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ORIGINAL REPORT

Pharmacogenetic Assessment of Toxicity and Outcome in Patients With Metastatic Colorectal Cancer Treated With LV5FU2, FOLFOX, and FOLFIRI: FFCD 2000-05

Valérie Boige, Jean Mendiboure, Jean-Pierre Pignon, Marie-Anne Loriot, Marine Castaing, Michel Barrois, David Malka, David-Alexandre Trégouët, Olivier Bouché, Delphine Le Corre, Isabelle Miran, Claire Mulot, Michel Ducreux, Philippe Beaune, and Pierre Laurent-Puig

From the Departments of Medicine, Biostatistics and Epidemiology, and Oncogenetics and Institut National de la Santé et de la Recherche Médicale (INSERM) U981, Institut Gustave-Roussy, Villejuif; Université Paris Descartes, INSERM UMR-S775; INSERM UMR-S 937, Pierre et Marie Curie University and Medical School, Paris; and Department of Hepatogastroenterology, Centre Hospitalier Universitaire de Reims, Reims, France.

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M.D., P.B., and P.L.-P. contributed equally to this work.

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Corresponding author: Valérie Boige, MD, PhD, Institut Gustave Roussy, 39 rue Camille Desmoulins, 94805 Villejuif Cedex, France; e-mail: boige@igr.fr.

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ABSTRACT

Purpose
The aim was to investigate whether germline polymorphisms within candidate genes known or suspected to be involved in fluorouracil (FU), oxaliplatin, and irinotecan pathways were associated with toxicity and clinical outcome in patients with metastatic colorectal cancer (mCRC).

Patients and Methods
Blood samples from 349 patients included in the Fédération Francophone de Cancérologie Digestive 2000-05 randomized trial, which compared FU plus leucovorin (LV5FU2) followed by FU, leucovorin, and oxaliplatin (FOLFOX) followed by FU, leucovorin, and irinotecan (FOLFIRI; sequential arm) with FOLFOX followed by FOLFIRI (combination arm) in terms of progression-free survival (PFS) and overall survival, were collected. Twenty polymorphisms within the *DPD*, *TS*, *MTHFR*, *ERCC1*, *ERCC2*, *GSTP1*, *GSTM1*, *GSTT1*, and *UGT1A1* genes were genotyped.

Results
The *ERCC2-K751QC* allele was independently associated with an increased risk of FOLFOX-induced grade 3 or 4 hematologic toxicity ($P = .01$). In the sequential arm, *TS-5′UTR3RG* and *GSTT1* alleles were independently associated with response to LV5FU2 ($P = .009$) and FOLFOX ($P = .01$), respectively. The effect of oxaliplatin on tumor response increased with the number of *MTHFR-1298C* alleles (test for trend, $P = .008$). The PFS benefit from first-line FOLFOX was restricted to patients with *2R/2R* (hazard ratio [HR] = 0.39; 95% CI, 0.23 to 0.68) or *2R/3R* (HR = 0.59; 95% CI, 0.42 to 0.82) *TS-5′UTR* genotypes, respectively. Conversely, patients with the *TS-5′UTR 3R/3R* genotype did not seem to benefit from the adjunction of oxaliplatin (HR = 0.96; 95% CI, 0.66 to 1.40; trend between the three HRs, $P = .006$).

Conclusion
A pharmacogenetic approach may be a useful strategy for personalizing and optimizing chemotherapy in mCRC patients and deserves confirmation in additional prospective studies.

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INTRODUCTION

The treatment of metastatic colorectal cancer (mCRC) is based on systemic chemotherapy. Several effective drugs are available and can be administered either sequentially or in combination. Adding oxaliplatin and irinotecan to fluorouracil (FU) improves objective response rate (ORR) and progression-free survival (PFS) in patients with mCRC compared with FU alone but increases toxicity.[1,2] In the vast majority of patients who are not amenable to curative surgery, whether such combination therapies should be systematically used as first-line treatment or, alternatively, as a second-line treatment after FU treatment failure remains unclear.[3,4] Patient therapeutic management could be driven by the interindividual variability of toxicity and efficacy of chemotherapeutic agents. Therefore, identifying biomarkers that could help select the most appropriate regimen for each patient would be useful.

Genetic polymorphisms in drug target genes, genes encoding DNA repair enzymes, and detoxification pathways may influence the toxicity and the activity of FU, oxaliplatin, and irinotecan. Variability in genes coding for dihydropyrimidine dehydrogenase (*DPD*) involved in FU metabolism, thymidylate synthase (*TS*) targeted by FU, and methylenetetrahydrofolate reductase (*MTHFR*), which converts 5,10-methylenetetrahydrofolate required for TS enzyme inhibition to 5-methyltetrahydrofolate, may modulate FU-induced toxicity and response. The variable

number of tandem repeat (VNTR) polymorphism in the *TS* 5′-untranslated region (5′-UTR), which consists of two (*2R*) or three (*3R*) 28-base pair repeated sequences, the G/C polymorphism in the *3R* allele resulting in two additional alleles at this locus (*3G* or *3C*), and the 6-base pair insertion/deletion (6+/6–) in the 3′-untranslated region (3′-UTR) influence *TS* gene expression and clinical response to FU.[5-8] Two linked *MTHFR* single nucleotide polymorphisms (SNPs; *677C>T* and *1298A>C*) result in decreased enzyme activity, leading to increased levels of 5,10-methylenetetrahydrofolate and thus affecting intracellular folate metabolites and possibly FU sensitivity.[9-11]

DNA repair proteins and the glutathione S-transferase (GST) isoenzyme family influence the activity of platinum compounds such as oxaliplatin.[12] Several putative functional polymorphisms in excision repair cross-complementing group 1 (*ERCC1*), in xeroderma pigmentosum group D (*XPD/ERCC2*), and in the GST family of isoenzyme genes have been associated with the efficacy and safety profile of platinum compounds.[13-15]

Glucuronidation is the main route of detoxification and elimination of SN-38, the active metabolite of irinotecan. Several polymorphisms in uridine diphosphate-glucuronosyltransferases (UGTs), especially the UGT1A1 isoform in the white population, have been shown to influence the glucuronidating capacity and consequently the pharmacokinetics and toxicity of irinotecan.[16-18]

In the present study, we analyzed a panel of 20 polymorphisms within nine candidate genes known or suspected to be involved, based on previously described associations or putative functional effects, in FU, oxaliplatin, and irinotecan pathways in patients with mCRC included in a multicenter randomized phase III trial (Fédération Francophone de Cancérologie Digestive [FFCD] 2000-05). The primary objective of this trial was to compare sequential chemotherapy with FU plus leucovorin (LV5FU2) followed by FU, leucovorin, and oxaliplatin (FOLFOX) followed by FU, leucovorin, and irinotecan (FOLFIRI) with the FOLFOX first-line combination followed by FOLFIRI in terms of PFS after two lines of chemotherapy.[19] We assessed the impact of the polymorphisms on toxicity and outcome, with the aim of identifying predictive and prognostic genetic factors in a large population of patients with mCRC.

PATIENTS AND METHODS

Patient Samples

The FFCD 2000-05 randomized phase III trial assigned 410 patients with mCRC to receive either first-line LV5FU2 followed by second-line FOLFOX6 followed by third-line FOLFIRI (sequential arm, arm A) or first-line FOLFOX6 followed by second-line FOLFIRI (combination arm, arm B).[19] Tumor response was evaluated every 8 weeks according to WHO criteria. The primary end point was PFS after two lines of chemotherapy. Toxicity was graded according to National Cancer Institute Common Toxicity Criteria version 2.0. Among the 410 patients included in this trial, 349 (85%) gave their informed consent for germline DNA analysis on blood samples that could be extracted in 346 patients (84%; Fig 1). Their main characteristics did not differ from those of the whole study population (Data Supplement).

Genotyping

Genotyping for 20 polymorphisms from nine genes involved in the FU (*DPD*, *TS*, and *MTHFR*), oxaliplatin (*ERCC1*, *ERCC2*, *GSTT1*, *GSTM1*, and *GSTP1*), and irinotecan (*UGT1A1*) pathways was performed using polymerase chain reaction and quantitative multiplex polymerase chain reaction of short fluorescent fragments blinded to treatment arm and clinical data (Data Supplement).

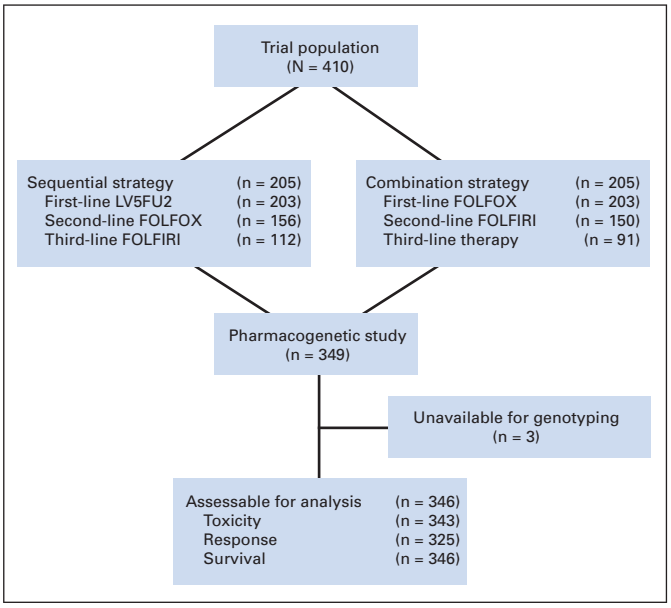

Fig 1. Study flow chart. Fluorouracil plus leucovorin (LV5FU2) alone or in combination with oxaliplatin (FOLFOX) or irinotecan (FOLFIRI) was administrated with modified de Gramont regimens.[20,21] Second-line treatment was FOLFOX in the sequential arm and FOLFIRI in the combination arm. FOLFIRI was administered as third-line treatment in the sequential arm.

Statistics

The end points of the study were severe toxicity (ie, grade 3 or 4 hematologic or GI toxicity or grade $\geq$ 2 cumulative peripheral neuropathy), 4-month ORR, PFS (for the first-line treatment), and overall survival (OS). Patients who received at least one and four treatment cycles were eligible for the analysis of toxicity and response, respectively. PFS was defined as the time from random assignment until the first occurrence of disease progression or death whatever the cause.

The analysis of prognostic factors (genotypes associated with a variation of toxicity/outcome regardless of treatment; ie, within a population receiving the same treatment) included, for FU, first-line patients in arm A; for oxaliplatin, first-line patients in arm B plus second-line patients in arm A, with stratification on the treatment arm; and for irinotecan, second-line patients in arm B plus third-line patients in arm A, with stratification on the treatment arm. The analysis of predictive factors for oxaliplatin (genotypes associated with a variation of the effect of a given treatment on toxicity/outcome; ie, assessment of the specific effect of oxaliplatin when added to LV5FU2) included first-line patients from both arms.

Genotype distributions were checked for agreement with those expected under Hardy-Weinberg equilibrium using a χ^2 test. The association of polymorphisms with ORR was tested using a logistic regression analysis. A Cox regression model (stratified by treatment arm) was used to test association with toxicity (in a time-dependent manner), PFS, and OS. The predictive values of genotypes were studied by testing the interaction between genotypes and the allocated treatment in the same model (ie, evaluating whether there was a significant trend between the hazard ratio [HR] or odds ratio [OR] in the combination arm compared with the sequential arm between genotypes harboring none, one, or two variant alleles). The test for trend assumes an ordering between the three categories. The test is most powerful if the mutated genes have additive effects, but the test is robust to deviation from this assumption and remains adequate if the mutation has a recessive or a dominant effect. The HRs and ORs have been estimated without the linearity constraint. Positive predictive values (PPVs) and negative predictive values (NPVs) were computed for polymorphisms significantly associated with ORR. Single locus analyses were then followed by haplotype association analyses using the THESIAS program[22] to account for the linkage disequilibrium (LD) between polymorphisms within the same gene. Multivariate analyses of toxicity were adjusted for age, sex, and performance status, and multivariate analyses of

Table 1. Baseline Patient Demographics and Clinical Characteristics, Toxicity, and Outcome

Demographic, Characteristic, Toxicity, or Outcome	Treatment Arm: Sequential Arm (n = 170) No. of Patients	%	Combination Arm (n = 176) No. of Patients	%
Age, years				
Median	67		68	
Range	37-80		34-83	
Male sex	102	60	112	64
Performance status				
0	79	47	75	45
1	66	39	75	43
2	25	15	26	15
No. of metastatic sites				
1	93	55	92	52
≥ 2	77	45	84	48
Köhne score*				
1	26	15	29	16
2	59	35	61	35
3	82	48	80	45
Severe toxicity†				
First line*	169		176	
GI	14	8	29	16
Hematologic	6	4	68	39
Neuropathy	—	—	111	63
Second line*	134		134	
GI	11	8	14	10
Hematologic	41	31	35	26
Neuropathy	66	49	46	34
Third line*	101		—	—
GI	7	7	—	—
Hematologic	30	32	—	—
Neuropathy	31	33	—	—
Response				
First line*	159		170	
Objective response	44	28	105	62
Second line*	125		121	
Objective response	30	24	16	13
Third line*	85		—	—
Objective response	8	9	—	—
Progression-free survival, months				
First line				
Median	5.7		7.7	
95% CI	4.9 to 6.5		6.9 to 8.5	
First plus second line				
Median	10.8		10.3	
95% CI	9.6 to 11.9		8.8 to 11.9	
Overall survival, months				
Median	16.8		15.9	
95% CI	15.8 to 19.4		14.4 to 18.2	

*Data not available for Köhne score in three and six patients in the sequential and combination arms, respectively; for first-line toxicity in one and one patient in the sequential and combination arms, respectively; for second-line toxicity in three and zero patients in the sequential and combination arms, respectively; for third-line toxicity in eight patients in the sequential arm; for first-line response in three and one patients in the sequential and combination arms, respectively; for second-line response in one and four patients in the sequential and combination arms, respectively; and for third-line response in two patients in the sequential arm.

†Severe toxicity denotes grade 3 or 4 toxicity (grade 2+ for cumulative peripheral neurologic toxicity) according to National Cancer Institute Common Toxicity Criteria version 2.0.

efficacy were adjusted for age, sex, and the Köhne prognostic score.[23,24] In this exploratory study, statistical significance was set at $P < .01$ (two-sided). For the purpose of judging significance, multivariate analysis was used.

RESULTS

Study Population

The main patient characteristics, toxicities, and outcomes are listed in Table 1. The 346 patients included in the pharmacogenetic study did not differ from the 64 other patients in terms of sex, performance status, number of metastatic sites, and Köhne prognostic score (Data Supplement). Among the clinical variables, multivariate analysis showed that only performance status and age were independent predictors for hematologic toxicity ($P = .01$ and $P = .008$, respectively), and only the Köhne score was an independent predictor for PFS ($P < .001$) and OS ($P = .004$; data not shown).

Genotypes and LD

All genotype distributions followed the Hardy-Weinberg equilibrium law and were similar to those previously reported in white populations. Strong LD was observed for pairs of polymorphisms within the same gene (Data Supplement). Estimated haplotype frequencies are provided in the Data Supplement.

Clinical Outcome and Genotypes

Toxicity. Because the *DPD-IVS14+1G>A* mutation was found heterozygously in only two patients (who both experienced FU-induced grade 4 neutropenia), *DPD* genotype was not included in the statistical analysis. No statistically significant association was found between any genotype and severe LV5FU2-induced toxicity, whether hematologic or GI (Data Supplement).

In the multivariate analysis, only the *ERCC2-K751QC* allele was significantly associated with an increased risk of FOLFOX-induced hematologic toxicity ($P = .01$; Data Supplement and Fig 2). No statistically significant association was found between any genotype and FOLFOX-induced severe GI or neurologic toxicity (Data

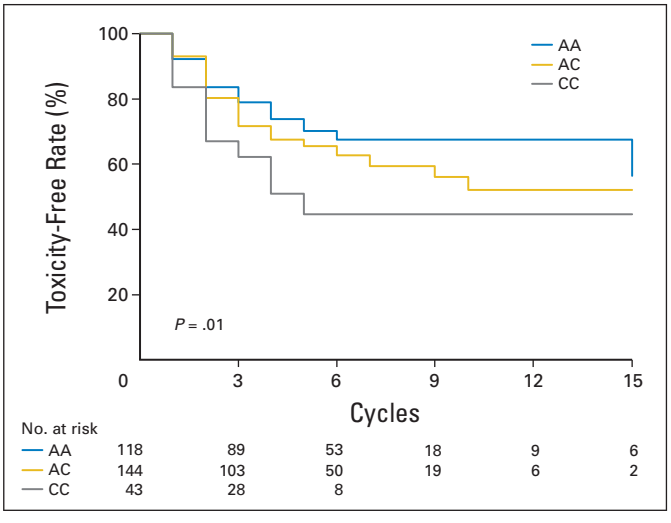

Fig 2. Grade 3 to 4 hematologic toxicity and *ERCC2–K751Q* polymorphism (time-dependent analysis). The *P* value indicated on Kaplan-Meier curves is the adjusted *P* value corresponding to the multivariate analysis. AA, homozygous wild-type; AC, heterozygous; CC, homozygous variant.

Supplement). No significant association was detected between *UGT1A1* genotypes and severe FOLFIRI-associated hematologic or GI toxicity (Data Supplement).

Response. In the multivariate analysis, a significant association was found between the presence of the *TS-5′UTR3RG* allele and a better ORR with LV5FU2 ($P = .009$; PPV = 39%; 95% CI, 27% to 51%; NPV = 80%; 95% CI, 72% to 81%) but not with FOLFOX ($P = .46$; Table 2; Data Supplement).

For FOLFOX, a treatment-dependent effect of *MTHFR-1298A>C* was found, as the effect of oxaliplatin on ORR increased with the number of *MTHFR-1298C* alleles (none: OR = 1.57; 95% CI, 0.97 to 2.56; one allele: OR = 2.61; 95% CI, 1.47 to 4.66; two alleles: OR = 6.35; 95% CI, 1.42 to 28.45; test for trend, $P = .008$ in multivariate analysis; Data Supplement). For patients treated with second-line FOLFOX (sequential arm), a significantly better ORR was observed for *ERCC1-IVS3+74G* allele carriers ($P = .01$ and $P = .02$ in the univariate and multivariate analyses, respectively; Table 2). Haplotype analysis further confirmed that the two haplotypes carrying the *ERCC1-IVS3+74G* allele were both associated with a better ORR ($P = .01$; data not shown). For patients treated with second-line FOLFOX (sequential arm), a significantly better ORR was also observed for *GSTT1*-positive allele carriers (≥ one allele; $P = .01$; PPV = 63%; 95% CI, 55% to 71%; NPV = 42%; 95% CI, 26% to 58%; Table 2). No significant association was detected between *UGT1A1* genotypes and ORR with FOLFIRI (data not shown).

Survival. No significant association was detected between any genotype and PFS, except for the *TS-5′UTR* polymorphism (test for trend, $P = .006$), because median PFS was better with first-line FOLFOX only in patients with *TS-5′UTR2R/2R* or *2R/3R* genotypes (HR = 0.39; 95% CI, 0.23 to 0.68; and HR = 0.59; 95% CI, 0.42 to 0.82, respectively; Table 3 and Fig 3) and, conversely, patients with the *TS-5′UTR3R/3R* genotype did not seem to benefit from first-line FOLFOX (HR = 0.96; 95% CI, 0.66 to 1.40). No significant association was detected between any genotype and OS, except for *GSTP1-341C>T* ($P = .01$ by multivariate analysis; Data Supplement). Haplotype analyses did not add significant information.

DISCUSSION

This study prospectively assessed 20 germline DNA polymorphisms in nine key genes involved in FU, oxaliplatin, and irinotecan pathways in 349 patients from a prospective, randomized, phase III trial. To our knowledge, this is the most comprehensive prospective pharmacogenetic study in mCRC published to date.

Unlike prior studies (mostly retrospective),[7,25] we did not confirm any significant impact of *TS* polymorphisms on severe FU-induced toxicity in a homogenous group of 168 patients treated with the LV5FU2 regimen. This discrepancy may be a result of methodologic heterogeneities among the studies, including variable doses and schedules of FU-based therapy, concomitant administration of other cytotoxic drugs, and variable tumor stages. In the prospective study by Schwab et al,[26] which included 683 patients with different tumor types treated with various FU monotherapy regimens, the *TS-5′UTR2R/3R* and *3R/3R* genotypes were associated with a lower risk for diarrhea. However, most patients received weekly high-dose infusional or bolus FU, and toxicity was mainly observed with those regimens whose toxicity is higher than that of LV5FU2 administered to patients in the present trial.[27]

No significant association was found between any genotype and severe oxaliplatin-induced cumulative neurotoxicity. In particular, we did not confirm the previously suggested negative impact of the *GSTP1-313A* allele[28,29] or the putative negative impact of the *GSTP1-313G* allele.[30]

We showed that the *ERCC2-K751QC* allele conferred a significantly higher risk for severe FOLFOX-induced hematologic toxicity. Variant *ERCC2-D312NA* or *ERCC2-K751QC* alleles may result in lower repair efficiency, as attested by higher DNA adduct levels,[31-33] suggesting that a decreased ability to repair platinum agent–induced damage to normal cells may lead to increased toxicity. Having sufficient nucleotide excision repair activity may be crucial for repairing damage to normal tissues during chemotherapy. If confirmed, these results could lead to specific dose adaptation and/or prevention therapy in patients harboring a high-risk genotype and treated with platinum agents.

As previously shown, *UGT1A1*28* and *UGT1A1-3156A* alleles tended to be associated with increased severe FOLFIRI-induced hematologic toxicity (Data Supplement).[17,18] The fact that this association did not reach statistical significance may be a result of the impact of other confounding factors on toxicity in our trial because FOLFIRI was only given in the second- and third-line setting.

We found a significant and independent association between the presence of the *TS-5′UTR3RG* allele and a better ORR in patients treated with LV5FU2. Previous analyses of the VNTR polymorphism alone in patients with mCRC treated with FU showed contradictory results. Indeed, higher ORRs have been found to be associated with either *TS-5′UTR2R/2R*[7,34] or *TS-5′UTR3R/3R*[8] genotypes, whereas other studies did not detect any significant impact of these polymorphisms.[25,35] In fact, the *G>C* SNP in the *TS-5′UTR3R* allele greatly influences *TS* expression, which may explain the previously mentioned discrepancies when studying the predictive impact of VNTR polymorphism alone. However, even when *TS* genotypes are dichotomized as high- and low-expression genotypes, based on the combined analysis of both VNTR and *G>C* SNPs,[5,36] it has been found that either low-expression *TS* genotypes were associated with a better ORR[36-38] or, alternatively, *TS*-expression genotypes did not significantly affect response to FU-based chemotherapy.[25,39] Differences in patient population, chemotherapy regimens (FU alone or in combination), or tissue material used for *TS* genotyping (ie, tumor *v* normal DNA, leading to different results as a result of loss of heterozygosity in 18p) are likely to contribute to these discrepancies. Additionally, these results and ours may also reflect a more complex reality, involving unknown genetic polymorphisms and gene-environmental interactions.

The design of our trial, which randomly compared LV5FU2 and FOLFOX, allowed us to evaluate a differential effect according to whether or not patients received oxaliplatin. We found that the *TS-5′UTR* polymorphism was not associated with ORR in the overall population treated with FOLFOX, in accordance with the results of a previous prospective pharmacogenetic study.[30]

We were unable to find any prognostic impact of the *MTHFR* polymorphisms on tumor response to LV5FU2. However, we observed a treatment-dependent effect of the *MTHFR-1298A>C* polymorphism, as the effect of oxaliplatin on ORR increased with the number of *MTHFR-1298C* alleles. Interestingly, the *MTHFR-1298A>C* polymorphism was not associated with response to FU alone in several previous studies[8,40] but

Table 2. Association Between Genotypes and Response to LV5FU2 and FOLFOX: Univariate and Multivariate Analysis

	LV5FU2				FOLFOX											
	Prognostic Analysis (n = 156)				Predictive Analysis (n = 325)				Prognostic Analysis (n = 293)				Prognostic Analysis (second line; n = 169)*			
			Multivariate	Univariate			Multivariate	Univariate			Multivariate	Univariate			Multivariate	Univariate
Genotype	OR	95% CI	P†	P†	OR‡	95% CI	P†	P†	OR	95% CI	P†	P†	OR	95% CI	P†	P†
TS																
TS-3′ UTR																
6+/6+	1		.73	.79	1.28	0.69 to 2.38	.84	.78	1		.60	.88	1		.64	.68
6+/6–	1.56	0.72 to 3.37			0.92	0.31 to 2.72			1.08	0.63 to 1.84			.87	.35 to 2.16		
6–/6–	0.83	0.23 to 3.02			2.28	1.33 to 3.93			1.29	0.51 to 3.24			1.88	0.45 to 7.76		
TS-5′ UTR																
3R/3R	1		.14	.15	1.81	1.01 to 3.24	.66	.47	1		.68	.93	1		.45	.31
2R/3R	0.83	0.38 to 1.83			2.39	1.45 to 3.94			1.32	0.74 to 2.35			0.93	0.34 to 2.57		
2R/2R	0.37	0.11 to 1.28			2.61	0.83 to 8.20			0.75	0.35 to 1.59			1.65	0.50 to 5.44		
TS-5′ UTR G/C																
2R/2R, 2R/3RC, 3RC/3RC	1		.009	.008	2.94	1.70 to 5.08	.12	.10	1		.46	.61	1		.71	.93
2R/3RG, 3RC/3RG	2.63	1.18 to 5.88			1.54	0.87 to 2.73			0.82	0.46 to 1.46			0.58	0.22 to 1.51		
3RG/3RG	4.33	0.89 to 21.15			1.37	0.43 to 4.31			2.38	0.81 to 7.03			2.37	0.29 to 19.05		
MTHFR																
677C>T																
C/C	1		.47	.39	2.51	1.44 to 4.38	.32	.018	1		.88	.74	1		.40	.37
C/T	1.68	0.78 to 3.66			1.93	1.15 to 3.23			1.17	0.68 to 2.02			1.19	0.48 to 2.96		
T/T	1.05	0.29 to 3.83			1.81	0.57 to 5.79			0.80	0.34 to 1.85			1.88	0.46 to 7.62		
1298A>C																
A/A	1		.04	.03	1.57	0.97 to 2.56	.008	.006	1		.26	.25	1		.77	.69
A/C	0.60	0.28 to 1.29			2.61	1.47 to 4.66			1.20	0.66 to 1.98			0.80	0.31 to 2.04		
C/C	0.25	0.05 to 1.19			6.35	1.42 to 28.45			1.59	0.69 to 2.09			0.92	0.25 to 3.36		
ERCC1																
IVS5+33C>A																
C/C					2.74	1.73 to 4.32	.04	.05	1		.93	.87	1		.81	.59
C/A					1.33	0.72 to 2.45‡			1.33	0.72 to 2.46			1.26	0.50 to 3.17		
A/A									0.20	0.02 to 1.83			—§			
N118NT>C																
T/T					2.02	1.10 to 3.69	.80	.99	1		.10	.14	1		.07	.06
T/C					2.38	1.44 to 3.94			1.45	0.82 to 2.57			1.06	0.38 to 2.96		
C/C					1.86	0.72 to 4.82			1.79	0.82 to 3.92			4.10	1.10 to 15.29		
IVS3+74C>G																
C/C					2.06	1.16 to 3.65	.91	.89	1		.09	.11	1		.02	.01
C/G					2.28	1.37 to 3.81			1.50	0.86 to 2.63			1.38	0.50 to 3.83		
G/G					1.90	0.68 to 5.36			1.83	0.82 to 4.06			5.98	1.55 to 23.15		
IVS4 + 86T>C																
T/T					1.65	1.07 to 2.55	.15	.21	1		.18	.14	1		.09	.08
C/T					4.25	1.98 to 9.16			1.39	0.81 to 2.41			1.37	0.53 to 3.50		
C/C					1.46	0.39 to 5.48			1.61	0.56 to 4.64			3.96	0.93 to 16.87		
ERCC2																
D312NG>A																
G/G					1.71	0.95 to 3.07	.34	.38	1		.04	.03	1		.09	.10
G/A					2.64	1.59 to 4.40			1.95	1.12 to 3.42			1.78	0.65 to 4.86		
A/A					1.68	0.62 to 4.55			1.86	0.77 to 4.49			3.06	0.82 to 11.51		
K751QA>C																
A/A					1.77	0.97 to 3.23	.70	.81	1		.16	.13	1		.08	.09
A/C					2.62	1.56 to 4.40			1.88	1.07 to 3.32			3.28	1.04 to 10.38		
C/C					1.64	0.69 to 3.91			1.39	0.63 to 3.09			3.19	0.78 to 13.04		
R156RA>C																
C/C					3.02	1.56 to 5.86	.40	.54	1		.04	.08	1		.03	.05
C/A					1.57	0.97 to 2.53			0.65	0.36 to 1.17			0.65	0.26 to 1.61		
A/A					2.79	1.11 to 6.98			0.47	0.22 to 0.98			0.17	0.03 to 0.87		
IVS19-70G>A																
G/G					1.61	0.96 to 2.71	.11	.12	1		.12	.08	1		.25	.21
G/A					2.71	1.59 to 4.63			1.41	0.82 to 2.43			1.03	0.40 to 2.66		
A/A					2.49	0.76 to 8.14			1.78	0.73 to 4.34			2.53	0.69 to 9.25		
GST																
GSTM1																
0 allele					2.31	1.41 to 3.78	.44	.48	1		.33	.48	1		.47	.43
1 allele					1.90	1.09 to 3.33			0.68	0.40 to 1.15			0.90	0.39 to 2.11		
≥ 2 alleles					1.51	0.40 to 5.73			1.05	0.31 to 3.54			—‖			
GSTP1-313A>G																
A/A					1.95	1.17 to 3.26	.76	.89	1		.42	.43	1		.27	.38
A/G					2.33	1.33 to 4.09			0.76	0.44 to 1.33			0.54	0.22 to 1.35		
G/G					2.19	0.76 to 6.37			0.81	0.35 to 1.88			0.63	0.15 to 2.61		
GSTP1-341C>T																
C/C					2.19	1.48 to 3.25	.82	.84	1		1.00	.96	1		.14	.18
C/T					1.80	0.71 to 4.52			1.00	0.45 to 2.21§			0.20	0.03 to 1.65		
T/T					—‖								—‖			
GSTT1																
0 allele					2.19	0.92 to 5.21	.85	.81	1		.16	.23	1		.01	.02
1 allele					1.97	1.22 to 3.17			1.92	0.95 to 3.87			7.15	0.87 to 58.44		
≥ 2 alleles					2.44	1.20 to 4.97			1.85	0.87 to 3.93			11.73	1.37 to 100.11		

Abbreviations: LV5FU2, FU plus leucovorin; FOLFOX, FU, leucovorin, and oxaliplatin; OR, odds ratio.
*Sequential arm only.
†Trend test.
‡OR of objective responses in combination arm compared with sequential arm.
§Two last categories grouped.
‖Category without event.

Table 3. Association Between Genotypes and Progression-Free Survival: Univariate and Multivariate Analysis

Genotype	No. of Patients	Prognostic Analysis				Predictive Analysis			
		HR	95% CI	Multivariate *P*†	Univariate *P*†	HR‡	95% CI	Multivariate *P*†	Univariate *P*†
TS									
TS-3′ UTR									
6+/6+	155	1		.85	.82	0.67	0.48 to 0.94	.98	.66
6+/6–	154	0.83	0.66 to 1.06			0.68	0.49 to 0.95		
6–/6–	32	1.23	0.83 to 1.82			0.70	0.34 to 1.45		
TS-5′ UTR									
3R/3R	119	1		.68	.98	0.96	0.66 to 1.40	.006	.005
2R/3R	156	0.93	0.72 to 1.20			0.59	0.42 to 0.82		
2R/2R	61	1.12	0.81 to 1.56			0.39	0.23 to 0.68		
TS-5′ UTR G/C									
2R/2R, 2R/3RC, 3RC/3RC	192	1		.17	.09	0.59	0.44 to 0.80	.08	.04
2R/3RG, 3RC/3RG	108	0.80	0.63 to 1.03			0.66	0.44 to 0.99		
3RG/3RG	25	0.88	0.57 to 1.37			1.45	0.60 to 3.50		
MTHFR									
677C>T									
C/C	157	1		.87	.78	0.58	0.41 to 0.82	.18	.25
C/T	150	0.88	0.70 to 1.12			0.68	0.48 to 0.96		
TT	38	1.11	0.76 to 1.61			0.98	0.50 to 1.92		
1298A>C									
A/A	168	1		.87	.84	0.85	0.62 to 1.16	.03	.08
A/C	143	0.91	0.72 to 1.15			0.51	0.35 to 0.73		
C/C	34	1.18	0.81 to 1.72			0.47	0.24 to 0.93		
ERCC1									
IVS5+33C>A									
C/C	260	1		.41	.35	0.65	0.50 to 0.84	.78	1.00
C/A	79	0.89	0.68 to 1.16			0.71	0.44 to 1.13		
A/A	6	0.93	0.41 to 2.12			0.07	0.01 to 0.40		
N118NT>C									
T/T	135	1		.78	.81	0.88	0.62 to 1.27	.04	.15
T/C	164	0.90	0.70 to 1.15			0.58	0.42 to 0.82		
C/C	45	1.02	0.72 to 1.44			0.45	0.24 to 0.83		
IVS3+74C>G									
C/C	144	1		.84	.81	0.82	0.58 to 1.16	.09	.23
C/G	159	0.97	0.77 to 1.23			0.56	0.39 to 0.78		
G/G	41	1.08	0.75 to 1.55			0.49	0.26 to 0.95		
IVS4+86T>C									
T/T	207	1		.35	.40	0.76	0.57 to 1.02	.14	.20
C/T	118	1.20	0.94 to 1.53			0.48	0.33 to 0.71		
C/C	20	0.99	0.61 to 1.62			0.57	0.23 to 1.43		
ERCC2									
D312NG>A									
G/G	145	1		.88	.80	0.78	0.55 to 1.11	.17	.28
G/A	164	0.91	0.72 to 1.15			0.60	0.43 to 0.83		
A/A	36	1.16	0.80 to 1.69			0.51	0.26 to 1.01		
K751QA>C									
A/A	134	1		.99	.78	0.71	0.49 to 1.03	.83	.91
A/C	162	0.91	0.71 to 1.16			0.58	0.42 to 0.81		
C/C	49	1.06	0.75 to 1.49			0.77	0.43 to 1.37		
R156RA>C									
C/C	109	1		.72	.92	0.54	0.37 to 0.81	.62	.88
C/A	163	0.90	0.70 to 1.16			0.76	0.55 to 1.05		
A/A	73	1.10	0.81 to 1.50			0.57	0.35 to 0.94		
IVS19-70G>A									
G/G	163	1		.46	.66	0.74	0.53 to 1.03	.36	.55
G/A	148	0.94	0.74 to 1.19			0.57	0.40 to 0.80		
A/A	32	1.36	0.92 to 2.00			0.63	0.31 to 1.28		

(continued on following page)

Table 3. Association Between Genotypes and Progression-Free Survival: Univariate and Multivariate Analysis (continued)

Genotype	No. of Patients	Prognostic Analysis				Predictive Analysis			
		HR	95% CI	Multivariate *P*†	Univariate *P*†	HR‡	95% CI	Multivariate *P*†	Univariate *P*†
GST									
GSTM1									
0 alleles	182	1		.03	.05	0.59	0.43 to 0.80	.56	.56
1 allele	142	0.88	0.70 to 1.11			0.74	0.52 to 1.04		
≥ 2 alleles	18	0.55	0.32 to 0.94			0.56	0.20 to 1.57		
GSTP1-313A>G									
A/A	159	1		.45	.75	0.71	0.51 to 1.00	.78	.71
A/G	148	1.09	0.84 to 1.39			0.57	0.40 to 0.80		
G/G	39	1.12	0.78 to 1.61			0.77	0.40 to 1.47		
GSTP1-341C>T									
C/C	301	1		.02	.04	0.68	0.54 to 0.87	.64	.68
C/T	41	0.63	0.44 to 0.91			0.51	0.26 to 1.00		
T/T	2	0.83	0.20 to 3.41			—§			
GSTT1									
0 alleles	65	1		.08	.05	0.52	0.30 to 0.91	.57	.83
1 allele	169	1.26	0.92 to 1.72			0.83	0.60 to 1.15		
≥ 2 alleles	109	1.36	0.97 to 1.90			0.51	0.34 to 0.76		
UGT1A1									
**28*									
6/6	168	1		.71	.45	0.59	0.42 to 0.82	.52	.59
6/7	140	1.18	0.93 to 1.50			0.75	0.53 to 1.06		
7/7	35	0.92	0.62 to 1.38			0.66	0.32 to 1.37		
3156G>A									
G/G	187	1		.98	.62	0.62	0.45 to 0.85	.82	.78
G/A	130	1.19	0.94 to 1.51			0.74	0.52 to 1.07		
A/A	29	0.80	0.52 to 1.23			0.57	0.26 to 1.25		

Abbreviation: HR, hazard ratio.
†Trend test.
‡HR of recurrence or death in combination arm compared with sequential arm.
§Category without event.

was recently found to be a prognostic factor for tumor response to FOLFOX in the OPTIMOX-2 trial.[41] This could be explained by the synergistic antitumor effect of FU-oxaliplatin combination observed in both preclinical and clinical studies,[42] pointing out the key role of *MTHFR* in the FU pathway.

Although we did not find any significant association between genotypes and ORR in the overall FOLFOX population, we found that *ERCC1-IVS3+74G* and *GSTT1*-positive allele carriers exhibited significantly higher ORRs when receiving second-line FOLFOX (sequential arm). Because responders to second-line FOLFOX (by definition in this trial, after failure of LV5FU2) are more likely to benefit specifically from oxaliplatin, this suggests a key role for *ERCC1* and *GSTT1* in oxaliplatin-induced tumor response. In the study by Ruzzo et al,[30] the *ERCC1* genotype was not correlated with ORR. However, the *ERCC1-N118NT* allele, which is in LD with *ERCC1-IVS3+74C*, was independently correlated with unfavorable PFS. This was also the case in the study by Stoehlmacher et al.[43] In our study, patients with the *ERCC1-N118NC* mutant allele marginally benefited from first-line FOLFOX (HR = 0.58; 95% CI, 0.42 to 0.82; and HR = 0.45; 95% CI, 0.24 to 0.83 for *T/C* and *C/C* genotypes, respectively; test for trend, $P = .04$; Table 3).

We found that patients with a positive *GSTT1* genotype had a better ORR when treated with FOLFOX after treatment failure on LV5FU2. Our observation is in agreement with previous reports that showed lower response to chemotherapy of *GSTT1* null genotypes.[29,44-47] However, because GSTs are involved in detoxification via direct glutathione conjugation of xenobiotics, one might expect that a null genotype resulting in no enzyme activity would lead to better tumor response to chemotherapy and a better outcome, as previously suggested in several cancers.[48] In fact, reduced GST activity leads to increased glutathione levels.[49,50] As glutathione has been shown to bind to cisplatin, elevated glutathione levels may lead to decreased DNA binding capability of platinum compounds.[47-50]

One of the major findings of our study is the predictive effect of the *TS-5′UTR* genotype on PFS. In fact, we found that the PFS benefit conferred by first-line FOLFOX over LV5FU2 was restricted to patients with the *TS-5′UTR2R/2R* or *2R/3R* genotypes, suggesting that only these patients benefited from first-line FOLFOX chemotherapy, whereas patients with the *TS-5′UTR3R/3R* genotype did not. Several studies have also reported a worse clinical outcome in case of *TS-5′UTR2R/2R* in tumor and/or normal tissue from patients with CRC receiving FU alone in the metastatic and adjuvant settings.[8,51,52] Collectively, these results suggest a worse outcome for patients with mCRC with the *2R/2R* genotype when treated with FU alone and support the use of oxaliplatin-based doublet therapy in the first-line setting rather than FU monotherapy.

Several randomized trials including the present one have recently shown that doublet therapies (a fluoropyrimidine with either irinotecan or oxaliplatin) do not significantly improve OS compared with the

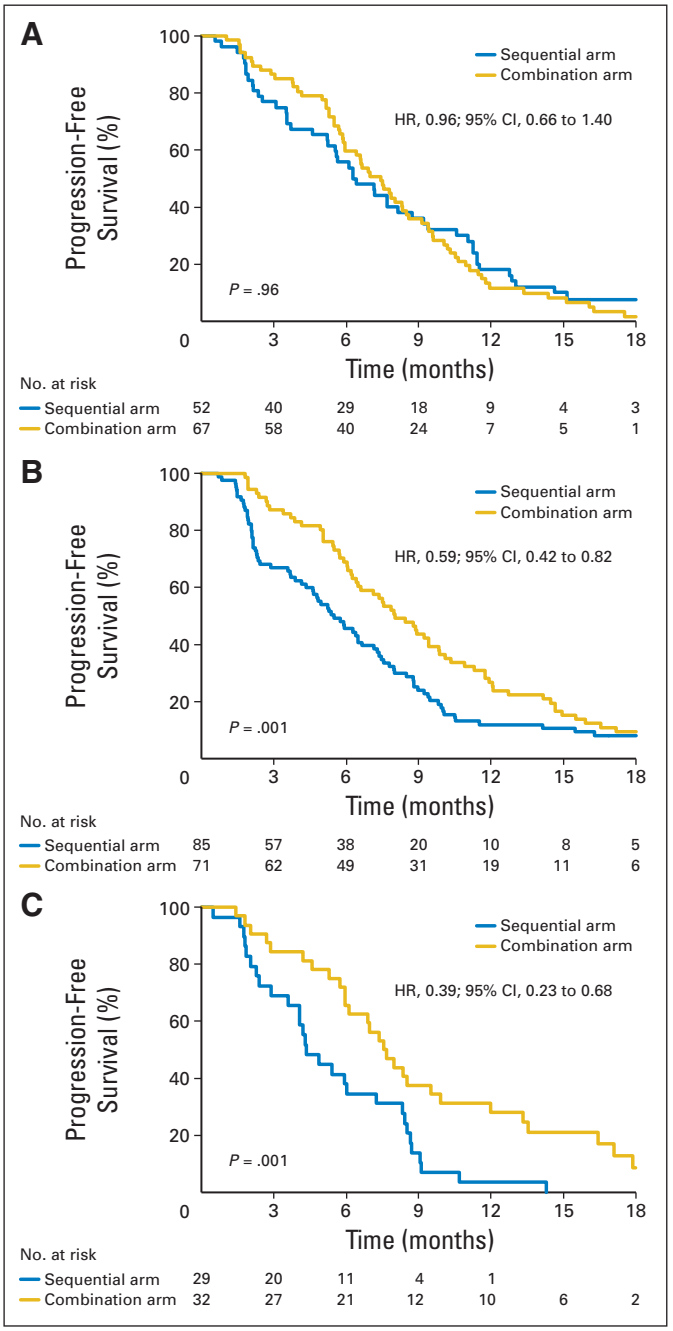

Fig 3. Progression-free survival and (A) *TS-5′ UTR3R/3R*, (B) *TS-5′ UTR2R/3R*, and (C) *TS-5′ UTR2R/2R* genotypes according to treatment arm. Hazard ratios (HR) of progression or death in the combination arm compared with the sequential arm are based on multivariate analysis. All *P* values indicated on Kaplan-Meier curves were adjusted *P* values corresponding to the multivariate analysis.

sequential use of cytotoxic drugs (starting with fluoropyrimidine alone) in nonresectable patients with mCRC.[3,4,19] Thus, sequential treatment remains a valid option for most patients with mCRC. In this respect, our findings represent a step toward personalized and optimized palliative chemotherapy in mCRC. We must emphasize the need to confirm our findings in large prospective trials. Given that approximately 200 tests have been performed, one would expect two false-positive findings, if one takes $P < .01$ as the limit of significance. In particular, further studies are warranted to confirm that first-line FOLFOX should be discouraged in patients with mCRC harboring a *3R/3R* genotype and that alternative treatments should be proposed. Finally, our findings may also be of interest to help in selecting patients with stage III CRC most likely to benefit from FOLFOX in the adjuvant setting.

AUTHORS' DISCLOSURES OF POTENTIAL CONFLICTS OF INTEREST

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AUTHOR CONTRIBUTIONS

Conception and design: Valérie Boige, Jean-Pierre Pignon, Philippe Beaune, Pierre Laurent-Puig
Provision of study materials or patients: Valérie Boige, Olivier Bouché, Michel Ducreux
Collection and assembly of data: Valérie Boige, Jean Mendiboure, Jean-Pierre Pignon, Marie-Anne Loriot, Michel Barrois, Delphine Le Corre, Isabelle Miran, Claire Mulot, Michel Ducreux
Data analysis and interpretation: Valérie Boige, Jean Mendiboure, Jean-Pierre Pignon, Marine Castaing, Michel Barrois, David Malka, David-Alexandre Trégouët, Philippe Beaune, Pierre Laurent-Puig
Manuscript writing: Valérie Boige, Jean Mendiboure, Jean-Pierre Pignon, Marine Castaing, Michel Barrois, David Malka, David-Alexandre Trégouët, Pierre Laurent-Puig
Final approval of manuscript: Valérie Boige, Jean Mendiboure, Jean-Pierre Pignon, Marie-Anne Loriot, Marine Castaing, Michel Barrois, David Malka, David-Alexandre Trégouët, Olivier Bouché, Delphine Le Corre, Isabelle Miran, Claire Mulot, Michel Ducreux, Philippe Beaune, Pierre Laurent-Puig

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