UGT1A1 Polymorphism Can Predict Hematologic Toxicity in Patients Treated with Irinotecan

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Abstract

Purpose: Irinotecan (CPT-11) is approved in metastatic colorectal cancer treatment and can cause severe toxicity. The main purpose of our study was to assess the role of different polymorphisms on the occurrence of hematologic toxicities and disease-free survival in high-risk stage III colon cancer patients receiving 5-fluorouracil (5FU) and CPT-11 adjuvant chemotherapy regimen in a prospective randomized trial.

Experimental Design: Four hundred patients were randomized in a phase III trial comparing LV5FU2 to LV5FU2 + CPT-11. DNA from 184 patients was extracted and genotyped to detect nucleotide polymorphism: 3435C>T for *ABCB1*, 6986A>G for *CYP3A5, UGT1A1*28* and -3156G>A for *UGT1A1*.

Results: Genotype frequencies were similar in both treatment arms. In the test arm, no significant difference was observed in toxicity or disease-free survival for *ABCB1* and *CYP3A5* polymorphisms. *UGT1A1*28* homozygous patients showed more frequent severe hematologic toxicity (50%) than *UGT1A1*1* homozygous patients (16.2%), $P = 0.06$. Moreover, patients homozygous for the mutant allele of -3156G>A *UGT1A1* polymorphism showed more frequent severe hematologic toxicity (50%) than patients homozygous for wild-type allele (12.5%), $P = 0.01$. This toxicity occurred significantly earlier in homozygous mutant than wild-type homozygous patients ($P = 0.043$). In a Cox model, the hazard ratio for severe hematologic toxicity is significantly higher for patients with the A/A compared with the G/G genotype [hazard ratio, 8.4; 95% confidence interval, 1.9–37.2; $P = 0.005$].

Conclusions: This study supports the clinical utility of identification of *UGT1A1* promoter polymorphisms before LV5FU2 + CPT-11 treatment to predict early hematologic toxicity. The -3156G>A polymorphism seems to be a better predictor than the *UGT1A1* $(TA)_6TAA>(TA)_7TAA$ polymorphism.

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Note: P. Laurent-Puig and M. Ychou contributed equally to this work.

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Irinotecan (7-ethyl-10-[4-(1-piperidino)-1-piperidino]-carbonyloxycamptothecin) (CPT-11) is a water-soluble analogue of 20(*S*)-camptothecin (CPT) and is an inactive prodrug. Its major metabolite, SN-38, is a potent active topoisomerase I inhibitor and is known to be toxic (1).

Due to its efficacy, CPT-11 is currently approved worldwide for use as first-line therapy in metastatic colorectal cancer, in combination with 5-fluorouracil (5FU) and leucovorin (LV; ref. 2). Adjuvant CPT-11 in combination with 5FU has recently been investigated in colorectal cancer. One limitation of CPT-11 is the unpredictable and occasionally fatal gastrointestinal and hematologic toxicity, which varies greatly between individuals. Predictive markers of CPT-11 toxicity may thus be deduced from the CPT-11 metabolic pathway.

CPT-11 is metabolized by carboxylesterase (CES), essentially the isoenzyme CES2, to active SN-38, then is further conjugated and detoxified by UDP-glucuronosyltransferase (UGT) 1A1 enzyme to yield its β-glucuronide, SN-38 G (3, 4). SN-38 G is excreted in the small intestine via the bile, where bacterial glucuronidase breaks down the glucuronide into SN-38 and glucuronic acid (5). Bilurubin undergoes the same glucuronidation by

Table 1. Distribution of the different genotypes

Gene	SNPs	Genotype			
		wt/wt, *n* (%)	wt/m, *n* (%)	m/m, *n* (%)	(*N* = 184)
UGT1A1	TA indel	79 (45)	81 (46)	16 (9)	176
UGT1A1	-3156G>A	83 (47)	80 (46)	13 (7)	176
CYP3A5	6986A>G	2 (1)	28 (15)	152 (84)	182
ABCB1	3435C>T	42 (23)	94 (53)	43 (24)	179

NOTE: The frequency expected for each genotype was evaluated on the basis of Hardy-Weinberg equilibrium proportions. None of these observed frequencies were significantly different from the expected frequencies.
Abbreviations: wt, wild-type allele; m, mutant allele.

UGT1A1 and is excreted into the bile (6). More than 50 genetic variants in the promoter and coding regions of the *UGT1A1* gene are currently known to affect enzyme activity (7), leading to different forms of unconjugated hyperbilirubinemias known as Crigler-Najjar syndrome types I and II and Gilbert's syndrome, a mild unconjugated hyperbilirubinemia with no structural liver disease or overt hemolysis (8). One of the most common genotypes in Gilbert's syndrome in Caucasian populations is the inheritance of a promoter region containing an extra TA dinucleotide in the $[A(TA)_6TAA]$ element, leading to 30% to 80% reduction in the expression of UGT1A1 protein (9, 10). Therefore, patients who are homozygous for this variant allele (designated as *UGT1A1*28*) exhibit a metabolic ratio of SN-38/SN-38 G higher than that observed for homozygous wild-type patients of an attenuated expression of *UGT1A1* and are predisposed to SN-38 initiated diarrhea (11, 12) and severe hematologic toxicity (11, 13). A more recently investigated promoter polymorphism, -3156G>A *UGT1A1*, seems to be a better predictor of the *UGT1A1* status than *UGT1A1*28* (14).

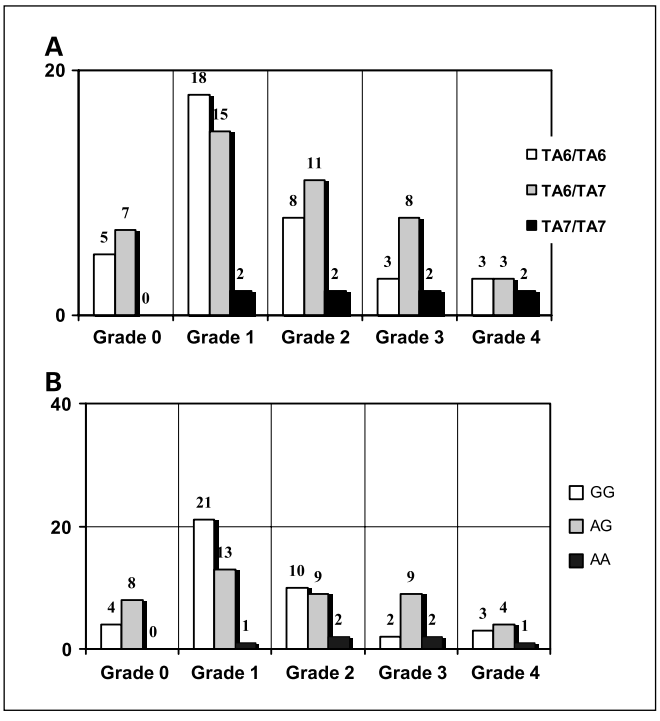

Fig. 1. *A,* distribution of the different genotypes TA indel according to hematologic toxicity in arm B (*N* = 89). *B,* distribution of the different genotypes -3156G>A according to hematologic toxicity in arm B (*N* = 89).

CPT-11 is also catalyzed by the cytochrome P450 (CYP) 3A subfamily, which catalyzes the metabolism of structurally diverse xenobiotics (15) and is the most abundant CYP enzyme in the human liver and small intestine (16). Substantial interindividual differences in CYP3A expression contribute to the variations in the oral bioavailability and systemic clearance of CYP3A substrates (17). In adults, the main CYP3A isoforms are CYP3A4 and CYP3A5. CYP3A5 plays a role in the elimination of CPT-11, forming the APC complex, a metabolite in which antitumor activity is 500 times less compared with SN-38. The polymorphism of *CYPA3A5* gene 6986A>G has already been described. Those with the *CYP3A5*3* allele display sequence variability in intron 3 that creates a cryptic splice site and encodes an aberrantly spliced mRNA with a premature codon stop, leading to the absence of protein expression (18, 19). Because CYP3A5 enzymes play a role in the elimination CPT-11, this polymorphism may partly explain the interindividual variability of CPT-11 toxicity.

In addition, CPT-11 and SN-38 can be transported out of the cell by the P-glycoprotein, a trans-membrane efflux pump (20, 21) that is a member of the ATP-binding cassette family. P-glycoprotein, also called *MDR1* (multidrug resistance), is encoded by the human *ABCB1* gene (ATP-binding cassette, subfamily B; ref. 22). Significant interindividual variations in the expression and function of P-glycoprotein may be a result of genetic factors. Various single nucleotide polymorphisms (SNP) have been identified within the *ABCB1* gene in the past few years (22). The SNP located on exon 26 3435C>T described by Hoffmeyer et al. (21) shows a correlation of this polymorphism with expression levels and function of *ABCB1*.

The main purpose of our study was to assess the role of different polymorphisms on the occurrence of hematologic toxicities and disease-free survival in high-risk stage III colon cancer patients receiving 5FU and CPT-11 adjuvant chemotherapy combined through the FOLFIRI regimen in a prospective randomized trial. The role of the following polymorphisms were investigated: two polymorphisms in the promoter region of *UGT1A1*, namely, *UGT1A1*28* (rs8175347) and the -3156G>A (rs10929302), the polymorphism 3435C>T for *ABCB1* (rs1045642) and 6986A>G for *CYP3A5* (rs776746).

Materials and Methods

Study design. Four hundred patients were randomized between November 1998 and September 2002 in 75 centers in France to the

Table 2. Severe hematologic toxicity according to TA indel (*UGT1A1*), -3156G>A (*UGT1A1*), 6986A>G (*CYP3A5*), and 3435C>T (*ABCB1*) polymorphisms in the B arm

Gene	SNPs	Severe hematologic toxicity						*P**	*N* = 93
		No			Yes				
		wt/wt, *n* (%)	wt/m, *n* (%)	m/m, *n* (%)	wt/wt, *n* (%)	wt/m, *n* (%)	m/m, *n* (%)		
UGT1A1	TA indel	31 (46)	33 (48)	4 (6)	6 (29)	11 (52)	4 (19)	0.06	89
UGT1A1	-3156G>A	35 (52)	30 (44)	3 (4)	5 (24)	13 (62)	3 (14)	0.01	89
CYP3A5	6986A>G	1 (2)	10 (14)	59 (84)	0 (0)	3 (14)	19 (86)	0.72	92
ABCB1	3435C>T	15 (22)	39 (57)	14 (21)	3 (13)	13 (57)	7 (30)	0.23	91

Abbreviations: wt, wild-type allele; m, mutant allele.
**P* for trend test.

phase III clinical trial FNCLCC Accord02/FFCD9802 comparing LV5FU2 alone versus LV5FU2 + CPT-11. All the patients signed an informed consent for the pharmacogenetic study.

Patients with high-risk stage III colon cancer were included (i.e., patients with postoperative N2 or N1 but with acute complication occlusion or perforation). They were randomized to either arm A, LV5FU2 (leucovorin 200 mg/m^2 as a 2-h infusion, 5FU 400 mg/m^2 bolus and 600 mg/m^2 22 h continuous infusion, d1-2); or arm B, LV5FU2 + CPT-11 (irinotecan 180 mg/m^2 90 min infusion d1+ LV5FU2) every 2 weeks for 12 cycles with no growth factors. Patients were stratified by center, by the delay between surgery and start of chemotherapy (≤28 days; >28 days), and by age (<65 years; ≥65 years).

From this clinical trial, paraffin-embedded samples from normal tissue for the pharmacogenetic study were obtained for 184 of the 400 patients from different centers in France, 91 from arm A (LV5FU2) and 93 from arm B (LV5FU2 + CPT-11).

Sample preparation and DNA extraction. Three 20-μm slices were cut from each normal paraffin-embedded block. Slices were deparaffinized twice with 1.2 mL toluene, vortexed and centrifuged, then washed twice with 1.2 mL of 100% ethanol. The samples were resuspended in 180 μL of Qiagen buffer ALT (Qiagen, Courtaboeuf, France) and 20 μL proteinase K (Roche Diagnostics, Mannheim, Germany). Samples were incubated overnight at 56°C with gentle shaking, and proteinase K was added twice. After 36 h, DNA was extracted from each sample using the QIAmp DNA Mini Kit from Qiagen according to the manufacturer's instructions. The final concentration of each DNA sample was adjusted to 25 ng/μL and stored at -20°C.

Determination of UGT1A1, CYP3A5, and ABCB1 gene polymorphism. The variant sequences of a two-nucleotide insertion (TA) within TATA box resulting in the sequence $(TA)_7TAA$ (-39 to -53; rs8175347) and the SNP -3156G>A for *UGT1A1* (rs10929302), 3435C>T for *ABCB1* (rs1045642) and 6986A>G for *CYP3A5* (rs776746) were characterized.

The TA indel variation of UGT1A1 was studied by fragment analysis. Briefly, a PCR was done in 15 μL containing 4 μL of DNA (25 ng/μL), 0.5 μmol/L each of forward (5′-5HEX-TTAACTTGGTG-TATCGATTGG-3′) and reverse (5′-CTTTGCTCCTGCCAGAGGT-3′) primer from Qiagen, 0.8 mmol/L of deoxynucleotide triphosphates, 1.5 μL of 10× PCR buffer from Qiagen, 0.3 μL of 5× Solution Q (Qiagen), 0.9 μL of 25 mmol/L $MgCl_2$ (Qiagen), 0.75 units Taq Hotstar (Qiagen), and 2.3 μL of water and run according to the following cycle profile: 95°C for 10 min, 40 cycles at 94°C for 30 s, 56°C for 30 s, 72°C for 30 s, and a final extension of 10 min at 72°C. The PCR was realized on thermal cycler PTC-100 (MJ Research Inc., Watertown, MA). For molecular analysis of $[A(TA)_nTAA]$, fluorescence-labeled PCR products were separated by automated capillary electrophoresis on the ABI PRISM 310 Genetic Analyzer (Applied Biosystems, Foster City, CA) and analyzed with GeneScan and Genotyper software (Applied Biosystems). The TA_5 allele corresponds to a 74-bp fragment, TA_6 allele corresponds to a 76-bp fragment, the TA_7 allele corresponds to a 78-bp fragment, and TA_8 corresponds to 80-bp fragments. For each run, positive controls were added, including patients with different genotype (i.e., TA_5/TA_6, TA_6/TA_6, TA_6/TA_7, TA_7/TA_7, TA_6/TA_8, and TA_7/TA_8).

For the SNPs -3156G>A of UGT1A1 (rs10929302), 6986A>G for CYP3A5 (rs776746) and 3435C>T for ABCB1 (rs1045642), alleles were determined by the use of TaqMan probes (Applied Biosystems). For the first two polymorphisms, primers and TaqMan probes were designed by Applied Biosystems (TaqMan Assays-by-Design, Applied Biosystems). For 3435C>T for *ABCB1*, primers and TaqMan probes were designed by us and synthesized by Applied Biosystems. The efficiency of this genotyping method was previously validated by sequencing (18). Sequences are available upon request. The PCR amplification was done in a volume of 8 μL containing 6 μL of reaction mix (Assays-by-Design, TaqMan Universal PCR Master Mix No AmpErase UNG, AmpliTaq Gold DNA polymerase, and water) and 2 μL of DNA (2.5 ng/μL) on an ABI

Table 3. Severe neutropenia according to TA indel (*UGT1A1*), -3156G>A (*UGT1A1*), 6986A>G (*CYP3A5*), and 3435C>T (*ABCB1*) polymorphisms in the B arm

Gene	SNPs	Severe neutropenia						*P**	*N* = 93
		No			Yes				
		wt/wt, *n* (%)	wt/m, *n* (%)	m/m, *n* (%)	wt/wt, *n* (%)	wt/m, *n* (%)	m/m, *n* (%)		
UGT1A1	TA indel	32 (46)	34 (49)	4 (6)	5 (26)	10 (53)	4 (21)	0.035	89
UGT1A1	-3156G>A	36 (51)	31 (44)	3 (4)	4 (21)	12 (63)	4 (20)	0.008	89
CYP3A5	6986A>G	1 (2)	11 (15)	60 (83)	0 (0)	2 (10)	18 (90)	0.426	92
ABCB1	3435C>T	15 (21.4)	40 (57.2)	15 (21.4)	3 (14)	12 (57)	6 (29)	0.382	91

Abbreviations: wt, wild-type allele; m, mutant allele.
**P* for trend test.

Table 4. Severe hematologic toxicity according to TA indel (*UGT1A1*), -3156G>A (*UGT1A1*), 6986A>G (*CYP3A5*), and 3435C>T (*ABCB1*) polymorphisms in the A arm

Gene	SNPs	Severe hematologic toxicity						P*	N = 91
		No			Yes				
		wt/wt, *n* (%)	wt/m, *n* (%)	m/m, *n* (%)	wt/wt, *n* (%)	wt/m, *n* (%)	m/m, *n* (%)		
UGT1A1	TA indel	40 (48)	35 (42)	8 (10)	2 (50)	2 (50)	0	0.73	87
UGT1A1	-3156G>A	41 (48)	36 (43)	6 (7)	2 (50)	1 (25)	1 (25)	0.60	87
CYP3A5	6986A>G	1 (1)	14 (16)	70 (82)	0	1 (20)	4 (80)	0.95	90
ABCB1	3435C>T	23 (28)	38 (46)	22 (27)	1 (20)	4 (80)	0	0.57	88

Abbreviations: wt, wild-type allele; m, mutant allele.
**P* for trend test.

PRISM 7900HT from Applied Biosystems according to the manufacturer's instructions. Genotypes were determined automatically using ABI Sequence Detection System software (SDS Software 2.1, Applied Biosystems). The ambiguous genotypes were analyzed by two independent observers (J.F. Côté, S. Kirzin), and discordant results were reamplified and reanalyzed.

Statistical analysis. The clinical trial data were managed and analyzed in the biostatistics unit of the Val d'Aurelle Regional Cancer Centre in Montpellier, France. Toxicities were graded according to National Cancer Institute-Common Toxicity Criteria v2. Severe hematologic toxicity consisted of either grade 3 or 4 neutropenia, thrombocytopenia, anemia, or leucopenia. For each genotype, association with hematological and gastrointestinal toxicity in each treatment arm was evaluated using a nonparametric test for trend across equally spaced ordered groups. Toxicity-free survival rates by cycle and disease-free survival rates from randomization were estimated using the Kaplan-Meier method. Univariate comparisons were done with the log rank test. Multivariate analyses, adjusted for important clinical variables, were done using the Cox proportional hazards model.

The deviations from the Hardy-Weinberg equilibrium of allele and genotype frequencies for the various SNPs were assessed by Fisher's exact test. Pairwise linkage disequilibrium between *UGT1A1*28* and *UGT1A1* -3156G>A was estimated by a log-linear model, and the extent of disequilibrium was expressed in terms of *D'*, which is the ratio of the unstandardized coefficient to its maximal/minimal value.

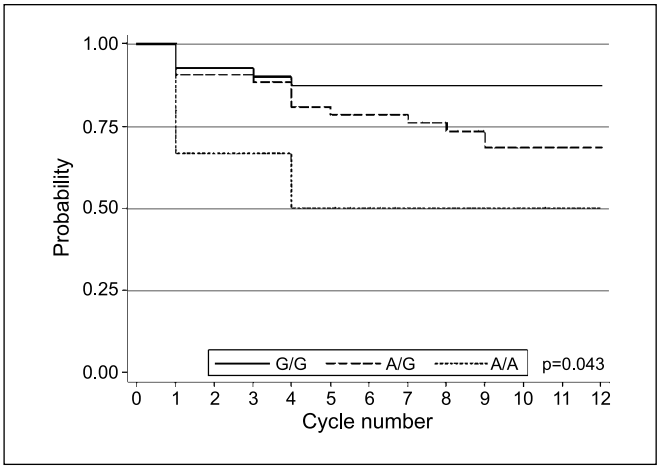

Fig. 2. Grade 3 to 4 hematologic toxicity-free survival curves estimated by the Kaplan-Meier method for patients from arm B according to chemotherapy cycle and the SNP -3156G>A (*UGT1A1*; *N* = 89).

All statistical analyses were done using Stata8 (StataCorp LP, College Station, TX) and Thesias for haplotype analysis provided by David Tregouet (Institut National de la Sante et de la Recherche Medicale U525, Paris, France) and were considered significant with a *P* value <0.05.

Results

Polymorphism frequencies. The FNCLCC Accord02/FFCD9802 trial included 400 patients. Normal DNA was available for 184 patients. Demographic and clinical data of this subset of patients did not differ significantly from the patients not selected for this analysis.

The frequencies of the variant tested alleles estimated on the entire series of 184 patients were 32.1%, 30.1%, 91.2%, and 50.3% for *UGT1A1* TA_7, *UGT1A1* -3156 A, *CYP3A5* 6986 G, and *ABCB1* 3435 T alleles, respectively. These frequencies are in accordance with those observed in Caucasian populations. Table 1 shows the genotype distribution of the different polymorphisms. All of these genotypes were distributed according to the Hardy-Weinberg equilibrium.

Distribution of the different UGT1A1 genotypes according to hematologic toxicity. Figure 1A and B shows the distribution of the different *UGT1A1* genotypes according to hematologic toxicity grades from patients in arm B. An increased frequency of homozygous variant genotypes was observed with increased hematologic toxicity. The *P* values were equal to 0.055 and 0.059 for *UGT1A1*28* and -3156G>A polymorphisms, respectively (trend test).

Hematologic toxicity and neutropenia. Analysis of severe hematologic toxic events showed more frequent toxicities

Table 5. Cox multivariate analysis of the occurrence of grade 3 to 4 hematologic toxicity in arm B (*N* = 89)

Variable	Hazard ratio	P*	95% confidence interval
Age	1.07	0.019	1.01-1.13
Gender (reference group M)	3.7	0.007	1.43-9.47
-3156 UGT1A1 wt/m	2.8	0.052	0.99-7.88
-3156 UGT1A1 m/m	8.4	0.005	1.90-37.19

Abbreviations: wt, wild-type allele; m, mutant allele.
**P* from Wald test.

Table 6. Frequency of the different haplotypes of *UGT1A1* gene according to severe hematologic toxicity

Haplotype		Severe hematologic toxicity	
-3156G>A	TA indel	No (n = 68)	Yes (n = 21)
G	TA_6	0.691	0.523
G	TA_7	0.044	0.025
A	TA_6	0.008	0.025
A	TA_7	0.257	0.427

(grade 3 or more) for patients with a homozygous TA_7 allele 4/8 (50%) than for patients homozygous for wild-type TA_6 allele 11/37 (16.2%), although this difference did not reach the threshold of statistical significance (P = 0.06, trend test, Table 2). However, a statistically significant difference was observed with *UGT1A1* -3156G>A SNP, with more frequent severe hematologic toxicities observed in patients homozygous for the mutant A allele 4/8 (50%) than for patients homozygous for wild-type G allele 5/40 (12.5%). Heterozygous patients showed an intermediate frequency of 30.2% (13/43, P = 0.01; trend test; Table 2). When only severe neutropenia was considered, these results remained in the same range (Table 3).

Regarding the 6986A>G (*CYP3A5*) and 3435C>T (*ABCB1*) polymorphisms, no statistically significant difference was found in the frequency of the different genotypes versus occurrence of severe hematologic toxicity or severe neutropenia (Tables 2 and 3).

Concerning the patients in arm A, no statistically significant difference was found in the frequency of the different genotypes versus the occurrence of severe hematologic toxicity according to the four SNP genotypes (Table 4).

Hematologic toxicity-free survival curves. Severe hematologic toxicity occurs significantly earlier in patients with AA genotype for *UGT1A1* -3156G>A polymorphism than for the other genotypes (Fig. 2, P = 0.043). When only severe neutropenia was considered, a similar result was observed with a P value of 0.024. Neither severe hematologic toxicity nor severe neutropenia was observed for patients with AA genotype after the fourth cycle (Fig. 2).

Cox multivariate analysis. A Cox multivariate analysis was done to estimate the hazard ratio of severe hematologic toxicity for the different *UGT1A1* -3156G>A genotypes. The hazard ratio for development of a grade 3 to 4 hematologic toxicity, after adjustment for age and gender was 8.4; 95% confidence interval, 1.9–37.2 for patients with the AA genotype of SNP -3156G>A *UGT1A1* compared with the GG genotype (Table 5, P = 0.005).

Haplotype analysis. Haplotype analysis combining the two *UGT1A1* polymorphisms was done. As expected, we found linkage disequilibrium between the two polymorphisms of the promoter region of *UGT1A1* gene. For patients receiving LV5FU2 + CPT-11, they were in nearly complete disequilibrium (D' = +0.94, $P < 10^{-4}$) and then generated two common haplotypes G-TA_2 and A-TA_2. Detailed haplotype frequency distribution in patients according to whether or not severe hematologic toxicity was observed is provided in Table 6.

Although the test for a difference of haplotype frequencies between the two groups of patients did not reach statistical significance (P = 0.081), the two haplotypes carrying the A allele tended to be at a higher frequency in patients with a severe hematologic toxicity than those without this allele. This is in agreement with the results observed in the univariate analysis.

Gastrointestinal toxicity. No significant statistical difference in the occurrence of severe gastrointestinal toxicity (grade 3 or more diarrhea, nausea, vomiting, or mucositis) was seen in 184 patients from either treatment arm, LV5FU2 alone, or in combination with CPT-11 in relation to SNPs of *UGT1A1* promoter TA indel, *UGT1A1* -3156G>A, *CYP3A5* 6986A>G, and *ABCB1* 3435C>T (Tables 7 and 8).

Dose relation CPT-11 and TA indel (UGT1A1), -3156G>A (UGT1A1) SNPs. No difference in median CPT-11 doses received was observed between the different genotypes.

Survival and polymorphisms. No significant survival difference was observed between patients in arm B according to different polymorphisms. There was a tendency for better disease-free survival for homozygous patients with the variant genotype of *UGT1A1*28* SNP with 3-year disease-free survival of 87% versus 52% and 42% for wild-type homozygous and heterozygous patients, respectively (P = 0.06; Fig. 3).

Discussion

This series of colon cancer patients shows the impact of four polymorphisms on irinotecan induced chemotherapy toxicity in an adjuvant chemotherapy situation. An association was found between two polymorphisms of the promoter region of *UGT1A1* and the occurrence of severe hematologic toxicity and, more specifically, severe neutropenia. These results confirm

Table 7. Gastrointestinal toxicity according to TA indel (*UGT1A1*), -3156G>A (*UGT1A1*), 6986A>G (*CYP3A5*), and 3435C>T (*ABCB1*) polymorphisms in the B arm

Gene	SNPs	Gastrointestinal toxicity						P*	N = 93
		No			Yes				
		wt/wt, n (%)	wt/m, n (%)	m/m, n (%)	wt/wt, n (%)	wt/m, n (%)	m/m, n (%)		
UGT1A1	TA indel	31 (45)	32 (46)	6 (9)	6 (30)	12 (60)	2 (10)	0.31	89
UGT1A1	-3156G>A	31 (45)	34 (49)	4 (6)	9 (45)	9 (45)	2 (10)	0.79	89
CYP3A5	6986A>G	0 (0)	10 (14)	60 (86)	1 (4.6)	3 (13.6)	18 (81.8)	0.38	92
ABCB1	3435C>T	14 (20)	41 (59)	14 (20)	4 (18)	11 (50)	7 (32)	0.40	91

Abbreviations: wt, wild-type allele; m, mutant allele.
*P for trend test.

Table 8. Gastrointestinal toxicity according to TA indel (*UGT1A1*), -3156G>A (*UGT1A1*), 6986A>G (*CYP3A5*) and 3435C>T (*ABCB1*) polymorphisms in the A arm

Gene	SNPs	Gastrointestinal toxicity						*P**	*N* = 91
		No			Yes				
		wt/wt, *n* (%)	wt/m, *n* (%)	m/m, *n* (%)	wt/wt, *n* (%)	wt/m, *n* (%)	m/m, *n* (%)		
UGT1A1	TA indel	41 (50)	33 (40)	8 (10)	1 (20)	4 (80)	0	0.50	88
UGT1A1	-3156G>A	41 (50)	34 (41)	7 (9)	2 (40)	3 (60)	0	0.96	88
CYP3A5	6986A>G	1 (1)	15 (18)	69 (81)	0	0	5 (100)	0.30	90
ABCB1	3435C>T	23 (28)	40 (48)	20 (24)	1 (20)	2 (40)	2 (40)	0.48	88

Abbreviations: wt, wild-type allele; m, mutant allele.
**P* for trend test.

those of Innocenti et al. (14) observed in 66 patients with advanced disease refractory to chemotherapy.

This toxicity is due to a deficit in glucuronidation as observed in Gilbert's syndrome. In our series, the frequency of patients homozygous for the *UGT1A1*28* allele partially responsible for this syndrome is similar to that observed in other Caucasian populations (7, 10).

The -3156G>A polymorphism is in strong linkage disequilibrium with *UGT1A1*28* polymorphism ($D' > 0.94$). In addition, -3156G>A polymorphism maybe a better predictor of hematologic toxicity than *UGT1A1*28* polymorphism, as suggested by Innocenti (14). The association with toxicity was significant only for the -3156G>A polymorphism. Haplotype analysis showed a higher frequency of severe hematologic toxicity for patients with the A allele of -3156G>A, regardless of the associated *UGT1A1* TA_6TAA/TA_7TAA. More data are necessary to explore the role of the *UGT1A1* haplotype in the occurrence of severe side effects and the relative predictive weight of each of the *UGT1A1* promoter polymorphisms.

This study showed that most severe hematologic or severe neutropenia toxicities occur in most mutant homozygous patients for -3156G>A polymorphism during the first cycle of chemotherapy and never after the fourth cycle (Fig. 2). Exploration of the -3156G>A *UGT1A1* polymorphism, before CPT-11 treatment to predict the early CPT-11 hematologic toxicity, seems interesting in the management of the patient. Specific studies are needed to validate a modification of the mode of administration of CPT-11 in these homozygous mutant patients. In particular, the concomitant administration of granulocyte colony-stimulating agent with CPT-11 should be tested to avoid adverse severe hematologic side effects in this subgroup of patients.

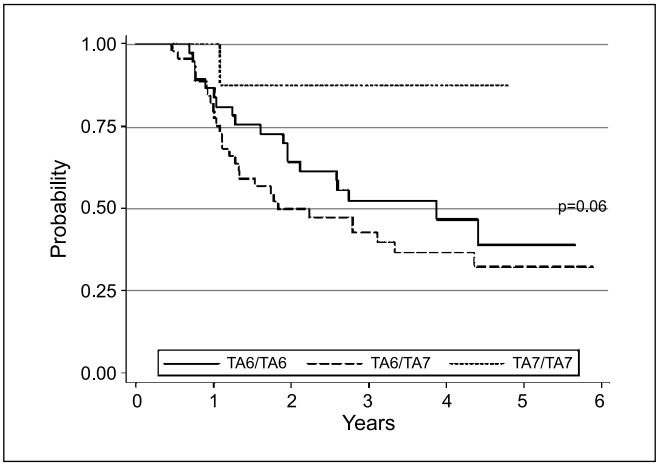

Fig. 3. Disease-free survival curves estimated by the Kaplan-Meier method for patients from arm B according to *UGT1A1* TA indel ($N = 89$).

Multivariate analysis showed a strong independent role of gender in the occurrence of severe hematologic toxicity. The association of gender and glucuronidation has already been reported (10, 14). Drugs metabolized by phase II enzymes (glucuronidation, conjugation, glucuronyltransferases, methyltransferases, and dehydrogenases) are usually cleared faster in men than in women (mg/kg basis; ref. 23). The clinical consequences of this interaction have never been shown in patients receiving CPT-11 and need to be further explored.

In our study, CPT-11 – induced gastrointestinal toxicity such as diarrhea showed no statistically significant relationship with the *UGT1A1* polymorphisms, in contrast to the series reported by Marcuello et al. (24). In their study of 95 metastatic colorectal cancer patients, the occurrence of severe diarrhea was more frequent in homozygous *UGT1A1*28* patients as compared with wild-type patients, possibly related to the higher doses of the CPT-11 regimen. In addition, a recent study by Massacesi et al. (25), showed that *UGT1A1* promoter polymorphism (TA indel) predicted the risk of diarrhea, emesis, and fatigue with CPT-11 and raltitrexed treatment. They were unable to evaluate the predictive role of *UGT1A1* promoter polymorphism (TA indel) for hematologic toxicity because they used a schedule for CPT-11, which reduced the number of grade 3 or 4 neutropenic events to only a few. CPT-11 was administered at a dose of 80 mg/m^2 (as a 30-min infusion) on days 1, 8, 15, 22, 36, 43, 50, and 57.

The absence of a significant association of severe hematologic toxicity or diarrhea with *ABCB1* and *CYP3A5* polymorphism is in agreement with previous published results (26) and suggests a major role of glucuronidation in the detoxification of the SN-38 compound. At least two other genes from the large family of ABC transporters (*ABCC2* and *ABCG2*) could also play a role in irinotecan metabolism, but no significant effects on the severity of adverse effects have been found thus far (27).

In conclusion, this study supports the clinical utility of identification of *UGT1A1* promoter polymorphisms before LV5FU2 + CPT-11 treatment to predict early hematologic toxicity. The -3156G>A polymorphism seems to be a better predictor than the *UGT1A1* $(TA)_6TAA>(TA)_7TAA$ polymorphism.

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