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ORIGINAL REPORT

The Role of UGT1A1*28 Polymorphism in the Pharmacodynamics and Pharmacokinetics of Irinotecan in Patients With Metastatic Colorectal Cancer

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ABSTRACT

Purpose
UGT1A1*28 polymorphism has been associated with decreased glucuronidation of SN38, the active metabolite of irinotecan. This could increase toxicity with this agent.

Patients and Methods
In a prospective study, 250 metastatic colorectal cancer patients were treated with irinotecan, fluorouracil, and leucovorin as first-line treatment. UGT1A1*28 polymorphism was investigated with respect to the distribution of hematologic and nonhematologic toxicity, objective response rate, and survival. Pharmacokinetics was investigated in a subgroup of patients (71 of 250) who had been analyzed with respect to toxicity and efficacy.

Results
UGT1A1*28 polymorphism was associated with a higher risk of grade 3 to 4 hematologic toxicity (odds ratio [OR], 8.63; 95% CI, 1.31 to 56.55), which was only relevant for the first cycle, and was not seen throughout the whole treatment period for patients with both variant alleles TA_7/TA_7 compared with wild-type TA_6/TA_6. The response rate was also higher in TA_7/TA_7 patients (OR, 0.32; 95% CI, 0.12 to 0.86) compared with TA_6/TA_6. A nonsignificant survival advantage was observed for TA_7/TA_7 when compared with TA_6/TA_6 patients (hazard ratio, 0.81; 95% CI, 0.45 to 1.44). Higher response rates were explained by a different pharmacokinetics with higher biliary index [irinotecan area under the curve (AUC)×(SN38 AUC/SN38G AUC)] and lower glucuronidation ratio (SN38G AUC/SN38 AUC) associated with the TA_7/TA_7 genotype and a higher response rate, indicating that the polymorphism is functionally relevant.

Conclusion
The results indicate that UGT1A1*28 polymorphism is of some relevance to toxicity; however, it is less important than discussed in previous smaller trials. In particular, the possibility of a dose reduction for irinotecan in patients with a UGT1A1*28 polymorphism is not supported by the result of this analysis.

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INTRODUCTION

Combinations of irinotecan, fluorouracil (FU), and leucovorin (LV) in a regimen (FOLFIRI) demonstrated superiority in overall response and survival as compared with FU/LV alone.[1]

Marked interpatient variability has been reported for the frequency of toxicity to FOLFIRI.[2] This may be due to the variability in levels of the active irinotecan metabolite SN38 in plasma and/or at the site of toxicity (ie, bone marrow). Several factors determine SN38 levels. In particular, the conversion of irinotecan to SN38 by the carboxylesterase enzymes[3] and the glucuronidation of SN38 to the inactive SN38 glucuronide (SN38G) by uridine diphosphate glucuronosyltransferase, most notably UGT1A1, the same enzyme that conjugates bilirubin.[4] Other metabolic or transport pathways can affect irinotecan and SN38 disposition; in particular, cytochrome p450 isoforms 3A4, 3A5,[5] and the adenosine triphosphate–binding cassette transporters.[6] The distribution qualities of SN38 versus irinotecan may also play an important role.

Impaired glucuronidation activity of the UGT1A1 enzyme, possibly due to the genetic polymorphism of the *UGT1A1* gene, has been thought to

have a crucial role in the variable toxicity reported. More than 50 genetic variations in the promoter and coding regions of the *UGT1A1* gene are currently known.[7] In particular, UGT1A1*28 (also known as TA indel) polymorphism, characterized by an extra TA repeat in the promoter region of the gene [$A(TA)_7TAA$] is believed to be involved in irinotecan toxicity. This polymorphism is believed to be associated with reduced glucuronidation of SN38 compared with wild-type *UGT1A1* [$A(TA)_6TAA$], possibly resulting in variable SN38 pharmacokinetics.[8-10]

The aim of this study was to investigate prospectively the role of UGT1A1*28 polymorphism in the development of toxicity in colorectal cancer (CRC) patients treated with the FOLFIRI regimen as first-line treatment for their metastatic disease. Furthermore, the study also investigated the effects of UGT1A1*28 genotype on irinotecan pharmacokinetics and tumor response.

PATIENTS AND METHODS

Study Design and Patient Eligibility

This prospective interinstitutional study involved 13 centers in Northeast Italy. The primary objective was to assess the relationship between the UGT1A1*28 allele and toxicity. Secondary objectives included investigation of the relationship between UGT1A1*28 and tumor response (complete response [CR], CR + partial response [PR]), clinical benefit (CR + PR + stable disease [SD]), time to progression (TTP), overall survival, and the pharmacokinetics of irinotecan. The study was coordinated and sponsored by the Centro di Riferimento Oncologico, National Cancer Center of Aviano (Aviano, Italy). The institutional review board of each participating institution approved the study protocol, and all patients signed a written informed consent before entering the study.

Eligibility criteria included histologically metastatic CRC; no prior chemotherapy for metastatic disease (adjuvant chemotherapy was allowed, except for irinotecan); age between 18 and 75 years; absolute neutrophil count $\geq$ 2,000 μL; platelets $\geq$ 100,000 μL; performance status (WHO) of 0 to 2; life expectancy more than 3 months; at least one measurable cancer lesion; normal renal function (creatinine clearance $>$ 65 mL/min by Cockcroft formula)[11]; ALT and AST less than 2.0$\times$ the upper limit of normal and total serum bilirubin less than 1.25$\times$ the upper limit of normal.

Treatment

Patients were treated with either the modified FOLFIRI regimen ($>$90% of patients) as described by Tournigand[12] (irinotecan 180 mg/m^2 intravenously for 2 hours on day 1 + FU 400 mg/m^2 bolus followed by FU 2,400 mg/m^2 continuous infusion during 46 hours + LV 200 mg/m^2 on day 1 every 2 weeks) or the FOLFIRI regimen (irinotecan 180 mg/m^2 intravenously for 2 hours on day 1 + FU 400 mg/m^2 bolus followed by FU 600 mg/m^2 continuous infusion during 22 hours on days 1 and 2 + LV 200 mg/m^2 on days 1 and 2 every 2 weeks).[1] Before starting irinotecan administration, patients were treated with atropine 0.5 mg, dexamethasone 8 mg, and granisetron 3 mg or ondansetron 8 mg. Diarrhea was treated promptly with loperamide 4 mg at the onset, and then with 2 mg every 2 hours until the patient was diarrhea free for at least 12 hours.

Efficacy and Toxicity Assessment

Objective clinical evaluation, blood counts, and hepatic and renal function tests were performed within 48 hours before each cycle. Patients were questioned specifically about nausea and vomiting, mucositis, diarrhea, malaise, and appetite at every cycle. Computed tomography scans of measurable lesions were assessed at baseline and then repeated at least every four cycles. Objective tumor response and duration of response were assessed by WHO criteria.[13] Patients with progressive disease (PD) could be dismissed from the study or could continue chemotherapy for two additional cycles according to the physician's decision.

Toxicity was evaluated according to National Cancer Institute Common Toxicity Criteria.[14] A single cycle of chemotherapy administration was considered sufficient for evaluation of acute toxicity, whereas response to treatment was evaluated only in patients who had received at least four cycles of chemotherapy. Clinical evaluations were performed blindly with respect to the genetic results, and clinical data were monitored by the study sponsor.

Chemotherapy was delayed until recovery if neutrophils were $\leq$ 1,500 μL or in the presence of significant, persisting, nonhematologic toxicity. In the event of grade 3 or 4 neutropenia, thrombocytopenia, and diarrhea, the irinotecan dose was reduced (from 180 mg/m^2) to 90 to 150 mg/m^2 based on the physician's assessment. Treatment was discontinued in the event of repeated grade 3 to 4 toxicity, despite dose reduction, or because of patient refusal.

UGT Genotyping Assays

Pyrosequencing (Biotage, Uppsala, Sweden) was used for genotyping genomic DNA extracted from peripheral blood using the High Pure PCR Template Preparation Kit (Roche Diagnostics GmbH, Mannheim, Germany). We used a reverse 5′-biotinylated PCR primer, 5′-GGC-AAA-GCC-ACA-GGT-CAG-C-3′, and a forward primer, 5′-TTT-TGG-CTC-GTG-CAG-GGT-GGA-C-3′. The reaction was performed in a 50-μL volume with 2 mmol/L $MgCl_2$, deoxynucleotide triphosphates 125 μmol/L each, 200 nmol/L each primer, and 1 unit of Taq polymerase for 35 cycles of amplification (15 seconds at 94°C, 30 seconds at 53.5°C, and 30 seconds at 72°C) obtaining a 98- to 100-bp fragment. The sequencing primer was 5′-GTG-GAC-TGG-CCT-CCT-TC-3′.

Drug Assays and Pharmacokinetic Analysis

Centro di Riferimento Oncologico and Noale Hospital participated in the collection of samples for pharmacokinetic evaluation in 71 patients (all treated with the modified FOLFIRI regimen). Heparinized serial blood samples were collected before drug administration, and at 0.5, 1.0, 1.5, 2.0, 2.25, 2.50, 2.75, 3.0, 3.50, 4.0, 6.0, 8.0, 10.0, 14.0, 26.0, and 50.0 hours after the start of the irinotecan infusion. Plasma was obtained immediately by centrifugation of the blood samples at 3,000 $\times$ *g* for 15 minutes at 4°C, and stored at −80°C. The total plasma concentration of irinotecan (lactone plus carboxylate) and its metabolites, SN38 and SN38G, were determined using high-performance liquid chromatography as described.[15,16] The intra- and interday variability was less than 10% for irinotecan and other metabolites.

Noncompartmental analysis was used for pharmacokinetics analysis. The apparent terminal elimination rate constant (*k*) was determined by log-linear regression analysis of the terminal phase of the plasma concentration–time curve. The terminal half-life was calculated as 0.693/*k*. A linear-log trapezoidal numerical integration method was used to calculate the area under the irinotecan, SN38, and SN38G plasma concentration–time curve ($AUC_{0\ to\ last}$) from time 0 to the last sampling time (C_{last}). Area under the irinotecan, SN38, and SN38G plasma concentration–time curves to infinite time (AUC) were calculated by adding C_{last}/k to $AUC_{0\ to\ last}$.

The extent of glucuronidation of SN38 to SN38G in plasma was defined as the ratio of SN38G AUC/SN38 AUC (glucuronidation ratio [GR]). The biliary index (BI) was defined as the product of the irinotecan AUC and the ratio of the SN38 AUC over the SN38G AUC.

Statistical Methods

Unconditional logistic regression models were used to assess the relative risk of clinical benefit/response and grade 3 to 4 toxicity between patients with different UGT1A1*28 polymorphic statuses and to control for confounding factors (including all available prognostic factors: sex, age, primary tumor sites, and prior adjuvant chemotherapy). The overall survival and TTP were estimated using the Kaplan and Meier product limit method. Cumulative survival probability was calculated at 24 months within each of the three genotypes. Differences were tested using the log-rank test.

To assess the relative excess risk of progression/death between patients with different UGT1A1*28 genotypes and to control for confounding factors, proportional hazards models (including sex, age, primary sites, and adjuvant chemotherapy) were fitted computing hazard ratios (HRs) and the corresponding 95% CIs. The proportional assumption was examined with log-log survival plots or by adding time-dependent interaction terms into the model.

Differences between pharmacokinetic parameters by genotype, clinical toxicity, and therapeutic outcome were assessed by the nonparametric Wilcoxon or Kruskal-Wallis tests.

RESULTS

Patient Characteristics, Treatment Tolerance, and Response

From July 2002 to October 2005, 267 white patients were enrolled. After the Monitoring Committee evaluation, 250 patients were found eligible and included in the final analysis (Table 1). In total, 2,151 2-week cycles were administered (median, eight; range, one to 20).

Table 1. Demographic and Clinical Characteristics at Study Entry

Characteristic	No.	%
Total	267	
Eligible	250	
Not eligible*	17	
Sex		
Male	162	64.8
Female	88	35.2
Age, years		
Mean	60.6	
SD	10.3	
Range	26-75	
Primary tumor site		
Colon	179	71.6
Right	79	44.1
Left	100	55.9
Rectum	71	28.4
Metastatic site		
Liver	181	
Lung	83	
Other	80	
Number of metastatic sites		
1	108	43.2
≥ 2	142	56.8
Radical surgery of earlier tumor		
Yes	200	80.0
No	50	20.0
Stage of disease at diagnosis†		
I	5	2.0
II	20	8.0
III	65	26.0
IV	160	64.0
Adjuvant radiotherapy (only for rectum)		
Yes	33	46.5
No	38	53.5
Adjuvant chemotherapy		
FU	10	12.2
FU + FA	66	80.5
FU + FA + irinotecan	1	1.2
Other‡	5	6.1

Abbreviations: SD, standard deviation; FU, fluorouracil; FA, folinic acid; FUDR, floxuridine.

*Ten patients had no measurable metastatic lesions at the time of the recruitment, five were previously treated for the metastatic disease, one did not have a histologic diagnosis of colorectal adenocarcinoma, and one had previously developed a second neoplasm.

†Evaluated by TNM scale.

‡Three patients were treated with capecitabine, one patient was treated with FUDR i.a., and one patient was treated with FU + FA + oxaliplatin.

Tolerance to treatment was evaluated at the first cycle (acute toxicity) and cumulatively at the end of therapy (Table 2). Severe toxicity (grade 3 to 4) of any kind was seen in 22 of 250 patients (8.8%) during the first cycle and in 68 of 250 patients (27.2%) during the entire course of chemotherapy. The most frequent severe toxicity was neutropenia, and the predominant nonhematologic toxicities were diarrhea and nausea and/or vomiting.

Objective response (CR + PR) was observed in 103 of 238 assessable patients (43.3%) and included 18 CRs (7.6%) and 85 PRs (35.7%). SD was observed in 66 patients (27.7%) and PD was observed in 69 patients (29.0%).

UGT1A1*28 Genotype Relationship to Toxicity, Response, and Survival

Homozygous TA_7/TA_7, heterozygous TA_6/TA_7, and wild-type TA_6/TA_6 genotype frequencies were 8.8% (n = 22), 45.6% (n = 114), and 45.6% (n = 114), respectively. Allele frequencies of TA_6 and TA_7 were 68.4% and 31.6%, respectively. No TA_5 or TA_8 alleles were detected.

During the first cycle of therapy, a significant association was observed between the TA_7 allele and grade 3 to 4 hematologic toxicity (Table 3). Patients with TA_7/TA_7 genotype had more than eight-fold increased risk of developing grade 3 to 4 hematologic toxicity as compared with TA_6/TA_6 patients (odds ratio [OR], 8.63; 95% CI, 1.31 to 56.55). Grade 3 to 4 neutropenia occurred in two of 114 (1.7%) TA_6/TA_6, six of 114 (5.3%) TA_6/TA_7, and three of 22 (13.6%) TA_7/TA_7 patients.

A four-fold increase in OR, although not statistically significant, was observed between the TA_7 allele and grade 3 to 4 nonhematologic toxicity (Table 3). Diarrhea was not associated with the TA indel polymorphism. Grade 3 diarrhea occurred in three TA_6/TA_6 and three TA_6/TA_7 patients. No grade 4 diarrhea was observed during the first cycle. One TA_6/TA_6 patient developed asthenia and vomiting and one patient developed alopecia; one TA_7/TA_7 patient had grade 3 nausea/vomiting, one TA_7/TA_7 patient had one grade 3 cardiologic event, and one TA_7/TA_7 patient developed a grade 3 infection without neutropenia. A similar trend to that observed for grade 3 to 4 hematologic toxicity was also seen for overall toxicity of any kind (Table 3).

In contrast to what was observed during the first cycle, evaluation of the hematologic and nonhematologic toxicities throughout the entire course of treatment (including toxicity during the first cycle and subsequent cycles) did not reveal any clear association with UGT1A1*28 polymorphism. In particular, grade 3 to 4 neutropenia occurred in four of 22 (18.2%) TA_7/TA_7, 20 of 114 (17.5%) TA_7/TA_6, and 11 of 114 (9.6%) TA_6/TA_6 patients. Grade 3 to 4 diarrhea occurred in one of 22 (4.5%) TA_7/TA_7, 14 of 114 (12.3%) TA_7/TA_6, and six of 114 (5.3%) TA_6/TA_6 patients.

Dose reduction occurred in 20 of 114 (17.5%), 26 of 112 (23.2%), and four of 22 (18.2%) of TA_6/TA_6, TA_7/TA_6, and TA_7/TA_7 patients, respectively, with no significant association with genotypes.

To assess the risk of toxicity after the first cycle of therapy and its relation to *UGT1A1* polymorphism under more homogeneous conditions, the cumulative toxicity between cycle 2 and 6 was compared with that observed during cycle 1. No significant association was found between the TA_7 allele and grade 3 to 4 toxicity occurring during the second to sixth cycles of therapy (Table 3).

The *UGT1A1* TA_7/TA_7 genotype seemed to be associated with increased clinical benefit and tumor response. Homozygous TA_7/TA_7

Table 2. Most Common Adverse Events (grades 1 to 4 and 3 to 4)

Adverse Event	First Cycle (n = 250)				Entire Course of Chemotherapy (n = 250)			
	Grade 1-4		Grade 3-4		Grade 1-4		Grade 3-4	
	Total	%	Total	%	Total	%	Total	%
Nonhematologic toxic effects								
Diarrhea	69	27.6	6	2.4	115	46.0	21	8.4
Nausea	57	22.8	2	0.8	103	41.2	4	1.6
Vomiting	27	10.8	2	0.8	57	22.8	7	2.8
Asthenia	25	10.0	1	0.4	60	24.0	3	1.2
Alopecia	8	3.2	1	0.4	35	14.0	8	3.2
Mucositis	17	6.8	0	0.0	60	24.0	7	2.8
Anorexia	3	1.2	0	0.0	8	3.2	0	0.0
Infection without concomitant grade 3-4 neutropenia	2	0.8	1	0.4	8	3.2	3	1.2
Hematologic toxic effects								
Anemia	33	13.2	1	0.4	59	23.6	3	1.2
Neutropenia	40	16.0	11	4.4	91	36.4	35	14.0
Leukopenia	27	10.8	2	0.8	61	24.4	15	6.0
Fever with concomitant grade 3-4 neutropenia	2	0.8	2	0.8	7	2.8	3	1.2
Thrombocytopenia	2	0.8	0	0.0	8	3.2	0	0.0

patients had a significantly reduced risk of PD or SD compared with the wild-type genotype (OR, 0.32; 95% CI, 0.12 to 0.86). Considering clinical benefit, the homozygous TA_7/TA_7 patients had a significantly lower risk of experiencing progression (OR, 0.19; 95% CI, 0.04 to 0.89; Table 4). Analysis of TTP showed a significant reduction for patients with the variant allele TA_7/TA_7 (HR, 0.52; 95% CI, 0.31 to 0.90) and TA_6/TA_7 (HR, 0.73; 95% CI, 0.55 to 0.98) compared with the wild-type genotype. Median TTPs were 316, 239, and 226 days for *UGT1A1* TA_7/TA_7, TA_6/TA_7, and TA_6/TA_6 patients, respectively.

Median follow-up of the study was 15 months (range, 1 to 31 months), 130 deaths (52%) occurred during this period in the 250 patients enrolled. Survival analysis showed a nonsignificant survival

Table 3. Association Between UGT1A1*28 Polymorphism and Grades 3 to 4 Toxicity According to NCI-CTC Classification for Cycle 1, During Cycles 2 to 6, and During the Entire Course of Chemotherapy

Polymorphism	Cycle 1 (n = 250)				Cycles 2 to 6 (n = 217*)				End of Therapy (n = 250)			
	No. of Patients With an Event	No. of Patients Without an Event	OR†	95% CI	No. of Patients With an Event	No. of Patients Without an Event	OR†	95% CI	No. of Patients With an Event	No. of Patients Without an Event	OR†	95% CI
Hematologic												
TA_6/TA_6	2	112	1‡		9	90	1‡		12	102	1‡	
TA_6/TA_7	7	107	3.47	0.69 to 17.34	14	83	1.65	0.65 to 4.16	21	93	1.93	0.89 to 4.23
TA_7/TA_7	3	19	8.63	1.31 to 56.55	2	19	1.08	0.21 to 5.70	4	18	1.97	0.56 to 6.99
χ^2 for trend, *P*			.02				.5				.1	
Nonhematologic												
TA_6/TA_6	5	109	1‡		7	92	1‡		18	96	1‡	
TA_6/TA_7	3	111	0.63	0.15 to 2.75	7	90	1.11	0.36 to 3.44	20	94	1.09	0.53 to 2.24
TA_7/TA_7	3	19	4.10	0.86 to 19.55	2	19	1.46	0.26 to 8.13	5	17	1.41	0.45 to 4.47
χ^2 for trend, *P*			.3				.7				.6	
Overall§												
TA_6/TA_6	7	107	1‡		13	86	1‡		25	89	1‡	
TA_6/TA_7	10	104	1.51	0.54 to 4.18	19	78	1.70	0.76 to 3.76	35	79	1.60	0.87 to 2.96
TA_7/TA_7	5	17	4.94	1.36 to 17.98	4	17	1.70	0.48 to 6.06	8	14	2.07	0.76 to 5.64
χ^2 for trend, *P*			.03				.2				.08	

Abbreviations: NCI-CTC, National Cancer Institute Common Toxicity Criteria; PD, progressive disease; CRC, colorectal cancer; OR, odds ratio.
*A total of 211 patients had completed the six cycles of chemotherapy and six patients discontinued therapy prematurely due to toxicity in the absence of PD. For the remaining 33 patients, treatment was discontinued prematurely for reasons other than toxicity (eg, PD); therefore, they were not included in the cycle 2 to 6 toxicity determination.
‡Reference category.
†Logistic regression model including terms for age, sex, location of CRC (right colon, left colon, and rectum), and adjuvant chemotherapy.
§Hematologic and nonhematologic.

Table 4. Association Between UGT1A1*28 Polymorphism and Failure to Respond to Treatment in 238 Advanced CRC Patients

Polymorphism	Complete Response		Partial Response		Stable Disease		Progressive Disease	
	No.	%	No.	%	No.	%	No.	%
UGT1A1*28								
TA_6/TA_6 (n = 109)	10	9.2	34	31.2	29	26.6	36	33.0
TA_6/TA_7 (n = 108)	5	4.7	40	37.0	32	29.6	31	28.7
TA_7/TA_7 (n = 21)	3	14.3	11	52.4	5	23.8	2	9.5

	Response*						Response†					
	Yes	No	OR‡	95% CI	OR	95% CI	Yes	No	OR	95% CI	OR	95% CI
TA_6/TA_6	73	36		1§		1§	44	65		1§		1§
TA_6/TA_7	77	31	0.77	0.42 to 1.39	0.65‖	0.36 to 1.16	45	63	0.92	0.53 to 1.56	0.77‖	0.46 to 1.31
TA_7/TA_7	19	2	0.19	0.04 to 0.89			14	7	0.32	0.12 to 0.86		
Overall	169	69					103	135				

Abbreviations: CRC, colorectal cancer; OR, odds ratio.
*Responder (yes), stable, partial and complete response; nonresponder (no), progression (seven patients experienced progression before the fourth cycle of chemotherapy).
†Responder (yes), partial and complete response; nonresponder (no), stable and experienced progression.
‡Logistic regression model including terms for age, sex, location of CRC (right colon, left colon, and rectum), and adjuvant chemotherapy.
§Reference category.
‖TA_6/TA_7 and TA_7/TA_7 are in the same category.

advantage for the variant allele TA_7 subgroup when compared with TA_6/TA_6 genotype. HRs were 0.81 (95% CI, 0.45 to 1.44) for the TA_7/TA_7 and 0.84 (95% CI, 0.58 to 1.21) for the TA_6/TA_7 subgroups. Median survival times were 686, 669, and 613 days for the TA_7/TA_7, TA_6/TA_7, and TA_6/TA_6 patients, respectively.

*UGT1A1*28 Polymorphism and Pharmacokinetics and Pharmacodynamics Relationships*

Pharmacokinetic analyses were performed during the first cycle. Patients who experienced grade 3 to 4 toxicity of any type (hematologic and nonhematologic) after the first chemotherapy cycle were characterized by a significantly lower level of GR ($P = .01$) and an increased BI ($P = .003$) compared with those with grade 0 to 2 toxicity (Table 5). No statistically significant associations were seen between toxicity and irinotecan AUC, SN38 AUC, or SN38G AUC. Sixty-six of the 71 patients undergoing pharmacokinetic analysis were assessable for tumor response. GR and BI ($P < .01$ by Kruskal-Wallis test) but not irinotecan AUC, SN38 AUC, or SN38G AUC were found to be significantly associated with tumor response (Table 5).

GR was significantly lower in patients with CR/PR (median, 3.05; range, 0.96 to 6.48) than in patients experiencing PD/SD (median, 4.01; range, 1.09 to 15.9; $P = .02$). Conversely, in patients with PD/SD, BI was lower (median, 4.15 μmol/L · h; range, 1.86 to 14.73 μmol/L · h) than in patients with PR/CR (median, 6.77 μmol/L · h; range, 2.02 to 15.13 μmol/L · h; $P = .045$). Moreover, in patients experiencing a clinical benefit, the median GR was 3.05 (range, 0.09 to 8.14), which was significantly lower ($P = .0003$) than in patients with PD (median, 4.61, range, 2.18 to 15.91). The median BI was 6.67 μmol/L · h (range, 2.02 to 15.13 μmol/L · h) versus 3.76 μmol/L · h (range, 1.86 to 12.14 μmol/L · h) in patients with clinical benefit and PD, respectively ($P = .001$).

Table 5 also summarizes the differences in the relevant irinotecan pharmacokinetic parameters as a function of the UGT1A1*28 genotype for the 71 patients investigated. A significant correlation was found between lower GR ($P = .01$) and higher BI ($P = .007$) and UGT1A1*28 polymorphism.

DISCUSSION

UGT1A1*28 polymorphism has been reported to be associated with an increased toxicity after irinotecan chemotherapy.[9] However, results from the studies conducted to date have been conflicting and often have generated opposite conclusions. This is most likely a consequence of the relatively low number of patients included in these studies, the different schedules of irinotecan treatment used, the patient type, or the use of retrospective analyses.[9,17,18] In light of the conflicting results, our trial was conducted prospectively in a homogeneous patient population and single treatment regimen. This trial constitutes the largest prospective study conducted to date to investigate the relationship between *UGT1A1* polymorphism and irinotecan used in the FOLFIRI regimen. Overall, the incidence of toxicity observed in our study was lower than that reported by Douillard et al,[1] but well in agreement with that published for the FOLFIRI schedule.[12,19] We observed a significantly increased risk of developing severe hematologic toxicity (primarily grade 3 to 4 neutropenia) among patients carrying the TA_7 allele, which was only relevant for the first cycle and not seen throughout the whole treatment period. This finding suggests that UGT1A1*28 polymorphism may be important in the development of hematologic toxicity at the beginning of therapy, but becomes less important during subsequent cycles. Other effects can result from the continuous administrations of irinotecan. The increased probability that normal cells will be exposed to the drug during DNA replication and/or clinical measures (ie, supportive therapy) may overcome the effects of UGT1A1*28 polymorphism.

It is also noteworthy that there was no apparent association between TA_7/TA_7 genotype and diarrhea during any cycle of treatment. This last finding is consistent with previous studies.[17]

The overall response rate achieved in our study was quite comparable to previously published data on irinotecan and FU combinations.[1] A better tumor response (CR + PR) and a reduced PD in patients with the TA_7/TA_7 genotype was seen in the present study. However, the positive effect on tumor response of the TA_7/TA_7 genotype had a

Table 5. Pharmacokinetics Parameters: Relationship to UGT1A1*28 Polymorphism and Pharmacodynamics (toxicity and response)

Polymorphism	Irinotecan AUC (μM · h)	SN38 AUC (μM · h)	SN38G AUC (μM · h)	GR	BI (μM · h)
UGT1A1*28*					
TA_6/TA_6 (n = 31)					
Median	17.78	0.86	3.40†	3.75‡	4.40‡
Range	10.88-52.07	0.24-3.76	1.99-52.72	1.61-14.01	1.45-15.13
TA_6/TA_7 (n = 32)					
Median	18.09	1.07	3.10†	3.32†	5.62†
Range	10.66-40.82	0.32-2.26	1.46-27.90	1.29-15.91	1.86-12.76
TA_7/TA_7 (n = 8)					
Median	16.24	1.20	1.89	1.86	9.83
Range	11.22-32.69	0.51-2.73	0.46-17.41	0.90-6.37	3.84-14.73
Kruskal-Wallis test	.8	.7	.04	.01	.007
Toxicity, grade					
0-2 (n = 63)					
Median	17.59	1.02	3.16	3.73	4.72
Range	10.66-52.07	0.24-3.76	0.46-52.72	0.90-15.91	1.45-15.13
3-4 (n = 8)					
Median	19.18	1.61	3.04	2.13	8.23
Range	12.36-40.82	0.83-1.87	1.46-7.02	1.09-7.68	5.31-14.73
Wilcoxon's test	.7	.08	.5	.01	.003
Response§					
Progression (n = 24)					
Median	19.75	0.98	4.84	4.61	3.76
Range	10.88-52.07	0.35-3.76	1.79-52.72	2.18-15.91	1.86-12.14
Stable disease (n = 14)					
Median	17.43	0.94	2.68	3.02†	6.60†
Range	13.00-26.75	0.63-1.73	1.49-6.86	1.09-8.14	2.05-14.73
Partial response (n = 24)					
Median	16.47	1.07	3.10	3.07†	6.21‡
Range	10.66-43.12	0.32-2.73	1.46-17.41	1.29-6.48	2.02-15.13
Complete response (n = 4)					
Median	20.78	0.72	2.37	2.66‡	8.05†
Range	11.22-31.21	0.51-1.39	0.46-3.70	0.90-4.08	7.17-12.50
Kruskal-Wallis test	.6	.6	.08	.004	.005

Abbreviations: AUC, area under the plasma concentration-time curve; GR, glucuronidation ratio; BI, biliary index; SD, stable disease; PR, partial response; CR, complete response; PD, progressive disease.

*Wild-type and TA_6/TA_7 *v* TA_7/TA_7 patients.

†$P < .05$ by Wilcoxon's test.

‡$P < .01$ by Wilcoxon's test.

§SD, PR, CR *v* PD.

nonsignificant impact on patient survival. The advantage was approximately 2 months comparing TA_7/TA_7 versus TA_6/TA_6.

The association of TA_7/TA_7 genotype with a higher response rate could be explained by a different pharmacokinetics. In our study, the TA_7/TA_7 genotype was associated with a significant decrease (approximately 50%) in GR compared with the wild-type (TA_6/TA_6) and heterozygous (TA_6/TA_7) genotypes, in accordance with previous studies.[10,20] We also found significant association between TA_7/TA_7 genotype and higher BI, but not with SN38 AUC, probably due to the complex pathways leading to SN38 production. It must be considered that BI takes into account SN38 AUC, SN38G AUC, and irinotecan AUC, and therefore could be a better predictive marker for irinotecan metabolism and hence a surrogate marker of toxicity and tumor response. A significant correlation was in fact observed between BI, toxicity, or response rate, respectively.

No additional information can be derived from our study about the role of other promoter variants linked in a disequilibrium with UGT1A1*28 (−3156G > A, −3279G > T) or the promoter haplotype and diplotype compositions that result from *UGT1A1*. A superimposable pattern of correlation, although less significant, was found between these polymorphisms/haplotypes and the toxicity or response to therapy compared with that found for UGT1A1*28 and the associated pharmacokinetics. The 3156G > A polymorphism had the same trend of correlation, whereas the 3279G > T was inversely associated with UGT1A1*28 (data not shown).

In conclusion, data from our study indicate that the UGT1A1*28 genotype was significantly associated with hematologic toxicity only during the first cycle of chemotherapy. However, this association seems to have marginal clinical implications, given that the observed toxicities can be managed during the course of chemotherapy. It has been suggested that genetic testing for UGT1A1*28 polymorphism may have utility as a predictor of toxicity in patients receiving irinotecan.[9] Drawing a definite conclusion on the role of UGT1A1*28 polymorphism, as a predictor of irinotecan toxicity in CRC patients, would

require a randomized trial, aimed at assessing whether genotype-adjusted dosages of this drug could help establish not only a well-tolerated dose, but also an effective dose for tumor response in patients with TA_6/TA_6, TA_6/TA_7, and TA_7/TA_7 genotypes. Data reported from the literature and the data from this study are still insufficient for recommending specific dose adjustments in patients treated with any irinotecan-containing regimen, including the FOLFIRI regimen used in this trial, based on *UGT1A1* genotype. The observed increased response rate in patients with lower GR and increased BI (indicative of a biochemical effect of a reduced UGT enzyme activity) and the trend toward increased tumor response and survival in TA_7/TA_7 patients suggest the need for careful consideration before irinotecan dose reduction in patients carrying the polymorphic TA_7 allele is recommended.

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