Relevance of Different UGT1A1 Polymorphisms in Irinotecan-Induced Toxicity: A Molecular and Clinical Study of 75 Patients

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ABSTRACT

Purpose: We wanted to assess polymorphisms in the uridine diphosphogluronosyl transferase 1A1 (UGT 1A1) gene: the TATA box polymorphism and UGT 1A1 G71R and Y486D mutations in the coding sequence, the main mutations characterizing Gilbert’s syndrome, as predictors of severe toxic event occurrence after irinotecan (CPT-11) administration. Therefore, we set up a rapid, sensitive, and reliable technique in routine practice to detect before CPT-11 treatment, the at-risk patients.

Experimental Design: Seventy-five patients with advanced colorectal cancer and treated with CPT-11 and 5-fluorouracil, entered the study. We used the Pyrosequencing technology a real-time sequencing method, to detect the UGT 1A1 TATA box polymorphisms and mutations in the coding regions. Patients were also assessed for both biochemical and clinical evaluation and tolerance to treatment.

Results: No G71R and Y486D mutations were found in our population. Frequencies for UGT 1A1 TATA box polymorphisms were 41, 47, and 9% for wild-type 6/6, heterozygous 6/7, and Gilbert’s syndrome 7/7, respectively. Tolerance to treatment decreased with increased number of TA repeat with 71% of the patients in 7/7 group who experienced grade 3/4 toxicity.

Conclusions: The method we set up is suitable for the detection of UGT 1A1 polymorphism in routine practice before irinotecan treatment. It could help to detect the patients homozygous or heterozygous for Gilbert’s syndrome, at-risk of CPT 11-induced toxicity, and thus could help to individualize the dose to optimize efficacy and limit toxicity.

INTRODUCTION

Irinotecan is used or under evaluation in a broad spectrum of solid tumors (1, 2). Its dose-limiting toxicities, grade 3/4 delayed diarrhea and neutropenia frequencies have been reported at 44.4 and 28.8%, respectively, in the so-called FOLFIRI regimen, compared with 25.6 and 2.4%, respectively, in LV5FU2 regimen (3). Besides, irinotecan pharmacokinetics parameters display a wide interpatient variability (4, 5), which is involved in those toxic side effects genesis (6).

Its major metabolite, SN-38, is both a potent topoisomerase I inhibitor, more active than the parent drug, and a toxic compound (7). SN-38 is additionally conjugated into the inactive SN-38 glucuronide (SN-38G) by uridine diphosphogluronosyl transferase 1A1 (UGT 1A1) and then eliminated via the bile (8, 9).

Bilirubin undergoes the same glucuronidation by UGT 1A1 and then is excreted into the bile (10). Different forms of unconjugated hyperbilirubinemia are a result of genetic disorders affecting this enzyme and are described as Crigler-Najjar syndrome types I and II and Gilbert’s syndrome, a mild unconjugated hyperbilirubinemia in the absence of structural liver disease and overt hemolysis (11, 12). The hepatic bilirubin UGT 1A1 activity of individuals with Gilbert’s syndrome is ∼30% of normal one (13, 14). In vitro analyses have revealed that the genetic UGT 1A1 isoforms result in variable activity of both SN-38 and bilirubin glucuronidation (8). Some authors observed irinotecan-induced severe toxicity in patients with Gilbert’s syndrome (10, 15–17) and suggested that baseline serum bilirubin values could be a valid predictor of severe irinotecan-induced toxic effects.

Genetically, in Caucasian populations, Gilbert’s syndrome from a polymorphism in the promoter region of the UGT 1A1. The polymorphism consists of a TA insertion in the TATAA element of the 5’-promoter region (11, 12, 18–20). The resulting mutant genotype is A(TA)nTAA as opposed to A(TA)nTAA for the wild-type. Genotypes are described as 6/6, 6/7, and 7/7 indicating the number of TA repeat in the two alleles for wild-type, heterozygous, and homozygous Gilbert’s individuals, respectively. Despite a high prevalence of Gilbert’s syndrome, reaching 5–10% in Caucasian populations, this syndrome is usually not detected because it does not affect normal life. Ethnic factors are strongly implicated in the type of mutations responsible of Gilbert’s syndrome (21). Whereas the TA insertion is most common in Caucasian and African population (11, 12, 22), mutations in the coding regions of exon 1 (G71R-G to A at nucleotide 211) and exon 5 (Y486D-T to G at nucleotide 1456) of UGT 1A1 have been more often reported in Asian populations (13).
Because of the large use of irinotecan in a wide panel of tumors and the high risk of severe irinotecan-induced toxicity in Gilbert’s syndrome patients, some relevant and worthwhile questions remain unanswered: (a) is there a sequencing method, currently available for rapid and accurate detection of the most common polymorphisms such as A(TA)$_7$TAA, A(TA)$_6$/7TAA, G71R, and Y486D in clinical practice, before irinotecan infusion? (b) in clinical practice, how is the accuracy of single nucleotide polymorphism (SNP) detection compared with that of free and total bilirubin in patients’ plasma in terms of sensitivity and specificity? (c) should we manage 6/6 and 7/7 UGT1A1 genotype patients differently? and (d) how do heterozygous 6/7, 5/6, and 5/7 individuals behave in terms of irinotecan-induced toxicity profile?

In the present study, we tested and evaluated the Pyrosequencing technique, a high-throughput sequencing method, for detecting the relevant polymorphisms in the UGT1A1 gene and its promoter. In a population of French-Caucasian patients treated with irinotecan, we looked for TA insertion and also for G71R and Y486D SNPs. Phenotypic assessment consisted of liver function evaluation and bilirubin plasma levels measurement before treatment and hematological and digestive-tract tolerance to treatment. Correlation between tolerance to irinotecan treatment and UGT 1A1 genotype was then evaluated.

PATIENTS AND METHODS

Patients
Clinical Characteristics. The retrospective study population consisted of 75 fully informed consent patients (33 women and 52 men; mean age, 62 years; range, 39–79 years), who underwent chemotherapy for metastatic colorectal cancer. They were treated in daily hospitalization with irinotecan-based chemotherapy according to two regimens: (a) IRIFUFO group: irinotecan (85 mg/m$^2$), 90-min weekly infusions plus 5-fluorouracil (5-FU) (1200 mg/m$^2$), 7-h weekly infusions, and 100 mg/m$^2$ bolus l-folinic acid (28 patients; Ref. 23). In this group, one patient received orally 300 mg/m$^2$ uracil-tegafur (in 500 µl of whole blood) using DNA Isolation kit for Blood/Bone Marrow/Tissue (Roche Molecular Diagnostics, Meylan, France). Each sample was controlled with respect to DNA isolation by UV transilluminat of ethidiun bromide-stained gels from subsequent electrophoresis separation in 1.2% agarose.

To assess whether or not statistical difference existed between two observations, i.e., between two percentages relative to toxic events occurrence, Khi 2 test was used. According to the number of patients in each group, Yates modification or non-parametric Kruskal-Wallis test could be applied.

Construction of Mutant Vectors for the Coding Regions of the UGT 1A1 Gene. Segments of the DNA of human UGT 1A1 containing the regions of potential mutations (G71R and Y486D) were amplified by PCR and inserted into pGEMt vector (pGEM-T vector System II; Promega, Madison, WI).

PCR conditions were as follows: an initial denaturation for 2 min at 94°C, followed by 35 cycles. A cycle profile consisted of denaturation for 30 s at 94°C, annealing for 30 s at 60°C, and extension for 1 min at 72°C. A final extension step at 72°C for 5 min completed the reaction. PCR products were then cloned into pGEMt and then competent bacteria were transformed. Mutations introduced were used to provide homozygous positive controls for additional pyrosequencing analysis.

Statistical Considerations

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Determination of UGT 1A1 Gene Polymorphism
Blood samples were obtained for DNA isolation and determination of genotypes. All procedures were reviewed and approved by accredited ethics review boards, and patients signed informed consent forms.

DNA was extracted from peripheral blood mononuclear cells (500 µl of whole blood) using DNA Isolation kit for Blood/Bone Marrow/Tissue (Roche Molecular Diagnostics, Meylan, France). Each sample was controlled with respect to DNA isolation by UV transilluminat of ethidiun bromide-stained gels from subsequent electrophoresis separation in 1.2% agarose.

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Pyrosequencing Analysis
PCR Conditions. PCRs were performed with an initial denaturation for 5 min at 95°C, followed by 50 cycles of denaturation for 30 s at 95°C, primer annealing for 30 s at 60°C, and extension for 1 min at 72°C, followed by a final extension for 5 min at 72°C. All amplification reactions were performed in a DNA Thermal Cycler 480 (Perkin-Elmer, Boston, MA) with 1 unit of Taq Polymerase (EuroBlue Taq; Eurobio, Les Ulis, France).

PCR conditions were the same for the three mutations
tested. Table 1 shows the different sets of primers used to
amplify the sequences of interest, including the polymorphisms
in the UGT 1A1 gene to be analyzed, TA insertion in the
TATAAA element of the 5′-promoter region of the UGT 1A1
gene, and SNPs in the coding regions of exon 1 (G71R-G to A
at nucleotide 211) and exon 5 (Y486D-T to G at nucleotide
1456). For the determination of the TATA box genotypes, two
PCR fragments were designed to make it possible to analyze the
sequence both in forward and reverse sense to increase the
reliability of the analysis.

Choice of the Sequencing Primers. Different sequenc-
ing primers were designed to carry out TATA box pyrosequenc-
ing analysis. Then a selection was made on the ability to provide
interpretable Pyrograms. DNA products consisted of amplified
genomic DNA from control subjects.

For the determination of the UGT 1A1 genotype in coding
region (G71R and Y486D), sequencing primers were also de-
designed. The DNA mutant constructs as previously described
provided control homozygous mutated sequences.

Sequencing primers and dispensation order of the nucleo-

tides are displayed in Table 1.

Conditions for the Pyrosequencing Analysis. Tem-
plates for the pyrosequencing analysis were prepared as recom-
mended by the manufacturer, and the reaction was performed at
28°C on an automated PSQ 96 instrument (Pyrosequencing AB,
Uppsala, Sweden). However, to apply the pyrosequencing tech-
nique to our work and to provide accurate results, different
parameters were modified and tested, especially the dispensa-
tion order of the nucleotides and the time interval between
addition of two nucleotides (e.g., 60 and 120 s).

Table 1  Sets of primers used for amplification prior to sequencing

<table>
<thead>
<tr>
<th>Site of mutation</th>
<th>Sequences</th>
</tr>
</thead>
<tbody>
<tr>
<td>TATA box (biotin reverse)</td>
<td>5'-CTGCTACCTTTGTGGACTGACA-3'</td>
</tr>
<tr>
<td>Set of primers</td>
<td>5'-biotin-CCAGGACAGTGGGGGC-3'</td>
</tr>
<tr>
<td>Sequencing primer</td>
<td>5'-CGATTTGCTTTGCCC-3'</td>
</tr>
<tr>
<td>Dispensation order of the nucleotides</td>
<td>CATATATATATCATCATATAG</td>
</tr>
<tr>
<td>TATA box (biotin forward)</td>
<td>5'-biotin-CTGCTACCTTTGTGGACTG</td>
</tr>
<tr>
<td>Set of primers</td>
<td>ACA-3'</td>
</tr>
<tr>
<td>5'-CCAGGACAGTGGGGGC-3'</td>
<td></td>
</tr>
<tr>
<td>Sequencing primer</td>
<td>5'-CCCTCTCCTACTATATGC-3'</td>
</tr>
<tr>
<td>Dispensation order of the nucleotides</td>
<td>CATATATATATCATG</td>
</tr>
<tr>
<td>G71R (biotin reverse)</td>
<td>5'-TGTTGATCCCAGTGGATGG634-3'</td>
</tr>
<tr>
<td>Set of primers</td>
<td>5'-biotin-CTGCTACCTTTGTGGACTG</td>
</tr>
<tr>
<td>5'-CCAGGACAGTGGGGGC-3'</td>
<td></td>
</tr>
<tr>
<td>Sequencing primer</td>
<td>5'-GCCTCCTCCTACTATATGC-3'</td>
</tr>
<tr>
<td>Dispensation order of the nucleotides</td>
<td>CATATATATATCATG</td>
</tr>
</tbody>
</table>

G71R and Y486D Mutations. To sequence the G71R and Y486D mutations, the primers designed were
suitable for our method, and no interfering peaks were generated as shown in Fig. 2.

Conditions for the Pyrosequencing Analysis

TATA Box. We did not use the classical analysis that
consists of detecting SNPs due to substitution of a nucleotide by
another one in a known sequence, our work consisted of detecting
the insertion or deletion of a couple of nucleotides (e.g., a TA
insertion) in the TATA box of UGT 1A1 gene and in detecting the
number of TA repeats in each allele for every patient. Conse-

Clinical and Biological Results

Patients’ Characteristics before Treatment and
Treatment Tolerance

A total of 75 patients receiving irinotecan-based treatment,
23 females and 52 males, entered the study and was assessed for
toxic event occurrence.

Table 2 displays patient’s characteristics before treatment.
Tolerance to treatment is displayed in Table 3.

Twenty-eight patients (37%) received IRIFUFOL and 47
patients (63%) received FOLFIRI. Total serum bilirubin level
was normal in all but two patients at 32 μM. Alkaline phos-
phatas were increased in approximately one-third of the pa-

Approximately 65% of the patients well tolerated the treat-
ments with only grade 0 or 1 hematological and gastrointestinal toxicity. Mucositis, a 5-FU-related toxicity, was very rare thanks to the 5-FU dose adjustment. We observed more severe diarrhea in the group of patients treated with weekly schedule, compared with biweekly regimen.

In case of serious toxic event, the mean delay for occurrence was three courses (range, 1–5 courses). We considered the worst grade of toxicity.

Mean treatment duration was closely similar whatever the regimen (4.6 ± 2.4 months) but with a relatively large range (1–10).

UGT1A1 Genotype Results and Clinical Correlation

The TATA box polymorphism and the G71R and Y486D mutations were investigated for every patient. Results are displayed in Table 4. Percentages of homozygous 6/6 and of heterozygous 6/7 patients were closely similar (41 and 47%, respectively). Seven patients were homozygous 7/7. It is noteworthy that we found no hyperbilirubinemia in those 7/7 homozygous patients. There was no significant gender difference in the frequency of the 7/7 genotype nor in the total bilirubin concentrations of 7/7 males and females.

In our population, two patients presented rare genotypes, 5/6 and 5/7 (Fig. 1), and none were G71R or Y486D. However, our mutant constructs for those two mutations provide mutant samples validating our method of G71R and Y486D mutation polymorphism detection.

We tempted to correlate genotype and tolerance to treatment. First, of the seven homozygous 7/7 patients, one patient died after 5 weeks of IRIFUFOL because of grade 4 diarrhea with dehydration, fever, and then collapsus induced by irinotecan treatment. Four patients presented grade 3/4 early neutro-

![Figure 1](typical_pyrograms_ugt1a1_tata_box.png)

**Fig. 1** Typical pyrograms obtained for UGT 1A1-TATA box sequencing. Homozygous wild-type 6/6, heterozygous 6/7, homozygous Gilbert’s syndrome 7/7, and heterozygous 5/6 and 5/7 pyrograms are displayed. Control pyrogram consisted of sequencing primer alone.
penia and/or diarrhea. Only two patients well tolerated the treatment, and of them, a woman received a very low total dose of irinotecan in comparison to the other patients because of a small body surface area (1.36 m²).

Two of 7 patients in 7/7 group experienced grade 4 diarrhea compared with 2 of 35 patients in 6/7 group and 1 of 31 patients in 6/6 group. Using Kruskal-Wallis test, we found no significant statistical difference between the three populations of patients (P = 0.559). Concerning hematological toxicity, 5 of 7 patients in the 7/7 group and 14 of 35 in the 6/7 group had severe neutropenia (grade 3 or 4) compared with 3 of 31 in the 6/6 group. With Kruskal-Wallis test, we found a significant statistical difference between the three populations of patients (P = 0.001). Then we compared 7/7 group to 6/7 and to 6/6 groups (P = 0.02 and P = 0.003, respectively). On the other hand, in the 31 patients of the 6/6 group, 25 presented grade 0 or 1 neutropenia compared with 18 of 35 in the 6/7 group (P = 0.01) and only 2 of 7 in the 7/7 group (P = 0.02).

Irinotecan courses had to be postponed in 5 of 7 patients in the 7/7 group versus 21 of 35 in the 6/7 group and 10 of 31 patients in the 6/6 group. Hospitalization because of toxicity occurred for 5 in the 7/7 group versus 3 of 35 in the 6/7 group and 0 of 31 in the 6/6 group.

We can assume that hematological and digestive toxic events were not due to 5-FU because only one patient had intron 14G1A dihydropyrimidine dehydrogenase heterozygosity, and all of them had their 5-FU dose individually adjusted to avoid severe 5-FU. One patient had toxicity; this patient, with intron 14G1A dihydropyrimidine dehydrogenase heterozygosity, was also 7/7 UGT 1A1. Despite a 25% initial 5-FU dose reduction, he presented a grade 4 febrile neutropenia and a grade 1 diarrhea.

**DISCUSSION**

Patients with Gilbert’s syndrome are at high risk of irinotecan—induced myelosuppression and delayed diarrhea (6)—but until recently, no reliable and quick method was available for its detection. Our purpose was to set up an accurate technology to easily detect genetic variants of UGT 1A1 and to correlate different genotypes in Caucasian population with clinical tolerance. Actually, total and unbound serum bilirubin lev-

![G71R control pyrogram](image1)

![G71R wild-type](image2)

![G71R mutant](image3)

![Y486D control pyrogram](image4)

![Y486D wild-type](image5)

![Y486D mutant](image6)

*Fig. 2* Typical pyrogram obtained for G71R and Y486D mutations. Wild-type and mutant are displayed. Control pyrograms consisted of control primer alone.
els have been previously proposed for detection of Gilbert syndrome, eventually after a 24-h restricted diet (12), but this approach is not comfortable for the patients and not reliable.

Some methods of UGT1A1 gene TATA box genotyping have been developed but were tedious and time-consuming (21, 23, 26) and therefore not suitable for rapid Gilbert’s syndrome detection in routine practice. A pyrosequencing method, close to the TATA box polymorphisms is quite similar to that reported in the literature: 41% for 6/6 genotype, 47% for 6/7, and 9% for 7/7 genotype, i.e., homozygous Gilbert’s syndrome (12, 18, 26, 28). We detected also two patients with rare 5/6 and 5/7 heterozygous genotypes.

Concerning diarrheas, we observed more severe diarrheas in the group of patients treated with weekly schedule, compared with biweekly regimen. Maybe, weekly 5-FU was less well tolerated by gastrointestinal tract than biweekly schedule. This would be in accordance with tolerance results reported by Saltz with weekly 5-FU combined with irinotecan, compared with FOLFIRI regimen.

Clearly, grade 3/4 neutropenias were significantly more frequent in 7/7 and 6/7 patients than in 6/6 patients. We did not include the two patients with rare genotype, 5/6 and 5/7, in the statistical analysis. Linked to toxic side effects, more patients in the 7/7 group had postponed cycles compared with 6/7 group and 6/6 group. However, the influence of the genotype was less clear on the grade of diarrhea. Sixty percent of the patients had no diarrhea whatever the group of patients. It seems that the

### Table 2 Patients’ characteristics before irinotecan treatment

<table>
<thead>
<tr>
<th></th>
<th>IRIFUFOL</th>
<th>FOLFIRI</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>No. of patients</td>
<td>28</td>
<td>47</td>
<td>75</td>
</tr>
<tr>
<td>Dose (mg) (mean ± SD)</td>
<td>166.1 ± 60.8</td>
<td>319.8 ± 50.5</td>
<td>61 ± 11</td>
</tr>
<tr>
<td>Range in yrs (mean ± SD)</td>
<td>63.5 ± 9.7</td>
<td>61 ± 11</td>
<td>62 ± 10</td>
</tr>
<tr>
<td>Male/Female (no. of patients)</td>
<td>39–79</td>
<td>38–78</td>
<td>38–79</td>
</tr>
<tr>
<td>Performance status 0–1/2–3 (no. of patients)</td>
<td>22/6</td>
<td>30/17</td>
<td>52/23</td>
</tr>
<tr>
<td>Primary tumor site (%)</td>
<td>50/43/3.5 +</td>
<td>28/57/4 +</td>
<td>36/52/4</td>
</tr>
<tr>
<td>Hepatocarcinoma: 1 patient</td>
<td>Unknown: 3 patients</td>
<td>Stomach: 1 patient</td>
<td>Anus: 1 patient</td>
</tr>
<tr>
<td>Liver</td>
<td>18</td>
<td>29</td>
<td>47</td>
</tr>
<tr>
<td>Lung</td>
<td>10</td>
<td>13</td>
<td>23</td>
</tr>
<tr>
<td>Other</td>
<td>5</td>
<td>17</td>
<td>22</td>
</tr>
<tr>
<td>Previous chemotherapy [no. of patients (%)]</td>
<td>11/17</td>
<td>18/29</td>
<td>29/46</td>
</tr>
<tr>
<td>Yes/No</td>
<td>1/10</td>
<td>5/13</td>
<td>6/23</td>
</tr>
<tr>
<td>Liver tests (mean ± SE) [&lt;&gt;N, &lt;Nx1.5, &gt;Nx1.5 (%)]</td>
<td>11.3 ± 4.1 (0, 100, 0, 0)%</td>
<td>9.9 ± 5.9 (0, 95, 5, 0)%</td>
<td>10.6 ± 5.3 (0, 97, 3, 0)%</td>
</tr>
<tr>
<td>Bilirubin total</td>
<td>136.3 ± 101.6 (0, 70, 11, 19)%</td>
<td>137.4 ± 133.7 (0, 75, 9, 16)%</td>
<td>138.3 ± 121.5 (0, 67, 14, 18)%</td>
</tr>
<tr>
<td>Alkaline phosphatase (N = 38–126 units/liter, 37°C)</td>
<td>24.2 ± 23.6 (8, 89, 0, 3)%</td>
<td>24.1 ± 19.7 (16, 80, 2, 2)%</td>
<td>25.7 ± 21.0 (11, 84, 1, 4)%</td>
</tr>
<tr>
<td>ALT (N = 9–65 units/liter, 37°C)</td>
<td>33.3 ± 18.5 (0, 89, 3, 8)%</td>
<td>28.1 ± 20.2 (0, 93, 0, 7)%</td>
<td>32.5 ± 19.6 (0, 88, 4, 8)%</td>
</tr>
<tr>
<td>AST (N = 8–50 units/liter, 37°C)</td>
<td>161.6 ± 283.5 (0, 55, 19, 25)%</td>
<td>141.2 ± 210.0 (0, 65, 9, 26)%</td>
<td>153.1 ± 239.2 (0, 57, 16, 27)%</td>
</tr>
<tr>
<td>γ-Glutamyl transpeptidase (N = 8–78 units/liter, 37°C)</td>
<td>141.2 ± 210.0 (0, 65, 9, 26)%</td>
<td>153.1 ± 239.2 (0, 57, 16, 27)%</td>
<td></td>
</tr>
<tr>
<td>Renal function (mean ± SE) [&lt;&gt;N, &lt;Nx1.5, &gt;Nx1.5 (%)]</td>
<td>4.2 ± 1.3 (8, 89, 3, 0)%</td>
<td>5.0 ± 1.8 (5, 85, 10, 0)%</td>
<td>4.5 ± 1.6 (7, 86, 7, 0)%</td>
</tr>
<tr>
<td>Urea (N = 2.50–7.00 mm)</td>
<td>75.6 ± 15.4 (0, 96, 4, 0)%</td>
<td>77.9 ± 20.9 (0, 90, 10, 0)%</td>
<td>76.6 ± 18.9 (0, 93, 7, 0)%</td>
</tr>
<tr>
<td>Creatinine clearance (N = 80–120 ml/min)</td>
<td>96.7 ± 38.1 (33, 45, 15, 7)%</td>
<td>85.2 ± 25.0 (40, 53, 7, 0)%</td>
<td>91.2 ± 31.1 (39, 45, 10, 6)%</td>
</tr>
<tr>
<td>DPD mutation (no. of patients)</td>
<td>1 heterozygous</td>
<td>1 heterozygous</td>
<td></td>
</tr>
</tbody>
</table>

Abbreviations: N, normal range; ALT, alanine aminotransferase; AST, aspartate aminotransferase; DPD, dihydropyrimidine dehydrogenase.
genotype would not impact the gastrointestinal toxicity. This finding had not been reported thus far to our knowledge. We hypothesize that severe diarrhea occurrence could be less SN38 concentration-dependent than neutropenia.

Despite the limited size of our population of patients, these results are in favor of differently management of 6/6 and 7/7 patients and also 6/7 patients. Little is known about the sensitivity of heterozygous 6/7 to irinotecan in the literature. Our patients and also 6/7 patients. Little is known about the sensitivity of heterozygous 6/7 to irinotecan in the literature. Our patients and also 6/7 patients. 

On the basis of our small population of patients, biochemical markers such as bilirubin and alkaline phosphatase, look less powerful than UGT1A1 genotype for detecting Gilbert’s syndrome before irinotecan treatment. Mean bilirubin serum levels did not significantly differ between groups (9.5 ± 6.0, 11.3 ± 5.1, and 10.4 ± 2.3 μM for 6/6, 6/7, and 7/7 group, respectively) and particularly was not higher in 7/7 group. Our results are in accordance with those of some authors who reported that genotypes found in Gilbert’s syndrome do not always cause hyperbilirubinemia, probably because of non-genetic factors, including diet and therapeutic drug use (12, 30).

Notice that we measured preferably serum total bilirubin level and not unconjugated bilirubin, but, except in three patients, total bilirubin never exceeded unconjugated bilirubin normal range.

Likewise, alkaline phosphatase, also considered as a risk factor of irinotecan-induced toxicity, did not appear convincing to predict the at-risk patients because none of the patients who developed a grade 4 toxicity in 6/6 and 6/7 groups, had their alkaline phosphatase value upper 1.5 times the normal range, and only two of the five patients in the 7/7 group who presented severe toxic event and had their alkaline phosphatase value upper 1.5× the normal range.

There are ethnic differences in the frequency of the different UGT 1A1 polymorphisms responsible for Gilbert’s syndrome. Prevalence of the TATA box polymorphism is very high.
in Caucasian (10% of the population; Ref. 12) and in African people (20–25%; Refs. 18, 21). In Asian population, TATA box polymorphism is quite rare compared with SNPs in the coding regions of the UGT 1A1 gene: G71R and P229Q on exon 1 and Y486D on exon 5 (13, 23, 30). Some Japanese studies have shown the correlation between this polymorphism and irinotecan toxicity and disposition (28, 30) and concluded that determination of UGT 1A1 gene polymorphism could be clinically useful. In our experience, all of the French patients eligible for our study were of Caucasian type, and none of them had the G71R or Y486D mutation. We did not select the P229Q SNP because it has always been related to the A(TA);TAA polymorphism (30). We did not study UGT1A7 nor UGT1A9 polymorphisms, which, besides UGT1A1, have been recently reported and mainly characterized in vitro. They could influence SN38 glucuronidation in vivo. Some authors identified several SNPs such as 766G > A, located in UGT1A9 exon 1, resulting in the amino acid substitution of D256N and in vitro in a reduced glucuronidation activity (31). On the same way, F83L has been reported as very rare in a Japanese population. Little is known about their importance in vivo (32).

Irinotecan dosage is still usually calculated according to body surface area, not taking into account UGT 1A1 polymorphisms. Gilbert’s syndrome is not accurately detected by simple unconjugated bilirubin, and yet, patients with this syndrome are at high risk of severe toxicity. The method we set up already, to detect UGT1A1 polymorphisms, is simple, rapid, reliable, and sensitive and can be widely used in populations with different ethnic origins. According to our results on a limited number of patients, it could become a useful tool to detect UGT1A1 polymorphisms and to further study and detect UGT1A7 and UGT1A9 SNPs of interest. Therefore, stratification of the patients before irinotecan administration could permit to avoid toxic side effects. In practice, for patients homozygous 7/7, we want to warn physicians against the risk of toxicity. Irinotecan dosage might be initially reduced and treatment performed under reinforced follow-up. For patients heterozygous 6/7, the risk of toxicity remains significant. The question of the dosage for patients 6/6 or 5/5, 5/6, and 5/7 is relevant, too. The maximum recommended dosage is probably too low for these patients when all genotypes are pooled together. Patients 6/6 or 5/5, 5/6, and 5/7 have probably a higher UGT 1A1 activity and, consequently, an accelerated rate of glucuronidation of SN-38 (11). Moreover, several authors reported Phase I and II trials with 40% of the patients well tolerating much higher irinotecan dosage up to 500–700 mg/m² (33, 34). Determination of UGT1A1 polymorphism might permit to increase irinotecan dosage in 6/6 and a fortiori in 5/5, 5/6, and 5/7 patients. However, we must keep in mind that clinical tolerance and metabolism of irinotecan depend on many factors such as the schedule, the several metabolic pathways, implying UGT1A1, and probably to a lesser extent UGT1A7 and UGT1A9, also submitted to a genetic polymorphism and, until now, characterized essentially in vitro. Other metabolic pathways such as cytochrome P450 3A4 and 3A5 and ABC membrane proteins, especially ABC-B1 (35), are under investigation. Thus, we are carrying on a clinical trial combining irinotecan population pharmacokinetics, determination of these metabolic pathways polymorphisms, and clinical outcome, with the purpose of setting up an individual adjustment of irinotecan dosage based on the most relevant metabolic parameters.

### Table 4 Distribution of the different UGT 1A1 genotypes and correlation to tolerance of treatment

<table>
<thead>
<tr>
<th>UGT 1A1 genotypes</th>
<th>5/6 or 5/7</th>
<th>6/6</th>
<th>6/7</th>
<th>7/7</th>
<th>G71R and Y486D</th>
</tr>
</thead>
<tbody>
<tr>
<td>No. of patients (%)</td>
<td>2 (3%)</td>
<td>31 (41%)</td>
<td>35 (47%)</td>
<td>7 (9%)</td>
<td>0</td>
</tr>
<tr>
<td>Serum bilirubin (N = 3–22 μM)</td>
<td>12.0 ± 2.8 (10–14)</td>
<td>9.5 ± 6.0 (3–32)</td>
<td>11.3 ± 5.1 (4–32)</td>
<td>10.4 ± 2.3 (7–13)</td>
<td></td>
</tr>
<tr>
<td>Neutropenia (no. of patients)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>NCI-CTC Grade 0–1</td>
<td>2</td>
<td>25</td>
<td>18</td>
<td>2</td>
<td></td>
</tr>
<tr>
<td>Grade 2</td>
<td>3</td>
<td>3</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Grade 3</td>
<td>7</td>
<td>1</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Grade 4</td>
<td>2</td>
<td>1</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Grade 4 + fever</td>
<td>5</td>
<td>3</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Diarrhea (no. of patients)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>NCI-CTC Grade 0–1</td>
<td>1</td>
<td>20</td>
<td>21</td>
<td>4</td>
<td></td>
</tr>
<tr>
<td>Grade 2</td>
<td>1</td>
<td>7</td>
<td>7</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td>Grade 3</td>
<td>3</td>
<td>5</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Grade 4</td>
<td>1</td>
<td>2</td>
<td>2</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mucositis (no. of patients)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>NCI-CTC Grade 0–1</td>
<td>2</td>
<td>29</td>
<td>32</td>
<td>7</td>
<td></td>
</tr>
<tr>
<td>Grade 2</td>
<td>1</td>
<td>3</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Grade 3</td>
<td>1</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Grade 4</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Hospitalization due to toxicity (no. of patients)</td>
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<td>5</td>
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<tr>
<td>Delayed treatment (no. of patients)</td>
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<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1 wk</td>
<td>4</td>
<td>12</td>
<td>3</td>
<td></td>
<td></td>
</tr>
<tr>
<td>1–2 wks</td>
<td>2</td>
<td>4</td>
<td>1</td>
<td></td>
<td></td>
</tr>
<tr>
<td>2–3 wks</td>
<td>5</td>
<td>1</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Irinotecan treatment stop (no. of patients)</td>
<td>3</td>
<td>2</td>
<td></td>
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</tbody>
</table>

Abbreviations: NCI-CTC, National Cancer Institute-Common Toxicity Criteria.
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Relevance of Different UGT1A1 Polymorphisms in Irinotecan-Induced Toxicity: A Molecular and Clinical Study of 75 Patients

Elisabeth Rouits, Michèle Boisdron-Celle, Agnès Dumont, et al.


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