A strong association between TTF-1 expression and interstitial lung disease in predicting the efficacy of PD-1 inhibitor for nonsquamous NSCLC patients

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PD-1 inhibitor for NSCLC patients with ILD is challenging due to irAEs. Those patients without TTF-1 expression noticeably showed poorer prognosis than those without ILD suggesting TTF-1 as selection marker especially for NSCLC patients with ILD. https://bit.ly/4cSBwkF

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Abstract

Background Thyroid transcriptional factor-1 (TTF-1) is associated with the development of interstitial lung disease (ILD) and is a mutational target in lung adenocarcinoma with ILD. TTF-1 expression is also associated with the efficacy of pemetrexed-based chemotherapy for nonsquamous nonsmall cell lung cancer (NS-NSCLC). However, the relationship between TTF-1 expression and the efficacy of immunotherapy using programmed cell death 1 inhibitor (PD-1i), especially in lung cancer patients with ILD, remains unclear.

Methods Medical data of NS-NSCLC patients treated with PD-1i at multiple centres were analysed retrospectively. Patients were divided into those with or without concomitant ILD, with the two cohorts further stratified by TTF-1 expression.

Results The study population included 62 NS-NSCLC patients, 34 with and 28 without ILD. Median progression-free survival (PFS) during PD-1i treatment was significantly shorter in TTF-1-negative than -positive patients (2.0 *versus* 12.1 months, p=0.004) in the ILD cohort but did not differ significantly in the non-ILD cohort (1.8 *versus* 2.6 months, p=0.63). Median overall survival (OS) was also significantly shorter in TTF-1-negative than -positive patients in the ILD cohort (14.5 *versus* 42.5 months, p=0.018) but not in the non-ILD cohort (33.7 *versus* 37.1 months, p=0.53). Cox regression analyses showed that absence of TTF-1 expression was an independent risk factor for PFS (hazard ratio (HR) 2.75, p=0.024) and OS (HR 2.81, p=0.012) in the ILD cohort.

Conclusion TTF-1 expression in NS-NSCLC patients with ILD may predict prognosis when treated with PD-1i.

Introduction

Interstitial lung disease (ILD) is a strong risk factor for lung carcinogenesis independent of smoking [1, 2]. The cumulative incidence rate of lung cancer within 10 years in patients with ILD is as high as 54.7% [3]. The biological characteristics of tumours in nonsmall cell lung cancer (NSCLC) patients with ILD, relative

to patients without ILD, include lower frequencies of druggable driver mutations, especially *EGFR* mutations, a higher level of programmed cell death ligand 1 (PD-L1), a higher tumour proportion score (TPS) and a higher tumour mutation burden [4, 5], these characteristics being associated with favourable responses to immune checkpoint inhibitors, such as programmed cell death 1 inhibitors (PD-1i) [6]. The efficacy of PD-1i was recently reported to be noninferior in NSCLC patients with ILD compared with those without ILD [7, 8]. However, the treatment of lung cancer patients with ILD remains challenging because any treatment approach, such as surgery and pharmacotherapy including with PD-1i, could trigger the exacerbation of ILD, worsening respiratory conditions [7, 9]. Although PD-1i may be an effective treatment option for NSCLC patients with ILD, rigorous patient selection is required to avoid the development of adverse events of PD-1i treatment in patients with low expectations of drug efficacy.

Thyroid transcription factor-1 (TTF-1) is a tissue-specific transcription factor that regulates the development of the lungs and thyroid. Dysfunction in *NKX2-1*, the gene encoding TTF-1, is associated with the development of ILD [10, 11]. TTF-1 expression distinguishes lung adenocarcinoma from other types of NSCLC [12, 13], and may be a biomarker predicting the efficacy of immune checkpoint inhibitors (ICIs) other than PD-L1 TPS in patients with nonsquamous NSCLC (NS-NSCLC), including lung adenocarcinoma [14–16]. Lung adenocarcinomas with ILD are characterised by the accumulation of mutations in pulmonary surfactant system genes (PSSGs), such as *NKX2-1/TTF-1*. These mutations reduce or eliminate the expression of their encoded proteins [4]. Many idiopathic interstitial pneumonia-associated pulmonary adenocarcinomas, especially tumours associated with bronchiolar metaplasia in honeycomb lesions, are negative for TTF-1 expression [17]. These results suggested that lung cancer with ILD is associated with the absence of TTF-1 expression, and that the combination of ILD with negative TTF-1 expression strongly contributes to the distinct carcinogenic pathways and biological characteristics of lung cancer with ILD.

Evaluation of tumoural TTF-1 expression as a predictor of the treatment efficacy of PD-1i for NS-NSCLC should include patients with NS-NSCLC stratified by both TTF-1 expression and the presence of ILD in noncancerous lung tissue. This is because biological characteristics of NS-NSCLC with and without ILD may differ, including their response to PD-1i. Furthermore, the tumour immune microenvironment potentially affecting the efficacy of PD-1i may differ in lung cancer patients with and without ILD [18, 19]. Clinically, many NS-NSCLC patients with ILD are negative for TTF-1 expression; therefore, careful consideration of PD-1i therapy is required because of its frequent adverse events. The present study therefore investigated the association between the efficacy of PD-1i and TTF-1 expression in NS-NSCLC patients with and without ILD to determine whether concomitant ILD could affect the course of PD-1i treatment.

Materials and methods

Patients

The study population consisted of NS-NSCLC patients with stage IIIA–IV disease for whom curative surgery or radiotherapy could not be performed, and who experienced post-operative recurrence of stage I–IIIA disease and underwent curative surgery. Patients who underwent tegafur-uracil or platinum-based adjuvant chemotherapies for post-operative recurrence were also included. Patients who were treated with radical thoracic radiotherapy and/or had other concomitant cancers were excluded. This study population consisted of two cohorts, consisting of patients with advanced or post-operative recurrent NS-NSCLC with or without ILD. ILD was diagnosed by pulmonologists according to American Thoracic Society guidelines [20]. The ILD cohort consisted of patients with advanced or post-operative recurrent NS-NSCLC who were treated with nivolumab or pembrolizumab at Tokyo Medical and Dental University (TMDU) Hospital or its six affiliated hospitals in Japan between January 2016 and September 2020. The patients in this cohort partially overlapped with those in our previous study [6]. The non-ILD cohort consisted of patients with advanced or recurrent NS-NSCLC without ILD who were treated with nivolumab or pembrolizumab at TMDU hospital during the same period. NS-NSCLC patients who received chemoradiation, followed by treatment with durvalumab, anticytotoxic T-lymphocyte-associated antigen 4 therapy or cytotoxic chemotherapy in combination with ICIs, were excluded. Also excluded were patients with unevaluable TTF-1 expression, as determined by immunohistochemistry. This study was performed in accordance with the Declaration of Helsinki and was approved by the Ethics Committee of TMDU (M2022-130), which waived the requirement for patient informed consent due to the retrospective, observational nature of the study.

Data collection

Demographic and clinical data retrospectively collected from patients' medical records included age, sex, smoking status, Eastern Cooperative Group Performance Status (ECOG-PS), tumour stage, histological types, TPS of PD-L1, TTF-1 expression, p40 expression, numbers of treatment lines with nivolumab or pembrolizumab, response to PD-1i, progression-free survival (PFS) and overall survival (OS). Lung cancer

stage and histological type were defined according to the World Health Organization (WHO) classification and the 7th edition of the lung cancer tumour/node/metastasis (TNM) classification of the International Association for the Study of Lung Cancer (IASLC) [21]. Tumour responses to PD-1i were estimated by using the Response Evaluation Criteria in Solid Tumours (RECIST) version 1.1. PFS was defined as the time from the date of initial administration of nivolumab or pembrolizumab until the date of first disease progression, the date of initial administration of the next regimen or the date of death due to any cause. OS was assessed from the date of diagnosis or recurrence until death from any cause or the date on which the latest survival information was available. The cut-off date was 31 March 2021.

Pathological evaluation and immunohistochemistry

Pathological specimens were immunostained with antibodies to TTF-1 (clone 8G7G3/1; Agilent, Tokyo, Japan, and Roche Diagnostic, Tokyo, Japan), p40 (clone BC28; Nichirei Biosciences Inc, Tokyo, Japan) and PD-L1 (clone 22C3 pharmDx assay; Agilent). TTF-1 and p40 expression was evaluated by an expert pathologist (I. Onishi), with positivity defined as staining of >10% of tumour cells with antibody to TTF-1 or p40. NS-NSCLC was evaluated morphologically, and TTF-1 diffuse positivity was defined according to the WHO classification [22]. The levels of expression of PD-L1 TPS were categorised as <1%, 1–49% and ⩾50% from outsourced reports (LSI Medience, Tokyo, Japan).

Evaluation of $CD8^+$ T-cell infiltration

The tumour microenvironment in patients with post-operative recurrence was assessed by immunostaining tissue samples with antibody to CD8 (C8/144B; Agilent), with the samples scanned using a digital slide scanner (Nanozoomer S210; Hamamatsu Photonics, Shizuoka, Japan). These data were imported into HALO version 3.3 (Indica Labs, Albuquerque, NM, USA), and the densities of $CD8^+$ T-cells were calculated in annotation areas (mm^2) as determined manually by both an expert pulmonologist (M. Ito) and an expert pathologist (I. Onishi).

Statistical analysis

Categorical variables in two groups were compared by chi-square or Fisher's exact-test, and continuous variables were compared by Mann–Whitney U-tests. The concordance between two variables was also analysed by Cramer's V test, with V <0.25 indicating weak, 0.25<V<0.75 indicating moderate and V>0.75 indicating strong association. PFS and OS were analysed by the Kaplan–Meier method, with differences between groups compared by log-rank tests. Correlations of risk factors with PFS and OS were evaluated by Cox regression analysis. All statistical analyses were performed using R software version 4.3.0 (www.R-project.org/), with p-values <0.05 considered statistically significant.

Results

Patient characteristics

This study included two cohorts of patients with NS-NSCLC, one with and the other without ILD; their consort diagrams are presented in figure 1. Because relatively few NS-NSCLC patients with ILD received PD-1i monotherapy, 705 patients from nine affiliated hospitals in Japan were screened, with 34 of these patients enrolled in the ILD cohort (figure 1a). For comparison, this study included a non-ILD cohort, consisting of 28 NS-NSCLC patients without ILD who were treated with PD-1i monotherapy at TMDU hospital during the corresponding period (figure 1b). The patients in the ILD cohort were subdivided into those who were positive (n=16, 47.1%) and negative (n=18, 52.9%) for TTF-1, and the patients in the non-ILD cohort were also subdivided into those positive (n=23, 82.1%) and negative (n=5, 18.9%). The percentage of TTF-1-positive tumours was significantly higher in the non-ILD than in the ILD cohort (p=0.0076).

The characteristics of patients in the ILD and non-ILD cohorts are summarised in table 1. Age, sex, ECOG-PS, smoking status, tumour stage and histological characteristics did not differ significantly between TTF-1-positive and -negative patients in both the non-ILD and ILD cohorts. Treatment history potentially associated with PFS and/or OS, such as administration of tyrosine kinase inhibitors, platinum doublets and pemetrexed, which is strongly correlated with longer PFS in TTF-1-positive NS-NSCLC [23], did not differ significantly between TTF-1-positive and -negative patients in both cohorts. The frequency of PD-1i (nivolumab or pembrolizumab) and the timing of treatment lines also did not differ significantly between TTF-1-positive and -negative patients in both the non-ILD and ILD cohorts. Previous treatments for patients who were treated with PD-1i after first-line treatment include cytotoxic chemotherapy.

Association between PD-L1 and TTF-1 expression

Because PD-L1 TPS of NSCLC is an established biomarker of the efficacy of PD-1i [24], but could be a confounding factor when assessing whether TTF-1 expression is a potential biomarker of PD-1i efficacy,

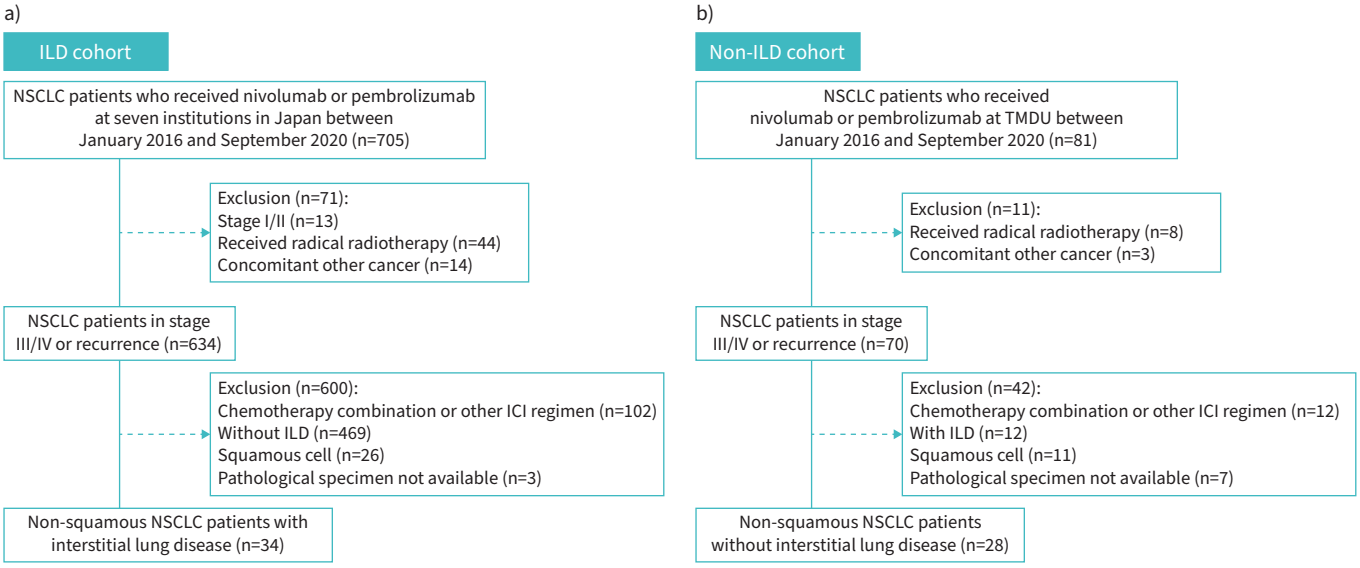

FIGURE 1 Consort diagrams of this study. a) Interstitial lung disease (ILD) cohort; b) non-ILD cohort. NSCLC: nonsmall cell lung carcinoma; ICI: immune checkpoint inhibitor; TMDU: Tokyo Medical and Dental University.

the association between immunohistochemistry expression of PD-L1 and TTF-1 was analysed using Cramer's V tests (supplementary table S1). Although the correlation between PD-L1 and TTF-1 expression in previous studies was not clear [15, 16, 25], the present study observed a moderate correlation between expression of PD-L1 and TTF-1.

Treatment and response based on status of TTF-1 and ILD

Analysis of PFS and OS in the non-ILD and ILD cohorts showed that the median PFS during PD-1i treatment was significantly shorter in TTF-1-negative than -positive patients in the ILD cohort (2.0 *versus* 12.1 months, p=0.004), but not in the non-ILD cohort (1.8 *versus* 2.6 months, p=0.63) (figure 2a). Response rate and disease control rate were significantly lower in TTF-1-negative than -positive patients in the ILD cohort (p=0.037 and=0.045, respectively) but did not differ significantly in the non-ILD cohort (p=1.0 each) (supplementary table S2). Median OS was also significantly shorter in TTF-1-negative than -positive patients in the ILD cohort (14.5 *versus* 42.5 months, p=0.018), but not in the non-ILD cohort (33.7 *versus* 37.1 months, p=0.53) (figure 2b). Cox regression analyses showed that absence of TTF-1 expression was a significant risk factor for PFS (hazard ratio (HR) 2.75; 95% CI 1.14–6.60, p=0.024) (table 2) and OS (HR 2.81; 95% CI 1.26–6.28, p=0.012) (table 3) in the ILD cohort but did not show significance in the non-ILD cohort.

Immune-related tumour microenvironments stratified by status of TTF-1 and ILD

Survival analyses showed that TTF-1-negative NS-NSCLC patients with ILD had a poor prognosis. To determine the underlying mechanism, the immune-related tumour microenvironment (TME) was assessed immunohistochemically by evaluating tumour infiltration of $CD8^+$ T-cells. Surgically resected specimens that included intra-tumoural and distant noncancerous areas were analysed from 10 and nine patients in the ILD and non-ILD cohorts, respectively (supplementary figure S1). The density of $CD8^+$ T-cells was generally higher in the ILD than the non-ILD cohort, with subgroup analysis showing that the $CD8^+$ T-cell densities of intra-tumoural areas were significantly higher in TTF-1-positive than -negative patients in the ILD, but not in the non-ILD, cohort (figure 3a). By contrast, $CD8^+$ T-cell densities did not differ significantly in distant noncancerous areas of both the ILD and non-ILD cohorts (figure 3b).

Discussion

The present study showed that PFS and OS were significantly shorter in TTF-1-negative than in TTF-1-positive NS-NSCLC patients with ILD who received PD-1i monotherapy, whereas these differences were not observed in NS-NSCLC patients without ILD. The carcinogenesis of lung adenocarcinoma with ILD is distinct, being characterised by inactivating mutations in PSSGs, including *NKX2-1/TTF-1* [4] and loss of TTF-1 expression. This loss of TTF-1 expression in particular also affected the efficacy of PD-1i,

TABLE 1 Patient characteristics

Characteristics	Non-ILD				ILD			
	All	TTF-1 positive	TTF-1 negative	p-value	All	TTF-1 positive	TTF-1 negative	p-value
Patients n	28	23	5		34	16	18	
Age, median (range)	65 (34–82)	65 (34–82)	69 (61–78)	0.44	70.5 (50–83)	69.5 (50–83)	70.5 (50–83)	0.79
Sex				1				0.65
Male	19 (67.9)	16 (69.6)	3 (60.0)		29 (85.3)	13 (81.4)	16 (88.9)	
Female	9 (32.1)	7 (30.4)	2 (40.0)		5 (14.7)	3 (18.6)	2 (11.1)	
PS				0.083				0.66
0–1	21 (75.0)	19 (82.6)	2 (40.0)		28 (82.4)	14 (87.5)	14 (77.8)	
2–3	7 (25.0)	4 (17.4)	3 (60.0)		6 (17.6)	2 (12.5)	4 (22.2)	
Smoking status				0.21				0.54
Never	5 (17.9)	3 (13.0)	2 (40.0)		5 (14.7)	3 (18.6)	2 (11.1)	
Former/current	23 (82.1)	20 (87.0)	3 (60.0)		28 (82.4)	13 (81.4)	15 (83.3)	
N/A	0	0	0		1 (2.9)	0	1 (5.6)	
Treatment for ILD								
Anti-fibrotic drug	0	0	0	1	0	0	0	1
Glucocorticoids	0	0	0	1	2 (5.9)	2 (12.5)	0	0.21
Supplemental oxygen therapy	0	0	0	1	2 (5.9)	0	2 (11.1)	0.49
Stage				0.79				0.50
III	2 (7.1)	2 (8.7)	0		12 (35.3)	4 (25.0)	8 (44.4)	
IV	16 (57.1)	13 (56.5)	3 (60.0)		11 (32.4)	6 (37.5)	5 (27.8)	
Post-surgical recurrence	10 (35.7)	8 (34.8)	2 (40.0)		11 (32.4)	6 (37.5)	5 (27.8)	
CNS metastasis	13 (46.4)	11 (47.8)	2 (40.0)	1	9 (26.5)	5 (31.3)	4 (22.2)	0.70
Histology				0.33				0.27
Adenocarcinoma	26 (92.9)	22 (95.7)	4 (80.0)		24 (70.6)	13 (81.4)	11 (61.1)	
Nonsquamous NSCLC	2 (7.1)	1 (4.3)	1 (20.0)		10 (29.4)	3 (18.6)	7 (38.9)	
Gene aberrations								
EGFR mutation	3 (10.7)	3 (13.0)	0		0	0	0	
ALK fusion	0	0	0		0	0	0	
Others	0	0	0		1 (2.9)	0	1 (5.6)	
TKI treatment	5 (17.9)	5 (21.7)	0	0.55	85	1 (6.3)	0	0.47
Platinum doublet treatment	21 (75.0)	17 (73.9)	4 (80.0)	1	24 (70.6)	9 (56.3)	15 (83.3)	0.13
Pemetrexed treatment	20 (71.4)	17 (73.9)	3 (60.0)	0.61	9 (26.5)	6 (37.5)	3 (16.7)	0.25
PD-1 inhibitors				0.33				
Nivolumab	14 (50.0)	13 (56.5)	1 (20.0)		15 (44.1)	6 (37.5)	9 (50.0)	0.51
Pembrolizumab	14 (50.0)	10 (43.5)	4 (80.0)		19 (55.9)	10 (62.5)	9 (50.0)	
Line number of PD-1 inhibitor treatment				0.096				0.13
1st	8 (28.6)	7 (30.4)	1 (20.0)		10 (29.4)	7 (43.8)	3 (16.7)	
2nd	11 (39.3)	7 (30.4)	4 (80.0)		10 (29.4)	5 (31.3)	5 (27.8)	
>3rd	9 (32.1)	9 (39.1)	0		14 (41.2)	4 (25.0)	10 (55.6)	

Data are presented as n (%) unless indicated otherwise. p-values were calculated using the chi-squared test, Fisher's exact test or the Mann–Whitney U-test, as appropriate. ILD: interstitial lung disease; TTF-1: thyroid transcription factor-1; PS: performance status; CNS: central nervous system; NSCLC: nonsmall cell lung cancer; TKI: tyrosine kinase inhibitor; PD-1: programmed cell death 1.

especially when compared with NS-NSCLC without ILD. The findings of this study may be especially useful for identifying NS-NSCLC patients with ILD who should avoid treatment with PD-1i.

TTF-1 is a transcriptional factor regulating lung formation. Penetrant inactivating mutations of *NKX2-1*, the gene encoding TTF-1, are associated with the development of ILD [11]. TTF-1 has also been associated with differentiation [26] and is expressed in most NS-NSCLCs [12, 13]. Clinically, TTF-1 expression is mainly used to diagnose the histological subtype of lung cancer and to distinguish primary from metastatic lung tumours. It could also be used as a potential marker of prognosis [27] or responses to treatment, including to pemetrexed-based chemotherapy [23, 28], immunotherapy [14, 16] and chemo-immunotherapy that includes pemetrexed [15, 25, 29]. Because pemetrexed-based chemotherapy has been established as the standard treatment for patients with NS-NSCLC [30], the evaluation of TTF-1 expression may have potential for determining tumour responses to pemetrexed-based chemotherapy and chemo-immunotherapy.

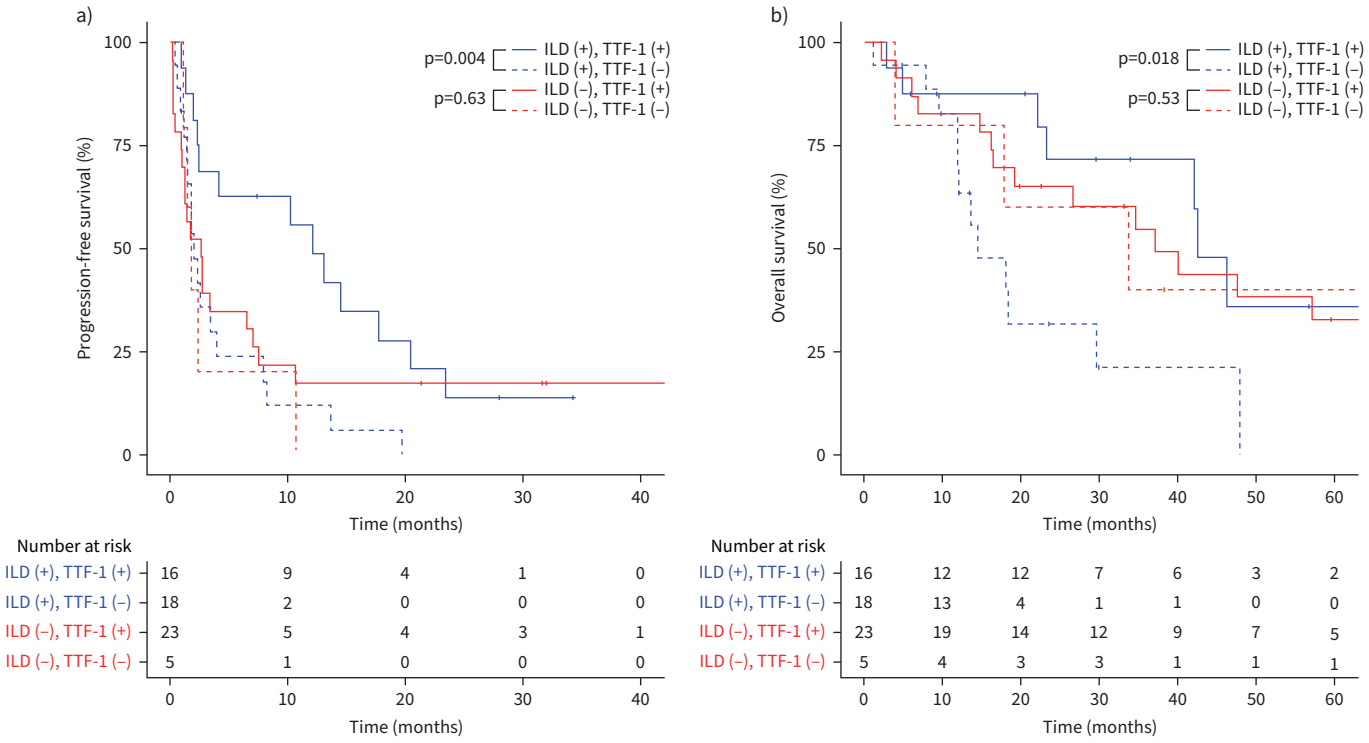

FIGURE 2 Kaplan–Meier analyses of a) progression-free survival and b) overall survival of patients in the ILD cohort (blue) and non-ILD cohort (red). Solid lines indicate TTF-1-positive patients and dashed lines indicate TTF-1-negative patients. p-values were calculated using log-rank tests, with the numbers at risk in each cohort also displayed. ILD: interstitial lung disease; TTF-1: thyroid transcription factor-1.

To date, only two retrospective studies have investigated the association between TTF-1 expression and the efficacy of ICIs. Along with *KRAS* mutations and PD-L1, TTF-1 expression was found to be independently prognostic of responses to immunotherapy, including treatment with anti-PD-L1, anti-PD-1, anti-CTLA4, and combinations of anti-PD-L1/1 and anti-CTLA-4 agents, in patients with NS-NSCLC [14]. Patients with TTF-1-positive lung adenocarcinoma were found to have better outcomes than those with TTF-1-negative tumours during ICI monotherapy, with TTF-1 and PD-L1 expression showing a positive association [16]. The binding of NKX2-1 to a region upstream of *CD274/PD-L1* regulated the expression of *CD274/PD-L1* mRNA in a lung cancer cell line [31]. Moreover, a subset of TTF-1-negative lung adenocarcinomas triggered by SRGN was reported to have an immunosuppressive phenotype, characterised by higher PD-L1 expression in tumours and T-cell infiltration of PD-1-positive tumours [32].

TABLE 2 Cox regression analysis of factors associated with progression-free survival

	Non-ILD cohort				ILD cohort			
	Univariate		Multivariate		Univariate		Multivariate	
Factor	HR (95% CI)	p-value	HR (95% CI)	p-value	HR (95% CI)	p-value	HR (95% CI)	p-value
Age: ⩾75 years *versus* <75 years	0.94 (0.28–3.17)	0.92			0.91 (0.42–1.94)	0.80		
PS: 2–3 *versus* 0–1	2.31 (0.94–5.64)	0.067	3.37 (0.84–13.57)	0.087	1.64 (0.66–4.07)	0.29	1.07 (0.39–2.95)	0.9
CNS metastasis: Yes *versus* No	2.06 (0.89–4.76)	0.093	1.82 (0.75–4.42)	0.18	0.87 (0.39–1.97)	0.74		
PD-L1 TPS: <50% *versus* ⩾50%	1.48 (0.64–3.44)	0.36			1.37 (0.66–2.83)	0.39		
Line number of PD-1 inhibitor treatments: ⩾3rd *versus* 1st/2nd	0.99 (0.42–2.33)	0.98			1.88 (0.91–3.88)	0.088	1.62 (0.75–3.48)	0.22
TTF-1 expression: Negative *versus* Positive	1.27 (0.47–3.43)	0.64	0.55 (0.12–2.51)	0.44	3.02 (1.38–6.63)	**0.0058**	2.75 (1.14–6.60)	**0.024**

p-values calculated using a Cox proportional hazards model. p-values in bold denote statistical significance. ILD: interstitial lung disease; PS: performance status; CNS: central nervous system; PD-L1: programmed cell death ligand 1; TPS: tumour proportion score; PD-1: programmed cell death 1; TTF-1: thyroid transcription factor-1.

TABLE 3 Cox regression analysis of factors associated with overall survival

	Non-ILD cohort				ILD cohort			
	Univariate		Multivariate		Univariate		Multivariate	
Factor	HR (95% CI)	p-value	HR (95% CI)	p-value	HR (95% CI)	p-value	HR (95% CI)	p-value
Age: ⩾75 years *versus* <75 years	1.71 (0.36–8.05)	0.50			1.64 (0.62–4.35)	0.32		
PS: 2–3 *versus* 0–1	2.79 (1.03–7.55)	0.044	1.54 (0.51–4.62)	0.44	1.2 (0.39–3.71)	0.75		
CNS metastasis: Yes *versus* No	1.18 (0.47–2.92)	0.73			1.68 (0.66–4.28)	0.28		
PD-L1 TPS: ⩾50% *versus* <50%	1.4 (0.55–3.60)	0.48			1.37 (0.66–2.83)	0.39		
Line number of PD-1 inhibitor treatments: ⩾3rd *versus* 1st/2nd	0.24 (0.074–0.76)	**0.016**	0.29 (0.081–1.02)	0.054	2.16 (0.84–5.52)	0.11	1.56 (0.76–3.33)	0.21
Platinum doublet treatment: No *versus* Yes	0.51 (0.17–1.52)	0.23			0.57 (0.25–1.28)	0.17		
Pemetrexed treatment: No *versus* Yes	0.59 (0.20–1.76)	0.35			1.86 (0.81–4.29)	0.15		
TTF-1 expression: Negative *versus* Positive	0.7 (0.23–2.14)	0.53			3.02 (1.38–6.63)	**0.0058**	2.81 (1.26–6.28)	**0.012**

p-values calculated using a Cox proportional hazards model. p-values in bold denote statistical significance. ILD: interstitial lung disease; PS: performance status; CNS: central nervous system; PD-L1: programmed cell death ligand 1; TPS: tumour proportion score; PD-1: programmed cell death 1; TTF-1: thyroid transcription factor-1.

The present study found that the expression of PD-L1 protein was moderately correlated with TTF-1 expression, suggesting a confounding relationship between PD-L1 and TTF-1 expression. More interestingly, Cox regression analysis showed that prognosis during PD-1i treatment in patients with NS-NSCLC was more strongly associated with TTF-1 than PD-L1 expression only when ILD was present. The presence of $CD8^+$ tumour-infiltrating lymphocytes (TIL) in the TME is also predictive of the efficacy of ICIs. The TME can be classified into four categories according to PD-L1 expression (TPS ⩾50% or <50%) and TIL status (high or low), with these categories predicting the PD-1/PD-L1 blockade response in a stratified manner [33]. Interestingly, PFS was longer in patients with "PD-L1 low and TIL high" tumours than in those with "PD-L1 high and TIL low" tumours, suggesting that TIL status has a prognostic impact on PFS. The present study showed that the density of $CD8^+$ TILs was higher in TTF-1-positive NS-NSCLC patients with ILD than in the other categories. Because only ~40 NSCLC

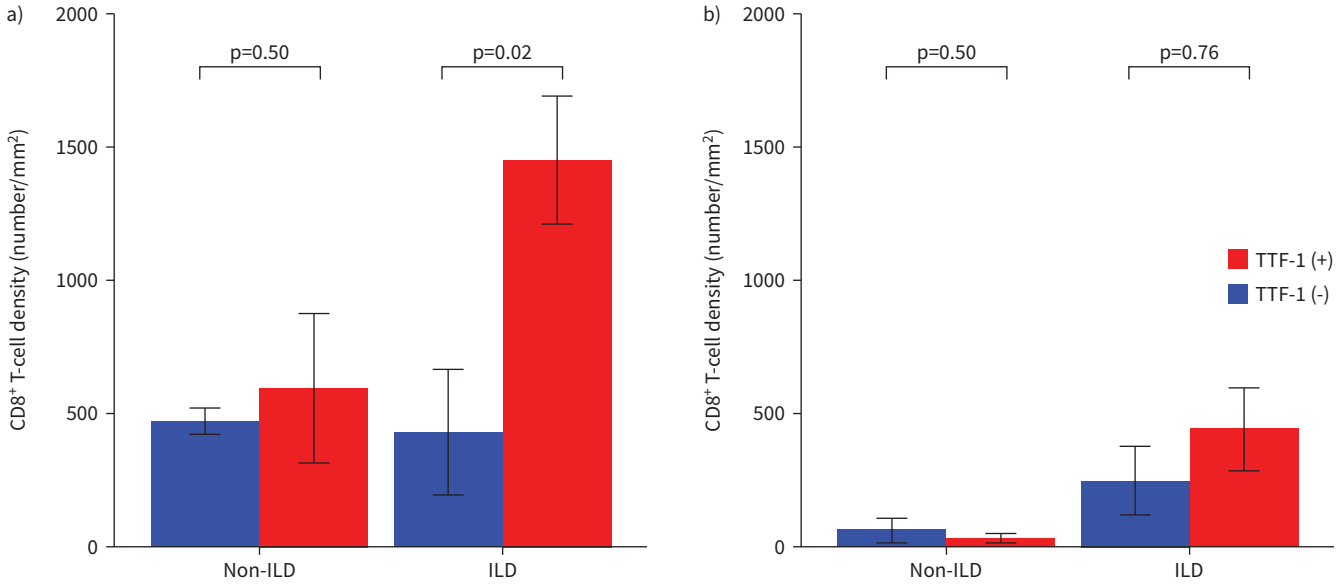

FIGURE 3 Densities of $CD8^+$ T-cells in tumour samples from the ILD and non-ILD cohorts. Densities of $CD8^+$ T-cells in a) intra-tumoural areas and b) distant noncancerous areas stratified by TTF-1 expression. Data are presented as mean±SEM. p-values were calculated using Mann–Whitney U-tests. ILD: interstitial lung disease; TTF-1: thyroid transcription factor-1.

patients with ILD have been analysed to date [19, 34], the dynamics of $CD8^+$ TILs in NSCLC with ILD have not been determined. Although the synergetic mechanism by which TTF-1 expression and ILD recruits TILs into intra-tumoural areas remains unclear, the present results showed a gradient of $CD8^+$ TIL density according to TTF-1 expression in the ILD cohort. This finding may explain the prognostic difference observed in the ILD cohort. Cox regression analysis showed that later line treatment with PD-1i was also associated with better OS in the non-ILD cohort. However, the underlying mechanism could not be determined in this study.

The frequency and severity of immune-related adverse events (irAEs) has been regarded as significantly higher in NSCLC patients with ILD than in those without ILD, although OS did not differ significantly between NSCLC patients with and without ILD [7]. Predicting the risk of developing irAEs has been challenging, even using clinical big data [35]. Even interstitial lung abnormality is associated with the development of symptomatic pneumonitis in NSCLC patients who underwent immunotherapy [36]. It would be important to request the assistance of pulmonologists to determine the risks of treatment and recognise the early signs of treatment-related complications, such as pneumonitis or acute exacerbations of the underlying ILD [37]. In this study, TTF-1 expression was not associated with the frequency or severity of drug-induced exacerbation of ILD (supplementary table 3). Our previous study suggested that radiological findings from high-resolution computed tomography may be useful in assessing irAEs that exacerbate ILD [38]. Indications for PD-1i treatment of NS-NSCLC patients with ILD should be based on careful patient selection, including predictions of the effects of TTF-1 expression and/or radiological risk assessment with the support of expert pulmonologists if possible.

The present study had several limitations. First, this study was a small and retrospective analysis, especially with respect to the number of TTF-1-negative patients in the non-ILD cohort. Second, although this study showed that TTF-1 expression was associated with the effect of PD-1i in NS-NSCLC patients with ILD, the mechanisms underlying this synergistic effect remain largely unclear. Our results should be carefully interpretated and additional studies are warranted.

In conclusion, the present study showed that, compared with expression of TTF-1, lack of expression of TTF-1 in NS-NSCLC patients with ILD was significantly associated with poorer prognosis when patients were treated with PD-1i monotherapy. Because PD-1i treatment of NS-NSCLC patients with ILD is challenging, it would be important to enlist the help of pulmonologists. The present results may help in selecting appropriate NS-NSCLC patients with ILD for treatment with PD-1i.

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