Pharmacogenetic Tailoring of Irinotecan-based First-line Chemotherapy in Metastatic Colorectal Cancer: Results of a Pilot Study

GILLES FREYER[1], AUDE DURET[1+], GERARD MILANO[2], ETIENNE CHATELUT[3], CHRISTINE REBISCHUNG[4], JEAN-PIERRE DELORD[5], YACINE MERROUCHE[6], GERARD LLEDO[7], MARIE-CHRISTINE ETIENNE[2] and CLAIRE FALANDRY[1]

[1]*Université de Lyon and Department of Medical Oncology, Centre Hospitalier Lyon Sud, Pierre-Bénite, France;*
[2]*Department of Pharmacology, Centre Antoine Lacassagne, Nice, France;*
Departments of [3]*Pharmacology and* [5]*Medical Oncology, Institut Claudius Régaud, Toulouse, France;*
[4]*Department of Medical Oncology, Hôpital Nord, Grenoble, France;*
[6]*Department of Medical Oncology, Institut de Cancérologie de la Loire, Saint-Priest-en-Jarez, France;*
[7]*Department of Medical Oncology, Clinique Saint-Jean, Lyon, France*

Abstract. *Background: Tolerability to irinotecan may be explained by pharmacogenomic polymorphisms. The purpose of this pharmacogenetic trial was to study the relevance of thymidylate synthase (TS) genotyping and of the isoform 1A1 of uridine diphosphate glucuronosyltransferase (UGT1A1) in order to tailor a combination chemotherapy regimen of 5-fluorouracil, leucovorin and irinotecan (FOLFIRI) in metastatic colorectal cancer. Patients and Methods: Patients with favourable TS and UGT1A1 profiles received high-dose (HD) FOLFIRI. Patients with TS-3R/3R could not receive HD-FOLFIRI, and those with UGT1A1-7/7 received standard FOLFIRI. The endpoints were overall response rate and safety. Results: Sixty-nine patients were enrolled in the study. Sixty-five patients received chemotherapy. Twenty patients (30.8%) achieved a partial response. The haematological toxicity was less in the HD-FOLFIRI subgroup. Patients having received HD-FOLFIRI did not experience increased levels of nausea-vomiting, asthenia or alopecia. Diarrhoea was more frequent with HD-FOLFIRI. Conclusion: The genotypic assessment allowed a safer use of HD-FOLFIRI. Further investigations may target patients who benefit from intensification.*

[+]Deceased.

Correspondence to: Professor Gilles Freyer, Département d'Oncologie Médicale, Centre Hospitalier Lyon Sud, 165 chemin du Grand Revoyet, 69495 Pierre-Bénite Cedex, France. Tel: +33 478864318, Fax: +33 478864319, e-mail: gilles.freyer@chu-lyon.fr

Key Words: Irinotecan, metastatic colorectal cancer, pharmacogenetics.

In most Western societies, colorectal cancer (CRC) is the second most common cause of cancer-related death. Approximately 35% of patients have stage IV disease at presentation, and 20% to 50% of patients with stage II or III disease will progress to stage IV. With the introduction of new therapies and the improvement of surgical techniques, the death rate continues to decline at a rate of approximately 1.8% per year. Until recently, the 5-fluorouracil-leucovorin regimen (5-FU/LV) was the standard treatment used, producing median survival times of approximately 12 months as first-line therapy for advanced CRC (1, 2). Starting in the mid-1990s, new efficient cytotoxic chemotherapeutic agents became available (3, 4).

In metastatic CRC (mCRC), first-line use of combination chemotherapy regimens is preferable to the use of single agents. New regimens with oxaliplatin and irinotecan have resulted in longer median survival times (3, 4). Two major regimens are currently used: FOLFIRI (5-FU/LV, irinotecan) and FOLFOX (5-FU/LV, oxaliplatin). The response rate with FOLFIRI and FOLFOX6 was 56% and 54%, respectively, while the median progression-free survival (PFS) time was of 8.5 and 8 months, respectively (4).

Given the similar efficacy between the FOLFIRI and FOLFOX regimens, the initial choice of which to use is largely governed by their differential toxicities. Neurotoxicity, neutropenia and thrombocytopenia are more frequent with FOLFOX, while febrile neutropenia, nausea-vomiting, stomatitis, alopecia and fatigue are more frequent with FOLFIRI. Grade 3-4 toxicities are more common with FOLFOX, whereas serious adverse events are more frequent with FOLFIRI (4). The use of irinotecan is often associated with unpredictable toxicities. These toxicities are the result of a direct toxic effect of SN-38, the main active metabolite

of irinotecan. Irinotecan is converted in SN-38 by tissue and serum carboxylesterase (CES), which is 100 to 1,000 times more cytotoxic than the parent irinotecan (5). Irinotecan-induced diarrhoea is thought to be a consequence of the direct enteric toxicity of SN-38. The level of SN-38 is regulated by the conversion of irinotecan by CES and by its glucuronidation in inactive SN-38 glucuronide (SN-38G) *via* uridine diphosphate glucuronosyltransferase (UGT). An important interpatient variability in the glucuronidation of SN-38 has been described experimentally, and isoform 1A1 of UGT (UGT1A1) has been identified as the main enzyme involved in the glucuronidation of SN-38 (6). It has been shown that the metabolism of irinotecan is substantially influenced by a nucleotide polymorphism in the TATA-box sequences of *UGT1A1*. A seventh TA-repeat (instead of six), named *UGT1A1**28, in one allele results in an approximately 70% reduction of transcriptional activity compared to wild-type (7). Such patients may be at increased risk for severe drug-related toxicities. In a small series of twenty patients treated with irinotecan, the polymorphism of *UGT1A1* was correlated with the occurrence of digestive and haematological toxicities (8). In patients having seven repeats, especially for homozygote status (7/7), the risk of grade 3-4 diarrhoea and neutropenia was higher than in patients with six repeats. This increase in toxicity was significantly correlated with lower levels of SN-38 glucuronidation. To date, it is recommended to treat patients bearing a *UGT1A1* 7/7 genotype with doses of irinotecan lower than 350 mg/m^2 every three weeks (9). This is consistent with the standard FOLFIRI regimen (irinotecan 180 mg/m^2 every two weeks), but this is not possible with the high-dose (HD) FOLFIRI previously described by Ducreux *et al*. (10).

Thymidylate synthase (TS) is the main target of 5-FU. The *TS* promoter contains two or three tandem repeats, so-called 2R or 3R, of a 28-base sequence that influence TS transcription level. The *TS* mRNA synthesis rate observed with a 3R promoter is significantly higher than that observed with a 2R promoter. Various genotypes (2R/2R, 2R/3R and 3R/3R) are well distributed and are partially able to predict response to 5-FU. Indeed, it has been shown that the 3R/3R genotype is associated with a lower response to fluorouracil-based chemotherapy (11-13).

In spite of a clear improvement in the management of mCRCs, the five-year survival rate remains at approximately 10%. The lack of efficacy of cytotoxic agents may be partly explained by a suboptimal use related to empirical design. It has been suggested that screening for *UGT1A1**28 variant before treatment may identify patients with lower glucuronidation rates and greater susceptibility to irinotecan-induced haematological and non-haematological toxicities (14). As interpatient tolerability and efficacy may be partially explained by gene polymorphisms, the purpose of the present trial was to study the benefit-to-risk ratio of a tailored FOLFIRI regimen in mCRC patients selected according to their *TS* and *UGT1A1* genotypes.

Patients and Methods

Study population. Patients aged at least 18 years and less than 85 years with histologically or cytologically proven measurable mCRC were eligible. Patients had to have at least one lesion ≥20 mm if measured with a computerised tomography (CT) or a magnetic resonance imaging (MRI) scan, or >10 mm if measured with spiral CT scan. Eligibility criteria included a World Health Organization performance status ≤2; adequate haematological (neutrophils ≥1.5×10^9/l, platelets ≥100×10^9/l), renal (serum creatinine ≤130 μmol/l) and hepatic (transaminases ≤2.5 the upper limit of normal [ULN], alkaline phosphatases ≤5 ULN and bilirubin ≤2 ULN) tests. A prior adjuvant non-irinotecan-based chemotherapy was allowed. Patients were not eligible if they presented contra-indications to irinotecan or 5-FU, if they had received prior adjuvant chemotherapy including irinotecan or if they had brain or meningeal metastases, documented dihydropyrimidine dehydrogenase (DPD) deficiency, intestinal obstruction or chronic inflammatory colorectal disease, history of previous cancer (except for treated cutaneous carcinomas, *in situ* carcinoma of the uterine cervix, breast cancer or bladder cancer), concurrent antitumour therapy and other significant medical conditions or any uncontrolled infection. Pregnant or breast-feeding women were excluded.

Potentially eligible patients underwent imaging assessments no more than four weeks before the onset of treatment and within the eight days of clinical and biological assessments. Written informed consent was obtained before enrolment in the study. The protocol was reviewed and approved by the Ethics Committee/Institutional Review Board and the study was conducted according to the Declaration of Helsinki and European Good Clinical Practice requirements. The trial was registered in the website http://www.ClinicalTrials.gov with reference identification NCT00138060.

Treatment regimens. The treatment was assigned according to the genotypic screening performed on blood DNA samples (Figure 1). Patients with favourable *TS* profile (*i.e*., genotype 2R/2R or 2R/3R) received either standard FOLFIRI or HD-FOLFIRI. Standard FOLFIRI consisted of irinotecan 180 mg/m^2 administered intravenously in 90 minutes, folinic acid (L-levofolinate 200 mg/m^2 or folinic acid 400 mg/m^2) delivered concurrently with irinotecan in 2 hours, intravenous 5-FU 400 mg/m^2 on day one (bolus or 15-minute continuous infusion), and intravenous 5-FU 2400 mg/m^2 on days one and two for 46 hours. HD-FOLFIRI consisted of irinotecan 260 mg/m^2 administered intravenously in 90 minutes, with folinic acid and 5-FU delivered similarly to standard FOLFIRI. Each cycle was delivered every 14 days. The dose of FOLFIRI was adjusted to the *UGT1A1* profile (Figure 1). Given the benefit of bevacizumab combined with standard FOLFIRI in terms of overall survival (15), bevacizumab was allowed in patients receiving standard FOLFIRI. Granulocyte colony-stimulating factor (G-CSF) was allowed in patients receiving HD-FOLFIRI.

The tolerability to chemotherapy was evaluated before each cycle. An absolute blood count was performed on day 14 and non-haematological toxicity was evaluated during the period between cycles. In case of dose reduction, the reduced doses were maintained for all subsequent cycles. Repeated grade 4 toxicities, in spite of a

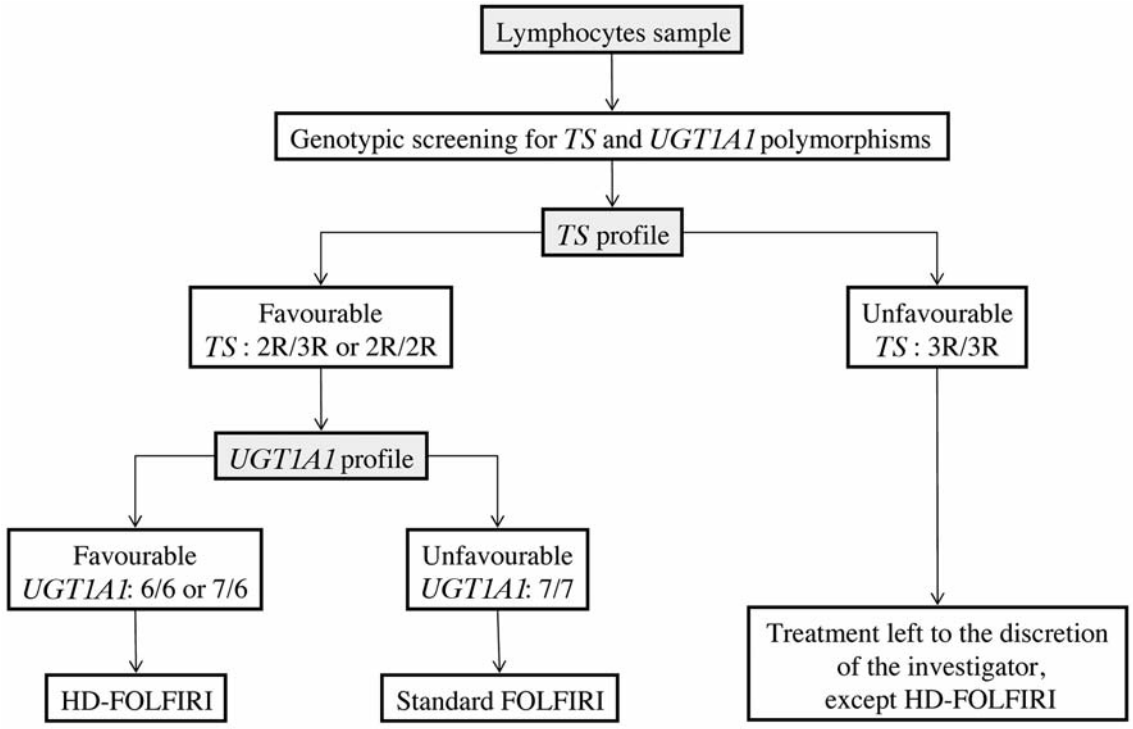


Figure 1. *Assignment of treatment according to the genotypic polymorphism of TS and UGT1A1.*

dose reduction (except for haematological toxicity and alopecia), led to treatment withdrawal.

Genotypic assessment. For *TS* assessment, Blood DNA was extracted on the PAXgene™ Blood DNA kit (PreAnalytiX GmbH, Hombrechtikon, Switzerland). The 28 bp repeat polymorphism in the 5' region of the *TS (TYMS)* gene was analysed by polymerase chain reaction (PCR) (3% agarose gel, 500 ng genomic DNA), as previously described (16). Expected fragment sizes were 220 bp for 2R and 248 bp for 3R.

For *UGT1A1* assessment, genomic DNA was extracted automatically from blood with the EZ1 processor (Qiagen, Courtaboeuf, France) according to the manufacturer's instructions. The TA repeat in the *UGT1A1* gene promoter (*UGT1A1**28 genotype, rs8175347) was analysed by PCR which were performed on genomic DNA using appropriate primers: forward 5'GCCAGTTCAACTGT TGTTGCC3', reverse 5'CCACTGGGATCAACAGTATCT3'. The expected fragments (320bp) were subjected to direct sequencing analysis with the Big dye terminator v3.1 cycle kit (Applied Biosystems, Warrington, UK) (17).

Statistical analysis. The current overall response rate (ORR) with a standard first-line treatment of mCRC is estimated to be close to 50% (3, 4). The hypothesis was to increase the ORR to 80% with a genotype-targeting strategy. This study was planned on the basis of a null hypothesis of 0.50 *versus* an alternative of 0.80, with a type I error (α) of 0.05 and a power of 90% (1-β). This hypothesis required at least 58 patients. In the study, eleven patients were added in order to prevent the risk of non-evaluable disease.

The primary endpoint was the ORR defined according to the RECIST criteria (18). Patients were evaluated after the fourth and the eighth cycles. The secondary endpoint was the safety. Toxicity was graded according to NCI-CTC criteria (version 3.0) (19), and serious adverse events were defined according to the guidelines of the International Conference on Harmonization (20).

Results

Patient and tumour characteristics. From 2005 to 2008, 69 patients with mCRC (37 men, 32 women) were enrolled from six French centres. The median age was 64 years (range, 38 to 83 years). The main patient characteristics are described in Table I and the distribution of *UGT1A1* and *TS* genotypes is presented in Figure 2.

Treatment. Based on the genotypic profile, eight patients were planned to receive standard FOLFIRI, 44 to receive HD-FOLFIRI and 17 to receive a chemotherapy regime of the investigator's choice. Among the latter 17 patients with a 3R/3R *TS* genotype, seven were planned to receive a standard FOLFIRI. Four patients were not able to receive chemotherapy; one died of a post-surgical septic shock, one had a pulmonary embolism, one had a general status impairment contraindicating chemotherapy and one had a psychiatric decompensation.

Table I. *Patient and tumour characteristics at baseline.*

Characteristic	Overall population	Standard FOLFIRI	HD-FOLFIRI	Other CT
No. of patients	69	15	44	10
PS, n (%)				
0	33 (47.8)	8 (53.4)	22 (50.0)	3 (30.0)
1	30 (43.5)	5 (33.3)	20 (45.5)	5 (50.0)
2	1 (1.5)	0 (0.0)	0 (0.0)	1 (10.0)
Unknown	5 (7.2)	2 (13.3)	2 (4.5)	1 (10.0)
TS, n (%)				
2R/2R	13 (18.8)	2 (13.3)	11 (25.0)	0 (0.0)
2R/3R	39 (56.5)	6 (40.0)	33 (75.0)	0 (0.0)
3R/3R	17 (24.7)	7 (46.7)	0 (0.0)	10 (100)
UGT1A1, n (%)				
6/6	26 (37.7)	2 (13.3)	20 (45.5)	4 (40.0)
6/7	35 (50.7)	6 (40.0)	24 (54.5)	5 (50.0)
7/7	7 (10.1)	6 (40.0)	0 (0.0)	1 (10.0)
Unknown	1 (1.5)	1 (6.7)	0 (0.0)	0 (0.0)
No. of metastatic sites, n (%)				
1	35 (50.7)	9 (60.0)	23 (52.3)	3 (30.0)
2	23 (33.3)	4 (26.6)	15 (34.1)	4 (40.0)
>2	9 (13.1)	1 (6.7)	6 (13.6)	2 (20.0)
Unknown	2 (2.9)	1 (6.7)	0 (0.0)	1 (10.0)
Metastatic sites, n (%)				
Local relapse	3 (4.3)	1 (6.7)	1 (2.3)	1 (10.0)
Lymph nodes	18 (26.1)	3 (20.0)	14 (31.8)	1 (10.0)
Liver	51 (73.9)	11 (73.3)	34 (77.3)	6 (60.0)
Peritoneal carcinomatosis	9 (13.1)	3 (20.0)	2 (4.5)	4 (40.0)
Lung	17 (24.6)	2 (13.3)	13 (29.5)	2 (20.0)
Other	11 (15.9)	0 (0.0)	8 (18.2)	3 (30.0)

PS, Performance status; CT, chemotherapy.

A total of 65 patients actually received chemotherapy: 14 received standard FOLFIRI (of which eight had a favourable *TS* genotype and six had an unfavourable *TS* genotype), 42 received HD-FOLFIRI and nine received other regimens consisting of FOLFOX-6 (n=4), FOLFOX-4 (n=1), TOMOX (n=2), XELOX (n=1), or capecitabine single-agent (n=1). The median number of delivered cycles was eight for standard FOLFIRI (range, 4 to 19) and eight for HD-FOLFIRI (range, 1 to 20). The mean dose intensity of irinotecan was 99.9% and 95.6%, for standard and HD-FOLFIRI, respectively.

Overall, 25 patients received G-CSF: 6 (42.9%) with standard FOLFIRI, 17 (40.5%) with HD-FOLFIRI and 2 (22.2%) with other regimens. Fifteen patients received bevacizumab; five, nine and one patient with standard FOLFIRI, HD-FOLFIRI and others regimens, respectively.

Safety. Safety analysis was conducted on the 65 treated patients. Two cases of febrile neutropenia were reported; one in standard FOLFIRI (*TS* 2R/3R, *UGT1A1* 7/7) and one in HD-FOLFIRI. Main toxicities according to genotypic profile and treatment regimen are summarised in Table II. The tailoring of FOLFIRI based on the genotypic profile led to a weaker haematological toxicity in the HD-FOLFIRI subgroup. Patients having received HD-FOLFIRI did not experience more nausea-vomiting, asthenia or alopecia than patients having received standard FOLFIRI or other chemotherapy regimens. In contrast, diarrhoea was more frequent with HD-FOLFIRI, but remained of grade 1-2 except for one patient. The other toxicities were infrequent and were always mild to moderate.

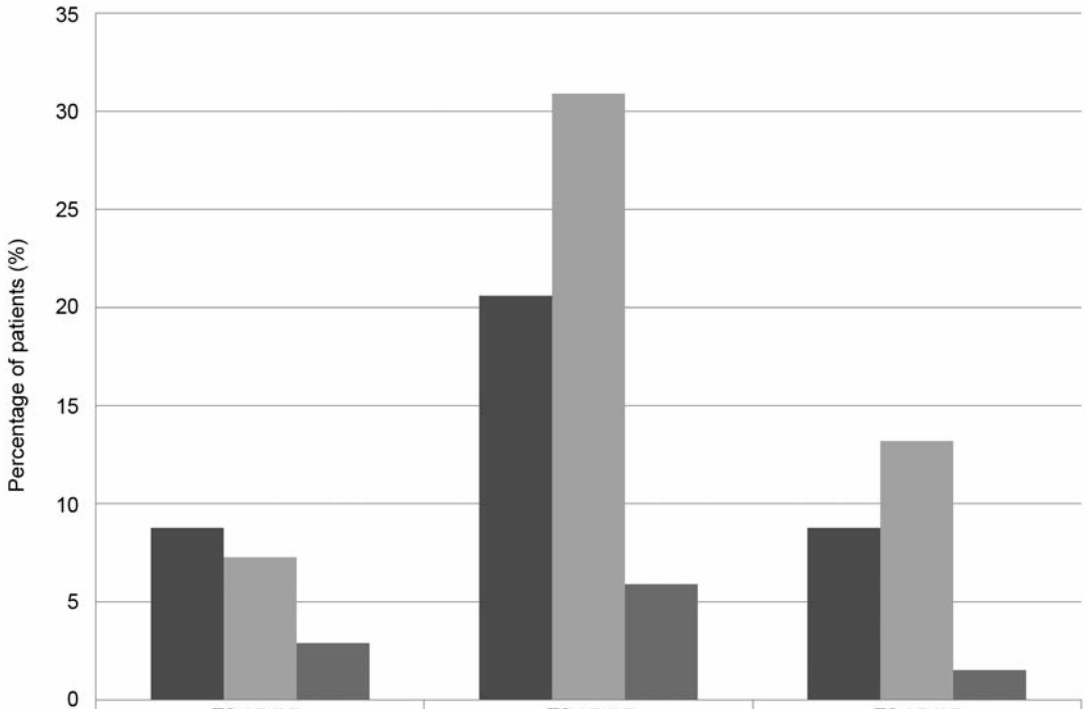
A serious adverse event was reported in eight HD-FOLFIRI patients (19%), two standard FOLFIRI patients (14.3%) and in one patient having received another chemotherapy regimen (11.1%). Overall, no toxic deaths occurred.

Disease outcome. Among the 65 treated patients, 20 (30.8%) achieved a partial response (PR) and 34 (52.3%) had a stable disease: 4 (28.6%) patients achieved a PR in the standard FOLFIRI subgroup, 14 (33.3%) in the HD-FOLFIRI subgroup and 2 (22.2%) in patients having received another chemotherapy regimen. The ORR and the rate of liver surgery according to genotypic profile and chemotherapy regimens are presented in Table III. The median duration of response was 11 months (range, 3.2 to 47.8 months) and the median duration of stabilisation was 8.6 months (range, 2.5 to 45.4 months).

After a median follow-up of 24 months, 61 patients (93.8%) had relapsed with a median PFS of 8 months (range, 1.4 to 47.8 months): the median PFS was 7.7 months for patients with a favourable *TS* profile and *UGT1A1* 6/6 or 6/7, 6 months for patients with *UGT1A1* 7/7, and 9.8 months for patients with *TS* 3R/3R. At the time of analysis, 30 patients (46.1%) died from disease progression with a median survival time of 18 months (range, 3 to 48 months): the median survival time was 18.8 months for patients with a favourable *TS* profile and *UGT1A1* 6/6 or 6/7, 12.3 months for patients with *UGT1A1* 7/7 and 19 months for patients with *TS* 3R/3R.

Discussion

The management of mCRC is becoming increasingly complex, with the development of innovative new therapies and further scope for combinations of active agents. One of the first advances in treatment was the introduction of new cytotoxic agents, such as irinotecan and oxaliplatin, combined with 5-FU (3, 4). The second step was to increase the dose of the cytotoxic agent to improve the efficacy of those regimens. The increase in the dose of irinotecan from 180 mg/m^2 to 260 mg/m^2 every two weeks led to an improvement of tumour growth control with 54% of PR (10). Another approach in treatment intensification was to combine three drugs instead of two in the triplet regimen 5-FU-irinotecan-oxaliplatin (FOLFOXIRI). A first phase III



	TS 2R/2R	*TS* 2R/3R	*TS* 3R/3R
UGT1A1 6/6	8.8	20.6	8.8
UGT1A1 6/7	7.3	30.9	13.2
UGT1A1 7/7	2.9	5.9	1.5

Figure 2. *Distribution of UGT1A1 and TS genotypes.*

study comparing FOLFOXIRI to FOLFIRI in 283 participants demonstrated more toxic side effects but no difference in outcome for the triple combination (21). A further phase III trial compared FOLFOXIRI with FOLFIRI in 244 persons and found a statistically significant overall survival advantage of 22.6 months *versus* 16.7 months (p=0.032) for the triplet arm with increased but manageable toxicities (22). More recently, targeted therapies are increasingly used combined with or as an alternative to chemotherapy. For mCRC, two monoclonal antibodies, bevacizumab and cetuximab, have entered routine clinical practice. Bevacizumab targets vascular endothelial growth factor (VEGF) overexpressed in approximately 50% of CRCs (23). Bevacizumab in combination with chemotherapy is now regarded as an appropriate first-line therapy for mCRC (15, 24). Cetuximab is directed against the extracellular domain of the epidermal growth factor receptor (EGFR). Although the *EGFR* gene is overexpressed or up-regulated in 60% to 80% of CRCs (25), response to cetuximab appears independent of EGFR expression (26, 27).

Such treatment progress has resulted in improvements in overall survival and the possibility of liver surgery. However, these improvements have been made at the price of higher toxicities, of which some can be predicted by the use of pharmacogenetics. With regard to the safety and efficacy of irinotecan, the most relevant pharmacogenetic analysis is that of screening based on the genotype of *TS* and *UGT1A1* (7, 8, 11-13). In the present study, the selection of patients for HD-FOLFIRI treatment based on *UGT1A1* genotype probably had a protective effect on haematological toxicity. The incidence of neutropenia was similar in patients of the HD-FOLFIRI subgroup receiving or not receiving G-CSF support, although HD-FOLFIRI is known to be strongly haematotoxic (10). The incidence of neutropenia was lower in the HD-FOLFIRI group, although the use of G-CSF was similar with that of the standard FOLFIRI group. Overall, patients experienced the same level of toxicity irrespective of treatment regimens, except for diarrhoea which remained mild to moderate and was probably independent of genotype. No additional severe diarrhoea with HD-FOLFIRI was observed compared with other regimens. Another issue was the treatment choice left to the discretion of investigators in the subset of patients having a *TS* 3R/3R genotype. All of those patients had received a TS inhibitor (5-FU,

Table II. *Toxicities according to genotypic profile and chemotherapy regimens in the 65 treated patients.*

	TS 2R/2R or 2R/3R				
	UGT1A1 6/6 or 6/7		UGT1A1 7/7	TS 3R/3R	
	HD-FOLFIRI	St. FOLFIRI	St. FOLFIRI	St. FOLFIRI	Other CT
No. of patients	42	2	6	6	9
Neutropenia, n (%)					
Grade 1-2	3 (7.1)	0 (0.0)	1 (16.7)	1 (16.7)	2 (22.2)
Grade 3-4	5 (11.9)	0 (0.0)	2 (33.3)	0 (0.0)	0 (0.0)
Diarrhoea, n (%)					
Grade 1-2	26 (61.9)	1 (50.0)	1 (16.7)	3 (50.0)	3 (33.3)
Grade 3	1 (2.4)	1 (50.0)	0 (0.0)	0 (0.0)	0 (0.0)
Nausea, n (%)					
Grade 1-2	20 (47.6)	2 (100)	2 (33.3)	3 (50.0)	3 (33.3)
Vomiting, n (%)					
Grade 1-2	7 (16.7)	1 (50.0)	0 (0.0)	1 (16.7)	1 (11.1)
Grade 3-4	0 (0.0)	0 (0.0)	0 (0.0)	1 (16.7)	0 (0.0)
Asthenia, n (%)					
Grade 1-2	18 (42.9)	0 (0.0)	2 (33.3)	3 (50.0)	3 (33.3)
Grade 3	1 (2.4)	1 (50.0)	1 (16.7)	0 (0.0)	1 (11.1)
Alopecia, n (%)					
Grade 1-2	9 (21.4)	0 (0.0)	2 (33.3)	2 (33.3)	1 (11.1)
Grade 3	1 (2.4)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)

CT, Chemotherapy.

Table III. *Response and liver surgery according to genotypic profile and chemotherapy regimens in the 65 treated patients.*

	TS 2R/2R or 2R/3R				
	UGT1A1 6/6 or 6/7		UGT1A1 7/7	TS 3R/3R	
n (%)	HD-FOLFIRI	St. FOLFIRI	St. FOLFIRI	St. FOLFIRI	Other CT
No. of patients	42	2	6	6	9
ORR	14 (33.3)	0 (0.0)	2 (33.3)	2 (33.3)	2 (22.2)
SD	19 (45.2)	2 (100)	3 (50.0)	3 (50.0)	7 (77.8)
PD	8 (19.0)	0 (0.0)	1 (16.7)	1 (16.7)	0 (0.0)
NE	1 (2.4)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)
Liver surgery	14 (33.3)	0 (0.0)	1 (16.7)	0 (0.0)	4 (44.4)

capecitabine or raltitrexed) and the disease outcome was not worse than in the subset of patients with a favourable TS profile. However, it has been shown that the response to raltitrexed is affected by a high *TS* mRNA expression (28) and patients with a *TS* 2R/2R are more likely to respond to capecitabine than patients with a 2R/3R or 3R/3R polymorphism (29). In the present study, genomic DNA was extracted from leukocytes. This approach may be debatable as the intratumoural profile may be different from the leukocyte profile.

These polymorphisms are well identified but their clinical relevance is not fully elucidated. A recent study did not support the clinical use of molecular markers, such as TS and UGT1A1, to predict the toxicity of irinotecan (30). A high level of topoisomerase-1 was associated with an overall survival benefit with first-line combination chemotherapy and may be a candidate to identify subpopulations that may benefit from irinotecan (30, 31). Among the specific gene polymorphisms known to be involved in the irinotecan metabolic pathway, the combination of at least one *SLCO1B1* 521 T allele, one *ABCB1* 1236 C allele and one *UGT1A1**28 variant 7 repeat demonstrated a statistically significant association with grade 3-4 toxicities in mCRC patients (32).

The present trial was one of the first to explore prospectively a screening of mCRC patients based on efficacy and safety biomarkers. In this multicenter study, the median time to obtain the genotype was approximately five days showing that this screening was feasible in routine practice. Although this approach failed to demonstrate any benefit of the selected markers, the genotypical assessment allowed a safer use of HD-FOLFIRI. It was noteworthy that 10% of patients had a *UGT1A1* 7/7 profile, requiring this to be taken into account in routine practice. It is acknowledged that such patients cannot tolerate the standard 360 mg/m^2 3-week regimen. In terms of dose intensity, HD-FOLFIRI is not acceptable in *UGT1A1* 7/7 patients. Patients should therefore be offered *UGT1A1* genotyping in routine practice and in the context of any clinical trial exploring the benefit of high-dose chemotherapy (33). Pharmacogenomics should be explored to optimise the tailoring of treatment according to the profile of each patient. This is crucial because of the combination of chemotherapy with targeted therapies. At present, two French trials are ongoing to select the treatment of CRC patients on their *UGT1A1* profile, namely FFCD 06-04 and FFCD 05-04 (www.ffcd.fr).

Acknowledgements

We thank Stéphane Nancey, Arnaud Tantin, Dominique Mille, Jean-Louis Gaudin, Magali Litor, Sophie Tartas, Véronique Trillet-Lenoir, Pascal Artru, and Raymonde Maraval-Gaget for their contribution. Isabelle Chapelle-Marcillac provided editorial assistance in the preparation of the manuscript. Pfizer France provided financial support for the medical writing.

References

1 Advanced Colorectal Cancer Meta-Analysis Project: Modulation of fluorouracil by leucovorin in patients with advanced colorectal cancer: evidence in terms of response rate. J Clin Oncol *10*: 896-903, 1992.

2 Meta-analysis Group In Cancer: Efficacy of intravenous continuous infusion of fluorouracil compared with bolus administration in advanced colorectal cancer. J Clin Oncol *16*: 301-308, 1998.
3 Goldberg RM, Sargent DJ, Morton RF, Fuchs CS, Ramanathan RK, Williamson SK, Findlay BP, Pitot HC and Alberts SR: A randomized controlled trial of fluorouracil plus leucovorin, irinotecan, and oxaliplatin combinations in patients with previously untreated metastatic colorectal cancer. J Clin Oncol *22*: 23-30, 2004.
4 Tournigand C, André T, Achille E, Lledo G, Flesh M, Mery-Mignard D, Quinaux E, Couteau C, Buyse M, Ganem G, Landi B, Colin P, Louvet C and de Gramont A: FOLFIRI followed by FOLFOX6 or the reverse sequence in advanced colorectal cancer: a randomized GERCOR study. J Clin Oncol *22*: 229-237, 2004.
5 Mathijssen RH, van Alphen RJ, Verweij J, Loos WJ, Nooter K, Stoter G and Sparreboom A: Clinical pharmacokinetics and metabolism of irinotecan (CPT-11). Clin Cancer Res *7*: 2182-2194, 2001.
6 Iyer L, King CD, Whitington PF, Green MD, Roy SK, Tephly TR, Coffman BL and Ratain MJ: Genetic predisposition to the metabolism of irinotecan (CPT-11). Role of uridine diphosphate glucuronosyltransferase isoform 1A1 in the glucuronidation of its active metabolite (SN-38) in human liver microsomes. J Clin Invest *101*: 847-854, 1998.
7 Iyer L, Hall D, Das S, Mortell MA, Ramírez J, Kim S, Di Rienzo A andRatain MJ: Phenotype-genotype correlation of in vitro SN-38 (active metabolite of irinotecan) and bilirubin glucuronidation in human liver tissue with *UGT1A1* promoter polymorphism. Clin Pharmacol Ther *65*: 576-582, 1999.
8 Iyer L, Das S, Janisch L, Wen M, Ramírez J, Karrison T, Fleming GF, Vokes EE, Schilsky RL and Ratain MJ: *UGT1A1**28 polymorphism as a determinant of irinotecan disposition and toxicity. Pharmacogenomics J *2*: 43-47, 2002.
9 Innocenti F, Undevia SD, Iyer L, Chen PX, Das S, Kocherginsky M, Karrison T, Janisch L, Ramírez J, Rudin CM, Vokes EE and Ratain MJ: Genetic variants in the UDP-glucuronosyltransferase 1A1 gene predict the risk of severe neutropenia of irinotecan. J Clin Oncol *22*: 1382-1388, 2004.
10 Ducreux M, Raoul JL, Marti P, Merrouche Y, Tigaud JM, Rebischung C and Boige V: High-dose irinotecan plus LV5FU2 or simplified LV5FU (HD-FOLFIRI) for patients with untreated metastatic colorectal cancer: a new way to allow resection of liver metastases? Oncology *74*: 17-24, 2008.
11 Villafranca E, Okruzhnov Y, Dominguez MA, García-Foncillas J, Azinovic I, Martínez E, Illarramendi JJ, Arias F, Martínez Monge R, Salgado E, Angeletti S and Brugarolas A: Polymorphisms of the repeated sequences in the enhancer region of the thymidylate synthase gene promoter may predict downstaging after preoperative chemoradiation in rectal cancer. J Clin Oncol *19*: 1779-1786, 2001.
12 Iacopetta B, Grieu F, Joseph D and 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 *85*: 827-830, 2001.
13 Marsh S, McKay JA, Cassidy J and McLeod HL: Polymorphism in the thymidylate synthase promoter enhancer region in colorectal cancer. Int J Oncol *19*: 383-386, 2001.
14 Ando Y, Saka H, Ando M, Sawa T, Muro K, Ueoka H, Yokoyama A, Saitoh S, Shimokata K and Hasegawa Y: Polymorphisms of UDP-glucuronosyltransferase gene and irinotecan toxicity: a pharmacogenetic analysis. Cancer Res *60*: 6921-6926, 2000.
15 Hurwitz H, Fehrenbacher L, Novotny W, Cartwright T, Hainsworth J, Heim W, Berlin J, Baron A, Griffing S, Holmgren E, Ferrara N, Fyfe G, Rogers B, Ross R and Kabbinavar F: Bevacizumab plus irinotecan, fluorouracil, and leucovorin for metastatic colorectal cancer. N Engl J Med *350*: 2335-2342, 2004.
16 Mandola MV, Stoehlmacher J, Muller-Weeks S, Cesarone G, Yu MC, Lenz HJ and Ladner RD: A novel single nucleotide polymorphism within the 5' tandem repeat polymorphism of the thymidylate synthase gene abolishes USF-1 binding and alters transcriptional activity. Cancer Res *63*: 2898-2904, 2003.
17 Rouits E, Boisdron-Celle M, Morel A and Gamelin E: Simple and sensitive high-performance liquid chromatography method for simultaneous determination of urinary free cortisol and 6β-hydroxycortisol in routine practice for CYP 3A4 activity evaluation in basal conditions and after grapefruit juice intake. J Chromatogr B Analyt Technol Biomed Life Sci *793*: 357-366, 2003.
18 Eisenhauer EA, Therasse P, Bogaerts J, Schwartz LH, Sargent D, Ford R, Dancey J, Arbuck S, Gwyther S, Mooney M, Rubinstein L, Shankar L, Dodd L, Kaplan R, Lacombe D and Verweij J: New response evaluation criteria in solid tumours: revised RECIST guideline (version 1.1). Eur J Cancer *45*: 228-247, 2009.
19 Common Terminology Criteria for Adverse Events v3.0 (CTCAE). Publish date: August 9, 2006. http://ctep.cancer.gov
20 International Conference on Harmonization Guidelines. http://www.ich.org.
21 Souglakos J, Androulakis N, Syrigos K, Polyzos A, Ziras N, Athanasiadis A, Kakolyris S, Tsousis S, Kouroussis Ch, Vamvakas L, Kalykaki A, Samonis G, Mavroudis D and Georgoulias V: FOLFOXIRI (folinic acid, 5-fluorouracil, oxaliplatin and irinotecan) *vs*. FOLFIRI (folinic acid, 5-fluorouracil and irinotecan) as first-line treatment in metastatic colorectal cancer (MCC): a multicentre randomised phase III trial from the Hellenic Oncology Research Group (HORG). Br J Cancer *94*: 798-805, 2006.
22 Falcone A, Ricci S, Brunetti I, Pfanner E, Allegrini G, Barbara C, Crinò L, Benedetti G, Evangelista W, Fanchini L, Cortesi E, Picone V, Vitello S, Chiara S, Granetto C, Porcile G, Fioretto L, Orlandini C, Andreuccetti M and Masi G; Gruppo Oncologico Nord Ovest: Phase III trial of infusional fluorouracil, leucovorin, oxaliplatin, and irinotecan (FOLFOXIRI) compared with infusional fluorouracil, leucovorin, and irinotecan (FOLFIRI) as first-line treatment for metastatic colorectal cancer: the Gruppo Oncologico Nord Ovest. J Clin Oncol *25*: 1670-1676, 2007.
23 Lee JC, Chow NH, Wang ST and Huang SM: Prognostic value of vascular endothelial growth factor expression in colorectal cancer patients. Eur J Cancer *36*: 748-753, 2000.
24 Kabbinavar FF, Hambleton J, Mass RD, Hurwitz HI, Bergsland E and Sarkar S: Combined analysis of efficacy: the addition of bevacizumab to fluorouracil/leucovorin improves survival for patients with metastatic colorectal cancer. J Clin Oncol *23*: 3706-3712, 2005.
25 Porebska I, Harlozinska A and Bojarowski T: Expression of the tyrosine kinase activity growth factor receptors (EGFR, ERB B2, ERB B3) in colorectal adenocarcinomas and adenomas. Tumour Biol *21*: 105-115, 2000.
26 Saltz LB, Meropol NJ, Loehrer PJ Sr, Needle MN, Kopit J and Mayer RJ: Phase II trial of cetuximab in patients with refractory colorectal cancer that expresses the epidermal growth factor receptor. J Clin Oncol *22*: 1201-1208, 2004.

27 Cunningham D, Humblet Y, Siena S, Khayat D, Bleiberg H, Santoro A, Bets D, Mueser M, Harstrick A, Verslype C, Chau I and Van Cutsem E: Cetuximab monotherapy and cetuximab plus irinotecan in irinotecan-refractory metastatic colorectal cancer. N Engl J Med *351*: 337-345, 2004.

28 Farrugia DC, Ford HE, Cunningham D, Danenberg KD, Danenberg PV, Brabender J, McVicar AD, Aherne GW, Hardcastle A, McCarthy K and Jackman AL: Thymidylate synthase expression in advanced colorectal cancer predicts for response to raltitrexed. Clin Cancer Res *9*: 792-801, 2003.

29 Park DJ, Stoehlmacher J, Zhang W, Tsao-Wei D, Groshen S and Lenz HJ: Thymidylate synthase gene polymorphism predicts response to capecitabine in advanced colorectal cancer. Int J Colorectal Dis *17*: 46-49, 2002.

30 Braun MS, Richman SD, Quirke P, Daly C, Adlard JW, Elliott F, Barrett JH, Selby P, Meade AM, Stephens RJ, Parmar MK and Seymour MT: Predictive biomarkers of chemotherapy efficacy in colorectal cancer: results from the UK MRC FOCUS trial. J Clin Oncol *26*: 2690-2698, 2008.

31 Kostopoulos I, Karavasilis V, Karina M, Bobos M, Xiros N, Pentheroudakis G, Kafiri G, Papakostas P, Vrettou E and Fountzilas G: Topoisomerase I but not thymidylate synthase is associated with improved outcome in patients with resected colorectal cancer treated with irinotecan-containing adjuvant chemotherapy. BMC Cancer *9*: 339, 2009.

32 Rhodes KE, Zhang W, Yang D, Press OA, Gordon M, Vallböhmer D, Schultheis AM, Lurje G, Ladner RD, Fazzone W, Iqbal S and Lenz HJ: *ABCB1, SLCO1B1* and *UGT1A1* gene polymorphisms are associated with toxicity in metastatic colorectal cancer patients treated with first-line irinotecan. Drug Metab Lett *21*: 23-30, 2007.

33 McLeod HL and Watters JW: Irinotecan pharmacogenetics: Is it time to intervene? J Clin Oncol *22*: 1356-1359, 2004.

Received October 19, 2010
Revised November 19, 2010
Accepted November 22, 2010