

UGT1A and Irinotecan Toxicity: Keeping It in the Family

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Irinotecan is widely used for the treatment of metastatic colorectal cancer. It can be administered as first-line therapy in combination with 5-fluorouracil/leucovorin, with or without bevacuzimab, and as second-line therapy of disease that is refractory to oxaliplatin and/or 5-fluorouracil-based therapies. In this setting, it can be administered as a single agent or as part of the fluorouracil, leucovorin, and irinotecan regimen, in both instances with or without cetuximab. In addition, it can be administered for disease that is refractory to irinotecan-based therapies when combined with cetuximab. However, the toxicity and activity of irinotecan remains unpredictable.¹

Irinotecan efficacy is dependent on activation by carboxyesterases to form the active metabolite SN-38, which is a potent poison of topoisomerase I. The major route of SN-38 elimination is via glucuronidation by the UGT1A enzymes, predominantly by hepatic UGT1A1. *UGT1A1*28* is a common allele with seven TA repeats in a TATA box of the promoter of *UGT1A1* compared with the wild-type allele (*UGT1A1*1*), which has six TA repeats. The allelic frequency of **28* in whites is approximately 39% and the frequency of the homozygous variant genotype (**28/*28*) is approximately 10%. Seven TA repeats is associated with decreased gene transcription and expression of UGT1A1 and reduced enzyme activity compared with six TA repeats.² Patients homozygous for *UGT1A1*28* have reduced glucuronidation of SN-38 and an elevated risk of neutropenia compared with patients with one or two wild-type alleles.³

In 2005, the US Food and Drug Administration recommended the manufacturers of irinotecan amend the package insert of the drug to warn of the elevated risk of neutropenia for patients homozygous for *UGT1A1*28*. The warning recommended that these patients should receive a reduced starting dose of irinotecan by at least one level. Adoption of *UGT1A1* testing for irinotecan dosing has been hampered for various reasons. The clinical action for the *UGT1A1* genotype test results is not as simple as treat or do not treat, as is the case for the *KRAS* mutation and cetuximab or panitumumab.^{4,5} The genotype-dependent actions for prescribing irinotecan are "standard dose" or "reduce dose" and it is not clear by how much the dose should be reduced. In addition, the association between genotype and hematologic toxicity seems to be influenced by the dose of irinotecan.⁶ Also, there is a lack of empirical evidence that dosing irinotecan on the basis

of genotype improves the safety of irinotecan without compromising the efficacy of the therapy.

The *UGT1A1*28/*28* genotype confers a high probability of hematologic toxicity at high irinotecan doses ($> 250 \text{ mg/m}^2$); however, it does not explain many cases of severe neutropenia.⁷ It is likely that other factors, genetic and nongenetic, contribute to a patient's risk of irinotecan-induced toxicity. Many studies have investigated the involvement of other polymorphisms in genes that regulate the pharmacokinetics and pharmacodynamics of irinotecan in the efficacy and safety of the drug.^{3,7-9}

In this edition of *Journal of Clinical Oncology*, Cecchin et al¹⁰ report results from a follow-up *UGT1A* genotyping study of 250 metastatic colorectal cancer patients treated with fluorouracil, leucovorin, and irinotecan as first-line therapy. The relationship between *UGT1A1*28* genotype and pharmacokinetics of irinotecan (subset of 71 patients), and irinotecan-related toxicity and efficacy were previously evaluated and reported.⁸ In the earlier study, the authors demonstrated that *UGT1A1*28/*28* genotype predicted a higher risk of hematologic toxicity in the first cycle but not during the entire course of therapy. Clinical response rate was higher for these patients and they tended to have a longer time to progression than other patients. In addition, *UGT1A1*28/*28* genotype and clinical response were associated with higher biliary indices and lower glucuronidation ratios, but not SN-38 area under the concentration time curve, suggesting genotype influences pharmacokinetics and in turn the efficacy of the anticancer agent. However, a lingering question for the field is the contribution of other members of the UGT1A family.

UGT1A enzymes are a family of glucuronosyltransferase enzymes responsible for the glucuronidation of endogenous substrates and xenobiotics. The enzymes catalyze the transfer of glycosyl (sugar) residues to lipophilic substrates, rendering them more water soluble, thereby facilitating their biliary or renal elimination. The *UGT1A* locus has been mapped to chromosome 2q37 and consists of 13 genes. There are nine unique first exons (and four pseudoexons) each preceded by a promoter, and each functional exon is spliced to four consecutive exons (exons 2 to 5) that are common to all UGT1A mRNAs.^{11,12} The enzymes have identical carboxy-terminal domains (because of shared exons 2 to 5), but variable amino-terminal regions (because of unique exon 1). Expression of the genes is tissue-specific

with transcription of UGT1A1, 1A3, 1A4, 1A6, and 1A9 being exclusive to the liver, UGT1A7 reported in the esophagus and stomach, UGT1A8 in the esophagus and colon, and UGT1A10 in the esophagus, stomach, colon, and bile ducts.¹¹ Considerable ethnic differences in the genetic variation of the *UGT1A* locus have been reported. For instance, East Asians have a lower allelic frequency of *UGT1A1**28 than whites and individuals of African descent. Indeed, the genetic variant primarily responsible for Gilbert's syndrome in East Asian populations is a nonsynonymous polymorphism at *UGT1A1* c.211G>A leading to a glycine-to-arginine substitution at amino acid 71 (*UGT1A1**6). Genotyping both *UGT1A1**28 and *UGT1A1**6 to predict irinotecan-induced hematologic toxicity is recommended in East Asian patients. Interestingly, *UGT1A1**6 is highly related to *UGT1A7**3 in East Asians ($r^2 = 0.84$) and coinheritance of the latter allele might contribute to the impaired SN-38 glucuronidation phenotype in patients homozygous for *UGT1A1**6. Important to note is that the frequency of the *UGT1A7**3 allele is considerably less in East Asian groups (Taiwan Chinese 0.15 and Japanese 0.26) than whites (0.36).¹³

In this study, patients were genotyped for additional promoter region polymorphisms in *UGT1A1* (*60, and *93), and polymorphisms in *UGT1A9* and *UGT1A7*. In multivariate analysis, only the *UGT1A7**3/*3 genotype significantly predicted hematologic toxicity in the first cycle. Haplotype I (protective) and gender (females more sensitive than males) together predicted hematologic toxicity during the entire course of therapy. *UGT1A1**28/*28 and the number of metastatic sites were significant predictors of response rate. Haplotype II was also associated with a better response rate. None of the other *UGT1A* variants or haplotypes were associated with time to progression. *UGT1A1**60 and *UGT1A7**3 were associated with pharmacokinetic markers of the extent of SN-38 glucuronidation. *UGT1A1**60 (-3279) was a better predictor of SN-38 glucuronidation than *UGT1A1**28.¹⁰ This allele should be assessed additionally to determine whether it is superior to *UGT1A1**28 at predicting SN-38 glucuronidation and also irinotecan-treatment related outcomes and may be of particular importance to individuals of African descent, as the frequency of the allele is higher in this ethnic group than in whites or East Asians.

To date, most irinotecan pharmacogenetic trials have focused on the *UGT1A1**28 allele, because of the early successes in demonstrating associations between the homozygous genotype and in vivo SN-38 pharmacokinetics and irinotecan-related toxicities. It has been thought that UGT1A1 is the predominant enzyme responsible for the metabolism of SN-38 glucuronidation in vivo. The findings of Cecchin et al suggest that alternative *UGT1A* alleles/haplotypes are more predictive of hematologic toxicity than *UGT1A1**28, specifically, *UGT1A7**3/*3 genotype and *UGT1A* haplotype I, which predict elevated risk of severe hematologic toxicity in the first cycle and during the entire course of therapy, respectively.

All irinotecan pharmacogenetic studies to date have suggested that *UGT1A1**28 explains up to 50% of the cases of severe neutropenia from irinotecan. Therefore, it is no surprise that other factors (genetic or otherwise) will have a role in mediating irinotecan activity. The results of Cecchin et al imply that UGT1A7 might be an important player in the metabolism of SN-38. This enzyme is extrahepatic with expression observed in lung, esophagus, stomach, and pancreas, making its role in the disposition of irinotecan, an IV-administered drug, unclear. The alleles associated with irinotecan effect might be merely

in linkage with as yet unidentified functional single nucleotide polymorphisms (SNPs). In-depth gene resequencing of this important *UGT1A* gene cluster followed by functional characterization of putatively functional SNPs may reveal the identity of previously unreported polymorphisms of possible functional significance. Because of the large size of the region (200 kb), most resequencing studies have focused on the first exons of the *UGT1A* genes, whereas few studies have investigated the promoter regions of the genes. Resequencing these areas may lead to the identification of regulatory SNPs responsible for the observed associations between *UGT1A7**3 and haplotype I and irinotecan-induced toxicity.

A recent meta-analysis demonstrated that *UGT1A1**28 genotype is moderately predictive of severe irinotecan-induced hematologic toxicity at intermediate doses of irinotecan and strongly predictive at high-doses (> 250 mg/m²), but at low doses these patients have a comparable incidence of toxicity to other patients.⁶ Patients in this study received an intermediate dose, 180 mg/m² biweekly. The relationships between *UGT1A* polymorphisms and haplotypes and irinotecan-related treatment outcomes warrant additional investigation in other irinotecan-containing dosage regimens to determine whether the relationships hold.

The data strongly implicates the *UGT1A* locus, rather than merely a single isolated SNP, as an important mediator of irinotecan-associated activity. The results of this study demonstrate the importance of genotyping multiple polymorphisms across *UGT1A1*, *UGT1A7*, and *UGT1A9* and assessing haplotypes for prediction of irinotecan-related toxicity rather than relying on the presence of a single polymorphism in *UGT1A1*. SNPs considered together may provide better prediction of those patients that are at highest risk of severe hematologic toxicity. *UGT1A7**3 and haplotype I should be studied additionally to assess whether they are superior to *UGT1A1**28 as a marker of hematologic toxicity.

AUTHORS' DISCLOSURES OF POTENTIAL CONFLICTS OF INTEREST

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