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ORIGINAL ARTICLE

Absence of thyroid transcription factor-1 expression is associated with poor survival in patients with advanced pulmonary adenocarcinoma treated with pemetrexed-based chemotherapy

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Abstract

Introduction Adenocarcinoma is the commonest histologic subtype of lung cancer and is often identified by immunohistochemical staining for thyroid transcription factor-1 (TTF-1). However, up to 20% of lung adenocarcinomas do not express TTF-1, and there is uncertainty regarding the significance of this. We aimed to evaluate the prognostic effect of TTF-1 expression status on survival in patients treated with pemetrexed-based chemotherapy for advanced adenocarcinoma of the lung.

Methods This retrospective study included patients treated with pemetrexed-based chemotherapy for stage IIIB/IV lung adenocarcinoma, who had known TTF-1 expression status. Clinical and demographic data were obtained from medical records. Overall survival (OS) was estimated using the Kaplan-Meier method, and differences in survival between groups assessed using the Cox proportional hazards model.

Results Forty-four patients were identified with documented TTF-1 expression: 35 with TTF-1-positive and 9 with TTF-1-negative disease. Patients in the TTF-1-negative group had poorer performance scores than those in the TTF-1-positive group (ECOG 2: 67 vs 20%, $p = 0.008$), and received less chemotherapy (median cycles 2 vs 4, $p = 0.009$), and were fewer in treatment with doublet regimens (22 vs 69%, $p = 0.013$). OS was significantly shorter in the TTF-1-negative group than in the TTF-1-positive group (2.4 vs 11.5 months, HR 8.38, $p < 0.0001$).

Conclusions In this group of patients treated with pemetrexed-based chemotherapy for advanced pulmonary adenocarcinoma, absence of TTF-1 expression was associated with an aggressive tumor phenotype, poorer performance status, and poor survival. This subgroup of patients should be recognized as having a distinct clinical course, with limited benefit from standard chemotherapy.

Keywords Biomarker · Chemotherapy · Non-small cell lung cancer · Pemetrexed · Thyroid transcription factor-1 · TTF-1

Introduction

For several decades, lung cancer has been the most common malignancy in the world, and remains the commonest cause of cancer death [1]. As smoking rates have declined, the relative incidence of adenocarcinomas, the commonest type of non-small cell lung cancer (NSCLC), has increased and now accounts for approximately 50% of all lung cancers [2]. Despite screening programs, the majority of patients present with advanced disease. Although a greater understanding of molecular biology has translated into novel treatments for patients with mutations in the epidermal growth factor receptor (*EGFR*) or rearrangements of the anaplastic lymphoma kinase (*ALK*) genes, cytotoxic chemotherapy remains the mainstay of treatment for many patients. Pemetrexed is a multi-targeted antifolate cytotoxic agent, which, in combination with cisplatin, improved survival versus gemcitabine-cisplatin in patients with non-squamous NSCLC [3]. Primarily based on these data, pemetrexed-platinum combinations have become the

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standard of care for patients with advanced pulmonary adenocarcinoma in many parts of the world. Aside from tumor histology, there are no currently accepted biomarkers predicting benefit from pemetrexed.

Thyroid transcription factor-1 (TTF-1), also known as NK 2 homeobox 1 (NKX2-1) is a nuclear transcriptional regulator, first identified as a promoter of thyroglobulin [4]. It is coded by *NKX2-1* located on chromosome 14 and has been identified as being important in pneumocyte differentiation and pulmonary branching morphogenesis [5, 6]. Immunochemical studies have shown that up to 80% of pulmonary adenocarcinomas are TTF-1 positive and it has become the marker of choice for identifying this disease subtype [7]. In vitro, TTF-1 has shown both pro- and anti-oncogenic effects, and its role in pulmonary carcinogenesis remains unclear [8, 9]. Although it has been suggested that TTF-1 expression is a positive prognostic variable, available data are inconsistent, possibly due to heterogeneous study populations and treatment regimens [10, 11]. Therefore, the aim of this retrospective study was to investigate TTF-1 as a prognostic variable in patients with advanced pulmonary adenocarcinoma treated with pemetrexed-based chemotherapy.

Methods

This single-institution analysis included patients treated with pemetrexed-based chemotherapy between July 2008 and December 2013. Patients who had received at least one dose of pemetrexed were identified from the institutional pharmacy database and were included whether they received single-agent chemotherapy, or in combination with carboplatin or cisplatin. Pathology reports were examined to exclude histology other than pulmonary adenocarcinoma and to document TTF-1 expression. Immunohistochemical TTF-1 positivity was defined as 1% or greater nuclear staining (i.e., not membranous or cytoplasmic), using a commercially available anti-TTF1 antibody (SPT24, mouse monoclonal antibody, Leica Biosystems, Nussloch, DE). Only patients with AJCC stage IIIB/IV disease were included and medical records were reviewed to establish patient demographics, treatment details (including chemotherapy regimen and number of cycles), and survival. Reports of computed tomography (CT) scans before and after chemotherapy were reviewed to assess tumor response. Exclusion criteria included inadequate data on patient demographics or TTF-1 expression, early-stage (AJCC I–IIIA) disease, uncertainty of lung cancer diagnosis and those who subsequently underwent radical surgery or radiotherapy.

The primary endpoint for the study was overall survival (OS) from commencement of palliative chemotherapy, with events defined as death from any cause. This was compared between patients with TTF-1-positive and TTF-1-negative advanced pulmonary adenocarcinoma. Survival was estimated with the Kaplan-Meier method and Cox proportional hazards model. Objective response, defined as $\geq 30\%$ decrease in tumor size measured by reporting radiologist, was a secondary endpoint. Differences between groups were tested using the Wilcoxon rank sum test or Fisher's exact test as appropriate, with p values < 0.05 considered statistically significant. Statistical analyses were performed using SAS v9.4 (SAS Institute Inc., Cary, NC, USA).

Results

Between July 2008 and December 2013, 103 patients were identified from the pharmacy database as having received at least one dose of pemetrexed, of whom 10 were excluded: 4 had mesothelioma, 2 had carcinoma of unknown

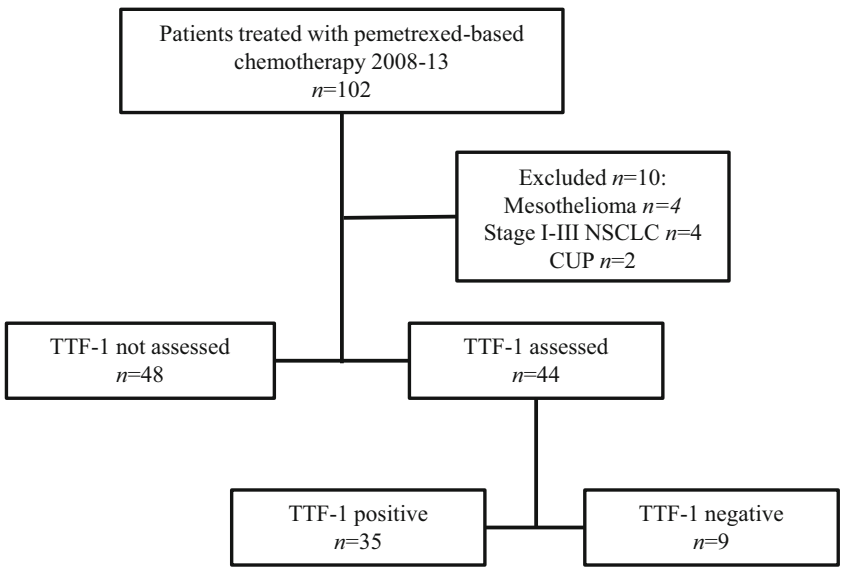

Fig. 1 Diagram of patients screened for study and allocation to the TTF-1-positive and TTF-1-negative groups. CUP, carcinoma of unknown primary; NSCLC, non-small cell lung cancer; TTF-1, thyroid transcription factor-1

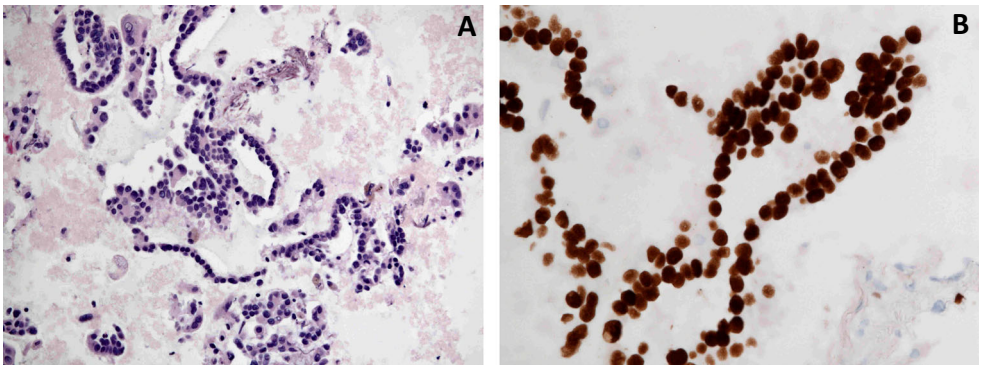

Fig. 2 Photomicrographs showing (**a**) pulmonary adenocarcinoma after H&E staining and (**b**) TTF-1-positive adenocarcinoma using Dako antibody

primary, and 4 did not have stage IIIB/IV disease (Fig. 1). Of the remaining 93 patients, TTF-1 expression was available in 44 (47%). Most patients (80%) had TTF-1-positive tumors; 9 (20%) had TTF-1-negative tumors (see Fig. 2 and Table 1). Treatment consisted of pemetrexed and carboplatin in 26 patients (59%) and pemetrexed alone in 18 patients (41%). The TTF-1-negative group was predominantly male (78%), but the difference in sex distribution between the groups was not significant ($p = 0.15$). In this sample, EGFR and ALK status was available in 9 patients: 8 in the TTF-1-positive group and 1 in the TTF-1-negative group. One patient in the TTF-1-positive group had an EGFR mutation; the remaining patients were EGFR and ALK negative. One patient had insufficient tissue for testing.

At a median follow-up of 28 months, there were 30 deaths in the TTF-1-positive group; all patients had died in the TTF-1-negative group. There was a significant difference observed in OS between the groups (Fig. 3): median survival in patients with TTF-1-positive disease was 11.5 months (95% CI 6.8–14.2 months), compared with 2.4 months in patients with TTF-1-negative tumors (95% CI 1.0–5.2 months, log-rank $p < 0.001$). The hazard ratio for OS in the TTF-1-negative group was 8.38 ($p < 0.001$). The 3-month and 6-month survival is summarized in Table 2. In total, 42 patients had more than one computed tomography (CT) scan and were evaluable for response. There was a trend toward a higher response rate in patients with TTF-1-positive tumors (52%) than in patients with TTF-1-negative tumors (22%); however, this difference was not statistically significant ($p = 0.12$).

Table 1 Clinical characteristics and treatment details of included patients

	All patients		TTF-1 positive		TTF-1 negative		*p*
	N = 44	%	*N* = 35	%	*N* = 9	%	
Age (years)							0.59
Median	61		61		63		
Range	36–78		39–78		36–73		
Sex							0.15
Male	24	56	17	50	7	78	
Female	20	44	18	50	2	22	
ECOG performance status*							0.008
0	6	14	6	17	0	0	
1	24	55	21	60	3	33	
2	13	30	7	20	6	67	
Disease stage							0.435
IIIB	9	20	8	22	1	11	
IV	35	80	27	78	8	89	
Chemotherapy							0.013
Pemetrexed-platinum	26	59	24	69	2	22	
Pemetrexed	18	41	11	31	7	78	
Median number of cycles	4		4		2		0.009
Range	1–6		1–6		1–5		

ECOG Eastern Cooperative Oncology Group, *TTF-1* thyroid transcription factor-1

*Performance status missing in one patient from the TTF-1-positive group

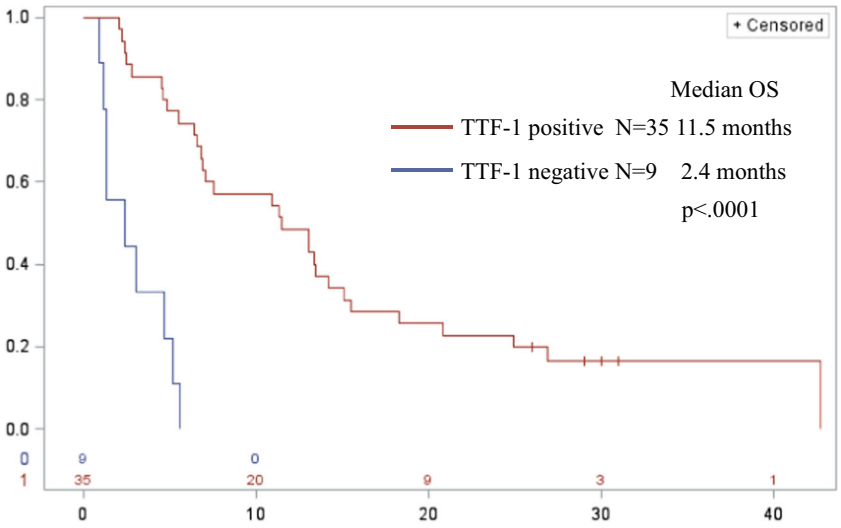

Fig. 3 Kaplan-Meier survival curve of patients with TTF-1-positive and TTF-1-negative tumors, following commencement of pemetrexed-based palliative chemotherapy. Patients with TTF-1-negative disease had significantly shorter survival than those with TTF-1-positive disease (HR 8.38, $p < 0.001$). OS, overall survival; TTF-1, thyroid transcription factor-1

Discussion

TTF-1 has been used for several years as the immunohistochemical biomarker of choice for identification of adenocarcinoma of pulmonary origin as it is expressed in approximately 80% of these tumors [7]. Despite this, the true relationship between TTF-1 and carcinogenesis remains unclear, with pro- and anti-oncogenic effects documented in preclinical studies [8, 9]. In the current study, we have highlighted the potential role of TTF-1 as a prognostic biomarker: patients with TTF-1-negative advanced pulmonary adenocarcinoma had significantly shorter survival than those with TTF-1-positive tumors.

The positive correlation between TTF-1 expression and prognosis has been seen in some, but not all studies [12–18]. Many of the published reports have included patients with early-stage disease, and only a small number have focused on advanced cancer. In 2009, Martins et al. reported on a group of patients from a Brazilian center treated with platinum-based chemotherapy and showed a non-significant trend to better survival in those with at least 1% TTF-1-positive tumors (HR 0.47, $p = 0.073$), with a significantly better survival in those with 60% TTF-1 positivity (HR 0.42, $p = 0.019$) [15]. A Norwegian study of 228 patients with all NSCLC histological subtypes, treated with carboplatin doublet chemotherapy, also reported longer survival in patients with TTF-1-positive tumors (HR 0.56, $p < 0.001$) [17]. Most recently, a study of 120 patients from Egypt treated with various chemotherapy regimens for advanced non-squamous NSCLC found that patients with TTF-1-positive tumors had longer overall survival than those with no TTF-1 expression (12.8 vs 5.8 months, $p = 0.011$), although TTF-1 was not an independent prognostic factor on multivariable analysis (HR 2.32, $p = 0.1$) [18].

A limitation of these studies is the inclusion of a heterogeneous range of NSCLC histology and older chemotherapy combinations. Increasingly, advances in our understanding of the heterogeneous molecular and genetic biology of lung cancer have led to more individualized therapies for a proportion of patients with this highly lethal disease. Pemetrexed is a cytotoxic chemotherapy agent, which was first synthesized as a thymidylate synthase (TS) inhibitor, but also acts on other components of folate metabolism including dihydrofolate reductase [19, 20]. It has been widely adopted as a standard therapy for advanced non-squamous NSCLC since the pivotal phase III study showed an improvement in OS for pemetrexed and cisplatin compared to gemcitabine and cisplatin [3]. Preclinical models have suggested that the difference in pemetrexed efficacy between squamous and non-squamous NSCLC is related to TS expression [21]. It has been also been suggested that pemetrexed maintenance might be associated with improved response rates and survival in patients with low tumor TS expression [22].

Data in support of TTF-1 as a prognostic biomarker in patients receiving pemetrexed come mainly from Asian

Table 2 Summary of overall survival in patients with TTF-1-positive and TTF-1-negative lung adenocarcinoma

	Median OS in months (95% CI)	Percentage surviving at 3 months (%)	Percentage surviving at 6 months (%)	Hazard ratio for OS (TTF-1 negative)	*p* value for comparison
TTF-1 positive	11.51 (6.79–14.16)	86	71	8.38	< 0.0001
TTF-1 negative	2.36 (0.95–5.18)	33	0		

OS overall survival, *TTF-1* thyroid transcription factor-1

populations. A study from Korea of 284 patients treated with pemetrexed-based chemotherapy for adenocarcinoma-NSCLC showed longer PFS in patients with TTF-1-positive disease (3.8 vs 1.3 months, $p < 0.001$) [16]. Arguably, the strongest evidence comes from a meta-analysis in 2006, which reported similar results to our study with longer OS in patients whose tumors express TTF-1 (HR 0.64, 95% CI 0.41–1.0), confirmed by a second meta-analysis in 2013 (HR 0.49, 95% CI 0.42–0.55) [10, 11]. There are limitations to the applicability of these data; however, they are based on small studies with heterogeneous patient populations and with often limited data on treatment. More recently, biomarker analysis of patients from a completed phase III trial comparing pemetrexed or gemcitabine with carboplatin in all histologic subtypes of NSCLC was reported [17]. The results of this study are in agreement with our own: patients with TTF-1-positive tumors by IHC had significantly longer OS at 10.4 months than those with no TTF-1 expression at 6 months. Only 47% of patients in this study were TTF-1 positive, due to the inclusion of patients with squamous or other histologic subtypes of NSCLC, and this heterogeneity limits the utility of these results. In this setting, our results add further strength to the body of data supporting the observation that TTF-1 is a positive prognostic variable, particularly as we focused on a European population with relatively homogeneous treatment. Importantly, we have identified a subgroup of patients with metastatic pulmonary adenocarcinoma without TTF-1 expression, with very poor outcomes. It appears as though these patients have a more aggressive tumor phenotype, leading to greater disease burden, greater deterioration in performance status, and ultimately shorter prognosis than patients with TTF-1-positive tumors. These patients are in need of novel treatment approaches.

Our study has limitations: the large number of patients with advanced NSCLC without TTF-1 testing limits our sample size which in turn affects the strength of our conclusions. Additionally, the rate of EGFR and ALK testing was too low to allow inclusion as a variable, although as no patients were treated with tyrosine kinase inhibitors, the effect of this variable on survival is likely to be less pronounced. Smoking history was also not recorded reliably enough to include as a variable. There are imbalances between patients with TTF-1-positive and TTF-1-negative tumors in regard to performance status and single-agent/doublet chemotherapy, but the small sample size precluded a multivariable analysis to adjust for these variables.

In conclusion, this study supports the hypothesis that in patients with advanced pulmonary adenocarcinoma treated with pemetrexed-based chemotherapy, lack of TTF-1 expression by immunohistochemistry is associated with significantly shorter survival than in patients whose tumors express this marker. Additional investigation of the molecular biology of TTF-1-negative lung cancer is warranted and specific clinical trials should be developed for this subgroup of patients.

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Compliance with ethical standards

This study involved human participants and was performed in accordance with the ethical standards of the Declaration of Helsinki.

Conflict of interest The authors declare that they have no conflict of interest.

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