Original Article
Middle East Journal of Cancer; January 2023; 14(1): 73-80

SLC19A1 and Thymidylate Synthase Polymorphisms Do not Predict Survival Outcome in Non-Small-Cell Lung Cancer Treated with Pemetrexed-Cisplatin Chemotherapy Regimen

Mohammad Hadi Abbasian*, MSc, Nafiseh Ansarinejad,***, MD, Tayeb Ramim****, MD, Farshid Fardad**,***, MD, Bahareh Abbasi*,***♦, MD**

**Department of Medical Genetics, National Institute for Genetic Engineering and Biotechnology, Tehran, Iran*
***Department of Hematology and Oncology, Hazrat Rasool-e Akram Hospital, Iran University of Medical Sciences, Tehran, Iran*
****Cancer Pharmacogenetics Research Group (CPGRG), Iran University of Medical Sciences, Tehran, Iran*
*****Department of Health Information Management, School of Health Management and Information Sciences, Iran University of Medical Sciences, Tehran, Iran*

Please cite this article as: Abbasian MH, Ansarinejad N, Ramim T, Fardad F, Abbasi B. SLC19A1 and thymidylate synthase polymorphisms do not predict survival outcome in non-small-cell lung cancer treated with pemetrexed-cisplatin chemotherapy regimen. Middle East J Cancer. 2023;14(1):73-80. doi: 10.30476/mejc.2022.89424.1600.

Abstract

Background: Lung cancer is the leading cause of cancer deaths worldwide. Pharmacogenomics plays an important role in tailoring cancer patients' treatment. Pemetrexed is widely used in first- and second-line chemotherapy of non-small cell lung cancer (NSCLC); however, there is no available predictive biomarker for pemetrexed treatment. The present study aimed to investigate the role of polymorphisms in thymidylate synthase and SLC19A1 polymorphisms with clinical outcome in patients with advanced NSCLC treated in first-line with pemetrexed or pemetrexed plus cisplatin.

Method: This cohort study included 40 metastatic lung cancer patients treated with pemetrexed plus cisplatin. We utilized the tetra-primer amplification refractory mutation system-polymerase chain (ARMS-PCR) reaction for genotyping of rs3788189 and rs1051298. TYMS 28-VNTR and rs16430 were genotyped in the patients via PCR amplification and PCR-RFLP, respectively. Fisher's exact test and Kaplan-Meier curve were used for statistical analysis.

Results: We recruited 40 patients in this research with a median age of 58.9 years. The median survival of all the 40 patients was 11.6 months. The overall survival of the patients, as well as their gender, age, and metastatic sites were not found to be statistically associated with rs1051298, rs3788189, TYMS VNTR, and rs16430.

Conclusion: Our study did not identify any associations between the SLC19A1 and TYMS VNTR and rs16430 and clinical outcomes in advanced NSCLC patients. However, further investigation will be conducive to finding effective clinical biomarkers for the treatment of patients with NSCLC.

♦Corresponding Author:
Bahareh Abbasi, MD
Department of Medical Genetics, National Institute for Genetic Engineering and Biotechnology, Tehran, Iran
Fax: +9821 44570799
Email: b.abbasi@nigeb.ac.ir

Keywords: Non-small cell lung cancer, Thymidylate synthase, SLC19A, Pemetrexed, Polymorphism

Received: May 01, 2021; Accepted: April 04, 2022

Introduction

Lung cancer remains the most frequently diagnosed cancer and is the leading cancer-related death worldwide with 1.8 million deaths in 2018.[1] Non-small cell lung cancer (NSCLC) and small cell lung cancer (SCLC) are the two main types of this malignancy. NSCLC accounts for approximately 85% of all lung cancer cases. Chemo- and radiotherapy are the backbones of lung cancer treatment. Nonetheless, tumor progression have been observed in the majority of patients. As a result, the five-year survival of lung cancer patients remains 15% and just <7% of lung cancer patients are alive 10 years following diagnosis.[2, 3]

Pemetrexed is a folate antimetabolite with anticancer activity. It is clinically useful for the treatment of first- and second-line or maintenance therapy in advanced NSCLC.[4] Pemetrexed plus platinum-based agents have been recommended for advanced lung cancer and Mesothelioma.[5] Inhibition of three key enzymes, namely thymidylate synthase (TS), glycinamide ribonucleotide formyltransferase (GARFT), and dihydrofolate reductase (DHFR), results in inhibition of DNA and RNA synthesis. Studies have shown that response to pemetrexed therapy depends on expression levels of some genes, including TS, 5,10- methylenetetrahydrofolate reductase (MTHFR), and Solute Carrier Family 19, member 1 (SLC19A1 or RFC1).[6] Accordingly, it is crucial to identify pharmacogenomics markers for predicting responders and non-responders to pemetrexed.

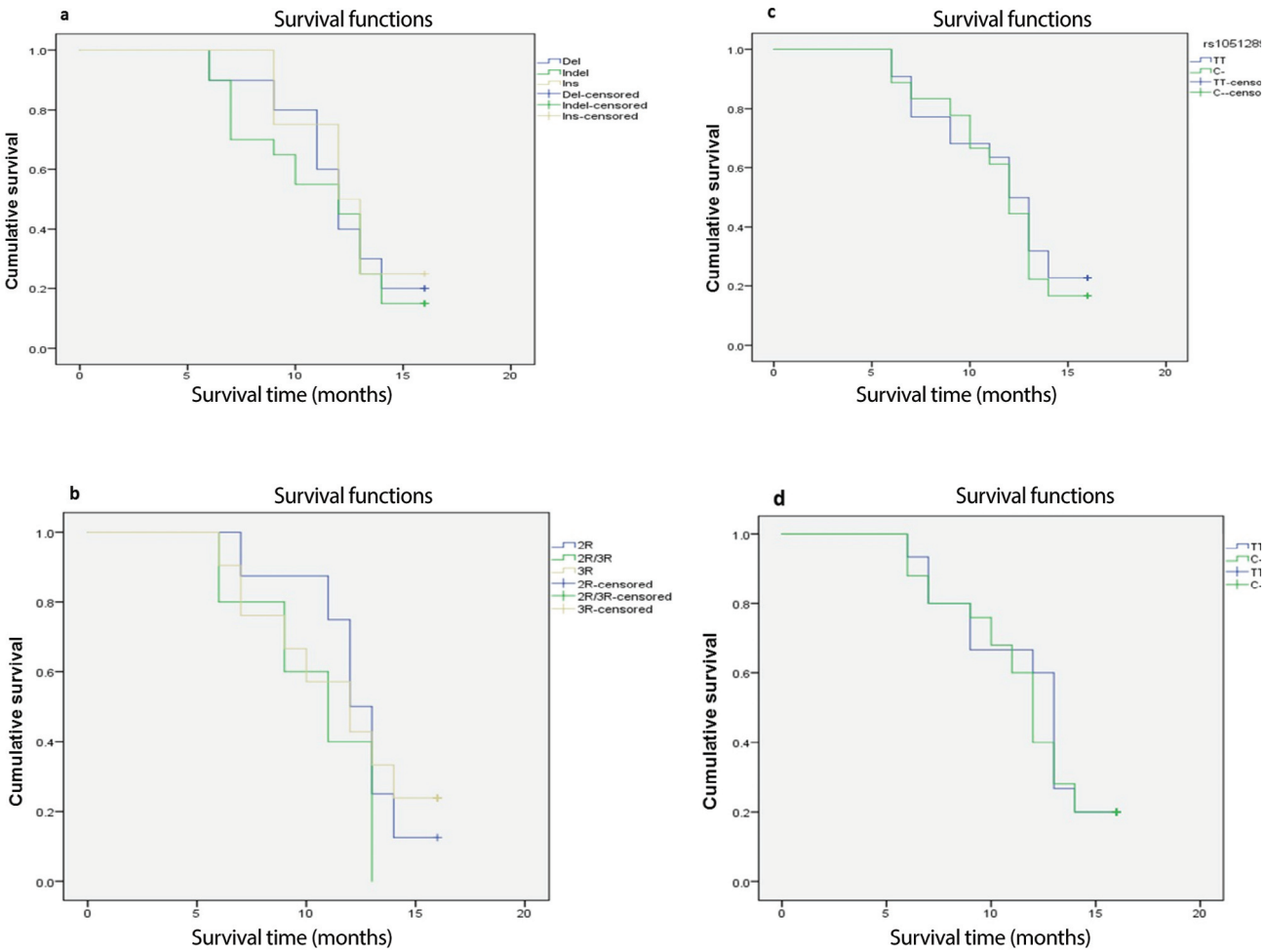

Figure 1. Kaplan-Meier OS curves according to genotyping. A) TYMS VNTR; B) rs16430; C) rs1051289; (12 vs. 12 months, $P = 0.67$) D) rs3788189 (13 vs. 12 months, $P = 0.72$).
OS: Overall survival

TS is an important target for several drugs, including 5-fluorouracil (5FU), methotrexate, pemetrexed, and raltitrexed (Tomudex).[7-9] Dysregulation of TYMS is linked to resistance against chemotherapy in several cancers and has a predictive value for the efficacy of TYMS targeted chemotherapy. The expression of TS mRNA level is regulated by a variable number of 28-base pair (bp) tandem repeats (VNTR) in the 5′regulatory region that consists of a double (2R) or triple (3R) repeat of a 28-base-pair (bp) tandem repeats.[10] Another polymorphism that affects TS mRNA level and TS protein expression is a 6-bp insertion at nucleotide 1494 in the 3′UTR. (rs16430).[11]

SLC19A1 encodes the reduced folate carrier protein (RFC1) which is the bi-directional transporter of 5-methyltetrahydrofolate in a cell membrane and plays a central role in folate concentration in cells. Previous studies have reported several polymorphisms in the SLC19A1 gene, which changed the transporter structure and function. A number of papers suggested that polymorphisms in the SLC19A1 are associated with idiopathic recurrent pregnancy loss and neural tube defects and MTX therapeutic outcome.[12-14] SLC19A1 rs3788189 G>T polymorphism, located in the intronic region of SLC19A1 was associated with overall breast cancer risk in African-American women, lung cancer disease-free survival (DFS), and overall survival (OS).[15, 16] Another polymorphism, rs1051298 (IVS2 (4935) G /A) correlated significantly with leukopenia.[17] The current work aimed to evaluate the effect of rs3788189 and rs1051298, TYMS 28-VNTR, and rs16430 on the efficacy of pemetrexed therapy in patients with advanced non-small-cell lung cancer.

Materials and Methods

In this cohort study, we recruited 40 patients who had been diagnosed with NSCLC from June 2018 to December 2019 in Hazrat Rasool-e Akram Hospital, Tehran, Iran. The Ethics Committee of the National Institute of Genetic Engineering and Biotechnology (NIGEB) approved this study (IR.NIGEB.EC1398.6.24C). All the patients provided written informed consent. The eligible subjects had no previous chemotherapy treatment. Their studied characteristics were age, gender, smoking history, histological type. The inclusion criteria were being diagnosed with advanced NSCLC that was treated with pemetrexed and cisplatin. The patients received 500 mg/m^2 of pemetrexed plus 75 mg/ m^2 cisplatin every three weeks.

Genotyping

Before the patients underwent the first chemotherapy cycle, 3mL of peripheral blood was collected into the EDTA tubes. Genomic DNA was isolated from the peripheral blood using the General extraction kit (South Korea). The TYMS VNTR polymorphism analysis was carried out in the same reaction mixture of 22 µl. The mixture contained: 1 µl of sample DNA (50 ng/ µl), 0.5 µM of each primer, 10 µl Taq DNA Polymerase 2x Master Mix RED (Amplicon, Denmark), and 10 µl water. Polymerase chain reaction (PCR)-restriction fragment length polymorphism (RFLP) analysis of rs16430 was performed with DraI fast digest enzymes (Thermo Fisher Scientific, the USA), as described previously.[18] The SLC19A1 rs3788189 and rs1051298 polymorphisms were selected owing to their association with pemetrexed therapeutic outcomes. We used tetra-primers ARMS-PCR for genotyping. The volume of the total PCR reaction system was 15 µl, including 0.5 µl of primers, 6 µl of DNA Polymerase Master Mix RED (Amplicon, Denmark), 6 µl dH2O, and 1 µl of DNA. The fragments were visualized in a 2% agarose gel. Table 1 represents the primers used for amplification.

Statistical analysis

The obtained data were analyzed via SPSS software version 24 (SPSS, Inc., Chicago, IL, USA). The correlations between each genotype and other categorical variables were compared using the x^2 test or Fisher's exact test. A *P*-value of less than 0.05 was considered to be statistically significant. Hardy-Weinberg equilibrium of genotype frequencies was estimated with chi-squared test. We measured OS from the starting date of pemetrexed therapy to the date of death

Table1. Primer sequence and PCR product length

SNP in dbSNP	Amino acid change	Types of variants	Gene	Primers	PCR product length
rs16430	6-bp deletion	deletion/insertion (indel)	TYMS	F caaarcrgagggagctgagtaaca R aaagcgtggacgaatgcaga	Del/del : 123bp Ins/ins : 60,63 Ins/del : 123,63,60
rs45445694	28-bp repeat	duplication	TYMS	F ctaagactctcagctgtggccctg R ccacaggcatggcgcggc	2R: 276 3R:304
rs3788189	G>A	substitution	SLC19A	F outer agagcagggtgaggagagccacagc R outer agcctcctgaaccctctgcacctcc G allele atgaccagaaggagcagcaccagtgg T allele gctgcgagaatcaggagagatgtggctaa	267bp 141bp 180bp
rs1051298	G>A	substitution	SLC19A	F outer gcatgtggcttctctgtctctgtggaaaag R outer gagtattcacatcacatcagatggtcccgc C allele gcacataccaaggccagcacgtacg T allele gaggggactggtcccggtcacagt	396bp 254bp 190bp

PCR: Polymerase chain reaction; SNP: Single nucleotide polymorphism; bp: Base pair; TYMS: Thymidylate synthetase; SLCA19A: Solute carrier family 6 member 19; F: Forward; R: Reverse; Del: Deletion; Ins: Insertion

or final follow-up. The survival data were assessed using a Kaplan-Meier curve.

Results

Patient characteristics and allele frequencies

Between June 2018 and December 2019, a total of 40 patients (25 males and 15 females) were enrolled in this study. The median age of the participants was 58.9 years (ranging from 40-77). Table 2 demonstrates the clinicopathological characteristics of the patients. Our subjects were treated with pemetrexed 500 mg/m^2 and cisplatin 75 mg/m^2 on day 1 of the 21-day cycle for four cycles. The data are available for 34 patients.

Genetic polymorphisms and survival

In this study, we explored the effect of several polymorphisms on clinicopathological characteristics, such as age, gender, smoking, and metastatic to different organs were not associated with a different type of polymorphisms. The chi-square test suggested that rs16430, TYMS VNTR, and rs3788189 were not in Hardy-Weinberg equilibrium (P-value > 0.05). The median survival of all the 40 patients was 11.6 months. No significant differences were observed concerning survival among the patients of different genders, ages, and metastatic sites. The OS time of the patients with rs1051298 TT alleles was not longer than that in the cases with TC and CC alleles (12 vs. 12 months, P = 0.67). No statistically significant association was observed between rs3788189 and OS (13 vs. 12 months, P = 0.72). Furthermore, different genotypes of TYMS 28-VNTR and rs16430 were not associated with the survival of the patients (Figure 1).

Discussion

To the best of our knowledge, this is the first report evaluating the role of TYMS and SLC19A1 gene polymorphisms and pemetrexed-based chemotherapy in Iranian advanced NSCLC patients. The results of this study revealed no correlations between TYMS 28-VNTR, rs16430, rs1051298, and rs3788189 and chemotherapy response in patients.

Pemetrexed is a methotrexate derivative that targets TS, DHFR, and GARFT, which are involved in pyrimidine and purine metabolism. Pemetrexed is transported into the cell via reduced folate carrier RFC which is encoded by the SLC19A1 gene and was approved in 2004 by the U.S. Food and Drug Administration (FDA) for treatment of non-resectable pleural mesotheliomas. Cisplatin/pemetrexed is also prescribed for the treatment of locally advanced NSCLC.[19] Previous studies have shown that genetic variations are responsible for drug response. Pharmacogenomics biomarker will improve the chemotherapy efficacy in cancer patients. In our previous study, MTHFR rs1801133 polymorphisms were not associated with survival in NSCLC patients treated with

Table 2. Distribution of TYMS and SLCA19 polymorphisms genotypes

dbSNP ID	Genotypes	No. of patients	Percentage (%)
rs16430			
Del		13	32.5
Indel		21	52.5
Ins		6	15.0
TYMS VNTR			
2R/2R		6	15
2R/3R		10	25
3R/3R		24	60
rs3788189			
TT		25	62.5
GG		5	12.5
TG		10	25.0
rs1051298			
CC		12	30.0
TC		6	15.0
TT		22	55.0

Del: Deletion; Ins: Insertion; TYMS: Thymidylate synthetase; VNTR: Variable number tandem repeats (VNTRs); No.: Number

pemetrexed-based chemotherapy.[20] Polymorphism in SLC19A1 is associated with adverse drug reaction occurrences and decreased survival of lung cancer patients. Pemetrexed plus cisplatin has been used to treat malignant pleural mesothelioma and NSCLC patients. Pemetrexed has been regarded as the first- and second-line treatment for advanced NSCLC patients. In our previous research, we also indicated that TS polymorphisms could be considered as a pharmacogenomics biomarker for colorectal cancer patients treated with fluoropyrimidine-based cancer chemotherapy.[21] Therefore, it is crucial to identify biomarkers in cancer patients who are most likely to benefit from pemetrexed treatment. The main objective of the present study was to investigate the association of four polymorphisms in the TYMS and SLC19A1 gene with survival in advanced NSCLC patients treated with pemetrexed-based chemotherapy.

Herein, TYMS 28-VNTR and rs16430 genotypes were not associated with the survival of NSCLC patients. In previous studies, TS activity was a predictor for sensitivity to 5FU and 5FU/LV in colon cancer cell lines.[22] Our previous study showed that TS polymorphisms were associated with an increased risk of grade III neurotoxicity and anemia in colorectal cancer patients treated with fluoropyrimidine-based cancer chemotherapy.[18] TS induction resulted in resistance against 5-FU in lung cancer. For the first time, Otake et al. showed that TS was overexpressed in NSCLC. They also proposed that TS may be involved in NSCLC tumorigenesis.[23] The upregulation of TS leads to resistance against pemetrexed in breast and colon cancer.[24, 25] These studies have suggested that TS activity is associated with resistance to pemetrexed and can be used as a biomarker for the prediction of tumor response to pemetrexed. On the other hand, TS expression could not be predicted through pemetrexed sensitivity in gastric cancer.[26] Gomez et al. studied 61 breast cancer patients treated with pemetrexed. According to their results, "low" pretreatment TS expression was associated with response to pemetrexed chemotherapy.[27] Nakagawa et al. showed that higher TS expression in tumor cells is associated with higher proliferative activity in NSCLC, especially in lung adenocarcinoma cells.[28] Wang et al. reported that progression-free survival (PFS) and OS were better in NSCLC patients with the (-6 bp/-6 bp) than those in patients with (-6 bp/+6 bp).[29] In their research, 60 histologically malignant pleural mesothelioma were treated with pemetrexed and pemetrexed/platinum agent. They suggested that TS protein levels were an independent prognostic factor for time to PFS and OS in patients. In human NSCLC tumors, an inverse correlation was observed between TS expression and tumor response and PFS in patients treated with pemetrexed-based chemotherapy.[30] The VNTR in the TYMS was a predictive factor for anemia and thrombocytopenia in non-squamous NSCLC

(non-sq NSCLC) patients treated with carboplatin and pemetrexed regimen.[31] Arévalo et al. found that 3R/3R genotype was significantly correlated with OS in advanced NSCLC patients receiving pemetrexed.[32] Li et al. indicated that the response rate was higher in patients with the TS 2R/2R, 2R/3C, or 3C/3C genotypes than that in the other groups. Furthermore, they found that the polymorphism of the 5' UTR of the TS gene is a predictor of survival in advanced NSCLC patients treated with pemetrexed.[33] Lee et al. studied the expressions of TS in pulmonary adenocarcinoma patients treated with pemetrexed/cisplatin, as the first-line treatment. Low TS expressions were significantly associated with better RR and longer PFS in these patients.[34] Inconsistent with our results, no significant correlation was found among different TS gene polymorphisms and disease control rate (DCR), objective response rate (ORR), and PFS of pemetrexed-based treatment in advanced NSCLC.[35] On account of these controversies, the well-designed study should evaluate the role of TS gene polymorphisms and expression on lung cancer genetic susceptibility and its role in predicting specific treatment regimens.

The results of our study suggested that the TT alleles in rs1051298, compared to TC and CC alleles, are not associated with the survival of NSCLC patients (12 vs. 12 months, $P = 0.67$). The study of Adjei et al. implied that C/T of rs1051298 was associated with longer PFS and predict survival in NSCLC patients treated with pemetrexed.[17, 36] In another study, Li et al. showed that rs1051298 CC genotype had a longer median OS than patients with the other two genotypes (12.2 vs. 8.9 and 7.3 months, respectively; log-rank $x2 = 2.957$; $P = 0.022$).[32] In the study of Corrigan et al., rs1051298 was associated with OS in NSCLS and mesothelioma patients who received pemetrexed/platinum doublet chemotherapy.[37] Most recently, Zhang et al. revealed that rs1051298 had significant associations with the risk of hepatotoxicity in patients who received pemetrexed treatment.[38] These results proposed that the C allele in rs1051298 was associated with pemetrexed drug toxicity and longer PFS and OS in lung cancer patients.

Our results demonstrated no associations between rs3788189 and NSCLC patients (13 vs. 12 months, $P = 0.72$). However, the study of Adjei et al. showed that rs3788189 was associated with OS in NSCLC patients receiving pemetrexed-based therapy.[36] Moreover, Dy et al. indicated that NSCLC patients were treated with perioperative chemotherapy with cisplatin and pemetrexed. The results of this study revealed a statistically significant association between SLC19A1 rs3788189 TT genotype ($P = 0.0821$) and DFS.[16]

The current study had several limitations; the major one was the small sample size. It is a possible explanation of why the studied polymorphisms were not associated with the OS of NSCLC patients in our study. Furthermore, to achieve reliable results concerning the prediction of the benefit of pemetrexed therapy in lung cancer patients, we investigated other important pharmacogenomics polymorphisms within genes, such as MTHFR, GGH, DHFR, and FOLR.[37] Finally, ADRs recorded data were not suitable for assessing drug efficacy. Given all these limitations, further investigation with different a variety of gene polymorphisms, larger sample size, and detailed clinical data is required.

Conclusion

Previous pharmacogenomics studies have identified markers of pemetrexed toxicity. The present research demonstrated that TYMS and SLC19A1 gene polymorphisms do not predict survival differences in pemetrexed-treated advanced NSCLC. Our results should be validated in larger studies using pemetrexed-based chemotherapy.

Acknowledgements

The authors appreciate all the patients who agreed to participate in this study. This study was funded by the National Institute of Genetic Engineering and Biotechnology (NIGEB), Tehran, Iran.

Conflict of Interest

None declared.

References

1. Bray F, Ferlay J, Soerjomataram I, Siegel RL, Torre LA, Jemal A. Global cancer statistics 2018: GLOBOCAN estimates of incidence and mortality worldwide for 36 cancers in 185 countries. *CA Cancer J Clin*. 2018;68(6):394-424. doi: 10.3322/caac.21492. Erratum in: *CA Cancer J Clin.* 2020;70(4):313.
2. Postmus P, Kerr K, Oudkerk M, Senan S, Waller D, Vansteenkiste J, et al. Early and locally advanced non-small-cell lung cancer (NSCLC): ESMO Clinical Practice Guidelines for diagnosis, treatment and follow-up. *Ann Oncol.* 2017;28:iv1-iv21. doi: 10.1093/annonc/mdx222.
3. Bilfinger T, Keresztes R, Albano D, Nemesure B. Five-year survival among stage IIIA lung cancer patients receiving two different treatment modalities. *Med Sci Monit.* 2016;22:2589-94. doi: 10.12659/msm.898675.
4. Hanna N, Shepherd FA, Fossella FV, Pereira JR, De Marinis F, Von Pawel J, et al. Randomized phase III trial of pemetrexed versus docetaxel in patients with non-small-cell lung cancer previously treated with chemotherapy. *J Clin Oncol.* 2004;22(9):1589-97. doi: 10.1200/JCO.2004.08.163.
5. Vogelzang NJ, Rusthoven JJ, Symanowski J, Denham C, Kaukel E, Ruffie P, et al. Phase III study of pemetrexed in combination with cisplatin versus cisplatin alone in patients with malignant pleural mesothelioma. *J Clin Oncol.* 2003;21(14):2636-44. doi: 10.1200/JCO.2003.11.136.
6. Krawczyk P, Kucharczyk T, Kowalski DM, Powrózek T, Ramlau R, Kalinka-Warzocha E, 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(12):2047-57. doi: 10.1007/s00432-014-1756-6.
7. Pullarkat ST, Stoehlmacher J, Ghaderi V, Xiong YP, Ingles SA, Sherrod A, et al. Thymidylate synthase gene polymorphism determines response and toxicity of 5-FU chemotherapy. *Pharmacogenomics J.* 2001;1(1):65-70. doi: 10.1038/sj.tpj.6500012.
8. Kumagai K, Hiyama K, Oyama T, Maeda H, Kohno N. Polymorphisms in the thymidylate synthase and methylenetetrahydrofolate reductase genes and sensitivity to the low-dose methotrexate therapy in patients with rheumatoid arthritis. *Int J Mol Med.* 2003;11(5):593-600.
9. Ozasa H, Oguri T, Uemura T, Miyazaki M, Maeno K, Sato S, et al. Significance of thymidylate synthase for resistance to pemetrexed in lung cancer. *Cancer Sci.* 2010;101(1):161-6. doi: 10.1111/j.1349-7006.2009.01358.x.
10. Horie N, Aiba H, Oguro K, Hojo H, Takeishi K. Functional analysis and DNA polymorphism of the tandemly repeated sequences in the 5'-terminal regulatory region of the human gene for thymidylate synthase. *Cell Struct Funct.* 1995;20(3):191-7. doi: 10.1247/csf.20.191.
11. Ulrich CM, Bigler J, Velicer CM, Greene EA, Farin FM, Potter JD. Searching expressed sequence tag databases: discovery and confirmation of a common polymorphism in the thymidylate synthase gene. *Cancer Epidemiol Biomarkers Prev.* 2000;9(12):1381-5.
12. Pei L, Zhu H, Ye R, Wu J, Liu J, Ren A, et al. Interaction between the SLC19A1 gene and maternal first trimester fever on offspring neural tube defects. *Birth Defects Res A Clin Mol Teratol*. 2015;103(1):3-11. doi: 10.1002/bdra.23257.
13. Mohtaram S, Sheikhha MH, Honarvar N, Sazegari A, Maraghechi N, Feizollahi Z, et al. An association study of the SLC19A1 gene polymorphisms/haplotypes with idiopathic recurrent pregnancy loss in an Iranian population. *Genet Test Mol Biomarkers.* 2016;20(5):235-40. doi: 10.1089/gtmb.2015.0230.
14. Bohanec Grabar P, Leandro-García LJ, Inglada-Pérez L, Logar D, Rodríguez-Antona C, Dožlan V. Genetic variation in the SLC19A1 gene and methotrexate toxicity in rheumatoid arthritis patients. *Pharmacogenomics.* 2012;13(14):1583-94. doi: 10.2217/pgs.12.150.
15. Gong Z, Yao S, Zirpoli G, David Cheng TY, Roberts M, Khoury T, et al. Genetic variants in one-carbon metabolism genes and breast cancer risk in E uropean A merican and A frican A merican women. *Int J Cancer.* 2015;137(3):666-77. doi: 10.1002/ijc.29434.
16. Dy GK, Bogner PN, Tan W, Demmy TL, Farooq A, Chen H, et al. Phase II study of perioperative chemotherapy with cisplatin and pemetrexed in non–small-cell lung cancer. *J Thorac Oncol.* 2014;9(2):222-30. doi: 10.1097/JTO.0000000000000062.
17. Adjei AA, Mandrekar SJ, Dy GK, Molina JR, Adjei AA, Gandara DR, et al. Phase II trial of pemetrexed plus bevacizumab for second-line therapy of patients with advanced non-small-cell lung cancer: NCCTG and SWOG study N0426. *J Clin Oncol.* 2010;28(4):614-9. doi: 10.1200/JCO.2009.23.6406.
18. Abbasian MH, Ansarinejad N, Abbasi B, Iravani M, Ramim T, Hamedi F, et al. The role of dihydropyrimidine dehydrogenase and thymidylate synthase polymorphisms in fluoropyrimidine-based cancer chemotherapy in an Iranian population. *Avicenna J Med Biotechnol.* 2020;12(3):157-64.
19. Scagliotti GV, Parikh P, Von Pawel J, Biesma B, Vansteenkiste J, Manegold C, 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(21):3543-51. doi: 10.1200/JCO.

2007.15.0375.
20. Abbasian MH, Ansarinejad N, Ramim T, Fardad F, Abbasi B. Methylenetetrahydrofolate reductase C677T (rs1801133) polymorphism and pemetrexed treatment outcome in patients with non–small-cell lung cancer. *Pers MJ*. 2021; 6(23):5-9. doi: 10.22034/pmj.2021.249035.
21. Abbasian MH, Abbasi B, Ansarinejad N, Motevalizadeh Ardekani A, Samizadeh E, Gohari Moghaddam K, et al. Association of interleukin-1 gene polymorphism with risk of gastric and colorectal cancers in an Iranian population. *Iran J Immunol.* 2018;15(4):321-8. doi: 10.22034/IJI.2018.39401.
22. Otake Y, Tanaka F, Yanagihara K, Hitomi S, Okabe H, Fukushima M, et al. Expression of thymidylate synthase in human non-small cell lung cancer. *Jpn J Cancer Res.* 1999;90(11):1248-53.
23. Sigmond J, Backus HH, Wouters D, Temmink OH, Jansen G, Peters GJ. Induction of resistance to the multitargeted antifolate Pemetrexed (ALIMTA) in WiDr human colon cancer cells is associated with thymidylate synthase overexpression. *Biochem Pharmacol.* 2003;66(3):431-8. doi: 10.1016/s0006-2952(03)00287-9.
24. Longley DB, Ferguson PR, Boyer J, Latif T, Lynch M, Maxwell P, et al. Characterization of a thymidylate synthase (TS)-inducible cell line: a model system for studying sensitivity to TS-and non-TS-targeted chemotherapies. *Clin Cancer Res.* 2001;7(11):3533-9.
25. Kim JH, Lee KW, Jung Y, Kim TY, Ham HS, Jong HS, et al. Cytotoxic effects of pemetrexed in gastric cancer cells. *Cancer Sci.* 2005;96(6):365-71. doi: 10.1111/j.1349-7006.2005.00058.x.
26. Gomez HL, Santillana SL, Vallejos CS, Velarde R, Sanchez J, Wang X, et al. A phase II trial of pemetrexed in advanced breast cancer: clinical response and association with molecular target expression. *Clin Cancer Res.* 2006;12(3):832-8. doi: 10.1158/1078-0432.CCR-05-0295.
27. Nakagawa T, Otake Y, Yanagihara K, Miyahara R, Ishikawa S, Fukushima M, et al. Expression of thymidylate synthase is correlated with proliferative activity in non-small cell lung cancer (NSCLC). *Lung Cancer.* 2004;43(2):145-9. doi: 10.1016/j.lungcan.2003.09.004.
28. Wang X, Wang Y, Wang Y, Cheng J, Wang Y, Ha M. Association of thymidylate synthase gene 3'-untranslated region polymorphism with sensitivity of non-small cell lung cancer to pemetrexed treatment: TS gene polymorphism and pemetrexed sensitivity in NSCLC. *J Biomed Sci.* 2013;20(1):1-8. doi: 10.1186/1423-0127-20-5.
29. Takezawa K, Okamoto I, Okamoto W, Takeda M, Sakai K, Tsukioka S, et al. Thymidylate synthase as a determinant of pemetrexed sensitivity in non-small cell lung cancer. *Br J Cancer.* 2011;104(10):1594-601. doi: 10.1038/bjc.2011.129.
30. Kanazawa K, Yokouchi H, Wang X, Ishida T, Fujita Y, Fujiuchi S, 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(6):1149-57. doi: 10.1007/s00280-014-2589-3.
31. Arévalo E, Castañón E, López I, Salgado J, Collado V, Santisteban M, et al. Thymidylate synthase polymorphisms in genomic DNA as clinical outcome predictors in a European population of advanced non-small cell lung cancer patients receiving pemetrexed. *J Transl Med.* 2014;12(1):1-9 doi: 10.1186/1479-5876-12-98.
32. Li WJ, Jiang H, Fang XJ, Ye HL, Liu MH, Liu YW, et al. Polymorphisms in thymidylate synthase and reduced folate carrier (SLC19A1) genes predict survival outcome in advanced non-small cell lung cancer patients treated with pemetrexed-based chemotherapy. *Oncol Lett.* 2013;5(4):1165-70. doi: 10.3892/ol.2013.1175.
33. Lee SH, Noh KB, Lee JS, Lee EJ, Min KH, Hur GY, et al. Thymidylate synthase and ERCC1 as predictive markers in patients with pulmonary adenocarcinoma treated with pemetrexed and cisplatin. *Lung Cancer.* 2013;81(1):102-8. doi: 10.1016/j.lungcan.2013.03.002.
34. Hu Q, Li X, Su C, Chen X, Gao G, Zhang J, et al. Correlation between thymidylate synthase gene polymorphisms and efficacy of pemetrexed in advanced non-small cell lung cancer. *Exp Ther Med.* 2012;4(6):1010-6. doi: 10.3892/etm.2012.730.
35. Adjei AA, Salavaggione OE, Mandrekar SJ, Dy GK, Ziegler KLA, Endo C, et al. Correlation between polymorphisms of the reduced folate carrier gene (SLC19A1) and survival after pemetrexed-based therapy in non-small cell lung cancer: a North Central Cancer Treatment Group-based exploratory study. *J Thorac Oncol.* 2010;5(9):1346-53. doi: 10.1097/JTO.0b013e3181ec18c4.
36. Corrigan A, Walker JL, Wickramasinghe S, Hernandez MA, Newhouse SJ, Folarin AA, et al. Pharmacogenetics of pemetrexed combination therapy in lung cancer: pathway analysis reveals novel toxicity associations. *Pharmacogenomics J.* 2014;14(5):411-7. doi: 10.1038/tpj.2014.13.
37. Zhang X, Zhang D, Huang L, Li G, Chen L, Ma J, et al. Discovery of novel biomarkers of therapeutic responses in Han Chinese pemetrexed-based treated advanced NSCLC patients. *Front Pharmacol.* 2019;10:944. doi: 10.3389/fphar.2019.00944.