Correlation Between Methylenetetrahydrofolate Reductase (MTHFR) C677T Polymorphisms and Pemetrexed Chemotherapy Efficacy/Toxicity in Non-Squamous Non-Small Cell Lung Cancer

Authors' Contribution:
Study Design A
Data Collection B
Statistical Analysis C
Data Interpretation D
Manuscript Preparation E
Literature Search F
Funds Collection G

AE   Gaochen Lan
ABD Lin Lin
CE   Xiong Chen
CF   Libin Chen
BCD Xi Chen

Department of Medical Oncology, Fuzhou General Hospital of Nanjing Military Command, Fuzhou, Fujian, P.R. China

Corresponding Author: Xi Chen, e-mail: cxifuzhou@163.com
Source of support: This work was supported by Wu Jieping Medical Foundation Special Fund for Clinical Research (No. 320.6750.14032)

Background: In the present study, we aimed to retrospectively analyze the correlation between toxicity of pemetrexed (PEM) chemotherapy and methylenetetrahydrofolate reductase (MTHFR) C677T polymorphisms in patients with advanced non-squamous non-small cell lung cancer (non-sq NSCLC).

Material/Methods: We used polymerase chain reaction, gene scanning, and restriction fragment length polymorphism to analyze MTHFR C677T in 51 patients with advanced non-sq NSCLC. The patients received chemotherapies with single-agent PEM (monotherapy group) or with PEM combined with cisplatin (joint group). The correlation between MTHFR C677T polymorphisms and chemotherapy efficacy/toxicity was also assessed.

Results: There were 40 patients in the monotherapy group and 11 patients in the joint group. Among the 40 patients received single-agent PEM chemotherapy, those with the CT/TT genotype had higher incidence of leukopenia, neutropenia, nausea, and fatigue compared to patients with the with wild-type genotype CC (all $P<0.05$). However, polymorphisms of MTHFR C677T were not significantly associated with other adverse events and clinical outcomes.

Conclusions: Compared with genotype CC (the wild type), patients with the CT/TT genotype had higher incidence of leukopenia, neutropenia, nausea, and fatigue. Therefore, the MTHFR C677T polymorphism could be a predictive factor for leukopenia, neutropenia, nausea, and fatigue toxicities in non-sq NSCLC patients treated with single-agent PEM.

MeSH Keywords: Carcinoma, Non-Small-Cell Lung • Medical Oncology • Methylenetetrahydrofolate Reductase (NADPH2) • Polymorphism, Single Nucleotide • Toxicity Tests

Abbreviations: PEM – pemetrexed; MTHFR – methylenetetrahydrofolate reductase; non-sq NSCLC – non-small cell lung cancer; ECOG PS – Eastern Cooperative Oncology Group performance status; CR – complete response; PR – partial response; SD – stable disease; PD – progressive disease; RECIST – Response Evaluation Criteria in Solid Tumor; NCI-CTC – National Cancer Institute Common Toxicity Criteria; CDDP – cisplatin

Full-text PDF: https://www.medscimonit.com/abstract/index/idArt/904836

📄 1843   ⊞ 6   📊 1   📖 29

Background

Lung cancer is the cancer with the highest incidence in the world, and non-small cell lung cancer (NSCLC) accounts for about 80% of all lung cancer cases [1,2]. The majority of NSCLC are diagnosed at unresectable stage [3], so chemotherapy, such as use of Paris Saponins and PEM, is the backbone of treatment in most patients [4]. Studies on chemotherapy of NSCLC were conducted and the PI3K/AKT Pathway was found to be associated with the chemo-resistance of NSCLC [4,5]. The poor survival of NSCLC patients was also found to be associated with aberrant expression of autophagy-related gene [6]. Pemetrexed (PEM) is frequently used for advanced non-squamous non-small cell lung cancer (non-sq NSCLC). Some studies show that when compared with other chemotherapies, the overall survival of patients receiving chemotherapy using PEM is increased for patients with advanced non-sq NSCLC [7,8]. Myelosuppression, which has symptoms such as leucopenia, neutropenia, anemia, and thrombocytopenia, is a common toxicity of PEM. In addition, the slight toxicities due to PEM are generally tolerable [9,10]. However, the efficacy and toxicity of PEM vary greatly for patients receiving different doses of the drug.

The great variation in efficacy and toxicity in treating non-sq NSCLC might be partly due to genetic polymorphisms [11,12]. Methylenetetrahydrofolate reductase (MTHFR) is the rate-limiting enzyme in folate metabolism and is the target of PEM, reducing 5,10-methyleneterahydrofolate to 5-methyleneterahydrofolate. As a carrier of one-carbon units within the cells, MTHFR is involved in synthesizing purine and pyrimidine, which act against chemotherapies such as PEM [13–15]. The MTHFR gene is located in the human chromosome 1p36.3, and harbors 11 exons and 10 introns, encoding known polymorphisms such as C677T, A1298C, and T1059C [16–18]. The C677T polymorphism is the most frequent missense mutation, with genotypes of wild-type CC, homozygous TT, and heterozygous CT [16]. CC is the wild-type genotype, TT (bi-allelic polymorphism) is the homozygous genotype, and CT (mono-allelic polymorphism) is the heterozygous genotype. The homozygous mutations for the *MTHFR C677T* are associated with significantly increased progression-free survival (PFS) when compared with patients with wild-type or heterozygous mutations [19]. A recent study showed that *MTHFR C677T* polymorphisms had no correlation with the treatment efficacy of PEM, and a correlation between *MTHFR C677T* polymorphisms and toxicity of PEM has been reported [20]. However, few studies reported that *MTHFR C677T* polymorphisms is correlation with the toxicity of PEM in non-sq NSCLC.

Thus, this study aimed to evaluate the efficacy and toxicity of PEM for non-sq NSCLC, and to analyze the correlation between the efficacy or toxicity of PEM and *MTHFR C677T* polymorphisms, which might be helpful in identifying a reliable biomarker to predict the effectiveness and safety of PEM for non-sq NSCLC.

Material and Methods

Patients

Patients had been diagnosed cytologically or histologically as having non-sq NSCLC. Their organ functions were assessed according to Eastern Cooperative Oncology Group performance status (ECOG PS), including adequate bone marrow, liver, and kidney function. They also had received more than 1 cycle of chemotherapy regimens for advanced non-sq NSCLC at Fuzhou General Hospital of Nanjing Military Command from January 2012 to December 2014. Out of the total of 55 patients in the study, 4 were lost during follow-up.

Treatments

The monotherapy group included 40 patients who received 500 mg/m^2 of PEM by intravenous (i.v.) administration per day. The joint group included 11 patients who received 500 mg/m^2 PEM by i.v. administration combined with 75 mg/m^2 of cisplatin per day. Three weeks were regarded as a cycle. All the patients were given folic acid and vitamin B12 to alleviate the toxicity. Dose adjustment and cycle delay of 21 days or less were permitted to resolve the toxicity effects. All patients signed the informed consent and the study was approved by the Ethics Committee of Fuzhou General Hospital of Nanjing Military Command.

Assessment of treatment response and toxicity

The tumor responses were assessed by computed tomography (CT) or magnetic resonance imaging (MRI) every 2 cycles. The clinical response was assessed as complete response (CR), partial response (PR), stable disease (SD), or progressive disease (PD), according to the guidelines of Response Evaluation Criteria in Solid Tumor (RECIST). The toxicities, including myelosuppression and gastrointestinal reactions, were assessed according to the National Cancer Institute Common Toxicity Criteria (NCI-CTC).

Genetic analysis

Genomic DNA was isolated from the peripheral blood (5 mL) using the QIAmp DNA extraction kit (QIAGEN, Hilden, Germany) [21]. DNA amplification was performed by using a 7500 real-time quantitative PCR device (Applied Biosystems, Foster City, CA, USA). The primers used for amplification and sequencing of *MTHFR C677T* were: sense: 5'-AAGGAGGAGCTGCTGAAGATG-3';

Table 1. Clinicopathologic characteristics of 51 non-sq NSCLC patients.

Characteristics	No. of patients (n, %)	
Age (years old)		
≤65	36	(70.60)
>65	15	(29.40)
Gender		
Male	34	(66.70)
Female	17	(33.30)
Stage		
IIIB	5	(9.80)
IV	46	(90.20)
Histology		
Adenocarcinoma	47	(92.20)
Large cell carcinoma	4	(7.80)
ECOG performance status		
0	16	(31.30)
1	35	(68.70)
Smoking history		
Yes	32	(62.80)
No	19	(37.20)
Treatment line/cycle		
PEM (i.v. 500 mg/m^2)	40	(78.40)
PEM+CDDP (i.v. 500 mg/m^2 PEM +75 mg/m^2 of CDDP)	11	(21.60)

ECOG – Eastern Cooperative Oncology Group;
PEM – pemetrexed; CDDP – cisplatin.

Table 2. Number of cases with different MTHFR genotypes.

Gene	Gene variants	No. of patients (%)	
MTHFR C677T	CC	21	(41.20)
	CT	20	(39.20)
	TT	10	(19.60)

MTHFR – methylenetetrahydrofolate reductase.

Table 3. Treatment response.

Tumor response	No. of patients (%)	
Complete response (CR)	0	(0.00)
Partial response (PR)	10	(19.60)
Stable disease (SD)	25	(49.00)
Progressive disease (PD)	16	(31.40)
Overall response rate (ORR)	10	(19.60)
Disease control rate (DCR)	35	(68.60)

ORR – CR+PR; DCR – PR+SD.

assessed by Kaplan-Meier test. A P value less than 0.05 was considered to be statistically significant.

Results

Patient characteristics

Four of 55 patients were lost during follow-up. Patients were diagnosed cytologically or histologically as having non-sq NSCLC. The clinicopathological characteristics of the 51 patients included in the study are listed in Table 1. The median age of patients was 57 years old, and 36 patients were less than 65 years old. Among them, 34 patients (66.7%) were males. Forty-six patients (90.2%) had stage IV disease and 47 patients (92.2%) were diagnosed with adenocarcinoma. Thirty-five patients (68.7%) had ECOG performance status of 1, and 32 patients (62.8%) had a smoking history. Forty (78.4%) patients received single-agent PEM (i.v. 500 mg/m^2) and 11 (21.6%) received joint chemotherapy using PEM+CDDP (i.v. 500 mg/m^2 PEM +75 mg/m^2 of cisplatin).

MTHFR C677T genotypes distribution among non-sq NSCLC patients

In 51 non-sq NSCLC patients, 21 (41.2%) were carrying the CC wild genotype, 20 (39.2%) were carrying the CT heterozygous genotype, and 10 (19.6%) were carrying the TT variability homozygous (Table 2). Moreover, *MTHFR C677T* polymorphisms were not in Hardy-Weinberg equilibrium.

antisense: 5'-CTTTGCCATGTCCACAGCATG-3'. The volume of total PCR reaction system was 5 μL, including 0.25 μL of primers and probes, 2.5 μL of PCR reaction solution, and 5 ng of DNA. The PCR reaction conditions were: pre-change at 95°C for 10 min; 40 cycles of denaturation at 92°C for 15 s, and extension at 60°C for 1 min.

Statistical analyses

Data were analyzed using SPSS version 16.0 (SPSS, Inc., Chicago, IL, USA). The correlations between genotypes and clinical response were analyzed by the χ^2 test or Fisher's exact test. The correlations between genotypes and chemotherapy-related toxicity were analyzed by Fisher's exact test or rank sum test. The analysis of correlation between genotype and PFS was

Table 4. Correlation of genotypes with response to PEM therapy.

	All patients N (%)	Single agent PEM		PEM combination with CDDP	
		CC	CT+TT	CC	CT+TT
Number (n,%)	51 (100.00)	16 (31.30%)	24 (47.10%)	5 (9.90%)	6 (11.70%)
ORR (n,%)	10 (19.60)	4 (7.80%)	3 (5.90%)	2 (3.90%)	1 (2.00%)
P		0.407		0.545	
DCR (n,%)	35 (68.6)	11 (21.60%)	15 (29.40%)	4 (7.80%)	5 (9.80%)
P		0.685		1	

PEM – pemetrexed; CDDP – cisplatin; ORR – overall response rate; DCR – disease control rate.
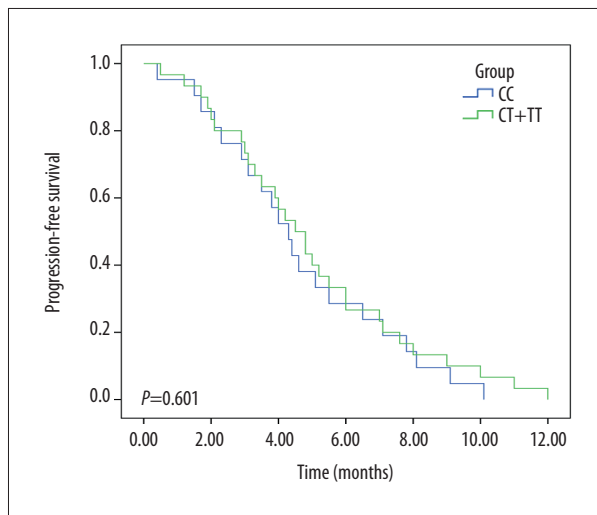
Figure 1. The correlation of MTHFR C667T polymorphisms with progression-free survival (PFS) in non-sq NSCLC patients treated with pemetrexed (PEM).

Correlation of *MTHFR C677T* genotypes with therapeutic response to PEM therapy

Ten of the patients had PR, and 25 had SD. However, in 16 patients the disease progressed, resulting in an ORR (overall response rate, =CR+PR) of 19.6% and a DCR (disease control rate, =PR+SD) of 68.6% (Table 3).

MTHFR C677T polymorphisms were not correlated with the therapeutic response in either group ($P>0.05$) (Table 4). The PFS of patients with homozygous mutation or heterozygous mutation for the *MTHFR C677T* was 4.5 months longer than in those with the wild type, but without significant differences ($P> 0.05$) (Figure 1).

General toxicity occurrence of PEM therapy among the non-sq NSCLC patients

In general, the frequency of PEM-related hematologic toxicity was much higher than that of non-hematologic toxicity

among the 51 patients (Table 5). The percentage of severe (grade 3 or 4) non-hematologic toxicity, except for liver toxicity, was generally less than 5%. For hematologic toxicity, the rates of severe hematologic toxicity were 9.8% with leukopenia, 13.7% with neutropenia, 7.8% with anemia, and 5.9% with thrombocytopenia.

Correlation of *MTHFR C677T* genotypes with toxicity of PEM therapy

In the single-agent PEM group, *MTHFR C677T* genotypes were significantly correlated with leukopenia, neutropenia, nausea, and fatigue ($P<0.05$). However, *MTHFR C677T* genotypes had no correlation with any toxicity in the joint group, including leucopenia, neutropenia, anemia, thrombocytopenia, liver toxicity, nausea, vomiting, and fatigue ($P>0.05$) (Table 6).

Discussion

PEM is a multi-targeted anti-folate drug that interrupts folate-dependent enzymes such as thymidylate synthase (TS), dihydrofolate reductase (DHFR), and glycinamide ribonucleotide formyl transferase (GARFT). The enzymes involved in purine and thymidine biosynthesis influence DNA synthesis of tumor cells and inhibit cell proliferation [22,23]. Some studies have shown that TS polymorphisms influences the outcomes of NSCLC patients [24,25]. MTHFR, targeted by PEM, is the rate-limiting enzyme in folate metabolism, and it acts as a carrier of one-carbon to regulate DNA synthesis and methylation [26]. The most common gene mutation of MTHFR, the C677T mutation (alanine → valine), influences the sensitivity and toxicity of PEM treatment [19,27].

Our study results showed that *MTHFR C677T* was not correlated with the treatment efficacy in either the monotherapy group or the joint group, and PFS was not significantly associated with *MTHFR C677T*, suggesting that *MTHFR C677T* might not be useful for predicting clinical outcomes of PEM treatment. This finding is slightly different from results of some

Table 5. Chemotherapy toxicity occurrence.

Toxicity	Grade (n)				Percentage of grade 3/4 (%)
	1	2	3	4	
Hematologic					
Leukopenia	5	7	3	2	9.8
Neutropenia	4	6	4	3	13.7
Anemia	6	2	2	2	7.8
Thrombocytopenia	6	5	2	1	5.9
Non-hematologic					
Liver toxicity	4	2	2	1	5.9
Infection	0	0	1	0	2
Nausea	12	6	2	0	3.9
Vomiting	4	3	1	0	2
Constipation	2	0	0	0	<0.1
Fatigue	16	4	1	1	3.9
Allergic reaction	3	0	0	0	<0.1
Alopecia	3	0	0	0	<0.1

Grade statement: the higher the grade, the more severe the toxicity. Patients graded as 3 or 4 is considered severe.

previous studies that reported a significant correlation between the *MTHFR C677T* and clinical outcomes [19,27]. However, our data are consistent with a recent report [20] that assessed the correlation between *MTHFR C677T* and chemotherapy efficacy or toxicity among Japanese non-sq NSCLC patients. There are 2 reasons for these different results. Firstly, the distribution of MTHFR differs by ethnicity [28]. For instance, the frequency of C677T allele was reported as 22~44% among Europeans, 7% among Sub-Saharan Africans, and up to 40% among Chinese [18]. Secondly, multiple key enzymes, including TS, DHFR, and GARFT, are involved in the metabolism of PEM. It is possible that the polymorphisms of the enzymes, together with those of MTHFR, influence the efficacy of PEM, but the specific mechanisms remain unknown.

In terms of toxicity, there were some significant correlations between *MTHFR C677T* and toxicity in the monotherapy group, including leucopenia, neutropenia, nausea, and fatigue. The results suggest that *MTHFR C677T* might be useful for predicting some hematologic symptoms in response to PEM. However, another study [29] found no significant correlations between *MTHFR C677T* and toxicity. To the best of our knowledge, the present study is the first to report a correlation between MTHFR polymorphisms and toxicity in PEM treatment for non-sq NSCLC patients. However, consistent with a previous report [20], we found no correlation between *MTHFR C677T* polymorphisms and any toxicities in the joint group, including leucopenia, neutropenia, anemia, thrombocytopenia, liver

toxicity, nausea, vomiting, and fatigue. We hypothesize that the results were influenced by CDDP, and we intend to conduct a study with a larger sample size to verify this assumption in the future.

The efficacy and toxicity of chemotherapy could vary with ethnicity and genetic makeup, so genetic analysis could help determine which patients are suitable for chemotherapy and to adjust the chemotherapy dose, thus improving the efficacy and controlling the toxicity of chemotherapy. Moreover, analyzing the genotypes of MTHFR could determine the optimal selection medium for individualized PEM treatment of non-sq NSCLC. However, we failed to find a consistent correlation between *MTHFR C677T* and clinical outcomes or toxicity to PEM.

Conclusions

In conclusion, compared with wild-type genotype CC, patients with CT/TT genotypes had higher incidence of leukopenia, neutropenia, nausea, and fatigue among the non-sq NSCLC patients. Therefore, *MTHFR C677T* polymorphism could be a predictive factor for leukopenia, neutropenia, nausea, and fatigue in non-sq NSCLC patients receiving single-agent PEM treatment.

Cnoflict of interests

None.

Table 6. Correlation of genotype of non-sq NSCLC patients and toxicity of PEM.

	Grade	Patients	Single agent PEM		PEM combination with CDDP	
			CC	CT+TT	CC	CT+TT
Number(n)		51	16	24	5	6
Leukopenia	1	5	1	3	0	1
	2	7	1	4	1	1
	3	3	0	2	0	1
	4	2	0	1	1	0
	P		0.042		1	
Neutropenia	1	4	0	2	1	1
	2	6	0	5	1	0
	3	4	1	2	0	1
	4	3	0	1	1	1
	P		0.022		0.847	
Anemia	1	6	1	3	1	1
	2	2	0	1	1	0
	3	2	0	1	0	1
	4	2	0	1	1	0
	P		0.118		0.369	
Thrombocytopenia	1	6	1	2	2	1
	2	5	0	2	1	2
	3	2	1	0	0	1
	4	1	0	1	0	1
	P		0.505		0.163	
Liver toxicity	1	4	1	0	2	1
	2	2	0	1	0	1
	3	2	1	0	0	1
	4	1	0	1	0	0
	P		0.711		0.481	
Vomiting	1	4	1	3	0	0
	2	3	1	0	0	2
	3	1	0	1	0	0
	4	0	0	0	0	0
	P		0.739		0.429	
Nausea	1	12	2	7	2	1
	2	6	1	4	0	1
	3	2	0	1	1	0
	4	0	0	0	0	0
	P		0.047		0.421	
Fatigue	1	16	2	12	1	1
	2	4	1	1	0	2
	3	1	0	0	0	1
	4	1	0	1	0	0
	P		0.029		0.59	

Non-sq NSCLC – non-squamous non-small cell lung cancer; PEM – pemetrexed; CDDP – cisplatin.

References:

1. Jemal A, Siegel R, Ward E et al: Cancer statistics, 2008. Cancer J Clin, 2008; 58: 71–96

2. Dela Cruz CS, Tanoue LT, Matthay RA: Lung cancer: Epidemiology, etiology, and prevention. Clin Chest Med, 2011; 32: 605–44

3. Jemal A, Bray F, Center MM et al: Global cancer statistics. Cancer J Clin, 2011; 61: 69–90

4. Zhu X, Jiang H, Li J et al: Anticancer effects of paris saponins by apoptosis and PI3K/AKT pathway in gefitinib-resistant non-small cell lung cancer. Med Sci Monit, 2016; 22: 1435–41

5. Wang S, Liu F, Zhu J et al: DNA repair genes ERCC1 and BRCA1 expression in non-small cell lung cancer chemotherapy drug resistance. Med Sci Monit, 2016; 22: 1999–2005

6. Zhang W, Sun J, Luo J: High expression of Rab-like 3 (Rabl3) is associated with poor survival of patients with non-small cell lung cancer via repression of MAPK8/9/10-mediated autophagy. Med Sci Monit, 2016; 22: 1582–88

7. Treat J, Scagliotti GV, Peng G et al: Comparison of pemetrexed plus cisplatin with other first-line doublets in advanced non-small cell lung cancer (NSCLC): A combined analysis of three phase 3 trials. Lung Cancer, 2012; 76: 222–27

8. Al-Saleh K, Quinton C, Ellis PM: Role of pemetrexed in advanced non-small-cell lung cancer: Meta-analysis of randomized controlled trials, with histology subgroup analysis. Curr Oncol, 2012; 19: e9–e15

9. Scagliotti G, Hanna N, Fossella F et al: The differential efficacy of pemetrexed according to NSCLC histology: A review of two Phase III studies. Oncologist, 2009; 14: 253–63

10. Scagliotti GV, Parikh P, von Pawel J et al: Phase III study comparing cisplatin plus gemcitabine with cisplatin plus pemetrexed in chemotherapy-naive patients with advanced-stage non-small-cell lung cancer. J Clin Oncol, 2008; 26: 3543–51

11. Kao SC, Phan VH, Clarke SJ: Predictive markers for haematological toxicity of pemetrexed. Curr Drug Targets, 2010; 11: 48–57

12. Sakamori Y, Kim YH, Yoshida H et al: Effect of liver toxicity on clinical outcome of patients with non-small-cell lung cancer treated with pemetrexed. Mol Clin Oncol, 2015; 3: 334–40

13. Choi SW, Mason JB: Folate and carcinogenesis: An integrated scheme. J Nutr, 2000; 130: 129–32

14. Tiseo M, Giovannetti E, Tibaldi C et al: Pharmacogenetic study of patients with advanced non-small cell lung cancer (NSCLC) treated with second-line pemetrexed or pemetrexed-carboplatin. Lung Cancer, 2012; 78: 92–99

15. Sohn KJ, Croxford R, Yates Z et al: Effect of the methylenetetrahydrofolate reductase C677T polymorphism on chemosensitivity of colon and breast cancer cells to 5-fluorouracil and methotrexate. J Natl Cancer Inst, 2004; 96: 134–44

16. Frosst P, Blom HJ, Milos R et al: A candidate genetic risk factor for vascular disease: A common mutation in methylenetetrahydrofolate reductase. Nat Genet, 1995; 10: 111–13

17. Viel A, Dall'Agnese L, Simone F et al: Loss of heterozygosity at the 5,10-methylenetetrahydrofolate reductase locus in human ovarian carcinomas. Br J Cancer, 1997; 75: 1105–10

18. Botto LD, Yang Q: 5,10-Methylenetetrahydrofolate reductase gene variants and congenital anomalies: A HuGE review. Am J Epidemiol, 2000; 151: 862–77

19. Smit EF, Burgers SA, Biesma B et al: Randomized phase II and pharmacogenetic study of pemetrexed compared with pemetrexed plus carboplatin in pretreated patients with advanced non-small-cell lung cancer. J Clin Oncol, 2009; 27: 2038–45

20. Kanazawa K, Yokouchi H, Wang X et al: Phase II trial of carboplatin and pemetrexed as first-line chemotherapy for non-squamous non-small cell lung cancer, and correlation between the efficacy/toxicity and genetic polymorphisms associated with pemetrexed metabolism: Hokkaido Lung Cancer Clinical Study Group Trial (HOT) 0902. Cancer Chemother Pharmacol, 2014; 74: 1149–57

21. Boom R, Sol CJ, Salimans MM et al: Rapid and simple method for purification of nucleic acids. J Clin Microbiol, 1990; 28: 495–503

22. Adjei AA: Pemetrexed (ALIMTA), a novel multitargeted antineoplastic agent. Clin Cancer Res, 2004; 10: 4276s–80s

23. Schultz RM, Patel VF, Worzalla JF, Shih C: Role of thymidylate synthase in the antitumor activity of the multitargeted antifolate, LY231514. Anticancer Res, 1999; 19: 437–43

24. Kaira K, Ohde Y, Nakagawa K et al: Thymidylate synthase expression is closely associated with outcome in patients with pulmonary adenocarcinoma. Med Oncol, 2012; 29: 1663–72

25. Hashimoto H, Ozeki Y, Sato M et al: Significance of thymidylate synthase gene expression level in patients with adenocarcinoma of the lung. Cancer, 2006; 106: 1595–601

26. Lee MS, Asomaning K, Su L, Wain JC et al: MTHFR polymorphisms, folate intake and carcinogen DNA adducts in the lung. Int J Cancer, 2012; 131: 1203–9

27. Krawczyk P, Kucharczyk T, Kowalski DM et al: Polymorphisms in TS, MTHFR and ERCC1 genes as predictive markers in first-line platinum and pemetrexed therapy in NSCLC patients. J Cancer Res Clin Oncol, 2014; 140: 2047–57

28. Martin DN, Boersma BJ, Howe TM et al: Association of MTHFR gene polymorphisms with breast cancer survival. BMC Cancer, 2006; 6: 257

29. Jung M, Lee CH, Park HS et al: Pharmacogenomic assessment of outcomes of pemetrexed-treated patients with adenocarcinoma of the lung. Yonsei Med J, 2013; 54: 854–64