCLC, non-small cell lung carcinoma.

Table 1 Patient Characteristics According to the Expression of TTF-1

	All n = 95 (%)	TTF-1 Positivity n = 58 (%)	TTF-1 Negativity n = 37 (%)	p value
Age (years old, median, (range))	71 (44–81)	71 (48–79)	69 (44–81)	0.93
Sex				
Male	70 (73.7)	40 (69.0)	30 (81.1)	
Female	25 (26.3)	18 (31.0)	7 (18.9)	0.24
ECOG PS score				
0	28 (29.5)	14 (24.1)	14 (37.8)	
1	62 (65.3)	41 (70.7)	21 (56.8)	
2	5 (5.3)	3 (5.2)	2 (5.4)	0.36
Stage				
III	9 (9.5)	6 (10.3)	3 (8.1)	
IV	70 (73.7)	42 (72.4)	28 (75.7)	
Recurrence	16 (16.8)	10 (17.2)	6 (16.2)	1
Histology				
Adenocarcinoma	74 (77.9)	42 (72.4)	32 (86.5)	
NSCLC favor adenocarcinoma	21 (22.1)	16 (27.6)	5 (13.5)	0.13
PD-L1 tumor proportion score				
<1%	26 (27.4)	8 (13.8)	18 (48.6)	
1–49%	36 (37.9)	26 (44.8)	10 (27.0)	
≥50%	20 (21.1)	14 (24.1)	6 (16.2)	< 0.01
Not Applicable	13 (13.7)	10 (17.2)	3 (8.1)	
Mutation status				
Negative	79 (83.2)	46 (79.3)	33 (89.2)	
EGFR	14 (14.7)	11 (19.0)	3 (8.1)	
ALK	1 (1.1)	1 (1.7)	0	
ROS-1	1 (1.1)	0	1 (2.7)	0.2
Immune checkpoint inhibitor				
Pembrolizumab	50 (52.6)	33 (56.9)	17 (45.9)	
Atezolizumab	34 (35.8)	19 (32.8)	15 (40.5)	
Nivolumab + Ipilimumab	11 (11.6)	6 (10.3)	5 (13.5)	0.6
Chemotherapy regimen				
Pemetrexed-based	61 (64.2)	39 (67.2)	22 (59.5)	
Taxane-based	34 (35.8)	19 (32.8)	15 (40.5)	0.51

Abbreviations: TTF-1, thyroid transcription factor-1; ECOG PS, Eastern Cooperative Group performance status; NSCLC, non-small cell lung carcinoma; PD-L1, programmed death ligand 1; EGFR, epidermal growth factor receptor; ALK, anaplastic lymphoma kinase; ROS-1, c-ros oncogene 1. Each parameter is expressed as the number (percentage) of patients and median (range).

characteristics associated with PFS, patients who were ≥75 years had worse PFS than those who were <75 years, and there were no significant differences in PFS between PS 0–1 and 2, between PD-L1 ≥50% and <50%, and between adenocarcinoma or NSCLC favor adenocarcinoma. No significant differences were observed in PFS between patients treated with taxane- and pemetrexed-based immunochemotherapy, and between each ICI.

TTF-1 Scoring and the Definition of TTF-1 Positivity for Predictive Factors

NJ conducted IHC staining on 95 samples, including 14 (14.7%) surgical samples, 74 (77.9%) biopsy samples, and seven (7.4%) cell block samples. The scoring of TTF-1 IHC staining is shown in Figure 3. In 74 patients with adenocarcinoma, 57 (77.0%) patients showed TTF-1 scores of ≥2.

Percentage of positive cells	Staining intensity: 0	1	2	
All, n = 95				
0 (0%)	17			n = 17
1 (1-9%)		1	0	n = 1
2 (10-49%)		5	2	n = 7
3 (≥ 50%)		12	58	n = 70
	n = 17	n = 18	n = 60	

Score	n (%)
0	17 (17.9)
2	1 (1.1)
3	5 (5.3)
4	14 (14.7)
5	58 (61.1)

Figure 3 TTF-1 scoring combined staining intensity and the percentage of stained cells. This scoring (0–5) was conducted by adding up the staining intensity score and percentage of positive cells score. The score of 0 was filled with a gray color, the score of 2 was filled with blue color, the score of 3 was filled with green color, the score of 4 was filled with yellow color, and the score of 5 was filled with Orange color.
Abbreviation: TTF-1, thyroid transcription factor-1.

As TTF-1 has previously been shown to be a predictive factor for pemetrexed,[16,17,21] we first compared PFS in patients treated with pemetrexed-based immunochemotherapy, with significant differences between TTF-1 scores of 5 and ≤4, but not between 0 and 2–4 (Figure 4). ROC curves for the association between TTF-1 score and PFS were plotted to assess cut-off values. The area under the curve (AUC) was calculated as 0.641 for the TTF-1 score in patients treated with pemetrexed-based immunochemotherapy, with a TTF-1 score of 5 corresponding to the highest combined sensitivity and specificity on the ROC curve (43.9% sensitivity and 87.1% specificity). Therefore, we defined the TTF-1 score of 5 as TTF-1 positivity, and the TTF-1 score of ≤4 as TTF-1 negativity in this study. The positivity rate of TTF-1 was 61.1%. As presented in Table 1, no significant differences were observed in patient characteristics between positive and negative TTF-1, excluding PD-L1 expression. In five patients with NSCLC favor adenocarcinoma and TTF-1 negativity, all their TTF-1 scores were ≥3 not 0. All patients who tested positive for EGFR/ROS-1 and negative for TTF-1 had a TTF-1 score of 4 not 0.

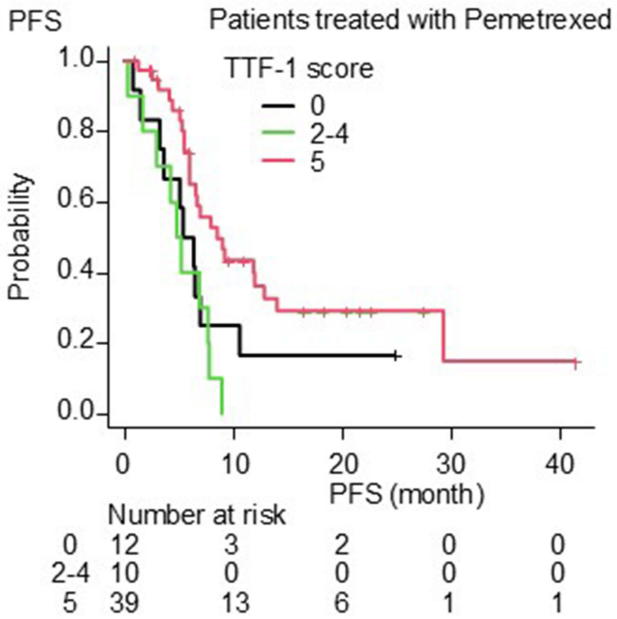

Figure 4 Kaplan–Meier survival curve analysis of PFS in patients who were treated with pemetrexed-based immunochemotherapy (n = 61) with TTF-1 scores of 0 (n = 12), 2–4 (n = 10), and 5 (n = 39). There were significant differences between TTF-1 scores of 5 and ≤ 4, but not between 0 and 2–4.
Abbreviations: PFS, progression-free survival; TTF-1, thyroid transcription factor-1.

Efficacy of Immunochemotherapy on TTF-1

All patients (n = 95); Patients with TTF-1 positivity tended to have improved ORR and PFS (Table 2 and Figure 5A).

EGFR/ALK/ROS-1-negative patients (n = 79); Excluding patients with EGFR/ALK/ROS-1 positivity, the median PFS was significantly longer in patients who tested positive rather than negative for TTF-1 (Figure 5B).

Patients with adenocarcinoma (n = 74); No significant differences were observed in ORR and PFS between the adenocarcinoma patients who tested positive and negative for TTF-1.

Patients treated with pemetrexed-based (n = 61) and with taxane-based immunochemotherapy (n = 34); The median PFS for patients treated with pemetrexed-based immunochemotherapy with TTF-1 positivity was significantly longer than TTF-1 negativity (Table 2 and Figure 5C). In the multivariate analysis of PFS in patients treated with pemetrexed-based immunochemotherapy incorporating age, PD-L1, PS, and TTF-1, TTF-1 was the only independent predictor of PFS (Table 3). On the other hand, the median PFS for patients treated with taxane-based immunochemotherapy with TTF-1 negativity was not inferior to those with TTF-1 positivity (Table 2 and Figure 5D).

Patients with TTF-1 negativity (n = 37); In patients who tested negative for TTF-1, patients tended to show better ORR and PFS when treated with taxane-based immunochemotherapy (n = 15) than with pemetrexed-based immunochemotherapy (n = 22) (ORR, 53.3% and 22.7%, respectively; p = 0.083. PFS, 6.4 months; 95% CI: 5.0–13.8 and 5.3 months; 95% CI: 3.2–6.9, respectively; p = 0.16). The patient characteristics of patients who tested negative for TTF-1 with respect to age, sex, PS, stage, histology and PD-L1 did not differ significantly between pemetrexed and taxane-based immunochemotherapy.

Patients treated with each ICI [pembrolizumab (n = 50), atezolizumab (n = 34), and nivolumab + ipilimumab (n = 11)]; The patients treated with pembrolizumab with TTF-1 positivity (n = 33) had significantly better ORR and longer PFS than those with TTF-1 negativity (n = 17) (ORR, 51.5% and 17.6%, respectively; p = 0.032. PFS, 8.5 months; 95% CI: 5.9–12.8 and 5.4 months; 95% CI: 1.6–7.6, respectively; p = 0.022), while no significant difference was shown in the outcomes of the patients treated with atezolizumab between the patients with TTF-1 positivity (n = 19) and negativity (n = 15). There were

Table 2 Efficacy of Immunochemotherapy on TTF-1

	TTF-1 Positivity	TTF-1 Negativity	HR (95% CI)	p value
All (n = 95)	58 (61.1)	37 (38.9)		
ORR, %	51.7	35.1	1.98 (0.85–4.6)	0.141
PFS, month (95% CI)	7.6 (5.9–9.2)	6.4 (5.0–6.9)	0.63 (0.39–1.0)	0.056
OS, month (95% CI)	22.9 (19.0-NA)	18.6 (11.4-NA)	0.65 (0.36–1.2)	0.17
EGFR/ALK/ROS-1-negative (n = 79)	46 (58.2)	33 (41.8)		
ORR, %	52.2	36.4	1.9 (0.76–4.8)	0.179
PFS, month (95% CI)	8.0 (5.9–12.0)	5.9 (4.8–7.6)	0.58 (0.34–0.98)	0.04
OS, month (95% CI)	21.4 (15.4-NA)	16.2 (11.4-NA)	0.66 (0.35–1.2)	0.197
Adenocarcinoma (n = 74)	42 (56.8)	32 (43.2)		
ORR, %	45.2	31.3	1.8 (0.69–4.8)	0.241
PFS, month (95% CI)	8.0 (5.5–12.0)	6.4 (4.8–6.9)	0.63 (0.37–1.1)	0.1
OS, month (95% CI)	NA (19.0-NA)	13.4 (9.7-NA)	0.54 (0.27–1.1)	0.079
Pemetrexed-based (n = 61)	39 (63.9)	22 (36.1)		
ORR, %	48.7	22.7	3.2 (0.99–10.5)	0.059
PFS, month (95% CI)	8.5 (5.9–12.8)	5.3 (3.2–6.9)	0.43 (0.23–0.79)	< 0.01
OS, month (95% CI)	21.4 (15.4-NA)	18.6 (10.5-NA)	0.67 (0.31–1.4)	0.298
Taxane-based (n = 34)	19 (55.9)	15 (44.1)		
ORR, %	57.9	53.3	1.2 (0.31–4.7)	1
PFS, month (95% CI)	7.3 (4.4–8.4)	6.4 (5.0–13.8)	1.3 (0.56–2.8)	0.587
OS, month (95% CI)	NA (11.2-NA)	16.2 (8.2-NA)	0.59 (0.20–1.7)	0.323

Abbreviations: TTF-1, thyroid transcription factor-1; OR, odds ratio; HR, hazard ratio; CI, confidence interval; ORR, objective response rate; PFS, progression-free survival; OS, overall survival.

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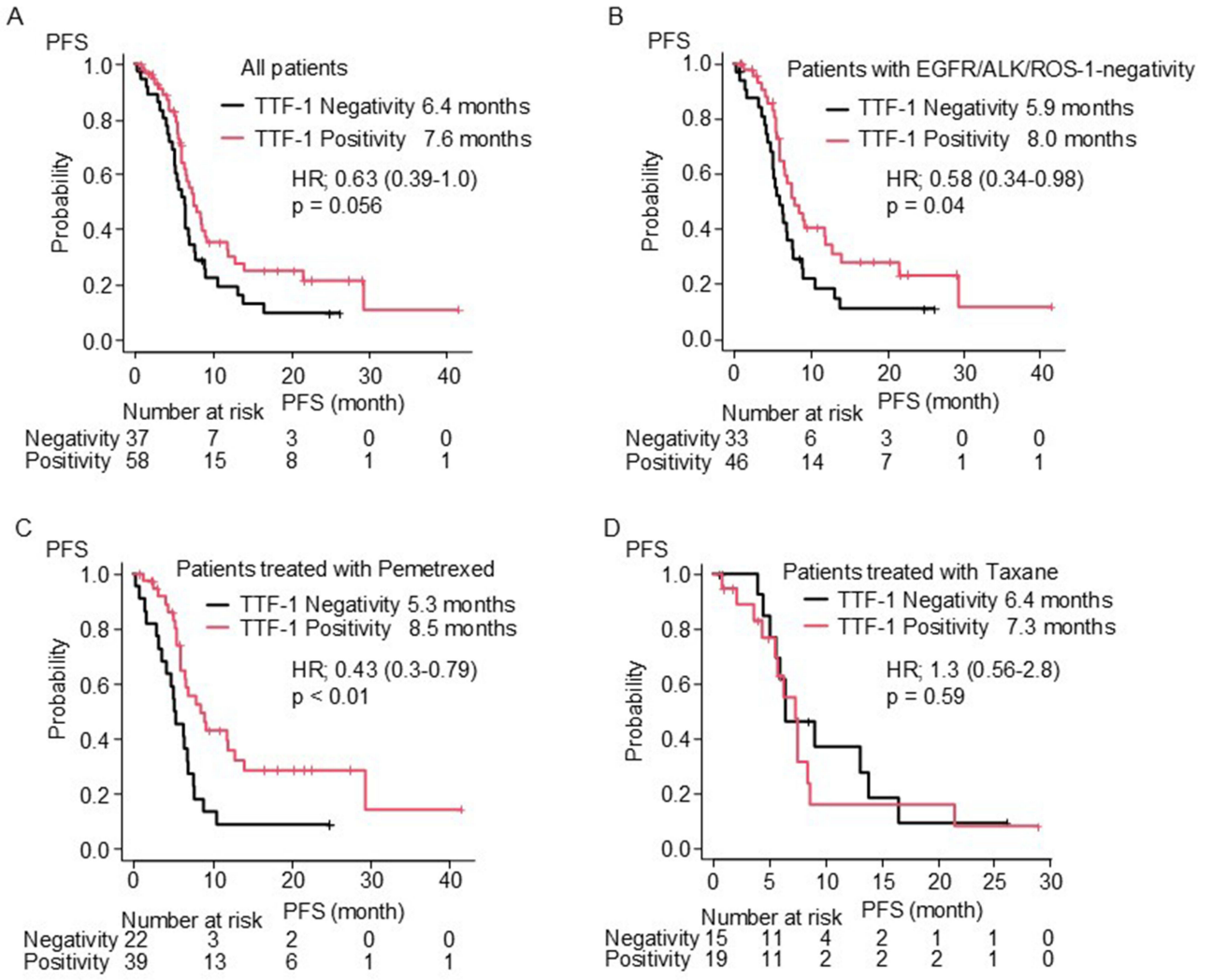

Figure 5 Kaplan–Meier survival curve analysis of PFS with 95% CI and HR with 95% CI. (**A**) In all patients (n = 95), patients with TTF-1 positivity (n = 58) with TTF-1 positivity tended to have longer PFS than those with TTF-1 negativity (n = 37). (**B**) In patients with EGFR/ALK/ROS-1 negativity (n = 79), patients with TTF-1 positivity (n = 46) had significantly longer PFS than those with TTF-1 negativity (n = 33). (**C**) In patients who were treated with pemetrexed-based immunochemotherapy (n = 61), patients with TTF-1 positivity (n = 39) had significantly longer PFS than those with TTF-1 negativity (n = 22). (**D**) In patients who were treated with taxane-based immunochemotherapy (n = 34), PFS for patients with TTF-1 negativity (n = 15) was not inferior to those with TTF-1 positivity (n = 19).
Abbreviations: PFS, progression-free survival; TTF-1, thyroid transcription factor-1; HR, hazard ratio; CI, confidence interval.

no significant differences in outcomes between the patients treated with nivolumab + ipilimumab between the patients with TTF-1 positivity (n = 6) and negativity (n = 5) (ORR, 33.3% and 40%, respectively; p = 1. PFS, 5.5 months; 95% CI: 2.5-NA and 5.0 months; 95% CI: 2.9-NA, respectively; p = 0.187), and in patients with TTF-1 negativity, no significant

Table 3 Multivariable Analysis of Factors Associated with PFS and OS of the Patients Who Were Treated with Pemetrexed-Based Chemotherapy (n = 61)

Variables	PFS, HR (95% CI)	p value	OS, HR (95% CI)	p value
TTF-1	0.51 (0.27–0.99)	0.045	0.83 (0.37–1.83)	0.64
Age ≥ 75	2.15 (0.94–4.91)	0.069	1.64 (0.58–4.65)	0.35
ECOG PS ≥ 2	1.33 (0.40–4.44)	0.64	2.92 (0.83–10.3)	0.096
PD-L1 TPS of ≥ 50%	0.86 (0.39–1.91)	0.71	0.74 (0.30–1.80)	0.5

Abbreviations: PFS, progression-free survival; HR, hazard ratio; CI, confidence interval; OS, overall survival; TTF-1, thyroid transcription factor-1; ECOG-PS, Eastern Cooperative Group performance status; PD-L1 TPS, programmed death ligand 1 tumor proportion score.

difference was observed in the outcomes between the patients treated with nivolumab + ipilimumab (n = 5) and other ICI (n = 32) (ORR, 40% and 34.4%, respectively; p = 1. PFS, 5.0 months; 95% CI: 2.9-NA and 6.4 months; 95% CI: 5.0–7.7, respectively; p = 0.37).

TTF-1 and PD-L1

As presented in Table 1, patients who tested negative for TTF-1 had a significantly higher proportion of PD-L1 TPS < 1%. The patients with both a PD-L1 TPS of <1% and TTF-1 score of 0 (n = 7) had significantly shorter PFS than those with both a PD-L1 TPS of ≥50% and TTF-1 score of 5 (n = 14) and those with others (n = 61) (4.2 months; 95% CI: 1.4-NA, 6.5 months; 95% CI: 3.6–9.2 and 6.9 months; 95% CI: 5.6–8.5, respectively; p = 0.034). The median PFS of patients with both a PD-L1 TPS of <1% and TTF-1 score of 0 was significantly worse than those of others (4.2 months; 95% CI: 1.4-NA and 6.7 months; 95% CI: 5.9–8.4, respectively; p < 0.01), while there was no significant difference in the PFS between patients with both a PD-L1 TPS of ≥50% and TTF-1 score of 5 and those with others (6.5 months; 95% CI: 3.6–9.2 and 6.4 months; 95% CI: 5.5–7.7, respectively; p = 0.73). In the multivariate analysis of PFS incorporating age, PS, and both a PD-L1 TPS of <1% and TTF-1 score of 0, the age and both a PD-L1 TPS of <1% and TTF-1 score of 0 were each independent predictors of PFS (Age, HR: 2.06; 95% CI: 1.09–3.88, p = 0.026. Both a PD-L1 TPS of <1% and TTF-1 score of 0, HR: 2.57; 95% CI: 1.05–6.28, p = 0.039).

Discussion

In this study, we defined diffuse and strong TTF-1 staining (TTF-1 score of 5) as a predictive factor of immunochemotherapy and showed that patients with TTF-1 positivity had significantly longer PFS than those with TTF-1 negativity excluding patients with mutation positivity. Diffuse and strong TTF-1 positivity may be useful for the predictive factor for pemetrexed-based immunochemotherapy. On the other hand, taxane-based immunochemotherapy was effective regardless of TTF-1 expression.

In previous reports on the association between ICI outcome and TTF-1,[18–20] no clear criteria were present for TTF-1 positivity. In this study, as one pathologist conducted and reviewed TTF-1 staining using the same device, same clone (8G7G3/1), and unified criteria, such as staining intensity and percentage of positive cells, a low margin of error was expected in the TTF-1 assessment. Moreover, we conducted TTF-1 scoring (0–5) by adding up the staining intensity score and percentage of positive cells score and clearly defined diffuse and strong TTF-1 staining as a predictive factor of immunochemotherapy. Lung adenocarcinoma is classified into terminal respiratory unit (TRU) and non-TRU adenocarcinomas.[27] TRU includes Clara cells and type II alveolar epithelial cells, is well differentiated, and expresses TTF-1, whereas non-TRU adenocarcinoma derived from mucous columnar cells does not express TTF-1 and has a worse prognosis than TRU.[28–30] Some tumors had intermediate morphological features between TRU and non-TRU adenocarcinomas.[30] Patients with partial positivity of TTF-1 were reported to have shorter OS for immunochemotherapy than patients with diffuse positivity.[24] Although partial positivity of TTF-1 is significant for diagnosis, considering that patients with no TTF-1 staining (TTF-1 score of 0) had similar PFS with pemetrexed-based immunochemotherapy to those with partial or weak TTF-1 staining (TTF-1 score of 2–4), TTF-1 was considered to be an indicator of a better response to pemetrexed-based immunochemotherapy, when it was diffuse and strongly stained.

Excluding those who tested positive for EGFR/ALK/ROS-1, the PFS of immunochemotherapy was significantly longer in patients who tested positive rather than negative for TTF-1. This result was similar to that of a previous report in patients treated with immunochemotherapy for the first line treatment.[20] TTF-1 negativity is associated with serine-threonine kinase 11 (STK11) loss,[31–33] and tumor-associated neutrophil recruitment,[34,35] which are associated with the tumor microenvironment and affect the response to ICI treatment. This might explain the tendency of poor outcomes in patients treated with immunochemotherapy who test negative for TTF-1 in this study.

We showed that TTF-1 negativity was significantly associated with worse outcomes in patients treated with pemetrexed-based immunochemotherapy, as previously reported.[21] In lung adenocarcinoma with TTF-1 negative, thymidylate synthase (TS) are highly expressed.[36] TS positivity is associated with poor outcomes in pemetrexed-based chemotherapy.[16,37] Moreover, it was reported that pemetrexed increased PD-L1 expression and created a favourable microenvironment for immunotherapy through the TS−reactive oxygen species−Nuclear factor κB (NF-κB) pathway, and that the failure to stimulate this pathway by pemetrexed resulted in failure to increase PD-L1 expression.[38] This could explain the poor outcomes of the patients treated with pemetrexed-based immunochemotherapy with TTF-1 negativity.

Conversely, the outcomes of the patients treated with taxane-based immunochemotherapy with TTF-1 negativity were not inferior to those with TTF-1 positivity. Additionally, in TTF-1 negativity, patients treated with taxane-based immunochemotherapy showed better ORR and PFS than those with pemetrexed-based immunochemotherapy. The patients treated with pembrolizumab with TTF-1 positivity had significantly better outcomes than those with TTF-1 negativity, this might be influenced by pemetrexed because all of the patients treated with pembrolizumab were treated with pemetrexed. On the other hand, the outcomes of the patients treated with nivolumab + ipilimumab with TTF-1 negativity were not inferior to those with TTF-1 positivity despite the treatment of pemetrexed. Further investigations of immunochemotherapy regimens for advanced lung adenocarcinoma with TTF-1 negativity are required.

In this study, patients with TTF-1 negativity had a significantly higher proportion of PD-L1 TPS < 1% than patients with TTF-1 positivity. Although their positive criteria differed from those of this study, previous reports have stated that TTF-1 expression was significantly associated with PD-L1 expression.[19,20] In basic studies, some have reported that TTF-1 expression correlates with PD-L1 expression,[39] while others have reported that TTF-1 negativity is more common in tumors with high PD-L1 expression.[40] This real-world data showed that patients who tested negative for TTF-1 had a significantly higher proportion of PD-L1 TPS < 1%. Similar to the present study, it was reported that outcomes were worse with ICI monotherapy for both TTF-1 and PD-L1 negativity,[18] and better with immunochemotherapy for both TTF-1 positivity and a PD-L1 TPS of ≥50%.[20] Further investigation into the association between TTF-1 and PD-L1 expression is required.

There were some limitations in this study. First, this study was retrospective and limited to a single-race. Second, the outcomes of immunochemotherapy were not centrally reviewed. Though the outcome was evaluated based on imaging findings according to RECIST version 1.1, the timing of the evaluation was not specified due to the retrospective nature and varied according to institutional standards. And then the sample size was not large, but the number of patients was sufficient to meet the initially calculated sample size. These limitations may affect the generalizability of our findings. While we observed trends in the association between TTF-1 expression and outcomes, these did not reach statistical significance. Although we have shown trends towards better outcomes with TTF-1 negativity when treated with taxane-based immunochemotherapy than with pemetrexed-based immunochemotherapy, there may be potential confounding factors or selection bias. These findings should be interpreted with caution, and further prospective studies with larger cohorts are required to confirm their clinical relevance. We participated in the Phase II clinical trial of TTF-1 negative advanced non-squamous NSCLC (jRCTs071220008). Despite these limitations, our study suggests that TTF-1 has the potential as biomarker for tailoring immunochemotherapy in lung adenocarcinoma. These biomarkers could help identify patients who are likely to benefit from specific regimens. Future research should focus on prospective validation of these findings in larger, multi-center cohorts, as well as exploring the underlying biological mechanisms. Additional subgroup analyses based on different treatment regimens and patient characteristics provided further support for our main findings. These observations underline the importance of comprehensive biomarker profiling in personalizing lung adenocarcinoma treatment.

Conclusions

Diffuse and strong TTF-1 staining may be useful for the predictive factor for patients with advanced lung adenocarcinoma or NSCLC favor adenocarcinoma treated with pemetrexed-based immunochemotherapy. TTF-1 staining may be desirable to develop a more optimal immunochemotherapy for lung adenocarcinoma.

Abbreviations

NSCLC, non-small cell lung carcinoma; TTF-1, thyroid transcription factor-1; IHC, immunohistochemical; ICI, immune checkpoint inhibitor; immunochemotherapy, immune checkpoint inhibitor therapy in combination with chemotherapy; SCC, squamous cell carcinoma; PFS, progression-free survival; ORR, objective response rate; OS, overall survival; PD-L1, programmed death ligand 1; ECOG-PS, Eastern Cooperative Oncology Group performance status; EGFR, epidermal growth factor receptor; ALK, anaplastic lymphoma kinase; ROS-1, c-ros oncogene 1; 95% CIs, 95% confidence intervals; HR, hazard ratio; OR, odds ratio; TPS, tumor proportion score; TRU, terminal respiratory unit; STK11, serine-threonine kinase 11; TS, thymidylate synthase.

Data Sharing Statement

Data included in article/supp. material/referenced in article. The datasets generated and/or analyzed during the current study are available from the corresponding author on reasonable request.

Ethics Approval and Informed Consent

The study conformed to the principles of the Declaration of Helsinki. This study was approved by the Institutional Ethics Committee of Kobe University Hospital on March 23, 2022 (B210309) and by the Institutional Ethics Committee of each institution. Informed consent was obtained using an opt-out method, where patients could refuse to participate after checking the study content through the hospital website.

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Author Contributions

All authors made a significant contribution to the work reported, whether that is in the conception, study design, execution, acquisition of data, analysis and interpretation, or in all these areas; took part in drafting, revising or critically reviewing the article; gave final approval of the version to be published; have agreed on the journal to which the article has been submitted; and agree to be accountable for all aspects of the work.

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