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ORIGINAL REPORT

Genotype-Driven Phase I Study of Irinotecan Administered in Combination With Fluorouracil/Leucovorin in Patients With Metastatic Colorectal Cancer

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ABSTRACT

Purpose
We aimed to identify the maximum-tolerated dose (MTD) of irinotecan in patients with cancer with UGT1A1*1/*1 and *1/*28 genotypes. We hypothesize that the patients without the *28/*28 genotype tolerate higher doses of irinotecan.

Patients and Methods
Patients undergoing first-line treatment for metastatic colorectal cancer (CRC) eligible for treatment with irinotecan plus infusional fluorouracil/leucovorin (FOLFIRI) were screened for the UGT1A1*28/*28 genotype and excluded from the study. Fifty-nine white patients with either the *1/*1 or the *1/*28 genotype were eligible for dose escalation of irinotecan. The starting dose of biweekly irinotecan was 215 mg/m^2 for both genotype groups, whereas the dose of infusional fluorouracil was fixed. Pharmacokinetic data of irinotecan and metabolites were also obtained.

Results
The dose of irinotecan was escalated to 370 mg/m^2 in patients with the *1/*28 genotype and to 420 mg/m^2 in those with the *1/*1 genotype. Dose-limiting toxicities (DLTs) were observed in two of four of *1/*28 patients at 370 mg/m^2 and in two of three of *1/*1 patients at 420 mg/m^2. No DLTs were observed in 10 *1/*28 patients at 310 mg/m^2 and in 10 *1/*1 patients at 370 mg/m^2; hence these dose levels were the MTD for each genotype group. The most common grade 3 to 4 toxicities were neutropenia and diarrhea. The pharmacokinetics of irinotecan and SN-38 exhibit linear kinetics.

Conclusion
The recommended dose of 180 mg/m^2 for irinotecan in FOLFIRI is considerably lower than the dose that can be tolerated when patients with the UGT1A1*28/*28 genotype are excluded. Prospective genotype-driven studies should test the efficacy of higher irinotecan doses in the FOLFIRI schedule.

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INTRODUCTION

Infusional fluorouracil/leucovorin (FU/LV) plus irinotecan (FOLFIRI) is one of the standard first-line options for patients with metastatic colorectal cancer (CRC).[1-3] FOLFIRI has the advantage of a favorable toxicity profile compared with bolus FU/LV combination therapy.[4,5] The dose of irinotecan in FOLFIRI is 180 mg/m^2 every 2 weeks.[6,7] However, dose-finding studies during the early development of irinotecan were conducted before the genetic basis of severe toxicity of irinotecan was established. Irinotecan has significant side effects, including myelosuppression and delayed-type diarrhea, and the UGT1A1*28 allele has been associated with the risk of severe neutropenia.[8-12] The UGT1A1*28 allele confers reduced UGT1A1-mediated inactivation of SN-38, the active metabolite of irinotecan, and the current US package insert includes homozygosity of UGT1A1*28 as a risk factor for severe neutropenia.[13]

We hypothesize that patients without the UGT1A1*28/*28 genotype are less sensitive to the toxic effects of the standard dose of irinotecan and can tolerate higher doses of irinotecan in the FOLFIRI regimen. Hence we performed a dose-finding study in patients with the UGT1A1*1/*1 and *1/*28 genotypes treated with escalated doses of irinotecan.

PATIENTS AND METHODS

Patient eligibility

This study involved seven Italian centers and enrolled patients with histologically proven metastatic CRC. Eligibility criteria included UGT1A1*1/*1 and *1/*28 genotypes, no prior chemotherapy for metastatic disease, age ≥ 18 or ≤ 75 years, absolute neutrophil count ≥ 2,000/μL, platelets ≥ 100,000/μL, performance status (Eastern Cooperative Oncology Group) of 0 to 2, life expectancy more than 3 months, creatinine clearance more than 65 mL/min (Cockcroft-Gault formula), ALT and AST less than 2× the upper limit of normal, and total serum bilirubin less than 1.25× the upper limit of normal. Patients with either *28/*28 or genotypes containing the *36 (TA_5) and *37 (TA_8) UGT1A1 alleles were not eligible.

Study Objectives

The primary objective was to perform a phase I study to assess the maximum-tolerated dose (MTD) and dose-limiting toxicity (DLT) of irinotecan in FOLFIRI at cycle 1 in patients with the *1/*1 and *1/*28 genotypes. Secondary objectives included (1) the safety of dose-escalated irinotecan during the overall duration of therapy, (2) the effect of higher doses of irinotecan and genotype on the efficacy of FOLFIRI (objective response rate [complete plus partial response], and time to progression [TTP]), and (3) the evaluation of the pharmacokinetics of irinotecan and its metabolites at higher irinotecan doses. The study was sponsored by the Centro di Riferimento Oncologico National Cancer Center of Aviano, Italy. The institutional review board of each participating institution approved the study protocol, and all patients signed a written informed consent.

Drug Administration and Dose Escalation

Patients were treated with FOLFIRI, with irinotecan administered at doses higher than the standard 180 mg/m^2 in patients with the *1/*1 and *1/*28 genotypes, whereas the dose of infusional FU/LV remained unchanged. Enrolling physicians were not blinded to the patient's genotype.

The initial dose of irinotecan for patients with the *1/*1 and *1/*28 genotypes was 215 mg/m^2 (a 20% increase from the standard every-2-weeks dose), administered as a 120-minute intravenous infusion every 2 weeks. The dosage of irinotecan was increased to 260, 310, 370 (*1/*28 and *1/*1), and 420 mg/m^2 (*1/*1 only) by 20% increases for both genotypes, with the exception of 420 mg/m^2 (14% in *1/*1). FU was administered as a 400-mg/m^2 bolus right after the end of the irinotecan infusion, followed by 2,400 mg/m^2 over a 46-hour continuous infusion plus 200 mg/m^2 LV as a 120-minute intravenous infusion during irinotecan administration every 2 weeks. One cycle was 28 days. Before starting irinotecan, patients were pretreated with standard doses of atropine, dexamethasone, granisetron, or ondansetron. Diarrhea was promptly treated 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. Growth factors (ie, granulocyte colony-stimulating factor) were given only to treat grade 3 to 4 neutropenia events.

DLT was defined as hematologic grade 4 toxicity or nonhematologic grade 3 to 4 toxicity recorded during cycle 1 that developed or persisted despite supportive measures. Three patients were enrolled at each dose level, and if the DLT was observed in fewer than one of three of them, the dose was escalated, and three additional patients were treated at the next dose level. If the DLT was observed in one of three of the patients, then three additional patients were enrolled at the same dose level, and the escalation to the next dose level continued if the DLT occurred in fewer than two of the six patients. If the DLT was observed in more than one of three or more than one of six patients treated at any given dose level, the dose escalation was stopped. Ten patients total were then enrolled at one dose level below to assess the safety and the interpatient pharmacokinetic variability, and if fewer than two of 10 patients experienced DLT, this dose level was declared the MTD. No intrapatient dose escalation was allowed.

UGT1A1*28 Genotyping Assay

Patients were genotyped for eligibility using a previously validated assay.[12]

Pharmacokinetics

Serial blood samples were collected into heparinized tubes before drug administration and at 0.5, 1.0, 1.5, 2.0, 2.25, 2.5, 2.75, 3.0, 3.5, 4.0, 6.0, 8.0, 10, 14, 26, and 50 hours after the start of the first irinotecan infusion at cycle 1. The total plasma concentration of irinotecan (lactone plus carboxylate) and its metabolites SN-38 and SN-38 glucuronide (SN-38G), were determined using high-performance liquid chromatography. Noncompartmental analysis was used for pharmacokinetics analysis.[12] The calculation of the plasma concentration-time curve (AUC) from time 0 to the last sampling time, irinotecan clearance, glucuronidation ratio, and the biliary index were calculated as previously reported.[12]

Efficacy and Toxicity Assessment

Blood counts were measured at baseline, weekly during cycle 1, and within 48 hours before each administration during the following cycles. Objective clinical evaluation and hepatic and renal function tests were performed at baseline and within 48 hours before each irinotecan administration, during which patients were questioned about nausea and vomiting, mucositis, diarrhea, malaise, and appetite. Patients were given a questionnaire to report the number of daily bowel movements. Computed tomography scans of measurable lesions were assessed at baseline and then repeated at least every two cycles. Objective tumor response and duration of response, limited to those patients with measurable disease at enrollment, were assessed according to WHO criteria.[14] TTP was measured from the time of drug administration to the occurrence of progressive disease. Tumor response to treatment was evaluated in patients who had received at least two cycles, with the exception of patients who experienced disease progression before the end of cycle 2.

Table 1. Patient Characteristics

Characteristic	No. of Patients	%
Assessable		
For safety	59	
For efficacy	44	
For pharmacokinetics	33	
Age, years		
Median	64	
Range	46-79	
Sex		
Male	34	57.6
Female	25	42.4
Body-surface area, m^2		
Median	1.8	
Range	1.4-2.4	
Adjuvant chemotherapy		
Yes	23	39.0
No	36	61.0
Performance status, ECOG		
0	22	37.3
1	37	62.7
Primary site		
Colon	35	59.3
Rectum*	24	40.7
No. of metastatic sites†		
1	13	22.0
≥ 2	42	71.2
Radical surgery		
Yes	51	86.5
No	8	13.5
Stage		
III	1	1.7
IV	58	98.3

Abbreviation: ECOG, Eastern Cooperative Oncology Group.
*Patients with rectal tumors received adjuvant radiotherapy.
†Four patients had no measurable lesions at the time of recruitment.

Toxicity was evaluated according to National Cancer Institute Common Toxicity Criteria version 3. Patients were treated at full dose of irinotecan in absence of major toxicity if the following criteria were met: full recovery from any nonhematologic toxicity, absolute neutrophil count ≥ 1,500/μL, and platelet count ≥ 100,000/μL. Patients experiencing hematologic grade 4 toxicity or nonhematologic grade 3 to 4 toxicity were allowed to continue irinotecan at a lower dose on the basis of the physician's assessment, without FU dosage modifications. FOLFIRI was discontinued because of disease progression, intolerable side effects, patient refusal, or physician assessment.

Statistics

Exploratory analyses were conducted on the secondary objectives of the study, and a *P* value less than .05 was considered of nominal statistical significance. The effect of irinotecan dose and UGT1A1*28 genotype on TTP was estimated using the Kaplan-Meier method, and differences were tested using the log-rank test. The effect of irinotecan dose and UGT1A1*28 genotype on response rate was evaluated using multivariate logistic regression modeling, adjusting for age, sex, and adjuvant chemotherapy. The correlation between irinotecan dose and pharmacokinetic parameters was tested by Spearman's rank correlation test. The Mann-Whitney test was used for two-group comparisons (UGT1A1*1/*1 and *1/*28 genotypes and dose-normalized AUCs).

RESULTS

Patient Characteristics and Dose Escalation

Sixty-three white patients were screened for UGT1A1*28, and 35 *1/*1, 24 *1/*28, and four *28/*28 genotype patients were identified (Hardy-Weinberg equilibrium, $P > .05$). The four *28/*28 patients were not eligible for dose escalation and were not enrolled onto the study. Patient characteristics are shown in Table 1. The dose of irinotecan was escalated from 215 to 370 mg/m^2 in *1/*28 patients and to 420 mg/m^2 in *1/*1 patients (Table 2).

Toxicity and DLT

At cycle 1, the most frequent severe hematologic toxicity (14 of 59 patients, 24%) was grade 3 to 4 neutropenia. One patient had grade 4 febrile neutropenia (Table 3). Among the severe nonhematologic toxicities, the most common were grade 3 diarrhea (7%), grade 3 to 4 asthenia (5%), and grade 3 anorexia (3%). Grade 3 was the worst grade of diarrhea. Over the entire course of therapy, the prevalence of grade 3 to 4 neutropenia was 37% (with 7% of grade 3 to 4 febrile neutropenia), and grade 3 diarrhea was found in 14% of patients (Table 3).

In the *1/*28 patients, based on the two of four patients with DLTs at 370 mg/m^2, the cohort was expanded to 10 patients at 310 mg/m^2. No DLT was observed in the expanded cohort of 10 patients treated at 310 mg/m^2, which is the MTD in *1/*28 patients. In *1/*1 patients, two DLTs occurred in the three patients treated at 420 mg/m^2, and no DLTs occurred in the 10 patients treated at 370 mg/m^2, which is the MTD in *1/*1 patients.

Among all treated patients, the most common DLTs were myelosuppression (n = 3), diarrhea (n = 3), and asthenia (n = 3; Table 2). One toxic death occurred in a *1/*1 female 49 year-old patient treated with 310 mg/m^2 of irinotecan at cycle 1 who experienced grade 4 febrile neutropenia, thrombocytopenia leukopenia, and grade 3 stomatitis. The pancytopenia experienced by this patient should be then regarded as grade 5. This patient had a pharmacokinetic profile suggestive of increased drug exposure (possibly due to impaired excretion) when compared with that of the group of patients treated at this dose level (AUCs of 44.85, 1.21, and 4.27 μmol/L*h for irinotecan, SN-38, and SN-38G, respectively; comparison shown in Table 4). All patients with grade 3 or worse toxicity were tested and were found to have a wild-type IVS14+1G>A polymorphism of the dihydropyrimidine dehydrogenase gene (data not shown).

The median number of FOLFIRI cycles was four (range, 0.5 to nine cycles). In the 20 *1/*1 and *1/*28 patients treated at the MTD, the median number of cycles of therapy was eight (range, two to 12 cycles), with a median dose-intensity of irinotecan of 90% (range, 57% to 100%, calculated in milligrams per square meter per week). The FU dosage was not modified.

Pharmacokinetics of Irinotecan and Its Metabolites

Data are available from 33 patients (Table 4). There was a significant ($P \leq .001$) correlation between dose per square meter and the AUC of irinotecan (Spearman $r = 0.60$), SN-38 ($r = 0.55$), SN-38G ($r = 0.60$), and biliary index ($r = 0.39$, $P < .05$). No significant correlation was observed with glucuronidation ratio ($P > .05$). The AUCs of irinotecan and SN-38 at 420 mg/m^2 were almost twice those at 215 mg/m^2 (Table 4), suggesting a linear relationship between dose

Table 2. Dose Escalation and DLT of Increased Irinotecan Doses in Patients Treated With FOLFIRI

Irinotecan Dose (mg/m^2)	No. of *1/*1 Patients	DLT		No. of *1/*28 Patients	DLT	
		No. of Patients With DLT	Type of DLT		No. of Patients With DLT	Type of DLT
215	4*	0		6	1	Grade 3 nausea, diarrhea, anorexia, and asthenia
260	12†	1	Grade 4 thrombocytopenia, grade 3 diarrhea	4‡	0	
310	6	1	Grade 3 stomatitis, grade 4 pancytopenia§	10	0	
370	10	0		4	2	Grade 4 neutropenia and leucopenia, grade 3 asthenia
420	3	2	Grade 3 anorexia, grade 4 asthenia, grade 3 diarrhea	—		

Abbreviations: DLT, dose-limiting toxicity; FOLFIRI, infusional fluorouracil and leucovorin plus irinotecan.
*One "excess patient" (see Discussion for the definition of excess patients).
†Six excess patients.
‡One excess patient.
§One toxic death (grade 5).

Table 3. Toxicity During the First Cycle and All Cycles

Toxicity	First Cycle								All Cycles							
	Grade 1		Grade 2		Grade 3		Grade 4		Grade 1		Grade 2		Grade 3		Grade 4	
	No.	%	No.	%	No.	%	No.	%	No.	%	No.	%	No.	%	No.	%
Anemia	17	29	5	8	1	2	0	0	21	36	7	12	1	2	0	0
Leukopenia	12	20	9	15	3	5	2*	3	13	22	11	19	5	8	2*	3
Neutropenia	1	2	8	14	12	20	2*	3	10	17	6	10	19	32	3*	5
Fever with concomitant neutropenia	1	2	0	0	0	0	1	2	1	2	1	2	2	3	2	3
Thrombocytopenia	1	2	0	0	1	2	2*	3	1	2	0	0	1	2	2*	3
Nausea/vomiting	18	31	10	17	1	2	0	0	21	36	15	25	1	2	0	0
Diarrhea	18	31	12	20	4	7	0	0	20	34	12	20	8	14	0	0
Stomatitis	4	7	4	7	1	2	0	0	9	15	4	7	4	7	0	0
Anorexia	3	5	2	3	2	3	0	0	5	8	2	3	2	3	0	0
Hepatic (transaminases)	4	7	1	2	1	2	0	0	5	8	1	2	1	2	0	0
Hepatic (bilirubin)	2	3	0	0	0	0	0	0	2	3	0	0	1	2	0	0
Alopecia	3	5	1	2	0	0	0	0	3	5	5	8	2	3	0	0
Asthenia	9	15	7	12	2	3	1	2	12	20	14	24	2	3	1	2
Other	14	24	5	8	0	0	0	0	17	29	13	22	1	2	0	0

*One toxic death (grade 5).

and exposure. Irinotecan clearance was unchanged across the dose levels (Spearman $r = 0.09$, $P > .05$, Table 4). No statistically significant difference was observed between *1/*1 and *1/*28 patients in relation to dose-normalized irinotecan and SN-38 AUC; actual AUCs (not dose/normalized) do not show any statistically significant difference (data not shown).

Tumor Response and TTP

Forty-four patients were assessable for tumor response, and the remaining patients were not assessable for the following reasons: DLT at cycle 1 without being rechallenged with a lower dose of irinotecan (n = 5), early withdrawal from the study (n = 6), and lack of measurable lesions (n = 4). Of the seven patients with DLT, two patients (*1/*28 at 215 mg/m^2, *1/*1 at 420 mg/m^2) were rechallenged with a 20% and 50% dose reduction of irinotecan, respectively, and they obtained a complete response.

The overall response rate (complete plus partial response) was 43% (n = 19). The response rate was higher in patients with either the *28 allele or treated at doses ≥ the MTD (Table 5). These two parameters tended to be independent from each other in multivariate analysis for response rate that included age, sex, and adjuvant chemotherapy (odds ratio for *1/*28 = 3.18, 95% CI, 0.80 to 12.67, $P = .099$; odds ratio for ≥ MTD = 4.38, 95% CI, 1.13 to 17.03, $P = .030$; $P = .007$ for the overall multivariate model). Median follow-up time was 338 days (range, 37 to 655 days), and median TTP was 219 days (range, 55 to 515 days). TTP was not different between ≥ MTD (median TTP, 294 days) and less than MTD (224 days; median hazard ratio of 0.85; 95% CI, 0.40 to 1.80).

DISCUSSION

This study tested a new approach for phase I drug development in the era of genomic medicine: the use of genetic information to escalate the drug dose in patients who are likely to be less sensitive to the toxic effects of chemotherapy. By dose escalating irinotecan only in patients without the high-risk UGT1A1*28/*28 genotype (10% on average in patients of European descent[15,16]), we demonstrated that the recommended dose of 180 mg/m^2 for irinotecan in FOLFIRI is considerably lower than the dose that can be tolerated by the non–UGT1A1*28/*28 patients. For approved drugs, the dosage recommendations obtained from traditional, non–genotype-directed, phase I studies should be revised in light of validated genetic markers of toxicity risk. In particular, for "classic" cytotoxic chemotherapy, the lack of patient stratification based on genotype might result in

Table 4. Pharmacokinetics Parameters of Irinotecan, SN-38, SN-38G, GR, and BI

Irinotecan Dose (mg/m^2)	No. of Patients	Irinotecan AUC (μmol/L*h)		Irinotecan CL (L/h)		SN-38 AUC (μmol/L*h)		SN-38G AUC (μmol/L*h)		GR		BI (μmol/L*h)	
		Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD
215	6	24.44	4.63	16.76	3.65	0.65	0.15	3.53	0.98	5.74	2.53	4.61	1.19
260	8	25.62	7.51	18.95	6.53	0.91	0.44	3.11	0.67	3.80	1.04	7.28	2.81
310	9	30.91	7.78	18.51	4.67	0.86	0.19	3.89	0.91	4.37	0.63	7.14	2.79
370	8	34.22	6.29	18.52	4.05	1.01	0.22	5.11	0.80	5.32	1.58	6.83	2.05
420	2	43.44	7.74	17.36	5.62	1.44	0.37	5.03	1.66	3.45	0.27	12.70	3.24
Spearman correlation coefficient	33	0.60		0.09		0.55		0.60		−0.05		0.39	
P		.0002		.6		.001		.0002		.8		.02	

Abbreviations: GR, glucuronidation ratio; BI, biliary index; SD, standard deviation.

Table 5. Response Rate: Effect of Irinotecan Dose and UGT1A1*28 Genotype

Response	No. of Patients	CR + PR				SD + PD				Odds Ratio	95% CI	*P*
		No.	%	CR	PR	No.	%	SD	PD			
Overall	44	19	43	7	12	25	57	14	11			
Doses < MTD*	24	6	25	2	4	18	75	13	5			
Doses ≥ MTD†	20	13	65	5	8	7	35	1	6	5.57	1.51 to 20.51	.014
*1/*1	27	8	30	2	6	19	70	13	6			
*1/*28	17	11	65	5	6	6	35	1	5	4.35	1.20 to 15.87	.031

Abbreviations: CR, complete response; PD, progressive disease; PR, partial response; SD, stable disease.
*Doses of 215, 260, and 310 mg/m^2 for the *1/*1 patients and 215 and 260 mg/m^2 for the *1/*28 patients.
†Doses of 370 and 420 mg/m^2 for the *1/*1 patients and 310 and 370 mg/m^2 for the *1/*28 patients.

significant underdosing of patient subgroups. Whether genotype-driven dosing will result in outcome differences needs to be tested prospectively.

Dose escalation of irinotecan according to genotype has established that 370 (*1/*1 genotype) and 310 (*1/*28 genotype) mg/m^2 can be safely administered every 2 weeks in patients undergoing first-line treatment for metastatic CRC treated with FOLFIRI. Although these dose levels are quite similar, 370 mg/m^2 seemed to not be tolerated in UGT1A1*1/*28 patients. In several studies of irinotecan pharmacogenetics, a gene-dosage effect (ie, the heterozygous being at intermediate risk of toxicity between the two homozygous) was apparent.[10,12,17-19] There is still uncertainty regarding the mode of inheritance of Gilbert's syndrome, but the molecular effects of the UGT1A1*28 allele are indicative of a trend toward reduced UGT1A1 function while increasing the number of *28 alleles (ie, UGT1A1 activity: *1/*1>*1/*28>*28/*28).[20-22]

From our study and preliminary data reported at recent American Society of Clinical Oncology Meetings, it emerges that the biweekly schedule is more amenable to dose escalation in genotype-selected patients than the three-weekly schedule with the standard bolus administration of FU, as the dose of irinotecan could not be increased significantly over the standard dose.[23,24] The reason might be that the three-weekly schedule uses 350 mg/m^2, a dose almost double than that of irinotecan in standard FOLFIRI. In addition, the risk of severe neutropenia in *28/*28 patients seems higher in three-weekly than in the biweekly regimen,[9] indicating an interaction between *28 and schedule, potentially limiting the dose escalation in *1/*28 in the three-weekly regimen. Doses higher than the standard dose have been previously tolerated in selected patients in Europe in various regimens,[25-27] but the lack of the genetic investigation on the UGT1A1*28 genotype in those studies precluded assessment of the basis for such increased tolerability.

In the first cycle, the DLTs of high-dose irinotecan are not dissimilar from those observed at conventional doses, with grade 3 to 4 neutropenia and diarrhea being the two most common severe side effects, consistent with the rates observed for FOLFIRI in patients with first-line metastatic disease.[1,6,28] The observed increase in the rates of severe toxicity during all administered cycles versus cycle 1 is suggestive of a cumulative toxic effect, as previously reported in the initial phase I of FOLFIRI.[7] The rates of grade 3 to 4 febrile neutropenia are comparable to those reported in larger studies of FOLFIRI, although the reported rates vary from 1% to 9%.[1,6,28,29] The most common nonhematologic severe toxicity in our study was diarrhea, with rates in the overall cycles comparable to those of recent studies,[6,28] probably reflecting improved management of diarrhea after the initial use of irinotecan.

No DLT was observed in the 10 patients enrolled in each genotype group when treated at the MTD (Table 2). For an effective regimen that is standard of care, higher doses should be delivered without negatively impacting dose-intensity as a result of intolerable cumulative toxicity. In the 20 patients treated at the MTD, the median number of cycles was eight (range, two to 12 cycles), consistent with the results from large first-line FOLFIRI studies.[1,6,28] At the MTD, the dose-intensity of irinotecan is also highly preserved (median, 90%; range, 57% to 100%). Despite these favorable features, the number of cycles that can be safely delivered in patients with metastatic CRC using higher doses of irinotecan remains to be evaluated in a large group of patients treated with FOLFIRI before the practice of treatment with FOLFIRI can be changed to genotype-directed and higher dosing of irinotecan.

Higher doses of irinotecan have a favorable pharmacokinetic profile, showing proportional increases in AUC of both irinotecan and SN-38. Dose linearity of irinotecan pharmacokinetics was previously reported.[30] In addition, patients receiving higher doses were more likely to achieve an objective response than patients treated at lower levels. However, this study cannot answer the question of whether higher irinotecan doses confer a survival advantage compared with standard dosing because of its small sample size and heterogeneity in dosing and tumor characteristics. Interethnic differences in the functional alleles of UGT1A1 represent a further limitation to the applicability of these findings. For example, the *6 allele (equivalent to the *28) is common in Asians (whereas the *28 allele is much less common) and is rare in other populations.[31]

One limitation of our study is that it focused only on *1/*1 and *1/*28 patients and was not designed to perform a dose-finding study also in *28/*28 patients. The safe dose of irinotecan in *28/*28 patients remains an open question that has not been addressed yet,[17-19,23,24] probably due to the difficulty in obtaining conclusive data from an adequate number of *28/*28 patients, who are approximately 10% of the overall European population.

Eight more patients were enrolled at lower doses when the cohort was already filled (ie, at 215 and 260 mg/m^2, Table 2). Due to the nature of a multisite phase I study, concomitant patient enrollment might result in "excess patients" enrolled right after the cohort has been already filled according to the 3 + 3 design. This should be avoided and prevented with better coordination among enrolling centers. In our study, however, the additional excess patients provided further evidence for the tolerability of irinotecan at lower doses when the *28/*28 patients are removed, as none of them developed DLT (Table 2).

The present study has identified the safe doses to administer when patients undergoing treatment with FOLFIRI are stratified by

the UGT1A1*28 genotype and the patients genetically at risk for toxicity (*28/*28) are excluded. Prospective FOLFIRI studies in a large population of patients with metastatic CRC should be performed to test whether higher irinotecan doses can increase the therapeutic index of FOLFIRI as compared with the standard dosing and whether other polymorphisms may be clinically important.[32,33]

AUTHORS' DISCLOSURES OF POTENTIAL CONFLICTS OF INTEREST

The author(s) indicated no potential conflicts of interest.

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