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The efficacy of pemetrexed as a third- or fourth-line therapy and the significance of thymidylate synthase expression in patients with advanced non-small cell lung cancer

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ABSTRACT

Background: Pemetrexed is one of the standard second-line therapies in advanced non-small cell lung cancer (NSCLC). Currently, there are no standard cytotoxic treatments beyond second-line therapy. We evaluated the efficacy and safety of pemetrexed as a salvage regimen in heavily pretreated NSCLC patients. We also analyzed thymidylate synthase (TS) expression in tumor tissues to determine whether TS expression is correlated with the clinical efficacy of pemetrexed.
Methods: One hundred and ten NSCLC patients who received pemetrexed as third- or fourth-line therapy at the Samsung Medical Center between June 2006 and June 2008 were retrospectively reviewed. TS expression was analyzed by immunohistochemical staining in 55 NSCLC tissue specimens. The relationships between TS expression and clinicopathological factors were evaluated. Univariate and multivariate analyses were performed to define the predictive factors and prognostic significances.
Results: The median age of patients in this study was 59 years (range: 24–84), 50.9% were men, and 27 (24.6%) were smokers or previous smokers. Sixty-five patients (59.1%) received pemetrexed as third-line treatment, and 95 (86.4%) had non-squamous cell carcinoma. Platinum-based chemotherapy (84.6%) was the most common first-line therapy, and EGFR TKIs [erlotinib (17.3%) or gefitinib (43.6%)] were a common second-line therapy. The median time from date of diagnosis to the date of the first pemetrexed treatment was 12.8 months (range: 1.8–62.2 months) and the median number of pemetrexed treatments was 4 (range 1–22). Eighteen patients achieved PR (16.3%), 41 patients SD (37.3%), and 43 patients PD (39.1%), with a disease control rate of 53.6%. The median follow-up duration was 16.1 months, the median progression-free survival (PFS) was 3.2 months (95% CI: 1.9–4.5 months), and the median overall survival (OS) was 11.6 months (95% CI: 9.0–14.1 months). Male gender was the only independent variable for poor PFS (HR = 1.673, 95% CI: 1.103–2.535), with poor performance status (HR = 2.454, 95% CI: 1.405–4.287) and history of smoking (HR = 1.856, 95% CI: 1.087–3.168) being independent adverse factors for OS. Thirteen of 55 tumor tissues (23.6%) showed TS expression; however, there were no significant correlations between TS expression and the clinicopathological factors.
Conclusion: Pemetrexed was suggested as a third- or fourth-line therapy due to its favorable efficacy and tolerable toxicity. Further studies are warranted to define the adequate sequence of salvage treatments, especially in patients with adenocarcinoma lung cancer.

1. Introduction

The standard first-line therapy for patients with advanced non-small cell lung cancer (NSCLC) is platinum-based doublet combination chemotherapy, which offers a modest survival advantage [1]. Unfortunately, all NSCLC patients eventually experience disease progression and require salvage therapy. Recently, ASCO and NCCN guidelines recommend docetaxel, pemetrexed, or erlotinib as second-line therapies. Shepherd et al. showed that docetaxel was superior to the best supportive care (median survival: 7.0 months vs. 4.6 months, respectively) [2]. Hanna et al. compared pemetrexed with docetaxel, which showed equivalent outcomes (median survival: 8.3 months vs. 7.9 months, respectively) [3]. In a BR.21 trial, erlotinib showed survival benefits when given as a second- or third-

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line therapy, compared to a placebo (overall survival: 6.7 months vs. 4.7 months, respectively) [4]. A recent retrospective study demonstrated that docetaxel and EGFR (epidermal growth factor receptor) tyrosine kinase inhibitor (TKI) were reasonable therapeutic options as third-line therapy [5]. Currently, there is no standard chemotherapeutic regimen for third-line or beyond therapy in NSCLC patients. However, EGFR TKI is frequently used in East Asia as a second-line therapy owing to its efficacy. Thus, pemetrexed is more often administered as a third-line or beyond therapy in Korea.

Given its mild toxicity profile and promising anti-tumor activity in previous trials, pemetrexed may be a good drug candidate for third-line or beyond therapy in NSCLC patients [6,7]. Pemetrexed, a multitargeted antifolate agent, inhibits at least three of the enzymes involved in DNA synthesis and folate metabolism: thymidylate synthase (TS), dihydrofolate reductase (DHFR) and glycinamide ribonucleotide formyl transferase (GARFT). Among them, TS is a key enzyme that catalyzes the methylation of fluoro-dUMP, the precursor of DNA synthesis, into dTMP [8]. Previous studies showed that higher TS levels were associated with poor prognosis in NSCLC patients [9,10], and could be a predictor of TS-inhibiting agents [11].

In view of these data, we retrospectively analyzed the efficacy and safety of pemetrexed as a salvage regimen in heavily pretreated NSCLC patients. In addition, we investigated the correlation between TS expression in tumor tissues and the clinical efficacy of pemetrexed.

2. Materials and methods

2.1. Patients and data collection

One hundred and ten patients with NSCLC who received pemetrexed as third- or fourth-line therapy at Samsung Medical Center between June 2006 and June 2008 constituted the study cohort. The relationships between TS expression and the clinicopathological factors were evaluated in available tissue specimens for additional immunohistochemical assays. Clinical data was retrospectively reviewed from medical records. Pathologic diagnoses of NSCLC and immunostained sections were reviewed by one of the authors (J Han). The patients were followed up for a minimum of 7 months to a maximum of 30.8 months (median follow-up: 16.1 months). This study was approved by the Institutional Review Board of Samsung Medical Center.

2.2. Treatment

All patients received pemetrexed 500 mg/m^2 every 21 days. Oral daily doses of folic acid (1000 μg) were given 1–2 weeks before the first dose of pemetrexed and for 3 weeks after therapy. Injections of vitamin B12 (1000 μg IM) were given 1–2 weeks before the first dose of pemetrexed and at 9-week intervals during treatment. Dexamethasone (4 mg, oral, twice daily) was given the day before, the day of, and the day after each dose of pemetrexed therapy.

2.3. Treatment response

Appropriate imaging studies, including chest CT scans, were performed every two cycles (or sooner if needed) to evaluate treatment responses for documentation of disease progression. Responses were classified according to the Response Evaluation Criteria in Solid Tumors (RECIST) criteria [12].

2.4. Immunohistochemistry

Tissue sections were deparaffinized in xylene and then rehydrated in serially graded alcohol. TS antigen retrieval consisted of microwave heating (three cycles of 5 min at 700 W) in 10 mM citrate buffer at pH 6.0 and cooling to room temperature for 20 min. After washing in distilled water, the slides were preincubated in 4% BSA and dextran solution for 30 min to reduce nonspecific binding. The slides were incubated 1 h at room temperature with mouse monoclonal anti-TS (Zymed Laboratories, CA) at a 1:100 dilution in a humidified chamber. After washing, the tissue section was observed with peroxidase-labeled polymer conjugated to goat anti-mouse immunoglobulins in Tris–HCl buffer (Envision plus System-HRP labeled polymer, Dako, Glostrup, Denmark) and

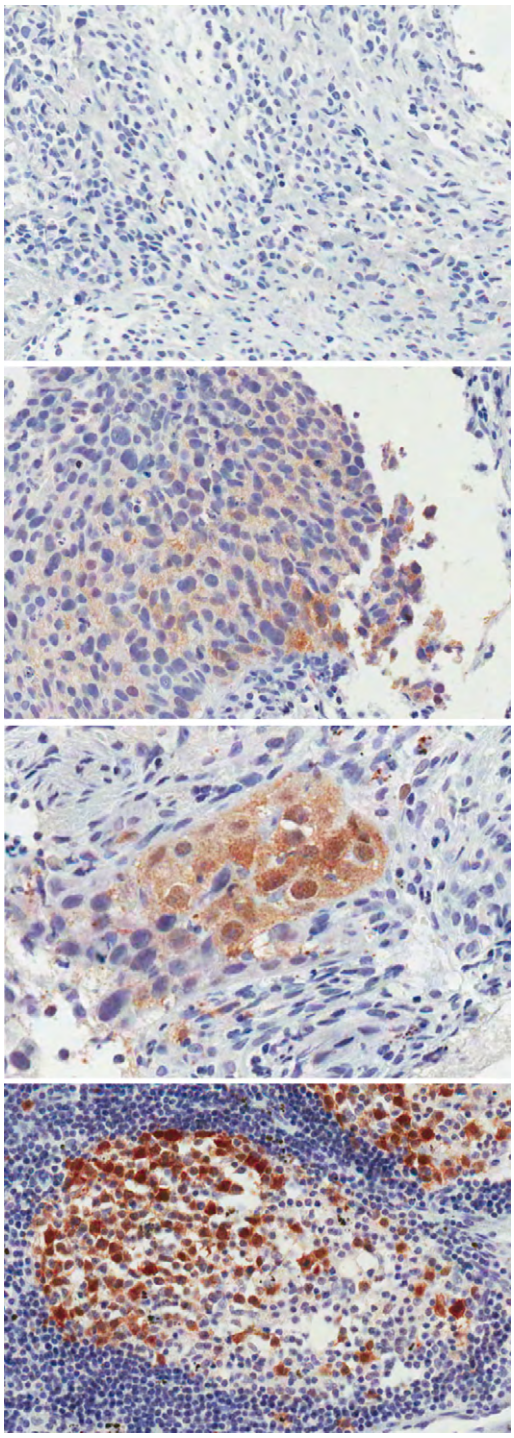

Fig. 1. Immunohistochemical staining for TS in tumor tissues.

Table 1
Characteristics of patients.

	Third line	Fourth line	*p*-Value	Total
Total	65	45		110
Median age (years)	61 (24–84)	56 (39–75)	0.219	59 (24–84)
Gender			0.007	
Male	26 (40%)	30 (67%)		56 (51%)
Female	39 (60%)	15 (33%)		54 (49%)
Disease status			0.868	
Recurred	15 (23%)	11 (24%)		26 (24%)
Advanced	50 (77%)	34 (76%)		84 (76%)
ECOG performance status			0.802	
0–1	53 (82%)	36 (80%)		89 (81%)
2–3	12 (19%)	9 (20%)		21 (19%)
Smoking history			0.012	
Never	53 (82%)	27 (60%)		80 (73%)
Former and current	10 (15%)	17 (38%)		27 (25%)
Unknown	2 (3%)	1 (2%)		3 (3%)
Histology			1.000	
Non-squamous cell carcinoma	57 (88%)	39 (86.7)		96 (87%)
Squamous cell carcinoma	8 (12%)	6 (13.3)		14 (13%)
First-line therapy				
Gemcitabine-platinum	48 (74%)	27 (60%)		75 (68%)
Other-platinum	8 (12%)	11 (24%)		19 (17%)
TKIs	6 (9%)	5 (11%)		11 (10%)
Others	3 (5%)	2 (4%)		5 (5%)
Second-line therapy				
TKIs	52 (80%)	15 (33%)		67 (61%)
Docetaxel	4 (6%)	15 (33%)		19 (17%)
Others	9 (14%)	15 (33%)		24 (22%)
Third-line therapy				
TKIs		25 (56%)		
Docetaxel		6 (13%)		
Others		14 (31%)		
Best response to first-line therapy			1.000	
PR-SD	44 (68%)	29 (65%)		73 (66%)
PD	20 (31%)	14 (31%)		34 (31%)
Unknown	1 (2%)	2 (4%)		3 (3%)

ECOG: Eastern Cooperative Oncology Group.

incubated for 30 min at room temperature. The slides were then washed and the chromogen was developed for 5 min with liquid 3,30-diaminbenzidine (Dako, Glostrup, Denmark). Finally, the sections were counterstained with Mayer hematoxylin. Negative controls were processed as above, without the primary antibody. To date, there are several commercially available anti-TS antibodies, but there are no validated scoring methods for interpreting the immunohistochemical staining. Colon cancer tissues were stained to serve as positive controls. Adjacent normal-appearing bronchial epithelium within each tissue section served as internal references. The intensity of the TS expression was scored on a scale of 0, 1+, 2+, 3+, where scores of 2+ and above were classified as high [10] (Fig. 1).

2.5. Statistical analyses

Progression-free survival (PFS) was defined as the time from the first date of pemetrexed therapy to the date of documented progression or death from any cause. The overall survival (OS) after pemetrexed use was measured from the first date of pemetrexed therapy to the date of death or the last follow-up. The Kaplan–Meier product-limit method was used to estimate PFS and OS. Survival rates were compared using the log-rank test. Multivariate analysis of the independent prognostic factors for survival was performed using the Cox proportional hazard regression model with a 95% confidence interval (CI).

3. Results

3.1. Patient characteristics

The main clinical characteristics of the patients are shown in Table 1. The median age was 59 years (range: 24–84), of which 50.9% were men, and most patients (81.8%) had a good Eastern Cooperative Oncology Group (ECOG) performance status of 0–1. Twenty-seven patients (24.6%) were either current or previous smokers. Sixty-five patients (59.1%) received pemetrexed as third-line therapy and 45 patients (40.9%) as fourth-line therapy. Ninety-five (86.4%) patients had non-squamous cell carcinoma (adenocarcinoma: $N = 90$, mucoepidermoid carcinoma: $N = 1$, undifferentiated carcinoma: $N = 3$, sarcomatoid carcinoma: $N = 1$) and 15 had squamous cell carcinoma. The most frequently administered

Table 2
Best responses to pemetrexed.

Best response	Number of patients (%)			
	Third line	Fourth line	Total	
Partial response	9 (13.8)	9 (20.0)	18 (16.3)	
Stable disease	27 (41.5)	14 (31.1)	41 (37.3)	
Progressive disease	25 (38.5)	18 (40)	43 (39.1)	
Disease control	36 (55.4)	23 (51.1)	59 (53.6)	$p = 0.770$
Not evaluable	4 (6.2)	4 (8.9)	8 (7.3)	

Table 3
Univariate and multivariate analyses for PFS and OS.

Feature	PFS (Mo)	Univariate (p)	Multivariate (p, HR)	OS (Mo)	Univariate (p)	Multivariate (p, HR)
All	3.22			11.57		
Age (years)		0.162			0.815	
≤65	4.67			11.57		
>65	2.56			12.95		
Gender		0.014	0.015		0.215	
Male	2.07		HR = 1.673	10.98		
Female	3.65		(1.103–2.535)	11.90		
ECOG		0.420			0.001	0.002
0–1	3.39			13.02		HR = 2.454
≥2	1.35			6.67		(1.405–4.287)
Smoking history		0.116			0.019	0.023
Has smoked	2.07			9.50		HR = 1.856
Never smoked	3.22			12.95		(1.087–3.168)
Histologic type		0.050			0.064	
Non-squamous	3.65			12.20		
Squamous	1.78			6.51		
Treatment line		0.979			0.213	
Third	3.22			11.05		
Fourth	3.19			13.02		
Response to first-line CT	0.129			0.158		
PR + SD	2.79			12.85		
PD	3.19			9.14		
Response to pemetrexed	<0.001			<0.001		
PR + SD (59)	6.08			15.45		
PD (43)	1.25			6.67		

first-line and second-line treatment regimens were platinum-based chemotherapy (85.5%) and EGFR-TKIs [erlotinib (17.3%) or gefitinib (43.6%)], respectively. The best responses to the first-line treatments were as follows: PR in 48 patients (44.8% of evaluable patients), SD in 25 patients (23.4%), and PD in 34 patients (31.8%). The median time from the date of diagnosis to the date of pemetrexed treatment was 12.8 months (range: 1.8–62.2 months). At the time of the pemetrexed treatment, 33 patients (30%) had treated, stable brain metastases.

3.2. Efficacy of pemetrexed

The median cycle of pemetrexed administration was 4 (range: 1–22). Nineteen patients (17.3%) received six cycles of pemetrexed. Of note, 26 patients (23.6%) received more than six cycles and 10 patients (9.1%) received 12–22 cycles. In this study cohort, the disease control rate was 53.6% with 18 (16.3%) PRs, 41 (37.3%) SDs and 43 (39.1%) PDs (Table 2). The median follow-up duration was 16.1 months, the median PFS was 3.2 months (95% CI: 1.9–4.5 months) and the median OS was 11.6 months (95% CI: 9.0–14.1 months) (Fig. 2). At the time of final analysis, 68 patients (61.8%) were dead and eight patients were lost to follow-up.

3.3. Univariate and multivariate analyses

With univariate analysis, male gender ($p = 0.014$) and squamous cell type ($p = 0.050$) were associated with a shorter PFS. Poor performance status ($p = 0.001$) and a history of smoking ($p = 0.019$) were significant predictive factors for poor survival (Table 3 and Fig. 3). There was no difference in PFS ($p = 0.979$) or OS ($p = 0.213$) between third- and fourth-line therapies.

Multivariate analysis using the Cox regression model was performed for PFS and OS. Male gender was the only independent variable in the model correlated with a poor PFS ($p = 0.015$, HR = 1.67, 95% CI: 1.10–2.54). Prognostic variables for a shorter OS were poor performance status ($p = 0.002$, HR = 2.45, 95% CI: 1.41–4.29) and a history of smoking ($p = 0.023$, HR = 1.86, 95% CI: 1.09–3.17).

3.4. Toxicity

Only one treatment-related death was observed, with the cause of death being pneumonia. Two patients developed herpes zoster infections. Scheduled chemotherapy was delayed in only two patients due to grade 3 neutropenia and grade 3 generalized edema, respectively (Table 4).

3.5. TS expression

TS expression was observed in 13 of 55 available tumor tissues (23.6%). The staining intensity scores were as follows: none (0) in 42 (76.4%) cases, weak (+1) in two (3.6%) cases, moderate (+2) in seven (12.7%) cases and strong (+3) in four (7.3%) cases (Table 5). There were no significant differences in PFS (low TS vs.

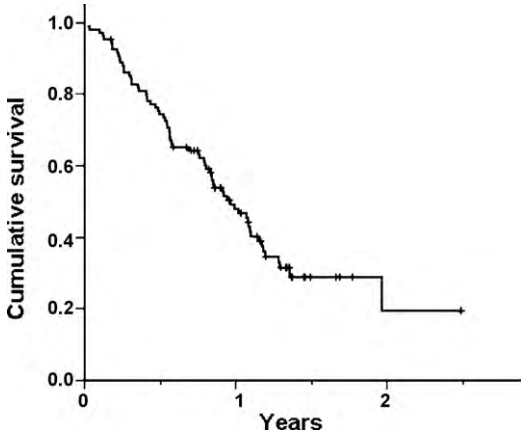

Fig. 2. Overall survival.

high TS, 2.4 months, 95% CI: 0.7–4.0 months vs. 1.3 months, 95% CI: 0.8–3.0 months, respectively; $p = 0.407$) or OS (low TS vs. high TS, 9.5 months, 95% CI: 5.9–13.2 months vs. 6.7 months, 95% CI: 5.7–7.9 months, respectively, $p = 0.688$), according to TS expression levels (Fig. 4). In addition, there were no significant associations between TS expression and the clinical parameters such as age, gender, performance status, history of smoking, histologic type, treatment line and response rate.

4. Discussion

In this study, we evaluated the efficacy and safety of pemetrexed as a third- or fourth-line therapy in patients with advanced NSCLC. The disease control rate was 53.6% with 18 (16.3%) PRs and 41 (37.3%) SDs. In addition, the median PFS and OS were 3.2 months and 11.6 months, respectively. The median PFS observed in this study was comparable to other reports in third-line settings [5,6,13]. However, the OS was longer in this study than those reported in previous studies [4,5,14]. One plausible explanation for such a favorable outcome may be attributed to a higher proportion of patients who had never smoked and of non-squamous cell

Table 4
Toxicity profiles.

	Third line ($N = 65$)			Fourth line ($N = 45$)		
	Gr 1/2	Gr 3	Gr 4/5	Gr 1/2	Gr 3	Gr 4/5
Neutropenia	0	1	0	1	0	0
Fatigue	16	0	0	7	0	0
Anorexia	10	0	0	4	0	0
Nausea	10	0	0	8	0	0
Mucositis	1	0	0	1	0	0
Constipation	1	0	0	1	0	0
Diarrhea	0	0	0	2	0	0
Pruritus	4	0	0	2	0	0
Rash	7	0	0	4	0	0
Facial edema	8	0	0	1	0	0
Edema	1	1	0	1	0	0
Neuropathy	3	0	0	2	0	0
Headache	6	0	0	1	0	0
Infection[a]	0	2	1	0	0	0
General ache	1	0	0	4	0	0
Indigestion	1	0	0	3	0	0

[a] The two patients (G3) had herpes zoster.

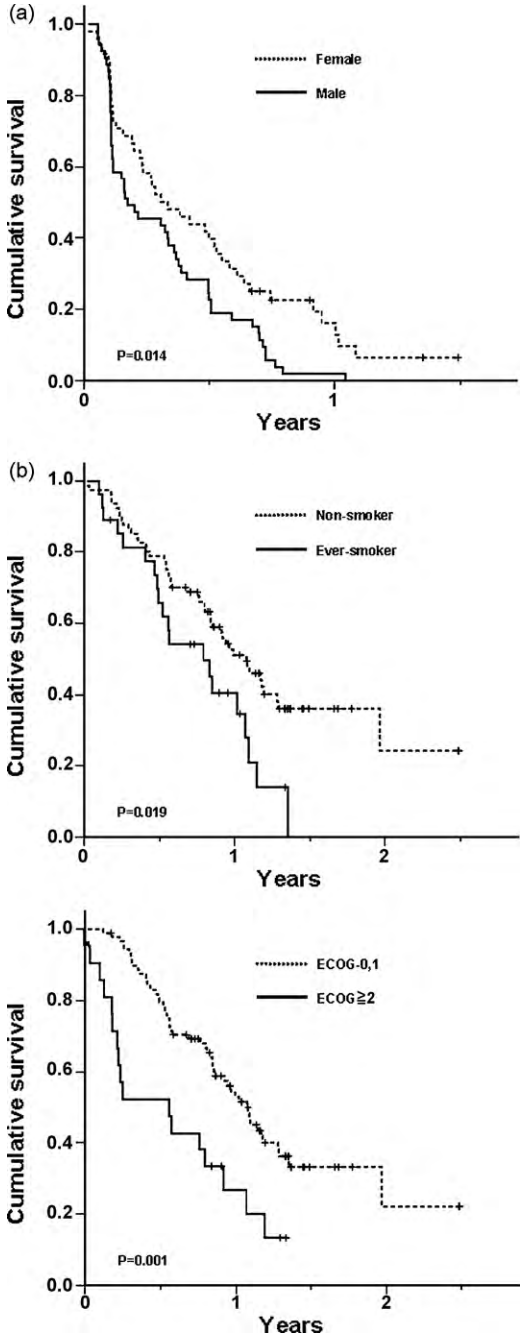

Fig. 3. (A) Comparison of PFS by gender, (B) comparison of OS by smoking history, and (C) Comparison of OS by performance status.

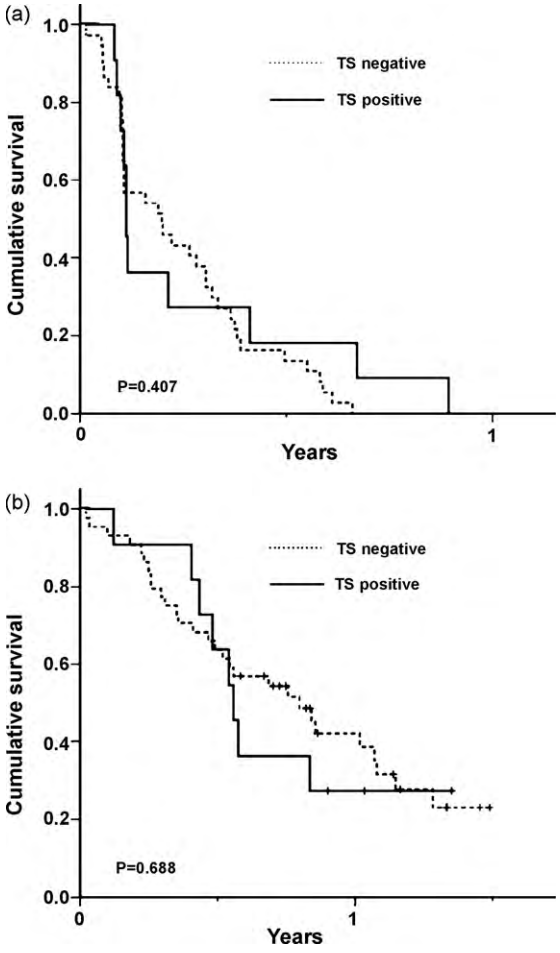

Fig. 4. (A) Comparison of PFS by TS expression and (B) Comparison of OS by TS expression.

Table 5
TS expressions in tumor tissues.

Intensity of stained tumor cells	Number (%)	Responder (CR + PR + SD)	Non-responder (PD)	Not evaluable
Grade 0	42 (76.4)	21	18	3
Grade 1	2 (3.6)	2	0	0
Grade 2	7 (12.7)	3	4	0
Grade 3	4 (7.3)	1	3	0

types in our series [15]. Another contributing factor might be ethnic differences, which have been considered a consistently good prognostic factor, as previously reported [16,17].

Intriguingly, although the fourth-line therapy group had more unfavorable factors (i.e., male, smoker) than the third-line group, there were no differences in response rates, PFSs and OSs. The overall survival was 13.0 months for the fourth-line therapy group and 11.0 months for the third-line therapy group. Furthermore, considering the toxicity profiles, most of the patients tolerated the treatment well, even in third or fourth-line therapy. Given that many patients were still in good performance status even after failure of the second- or third-line therapies and were willing to receive further anti-cancer treatment, pemetrexed could be recommended as salvage therapy. Moreover, 20% of patients with poor performance status enrolled in this study also tolerated pemetrexed well.

A recent phase III study showed a significant improvement in survival with first-line pemetrexed and cisplatin in NSCLC patients with adenocarcinoma [16]. We also recently reported the excellent outcome of Korean adenocarcinoma patients in second-line therapy with pemetrexed [18]. Although adenocarcinoma was not a significant prognostic factor in this study, there was a trend favoring non-squamous histology. Median PFSs in the non-squamous and squamous histology group were 3.7 and 1.8 months, respectively ($p = 0.05$), and the median OSs were 12.2 and 6.5 months, respectively ($p = 0.06$). This result might be due to the small number of squamous histology (12%) samples in this cohort.

Based on currently available data, NSCLC patients presenting an increased TS expression appear to have poor prognoses, especially in squamous cell carcinoma types [11]. Conversely, expression of TS neither influenced the prognosis nor predicted the response to pemetrexed in our series. One plausible explanation for such a negative finding could be the small sample size utilized in this study. Furthermore, the heterogeneity of TS staining within the tumors and lack of a standardized scoring system in NSCLC may have contributed to our results. Several studies have reported various percentages of patients with NSCLC – ranging from 29.6% to 72.5% – presenting TS positivity [10,11,19–22]. In our study, TS positivity (20%) was lower than in other studies. Third, there may be a discrepancy between TS enzymatic activity and protein expression by immunohistochemistry, as previously reported [22]. Thus, RT-PCR should be considered as an alternative method to determine the level of TS expression instead of protein expression [23,24]. Fourth, the level of reduced folate carrier (RFC), as well as that of folate binding protein (FBP), may influence the uptake and the effectiveness of this drug. As pemetrexed is a prodrug, the activity of folylpolyglutamate synthetase, the enzyme that converts pemetrexed to polyglutamate forms, may be an important determinant of the response. Therefore, in order to elucidate the correlation between TS expression and treatment outcome, further biomarker analyses should be conducted in larger numbers of patients.

In conclusion, pemetrexed was suggested as a third- or fourth-line therapy due to its favorable efficacy and tolerable toxicity, especially in women, patients with good performance status or non-smokers. Given the frequent use of EGFR-TKIs as second-line therapy in Korean NSCLC patients, further studies are warranted to define the adequate sequence of salvage treatments, especially in patients with adenocarcinoma lung cancer.

Conflict of interest statement

None declared.

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