HHS Public Access

Author manuscript

J Thorac Oncol. Author manuscript; available in PMC 2017 December 19.

Published in final edited form as:

J Thorac Oncol. 2010 September ; 5(9): 1346–1353. doi:10.1097/JTO.0b013e3181ec18c4.

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

Araba A. Adjei, PhD[*], **Oreste E. Salavaggione, MD**[*], **Sumithra J. Mandrekar, PhD**[†], **Grace K. Dy, MD**[*], **Katie L. Allen Ziegler, BS**[†], **Chiaki Endo, MD**[‡], **Julian R. Molina, MD, PhD**[†], **Steven E. Schild, MD**[§], and **Alex A. Adjei, MD, PhD**[*]

[*]Departments of Pharmacology & Therapeutics; and Medicine, Roswell Park Cancer Institute, Buffalo, New York

[†]Division of Biomedical Statistics and Informatics; and Medical Oncology, Mayo Clinic, Rochester, Minnesota

[‡]Department of Thoracic Surgery, Tohoku University, Aoba-ku, Sendai, Japan

[§]Division of Radiation Oncology, Mayo Clinic, Phoenix, Arizona

Abstract

Purpose—To correlate polymorphisms in genes involved in the transport, activation, and inactivation of pemetrexed with the outcome of patients with advanced non-small cell lung cancer (NSCLC) treated with pemetrexed.

Experimental Design—Data from a phase II NSCLC trial evaluating the optimal schedule of gemcitabine and pemetrexed were used. All patients with available DNA were genotyped for polymorphisms in *FPGS, GGH*, and *SLC19A1* genes. Patients with various genotypes were compared for efficacy and adverse events resulting from pemetrexed.

Results—Fifty-four patients had genotype results for all polymorphisms studied. Patients with the homozygous variant genotypes for *SLC19A1* IVS4(2117) C>T, IVS5(9148) C>A, and wild-type genotype for exon6(2522) C>T had a significantly better overall survival compared with their counterparts (median overall survival in months: 8.9 [CC] versus 14.0 [CT] versus 16.7 [TT]; 9.4 [CC] versus 10.3 [CA] versus 22.7 [AA]; and 22.7 [CC] versus 10.3 [CT] versus 9.4 [TT] respectively; all log rank $p = 0.03$). Patients with the heterozygous TC genotype for *GGH* IVS5(1042) T>C had greater rates of confirmed response + stable disease compared with the TT genotype (85% versus 60%; odds ratio = 4.0; $p = 0.06$). A greater risk for grade 3/4 SGPT (ALT) elevation was observed in patients heterozygous (GA) for the *FPGS* IVS1 (28) G>A polymorphism compared with the GG genotype (43% versus 13%; odds ratio = 5.0, $p = 0.07$). All results were largely consistent within patients with nonsquamous ($n = 40$) histology.

Address for correspondence: Alex A. Adjei, MD, PhD, Roswell Park Cancer Institute, Elm & Carlton Streets, Buffalo, NY 14263. alex.adjei@roswellpark.org.

Disclosure: The authors declare no conflicts of interest.

Conclusion—Polymorphisms in *SLC1A91* seem to predict for survival differences in pemetrexed-treated NSCLC. Additionally, polymorphisms in *GGH* and *FPGS* have marginal associations with response and adverse event. These results should be validated in larger prospective studies using pemetrexed.

Keywords

Polymorphisms; Reduced folate carrier gene; *SLC19A1*; Pemetrexed; Non-small cell lung cancer

The potency of pemetrexed, *N*-[4-[2-(2-amino-3,4-dihydro-4-oxo-7H-pyrrolo[2,3-day]pyrimidin-5-yl)-ethyl]-benzoyl]-L-glutamic acid, compared with numerous other classic antifolates including methotrexate is due to pemetrexed's rapid transport into cells by the reduced folate carrier protein, RFC, encoded by the gene *SLC19A1*. Pemetrexed is transported into cells about two times faster than methotrexate[1,2] and binds to the folate receptor-α with a very high affinity, similar to that of folic acid.[3] Pemetrexed has been shown to be one of the best substrates for folylpolyglutamate synthetase protein encoded by *FPGS*, when compared with numerous other classic antifolates.[4] Once it enters cells, pemetrexed is polyglutamated to the active pentaglutamate. Steady-state accumulation of pemetrexed polyglutamates depends primarily on activation/inactivation processes by FPGS and gamma glutamyl hydrolase encoded by *GGH*. The pentaglutamate form of pemetrexed is the predominant intracellular form and is >60-fold more potent in its inhibition of thymidylate synthase the target protein, than the monoglutamate.[5] Polyglutamation traps pemetrexed and enhances its intracellular retention. This polyglutamation competes with the hydrolysis of the accumulated pentaglutamate tails catalyzed by the GGH enzyme, thus allowing the efflux of pemetrexed out of the cells.[6]

Pemetrexed inhibits multiple folate-dependent enzyme targets most notably thymidylate synthase, resulting in decreased thymidine necessary for DNA synthesis.[7–9] It also inhibits dihydrofolate reductase and glycinamide ribonucleotide formyltransferase.[5]

Pemetrexed was first approved for second-line treatment of non-small cell lung cancer (NSCLC) in 2005.[10] It has also shown activity in a variety of solid tumors such as colorectal, gastric, breast, cervical and bladder cancers, in combination with other chemotherapeutic agents.[11] In the past year, pemetrexed has been approved for the first-line therapy for nonsquamous NSCLC.[12,13]

The molecular mechanism underlying the resistance to pemetrexed[6,14–18] may in part be due to alterations in the genes encoding the FPGS, GGH, and SLC19A1 proteins, which are responsible for the transport, activation, and inactivation of pemetrexed. These alterations might result in variations in clinical response and/or toxicity. Therefore, several or all these enzymes may play a part in determining the effectiveness of pemetrexed therapy in patients with NSCLC.

Based on the above, we focused on genetic variations that might affect the genes transporting, activating, and inactivating pemetrexed. We hypothesized that germ-line single-nucleotide polymorphisms (SNPs) in *SLC19A1*(transport), *FPGS* (activation), and *GGH* (inactivation), either singly or in combination, may play a role in the efficacy and/or toxicity

of pemetrexed. Thus, we examined the association between tag SNPs of *FPGS, GGH*, and *SLC19A1* and outcomes in patients who received pemetrexed on a North Central Cancer Treatment Group phase II NSCLC trial.[19]

PATIENTS AND METHODS

Patients and Study Design

A randomized three-arm phase II study was undertaken to evaluate the optimal administration schedule of pemetrexed and gemcitabine in chemo-naive patients with NSCLC.[19] Briefly, patients were enrolled into one of the following three schedules: (a) pemetrexed followed 90 minutes later by gemcitabine on day 1 and gemcitabine on day 8; (b) gemcitabine followed 90 minutes later by pemetrexed on day 1 and gemcitabine on day 8; or (c) gemcitabine on day 1 and pemetrexed followed 90 minutes later by gemcitabine on day 8. Patients provided written informed consent, and the study protocol was approved by each participating site's institutional review board. A total of 152 patients from the three schedules (A: 59, B: 31, and C: 62) were evaluable, and the results of the primary endpoint have been previously reported.[19] Approximately 94% of the patients were of white origin. No statistically significant differences were observed among the three treatment schedules in the distribution of age, sex, race, or stage. Schedule A achieved a response rate of 31% compared with 6.5% with schedule B and 16% with C. Of these, only schedule A met the protocol-defined response criteria for being worthy of further evaluation. Schedule B was discontinued after interim analysis because of the poor response rate. The median survival time (schedules A, B, and C: 11.4, 10.3, and 11.8 months, respectively) and time to disease progression (schedules A, B, and C: 4.7, 4.1, and 4.4 months, respectively) were similar among the three schedules.

Selection of Tag SNPs

We used our previous resequencing data from the white population to derive a combination of haplotype (ht)-tag and linkage disequilibrium (LD)-tag SNPs for *FPGS*.[20] We then searched the HapMap white database and obtained polymorphism (SNP) information for *GGH* and *SLC19A1*. Specifically, we loaded the SNP genotype datasets for the genes into Haploview, and polymorphisms with frequencies greater than 5% were selected for haplotype analysis at an r^2 threshold ≥ 0.8. Haplotypes close to or above 2% frequency were organized into single blocks from which the ht-SNPs were derived. Tag SNPs were also generated from the same data using the tagger software in Haploview v 4.1. SNPs that did not overlap from either analysis were included in the list of tag SNPs (Table 1) to provide a more complete coverage of the genetic variability in these three genes. A total of 19 loci were derived for genotyping.

DNA Extraction and Genotyping

Ten milliliters of blood was drawn into ethylenediaminetetraacetic acid (EDTA) tubes and shipped to the North Central Cancer Treatment Group central laboratory, where DNA was extracted and batched until the end of the study for the genotyping assay described later.

DNA samples were available for 61 of 152 patients on study because the protocol was amended to collect blood specimens for pharmacogenetics part way through the study, and participation in the translational component was optional. Three patients with DNA samples available were ineligible (central pathology review determined that patients had small cell lung cancer), and four samples failed during genotyping. Therefore, a total of 54 samples were successfully genotyped for the 19 loci in *FPGS, GGH*, and *SLC19A1*.

Genotyping was performed using the MassARRAY Compact system (Sequenom) in the Microarray and Genomics Core Facility at Roswell Park Cancer Institute. All genotyping was polymerase chain reaction (PCR) based and was performed using established methods at Roswell Park Cancer Institute. The primary genotyping platform for SNP analysis uses the Sequenom MassARRAY platform. This system uses mass spectrometry in the detection and analysis of primer-extended PCR products.[21] All reactions were carried out in a PTC225 thermal cycler (MJ Research, Inc.). DNA samples selected from the Centre d'Etude du Polymorphisme Humain (CEPH) reference families in addition to DNA samples from the white population set from the Coriell Cell Repository served as control samples for genotyping.

Statistical Analysis

Overall survival (OS) was defined as the time from randomization to death from any cause. Progression-free survival was defined as the time from randomization to the earlier of disease progression or death from any cause. Adverse events (AEs) were graded according to the National Cancer Institute Common Toxicity Criteria (version 2). Confirmed response (CR) was defined as either a complete or partial response (PR) per RECIST, observed on two consecutive evaluations at least 4 weeks apart. Fisher's exact test and logistic regression models adjusted for treatment arm were used to explore the differences in CR and AE rates between the genotype variant subgroups. Kaplan-Meier survival curves,[22] log-rank tests,[23] and Cox proportional hazards models adjusted for treatment arm were used to compare the time to event distributions for the various genotype subgroups. In view of the fact that 17 of the 19 SNPs were analyzed, p values <0.0003 would be deemed statistically significant using the Bonferroni method for multiple comparisons. However, given the limited sample size of 54 patients, and the various outcomes explored, all analyses were considered exploratory with no adjustments for multiple comparisons. Each SNP was explored by itself. Gene-gene interactions and simultaneous exploration of more than one SNP was not performed because of the sample size limitation.

RESULTS

Patient Characteristics

There were no significant differences in the baseline characteristics of the 54 patients included in this analysis compared with the rest of the 152 evaluable patients from the trial. The median age was 65 years (range: 43–85). Ninety-three percent of the patients had stage IV disease: 54% were men and 59% had a performance status of 1. Fourteen (26%) of the 54 patients had squamous cell histology. See Table 2 for details. These patient numbers were too few for specific analyses. However, tumor histology has been reported to be a

determinant of pemetrexed efficacy in NSCLC; therefore, analyses were performed with and without the 14 squamous samples (nonsquamous only), and the results remained consistent.

Genetic Polymorphisms: Tag SNPs and Distribution of Variants

A total of 19 tag SNPs from the three genes *FPGS, GGH*, and *SLC19A1* were derived through LD and haplotype analyses. Seventeen of the19 loci passed the genotyping quality control test and two failed. Table 1 shows the SNP identities and the genotype and minor allele frequencies (MAFs) for the study samples. All the SNPs were in Hardy-Weinberg equilibrium for the patient and control samples used. Frequency distributions were similar between our study samples and those reported in the HapMap database for white samples. Except for *FPGS* IVS1(28)G>A with MAF of 8.3%, all other SNPs included in the analyses had MAFs >10%. Genotypes of patient samples with variant distribution ≤7% ($N \leq 4$) were excluded from the analysis with clinical outcomes because of their low numbers. Also, analysis with squamous only samples were not performed because of the low number, $N = 14$.

Genetic Polymorphisms and Survival

Differences in OS were observed in patients harboring four SNPs in *SLC19A1* namely IVS2(4935)G>A, IVS4(2117)C>T, IVS5(9148)C>A, and exon6(2522)C>T. Three of these SNPs, IVS4(2117)C>T, IVS5(9148)C>A, and exon6(2522)C>T, were significantly associated with OS, log-rank $p = 0.03$ (Table 3, Figures 1 and 2). For patients with IVS4(2117)C>T polymorphism, the OS for those with the homozygous variant genotype TT was 16.7 months, the heterozygous CT was 14 months, and the wild-type CC genotype was 8.9 months, log-rank $p = 0.03$ (Figure 1, Table 3). The frequency for the wild-type CC genotype in IVS5(9148)C>A polymorphism was 22.2%, same as that for variant TT genotype in exon6(2522)C>T. Similarly, frequency for the variant AA genotype in IVS5(9148)C>A was 33.3%, same as that for wild-type CC genotype in exon6(2522)C>T (Table 2). Thus, the nucleotide frequency distribution at these two loci for the patients was similar. The OS of the patients was similarly distributed for the genotypes. Thus, patients with variant AA genotype in IVS5(9148)C>A and wild-type CC genotype in exon6(2522)C>T had median survival of 22.7 months compared with their counterparts with heterozygous genotypes of 10.3 months and CC or TT genotypes of 9.4 months, respectively, log-rank $p = 0.03$ (Table 3, Figures 2*A, B*). All these results were consistent after adjusting for treatment arm in Cox proportional hazards models (Table 3). At a log-rank $p = 0.09$, the association of *SLC19A1* IVS2(4935)G>A polymorphism with OS in patients was marginal. Patients with GG genotype had a median survival of 18.6 months, 10.6 months for those with the GA genotype, and 9.3 months for the AA variant genotype (Table 3, Figure 3). After excluding the squamous cell patients harboring these polymorphisms, the results in Table 3 remained consistent with the exception of a significant association for *SLC19A1* IVS2(4935)G>A polymorphism with OS, a log-rank change from $p = 0.09$ ($N = 54$) to $p = 0.02$ ($N = 40$) probably due to the fewer sample numbers. There was a nonsignificant trend toward a more prolonged PFS for the wild-type *SLC19A1* exon6(2522) CC genotype; however, in general, no difference in PFS was observed for the polymorphisms both overall and within the nonsquamous histology patients.

Genetic Polymorphisms and AEs

Grade 3/4 AEs, regardless of attribution, were observed in approximately 15% of the patients who had complete genotype data. The common (occurring in 10% or more of patients) grade 3/4 AEs included fatigue, observed in 20% of the patients, neutropenia (69% of patients), leukopenia (50% of patients), thrombocytopenia (15% of patients), dyspnea (19% of patients), and SGPT (alanine aminotransferase [ALT]) elevation (17% of patients). No statistically significant association with AEs was observed for any of the SNPs with the exception of a few trends noted below.

For *FPGS*, patients with the IVS1(28)G>A polymorphism showed a high risk of grade 3/4 SGPT (ALT) (odds ratio [OR] = 5.0, $p = 0.07$). Specifically, a higher percentage of patients with heterozygous GA had elevated SPGT (43%) than those homozygous for the wild-type genotype GG (13%) (Fisher's exact test $p = 0.09$) (Table 3). Similar to *FPGS, GGH* showed no difference in most of the severe AEs; however, patients with the AT genotype for the IVS3(1950)A>T polymorphism had a lower risk for grade 3/4 fatigue (OR = 0.1, $p = 0.08$): the wild-type AA genotype was associated more with fatigue than the AT genotype (29% versus 6%; Fisher's exact test $p = 0.07$) (Table 3). Patients with the *GGH* IVS2(2442)T>C polymorphism showed a significantly higher risk for grade 3/4 dyspnea (OR = 7.3, $p = 0.04$): more patients with the TC genotype had grade 3 + dyspnea compared with those with the TT genotype (40% versus 14%; Fisher's exact test $p = 0.08$) (Table 3). The results were consistent within the patients with nonsquamous histology.

Genetic Polymorphisms and Tumor Response

No statistically significant association was observed for any of the polymorphisms in *FPGS, SLC19A1*, and *GGH* with CR and clinical benefit (CB) rates (defined as CR plus stable disease), even when analysis were performed with only nonsquamous histology samples. However, a trend toward a higher CR and CB rates was seen in patients with the TC heterozygous genotype compared with the TT genotype for *GGH* IVS5(1042)T>C (CR: 40% versus 17%, Fisher's exact test $p = 0.10$; CB: 85% versus 60%, Fisher's exact test $p = 0.07$). These results were consistent after adjusting for treatment arm in logistic models, where patients with TC genotype were more likely to have a CR and CB (CR: TT versus TC: OR = 3.9, $p = 0.06$; CB: TT versus TC: OR = 4.0, $p = 0.06$) (Table 3). The discordance between polymorphisms and association with CR and OS is unclear. It might be due to the small sample size; however, in NSCLC, response rates do not always correlate with OS.

DISCUSSION

Germ-line polymorphisms have been reported to have major effects on efficacy and toxicity in patients undergoing treatment with anticancer drugs such as erlotinib, gefitinib, gemcitabine, cisplatin, taxanes, and platinum agents.[24–27] In this study, we evaluated the association of polymorphisms in pemetrexed-related genes with outcomes from combination therapy with gemcitabine. We realize that the overall toxicity and efficacy profile of the treatment outcome might be influenced by gemcitabine's contribution to the therapy, but there are studies that have evaluated single-drug (irinotecan or 5-fluorouracil)-related

polymorphisms in combination therapies where relevant polymorphisms have been found to correlate with toxicity and treatment outcomes.[28,29]

This study was based on accumulating data suggesting that resistance to antifolates, such as pemetrexed, may be due in part to decreased SLC19A1 transport, increased GGH expression or decreased FPGS expression, and/or inactivating mutations,[14–18] hence the molecular mechanism underlying this resistance might be due to genetic variations in these genes. Therefore, we focused our study on several tag SNPs in the genes responsible for transporting (*SLC19A1*), activating (*FPGS*), and inactivating (*GGH*) pemetrexed. Very few studies have examined the role of genetic polymorphisms in pemetrexed therapy for NSCLC. Therefore, we used samples from a randomized three-arm phase II study performed to evaluate the optimal administration schedule of pemetrexed and gemcitabine in chemo-naive patients with NSCLC[19] to evaluate potential associations between germ-line polymorphisms of *SLC19A1, FPGS*, and *GGH* and the efficacy and toxicity of pemetrexed.

Three tag SNPs in *SLC19A1* namely IVS4(2117)C>T, IVS5C>A, and exon6(2522)C>T were significantly associated with OS ($p = 0.03$), whereas the fourth SNP, IVS2(4935)G> A showed a marginally significant association ($p = 0.09$). Patients with the variant AA genotype for IVS5(9148) and those with wild-type CC genotype at exon6(2522) had a significantly longer survival with a median of 22.7 months than their counterparts with homozygous and heterozygous genotypes (9.4 and 10.3 months, respectively). Additionally, the variant genotype TT in IVS4(2117)C>T polymorphism predicted for improved OS. Although there were no significant associations between any of the polymorphisms and AEs or response, certain SNPs demonstrated a trend toward improved response rates (heterozygous TC genotype for *GGH* IVS2(1042) T>C were more likely to respond compared with those with the TT genotype; OR p value = 0.06) and elevation of transaminase activity (presence of variant allele in *FPGS* IVS1(28)G>A; OR p value = 0.07). These findings have to be interpreted with caution given the approximately 100 comparisons explored in this study (17 SNPs and six different outcomes). At a threshold of 5%, this means at least five of these 100 comparisons would likely have resulted in a significant finding by chance alone. If the p values were adjusted for the multiple comparisons, only p values less than 0.0005 would be deemed statistically significant. Given that the p values were on the order of 0.01 to 0.09, the “significant” and the “marginally significant” findings from this analysis should be viewed as promising.

In a recent study, the *SLC19A1* exon6(2522)CC genotype correlated with longer median PFS and 3-month progression in patients undergoing pemetrexed-bevacizumab combination therapy.[30] In this study, it predicted for OS, $p = 0.03$. This exon6(2522)C>T polymorphism is in LD with *SLC19A1* SNP rs1051266, an exon2(80)G>A (R27H) polymorphism ($D' = 0.963$, $r^2 = 0.837$, log of the likelihood odds ratio (LOD) = 24.42), whose homozygous AA variant genotype has been associated with worse outcome in children with acute lymphoblastic leukemia treated with methotrexate.[31,32] This G80A polymorphism is also in LD with *SLC19A1* IVS5(9148)C>A polymorphism ($D' = 0.949$, $r^2 = 0.753$, LOD = 12.64) whose AA genotype also predicted for OS, $p = 0.03$, in our study. This observation suggests that genetic variations in *SLC19A1* might predict for clinical outcome in pemetrexed therapy. Smit et al.[33] recently reported that there was no association between the *SLC19A1*

G80A SNP and PFS for patients on pemetrexed-carboplatin versus pemetrexed. In this study, it is the wild-type CC genotype of *SLC19A1* exon6 (2522) that demonstrated a trend toward a longer PFS, an observation also seen in a previous pemetrexed-bevacizumab combination therapy study,[30] suggesting that studies with larger patient cohorts are necessary.

The variant C allele in *GGH* IVS2(1042)T> C was associated with increased CB and response in this study, and it had also shown to be beneficial for decreased grade 4 AE in another study involving pemetrexed.[30] This suggests that the *GGH* INV2(1042)T>C polymorphism also plays a role in pemetrexed treatment outcome. We were not able to analyze these tag SNPs to assess their role in the differences in response rates between the different regimens because of small sample numbers. Therefore, we are unable to report whether a specific regimen might have influenced an association with the tag SNPs. However, all the polymorphisms that were associated with efficacy or toxicity of pemetrexed had allele frequencies that were relatively common; *FPGS* (8%), *GGH* (11–26%), and *SLC19A1* (42–56%), suggesting that inherited genetic variants, particularly for *SLC19A1*, could potentially contribute to clinical outcome after pemetrexed therapy, in a large proportion of the population.

In summary, our preliminary observations suggest that germ-line polymorphisms in *FPGS* and *GGH* are important determinants of pemetrexed response and toxicity, whereas polymorphisms in *SLC19A1* seem to predict for OS differences. Because this study had a small sample size, these hypotheses-generating findings are being verified in an ongoing larger patient (n = 1000) observational study of single-agent pemetrexed.

References

1. Westerhof GR, Schornagel JH, Kathmann I, et al. Carrier- and receptor-mediated transport of folate antagonists targeting folate-dependent enzymes: correlates of molecular-structure and biological activity. Mol Pharmacol. 1995; 48:459–471. [PubMed: 7565626]
2. Wang Y, Zhao R, Goldman ID. Characterization of a folate transporter in HeLa cells with a low pH optimum and high affinity for pemetrexed distinct from the reduced folate carrier. Clin Cancer Res. 2004; 10:6256–6264. [PubMed: 15448015]
3. Theti DS, Jackman AL. The role of alpha-folate receptor-mediated transport in the antitumor activity of antifolate drugs. Clin Cancer Res. 2004; 10:1080–1089. [PubMed: 14871988]
4. Habeck LL, Shih C, Gossett LS, et al. Substrate specificity of mammalian folylpolyglutamate synthetase for 5,lO-dideazatetrahydrofolate analogs. Mol Pharmacol. 1995; 48:326–333. [PubMed: 7651366]
5. Shih C, Chen VJ, Gossett LS, et al. LY231514, a pyrrolo[2,3-d]pyrimidine-based antifolate that inhibits multiple folate-requiring enzymes. Cancer Res. 1997; 57:1116–1123. [PubMed: 9067281]
6. Schneider E, Ryan TJ. Gamma-glutamyl hydrolase and drug resistance. Clin Chim Acta. 2006; 374:25–32. [PubMed: 16859665]
7. Schilsky, RL. Antimetabolites. In: Perry, MC., editor. The Chemotherapy Source Book. Baltimore, MD: Williams & Wilkins; 1992. p. 301-315.
8. Schultz RM, Patel VF, Worzalla JF, et al. Role of thymidylate synthase in the antitumor activity of the multitargeted antifolate, LY231514. Anticancer Res. 1999; 19:437–443. [PubMed: 10226579]
9. Grem, JL. Fluorinated pyrimidines. In: Chabner, BA., Collins, JM., editors. Cancer Chemotherapy: Principles and Practice. Philadelphia, PA: JB Lippincott; 1990. p. 180-224.
10. Cohen MH, Johnson JR, Wang YC, et al. FDA drug approval summary: pemetrexed for injection (Alimta) for the treatment of non-small cell lung cancer. Oncologist. 2005; 10:363–368. [PubMed: 15967829]

11. Adjei AA. Pemetrexed (Alimta), a novel multitargeted antineoplasticagent. Clin Cancer Res. 2004; 10:4276S–4280S. [PubMed: 15217974]
12. [Accessed January 15, 2010] Pemetrexed, Summary of Product Characteristics. Available at: http://www.emea.europa.eu/humandocs/PDFs/EPAR/alimta/H-564-PI-en.pdf
13. [Accessed January 15, 2010] FDA Grants Lilly's ALIMTA(R) (Pemetrexed for Injection) Third U.S. Approval. Available at: http://www.prnewswire.com/cgi-bin/micro_stories.pl?ACCT=916306&TICK=LLY&STORY=/www/story/09-29-2008/0004893937&EDATE=Sep+29,+2008
14. Gorlick R, Goker E, Trippett T, et al. Defective transport is a common mechanism of acquired methotrexate resistance in acute lymphocytic leukemia and is associated with decreased reduced folate carrier expression. Blood. 1997; 89:1013–1018. [PubMed: 9028333]
15. Gorlick R, Cole P, Banerjee D, et al. Mechanisms of methotrexate resistance in acute leukemia. Decreased transport and polyglutamylation. Adv Exp Med Biol. 1999; 457:543–550. [PubMed: 10500832]
16. Wong SC, Zhang L, Witt TL, et al. Impaired membrane transport in methotrexate-resistant CCRF-CEM cells involves early translation termination and increased turnover of a mutant reduced folate carrier. J Biol Chem. 1999; 274:10388–10394. [PubMed: 10187828]
17. Rhee MS, Wang Y, Nair MG, et al. Acquisition of resistance to antifolates caused by enhanced gamma-glutamyl hydrolase activity. Cancer Res. 1993; 53:2227–2230. [PubMed: 7683570]
18. Liani E, Rothem L, Bunni MA, et al. Loss of folylpoly-gamma-glutamate synthetase activity is a dominant mechanism of resistance to polyglutamylation-dependent novel antifolates in multiple human leukemia sublines. Int J Cancer. 2003; 103:587–599. [PubMed: 12494465]
19. Ma CX, Nair S, Thomas S, et al. Randomized phase II trial of three schedules of pemetrexed and gemcitabine as front-line therapy for advanced non-small-cell lung cancer. J Clin Oncol. 2005; 23:5929–5937. [PubMed: 16135464]
20. Leil TA, Endo C, Adjei AA, et al. Identification and characterization of genetic variation in the folylpolyglutamate synthase gene. Cancer Res. 2007; 67:8772–8782. [PubMed: 17875718]
21. Tang K, Fu DJ, Julien D, et al. Chip-based genotyping by mass spectrometry. Proc Natl Acad Sci USA. 1999; 96:10016–10020. [PubMed: 10468554]
22. Kaplan EL, Meier P. Nonparametric estimation from incomplete observations. J Am Stat Assoc. 1958; 53:457–481.
23. Mantel N. Evaluation of survival data and two new rank order statistics arising in its consideration. Cancer Chemother Rep. 1966; 50:163–170. [PubMed: 5910392]
24. Rudin CM, Liu W, Desai A, et al. Pharmacogenomic and pharmacokinetic determinants of erlotinib toxicity. J Clin Oncol. 2008; 26:1119–1127. [PubMed: 18309947]
25. Liu G, Gurubhagavatula S, Zhou W, et al. Epidermal growth factor receptor polymorphisms and clinical outcomes in non-small-cell lung cancer patients treated with gefitinib. Pharmacogenomics J. 2008; 8:129–138. [PubMed: 17375033]
26. Chew HK, Doroshow JH, Frankel P, et al. Phase II studies of gemcitabine and cisplatin in heavily and minimally pretreated metastatic breast cancer. J Clin Oncol. 2009; 27:2163–2169. [PubMed: 19307510]
27. Hildebrandt MA, Yang H, Hung MC, et al. Genetic variations in the PI3K/PTEN/AKT/mTOR pathway are associated with clinical outcomes in esophageal cancer patients treated with chemoradiotherapy. J Clin Oncol. 2009; 27:857–871. [PubMed: 19164214]
28. Omura K. Clinical implications of dihydropyrimidine dehydrogenase (DPD) activity in 5-FU-based chemotherapy: mutations in the DPD gene, and DPD inhibitory fluoropyrimidines. Int J Clin Oncol. 2003; 8:132–138. [PubMed: 12851836]
29. Côté JF, Kirzin S, Kramar A, et al. UGT1A1 polymorphism can predict hematologic toxicity in patients treated with irinotecan. Clin Cancer Res. 2007; 13:3269–3275. [PubMed: 17510208]
30. Adjei AA, Mandrekar SJ, Dy GK, et al. A phase II trial of pemetrexed plus bevacizumab for second-line therapy of patients with advanced non-small cell lung cancer (NSCLC): an NCCTG and SWOG Study N0426. J Clin Oncol. 2010; 28:614–619. [PubMed: 19841321]

31. Laverdière C, Chiasson S, Costea I, et al. Polymorphism G80A in the reduced folate carrier gene and its relationship to methotrexate plasma levels and outcome of childhood acute lymphoblastic leukemia. Blood. 2002; 100:3832–3834. [PubMed: 12411325]

32. Whetstine JR, Gifford AJ, Witt T, et al. Single nucleotide polymorphisms in the human reduced folate carrier: characterization of a high-frequency G/A variant at position 80 and transport properties of the His(27) and Arg(27) carriers. Clin Cancer Res. 2001; 11:3416–3422.

33. 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; 27:2038–2045. [PubMed: 19307503]

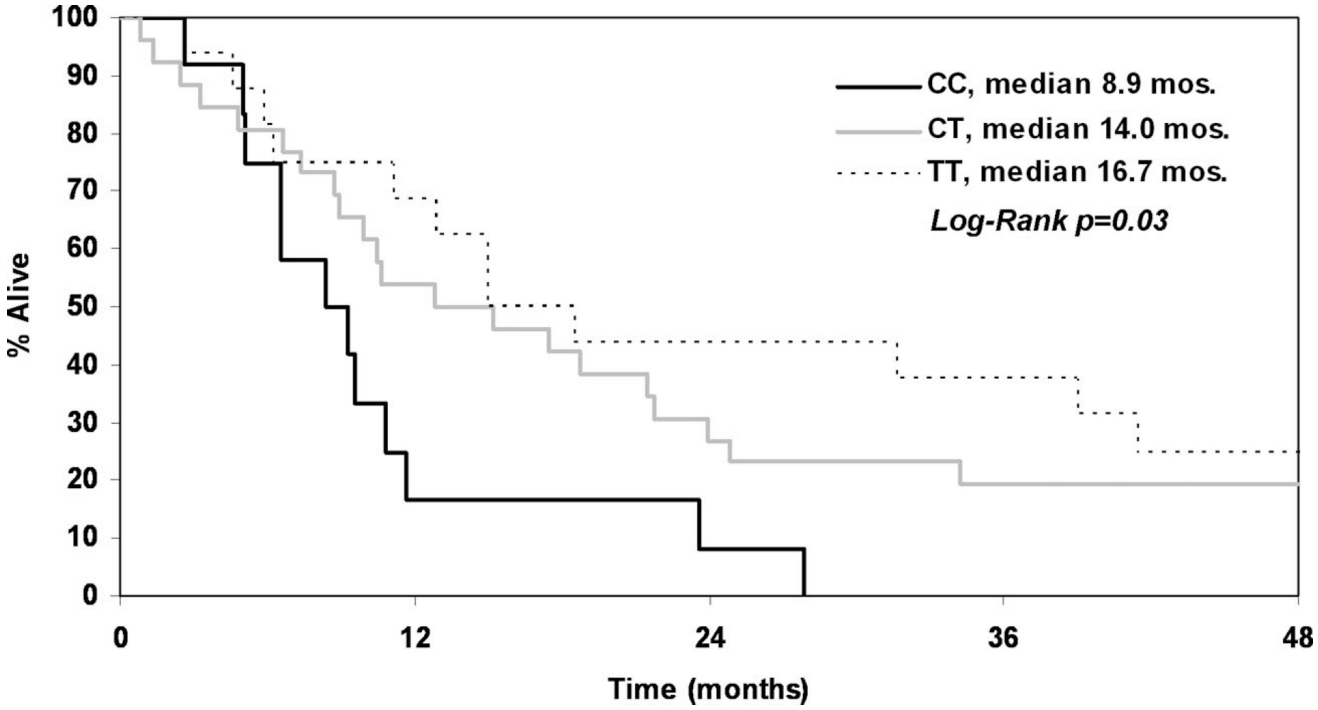

FIGURE 1.

Kaplan-Meier estimates for patients with non-small cell lung cancer (NSCLC) undergoing pemetrexed and gemcitabine combination therapy. Median overall survival (OS) was plotted by *SLC19A1* IVS4(2117)C>T polymorphism (rs2838958): CC versus CT versus TT genotypes; CC: *N*= 12, number of events = 12; CT: *N*= 26, number of events = 21; and TT: *N*= 16, number of events = 12.

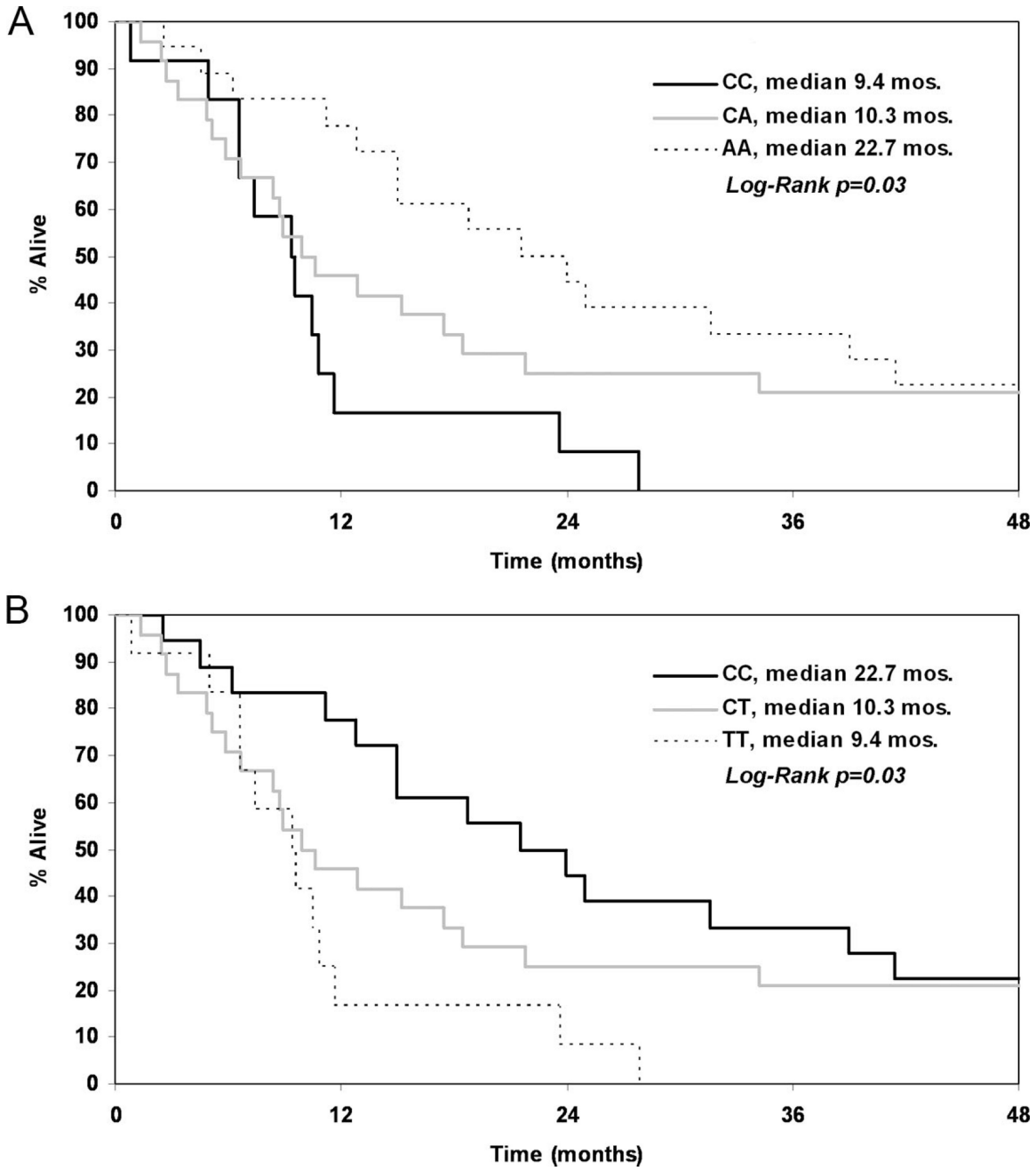

FIGURE 2.

Kaplan-Meier estimates for patients with non-small cell lung cancer (NSCLC) undergoing pemetrexed and gemcitabine combination therapy. *A*, Median overall survival (OS) was plotted by *SLC19A1* IVS5(9148)C>A polymorphism (rs3788189): CC versus CA versus AA genotypes; CC: *N*= 12, number of events = 12; CA: *N*= 24, number of events = 19; and AA: *N*= 18, number of events = 14. *B*, Median OS was plotted by *SLC19A1* exon6(2522)C>T polymorphism (rs1051298): CC versus CT versus TT genotypes; CC: *N*= 18, number of events = 14; CT: *N*= 24, number of events = 19; and TT: *N*= 12, number of events = 12. Frequency distributions of genotypes at loci were equal.

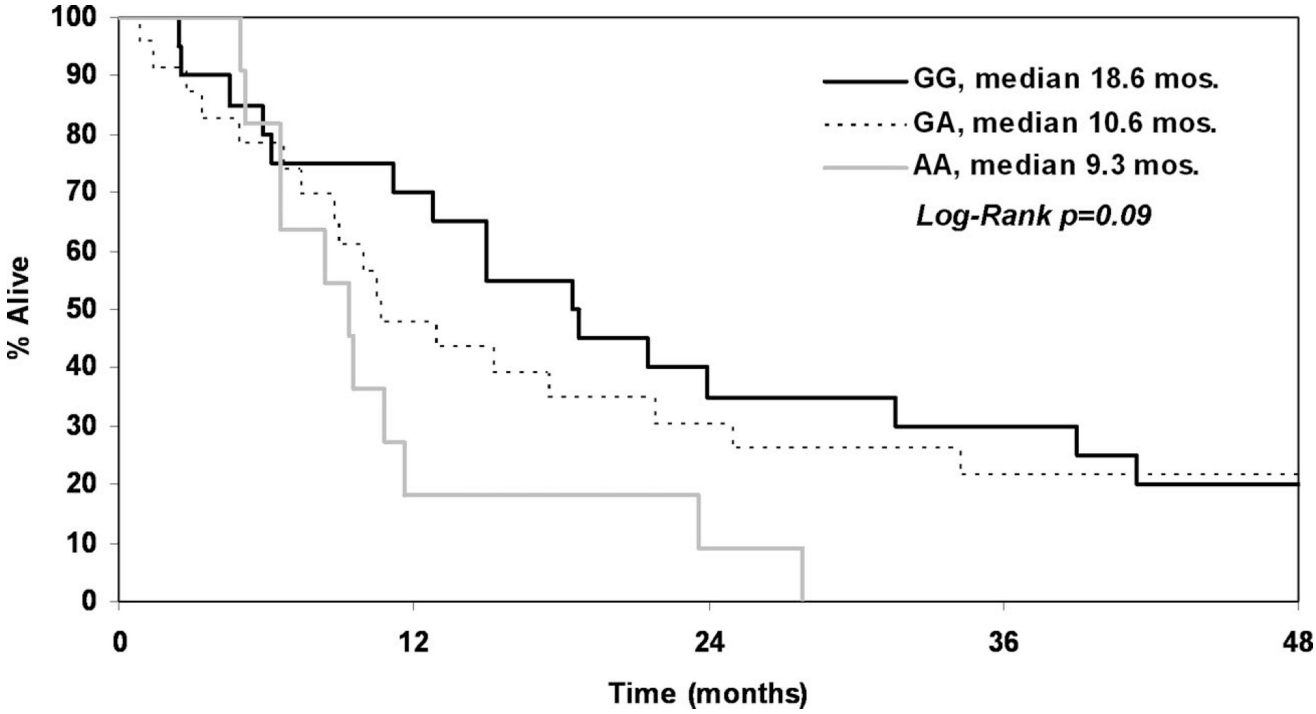

FIGURE 3.

Kaplan-Meier estimates for patients with non-small cell lung cancer (NSCLC) undergoing pemetrexed and gemcitabine combination therapy. Median overall survival (OS) was plotted by *SLC19A1* IVS2(4935)G>A polymorphism (rs914232): GG versus GA versus AA genotypes: GG: *N*= 20, number of events = 16; GA: *N*= 23, number of events = 18; and AA: *N*= 11, number of events = 11.

TABLE 1

Distribution of Genotype and Allele Frequencies in Patients

id	Gene	Gene ID/Chromosomal Location	dbSNP id	SNP Location/ Genotype	Genotype (Frequency, %) (*N* = 54)			Minor Allele Frequency (MAF), Study Sample
					Wild Type	Heterozygous	Variant	
1	*FPGS*	2356/9q34.1	rs36204706	***5FR (-747) C> T***	***CC (98.2)***	***CT (1.8)***	***TT (0.0)***	0.009
2			na	5FR(-573) C>G	CC (77.8)	CG (20.4)	***GG (1.8)***	0.120
3			na	IVS1(28) G>A	GG (85.2)	GA (13.0)	***AA (1.8)***	0.083
4			rs41307463	***IVS9(48) C>T***	***CC (96.3)***	***CT (3.7)***	***TT (0.0)***	0.019
5	*GGH*	8836/8q12.3 minus strand nucleotides shown for *GGH*	rs11545077[c]	Exon1(+91) C>T **Ala31Thr**	CC (51.9)	CT (44.4)	***TT (3.7)***	0.259
6			rs3780126	IVS1(1307) C>T	CC (33.3)	CT (51.9)	TT (14.8)	0.407
7			rs11990678	IVS2(2442) T>C	TT (79.6)	TC (18.5)	***CC (1.9)***	0.111
8			rs3780130	IVS3(1950) A>T	AA (63.0)	AT (33.3)	***TT (3.7)***	0.204
9			rs7010484	IVS5(1042) T>C	TT (55.6)	TC (37.0)	***CC (7.4)***	0.259
10			rs11995525	IVS7(1478) G>A	GG (48.2)	GA (44.4)	***AA (7.4)***	0.296
11			rs12677953	IVS7(6177) C>A	CC (75.9)	CA (24.1)	***AA (0.0)***	0.120
12	*SLC19A1*[a]	6573/21q22.3	rs914232	IVS2(4935) G>A	GG (37.0)	GA (42.6)	AA (20.4)	0.417
13			rs2838958	IVS4(2117) C>T	CC (22.2)	CT (48.2)	TT (29.6)	0.537
14			rs2297291	IVS5(391) C>T	CC (37.1)	CT (44.4)	TT (18.5)	0.407
15			rs2838956	IVS5(707) C>T	CC (16.7)	CT (48.1)	TT (35.2)	0.592
16[b]			rs3788189	IVS5(9148) C>A	CC (22.2)	CA (44.5)	AA (33.3)	0.556
17[b]			rs1051298	Exon6(2522) C>T (3'UTR)	CC (33.3)	CT (44.5)	TT (22.2)	0.444

Bold italicized items were not included in the correlations with clinical outcomes based on the distribution of the variants.

[a] Nucleotides shown for *SLC19A1* are on plus strand.

[b] Frequency distribution of wild-type CC in IVS5(9148) is the same as for variant TT in Exon6(2522) and vice versa; CC = TT, CA = CT, AA = CC.

[c] Previously known as rs13270305.

N: number of patient samples; na: no dbSNP id available.

TABLE 2

Baseline Characteristics

Characteristics	All Patients (*N* = 152)		Patients with DNA Genotyped (*N* = 54)		*p*[a]
	N	Percentage	*N*	Percentage	
Age, median (range)	65 (39–85)		65 (43–85)		0.55[b]
Gender					
Female	60	39.5	25	46.3	0.42
Male	92	60.5	29	53.7	
Homocysteine					
<12	94	61.8	34	63.0	0.65
≥12	25	16.4	11	20.4	
Unknown	33	21.7	9	16.7	
Performance score					
0	55	36.2	22	40.7	0.62
1	97	63.8	32	59.3	
Dominant disease status					
Soft tissue	31	20.4	9	16.7	0.60
Osseous	5	3.3	3	5.6	
Visceral	116	76.3	42	77.8	
Stage					
IIIB	20	13.2	4	7.4	0.33
IV	132	86.8	50	92.6	
Histology					
Squamous	31	20.4	14	25.9	0.45
Nonsquamous	117	77.0	40	74.1	
Missing	4	2.6	0	0.0	

[a] Fisher's exact test.

[b] Rank-Sum Test.

TABLE 3

Genotype and Outcomes

Adverse Event	Genotype	Frequency (%)	p^a	Odds Ratio (p)^b
Grade 3+ SGPT (ALT)	*FPGS* IVS1(28) G>A		0.09	
	GG	13		1.0 (—)
	GA	43		5.0 (0.07)
Grade 3+ fatigue	*GGH* IVS3(1950) A>T rs3780130		0.07	
	AA	29		1.0 (—)
	AT	6		0.1 (0.08)
Grade 3+ dyspnea	*GGH* IVS2(2442) T>C rs11990678		0.08	
	TT	14		1.0 (—)
	TC	40		7.3 (0.04)
Response	**Genotype**	**Frequency (%)**	p^a	**Odds Ratio (p)**^{b}
Confirmed response	*GGH* IVS5(1042) T>C rs7010484		0.10	
	TT	17		1.0 (—)
	TC	40		3.9 (0.06)
Clinical benefit (confirmed response + stable disease)	*GGH* IVS5(1042) T>C rs7010484		0.07	
	TT	60		1.0 (—)
	TC	85		4.0 (0.06)
Adverse Event	**Genotype**	**Median (months)**	p^c	**Hazard Ratio (p)**^{d}
Overall survival	*SLC19A1* IVS2(4935)G>A rs914232		0.09	
	GG	18.6		1.0 (—)
	GA	10.6		1.2 (0.71)
	AA	9.3		2.3 (0.05)
	SLC19A1 IVS4(2117)C>T rs2838958		0.03	
	CC	8.9		1.0 (—)
	CT	14.0		0.5 (0.04)
	TT	16.7		0.4 (0.02)
	SLC19A1 IVS5(9148)C>A rs3788189		0.03	
	CC	9.4		1.0 (—)
	CA	10.3		0.5 (0.08)
	AA	22.7		0.3 (0.01)
	SLC19A1 Exon6(2522)C>T rs1051298		0.03	
	CC	22.7		1.0 (—)
	CT	10.3		1.5 (0.27)
	TT	9.4		3.0 (0.01)

[a] Fisher's exact test.

[b] Logistic regression model adjusted for treatment arm. Frequency distribution of wild type CC in IVS5(9148) is the same as for variant *TT* in Exon6(2522) and vice versa; CC = TT, CA = CT, AA = CC.

[c] Log-rank test.

[d] Cox proportional hazards model adjusted for treatment arm.