Anaplastic Lymphoma Kinase Translocation

A Predictive Biomarker of Pemetrexed in Patients with Non-small Cell Lung Cancer

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Introduction: This study compared the efficacy of pemetrexed in patients with anaplastic lymphoma kinase (*ALK*)-positive versus *ALK*-negative (epidermal growth factor receptor [*EGFR*] mutant or wild type [WT] for both *ALK* and *EGFR*) non-small cell lung cancer (NSCLC).
Methods: Patients with advanced NSCLC who received second-line pemetrexed and beyond between March 2007 and April 2010 were screened for *EGFR* mutations and *ALK* rearrangements at Seoul National University Hospital. The clinical and in vitro efficacy of pemetrexed was evaluated for each genotypic group.
Results: Ninety-five NSCLC patients were genotyped as follows: 43 (45%) *EGFR* mutation, 15 (16%) *ALK* translocation, and 37 (39%) WT. The overall response rate was superior in *ALK*-translocated patients compared with *EGFR* mutant or WT patients (46.7 versus 4.7 versus 16.2%, $p = 0.001$). *ALK*-positive patients showed longer time to progression than *EGFR* mutant or WT patients (9.2 versus 1.4 versus 2.9 months, $p = 0.001$). *ALK* positivity alone was a significant predictor for overall response rate (hazard ratio [HR] = 0.07, 95% confidence interval [CI]: 0.01–0.32; $p = 0.001$) and time to progression (HR = 0.44, 95% CI: 0.24–0.80; $p = 0.007$). *ALK* positivity remained independently significant regardless of treatment line (HR = 0.43, 95% CI: 0.24–0.77; $p = 0.005$). Thymidylate synthase mRNA levels in *ALK*-positive cells were significantly lower compared with control cells ($p < 0.05$).
Conclusion: Pemetrexed is an effective treatment in patients with *ALK*-positive NSCLC. *ALK* positivity was independently predictive of pemetrexed efficacy in NSCLC patients.

Key Words: Lung cancer, ALK, Pemetrexed.

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Lung adenocarcinoma is heterogeneous with diverse somatic mutations associated with carcinogenesis.[1] Most East Asian patients with lung adenocarcinoma who have never smoked harbor targetable oncogenic mutations, including epidermal growth factor receptor (*EGFR*) mutations, fusions of echinoderm microtubule-associated protein-like 4 (*EML4*) and anaplastic lymphoma kinase (*ALK*), and human epidermal growth factor receptor 2.[2] The discovery of these mutations led to the era of targeted therapies. Recent phase III studies showed that first-line treatment with gefitinib lengthened survival time in patients with lung adenocarcinoma with *EGFR* mutations.[3,4]

An *EML4-ALK* fusion transcript derived from a small inversion within chromosome 2p has a transforming activity.[5] Tumors of *EML4-ALK* transgenic mice were effectively cleared by an *ALK* inhibitor, indicating that *ALK* inhibition shows strong therapeutic potential in patients with *ALK*-rearranged non-small cell lung cancer (NSCLC) (overall response rate [ORR], 57%; disease control rate (DCR) at 8th week, 87%; and 6-month progression-free survival, 72%).[6]

Several studies have shown that *ALK*-translocated NSCLC was most common in patients of younger age, patients with adenocarcinoma, and patients who never or lightly smoked.[5,7–10] Only one study has examined treatment outcomes of patients with *ALK* translocation.[7] *ALK*-positive NSCLC patients did not respond to *EGFR* TKIs and showed a similar response rate to platinum-based agents compared with wild-type (WT) patients. Therefore, typical clinical and pathologic findings, as well as responsiveness to *EGFR* TKIs, are useful in identifying *ALK*-translocated NSCLC patients. However, considering the low frequency of *EML4-ALK* translocation in NSCLC (3–13%),[5,7,8] it is crucial to find novel clinical features for *ALK*-positive lung cancer.

Pemetrexed is a multitargeted antifolate that inhibits thymidylate synthase (TS), dihydrofolate reductase, glycinamide ribonucleotide formyltransferase, and aminoimidazole carboxamide ribonucleotide formyltransferase. Pemetrexed prevents the formation of precursor pyrimidine and purine nucleotides.

Pemetrexed is currently approved for treatment of patients with nonsquamous cell histology as a first-line treatment in

combination with platinum,[11,12] as a second-line single agent,[13] and as maintenance therapy after first-line platinum-based chemotherapy.[14] In addition, pemetrexed showed modest efficacy as a third- or fourth-line treatment.[15,16]

A recent study demonstrated that pemetrexed plus cisplatin significantly prolonged time to progression (TTP) (9 versus 6.2 months) and overall survival (17 versus 11 months) in *ALK*-positive NSCLC patients, compared with *ALK*-negative patients, suggesting a potential role of pemetrexed in *ALK*-rearranged cases.[17] Therefore, this retrospective study was designed to compare the efficacy of pemetrexed between *ALK*-positive and *ALK*-negative cases and to identify features associated with treatment outcome in *ALK*-translocated NSCLC.

PATIENTS AND METHODS

Study Population

Korean patients with advanced NSCLC who received pemetrexed between March 2007 and April 2010 were identified from the database at Seoul National University Hospital. Patients were invited to participate in this study if they met all of the following inclusion criteria: (1) single-agent pemetrexed therapy, as a second-line, third-line, or subsequent treatment; (2) genotypic screening for *EGFR* mutation and *EML4-ALK* fusion; (3) histologically confirmed NSCLC at stage IIIB/IV or relapse; (4) at least one prior systemic chemotherapy, including platinum-based doublet; and (5) adequate bone marrow, renal, and hepatic functions. Ten and three *ALK*-positive patients were enrolled in phase I (NCT00585195)[6] and phase II (NCT00932451) trials of crizotinib (PF-02341066), respectively. Patients enrolled in the phase III trial (NCT00932893) were excluded because it was designed to compare the efficacy of PF-02341066 versus pemetrexed or docetaxel. Patients were categorized by their smoking histories. A never-smoker was defined as a patient who had smoked ≤100 cigarettes in their lifetime. Former light-smokers were defined as patients who had a history of ≤10 pack-years of smoking.[7] This study was approved by the Institutional Review Board of Seoul National University Hospital.

Determination of Molecular Subtypes

Patients were divided to three groups according to molecular subtype: *EGFR* mutation, *ALK* translocation, and WT. *ALK* positivity was defined as split signals ≥15% by

TABLE 1. Patients' Characteristics Based on Genotype

Characteristic	No. of Patients (%)				
	Total (n = 95)	*ALK* Translocation (n = 15)	*EGFR* Mutation (n = 43)	Wild Type (n = 37)	p
Age, yr					
Median	58	52	63	56	0.043
Range	28–79	34–67	34–79	28–79	
Sex					
Male	44 (46.3)	7 (46.7)	20 (46.5)	17 (45.9)	0.998
Female	51 (53.7)	8 (53.3)	23 (53.5)	20 (54.1)	
PS					
0–1	91 (95.8)	14 (93.3)	42 (97.7)	35 (94.6)	0.695
2–3	4 (3.2)	1 (6.7)	1 (2.3)	2 (5.4)	
Smoking					
Never	59 (62.1)	8 (53.3)	28 (65.1)	23 (62.2)	0.099
Light	7 (7.4)	0	6 (14.0)	1 (2.7)	
Former/current	29 (30.5)	7 (46.7)	9 (20.9)	13 (35.1)	
Histology					
Adenocarcinoma	78 (82.1)	14 (93.3)	37 (86.0)	27 (73.0)	0.282
Nonadenocarcinoma[a]	17 (17.9)	1 (6.7)	6 (14.0)	10 (27.0)	
Stage					
IA	1 (1.1)	0	1 (2.3)	0	0.408
IB	8 (8.4)	3 (20.0)	3 (7.0)	2 (5.4)	
IIB	2 (2.1)	0	2 (4.7)	0	
IIIA	6 (6.3)	1 (6.7)	2 (4.7)	3 (8.1)	
IIIB	7 (7.4)	0	2 (4.7)	5 (13.5)	
IV	71 (74.7)	11 (73.3)	33 (76.6)	27 (73.0)	
Pemetrexed					
Second-line	38 (40.0)	6 (40.0)	6 (14.0)	26 (70.3)	<0.001
≥ Third-line	57 (60.0)	9 (60.0)	37 (86.0)	11 (29.7)	

[a] Nonadenocarcinoma included adenosquamous, squamous, large cell carcinoma, and non-small cell lung cancer, not otherwise specified (NSCLC, NOS). One wild-type patient had squamous cell carcinoma.

ALK, anaplastic lymphoma kinase; *EGFR*, epidermal growth factor receptor; PS, performance status.

break-apart fluorescent in situ hybridization (FISH).[6] The *EGFR* mutant group harbored an activating mutation on exon 19 or 21. WT patients were neither *EGFR* mutant nor *ALK* positive. *EGFR* mutations on exons 18, 19, 20, and 21 were determined by direct DNA sequencing.[18]

Dual-probe hybridization for *ALK* was performed using the LSI *ALK* break-apart probe set (Vysis, Downers Grove, IL). The probe mixture was applied to the slides, which were then incubated in a humidified atmosphere with HYBrite (Vysis) at 77°C for 5 minutes to simultaneously denature the probe and target DNA. An additional 16-hour incubation at 37°C was required for hybridization. Next, the slides were immersed in 0.3% NP-40/0.4× SSC for 5 minutes at room temperature, followed by 0.3% NP-40/0.4× SSC for 5 minutes at 72°C. Nuclei were counterstained with DAPI (4′,6-diamidino-2-phenylindole).

ALK FISH was considered positive when more than 15% of 50 or more analyzed cells showed splitting apart of the fluorescent probes flanking the *ALK* locus. Immunohistochemistry (IHC) by a mouse monoclonal antibody for *ALK* (Novocastra, Clone 5A4, Newcastle upon Tyne, United Kingdom) was performed using a Bond-max automated immunostainer (Leica Microsystems, Milton Keynes, United Kingdom). Various normal and cancer tissue microarray blocks were included as positive and negative controls. *ALK* IHC was considered positive if moderate staining was identified in 10% or more of the tumor cells.[19] The *EGFR* mutation, *ALK* break-apart FISH, and *ALK* IHC were analyzed by experienced pathologists (Y.-K.J., D.H.C., and W.-H.K.).

Pemetrexed Treatment and Response

All patients received pemetrexed alone at a dose of 500 mg/m^2 every 21 days. Folic acid supplementation (1000 mg) was taken orally daily beginning 1 to 2 weeks before the first dose of pemetrexed and continued until 3 weeks after treatment ended. Dexamethasone (4 mg) was taken twice daily on the day before, the day of, and the day after each dose of pemetrexed. An injection of vitamin B_{12} (1000 μg) was given 1 to 2 weeks before the first dose of pemetrexed and was repeated approximately every 9 weeks during treatment. Treatment was continued until disease progression warranted termination, unacceptable toxicity was found, or until the patient or physician decided to discontinue therapy. Tumor response was evaluated every two cycles, or earlier if there were clinical signs of progression, by the Response Evaluation Criteria in Solid Tumors version 1.0.[20]

In Vitro Cytotoxicity of Pemetrexed

A modified MTT (3-[4,5-dimethylthiazol-2-yl]-2,5-diphenyltetrazolium bromide) assay was analyzed using CCK-8 (Dojindo, Rockville, MD). The inhibitory concentration at 50% (IC_{50}) was calculated. *ALK*-positive cells included NCI-H3122, kindly provided by Pasi A. Jänne at the Dana Farber Cancer Institute, and NCI-H2228. *EGFR* mutant cells (PC-9) were kindly provided by Mayumi Ono at Kyushu University. *ALK*-positive cells (NCI-H3122 and NCI-H2228), *EGFR* mutant cells (PC-9), WT cells (NCI-H1666), and control cells (NCI-H157, squamous cell carcinoma) were continuously exposed to pemetrexed at a concentration of 0.001 to 1 μM for 48 or 96 hours. After 1 hour of incubation at 37°C, the absorbance was measured at 450 nm in a microplate reader. TS mRNA expressions of NSCLC tumor cells were quantified by reverse-transcriptase polymerase chain reaction. All experiments were repeated three times. A two-sided *t* test was used to evaluate group differences.

Statistical Analysis

Analyzed variables include age, sex, performance status, smoking history, histology, stage, pemetrexed treatment line, and molecular subtypes. Pearson's χ^2 and one-way analysis of variance tests were performed to assess differences in clinical and pathological characteristics between the three molecular subgroups. The association between clinical factors and the response rate to pemetrexed was analyzed using Pearson's χ^2 test or Fisher's exact test, as appropriate. TTP was calculated

TABLE 2. Efficacy of Pemetrexed Based on Genotype

Molecular Subtypes	No. of Patients (%) *ALK* Translocation (n = 15)		*EGFR* Mutation (n = 43)		Wild Type (n = 37)		p
Cycle							
Median (range)	9 (1–36)		2 (1–23)		4 (1–33)		
Best response							
PR	7	46.7	2	4.7	6	16.2	
SD	6	40.0	9	20.9	15	40.5	
PD	2	13.3	32	74.4	16	43.2	
ORR	7	46.7	2	4.7	6	16.2	0.001
DCR (PR + SD)	13	86.7	11	25.6	21	56.8	0.000
Median TTP, mo (95% CI)	9.2 (4.65–13.74)		1.4 (1.27–1.52)		2.9 (0.51–5.28)		0.001

ALK, anaplastic lymphoma kinase; *EGFR*, epidermal growth factor receptor; PR, partial response; SD, stable disease; PD, progressive disease; ORR, overall response rate; DCR, disease control rate; CI, confidence interval; TTP, time-to-progression.

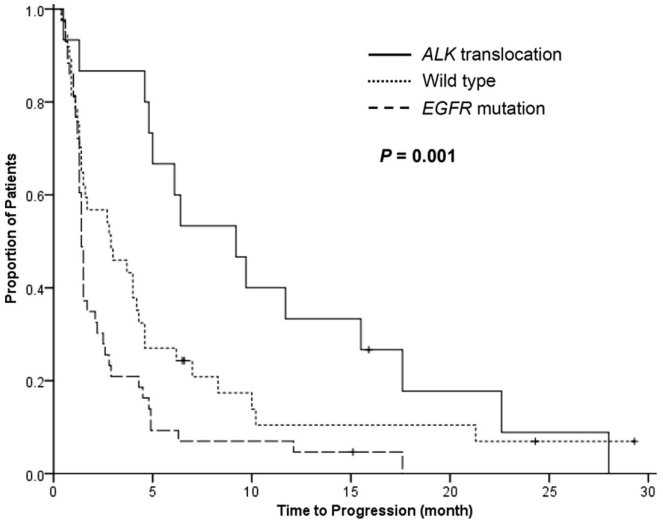

FIGURE 1. Kaplan-Meier plot of time to progression in all patients.

from the first date of pemetrexed therapy to the date of documented progression. Survival curves were plotted by the Kaplan-Meier method.[21] Differences between groups were compared using the log-rank test. Factors independently associated with TTP and response to pemetrexed were identified by multivariate analysis using the Cox's proportional hazards regression model and binary logistic regression, respectively. Two-sided p values less than 0.05 were considered significant. All analyses were performed using SPSS, version 12.0 (Chicago, IL).

RESULTS

Patients and Molecular Subtypes

Of 142 NSCLC patients who were treated with pemetrexed alone and underwent molecular screening for *EGFR* or *ALK*, 95 met the study inclusion criteria. Patients were segregated into *EGFR* mutant ($n = 43$), *ALK*-translocated ($n = 15$), and WT ($n = 37$) groups. Twenty-five patients had an exon 19 deletion, whereas 18 patients harbored an L858R mutation. Baseline characteristics are summarized in Table 1.

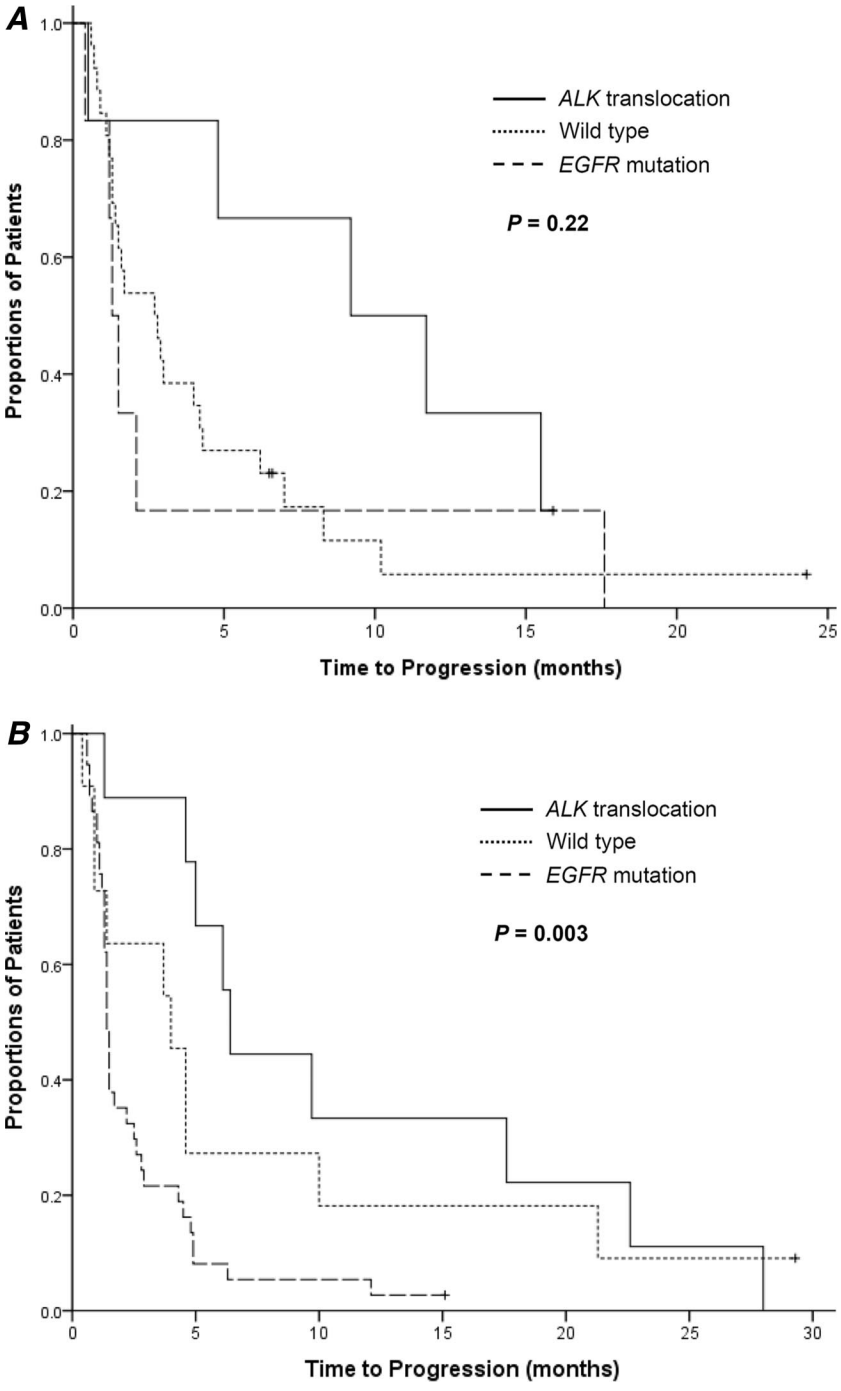

FIGURE 2. Kaplan-Meier plots of time to progression in patients treated with second-line pemetrexed (*A*) and in those treated with third-line pemetrexed and beyond (*B*).

TABLE 3. Summary for *ALK*-Positive NSCLC Patients

Pt	Age (yr)	Sex	Histology	Smoking (Pack-Year)	Surgery	RFS (mo)	Pemetrexed					OS[b] (mo)	Status
							Linex	Cycle	BR	TTP (mo)	OS[a] (mo)		
1	52	F	Ad	0			5	24	PR	17.6	39.3	133.9	Alive
2	49	F	Ad	25	Yes	16	2	6	SD	4.8	25.4	46.1	Death
3	45	M	Ad[c]	20			3	36	PR	28.0	35.2	60.4	Alive
4	54	M	Ad	20	Yes	109	5	23	SD	22.6	34.3	263.3	Alive
5	34	M	NSCLC, NOS	10			6	13	SD	9.7	33.6	62.9	Alive
6	64	M	Ad	0			3	6	SD	6.1	31.3	38.3	Alive
7	67	M	Ad	0			2	9	PR	15.5	25.6	34.8	Alive
8	60	F	Ad	0	Yes	17	3	5	SD	5.0	24.7	52.4	Alive
9	48	F	Ad	0			4	6	PR	4.6	22.5	30.6	Death
10	54	M	Ad	30			3	2	PD	1.3	10.5	19.6	Death
11	63	M	Ad	17			2	22	PR	15.9	17.9	23.3	Alive
12	55	F	Ad	20			2	12	SD	9.2	13.1	61.5	Alive
13	49	F	Ad	0			2	1	PD	0.5	12.9	20.0	Alive
14	51	F	Ad[c]	0			2	15	PR	11.7	11.7	17.7	Death
15	43	F	Ad	0	Yes	49	3	9	PR	6.4	7.5	75.8	Alive

[a] The time from the first date of pemetrexed treatment to the date of death or the last visit of the patient.
[b] The time from diagnosis to the date of death or the last visit of the patient.
[c] Adenocarcinoma with signet ring cell component.
Pt, patient; RFS, relapse-free survival; BR, best response; Ad, adenocarcinoma; SRC, signet ring cell; NSCLC NOS, non-small cell lung cancer not otherwise specified; TTP, time to progression.

There were no significant differences between the groups with respect to sex, performance status, smoking history, histology, and stage. *ALK*-positive patients were younger than others ($p = 0.043$). Regarding treatment line, more patients with WT and *ALK* translocation received pemetrexed as a second-line treatment than those with *EGFR* mutation ($p < 0.001$). Of 43 *EGFR*-mutant patients, 39 (90.7%) received *EGFR* TKIs before pemetrexed treatment.

Efficacy of Pemetrexed

After a median follow-up of 11.2 months (range, 0.9–39.3) from the start of pemetrexed treatment, 44 patients were still alive. Five patients continued to receive pemetrexed at the cutoff date for data collection (November 30, 2010). All patients could be evaluated for tumor response and treatment outcomes based on subtypes were described in Table 2. ORR was 15.8%, whereas DCR (partial response [PR] + stable disease [SD]) was 47.4%. Response rates were similar between second-line and ≥ third-line pemetrexed groups (ORR, 21.1 versus 12.3%, $p = 0.251$; and DCR, 52.6 versus 43.9%, $p = 0.402$, respectively). The median TTP in all patients was 2.2 months (95% confidence interval [CI]: 1.36–3.03).

There was no significant difference in TTP between second-line and ≥ third-line pemetrexed groups (2.7 versus 1.7 months; $p = 0.618$). Patients with *ALK* translocation showed higher response rates than those with *EGFR* mutation or WT (Table 2). In 38 patients treated with second-line pemetrexed, *ALK*-positive patients showed higher response rates, compared with *EGFR* mutated and WT patients (ORR, 50.0 versus 0 versus 19.2%; $p = 0.096$; and DCR, 83.3 versus 16.7 versus 53.8%; $p = 0.067$, respectively). For the ≥ third-line pemetrexed group (57 patients), ORR and DCR were significantly higher in *ALK* translocation patients than in *EGFR* mutation and WT patients (ORR, 44.4 versus 5.4 versus 9.1%; $p = 0.006$; and DCR, 88.9 versus 27.0 versus 63.6%; $p = 0.001$, respectively). *ALK* positivity was the only significant factor correlated with favorable ORR in univariate ($p < 0.001$) and multivariate analyses (hazard ratio [HR] = 0.07, 95% CI: 0.01–0.32; $p = 0.001$).

ALK-translocated patients showed superior TTP compared with *EGFR* mutant or WT patients (regardless of treatment line, 9.2 versus 1.4 versus 2.9 months, $p = 0.001$, Figure 1; and third line or beyond, 6.4 versus 1.4 versus 4.0 months, $p = 0.003$, Figure 2*B*). Second-line pemetrexed tended to prolong TTP in NSCLC patients with *ALK* translocation as compared with those with *EGFR* mutation and WT (9.2 versus 1.3 versus 2.7 months; $p = 0.224$, Figure 2*A*). *ALK* positivity was a significant predictor for TTP, as determined by univariate ($p = 0.004$) and multivariate analyses (HR 0.44, 95% CI, 0.24–0.80; $p = 0.007$). *ALK* positivity remained independently significant in treatment-line stratified multivariate analysis (HR = 0.43, 95% CI: 0.24–0.77; $p = 0.005$). Other clinical and pathologic factors were not predictive of TTP ($p > 0.05$). Treatment outcomes of pemetrexed in *ALK*-translocated NSCLC are detailed in Table 3. At the time of data cutoff, 11 patients were alive and the median overall survival had not been reached.

In Vitro Cytotoxicity of Pemetrexed

Although the IC_{50} values (mean ± SD) were significantly lower in *ALK*-positive cells (H3122, 68.7 ± 21.1 nM; H2228, 33.0 ± 10.7 nM) than in control cells (H157, >1 μM), there were no statistical differences between *ALK*-positive cells and WT cells (H1666, 50.9 ± 30.2; Figure 3*A*). The TS/GAPDH mRNA ratio of *ALK*-positive cells (H3122, 0.13 ± 0.10; H2228, 0.48 ± 0.08) was significantly lower

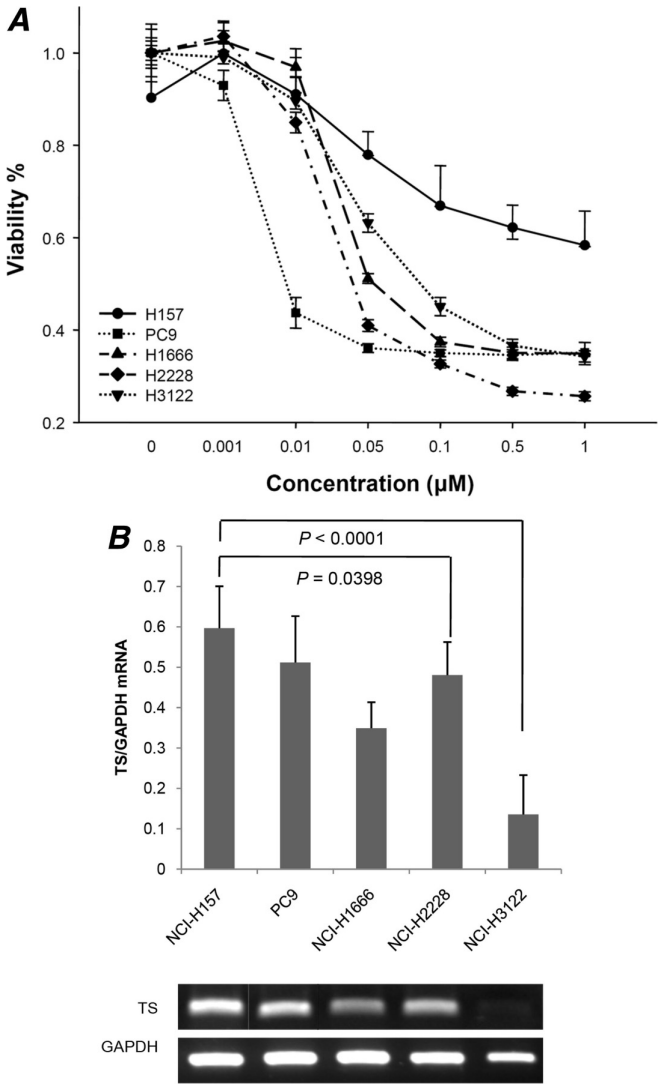

FIGURE 3. In vitro cytotoxicity of pemetrexed (*A*) and thymidylate synthase mRNA level (*B*) in non-small cell lung cancer cells.

compared with that of control cells (H157, 0.60 ± 0.10; Figure 3*B*). However, the TS/GAPDH mRNA ratio was similar between H2228 and *ALK*-negative cells (PC9, 0.51 ± 0.11; H1666, 0.35 ± 0.06).

DISCUSSION

Our study demonstrates that pemetrexed treatment produced significantly better outcomes in *ALK*-translocated NSCLC patients than in *EGFR* mutant or WT patients. DCRs, as well as overall response rates, were excellent in *ALK*-positive patients (86.7% of DCR and 46.7% of ORR). In addition, median TTP was nearly sixfold higher in *ALK*-positive NSCLC than in *ALK*-negative patients. *ALK* positivity alone was an independent predictor for the efficacy of pemetrexed treatment.

EGFR TKIs were ineffective in treating *ALK*-rearranged NSCLC.[7] However, pemetrexed significantly delayed TTP in *ALK*-positive patients compared with *ALK*-negative patients. Considering that the median progression-free survival times of NSCLC is 2.9 months when pemetrexed is used as a second-line therapy[13] and 3.0 months as a third-line treatment,[15] it is encouraging that *ALK*-positive patients remained progression-free at 9.2 months with second-line pemetrexed treatment and 6.4 months for third- or greater-line treatment in this study. Furthermore, the response rate to pemetrexed in *ALK*-positive NSCLC was more than 45%, which is superior to the ORR of 9.1 to 12.1% in the literature.[13,15] Disease was controlled in approximately 90% of *ALK*-positive patients after pemetrexed, regardless of treatment line.

In addition, two of four patients relapsed at 49 and 109 months after surgery and survived for more than 6 and 21 years, respectively. This is comparable with published reports of disease-free intervals of 73 months[22] and 20 years[23] in *ALK*-positive Japanese patients. This indicates an indolent clinical course. Less differentiated tumor grade and a low frequency of p53 mutations might reflect favorable prognosis in *EML4-ALK* translocated NSCLC.[24]

Because cytotoxicities of pemetrexed are similar in lung adenocarcinoma cells regardless of *ALK* positivity, an indolent clinical course rather than a direct cytotoxic effect of pemetrexed may be the mechanism by which pemetrexed prolongs survival time in *ALK*-positive NSCLC.

Recently, a heat-shock protein 90 (HSP90) inhibitor was found to suppress *ALK*-translocated NSCLC, both preclinically[25] and in an early-phase clinical trial.[26] The HSP90 inhibitor reduced *EML4-ALK*-driven tumor by disrupting phospho (p)-AKT and p-ERK 1/2 signals.[25]

Although the effect of pemetrexed on HSP90 is unknown, pemetrexed significantly downregulated p-AKT in NSCLC cells.[27] Therefore, pemetrexed may work on *ALK*-rearranged NSCLC cells by suppressing a downstream signal, p-AKT, shared by *ALK*. However, the exact mechanism of pemetrexed on *ALK*-translocated NSCLC cells is unknown. In addition, the role of first-line or maintenance pemetrexed should be resolved, even though pemetrexed plus cisplatin significantly prolonged survival in *ALK*-positive NSCLC patients.[17] Efficacy of pemetrexed in *ALK*-positive NSCLC will be shown as a second-line treatment (NCT00932893)[28] and first-line combination chemotherapy (NCT01154140).[29]

High TS expression has been considered as a resistance mechanism in NSCLC[30–32] and may be a predictive biomarker of pemetrexed sensitivity. TS mRNA levels were relatively low in *ALK*-positive cells in our study. However, it is difficult to conclude the mechanism of pemetrexed sensitivity based on TS mRNA levels because of the lack of TS evaluation in tumor tissue. However, *ALK* positivity can be a predictive biomarker for pemetrexed sensitivity in NSCLC in nonsquamous histology. Reversely, pemetrexed effectiveness can be used as one of selection criteria for *ALK* screening in lung cancer. Retrospective analysis of response to chemotherapy and prospective tracking of a small number of *ALK*-positive patients may weaken the association between pemetrexed sensitivity and *ALK* positivity in NSCLC. However,

it is worthwhile to elucidate the efficacy of pemetrexed based on the molecular subtypes.

In conclusion, pemetrexed significantly delayed TTP and showed excellent antitumor effects in patients with *ALK*-translocated NSCLC. *ALK* positivity could be a predictive biomarker for pemetrexed efficacy in patients with NSCLC. Nevertheless, caution is warranted in interpreting these findings, because of the unbalanced number of patients treated with pemetrexed in the *EGFR* mutant group. This can be attributed to the second-line use of *EGFR* TKIs in most cases. Future studies should focus on determining the mechanism of pemetrexed action on *ALK*-positive NSCLC. Moreover, the efficacy of pemetrexed in *ALK*-positive patients needs to be further investigated using prospective clinical trials.

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