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ORIGINAL ARTICLE

The value of genetic polymorphisms to predict toxicity in metastatic colorectal patients with irinotecan-based regimens

M. J. Lamas · G. Duran · E. Balboa · B. Bernardez · S. Candamio · Y. Vidal · A. Mosquera · J. M. Giraldez · R. Lopez · A. Carracedo · F. Barros

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Abstract
Purpose We are trying to identify predictive factors of high risk of toxicity by analyzing candidate genes in the irinotecan pathways in order to identify useful tools to improve mCRC patient management under real practice conditions.
Methods Genomic DNA was genotyped for UGT1A1 (*28, *60 and *93) from all 101 patients, and irinotecan dose was 180 mg/m^2 every second week. Clinical data were obtained by retrospective chart review. The primary endpoint is to find out whether the pharmacogenetic test in the clinical practice may predict toxicity.
Results Grade 3/4 diarrhea occurred in twelve patients and required dose reduction in six patients, and neutropenia reached grade 3/4 in 19 patients (only one patient with *28/*28 genotype). The UGT1A1*93 seemed to relate with grade 3/4 neutropenia but only in the heterozygote state (G/A), $p = 0.071$, and UGT1A*60 showed no association with neutropenia. Twenty-eight percentage of patients required the use of G-CSF; 64.3 % of them harbored *1/*28 or *28/*28 genotypes, $p = 0.003$. Thirty-seven (36.6 %) patients required dose reduction of irinotecan and/or 5-FU owing to toxicity, mainly neutropenia and diarrhea. No significant association was detected between *28, *60 and *93 *UGT1A* variants and severe irinotecan-associated hematologic or GI toxicity.
Conclusion The impact of increased risk of toxicity attributed to the UGT1A variants may be offset by irinotecan in clinical practice by dose reduction or the use of colony-stimulating factor.

Keywords Polymorphisms · Pharmacogenetic · Toxicity · Colorectal cancer · Irinotecan

M. J. Lamas (✉) · G. Duran · B. Bernardez · A. Mosquera · J. M. Giraldez
Oncology Pharmacy Unit, Complejo Hospitalario Universitario of Santiago, Choupana S/N, 15706 Santiago de Compostela, Spain
e-mail: mlamasd@yahoo.es

E. Balboa · A. Carracedo · F. Barros
Molecular Medicine Unit, Fundación Pública Galega de Medicina Xenómica, Choupana S/N, 15706 Santiago de Compostela, Spain

S. Candamio · Y. Vidal · R. Lopez
Department of Medical Oncology, Complejo Hospitalario Universitario of Santiago, Choupana S/N, 15706 Santiago de Compostela, Spain

A. Carracedo
Genomic Medicine Group-CIBERER, University of Santiago de Compostela, Calle San Fransisco S/N, 15705 Santiago de Compostela, Spain

Introduction

Irinotecan is approved for use in metastatic colorectal cancer (mCRC). The most usual combination with infusional 5-fluorouracil and leucovorin (FOLFIRI) has become a key regimen for first- or second-line treatment of metastatic colorectal cancer. Best benefit is obtained when both FOLFOX (oxaliplatin 85 mg/m^2 + leucovorin 200 mg/m^2 + 5FU 400 mg/m^2 bolus + infusional 5FU 2.400 mg/m^2 in 46 h) and FOLFIRI (irinotecan 180 mg/m^2 + leucovorin 200 mg/m^2 + 5FU 400 mg/m^2 bolus + infusional 5FU 2.400 mg/m^2 in 46 h) are administered independently of the order [1]. Monoclonal antibodies, bevacizumab and cetuximab, added to one or other chemotherapy regimen, increase benefit although their role is not well established yet [2–7]. Irinotecan is activated by

carboxylesterases to form the active metabolite SN-38. The major route of carboxylesterases to the active 7-ethyl-10-hydroxycamptothecin (SN-38) elimination is via glucuronidation by hepatic UDP-glucuronosyltransferase (UGT) 1A enzymes [8].

UGT1A1*28 is a common polymorphic allele with seven TA repeats in the TATA box of the UGT1A1 promoter, while the wild-type allele has six TA repeats (UGT1A1*1). These are named *1 (the "wild" sequence of (TA)6TAA) and *28 (with an extra TA repeat or (TA)7TAA). UGT1A1*28 has shown to be associated with decreased SN-38 glucuronidation in humans [9]. The association between the UGT1A1*28 genotype and irinotecan-induced neutropenia has been extensively studied. Some of these studies found that UGT1A1*28/*28 patients had an elevated risk of neutropenia compared with those carrying UGT1A1*1 allele(s) [10–12]. The US Food and Drug Administration (FDA) in 2005 recommended that Pfizer amended the package insert of irinotecan to warn of the elevated risk of neutropenia for UGT1A1*28/*28 patients. Although initial studies found UGT1A1*28 genotype to be strongly associated with risk of toxicity, results of subsequent studies [13, 14] were inconsistent. In a metanalysis carried out in 2007, which included 878 patients, the authors found that the irinotecan dose delivered modulated the association between UGT1A1*28 genotype and irinotecan-induced hematologic toxicity and that the interaction was clinically important only at higher irinotecan doses [15]. At lower irinotecan doses, factors other than UGT1A1*28 genotype, either genetic or non-genetic, seemed likely to determine a patient's risk of hematologic toxicity, whereas at higher drug doses, UGT1A1*28 genotype appeared to be an important determinant. More recently, a new meta-analysis based on a large sample size (1,998 patients) indicates that UGT1A1*28/*28 patients are at an increased risk of neutropenia not only if they are being treated with medium (150–200 mg/m^2) (Relative Risk RR, 2.00) or high doses $\geq$ 250 mg/m^2 (RR, 7.22) of irinotecan but also if they are being treated with low doses (RR, 2.43; 80–145 mg/m^2) [16]. Diarrhea is another important side effect related to irinotecan administration. The latter meta-analysis stated that UGT1A1*28/*28 patients were at an increased risk of diarrhea at medium (RR, 1.79; 95 % CI, 1.08–2.97) or high doses (RR, 2.32; 95 % CI, 1.25–4.28) of irinotecan, but not at low doses (RR, 0.65; 95 % CI, 0.27–1.58) [17]. In addition, other two variants (3156G > A-*UGT1A1*93* and 3279T > G-*UGT1A1*60*) have been found in linkage disequilibrium (LD) with *UGT1A1*28* and, thereafter, have been proposed as potential predictors of hematologic toxicity [10, 18].

In spite of the results mentioned previously, UGT1A1 genotyping is not yet a routine test in clinical practice. The usual practice of dose reductions or use of filgrastrim may hide the need of previous UGT1A1 test. In addition, DPYD, MTHFR and TYMS polymorphisms keep on being exploratory biomarkers in relation to 5-FU treatments.

We have selected a comprehensive panel of SNPs to use in patients with newly diagnosed metastatic colorectal cancer who could receive FOLFIRI along their illness. We are trying to evaluate the impact of these known polymorphisms in real practice conditions in order to identify useful tools to improve mCRC patient management.

Materials and methods

All patients in this study had histologically confirmed diagnosis of advanced stage adenocarcinoma of the colon or rectum, with a performance status of 0–2 according to the WHO scale. These patients were treated with 5-FU/irinotecan-based chemotherapy. Irinotecan (180 mg/m^2) was administered by intravenous (i.v) 2-h infusion concurrent with leucovorin (200 mg/m^2) and immediately followed by 5-FU (400 mg/m^2) given as an i.v bolus on day 1 and followed by 5-FU (2,400 mg/m^2) as a continuous 46-h infusion. The sums of irinotecan doses and of 5-FU doses administered during the cycles of FOLFIRI therapy were captured, as well as the number of cycles, total cumulative drug dose and description of treatment delays and/or reductions. Treatment was repeated every 2 weeks until progression or unacceptable adverse reactions. All toxicities were graded according to the National Cancer Institute Common Toxicity Criteria CTC v3.1. For each patient, we recorded the maximum observed toxicity grade (anemia, neutropenia, thrombocytopenia, diarrhea, neurotoxicity and nausea or vomiting). Granulocyte colony-stimulating factor (G-CSF) support was allowed if neutropenia compromised treatment schedule. We evaluated response according to Response Evaluation Criteria in Solid Tumors criteria (RECIST) by CT scan. Objective response was evaluated after 3 cycles of treatment and every 3 months. Response was classified as complete response (CR), partial response (PR), stable disease (SD) or progressive disease (PD) according to the RECIST criteria. For this study, patients with CR or PR were classified as responders, and patients with SD or PD were classified as nonresponders. Relevant clinical data were obtained from clinical records (gender, age, performance status, number of metastatic sites, colon vs. rectum involvement). The local ethics committee approved the pharmacogenetic study protocol, and all subjects signed an informed consent before participating in the study. Clinical data were obtained by retrospective chart review.

The primary endpoint is to find out whether the pharmacogenetic test before FOLFIRI treatment in the clinical practice may predict toxicity and in this case incorporating it to routine practice in our center.

Secondary endpoints include evaluating the utility of other related biomarkers to predict efficacy in terms of objective responses and progression-free survival (PFS).

Molecular analyses

DNA was extracted from peripheral blood samples collected from 101 colorectal cancer patients on day 1 of treatment.

Analysis of TA-repeat variability in the promoter region of UGT1A1 (UGT1A1*28) was analyzed by fluorescent DNA length fragment analysis using forward (5′ TCACG TGACACAGTCAAACATT 3′) and reverse (5′ CAGCAT GGGACACCACTG 3′) primers with an Applied Biosystems ABI 3730XL Genetic Analyzer (Life Technologies, Foster City, CA, USA).

Allelic discrimination of the upstream UGT1A1 −3279 G > T and −3156 G > A polymorphisms named *60 and *93, respectively, was performed by direct sequence analysis on the 3730XL DNA Analyzer using a ABI-PRISM Big-Dye Terminator v3.1 Ready-Reaction Cycle sequencing kit (Applied Biosystems).

The *TS* promoter region polymorphism (5′VNTR) was analyzed by sequencing. The *TS* 1494del6 polymorphism and *EGFR* (CA)*n* repeat polymorphism were analyzed by fluorescent fragment analysis. Polymorphisms at *MTHFR, XRCC1, ERCC1* and *DPYD* were analyzed by SnapShot method. Conditions and techniques have been recently described in a paper of our group [19].

Statistical analysis

The purpose of this analysis was to evaluate the association between the polymorphism and demographics data, toxicity, response to chemotherapy and survival time. The X^2 test was used to compare the observed genotype distributions with those expected by the Hardy–Weinberg equilibrium. Linkage disequilibrium (LD) was assessed using the program Haploview. Associations between genes and toxicity/response were studied through contingency tables. The X^2 and Fischer's exact test were used for the categorical variables to evaluate the association between the expression of genetic markers and the response to chemotherapy. In addition, binomial logistic regression methods were used to determine the strength of influence of the selected SNPs upon response prediction and coded with dummy indicator variables. Odds ratios (OR) and 95 % confidence intervals (95 % CI) were calculated for each genotype and haplotype compared with the homozygous for the major allele (the allele with greater frequency among controls), which were set as the reference genotype. Multivariate binary logistic regression analysis was used to determine the association between genetic markers and response. Age, sex, performance status and dose, as clinically significant variables, were included in the regression calculations, and the adjusted odds ratios (ORs) were calculated. The association between genotypes and PFS was estimated by computing hazard ratios and their 95 % CI from Cox proportional hazards regression models. PFS curves were estimated using the Kaplan–Meier method, and the log-rank test was used to compare the curves. $p < 0.05$ was considered statistically significant. Analyses were done using SPSS version 17.0 (SPSS Inc, Chicago, IL, USA).

Results

Patient characteristics

Baseline patient characteristics are listed in Table 1. Median age was 67 years (range, 30–87 years). The number of patients who received FOLFIRI as first-line chemotherapy was 67 (66.3 %) and as second line was 34 (33.7 %).

The frequencies of *1/*1, *1/*28 and *28/*28 genotypes were 58.4, 31.7 and 8.9 %, respectively, for UGT1A1*28. We constructed haplotypes using three polymorphisms (*UGT1A1*28*, *UGT1A1*60* and *UGT1A1*93*) to examine the effects of these key single-nucleotide polymorphisms and found five haplotypes with a frequency >1 %. Considering the two most frequent haplotypes, I and II, *1 is found with T at −3279 and G at −3156 (haplotype I), while *28 is found with the alternative alleles at −3279 and −3156 (haplotype II). We designate as haplotype III to all the other combinations. High LD was observed among the *UGT1A *28*, *UGT1A *93* and *UGT1A *60* variants ($0.93 < D' < 1.00$; $0.46 < r^2 < 0.76$).

DPYD IVS14 + 1G > A mutation was not found in any patient, as it could be expected given the low frequency of the polymorphism in general population. That is why *DPYD* genotype was not included in the statistical analysis. All variants were in Hardy–Weinberg equilibrium.

Thirty-eight patients had received adjuvant chemotherapy: Fifteen patients received 5-FU plus leucovorin or oral fluoropyrimidine, and twenty-three patients had received oxaliplatin (FOLFOX). Sixty-four patients (63.4 %) received the original full-dose FOLFIRI; in 5 patients, irinotecan was reduced below 75 %.

Safety

The median number of cycles administered was 4 (range, 3–12 cycles). Toxicity in all patients is shown in Table 2.

Tolerance was satisfactory, with grade 3–4 toxicity observed in 18.9 % of patients for neutropenia, 11.9 % for

Table 1 Baseline characteristics of patients included in the pharmacogenetics analysis

	n	%
Age, years		
Median	67	
Range	30–87	
Sex		
Male	64	63.4
Female	37	36.6
PS score (ECOG)		
0–1	96	93.1
2	5	6.9
Primary tumor		
Colon	40	39.6
Sigma	25	24.8
Rectum	36	35.6
Line of treatment		
First line	67	66.3
Second line	34	33.7
Chemotherapy regimens		
FOLFIRI	33	32.7
FOLFIRI-Cetuximab	6	5.9
FOLFIRI-Bevacizumab	58	57.4
Irinotecan-Cetuximab	4	4.0
Number of metastatic sites		
1	14	13.8
2	42	41.6
>2	45	44.6
Outcome		
Complete response	7	6.9
Partial response	27	26.7
Stable response	40	39.6
Disease progression	27	26.8

diarrhea, 4 % for leukopenia, 1 % mucositis, 4 % for anemia and no grade 3–4 for thrombocytopenia and nausea or vomiting. Five patients had febrile neutropenia. Toxicity was recorded in each patient, and we have not found any significant association between toxicity and genotype. There were no treatment-related deaths. Although most treatments were conducted in an outpatient setting, two patients required admission because of toxicity. Grade 3/4 diarrhea occurred in twelve patients and required dose reduction in six patients, and neutropenia reached grade 3/4 in 19 patients. Twenty-eight patients required the use of G-CSF and 18 (64.3 %) patients with *1/*28 or *28/*28 genotypes, $p = 0.003$.

None of the UGT1A genotypes analyzed in this study had a significant association with grade 3 diarrhea and neutropenia (Table 3). Only one patient was UGT1A1*28/*28, and he did not present any adverse events during this study.

We have found a trend in the association of UGT1A1 *93 with neutropenia grade 3/4, but only in the heterozygous state (G/A), $p = 0.071$, while UGT1A *60 showed no relation with neutropenia.

No significant association was detected between *28,*60 and *93 *UGT1A* variants and severe irinotecan-associated hematologic or GI toxicity.

Dose intensity (DI)

Thirty-seven (36.6 %) of the 101 patients required dose reduction of irinotecan and/or 5-FU because of toxicity, mainly neutropenia and diarrhea.

The median number of cycles at the time when dose was reduced for the first time was 5.5 (range, 2–11 cycles). Thirty-two (31.7 %) of the 101 patients required dose reduction to 80–75 %, and 5 patients received a reduced dose of irinotecan less than 75 %. Dose reduction occurred in 18 of 36 patients with *1/*28 or *28/*28 genotypes ($p = 0.17$) (Table 4).

Dose reduction after first cycle was more frequent in patients with the UGT1A1*93-AA genotype than in those with the –GG genotype (OR, 8,44; 95 % CI, 1.86–38.19; $p = 0.006$). Using the UGT1A1*60-TT group as a reference, the –GG genotype showed a 5.33-fold (95 % CI, 1.23–23.09; $p = 0.025$) increased risk of dose reduction after first cycle.

Patients aged over 70 years did not exhibit poor tolerance for the regimen, and women did not suffer more delays in scheduled chemotherapy.

Efficacy

Objective response (CR + PR) was observed in 34 of 101 assessable patients (33.6 %) and included 7 CR (6.9 %) and 27 PR (26.7 %). SD was obtained in 40 patients (39.6 %) and PD in 27 patients (26.8 %). A logistic regression model was performed to ascertain whether UGT1A variants were independently related to response, see Table 5. No significant association with outcome was observed with other UGT1A variants or their haplotypes. There were no significant differences in response according to other genes.

We have added clinical items (age, sex, performance status and dose) to the table of correlations with efficacy, and as you can see, there is no significant association except for the TS 3′-UTR polymorphism (test for trend, $p = 0.04$).

Table 2 Adverse events in patients receiving combination chemotherapy with irinotecan

	First cycle						All cycles					
	Grade 0–1		Grade 2		Grade 3–4		Grade 0–1		Grade 2		Grade 3–4	
	No.	%	No.	%	No.	%	No.	%	No.	%	No.	%
Toxicity												
Anemia	89	88.1	9	8.9	3	3.0	82	81.2	15	14.9	4	4.0
Leukopenia	94	83.0	5	5.0	2	2.0	86	85.1	11	10.9	4	4.0
Neutropenia	73	72.3	16	15.8	12	11.9	58	57.4	24	23.8	19	18.8
Thrombocytopenia	101	100.0	0	0.0	0	0.0	101	100.0	0	0.0	0	0.0
Nausea/vomiting	99	98.1	2	1.9	0	0.0	97	96.0	4	4.0	0	0.0
Diarrhea	91	90.1	9	8.9	1	1.0	67	66.3	22	21.8	12	11.9
Mucositis	99	98.1	2	1.9	0	0.0	93	92.1	7	9.7	1	4.2

Table 3 Associations between UGT1A1 genotypes and grade 3–4 toxicities

UGT1A1	Grade diarrhea		Grade neutropenia		Use G-CSF	
	G.0–2	G.3–4	G.0–2	G.3–4	Yes	No
*28						
*1/*1	50 (84.7)	9 (15.3)	51 (86.4)	8 (13.6)	10 (17.0)	49 (83.0)
*1/*28	29 (90.6)	3 (9.4)	23 (71.8)	9 (28.2)	12 (37.5)	20 (62.5)
*28/*28	9 (100.0)	0 (0.0)	8 (88.9)	1 (11.1)	6 (66.7)	3 (33.3)
Missing	1	–	1	–	1	–
*93						
G/G	9 (100.0)	0 (0.0)	8 (88.9)	1 (11.1)	6 (66.7)	3 (33.3
G/A	26 (89.7)	3 (10.3)	20 (69.0)	9 (31.0)	11 (37.9)	18 (62.1)
A/A	53 (85.5)	9 (14.5)	53 (85.5)	9 (14.5)	12 (19.4)	50 (80.6)
Missing	1	–	1	–	–	1
*60						
T/T	30 (83.3)	6 (16.7)	30 (83.3)	6 (16.7)	8 (22.2)	28 (77.8)
G/T	42 (87.5)	6 (12.5)	38 (79.2)	10 (20.8)	13 (27.1)	35 (72.9)
G/G	15 (100.0)	0 (0.0)	12 (80.0)	3 (20.0)	8 (53.3)	7 (46.7)
Missing	2	–	2	–	–	2

Table 4 Dose reduction of irinotecan by UGT1A1*28 genotypes

UGT1A1						
	*1/*1		*1/*28		*28/*28	
	n	%	*n*	%	*n*	%
100 %	41	(69.5)	19	(59.4)	4	(44.4)
Dose 80–75 %	16	(27.1)	11	(34.4)	4	(44.4)
<75 %	2	(3.4)	2	(6.2)	1	(1.2)

Survival

The median PFS time was 10 months (95 % CI, 9.6–10.8). For patients treated in first line with FOLFIRI, the median PFS was 10.5 months, and for patients in second line, mPFS was 9.9 months. There was no statistical association between UGT1A genotypes and haplotypes and PFS (Fig. 1). None of the other genotypes analyzed were significantly associated with PFS.

Discussion

The present study allowed us to evaluate UGT1A polymorphisms. In our series, UGT1A1 could not predict toxicity to FOLFIRI, either UGT1A1*28, UGT1A*93 or UGT1A*60. Our study differs from others [20, 21] that found an association between different UGT1A1 variants and hematologic toxicity or response. The authors even suggested that the UGT1A1 haplotypes might be a better

Table 5 Association between genotypes and response

Polymorphisms	Response rate (%) (CR + PR)	Poor responsive (%) (SD + PD)	OR (95 % CI)	*p*
UGT1A1				
*28				
*1/*1	18 (30.5)	41 (69.5)	1.00 ref	
*1/*28	13 (40.6)	19 (59.4)	1.56 (0.63–3.82)	0.33
*28/*28	3 (33.3)	6 (66.7)	1.14 (0.26–5.06)	0.86
Missing	–	–		
*93				
G/G	18 (29.0)	44 (71.0)	1.00 ref	
G/A	14 (48.3)	15 (51.7)	2.28 (0.92–5.68)	0.07
A/A	2 (22.2)	7 (77.8)	0.69 (0.13–3.69)	0.67
Missing		1		
*60				
T/T	12 (33.3)	24 (66.7)	1.00 ref	
G/T	14 (29.2)	34 (70.8)	0.82 (0.32–2.09)	0.68
G/G	7 (46.7)	8 (53.3)	1.75 (0.51–5.98)	0.37
Missing	1	1		
Haplotype				
I	13 (35.1)	24 (64.9)	1.00 ref	
II	14 (38.9)	22 (61.1)	1.17 (0.45–3.04)	0.74
III	7 (25.9)	20 (74.1)	0.65 (0.22–1.93)	0.43
Missing	–	1		
XRCC1 Arg399Gln				
G/G	14 (36.8)	24 (63.2)	1.0 ref	
G/A	11 (23.4)	36 (76.6)	0.52 (0.20–1.35)	0.18
A/A	9 (56.2)	7 (43.8)	2.20 (0.67–7.23)	0.19
ERCC1 Asn118Asn				
C/C	3 (17.6)	14 (82.4)	1.0 ref	
C/T	14 (34.1)	27 (65.9)	2.42 (0.59–9.85)	0.22
T/T	17 (39.5)	26 (60.5)	3.05 (0.76–12.23)	0.11
XPD-751				
A/A	16 (33.3)	32 (66.7)	1.0 ref	
A/C	17 (39.5)	26 (60.5)	1.31 (0.55–3.08)	0.54
C/C	1 (10.0)	9 (90.0)	0.22 (0.03–1.91)	0.17
MTHFR C677T				
C/C	10 (25.0)	30 (75.0)	1.0 ref	
C/T	19 (40.4)	28 (59.6)	2.04 (0.81–5.12)	0.13
T/T	5 (35.7)	9 (64.3)	1.67 (0.45–6.16)	0.44
MTHFR A1298C				
A/A	14 (35.0)	26 (65.0)	1.0 ref	
A/C	16 (39.0)	25 (61.0)	1.19 (0.48–2.93)	0.71
C/C	4 (20.0)	16 (80.0)	0.46 (0.13–1.66)	0.24
TS 1494del6[a]				
+ 6 bp/+ 6 bp	21 (42.0)	29 (58.0)	1.00 Ref	
+ 6 bp/−z6 bp	9 (22.0)	32 (78.0)	0.39 (0.15-0.98)	0.05
−6 bp/−6 bp	4 (40.0)	6 (60.0)	0.92 (0.23-3.67)	0.91
TS 5′UTR VNTR				
2R/2R,2R/3C,3C/3C	24 (39.3)	37 (60.7)	0.54 (0.21–1.24)	0.14
2R/3G,3C/3G,3G/3G	10 (25.0)	30 (75.0)		

CR complete response, *PR* partial response, *SD* stable disease, *PD* progression disease, *OR* odds ratio, *UGT1A1* UDP-glucuronosyltransferase 1A1; *XRCC1* X-ray cross-complementing group 1, *ERCC1* excision repair cross-complementing group 1, *MTHFR* methylenetetrahydrofolate reductase, *TS* thymidylate synthase, *UTR* untranslated region, *VNTR* variable number tandem repeat

[a] Odds ratio, 0.38; 95 % CI, 0.14–0.97; $p = 0.04$ (binary logistic regression model after adjustment for sex, age, performance status and dose)

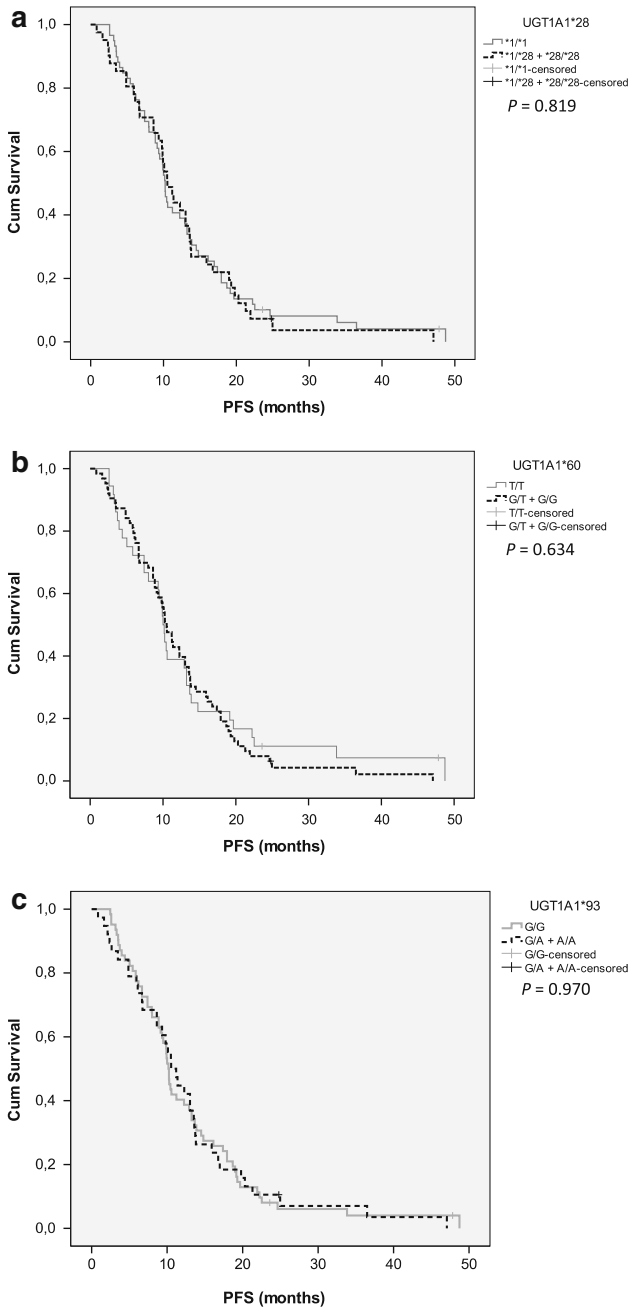

Fig. 1 Progression-free survival of patients with "wild-type" UGT1A1 (*continuous line*) and patients heterozygous and homozygous for: **a** UGT1A*28, **b** UGT1A*60, **c** UGT1A*93

predictor of the UGT1A1 status than the UGT1A1*28. From our point of view, this possibility remains to be proven.

Other predictors of irinotecan-induced toxicity could be nongenetic factors (neutrophil baseline levels or sex) or genetic factors (UGT1A1*93, ABCC1 IVS11 −48 C > T or SLCO1B1*1b) [22] that we have not explored.

Of the 60 or more UGT1A1 gene variants, two are responsible for 98–99 % of the genotypes found in the US white population: UGT1A1*1 and *28. 44 % of the US white population are homozygous for *1 (genotype *1/*1), 45 % are heterozygous (genotype *1/*28), and 11 % (about 1 in 10) are homozygous for *28 (genotype *28/*28). Other members of the family UGT also have some role (UGT1A 6, 7, 9, 10) [21, 23].

Previous studies seem to show a clear relationship between the most common *UGT1A1*28* genotype and severe neutropenia (and some evidence of a relationship with severe diarrhea), but there is no direct or indirect evidence to support the clinical utility of modifying an initial and/or subsequent dose of irinotecan in patients with mCRC as a way to change the rate of adverse drug events (e.g., severe neutropenia). Even if adverse drug-related events were reduced, this risk reduction may come at the expense of a reduction in tumor responsiveness in *28 homozygotes, leading to an overall net harm. The use of filgrastrim may mask the need of reduction doses. Our study found no independent genetic factors that predicted toxicity. We found no significant association between the UGT1A1 variants and grade 3 or 4 toxicity, and we observed the same trend as the results of Cecchin et al. [20].

Recently, several genotype-directed phase I clinical trials have found 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 [24, 25]. This reveals an important unsolved question, should UGT1A1*1 patients receive higher doses of irinotecan than usual? In a previous work, Marcuello et al. [14] observed that the presence of one or two alleles of UGT1A1*28 was not associated with a significantly higher response rate or a survival advantage in patients with mCRC who received irinotecan. Our results are consistent with their study, but controversy still remains. We observed a significantly increased risk of reducing dosage after first cycle among patients carrying the UGT1A1*93-AA and UGT1A1*60-GG, which was only relevant for the first cycle and not seen throughout the whole treatment period. As other authors, these variants do not add predictive power. Dose reductions were due mainly to diarrhea and neutropenia, but we did not find a significant association between UGT1A1*60 and toxicity, these results may be due to chance, or dose reductions could be an indirect proof of increased toxicity. In the article of Cecchin, the –GG genotype was associated with severe (grade 3–4) hematologic toxicity, but our patients only developed this toxicity in 18 %, probably because supportive therapies may well hide the possible cause.

Our results do not confirm that patients receiving irinotecan 180 mg/m^2 biweekly were at a higher risk of developing severe hematologic toxicity. Thus, evaluation of UGT1A1 variants does not improve the ability to predict

toxicities in the clinical setting of standard-dose irinotecan chemotherapy. Dose intensity in patients with UGT1A1*28 was lower than for UGT1A1*1, and use of filgrastrim was higher; differences were not statistically different, but the size of groups in every variant was small, and its provoking to think this maneuvers could underlie the lack of predictive effect in hematologic toxicity. The current guidelines of the American Society of Clinical Oncology (ASCO) and European Organization for Research and Treatment of Cancer (EORTC) recommend the use of a granulocyte colony-stimulating factor as secondary prophylaxis when it is judged important to maintain the dose density or dose intensity of chemotherapy for achieving survival benefits, by preventing dose reduction below threshold and delay of chemotherapy. Our goal was to find a relationship with neutropenia, and we have failed on it, but the use of G-CSF according to the aforementioned guidelines could mask the increased risk of neutropenia. As the nature of this study is exploratory, we find relevant to suggest the association with potential indirect indicators of toxicity, especially because this represents the real clinical practice.

TS is a controversial pharmacogenetic target. High-expression haplotypes or polymorphisms have been associated with poor response as it could be expected if this indicated resistance to 5-FU, and on the other side, our group has also found that high expression was related to a better response to 5-FU chemoradiotherapy [26]. In this latter case, 5-FU was the only drug, and the potential role of TS as a prognostic factor remained unclear. In our current work, high-expression genotypes were not related to a best PFS and objective response. A recent study [27] reported a strong correlation between TYMS 5′TRP genotypes and response treatment but no significant association with toxicity. Carlini et al. [28] also evaluated the relationship between TYMS polymorphism and response to capecitabine and irinotecan in metastatic CRC patients, but no significant association was noted. The 6-base pair insertion/deletion (6+/6−) in the 3′-untranslated region (3′-UTR) is associated with decreased mRNA stability and lower intratumoral TS expression in vitro. The value of this polymorphism as a predictor of clinical response to 5-FU-based therapy is not clearly established and requires further investigation [29, 30].

In another study [31], XRCC1 codon 399 Arg/Arg genotype patients present longer survival than patients with Gln/Arg and Gln/Gln who are treated with irinotecan. They also suggest that polymorphisms in the XPD gene may be associated with the overall survival in these patients. We do not find associations in our study between these polymorphisms and response or survival.

NCCN clinical guidelines does not support the use of the test by the moment; however, experts point out that initial reduction must be considered with elevated bilirubin levels or Gilbert's disease.

Clearly, this study is limited by the retrospective design and the limited power to detect haplotype effects of the UGT1A genes. But, despite the limited sample design, an effect of UGTA1*28 on severe toxicity as previously reported [20] would be confirmed (Power $(1\text{-}\beta) > 0.8$). Other germline variants in genes that might affect the efficacy and toxicity of fluorouracil (TS, MTHFR or XPD) have been taken into account, and no clinical effects were observed; these genes are unlikely to affect the pharmacogenetics of the FOLFIRI regimen.

The real value of the study lies in the search of the utility of pharmacogenetic analysis in real clinical practice conditions. In this setting, patients are instructed in the clinic to act early on the onset of diarrhea, and dose reductions or using filgrastrim is usual tool to keep dose intensity. So, we think that the impact of increased risk of toxicity attributed to the UGT1A variants may be offset by these conditions.

Our opinion is that the following point to be solved is not to test or not to test, but what to do next. The clinical utility of UGT1A1 testing remains uncertain and should not be incorporated in clinical practice until guidelines indicate how to use irinotecan if the patient is homozygous or heterozygous for UGT1A1*28 allele.

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Ethical conduct of research The authors state that they have obtained appropriate institutional review board approval or have followed the principles outlined in the Declaration of Helsinki for all human or animal experimental investigations. In addition, for investigations involving human subjects, informed consent has been obtained from the participants involved.

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