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 diphosphoglucuronosyl transferase 1A1 (UGT1A1) gene: the TATA box polymorphism and UGT1A1 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 UGT1A1 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 UGT1A1 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 the 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 diphosphoglucuronosyl transferase 1A1 (UGT1A1) and then eliminated via the bile (8, 9).

Bilirubin undergoes the same glucuronidation by UGT1A1 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 results 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 repeats 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 AT7TA, AT9TA, ATG7, and ATG8 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 UGT1A1 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) IFUROL regimen: irinotecan (85 mg/m²), 90-min weekly infusions plus 5-fluorouracil (5-FU) (1200 mg/m²), 7-h weekly infusions, and 100 mg/m² bolus l-folinic acid (28 patients; Ref. 23). In this group, one patient received orally 300 mg/m² uracil-tegafur (in 100 mg-tablet form) instead of 5-FU; (b) FOLFIRI regimen: 180 mg/m² irinotecan biweekly infusion over 90 min plus 2500 mg/m² 5-FU, 46-h continuous infusion, and 400 mg/m² bolus l-folinic acid (47 patients; Ref. 3). As done in routine practice at our institution to limit severe toxic side effects due to 5-FU, we looked for eight major SNPs of dihydropyrimidine dehydrogenase involved in reduced activity of this enzyme: intron G14A1, A2846T, T85C, del TCAT295-298, G1156T, G2657A, G2983, and T 1590C (24). Moreover, 5-FU dose was individually adjusted based on a dose adjustment chart previously published (25).

The liver function of the patients included in the study was also characterized: transaminases, total bilirubin, alkaline phosphates, and γ-glutamyl transpeptidase were measured.

**Efficacy and Toxicity Assessment.** According to the standard practice at our institution, tumor response was evaluated after 3 months of treatment and was classified according to WHO criteria. Confirmation of response at least 1 month after first evaluation was not systematically done.

In attempt to correlate genotype and tolerance to treatment, myelotoxicity and gastrointestinal tract toxicity were precisely evaluated through a per patient analysis, either weekly in IRIFUROL group or every 2 weeks in FOLFIRI regimen and graded according to the National Cancer Institute-Common Toxicity Criteria scale. We considered the worst grade of toxicity for each patient. Furthermore, irinotecan dose reductions or postponed treatments were listed.

**Statistical Considerations**

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.

**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 (50 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 transillumination of ethidium bromide-stained gels from subsequent electrophoretic separation in 1.2% agarose.

**Construction of Mutant Vectors for the Coding Regions of the UGT 1A1 Gene.** Segments of the DNA of human UGT1A1 containing the regions of potential mutations (G71R and Y486D) were amplified by PCR and inserted into pGEM 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 vector (pGEM-T vector System II; Promega, Madison, WI).

**Determination of UGT1A1 Polymorphisms in Irinotecan-Induced Toxicity**

**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 sequencing primers were designed to carry out TATA box pyrosequencing 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 designed. The DNA mutant constructs as previously described provided control homozygous mutated sequences.

Sequencing primers and dispensation order of the nucleotides are displayed in Table 1.

Conditions for the Pyrosequencing Analysis. Templates for the pyrosequencing analysis were prepared as recommended 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 technique to our work and to provide accurate results, different parameters were modified and tested, especially the dispensation order of the nucleotides and the time interval between addition of two nucleotides (e.g., 60 and 120 s).

RESULTS
Technical Results
Pyrosequencing

Choice of the Sequencing Primer
Primers for the TATA Box. The selected sequencing primer matched just before the TATA box and provided interpretable and reproducible sequence, easy to analyze.

Matching just before the beginning of the TATA box, this primer provided the theoretical sequence ATATATATATATATAAGTAGG. Then we designed a primer for the reverse sequencing, which matched at the beginning of the TATA box and on the first couple of TA sequence, providing the theoretical sequence ATATATATATAGG. Fig. 1 shows that, with those sequencing primers, no interfering peaks were generated as obtained on control pyrograms.

Primers for G71R an 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. Consequently, our type of analysis needed to be optimized for the parameters pointed with PSQ SNP Software. In that aim, conditions of cycle time were explored, and 120 s provided more accurate pyrograms than 60 s (standard parameter).

Fig. 1 shows typical pyrograms obtained for 5/6, 5/7, 6/6, 6/7, and 7/7 genotypes with 120 s as cycle time. Moreover, those results were obtained with appropriate dispensation order of nucleotides providing the best pyrograms.

G71R and Y486D Mutations. The detection of G71R and Y486D mutations used standards parameters and provided satisfactory results (Fig. 2) both for the wild-type detected in patients as for the mutated sequence from the mutant construct.

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 phosphatases were increased in approximately one-third of the patients and 40% had γ-glutamyl transpeptidases upper the normal range due to liver metastasis. Eighty-five percent of the patients presented transaminases in the normal range.

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-
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).

None of the patients in the 6/6 group, with high (one patient) or upper bilirubin level (one patient) and/or high alkaline phosphatase level presented severe toxic event, whereas one patient in the 6/7 group, with total bilirubin level upper the normal range (32 µm) but without cholestasis, experienced grade 4 neutropenia.

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 technique 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-
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 UGT 1A1 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 ours, has been reported in last few months on UGT 1A1 genotyping on a small population of patients, but no correlation to clinical outcome was sought (27).

In the present study, we were able to determine easily and rapidly the genotype of the TATA box of the UGT 1A1 gene and some of the SNPs in the coding regions of the gene itself, and we validated that approach in a population of 75 patients. This technique is completely suitable to a clinical practice. Sample preparation time to extract genomic DNA and PCR lasted 4 h, as with other methods previously reported. However, pyrosequencing and thus sample analysis time last 50 min for 96 samples. Then results can be given within the day.

In our population, the distribution of the UGT1A1 gene 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>
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<tr>
<th>Table 2</th>
<th>Patients’ characteristics before irinotecan treatment</th>
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<tbody>
<tr>
<td></td>
<td>IRIFUFOL</td>
</tr>
<tr>
<td>No. of patients</td>
<td>28</td>
</tr>
<tr>
<td>Dose (mg) (mean ± SD)</td>
<td>166.1 ± 60.8</td>
</tr>
<tr>
<td>Age in yrs (mean ± SD)</td>
<td>63.5 ± 9.7</td>
</tr>
<tr>
<td>Range in yrs</td>
<td>39–79</td>
</tr>
<tr>
<td>Male/Female (no. of patients)</td>
<td>22/6</td>
</tr>
<tr>
<td>Performance status 0–1/2–3 (no. of patients)</td>
<td>26/2</td>
</tr>
<tr>
<td>Primary tumor site (%) colon/rectum/both</td>
<td>Hepatocarcinoma: 1 patient</td>
</tr>
<tr>
<td></td>
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<tr>
<td></td>
<td></td>
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<td></td>
<td></td>
</tr>
<tr>
<td>Metastatic locations [no. of patients (%)]</td>
<td></td>
</tr>
<tr>
<td>Liver</td>
<td>18</td>
</tr>
<tr>
<td>Lung</td>
<td>10</td>
</tr>
<tr>
<td>Other</td>
<td>5</td>
</tr>
<tr>
<td>Previous chemotherapy [no. of patients (%)]</td>
<td>Yes/No</td>
</tr>
<tr>
<td>FOLFOX regimen/Other</td>
<td>1/10</td>
</tr>
<tr>
<td>Liver tests (mean ± SE) [N = 3–22 μM, 37°C] (%)</td>
<td>Bilirubin total</td>
</tr>
<tr>
<td></td>
<td>Alkaline phosphatases</td>
</tr>
<tr>
<td></td>
<td>ALT (N = 38–126 units/liter, 37°C)</td>
</tr>
<tr>
<td></td>
<td>AST (N = 8–50 units/liter, 37°C)</td>
</tr>
<tr>
<td></td>
<td>γ-Glutamyl transpeptidase (N = 8–78 units/liter, 37°C)</td>
</tr>
<tr>
<td>Renal function (mean ± SE) [N = 3–22 μM, 37°C] (%)</td>
<td>Urea (N = 2.50–7.00 mm)</td>
</tr>
<tr>
<td></td>
<td>Serum creatinine (N = 40–110 μM)</td>
</tr>
<tr>
<td></td>
<td>Creatinine clearance (N = 80–120 ml/min)</td>
</tr>
<tr>
<td>DPD mutation (no. of patients)</td>
<td>1 heterozygous</td>
</tr>
</tbody>
</table>

Abbreviations: N, normal range; ALT, alanine aminotransferase; AST, aspartate aminotransferase; DPD, dihydropyrimidinidem 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-dependant 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 observations are consistent with Raijmakers et al. and Iyer et al. in vitro and in vivo results (11, 28, 29). Ando et al. (30) stressed that the 7/7 and 6/7 genotypes would be a significant risk factor for severe irinotecan toxicity.

In our population of patients, 5-FU was certainly not involved in hematological and digestive tract toxicity because all of them had their 5-FU dose individually adjusted to avoid severe 5-FU toxicity, as commonly carried out in current clinical practice at our institution (25, 31). Furthermore, we systematically searched for the eight most important dihydropyrimidine dehydrogenase gene SNPs. We found in only one patient, the intron 14G1A dihydropyrimidine dehydrogenase heterozygosity, known to be responsible for partial dihydropyrimidine dehydrogenase deficiency, and moreover, it was combined to 7/7 UGT 1A1 genotype. Despite an initial 5-FU dose reduction, the patient died of febrile grade 4 neutropenia. We must emphasize that UGT1A1 and dihydropyrimidine dehydrogenase deficiencies can be combined and could potentiate each other in terms of toxic side effect.

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 μmol/L 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 nongenetic factors, including diet and therapeutic drug use (12, 30).

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 1.5× upper 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
UGT1A1 Polymorphisms in Irinotecan-Induced Toxicity

such as 766G/H11022 glucuronidation in vivo and mainly characterized polymorphisms, which, besides UGT1A1 in vitro amino acid substitution of D256N and about their importance reported as very rare in a Japanese population. Little is known glucuronidation activity (31). On the same way, F83L has been body surface area, not taking into account UGT 1A1 detect at high risk of severe toxicity. The method we set up already, to unconjugated bilirubin, and yet, patients with this syndrome are Gilbert’s syndrome is not accurately detected by simple ethnic origins. According to our results on a limited number of UGT1A7 morphisms and to further study and detect 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>
<thead>
<tr>
<th>Table 4</th>
<th>Distribution of the different UGT 1A1 genotypes and correlation to tolerance of treatment</th>
</tr>
</thead>
<tbody>
<tr>
<td>UGT 1A1 genotypes</td>
<td>5/6 or 5/7</td>
</tr>
<tr>
<td>No. of patients (%)</td>
<td>2 (3%)</td>
</tr>
<tr>
<td>Serum bilirubin level (N = 3–22 μM)</td>
<td>12.0 ± 2.8 (10–14)</td>
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<tr>
<td>Neutropenia (no. of patients)</td>
<td></td>
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<tr>
<td>NCI-CTC Grade 0–1</td>
<td>2</td>
</tr>
<tr>
<td>Grade 2</td>
<td>3</td>
</tr>
<tr>
<td>Grade 3</td>
<td>7</td>
</tr>
<tr>
<td>Grade 4</td>
<td>2</td>
</tr>
<tr>
<td>Grade 4 + fever</td>
<td></td>
</tr>
<tr>
<td>Diarrhea (no. of patients)</td>
<td></td>
</tr>
<tr>
<td>NCI-CTC Grade 0–1</td>
<td>1</td>
</tr>
<tr>
<td>Grade 2</td>
<td>1</td>
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<tr>
<td>Grade 3</td>
<td>3</td>
</tr>
<tr>
<td>Grade 4</td>
<td>1</td>
</tr>
<tr>
<td>Mucositis (no. of patients)</td>
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<tr>
<td>NCI-CTC Grade 0–1</td>
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<td>Grade 2</td>
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<td>Grade 3</td>
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<tr>
<td>Grade 4</td>
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<tr>
<td>Hospitalization due to toxicity (no. of patients)</td>
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<tr>
<td>Delayed treatment (no. of patients)</td>
<td></td>
</tr>
<tr>
<td>1 wk</td>
<td>4</td>
</tr>
<tr>
<td>1–2 wks</td>
<td>2</td>
</tr>
<tr>
<td>2–3 wks</td>
<td>5</td>
</tr>
<tr>
<td>Irinotecan treatment stop (no. of patients)</td>
<td>3</td>
</tr>
</tbody>
</table>

Abbreviations: NCI-CTC, National Cancer Institute-Common Toxicity Criteria.
ACKNOWLEDGMENTS

We thank the Comité Départemental du Maine-et-Loire de La Ligue Contre le Cancer for his financial support and Jenny Dunker for reading and commenting on the manuscript. We also thank Charline Jamet for her precious technical assistance and her availability.

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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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