DOI 10.1515/pterid-2013-0021 — Pteridines 2013; 24(1): 69–79

Ana Barcelos, Elisa Giovannetti*, Robert de Jonge, Mina Maftouh, Pieter Griffioen, Axel R. Hanauske and Godefridus J. Peters

Polymorphisms correlated with the clinical outcome of locally advanced or metastatic colorectal cancer patients treated with ALIRI vs. FOLFIRI

Abstract: Leucovorin-modulated 5-fluorouracil (5-FU) plus irinotecan (FOLFIRI) is the most common treatment of metastatic colorectal cancer (CRC). 5-FU inhibits thymidylate synthase (TS) and irinotecan topoisomerase I, leading to inhibition of DNA replication and repair. FOLFIRI efficacy suggested that other TS inhibitors might synergize with irinotecan, and Phase I/II studies for second-line treatment showed promising results of combinations with the multitargeted antifolate pemetrexed (PMX), which exerts its effects primarily via TS inhibition. However, a randomized Phase II trial of PMX + irinotecan (ALIRI) showed similar efficacy and safety, but significantly shorter progression-free survival (PFS) compared with FOLFIRI in locally advanced or metastatic CRC. In our previous aCGH study, we evaluated genome-wide copy number variations, whereas in the current study we evaluated relationships between functional polymorphisms and PFS. Candidate polymorphisms were studied by restriction fragment length polymorphism-polymerase chain reaction (RFLP-PCR) (TSER-2R/3R) or Taqman-PCR (*MTHFR-1958G>A, MTR-2756A>G, MTHFR-1298A>C, SHMT1-1420C>T, ATIC-347C>G, AMPD-134C>T, MTRR-66A>G* and *SLC-19A180G>A*) in 84 patients (40 FOLFIRI, 44 ALIRI). The Kaplan-Meier method was used to plot PFS, and the log-rank test to compare curves. At univariate analysis the homozygous variants of both *MTR-2756A>G* and *SHMT1-1420C>T* were associated with significantly shorter PFS. Conversely, a significantly longer PFS (7.3 months) was observed when *ATIC-347C>G CC+CG* genotypes were grouped vs. *GG*. At multivariate analysis the genotypes *MTR-2756A>G AA+AG, SHMT1-1420C>T TT+CT* and *ATIC-347C>G CC+CG* emerged as significant predictors for PFS. Because MTR, SHMT1 and ATIC are all involved in folate pathways, we further explored the effect of a combination of their risk genotypes on PFS, showing that patients carrying two risk genotypes had a significantly shorter PFS (3.9 months, $p<0.001$). The correlations of polymorphisms in genes with clinical outcome underscore the importance of a candidate gene-based approach. Ultimately, the validation of the role of these polymorphisms in prospective multicenter trials might optimize currently available treatments in selected CRC patients (e.g., FOLFIRI) or PMX-based treatments in other tumor types.

Keywords: 5-fluorouracil; clinical outcome; colorectal cancer; pemetrexed; polymorphisms.

***Corresponding author: Elisa Giovannetti,** Department of Medical Oncology, VU University Medical Center, Cancer Center Amsterdam, CCA 1.52, De Boelelaan 1117, 1081 HV Amsterdam, The Netherlands, Phone: +31-20-4442267, Fax: +31-20-4443844, E-mail: elisa.giovannetti@gmail.com

Ana Barcelos, Mina Maftouh and Godefridus J. Peters: Department Medical Oncology, VU University Medical Center, Amsterdam, The Netherlands

Robert de Jonge and Pieter Griffioen: Department of Clinical Chemistry, University Medical Center Rotterdam, Rotterdam, The Netherlands

Axel R. Hanauske: Eli Lilly and Company, Indianapolis, IN, USA

Introduction

Colorectal cancer (CRC) is the third most common cancer and the second cause of cancer-related death in the Western world [1]. The prognosis of CRC depends on tumor stage and treatment. For more than 50 years 5-fluorouracil (5-FU)-based therapy has remained the treatment of choice in both the adjuvant and palliative setting of CRC therapy. Currently, 5-FU or its prodrug capecitabine are given as combined therapeutic regimens including oxaliplatin, leucovorin, irinotecan, cetuximab and bevacizumab, which increased responses in first-line therapy up to 40% [2]. However, the overall prognosis is still poor, and no markers can reliably predict the subset of patients

who will respond to cytotoxic drugs [3]. Therefore, the main challenges in CRC chemotherapy rely both on the development of new combinations and on the identification of markers to predict drug sensitivity.

The leucovorin-modulated 5-FU plus irinotecan (FOLFIRI) is the most common chemotherapeutic regimen used for first-line treatment of metastatic CRC. In this regimen, cytotoxicity is achieved through thymidylate synthase (TS) inhibition by 5-FU, which leads to disturbance on DNA replication and repair, and through topoisomerase I inhibition by irinotecan, which leads to double-strand breaks. The efficacy of this regimen as well as capecitabine plus irinotecan suggested that other TS inhibitors might synergize with irinotecan, and Phase I/II studies for second-line treatment of CRC showed promising results of combinations with the multitargeted antifolate pemetrexed, which exerts its effects primarily through TS inhibition [4, 5]. However, a randomized

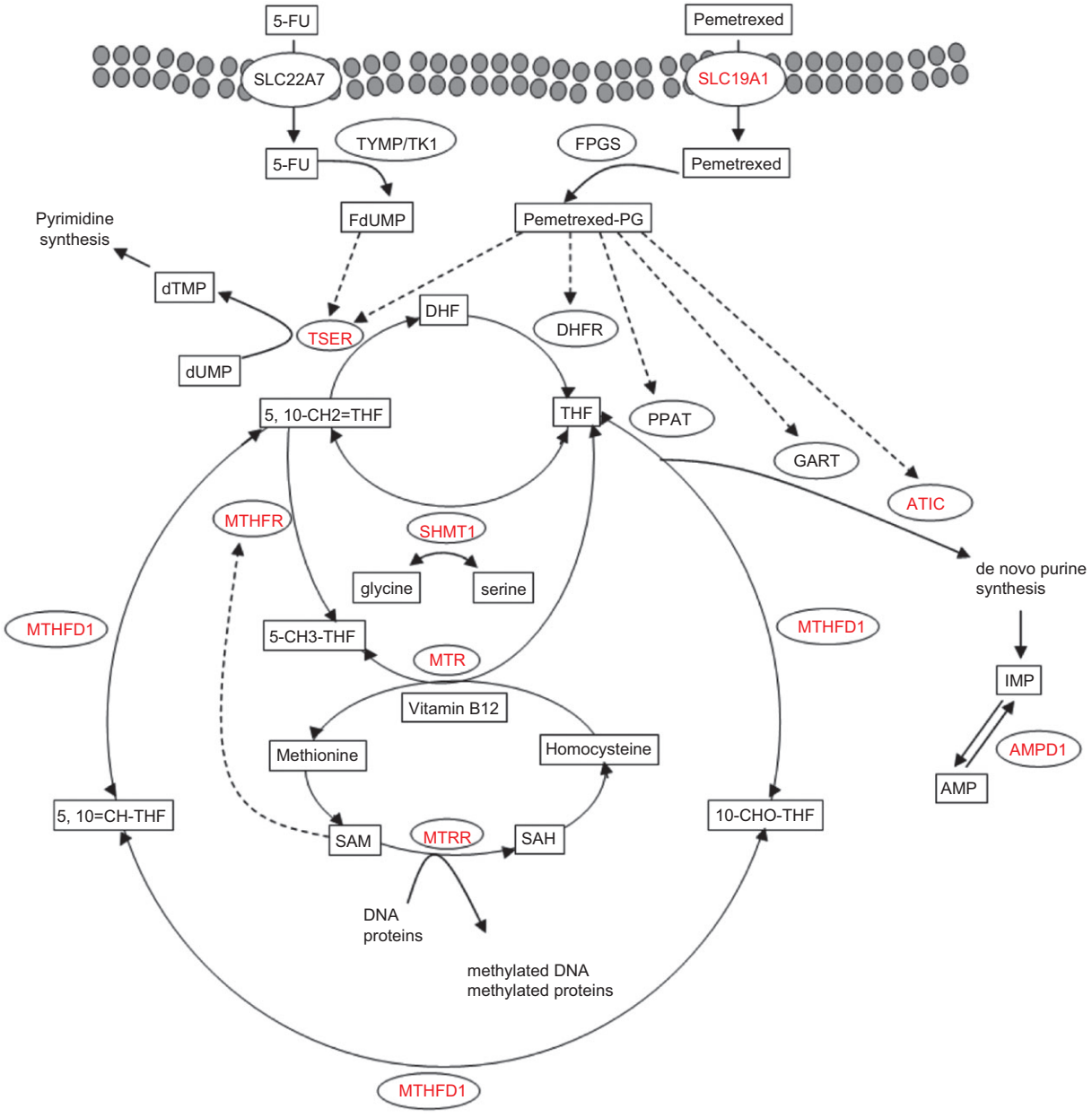

Figure 1 Scheme showing proteins involved in Pemetrexed and 5FU activity. All the enzymes encoded by the candidate genes/polymorphisms assessed in this study are marked in red. Pemetrexed is transported into the cells through SLC19A1 and is metabolized by FPGS to its active metabolite. Pemetrexed polyglutamate forms inhibit TS, DHFR, GARFT, ATIC and MTHFD1L, fundamental for the de novo biosynthesis of thymidine and purine nucleotides. See for further explanation the text.

Phase II trial of pemetrexed + irinotecan (ALIRI) showed similar efficacy and safety, but significantly shorter progression-free survival (PFS) compared with FOLFIRI treatment [4, 6].

These results prompted further molecular studies on key determinants of activity for different TS inhibitors (Figure 1). In particular, pemetrexed is an antifolate agent that acts by disturbing critical folate-dependent metabolic processes. Folates are required for the synthesis of thymidine and purines and also to generate methionine, which plays an important role in protein synthesis, polyamine synthesis and transmethylation, essential for cell proliferation [7].

Several proteins can affect pemetrexed activity (Figure 1). This drug is transported into the cells mainly through the reduced folate carrier (SLC19A1) system and the proton coupled folate transporter (PCFT). It is extensively metabolized by folylpolyglutamate synthetase to more active metabolites. In vitro studies have shown that pemetrexed polyglutamation dramatically increases its affinity to TS, dihydrofolate reductase (DHFR), and glycinamide ribonucleotide formyltransferase (GARFT), and in a lesser extent to aminoimidazole carboxamide ribonucleotide formyltransferase (ATIC) and methylenetetrahydrofolate dehydrogenase ($NADP^+$-dependent) 1-like (MTHFD1L), thus inhibiting their enzymatic activity [8–10]. All these folate-dependent enzymes are fundamental for de novo biosynthesis of thymidine and purine nucleotides. TS catalyzes the methylation of deoxyuridylate (dUMP) to deoxythymidylate (dTMP) using 5,10-methylenetetrahydrofolate (methylene-THF) as a cofactor. This function maintains the dTMP (thymidine-5′ monophosphate) pool critical for DNA replication and repair. DHFR converts dihydrofolate into tetrahydrofolate (THF), a methyl group shuttle required for de novo synthesis of purines, dTMP and certain amino acids. The protein encoded by *GARFT* is a trifunctional polypeptide, which has phosphoribosylglycinamide formyltransferase, phosphoribosylglycinamide synthetase and phosphoribosylaminoimidazole synthetase activity that are also required for de novo purine biosynthesis. The *ATIC* gene encodes a bifunctional protein that catalyzes the last two steps of the de novo purine biosynthetic pathway. The N-terminal domain has phosphoribosylaminoimidazole-carboxamide formyltransferase activity, and the C-terminal domain has IMP cyclohydrolase activity. MTHFD1L is an enzyme involved in THF synthesis in mitochondria. One-carbon substituted forms of THF are involved in de novo synthesis of purines and thymidylate, as well as in cellular methylation reactions through the regeneration of methionine from homocysteine [11].

The identification of genetic variables that predict resistance, sensitivity or toxicity to chemotherapy is of major interest in order to determine which patients would benefit most from standard therapy in terms of safety and efficacy. Moreover, although pemetrexed is not used for the treatment of CRC, the analysis from patients treated with ALIRI is an important step for the development of clinical pharmacogenetic models to understand genetic variations influencing the impact of this TS inhibitor that might apply to other tumor types.

Gene polymorphisms of *TSER, MTHFR-677C>T, MTHFR-1298A>C, MTHFD-1958G>A, MTR-2756A>G, MTRR-66A>G, SHMT-1420C>T* and *ATIC-347C>G* have been correlated with the clinical outcome in patients receiving different chemotherapeutic agents including antifolates and TS inhibitors [12–21]. Nevertheless, few data were published relating these polymorphisms to treatment outcome in patients with CRC treated with 5-FU or pemetrexed. Similarly, although so far no data have been published relating treatment outcome to *SLC-19A180G>A* and *AMPD-134C>T* polymorphisms, these two polymorphisms also play an important role in pemetrexed transport and purine nucleotide cycle and might influence clinical outcome.

Therefore, the main aim of this study was to identify a possible relation between these polymorphisms and PFS in patients with locally advanced or metastatic CRC treated with pemetrexed and/or 5-FU based therapy. The secondary aims were to identify a possible relation of these polymorphisms with treatment toxicity or clinical response.

Materials and methods

Patients

This multicenter pharmacogenetic study involved a number of patients enrolled from February 2004 to September 2005 in Australia, Germany, Greece, The Netherlands, Spain and Russia [6]. The clinical outcome of this study (JMAZ) has been published separately [6]. Patients ≥ 18 years of age with histologically or cytologically confirmed adenocarcinoma of the colon or rectum with locally advanced or metastatic disease were enrolled. Eligible patients had at least one unidimensional measurable lesion defined by response evaluation criteria in solid tumors (RECIST) [22], a life expectancy ≥12 weeks, an Eastern Cooperative Oncology Group performance status (ECOG PS) of 0–2, and adequate marrow reserve, hepatic and renal function. No previous chemotherapy for advanced disease was allowed. Prior adjuvant therapy (except irinotecan) was allowed if given ≥12 months before enrolment. All patients provided written informed consent. The study was performed according to the Declaration of Helsinki, and the protocol was reviewed and approved by the appropriate ethical review board.

Study design and treatment

This pharmacogenetic study assessed data from patients enrolled during the Randomized Phase II Trial of Pemetrexed plus Irinotecan (ALIRI) vs. Leucovorin-modulated 5-FU plus Irinotecan (FOLFIRI) in first-line treatment of locally advanced or metastatic colorectal cancer (clinicaltrial.gov identifier NCT00079872) [6]. Eligible patients were randomized in a 1:1 manner to receive pemetrexed 500 mg/m^2 and irinotecan 350 mg/m^2 on day 1 of each 21-day cycle (ALIRI arm) or 5-FU (400 mg/m^2 bolus and 600 mg/m^2 as a 22-h infusion) and leucovorin 200 mg/m^2 on days 1, 2, 15, 16, 29 and 30 of a 42-day cycle and irinotecan 180 mg/m^2 on days 1, 15 and 29 (FOLFIRI arm).

Baseline and treatment assessment

Pretreatment evaluation included a complete medical examination, ECOG PS, baseline tumor measurement via magnetic resonance imaging and clinical laboratory tests. Tumor measurements were repeated before every other cycle for patients receiving ALIRI and before every cycle for patients receiving FOLFIRI. Body surface area calculations, body weight, clinical laboratory tests and toxicity assessments according to the National Cancer Institute (NCI) Common Toxicity Criteria, version 2.0, were completed before each cycle.

Candidate polymorphisms

Candidate genes/polymorphisms were selected via a functional approach in order to evaluate determinants known to modulate drug metabolism and mechanism of action (Figure 1, Table 1). Therefore, we used the following criteria: (i) that the gene was part of a pathway for which there is a credible scientific basis to support its involvement in the study of drug activity; (ii) that the gene had an established, well-documented genetic polymorphism; (iii) that the frequency of the polymorphism was high enough that its effect on clinical outcome would be meaningful; and (iv) that the polymorphism had some degree of likelihood to alter the function of the gene in a biologically relevant manner. The *SLC1-19A180G>A* polymorphism might affect pemetrexed transport into malignant cells [23], whereas *TSER* genotypes might be associated with tumoral TS level; nevertheless, their value as a predictor of response to treatment with 5-FU is still controversial [20, 24, 25]. The *ATIC-347C>G* polymorphism might play an important role in sensitivity to the drug and in purine biosynthesis [26], whereas the *MTHFR-677C>T* and *1298A>C* polymorphisms might affect folate distribution either to DNA synthesis or to homocysteine remethylation [27]. Furthermore, the *MTHFD1-1958G>A* polymorphism might affect *MTHFD* expression which is involved in folate and methionine metabolism [7], whereas the *MTR-2756A>G* and *MTRR-66A>G* polymorphisms might affect the expression of *MTR* and *MTRR*, respectively, which are critical in methionine synthesis [28]. Finally, the *SHMT1-1420C>T* polymorphism might affect *SHMT* expression, which plays a pivotal role in the reversible conversion of serine and THF to glycine and methylene-THF [28], whereas AMPD catalyzes the irreversible deamination of adenosine monophosphate to inosine monophosphate [29] and plays a role in the purine nucleotide cycle.

Genotyping

DNA was isolated from pretreated blood samples using the QIAamp DNA mini kit (Qiagen, Venlo, the Netherlands) according to the manufacturer's instructions, and DNA yields and integrity were checked at 260–280 nm with the NanoDrop®-1000-Detector (NanoDrop Technologies, Wilmington, DE, USA). *TSER* and *MTHFR-677C>T* polymorphisms were examined by the Laboratory of Medical Oncology (VU University Medical Center, Amsterdam, The Netherlands), whereas *MTHFR-1298A>C, MTHFD1-1958G>A, MTR-2756A>G, MTRR-66A>G, SHMT1-1420 C>T, SLC-19A180G>A, ATIC-347C>G* and *AMPD1–34C>T*

Table 1 Clinical outcome according to polymorphisms.

Polymorphism	Genotype	Patients n (%)	PFS month (95% CI)	p-Value
TSER	*2R*	19 (24.4)	7.3 (6.4–8.2)	0.184
	3R	32 (41.0)	7.2 (4.0–10.4)	
	2R/3R	27 (34.6)	6.9 (6.2–7.7)	
	3R+2R/3R	59 (75.6)	7.2 (6.3–8.1)	0.795
MTHFR-677 C>T	*CC*	40 (48.2)	6.8 (5.2–8.3)	0.183
	TT	9 (10.8)	9.6 (6.6–12.6)	
	CT	34 (41.0)	7.7 (6.4–8.0)	
	CC+CT	74 (89.1)	6.9 (6.1–7.7)	0.065
MTHFR-1298 A>C	*AA*	41 (49.4)	7.7 (6.7–8.6)	0.712
	CC	5 (6.0)	4.8 (1.6–8.0)	
	AC	37 (44.6)	6.9 (6.4–8.5)	
	AA+AC	78 (79.0)	7.3 (6.6–8.0)	0.970
MTHFD-1958 G>A	*AA*	16 (19.0)	7.4 (6.2–8.5)	0.915
	GG	27 (32.1)	7.3 (6.1–8.5)	
	GA	41 (48.8)	6.8 (5.0–8.5)	
	GG+GA	68 (80.9)	7.2 (6.2–8.2)	0.739
MTR-2756 A>G	*AA*	50 (59.5)	7.2 (6.6–7.9)	0.012
	GG	7 (8.3)	4.8 (4.0–5.6)	
	AG	27 (32.1)	9.0 (6.0–12.0)	
	AA+AG	77 (91.7)	7.3 (6.6–8.0)	0.034
MTRR-66 A>G	*AA*	16 (19.5)	7.3 (7.1–7.5)	0.409
	GG	22 (26.8)	5.7 (4.8–6.6)	
	AG	44 (53.7)	8.8 (6.6–11.1)	
	AA+AG	60 (73.2)	7.6 (6.5–8.8)	0.205
SHMT1-1420 C>T	*CC*	39 (47.0)	5.6 (4.3–7.0)	0.039
	TT	4 (4.8)	7.8 (1.4–14.1)	
	CT	40 (48.2)	8.3 (7.1–9. 6)	
	TT+CT	44 (95.2)	8.3 (7.0–9.6)	0.025
SLC19A1-80 G>A	*AA*	15 (18.3)	8.0 (5.6–10.4)	0.326
	GG	24 (29.3)	6.9 (4.1–9.6)	
	GA	43 (52.4)	7.2 (6.2–8.3)	
	GG+GA	67 (81.7)	7.2 (6.5–7.8)	0.136
ATIC-347 C>G	*CC*	37 (44.6)	7.3 (6.5–8.1)	0.057
	GG	5 (6.0)	3.9 (2.6–5.2)	
	CG	41 (49.4)	7.2 (4. 9–9.6)	
	CC+CG	78 (94.0)	7.3 (6.6–8.0)	0.020
AMPD-134 C>T	*CC*	62 (74.7)	7.4 (6.4–8.3)	0.415
	TT	1 (1.2)	6.7 (5.4–8.8)	
	CT	20 (24.1)	7.1 (6.4–8.0)	
	CC+CT	82 (98.8)	7.2 (6.4–8.0)	0.531

Genotyping for *MTHFR-1958 G>A* and *MTR-2756 A>G*, was successfully performed in all the available blood samples, while a total of 83 samples out of the 84 patients were evaluable for *MTHFR-677 C>T, MTHFR-1298 A>C, SHMT1-1420 C>T, ATIC-347 C>G* and *AMPD-134 C>T*, 82 out of the 84 patients for *MTRR-66 A>G* and *SLC19A1-80 G>A* and 78 out of 84 patients for *TSER*.

polymorphisms were assessed by the Laboratory of Clinical Chemistry (Erasmus MC, Rotterdam, The Netherlands).

The *TSER* genotype was assessed by polymerase chain reaction (PCR) amplification of the enhancer region (5′UTR) of the TS gene using a forward primer (5′-*GTG GCT CCT GCG TTT CCC CC*-3′) and a reverse primer (5′-*GCT CCG AGC CGG CCA CAG GCA TGG CGC GG*-3′) as described previously [14, 24].

All other polymorphisms were evaluated with Taqman® probes based assays using the ABI PRISM-7000 Sequence Detection System instrument equipped with SDS version 1.3.0 software (Applied Biosystems, Leusden, the Netherlands). These PCR reactions were performed using 1.2 µL DNA sample and 5 µL reagents, in a total reaction volume of 6.2 µL. The reaction mixture underwent the following thermocycling conditions: 95°C for 10 min, 40 amplification cycles at 92°C for 15 s followed by a final annealing and extension step at 60°C for 1 min. Details of the genetic variants, primers and probes are in Supplemental Table 1.

Statistical analysis

The primary endpoint was to investigate the association between pharmacogenetic data and PFS, whereas the secondary endpoints included the association between polymorphisms and clinical response, clinical benefit and treatment toxicity. All analyses of the samples were done in a blinded manner relative to clinical outcome.

Genotype frequencies were checked for agreement with those expected under the Hardy-Weinberg equilibrium with the web-based SNP analyzer software (http://snp.istech21.com/snpanalyzer/2.0/).

PFS was defined as the time from study enrolment to disease progression or death from any cause, as appropriate. As this study was not designed to estimate overall survival (OS), no follow-up was performed on patients after relapse and second-line therapy. The Kaplan-Meier method was used to plot PFS, and the log-rank test to compare curves.

Objective response was evaluated according to RECIST criteria. Toxicity was assessed by the NCI Common Toxicity Criteria, version 2.0.

Demographic and clinical information were compared by treatment group, using Pearson's χ^2 two-sided test. This test was also used to compare clinical information (assessed as PFS, clinical response and toxicity) across genotype. The univariate analysis included different baseline demographic and clinical characteristics, such as gender, age, PS and clinical stage. Statistical analyses were performed grouping patients according to three different genotypes for each polymorphism. Further analyses were performed: (i) collapsing the homozygous and heterozygous genotypes (for all the population) when they had the same direction of effect (e.g., both had reduced/increased PFS compared with the reference group) and (ii) combining variant alleles associated with worse outcome (risk genotype). The significant prognostic variables in the univariate analysis were included in multivariate analyses, using Cox's proportional hazards model.

Data were analyzed using SPSS-16 software (SPSS, Inc., Chicago, IL, USA). All analyses were two-sided and a p-value< 0.05 was considered statistically significant.

As this is an exploratory study, no correction for multiple testing was performed.

Results

Genotyping analyses were performed in 84 patients. Patient age ranged from 33 to 78 years. Five of these patients had no metastatic disease, whereas ECOG PS was rated 0 for 25, 1 for 52, and 2 for 7 patients, respectively. Forty patients underwent treatment with the FOLFIRI regimen, and 44 were treated the ALIRI regimen. No significant difference in any clinical feature was observed between patients treated with these regimens, and no significant difference was observed between each regimen and PFS (Figure 2A).

Polymorphisms and outcome

Genotyping was successfully performed in the vast majority of DNA samples. All polymorphisms except *TSER*

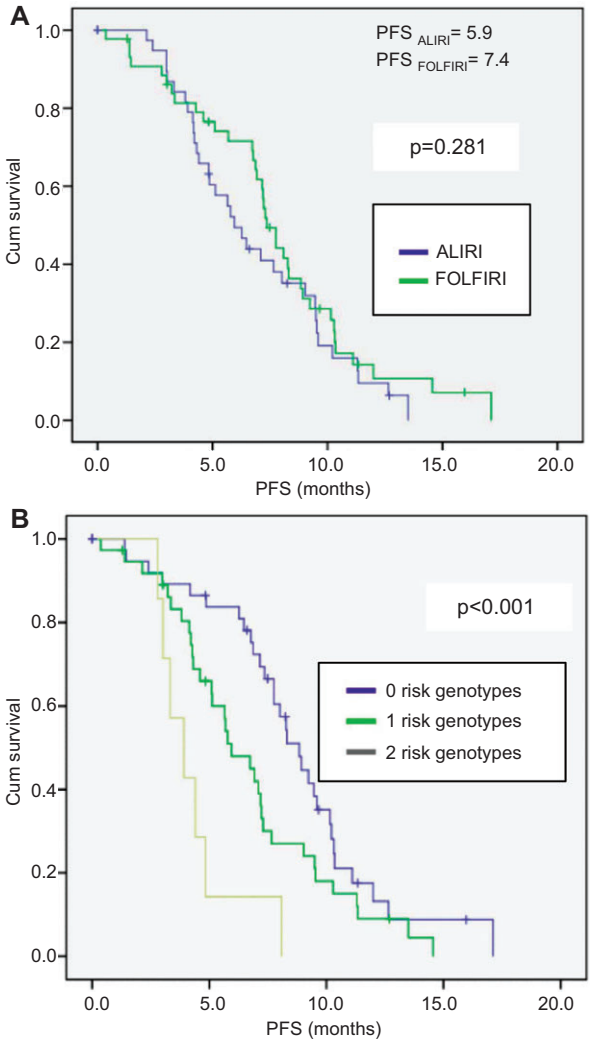

Figure 2 Kaplan-Meier curves showing progression free survival according to treatment group (A) ALIRI or FOLFIRI (A); or according to (B) number of risk genotypes present.

(p=0.04) followed the Hardy-Weinberg equilibrium. As this analysis was performed in individuals from different geographic areas (e.g., 64% from Australia and Russia), population substructure is the most likely explanation for this deviation. However, the allelic frequencies of all the studied polymorphisms are consistent with those observed in previous reports and in the NCBI and NCI-SNP500 databases (Table 1).

At univariate analysis, polymorphisms of *MTR-2756A>G, SHMT1-1420C>T* and *ATIC-347C>G* were significantly associated with PFS (Table 2). In particular, a significantly shorter PFS was observed in patients harboring the *MTR-2756A>G GG* genotype, who had a median PFS of 4.8 months [95% confidence interval (CI), 1.6–8.0 months], in comparison with patients carrying the *AA or AG* genotype (median PFS, 7.3 months; 95% CI, 6.6–8.0 months). Similarly, shorter PFS (5.6 months; 95% CI, 4.3–7.0 months) was observed in patients harboring the *SHMT1-1420C>T CC* genotype, in comparison with patients carrying the *SHMT1-1420C>T CT+TT* genotype

Table 2 Polymorphisms impact on PFS according to treatment.

Polymorphism	Genotype	ALIRI			FOLFIRI		
		Patients n (%)	PFS month	p-Value	Patients n (%)	PFS month	p-Value
TSER	*2R*	8	4.9 (4.4–5.3)	0.027	11	8.1 (5.8–10.4)	0.304
	3R	16	9.0 (4.7–13.3)		16	7.2 (5.3–9.0)	
	2R/3R	13	5.6 (3.0–8.3)		14	7.2 (6.4–8.0)	
	3R+2R/3R	29	7.6 (5.0–10.3)	0.57	30	7.2 (6.6–7.8)	0.186
MTHFR-677 C>T	*CC*	19	5.1 (3.9–6.3)	0.121	21	7.4 (5.7–9.0)	0.704
	TT	5	11.3 (4.4–18.2)		4	8.8 (6.2–11.5)	
	CT	16	6.5 (2.8–10.1)		18	7.8 (6.6–8.9)	
	CC+CT	35	5.8 (4.3–7.2)	0.055	39	7.4 (6.7–8.0)	0.553
MTHFR-1298 A>C	*AA*	22	7.6 (5.6–9.7)	0.007	19	7.3 (6.6–8.0)	0.515
	CC	3	3.3 (1.4–5.2)		2	6.8 (–)	
	AC	15	5.6 (3.5–7.8)		22	7.8 (5.4–10.1)	
	AA+AC	37	6.5 (4.6–8.3)	0.003	41	7.8 (7.0–8.5)	0.249
MTHFD-1958 G>A	*AA*	6	4.1 (0.5–7.8)	0.626	10	7.8 (6.4–9.1)	0.984
	GG	11	5.9 (2.0–9.9)		16	7.3 (5.6–9.0)	
	GA	23	5.8 (3.9–7.6)		18	7.2 (6.0–8.5)	
	GG+GA	34	5.9 (4.8–7.1)	0.374	34	7.3 (6.5–8.1)	0.866
MTR-2756 A>G	*AA*	23	5.6 (3.3–8.0)	0.067	27	7.8 (7.0–8.4)	0.080
	GG	2	4.4 (–)		5	5.1 (1.2–9.0)	
	AG	15	9.0 (5.4–12.6)		12	6.9 (0.0–14.1)	
	AA+AG	38	6.3 (4.4–8.1)	0.205	39	7.8 (6.6–9.0)	0.058
MTRR-66 A>G	*AA*	3	5.9 (–)	0.747	13	7.3 (7.1–7.5)	0.682
	GG	16	5.6 (4.5–6.8)		6	6.9 (3.5–10.4)	
	AG	21	7.1 (0.0–15.8)		23	8.8 (7.1–10.6)	
	AA+AG	24	7.1 (2.4–11.8)	0.524	36	7.8 (6.6–8.9)	0.586
SHMT1-1420 C>T	*CC*	22	5.1 (3.5–6.7)	0.632	17	5.7 (1.6–9.8)	0.022
	TT	–	–		4	7.8 (1.4–14.1)	
	CT	18	8.0 (5.0–11.0)		22	8.8 (7.9–9.8)	
	TT+CT	18	8.0 (5.0–11.0)	0.632	26	8.8 (7.8–9.9)	0.009
SLC19A1-80 G>A	*AA*	6	4.2 (0.0–9.0)	0.533	9	9.2 (4.4–14.1)	0.076
	GG	11	6.3 (1.0–11.5)		13	6.9 (3.9–9.9)	
	GA	22	5.9 (4.8–7.1)		21	7.8 (7.0–8.5)	
	GG+GA	33	5.9 (4.8–7.0)	0.359	34	7.4 (6.7–8.0)	0.048
ATIC-347 C>G	*CC*	16	4.9 (2.0–7.7)	0.069	21	7.4 (6.6–8.1)	0.549
	GG	5	3.9 (2.6–5.2)		–		
	CG	19	7.1 (5.0–9.2)		22	7.8 (4.2–11.3)	
	CC+CG	35	6.5 (4.7–8.2)	0.024	43	7.8 (6.7–8.8)	[a]
AMPD-134 C>T	*CC*	30	5.8 (3.8–7.8)	0.643	32	7.8 (6.5–9.0)	0.316
	TT	–	–		1	6.7 (–)	
	CT	10	5.9 (3.7–8.2)		10	7.2 (3.5–10.9)	
	CC+CT	40	5.9 (4.4–7.5)	[a]	42	7.8 (6.7–8.8)	0.250

[a]No comparison analysis performed because no patients in the FOLFIRI arm have the GG genotype

(median PFS of 8.3 months; 95% CI, 7.0–9.6 months). Finally, a significantly longer PFS (7.3 months; 95% CI, 6.6–8.0 months) was also observed when *ATIC-347C>G CC+CG* genotypes were grouped vs. *ATIC-347 GG*.

At multivariate analysis the genotypes *MTR-2756A>G AA+AG, SHMT1-1420C>T TT+CT* and *ATIC-347C>G CC+CG* emerged as significant predictors for PFS (Table 3).

Because *MTR, SHMT1* and *ATIC* are all involved in folate pathways, we further explored the effect of a combination of their risk genotypes on PFS. The deleterious genotypes included *MTR-2756A>G GG, SHMT1-1420C>T CC* and *ATIC-347C>G GG*, all of which were related to shorter PFS (Figure 2B). These combinations were analyzed in 81 patients, and patients carrying two risk genotypes had a significantly shorter PFS (3.9 months; 95% CI, 2.4–5.4 months; p<0.001). Patients with two risk genotypes were *MTR-2756A>G GG/SHMT-1420C>T CC* or *SHMT-1420C>T CC/ATIC-347C>G GG* carriers. However, no significant difference on PFS was observed between these two groups.

At univariate analysis by treatment group (Table 2), polymorphisms of *TSER, MTHFR-1298A>C* and *ATIC-347C>G* were associated with significantly differential PFS in the ALIRI group. In particular, the less common variant genotypes of *MTHFR-1298A>C* and *ATIC-347C>G* polymorphisms were associated with decreased PFS. However, the more common polymorphic variant of *SHMT1-1420C>T* and the less common variant of *SLC19A180G>A* were associated with significantly shorter PFS in the FOLFIRI group.

The analysis of the correlation with clinical response (Table 4) revealed a significant association with the polymorphism *MTHFD-1958G>A*. Patients harboring the *MTHFD-1958G>A AA* genotype achieved a significantly higher rate of clinical response (56%), in comparison with patients carrying the *MTHFD-1958 G>A GG* and *GA* genotypes (26% and 19.5%, respectively; p=0.025), or when compared with the grouped *MTHFD-1958G>A GG+GA* genotype (22%; p=0.020).

The results of the correlation with toxicity are described in Table 5, showing that the polymorphism *MTHFR-677C>T* was significantly associated with blood and bone marrow grade 3–4 toxicity. In particular, the patients carrying the *CC* genotype experienced a significantly higher frequency of blood and bone marrow grade 3–4 toxicities (50%) compared with *MTHFR-677C>T TT+CT* grouped genotypes (25.6%; p=0.038).

Table 3 Multivariate analysis.

Covariates for PFS	HR (95% CI)	Wald p-Value
MTR-2756AG polymorphism: *AA+AG vs. GG*	0.4 (0.2–1.0)	0.047
SHMT1-1420CT polymorphism: *TT+CT vs. CC*	0.6 (0.4–0.9)	0.031
ATIC-347CG polymorphism: *CC+CG vs. GG*	0.3 (0.1–0.7)	0.011

HR, Hazard ratio; PFS, progression free survival.

Table 4 Patients with clinical response by polymorphisms.

Polymorphism	Genotype	Patients n (%)	p-Value
TSER	*2R*	19 (24.4)	0.813
	3R	32 (41.0)	
	2R/3R	27 (34.6)	
	3R+2R/3R	59 (75.6)	0.506
MTHFR-677 C>T	*CC*	40 (48.2)	0.807
	TT	9 (10.8)	
	CT	34 (41.0)	
	CC+CT	74 (89.2)	0.758
MTHFR-1298 A>C	*AA*	41 (49.2)	0.833
	CC	5 (6.0)	
	AC	37 (44.6)	
	AA+AC	78 (94.0)	0.758
MTHFD-1958 G>A	*AA*	16 (19.0)	0.025
	GG	27 (32.1)	
	GA	41 (48.8)	
	GG+GA	68 (81.0)	0.020
MTR-2756 A>G AA	*AA*	50 (59.5)	0.106
	GG	7 (32.1)	
	AG	27 (32.1)	
	AA+AG	77 (91.7)	0.823
MTRR-66 A>G	*AA*	16 (19.5)	0.194
	GG	22 (26.8)	
	AG	44 (53.7)	
	AA+AG	60 (73.2)	0.052
SHMT1-1420 C>T	*CC*	39 (47.0)	0.139
	TT	4 (4.8)	
	CT	40 (48.2)	
	CC+CT	79 (95.2)	0.592
SLC19A1-80 G>A	*AA*	15 (18.3)	0.909
	GG	24 (29.3)	
	GA	43 (52.4)	
	GG+GA	67 (81.7)	0.707
ATIC-347 C>G	*CC*	37 (44.6)	0.833
	GG	5 (6.0)	
	CG	41 (49.4)	
	CC+CG	78 (94.0)	0.758
AMPD-134 C>T	*CC*	62 (74.7)	0.384
	TT	1 (1.2)	
	CT	20 (24.1)	
	CC+CT	82 (98.2)	0.288

Discussion

This study evaluated the impact of functional polymorphisms on clinical outcome in locally advanced

Table 5 Blood and bone marrow grade 3-4 toxicity by polymorphisms.

Polymorphism	Genotype	n (% within genotype)	p-Value
TSER	*2R*	7 (36.8)	0.955
	3R	12 (37.5)	
	2R/3R	11 (40.7)	
	3R+2R/3R	23 (39.0)	0.868
MTHFR-677 C>T	*CC*	20 (50.0)	0.062
	TT	3 (33.3)	
	CT	8 (23.5)	
	TT+CT	11 (25.6)	0.038
MTHFR-1298 A>C	*AA*	17 (41.5)	0.707
	CC	2 (40.0)	
	AC	12 (32.4)	
	AA+AC	29 (37.2)	1.000
MTHFD-1958 G>A	*AA*	3 (18.8)	0.209
	GG	10 (37.0)	
	GA	18 (43.9)	
	GG+GA	28 (41.2)	0.166
MTR-2756 A>G	*AA*	19 (38.0)	0.420
	GG	1 (14.3)	
	AG	11 (40.7)	
	AA+AG	30 (39.0)	0.375
MTRR-66 A>G	*AA*	8 (50.0)	0.433
	GG	8 (36.4)	
	AG	14 (31.8)	
	GG+AG	22 (33.3)	0.341
SHMT1-1420 C>T	*CC*	14 (35.9)	0.279
	TT	3 (75.0)	
	CT	14 (35.0)	
	CC+CT	28 (35.0)	0.277
SLC19A1-80 G>A	*AA*	5 (33.3)	0.912
	GG	9 (37.5)	
	GA	17 (39.5)	
	GG+GA	26 (38.8)	0.920
ATIC-347 C>G	*CC*	15 (40.5)	0.666
	GG	1 (20.0)	
	CG	15 (36.6)	
	CC+CG	30 (38.5)	0.726
AMPD-134 C>T	*CC*	24 (38.7)	0.707
	TT	0 (0)	
	CT	7 (35.0)	
	CC+CT	31 (37.3)	1.000

or metastatic CRC patients treated with ALIRI and/or FOLFIRI, and to our knowledge, is the first study to suggest the role of *MTR-2756A>G*, *SHMT1-1420C>T* and *ATIC-347C>G* polymorphisms as possible predictive factors for PFS. It is also the first study to show the correlation of the *SHMT1-1420C>T* and *ATIC-347C>G* polymorphisms with PFS in patients treated with FOLFIRI and ALIRI regimens, respectively.

Of note, in patients evaluated for polymorphism analysis no significant difference of median PFS was observed between the two regimens. Probably due to smaller sample size, this result was different from that obtained by Underhill et al. [6] where a significant longer PFS was observed on patients submitted to the FOLFIRI regimen. However, the lack of significant differences between PFS allowed us to perform all assessments on the pooled data, except for the analysis of polymorphisms on PFS by specific treatment group.

The most important results observed in this study include: (i) the shorter PFS for *MTR-2756A>G GG*, *SHMT-1420C>T CC* and *ATIC-347C>G GG* polymorphisms, at univariate analysis; (ii) the significant impact of *MTR-2756A>G AA+AG*, *SHMT1-1420C>T TT+CT* and *ATIC-347C>G CC+CG* polymorphisms on risk of progression, as observed at multivariate analysis; and (iii) the strong combined effect on PFS for *MTR-2756A>G*, *SHMT1-1420C>T* and *ATIC-347C>G* polymorphisms when combined risk genotypes were analyzed. These results are in accordance with the fact that all mentioned genes have a significant role in the folate pathways and consequent DNA synthesis.

The *SHMT1-1420C>T* polymorphic variant *"T"* produces a modified protein (L474F) that, although not affecting catalytic activity, impairs *SHMT1* nuclear transport and, subsequently, TS activity. This modified protein accumulates in the cytoplasm where it inhibits cellular methylation reactions by sequestering 5-methyl-THF [30]. 5-Methyl-THF is required to convert homocysteine into methionine, which is needed for the synthesis of S-adenosylmethionine (SAM). SAM plays an important role in intracellular methylation reactions, including DNA methylation, which is one of the critical molecular mechanisms for DNA stability [31]. Furthermore, the lack of 5-methyl-THF due to sequestration by L474F leads to an increase of homocysteine concentrations which has been related to increased DNA damage [32]. All these mechanisms can explain why homozygous *TT* carriers might be more sensitive to cytotoxic activity of TS inhibitors and antifolates, resulting in significantly longer PFS.

To date, no association was found between *ATIC* polymorphisms and the effect of pemetrexed in cancer. However, a correlation with the activity of MTX was hypothesized by Weisman et al., suggesting that *ATIC-347C>G GG* encoded for a protein causing decreased de novo purine synthesis [33]. The results obtained in the present study are in accordance with previous studies in rheumatoid arthritis and psoriasis, showing that the "*C*" allele was associated with lower disease activity [16, 34, 35].

When the impact of polymorphisms on PFS was assessed by treatment group, *TSER-3R*, *MTHFR-1298A>C AA* and *ATIC-347C>G CC+CG* polymorphisms were significantly associated with longer PFS in patients treated with ALIRI. Several studies reported controversial data about

the impact of *TSER* polymorphisms on clinical outcome in CRC patients treated with 5-FU/capecitabine-based chemotherapy [24, 36–44], whereas recent studies did not find a correlation of this polymorphism with outcome after pemetrexed-based therapies in mesothelioma and non-small cell lung cancer (NSCLC) [12, 36].

To our knowledge, there are no data on *MTHFR-1298A>C* polymorphisms on CRC patients treated with pemetrexed; nevertheless, the present results are in accordance with data on advanced gastric cancer patients treated with pemetrexed, showing a favorable prognostic role of the wild-type genotype [45]. These data are also similar to the results of the clinical/pharmacogenetic study by Smit and colleagues in NSCLC patients treated with pemetrexed. In this study, homozygous mutation for *MTHFR-1298A>C* was shown to have a trend towards a significant association with shorter PFS when compared with wild-type or heterozygous mutations [12]. The longer PFS observed in *MTHFR-1298A>C AA* carriers might be explained by the role of MTHFR on methylene-THF conversion to 5-methyl-THF. The *MTHFR-1298A>C* polymorphism is within the regulatory region of *MTHFR* and is known to reduce enzyme activity leading to a decreased pool of 5-methyl-THF, which leads in turn to an increase of homocysteine concentrations that have been related to increased DNA damage [32, 46].

In the FOLFIRI group, *SHMT1-1420C>T* and *SLC-19A180G>A* polymorphisms have shown a significant association with differential PFS. Once again, the significantly longer PFS observed in *SHMT1-1420C>T TT* carriers might be explained by the increase of homocysteine concentration which has been related to increased DNA damage [32]. Surprisingly, the *SLC-19A1180G>A GG+GA* polymorphism showed a significant association with shorter PFS in patients treated with FOLFIRI, but not in the ALIRI group. A previous study suggested that 5-FU transport may be mediated by SLC22A7 [47], while it is well established that antifolates such as methotrexate and pemetrexed are actively transported into cells by SLC-19A1 [23, 47]. Furthermore, *SLC-19A1*-IVS4(2117)C>T, IVS5(9148) C>A and exon6(2522)C>T polymorphisms were recently correlated to survival after pemetrexed-based therapy [48]. Nevertheless, there are no data correlating *SLC-19A180G>A* polymorphism to pemetrexed survival or PFS [48]. A possible explanation for the significant association found in our dataset is that leucovorin, used to enhance 5-FU activity, is also transported into the cell by SLC-19A1. The protein codified by the *SLC-19A180G>A AA* polymorphic gene might have higher transport activity leading to an increased concentration of intracellular leucovorin resulting in an increase in 5-FU activity. Furthermore, the effect of both *MTHFR* and *SLC-19A1* polymorphisms on PFS might be mediated by the influence on total homocysteine levels [49], which have a crucial impact on DNA damage.

To our knowledge, this is also the first study reporting a significant correlation of *MTHFD-1958G>A* polymorphisms with clinical response. To date, the *1958G>A* transition of *MTHFD1* has been linked with genetic higher risk of having a child with neural tube defects ("*AA*" carriers) [50] or schizophrenia ("*AA*" or "*AG*" carriers) [51]. Its association with response to cytotoxic drugs may result from the effect on de novo biosynthesis of N^5-methyltetrahydrofolate and subsequent reduction of SAM concentration, favoring DNA damage.

The identification of genetic variables that predict toxicity to chemotherapy is of major interest in patient selection avoiding the use of harmful therapies, and in the present study specific polymorphisms of *MTHFR* were identified as significant predictors for appearance of grade 3–4 blood and bone marrow toxicity. In particular, carriers of the *MTHFR-677C>T TT+CT* genotypes had a significantly lower risk to experience this severe toxicity compared with patients harboring the *MTHFR-677C>T CC* genotype. These results are in accordance with data from a clinical investigation on advanced gastric cancer patients treated with 5-fluoropyrimidine-based chemotherapy, which reported a significant association between the *TT* genotype with higher treatment-related general toxicity [52]. Conversely, other studies showed that the *CC* genotype was associated with grade 3–4 infections and diarrhea [52, 53]. However, a few studies analyzed the role of the *MTHFR-677C>T* polymorphism on toxic side effects after fluoropyrimidine-based therapy in CRC, drawing inconclusive results, while no data are available on the possible correlation with pemetrexed toxicity [54], and thus further studies are warranted.

In conclusion, although the present exploratory study was limited by sample size, and no correction for multiple testing was performed, the data on the correlation of polymorphisms in genes involved in the folate pathway or in antifolate chemotherapy with clinical outcome underscore the importance of a candidate gene and pathway-based approach in a genotyping investigation. These analyses should be integrated with genome-wide analysis, as described in our previous study [55].

Ultimately, the validation of the role of these polymorphisms in prospective multicenter trials might offer new tools for treatment optimization of both currently available regimens in selected CRC patients (e.g., FOLFIRI) and of pemetrexed-based treatment in other tumor types.

Received February 12, 2013; accepted April 8, 2013; previously published online May 11, 2013

References

1. Jemal A, Siegel R, Xu J, Ward E. Cancer statistics, 2010. CA Cancer J Clin 2010;60:277–300.
2. Meyerhardt JA, Mayer RJ. Systemic therapy for colorectal cancer. N Engl J Med 2005;352:476–87.
3. Walther A, Johnstone E, Swanton C, Midgley R, Tomlinson I, Kerr D. Genetic prognostic and predictive markers in colorectal cancer. Nat Rev Cancer 2009;9:489–99.
4. Britten CD, Izbicka E, Hilsenbeck S, Lawrence R, Davidson K, Cerna C, et al. Activity of the multitargeted antifolate LY231514 in the human tumor cloning assay. Cancer Chemother Pharmacol 1999;44:105–10.
5. Adjei AA. Pemetrexed (ALIMTA), a novel multitargeted antineoplastic agent. Clin Cancer Res 2004;10:4276s–80s.
6. Underhill C, Goldstein D, Gorbounova VA, Biakhov MY, Bazin IS, Granov DA, et al. A randomized phase II trial of pemetrexed plus irinotecan (ALIRI) versus leucovorin-modulated 5-FU plus irinotecan (FOLFIRI) in first-line treatment of locally advanced or metastatic colorectal cancer. Oncology 2007;73:9–20.
7. Stankova J, Lawrance AK, Rozen R. Methylenetetrahydrofolate reductase (MTHFR): a novel target for cancer therapy. Curr Pharm Des 2008;14:1143–50.
8. Shih C, Habeck LL, Mendelsohn LG, Chen VJ, Schultz RM. Multiple folate enzyme inhibition: mechanism of a novel pyrrolopyrimidine-based antifolate LY231514 (MTA). Adv Enzyme Regul 1998;38:135–52.
9. Leil TA, Endo C, Adjei AA, Dy GK, Salavaggione OE, Reid JR, et al. Identification and characterization of genetic variation in the folylpolyglutamate synthase gene. Cancer Res 2007;67:8772–82.
10. Shih C, Chen VJ, Gossett LS, Gates SB, MacKellar WC, Habeck LL, et al. LY231514, a pyrrolo[2,3-d]pyrimidine-based antifolate that inhibits multiple folate-requiring enzymes. Cancer Res 1997;57:1116–23.
11. The Weizmann Institute of Science. The human gene compendium. Rehovot, Israel: The Weizmann Institute of Science, 2010.
12. Smit EF, Burgers SA, Biesma B, Smit HJ, Eppinga P, Dingemans AM, 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.
13. Etienne-Grimaldi MC, Milano G, Maindrault-Goebel F, Chibaudel B, Formento JL, Francoual M, et al. Methylenetetrahydrofolate reductase (MTHFR) gene polymorphisms and FOLFOX response in colorectal cancer patients. Br J Clin Pharmacol 2010;69:58–66.
14. Giovannetti E, Ugrasena DG, Supriyadi E, Vroling L, Azzarello A, de Lange D, et al. Methylenetetrahydrofolate reductase (MTHFR) C677T and thymidylate synthase promoter (TSER) polymorphisms in Indonesian children with and without leukemia. Leuk Res 2008;32:19–24.
15. Hughes LB, Beasley TM, Patel H, Tiwari HK, Morgan SL, Baggott JE, et al. Racial or ethnic differences in allele frequencies of single-nucleotide polymorphisms in the methylenetetrahydrofolate reductase gene and their influence on response to methotrexate in rheumatoid arthritis. Ann Rheum Dis 2006;65:1213–8.
16. Wessels JA, van der Kooij SM, le Cessie S, Kievit W, Barerra P, Allaart CF, et al. A clinical pharmacogenetic model to predict the efficacy of methotrexate monotherapy in recent-onset rheumatoid arthritis. Arthritis Rheum 2007;56:1765–75.
17. de Jonge R, Hooijberg JH, van Zelst BD, Jansen G, van Zantwijk CH, Kaspers GJ, et al. Effect of polymorphisms in folate-related genes on in vitro methotrexate sensitivity in pediatric acute lymphoblastic leukemia. Blood 2005;106:717–20.
18. Krajinovic M, Lemieux-Blanchard E, Chiasson S, Primeau M, Costea I, Moghrabi A. Role of polymorphisms in MTHFR and MTHFD1 genes in the outcome of childhood acute lymphoblastic leukemia. Pharmacogenomics J 2004;4:66–72.
19. James HM, Gillis D, Hissaria P, Lester S, Somogyi AA, Cleland LG, et al. Common polymorphisms in the folate pathway predict efficacy of combination regimens containing methotrexate and sulfasalazine in early rheumatoid arthritis. J Rheumatol 2008;35:562–71.
20. Nannizzi S, Veal GJ, Giovannetti E, Mey V, Ricciardi S, Ottley CJ, et al. Cellular and molecular mechanisms for the synergistic cytotoxicity elicited by oxaliplatin and pemetrexed in colon cancer cell lines. Cancer Chemother Pharmacol 2010;66:547–58.
21. Patino-Garcia A, Zalacain M, Marrodan L, San-Julian M, Sierrasesumaga L. Methotrexate in pediatric osteosarcoma: response and toxicity in relation to genetic polymorphisms and dihydrofolate reductase and reduced folate carrier 1 expression. J Pediatr 2009;154:688–93.
22. Therasse P, Arbuck SG, Eisenhauer EA, Wanders J, Kaplan RS, Rubinstein L, et al. New guidelines to evaluate the response to treatment in solid tumors. European Organization for Research and Treatment of Cancer, National Cancer Institute of the United States, National Cancer Institute of Canada. J Natl Cancer Inst 2000;92:205–16.
23. Matherly LH, Hou Z, Deng Y. Human reduced folate carrier: translation of basic biology to cancer etiology and therapy. Cancer Metastasis Rev 2007;26:111–28.
24. Mauritz R, Giovannetti E, Beumer IJ, Smid K, Van Groeningen CJ, Pinedo HM, et al. Polymorphisms in the enhancer region of the thymidylate synthase gene are associated with thymidylate synthase levels in normal tissues but not in malignant tissues of patients with colorectal cancer. Clin Colorectal Cancer 2009;8:146–54.
25. Mauritz R, Peters GJ. Pharmacogenetics of colon cancer and potential implications for 5-fluorouracil-based chemotherapy. Curr Pharmacogenomics 2006;4:57–67.
26. Sharma S, Das M, Kumar A, Marwaha V, Shankar S, Singh P, et al. Purine biosynthetic pathway genes and methotrexate response in rheumatoid arthritis patients among north Indians. Pharmacogenet Genomics 2009;19:823–8.
27. Ueland PM, Hustad S, Schneede J, Refsum H, Vollset SE. Biological and clinical implications of the MTHFR C677T polymorphism. Trends Pharmacol Sci 2001;22:195–201.
28. Steck SE, Keku T, Butler LM, Galanko J, Massa B, Millikan RC, et al. Polymorphisms in methionine synthase, methionine synthase reductase and serine hydroxymethyltransferase, folate and alcohol intake, and colon cancer risk. J Nutrigenet Nutrigenomics 2008;1:196–204.

29. Safranow K, Czyzycka E, Binczak-Kuleta A, Rzeuski R, Skowronek J, Wojtarowicz A, et al. Association of C34T AMPD1 gene polymorphism with features of metabolic syndrome in patients with coronary artery disease or heart failure. Scand J Clin Lab Invest 2009;69:102–12.
30. Wernimont SM, Raiszadeh F, Stover PJ, Rimm EB, Hunter DJ, Tang W, et al. Polymorphisms in serine hydroxymethyltransferase 1 and methylenetetrahydrofolate reductase interact to increase cardiovascular disease risk in humans. J Nutr 2011;141:255–60.
31. Prinz-Langenohl R, Fohr I, Pietrzik K. Beneficial role for folate in the prevention of colorectal and breast cancer. Eur J Nutr 2001;40:98–105.
32. Fenech M. Folate, DNA damage and the aging brain. Mech Ageing Dev 2010;131:236–41.
33. Weisman MH, Furst DE, Park GS, Kremer JM, Smith KM, Wallace DJ, et al. Risk genotypes in folate-dependent enzymes and their association with methotrexate-related side effects in rheumatoid arthritis. Arthritis Rheum 2006;54:607–12.
34. Campalani E, Arenas M, Marinaki AM, Lewis CM, Barker JN, Smith CH. Polymorphisms in folate, pyrimidine, and purine metabolism are associated with efficacy and toxicity of methotrexate in psoriasis. J Invest Dermatol 2007;127:1860–7.
35. Lee YC, Cui J, Costenbader KH, Shadick NA, Weinblatt ME, Karlson EW. Investigation of candidate polymorphisms and disease activity in rheumatoid arthritis patients on methotrexate. Rheumatology (Oxford) 2009;48:613–7.
36. Zucali PA, Giovannetti E, Destro A, Mencoboni M, Ceresoli GL, Gianoncelli L, et al. Thymidylate synthase and excision repair-cross-complementing group-1 as predictors of responsiveness in mesothelioma patients treated with pemetrexed-carboplatin. Clin Cancer Res 2011;17:2581–90.
37. Pullarkat ST, Stoehlmacher J, Ghaderi V, Xiong YP, Ingles SA, Sherrod A, et al. Thymicylate synthase gene polymorphism determines response and toxicity of 5-FU chemotherapy. Pharmacogenomics J 2001;1:65–70.
38. Park DJ, Stoehlmacher J, Zhang W, Tsao-Wei D, Groshen S, Lenz HJ. Thymidylate synthase gene polymorphism predicts response to capecitabine in advanced colorectal cancer. Int J Colorectal Dis 2002;17:46–9.
39. Marsh S, McKay JA, Cassidy J, McLeod HL. Polymorphism in the thymidylate synthase promoter enhancer region in colorectal cancer. Int J Oncol 2001;19:383–6.
40. Etienne MC, Chazal M, Laurent-Puig P, Magne N, Rosty C, Formento JL, et al. Prognostic value of tumoral thymidylate synthase and p53 in metastatic colorectal cancer patients receiving fluorouracil-based chemotherapy: phenotypic and genotypic analyses. J Clin Oncol 2002;20:2832–43.
41. Lecomte T, Ferraz JM, Zinzindohoue F, Loriot MA, Tregouet DA, Landi B, et al. Thymidylate synthase gene polymorphism predicts toxicity in colorectal cancer patients receiving 5-fluorouracil-based chemotherapy. Clin Cancer Res 2004;10:5880–8.
42. Schwab M, Zanger UM, Marx C, Schaeffeler E, Klein K, Dippon J, et al. Role of genetic and nongenetic factors for fluorouracil treatment-related severe toxicity: a prospective clinical trial by the German 5-FU Toxicity Study Group. J Clin Oncol 2008;26:2131–8.
43. Iacopetta B, Grieu F, Joseph D, Elsaleh H. A polymorphism in the enhancer region of the thymidylate synthase promoter influences the survival of colorectal cancer patients treated with 5-fluorouracil. Br J Cancer 2001;85:827–30.
44. Tsuji T, Hidaka S, Sawai T, Nakagoe T, Yano H, Haseba M, et al. Polymorphism in the thymidylate synthase promoter enhancer region is not an efficacious marker for tumor sensitivity to 5-fluorouracil-based oral adjuvant chemotherapy in colorectal cancer. Clin Cancer Res 2003;9:3700–4.
45. Chen JS, Chao Y, Bang YJ, Roca E, Chung HC, Palazzo F, et al. A phase I/II and pharmacogenomic study of pemetrexed and cisplatin in patients with unresectable, advanced gastric carcinoma. Anticancer Drugs 2010;21:777–84.
46. Chandy S, Sadananda Adiga MN, Ramachandra N, Krishnamoorthy S, Ramaswamy G, Savithri HS, et al. Association of methylenetetrahydrofolate reductase gene polymorphisms and colorectal cancer in India. Indian J Med Res 2010;131:659–64.
47. Kobayashi Y, Ohshiro N, Sakai R, Ohbayashi M, Kohyama N, Yamamoto T. Transport mechanism and substrate specificity of human organic anion transporter 2 (hOat2 [SLC22A7]). J Pharm Pharmacol 2005;57:573–8.
48. Adjei AA, Salavaggione OE, Mandrekar SJ, Dy GK, Ziegler KL, 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;5:1346–53.
49. Chango A, Emery-Fillon N, de Courcy GP, Lambert D, Pfister M, Rosenblatt DS, et al. A polymorphism (80G→A) in the reduced folate carrier gene and its associations with folate status and homocysteinemia. Mol Genet Metab 2000;70:310–5.
50. Parle-McDermott A, Mills JL, Kirke PN, Cox C, Signore CC, Kirke S, et al. MTHFD1 R653Q polymorphism is a maternal genetic risk factor for severe abruptio placentae. Am J Med Genet A 2005;132:365–8.
51. Kempisty B, Sikora J, Lianeri M, Szczepankiewicz A, Czerski P, Hauser J, et al. MTHFD 1958G>A and MTR 2756A>G polymorphisms are associated with bipolar disorder and schizophrenia. Psychiatr Genet 2007;17:177–81.
52. Lu JW, Gao CM, Wu JZ, Sun XF, Wang L, Feng JF. [Relationship of methylenetetrahydrofolate reductase C677T polymorphism and chemosensitivity to 5-fluorouracil in gastric carcinoma]. Ai Zheng 2004;23:958–62 (in Chinese).
53. Afzal S, Jensen SA, Vainer B, Vogel U, Matsen JP, Sorensen JB, et al. MTHFR polymorphisms and 5-FU-based adjuvant chemotherapy in colorectal cancer. Ann Oncol 2009;20:1660–6.
54. De Mattia E, Toffoli G. C677T and A1298C MTHFR polymorphisms, a challenge for antifolate and fluoropyrimidine-based therapy personalisation. Eur J Cancer 2009;45:1333–51.
55. Leon LG, Giovannetti E, Smid K, van Houte BP, Hanauske AR, Giaccone G, et al. DNA copy number profiles correlate with outcome in colorectal cancer patients treated with fluoropyrimidine/antifolate-based regimens. Curr Drug Metab 2011;12:956–65.