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Methylenetetrahydrofolate reductase (MTHFR) gene polymorphisms and FOLFOX response in colorectal cancer patients

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WHAT IS ALREADY KNOWN ABOUT THIS SUBJECT

- Numerous clinical studies, including a few prospective ones, have reported conflicting results on the impact of gene polymorphisms related to fluorouracil (FU) and oxaliplatin pharmacodynamics.

WHAT THIS STUDY ADDS

- This prospective study is the first to report that clinical response to FOLFOX is significantly related to methylenetetrahydrofolate reductase (*MTHFR*) gene polymorphisms (677C→T and 1298A→C), with a response rate of 37, 53, 63 and 80% in patients harbouring no, one, two or three favourable MTHFR alleles, respectively.
- Only polymorphisms of genes related to oxaliplatin pharmacodynamics (GSTπ 105Ile→Val and XPD 751Ly→Gln) influenced progression-free survival.
- These results corroborate the observation that response was related to the cumulative FU dose, whereas progression-free survival was related to the cumulative oxaliplatin dose.

AIMS

To test prospectively the predictive value of germinal gene polymorphisms related to fluorouracil (FU) and oxaliplatin (Oxa) pharmacodynamics on toxicity and responsiveness of colorectal cancer (CRC) patients receiving FOLFOX therapy.

METHODS

Advanced CRC patients (n = 117) receiving FOLFOX 7 therapy were enrolled. Gene polymorphisms relevant for FU [thymidylate synthase (TYMS, 28 bp repeats including the G→C mutation + 6 bp deletion in 3′UTR), methylenetetrahydrofolate reductase (MTHFR, 677C→T, 1298A→C), dihydropyrimidine deshydrogenase (IVS14+1G→A) and Oxa: glutathione S-transferase (GST) π (105Ile→Val, 114Ala→Val), excision repair cross-complementing group 1 (ERCC1) (118AAT→AAC), ERCC2 (XPD, 751Lys→Gln) and XRCC1 (399Arg→Gln)] were determined (blood mononuclear cells).

RESULTS

None of the genotypes was predictive of toxicity. Response rate (54.7% complete response + partial response) was related to FU pharmacogenetics, with both 677C→T (P = 0.042) and 1298A→C (P = 0.004) MTHFR genotypes linked to clinical response. Importantly, the score of favourable MTHFR alleles (677T and 1298C) was positively linked to response, with response rates of 37.1, 53.3, 62.5 and 80.0% in patients bearing no, one, two or three favourable alleles, respectively (P = 0.040). Polymorphisms of genes related to Oxa pharmacodynamics showed an influence on progression-free survival, with a better outcome in patients bearing GSTπ 105 Val/Val genotype or XPD 751Lys-containing genotype (P = 0.054).

CONCLUSIONS

These results show that response to FOLFOX therapy in CRC patients may be driven by MTHFR germinal polymorphisms.

Introduction

Standard chemotherapies in advanced colorectal cancer (CRC) patients have evolved from simple fluorouracil (FU)-based treatment to FU-folinic modulation, FU combination with oxaliplatin (FOLFOX) or irinotecan (FOLFIRI), and finally to associations of FU-containing chemotherapies with biological targeted therapies [1–6]. The wide range of treatment options creates a need for individual predictive factors in order to choose the optimal treatment for a given patient. To this end, tumour molecular markers constitute a valuable approach. Recent data on metastatic CRC patients have clearly demonstrated the predictive value of the absence of K-Ras somatic mutations for selecting patients likely to benefit from the addition of antiepidermal growth factor receptor (EGFR) therapies [7]. However, the benefit of EGFR-targeted therapy in chemotherapeutic care is relatively limited at close to 10% [8]. Moreover, K-Ras status is not relevant when discriminating response to chemotherapy alone [9]. There is still a need for reliable predictive markers aimed at orienting medical treatment in advanced CRC. In this context, two complementary approaches can be considered: tumour markers on the one hand and host-dependent biological factors on the other. Pharmacogenetics belongs to this latter category. Pharmacogenetics, which examines the links between germinal gene polymorphisms and the variability of drug pharmacodynamics, is thus of special interest for anticancer agents. The purpose of the present study was to evaluate prospectively the predictive value of gene polymorphisms potentially related to FU and oxaliplatin pharmacodynamics, taking into account toxicity, response rate and progression-free survival (PFS). The chemotherapeutic protocol was FOLFOX, which is considered a standard in CRC in both advanced disease and the adjuvant setting.

Methods

Patients

One hundred and seventeen patients with advanced CRC were enrolled in this prospective ancillary pharmacogenetic study as part of the multicentre Phase II OPTIMOX 2 trial by the GERCOR group [10]. The study was carried out with ethics committee approval. Patient characteristics are shown in Table 1. All patients received FOLFOX 7 therapy. The main goal of the trial was to assess a new strategy with chemotherapy interruptions in an attempt to improve survival and quality of life. Therefore, patients were randomized in order to receive either six cycles of modified FOLFOX 7 regimen [mFOLFOX 7, 2-h infusion of oxaliplatin 100 mg m^{-2} + 400 mg m^{-2} leucovorin (LV) followed by 46-h infusion of FU 3 g m^{-2}, day 1 = day 15] (arm 2, $n = 58$) or six cycles of mFOLFOX 7 followed by LV–5FU maintenance therapy consisting of a simplified bimonthly regimen from cycle 7 until progression (2-h infusion of LV 400 mg m^{-2} followed by a bolus of FU 400 mg m^{-2} and then a 46-h infusion of FU 3 g m^{-2}) (arm 1, $n = 59$). The number of mFOLFOX 7 cycles was not significantly different between arm 1 and arm 2. The sum of oxaliplatin doses and of FU doses administered during the cycles of FOLFOX 7 therapy were computed. This cumulative oxaliplatin dose and this cumulative FU dose were not significantly different between arm 1 and arm 2.

Table 1

Patient characteristics

Age	Median, extremes	67 (31–80)
Sex	Men	65
	Women	52
Arm	1	59
	2	58
Arm 1		
Number of cycles*	Median, extremes	6 (2–8)
Total cumulative oxaliplatin dose*†	Median, extremes (mg)	1000 (320–1200)
Total cumulative FU dose*‡	Median, extremes (g)	29.8 (9.6–39.2)
Arm 2		
Number of cycles*	Median, extremes	6 (3–7)
Total cumulative oxaliplatin dose*†	Median, extremes (mg)	983 (600–1200)
Total cumulative FU dose*‡	Median, extremes (g)	29.5 (13.5–68.4)
Previous adjuvant therapy§	None	96
	Chemotherapy	14
	Radiotherapy	13
WHO performance status	0	60
	1	51
	2	6
Primary localization	Colon	74
	Rectum	40
	Both	3
Metastasis site§	Liver	97
	Lung	52
	Peritoneum	24
	Lymph node	19
	Bone	3
	Others	13

*The number of cycles and the cumulative oxaliplatin and FU doses administered during the FOLFOX 7 therapy were not significantly different between arm 1 and arm 2. †Cumulative oxaliplatin dose during initial FOLFOX 7 (cycles 1 to *n*). ‡Cumulative FU dose given as continuous infusion during initial FOLFOX 7 therapy (cycles 1 to *n*). §Sum not equal to 117 due to multiple choice.

Toxicity evaluation

For each toxicity pattern (neutropenia, thrombocytopenia, anaemia, nausea, vomiting, mucositis, diarrhoea, hand-foot syndrome, neurotoxicity and alopecia), the maximum observed toxicity grade was recorded (National Cancer Institute Common Terminology Criteria for Adverse Events grading). For each patient, we considered the maximum observed toxicity grade (whatever the toxic pattern) and the toxicity score (sum of each toxicity pattern grade).

Efficacy evaluation

Objective tumour response was assessed according to Response Evaluation Criteria in Solid Tumors criteria. The

best response was analysed, as well as PFS and overall survival (both computed from randomization). At time of analysis, 79 patients had died and median follow-up was 37.8 months (reverse Kaplan–Meier method).

Pharmacogenetic analyses

Constitutional gene polymorphisms were analysed on DNA extracted from a 9 ml blood sample (Paxgene Blood DNA kit; Preanalytics). Germinal polymorphisms of genes relevant for FU, i.e. thymidylate synthase (TYMS), methylenetetrahydrofolate reductase (MTHFR), dihydropyrimidine deshydrogenase (DPYD), and for oxaliplatin, i.e. glutathione S-transferase (GST) π, excision repair cross-complementing group 1 (ERCC1), ERCC2 (XPD) and XRCC1 were analysed as follows:

- TYMS: (i) 28 bp repeat polymorphisms (2R or 3R) in the 5'UTR [polymerase chain reaction (PCR)], along with the G→C mutation in the second repeat of the 3R allele [PCR-restriction fragment length polymorphism (RFLP)] [11]. The 2R allele presents one E-box binding site for upstream stimulatory factor (USF), whereas the 3R allele presents two E-box binding sites for USF. The presence of a G→C mutation in the 3R allele alters the USF binding, so that the 3RC allele exhibits a single functional E-box. Thus, TYMS genotype was classified as a function of the number of theoretical E-box binding sites likely to bind USF proteins: class 2 (2R2R or 2R3RC or 3RC3RC), class 3 (2R3RG or 3RC3RG), class 4 (3RG3RG). (ii) 6 bp deletion at position 1494 in the 3'UTR (PCR + electrophoresis) [12].
- MTHFR: 677C→T and 1298A→C (melting curve analysis) [12].
- DPYD: IVS14+1G→A (PCR-RFLP using the *Nde I* restriction enzyme) [11].
- GSTπ (105Ile→Val and 114Ala→Val), ERCC1 (118AAT→AAC), XPD (751Lys→Gln) and XRCC1 (399Arg→Gln) genotypes were determined by PCR-RFLP using restriction enzymes *Alw*26I, *Aci*I, *Bsr*DI, *Pst*I and *Mspl*, respectively, as previously described [13, 14]. Wild-type (wt) and mutated cell lines were used as controls.

Statistics

The exact *P*-values for Hardy–Weinberg equilibrium were tested on http://innateimmunity.net/IIPGA2. The influence of each gene polymorphism was evaluated on toxicity, tumour response, PFS and overall survival. For TYMS genotypes, these analyses were conducted in three different ways by considering: (i) the 6-bp deletion (wt/wt *vs.* wt/del *vs.* del/del), (ii) the 28-bp repeats (2R2R *vs.* 2R3R *vs.* 3R3R), and (iii) the 28-bp repeats including the G→C mutation (class 2 *vs.* class 3 *vs.* class 4, as previously defined). Non-parametric tests were performed for comparisons (Mann–Whitney or Kruskal–Wallis). χ^2 tests were applied for categorical variables. A logistic model was applied for multivariate analysis of response predictors [1 = complete response (CR) + partial response (PR), 0 = stable disease (SD) + progressive disease (PD)]. Survival curves were plotted according to the Kaplan–Meier method. The influence of the various tested parameters on PFS and overall survival was assessed by means of log rank test, or Cox analysis for continuous variables. Statistics were performed on SPSS software (version 15.0; SPSS Inc., Chicago, IL, USA)).

Results

Description of analysed genotypes

Table 2 depicts the frequency of analysed genotypes. All patients (n = 117) exhibited the wt genotype for the IVS14+1G→A polymorphism of the DPYD gene. With the exception of MTHFR 1298A→C polymorphism, all genotypes agree with those predicted by the Hardy–Weinberg equilibrium. Linkage disequilibriums were observed between TYMS 28-bp repeats and 6-bp deletion (association between 3RG3RG and 6 bp del, $P < 0.001$), MTHFR 677C→T and 1298A→C (no homozygous patients for both variants, $P < 0.001$), GSTπ 105Ile→Val and 114Ala→Val (association between 105Val and 114Val, $P = 0.003$) and

Table 2

Genotypes distribution

Gene	Genotype		*n*
TYMS	28 bp repeats	2R2R	28
		2R3R	53
		3R3R	30
	Class including G→C	2 (2R2R or 2R3RC or 3RC3RC)	66
		3 (2R3RG or 3RC3RG)	38
		4 (3RG3RG)	7
	6 bp deletion	wt/wt	61
		wt/del	43
		del/del	12
MTHFR	677C→T	CC	44
		CT	58
		TT	14
	1298A→C	AA	50
		AC	61
		CC	5
GSTπ	105Ile→Val	Ile/Ile	56
		Ile/Val	49
		Val/Val	12
	114Ala→Val	Ala/Ala	104
		Ala/Val	11
		Val/Val	0
ERCC1	118AAT→AAC	TT	32
		TC	62
		CC	23
XPD	751Lys→Gln	Lys/Lys	41
		Lys/Gln	58
		Gln/Gln	16
XRCC1	399Arg→Gln	Arg/Arg	56
		Arg/Gln	52
		Gln/Gln	6

between XPD 751Lys/Gln and ERCC1 118AAT→AAC (association between XPD 751Gln and ERCC1 118AAC, $P = 0.001$).

Impact of gene polymorphisms on toxicity

Tolerance was satisfactory, with grade 3–4 toxicity observed in 13% of patients for neutropenia, 4.3% for thrombocytopenia and nausea, 3.4% for diarrhoea, 2.6% for vomiting, 0.9% for mucositis and no grade 3–4 for anaemia, hand-foot syndrome, alopecia or neurotoxicity. Whatever the toxicity pattern, grade 3–4 was recorded in 22.9% of patients and the toxicity score ranged from 2 to 15 (median 7). Toxicity was not statistically different according to the treatment arm. None of the analysed gene polymorphisms were predictive of toxicity considered either as the maximum observed grade, or as the toxicity score.

Impact of gene polymorphisms on response

Best response was CR in two patients, PR in 62 patients, SD in 45 patients and PD in eight patients, accounting for a total of 54.7% clinical responses (CR+PR). Best response was not statistically different between the two arms (55.9% in arm 1 *vs.* 53.4% in arm 2, $P = 0.79$), but was significantly related to the total cumulative FU dose administered during the cycles of FOLFOX 7 therapy (median 27 and 30.4 g in SD+PD and CR+PR, respectively, $P = 0.021$) and total cumulative oxaliplatin dose (median 960 and 1020 mg in SD+PD and CR+PR, respectively, $P = 0.037$).

Both the MTHFR 677C→T and 1298A→C genotypes (Figure 1) were linked to clinical response ($P = 0.042$ and 0.004, respectively), with the rare allele linked to improved response. We defined a score of favourable MTHFR alleles corresponding to the sum of 677T and 1298C alleles. Importantly, tumour response continuously increased with the score of favourable MTHFR alleles (Table 3, $P = 0.040$). Response rates were 37.1, 53.3, 62.5 and 80.0% in patients bearing no, one, two or three favourable alleles, respectively. None of the other genotypes was linked to tumour response.

Finally, a multivariate approach including cumulative FU and oxaliplatin doses revealed that both the score of favourable MTHFR alleles ($P = 0.024$) and the cumulative FU dose administered during the cycles of FOLFOX 7 therapy ($P = 0.017$) were significantly related to tumour response.

Impact of gene polymorphisms on survival

At time of analysis, 111 patients had progressed and median PFS was 7.5 months. PFS was influenced by the treatment arm (median 9.1 *vs.* 6.3 months in arms 1 and 2, respectively, $P = 0.015$) and by the cumulative oxaliplatin dose ($P = 0.031$). None of the analysed genotypes significantly influenced PFS, including the score of favourable MTHFR alleles. However, PFS plots according to GSTπ and XPD polymorphisms revealed an improved PFS in patients bearing GSTπ 105 Val/Val genotype or XPD 751 Lys-

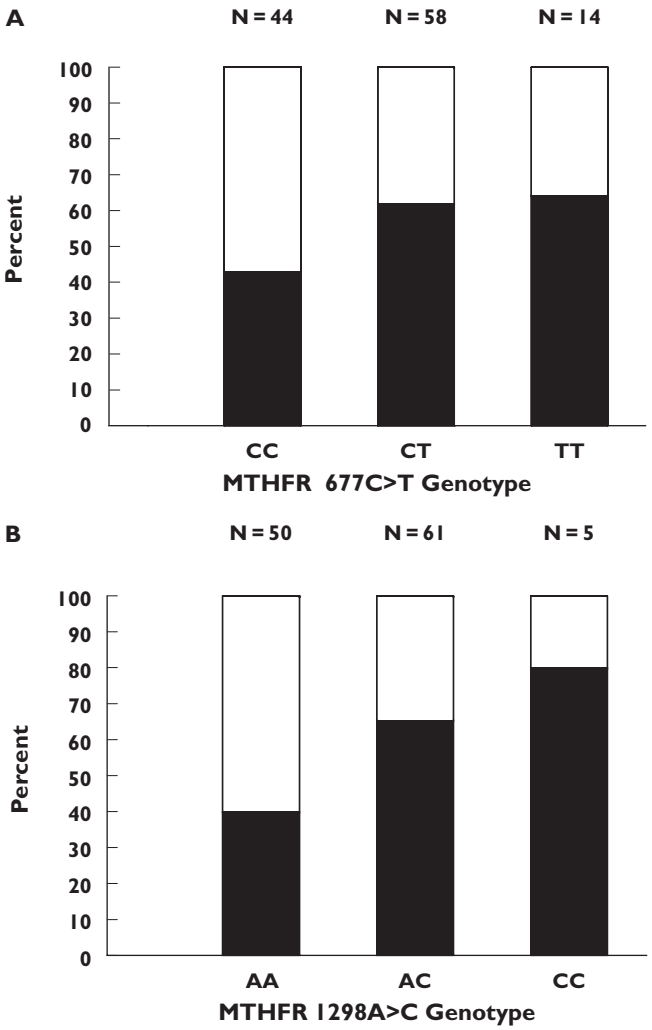

Figure 1

(a) Influence of methylenetetrahydrofolate reductase (MTHFR) 677C→T genotype on objective tumour response. Tumour response was 43.2, 62.1 and 64.3% in CC, CT and TT patients, respectively. χ^2 tests: CC *vs.* CT *vs.* TT, $P = 0.126$; CC *vs.* CT+TT, $P = 0.042$. (b) Influence of MTHFR 1298A→C genotype on objective tumour response. Tumour response was 40.0, 65.6 and 80.0% in AA, AC and CC patients, respectively. χ^2 tests: AA *vs.* AC *vs.* CC, $P = 0.014$; AA *vs.* AC+CC, $P = 0.004$. CR+PR (■); SD+PD (□)

containing genotype (see Figures S1 and S2). Therefore, we built a score of favourable genotype taking into account both polymorphisms of GSTπ (105Val/Val corresponding to the favourable genotype) and XPD (751Lys/Lys or Lys/Gln corresponding to the favourable genotype). As shown in Figure 2, the score of favourable genotype tends to discriminate PFS with a median of 6.0, 7.6 and 9.8 months in patients bearing no, one or two favourable genotypes, respectively ($P = 0.054$). The score of favourable genotypes exhibited a similar pattern in both arm 1 and arm 2; however, adjustment of treatment arm did not improve its statistical significance ($P = 0.065$).

At time of analysis, 79 patients had died and median overall survival was 22.8 months. Overall survival was not

Table 3

Influence of MTHFR genotype on tumour response

Score of favorable MTHFR alleles	*n*	Best response (CR+PR)	
Score 0	35	37.1% (13)	
Score 1	15	53.3% (8)	chi^2
Score 2	56	62.5% (35)	$P = 0.040$
Score 3	10	80.0% (8)	

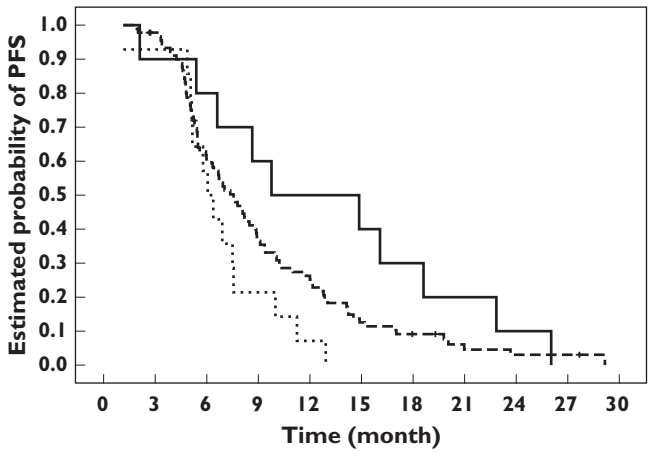

Figure 2

Progression-free survival (PFS) probability according to the score of favourable genotypes, including glutathione S-transferase (GST) π 105Ile→Val and XPD 751Lys→Gln polymorphisms. Favourable genotypes correspond to GSTπ 105 Val/Val, and XPD 751Lys/Lys or 751Lys/Gln. Median PFS was 6.0 months for score 0 (14 patients, 14 events), 7.6 months for score 1 (91 patients, 85 events) and 9.8 months for score 2 (10 patients, 10 events). Log rank test: $P = 0.054$. Score 0 (····); Score 1 (– – –); Score 2 (—)

influenced by the treatment arm, nor by the cumulative oxaliplatin or FU dose. None of the analysed genotypes had a significant impact on overall survival, including the score of favourable MTHFR alleles and the score of favourable GSTπ-XPD alleles.

Discussion

The present pharmacogenetic study was conducted on a prospective cohort of 117 advanced CRC patients, all receiving FOLFOX therapy. This study included two treatment modalities differing according to a chemotherapy interruption strategy (six mFOLFOX 7 cycles ± simplified LV5FU2 regimen). Importantly, the number of mFOLFOX 7 cycles, the cumulative oxaliplatin dose and the cumulative continuous FU dose administered during the cycles of FOLFOX 7 therapy were similar in both treatment arms (Table 1). Also, toxicity and tumour response were not significantly different between treatment arms. The aim of the present study was to examine the role of various germinal polymorphisms on toxicity, response and PFS. The selected genes included both genes relevant for FU (TYMS, MTHFR, DPYD) and oxaliplatin (GSTπ along with DNA repair enzymes ERCC1, XPD and XRCC1).

Tolerance was good, with 19.3% of patients exhibiting grade 3 toxicity and 3.7% grade 4 toxicity. None of the genes related to oxaliplatin pharmacodynamics was linked to toxicity. Also, TYMS and MTHFR polymorphisms were not predictive of toxicity, and all patients exhibited the common IVS14+1G variant for DPYD gene. The absence of impact of MTHFR polymorphism on toxicity concords with recent results obtained on >600 cancer patients receiving FU monotherapy [15].

A clinical response was observed in 54.7% of patients. Among all analysed polymorphisms, only MTHFR gene polymorphisms were related to tumour response (Figure 1, Table 3). The gene coding for TYMS is carried by chromosome 18p, frequently prone to loss of heterozygosity in CRC, and Uchida *et al.* [16] have clearly reported that TYMS germinal polymorphism does not faithfully reflect tumoral polymorphism. The fact that TYMS polymorphisms were analysed on blood mononuclear cells may thus explain the lack of association with tumour response. The MTHFR enzyme is located at a major folate metabolic cross-roads, irreversibly converting 5–10 methylenetetrahydrofolate (CH2FH4) into 5-methyltetrahydrofolate (CH3FH4). FU acts mainly via fluorodeoxyuridine monophosphate (FdUMP), which inhibits TYMS and subsequent DNA synthesis through the formation of an inactive ternary complex between TYMS, FdUMP and the methyl donor CH2FH4. Experimental [17] and clinical [18] studies have shown that optimal FU cytotoxicity requires elevated CH2FH4 tumoral concentrations. Accordingly, clinical studies have demonstrated higher efficacy when FU is associated with folinic acid, a precursor of CH2FH4 [19, 20]. The MTHFR gene is subject to several polymorphisms, of which the 677C→T (Ala to Val at codon 222) [21] and 1298A→C (Glu to Ala at codon 428) [22] single nucleotide polymorphisms are the two most commonly linked with altered enzyme activity and increased homocysteine levels [23]. Even though the impact of MTFHR genotype on tumoral CH2FH4 concentrations has not been clearly established, deficient MTHFR genotypes may theoretically favour an increase in intracellular CH2FH4 concentrations. It can thus be hypothesized that tumors exhibiting deficient MTHFR variants (677T or 1298C) may be more sensitive to FU cytotoxicity than tumours bearing the common MTHFR variants (677C or 1298A).

The impact of MTHFR polymorphisms on FU efficacy has been previously reported *in vitro* and *in vivo*. An experimental study on 19 human cancer cell lines of various origins has reported a greater FU efficacy in cell lines homozygous for the 1298C variant compared with cells

homozygous for the 1298A variant [12]. Also, Sohn *et al.* [24] demonstrated, on human cancer cell lines transfected with 677C or 677T MTHFR cDNA, significantly higher sensitivity to FU in the 677T cell lines relative to the 677C cell lines. The impact of MTHFR gene polymorphisms on treatment outcome in the clinical setting is more varied. Cohen *et al.* [25] were the first to describe a link between the 677C→T MTHFR polymorphism and tumour response to FU-based chemotherapy. In this study conducted on 43 metastatic CRC patients, all five 677TT patients responded to treatment, whereas response rate was approximately 50% in 677CC patients [25]. In a retrospective study from our group including 98 CRC patients with liver metastases receiving FUFOL, responsiveness was significantly linked to 677C→T genotype, with an increased response rate in 677TT tumours relative to 677CC (odds ratio = 1.88) [26]. In contrast, a study by Marcuello *et al.* [27] failed to show a link between MTHFR polymorphisms and clinical response in 94 metastatic CRC patients receiving FU associated with irinotecan or oxaliplatin. Also, Suh *et al.* [28] reported that MTHFR 677C→T polymorphism was not a significant predictor of response in 54 patients receiving FOLFOX treatment. More recently, Ruzzo and coworkers [29] have reported the absence of influence of 677C→T and 1298A→C MTHFR genotypes on objective response in 166 advanced CRC patients receiving first-line FOLFOX chemotherapy. Of note, the FOLFOX regimens differed among these studies according to the FU and oxaliplatin dose intensity. This could explain some of the discrepancies. In contrast, the present study clearly shows that both 677C→T and 1298A→C polymorphisms were significantly linked to FOLFOX responsiveness (Figure 1). Moreover, response rate continuously increased with the score of favourable MTHFR allele (Table 3), with only 37% of response in patients without any favourable allele, up to 80% in patients bearing three favourable alleles (i.e. homozygous for one variant and heterozygous for the second). None of the patients were homozygous for both 677T and 1298C. The divergence between present results and those of Ruzzo [29], both obtained on relatively large prospective cohorts of patients receiving FOLFOX therapy, is difficult to explain. Numerous studies on the role of MTHFR 677C→T and 1298C→A polymorphisms in CRC risk have clearly shown that physio-pathological consequences of a deficient MTHFR genotype are closely dependent on the folate status intake. It can thus be hypothesized that the influence of MTHFR polymorphisms on FU-based responsiveness in the Ruzzo study may have been blurred by a wide interpatient variability in folate status. Unfortunately, the Ruzzo study, like ours, did not provide information on the folate dietary status.

We previously reported in a previous study from our group [26] on 98 metastatic CRCs that MTHFR-deficient patients (1298CC genotype) had the shorter specific survival, suggesting a prognostic value of MTHFR polymorphism, probably independent of FU-based therapy. Also, a recent study by Zhang [30] reported a sex-specific influence of MTHFR 1298A→C polymorphism on overall survival in metastatic CRC women, with greater overall survival in MTHFR nondeficient women (1298AA genotype). Present data do not confirm the impact of MTHFR polymorphism on overall survival. A subgroup analysis in women did not reveal any prognostic value of 1298A→C genotype (49 events/52 women, data not shown). None of the analysed genotypes had a significant impact on overall survival.

Of note, PFS was related only to genes involved in oxaliplatin pharmacodynamics, with a tendency for a better outcome in patients bearing GSTπ 105 Val/Val genotype or XPD 751Lys-containing genotype (Figure 2). GSTπ is a phase II metabolic enzyme that inactivates platinum derivatives by adding a glutathione to its electrophile group. It has been shown that lymphocytic activity of GSTπ was significantly reduced in GSTπ 105 Val/Val patients compared with GSTπ 105 Ile/Ile patients [31]. Accordingly, Stoehlmacher *et al.* [32] reported in CRC patients receiving FOLFOX therapy that individuals bearing the GSTπ 105 Val/Val genotype had a better PFS and overall survival than patients bearing the GSTπ 105 Ile allele. XPD is a DNA repair enzyme of the ERCC2 group. The functional impact of XPD 751 Lys→Gln at the protein level is not clearly established. However, numerous clinical studies on CRC patients receiving oxaliplatin have reported a significantly improved PFS and/or survival in 751 Lys/Lys patients [29, 32–34]. The presently reported influence of GSTπ and XPD polymorphisms agrees well with the literature data.

Of particular interest is the comparison between the influence of cumulative drug doses and of pharmacogenetics on clinical end-points. On one hand, tumour response was significantly associated with elevated cumulative FU doses administered during the cycles of FOLFOX 7 therapy, suggesting that FU plays a preponderant role in tumour response. This observation closely concords with the pharmacogenetic results showing a major role of MTHFR polymorphisms in tumour response. Interestingly, the score of favourable MTHFR alleles and the cumulative FU dose were independent significant predictors of response. On the other hand, as previously reported [35], PFS was related to the cumulative oxaliplatin dose (and not to the cumulative FU dose) and the impact of the oxaliplatin dose was sustained by the influence of GSTπ and XPD polymorphisms on PFS. Even though PFS was linked to tumour response (data not shown), the influence of MTHFR genotypes on tumour response does not translate into an impact on PFS. Conversely, GSTπ and XPD genotypes do not influence tumour response while having an impact on PFS. These observations suggest that PFS is controlled by additional factors, including factors related to oxaliplatin pharmacodynamics.

The clinical usefulness of a routine pharmacogenetic approach to treatment adjustment is not yet established. Analysis of somatic K-Ras mutation is now applied in

routine practice to identify CRC patients who will benefit from anti-EGFR therapy. However, more predictive factors are needed in this context since fluoropyrimidines still constitute the core of the treatment. Present data establish the role of MTHFR germinal polymorphism as a potential strong predictor of response to FOLFOX therapy and show for the first time that the response rate to FOLFOX increases continuously with the number of favourable MTHFR alleles. If confirmed in further studies, a possible application of this result may be to propose an alternative regimen containing no FU in the 30% of patients without a favourable MTHFR allele (i.e. score 0). MTHFR polymorphisms may provide a useful marker that presents the advantage of large-scale feasibility of routine analysis based on an easy-to-perform blood sample, combined with relatively low cost.

Competing interests

T.A. received a speaker's honorarium from Sanofi Aventis.

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Supporting information

Additional Supporting Information may be found in the online version of this article:

Figure S1 Progression-free survival (PFS) probability according to the glutathione S-transferase (GST) π

105Ile→Val genotype. Median PFS was 7.36 months for Ile/Ile (56 patients, 53 events), 7.56 months for Ile/Val (49 patients, 46 events) and 9.76 months for Val/Val (12 patients, 12 events). Log rank test: $P = 0.20$ ($P = 0.22$ after adjustment on treatment arms)

Figure S2 Progression-free survival (PFS) probability according to the XPD 751Lys→Gln genotype. Median PFS was 6.38 months for Gln/Gln (16 patients, 16 events), 8.01 months for Lys/Gln (58 patients, 55 events) and 6.41 months for Lys/Lys (41 patients, 38 events). Log rank test: $P = 0.33$ ($P = 0.29$ after adjustment on treatment arms)