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# Sorafenib Is an Inhibitor of UGT1A1 but Is Metabolized by UGT1A9: Implications of Genetic Variants on Pharmacokinetics and Hyperbilirubinemia

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## Abstract

**Purpose:** Several case reports suggest sorafenib exposure and sorafenib-induced hyperbilirubinemia may be related to a $(TA)_{5/6/7}$ repeat polymorphism in *UGT1A1*28* (UGT, uridine glucuronosyl transferase). We hypothesized that sorafenib inhibits UGT1A1 and individuals carrying *UGT1A1*28* and/or UGT1A9 variants experience greater sorafenib exposure and greater increase in sorafenib-induced plasma bilirubin concentration.

**Experimental Design:** Inhibition of UGT1A1-mediated bilirubin glucuronidation by sorafenib was assessed *in vitro*. *UGT1A1*28* and *UGT1A9*3* genotypes were ascertained with fragment analysis or direct sequencing in 120 cancer patients receiving sorafenib on five different clinical trials. Total bilirubin measurements were collected in prostate cancer patients before receiving sorafenib ($n = 41$) and 19 to 30 days following treatment and were compared with *UGT1A1*28* genotype.

**Results:** Sorafenib exhibited mixed-mode inhibition of UGT1A1-mediated bilirubin glucuronidation ($IC_{50} = 18$ μmol/L; $K_i = 11.7$ μmol/L) *in vitro*. Five patients carrying *UGT1A1*28/*28* ($n = 4$) or *UGT1A9*3/*3* ($n = 1$) genotypes had first dose, dose-normalized areas under the sorafenib plasma concentration versus time curve (AUC) that were in the 93rd percentile, whereas three patients carrying *UGT1A1*28/*28* had AUCs in the bottom quartile of all genotyped patients. The Drug Metabolizing Enzymes and Transporters genotyping platform was applied to DNA obtained from six patients, which revealed the *ABCC2-24C>T* genotype cosegregated with sorafenib AUC phenotype. Sorafenib exposure was related to plasma bilirubin increases in patients carrying 1 or 2 copies of *UGT1A1*28* alleles ($n = 12$ and $n = 5$; $R^2 = 0.38$ and $R^2 = 0.77$; $P = 0.032$ and $P = 0.051$, respectively). *UGT1A1*28* carriers showed two distinct phenotypes that could be explained by *ABCC2-24C>T* genotype and are more likely to experience plasma bilirubin increases following sorafenib if they had high sorafenib exposure.

**Conclusions:** This pilot study indicates that genotype status of *UGT1A1*, *UGT1A9*, and *ABCC2* and serum bilirubin concentration increases reflect abnormally high AUC in patients treated with sorafenib. *Clin Cancer Res; 18(7); 2099–107. ©2012 AACR.*

## Introduction

Sorafenib tosylate inhibits VEGF receptors and several tyrosine kinases and thus is considered an antiangiogenic agent with dual multikinase activity (1). Although sorafenib is currently approved for the treatment of renal cell and unresectable hepatocellular carcinomas, it may also have activity in other solid tumors, which led to the initiation of several sorafenib-based clinical trials (2–5). There is wide variation in the response and toxicity between patients following sorafenib treatment that seems to be, at least in part, related to cumulative drug exposure (3). For this reason, studies investigating the sources of interindividual variation in sorafenib exposure are needed.

In humans, the majority (77%) of the sorafenib dose is either not absorbed or is eliminated through the hepatobiliary route (50% unchanged), whereas 19% of the dose (mostly glucuronides) is excreted in urine (6). Both routes of elimination require glucuronidation catalyzed by UGT1A9 (7, 8), although it remains unclear if other uridine

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## Translational Relevance

We investigated UGT1A-mediated (UGT, uridine glucuronosyl transferase) sorafenib glucuronidation *in vitro* and inhibition of UGT1A1 bilirubin conjugation by sorafenib, and ascertained whether patients carrying *UGT1A1*28* treated with sorafenib had increased sorafenib exposure or increased risk of developing hyperbilirubinemia. We also investigated genetic variation in genes encoding sorafenib-metabolizing enzymes (*UGT1A9*, *CYP3A4/5*) and the glucuronide transporter, *ABCC2*. *In vitro* data show that sorafenib inhibits UGT1A1. Patients carrying *UGT1A1*28/*28* had abnormally low exposure to sorafenib if they also carried the −24C>T variant in *ABCC2*, but had abnormally high exposure to sorafenib if they were wild-type for *ABCC2*. Patients carrying *UGT1A1*28/*28* also had greater increases in total bilirubin if sorafenib exposure was high. The clinical data suggest that sorafenib can cause hyperbilirubinemia in patients with Gilbert's syndrome, which can lead to abnormally high or low sorafenib exposure. Therefore, serum bilirubin concentration increases reflect high area under the sorafenib plasma concentration versus time curve in patients harboring *UGT1A1**28.

glucuronosyl transferases (UGT) are responsible for glucuronidation of oxidized sorafenib metabolites formed through CYP3A4/5 metabolism. Moreover, these enzymes show phenotypic variability based on multiple polymorphisms [i.e., *UGT1A9*3*, *UGT1A9* -118$_{dT9/10}$, *UGT1A9* IVS+1 399 C>T (9); *CYP3A4*1b*; and *CYP3A5*3C* (10]. Once in the gut, intestinal microflora deglucuronidate and reduce sorafenib resulting in enterohepatic circulation allowing systemic reexposure (10, 11). However, renal elimination seems to be irreversible and individuals with low creatinine clearance (CrCl < 60 mL/min) require more sorafenib dose reductions than patients with normal renal function (12). Thus, sorafenib glucuronidation is a significant route of sorafenib metabolism (6, 8) and can potentially alter sorafenib exposure.

It is known that 7 TA nucleotide repeats in the (TA)$_n$TAA promoter region of *UGT1A1* (*UGT1A1*28*) leads to decreased expression of UGT1A1, resulting in high plasma bilirubin levels and is often diagnosed as Gilbert's syndrome (13). Previous reports suggested that bilirubin concentrations were elevated by sorafenib (14, 15). Interestingly, 1 report suggested that sorafenib induced jaundice in individuals carrying *UGT1A1*28* alleles due to a proposed UGT1A1 inhibition (14). This is consistent with 3 additional reports that also suggested sorafenib might inhibit UGT1A1-mediated bilirubin glucuronidation resulting in elevated bilirubin concentration (14–17). Another study profiled a patient receiving sorafenib who had yellow skin coloration despite a normal serum concentration of bilirubin and determined that the outcome was likely, if not definitely, attributable to sorafenib treatment (18). Furthermore, a phase I dose-escalation trial ($n = 34$) of sorafenib with irinotecan, a UGT1A1 and UGT1A9 substrate, resulted in elevated irinotecan and SN-38 exposure with the highest sorafenib dose (400 mg twice daily; 17). In that study, sorafenib was reported to have an *in vitro* inhibitor constant ($K_i$) of 2.7 μmol/L in human liver microsomes. This suggested that the increased SN-38 exposure was due to sorafenib-induced inhibition of UGT1A1- and/or UGT1A9-mediated SN-38 glucuronidation. However, none of the above case reports evaluated sorafenib plasma concentration in these patients, thus further confirmation of these results in larger patient cohorts undergoing sorafenib treatment is needed.

Herein, we present a case report of a child with Gilbert's syndrome who underwent sorafenib treatment and experienced abnormally high sorafenib exposure. On the basis of this observation and the aforementioned case studies, we hypothesized that sorafenib and/or CYP3A4/5-mediated sorafenib oxide may be glucuronidated by UGT1A1 and/or UGT1A9, and that sorafenib acts as an inhibitor of UGT1A1-mediated bilirubin glucuronidation. In addition, the area under the sorafenib plasma concentration versus time curve (AUC), which is a measure of the exposure of a drug, and sorafenib-induced hyperbilirubinemia might be related to the *UGT1A1*28* allele that is responsible for most cases of Gilbert's syndrome. Because UGT1A9 and CYP3A4/5 are known to metabolize sorafenib (6, 8), we hypothesized that sorafenib exposure would also be related to allelic variation in the genes encoding these enzymes (19). To this end, we compared sorafenib AUC with genetic variation in *CYP3A4/5* and *UGT1A1/9* in patients with various solid tumors undergoing sorafenib therapy, as well as with sorafenib-related toxicities (hand-foot skin reaction; HFSR) and clinical outcome.

## Materials and Methods

### Materials

The following chemicals were purchased from their respective suppliers: sorafenib tosylate (CTEP, c/o Bayer Schering Pharma); Human CYP3A4 supersomes containing CYP450 oxidoreductase and NADPH generating system, 0.5 mol/L potassium phosphate, pH 7.4, 100 mmol/L Tris buffer, pH 7.4, Human UGT1A1 and UGT1A9 Supersomes and UGT Reaction Mix (BD Biosciences), methanol and acetonitrile (Optima grade, Fisher Scientific), formic acid and acetic acid (Sigma-Aldrich). β-glucuronidase was purchased from Roche (Roche Diagnostics). All water used was deionized and purified with a Millipore system.

### *In vitro* studies

Sorafenib and cytochrome P450 (CYP)-mediated oxidized sorafenib (sorafenib-N-oxide; M-2) were subjected to UGT-catalyzed glucuronidation by members of the UGT1A family, UGT1A1 and UGT1A9. Furthermore, the role of sorafenib as an inhibitor of UGT1A1-mediated



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bilirubin glucuronidation was also studied. Details are discussed in Supplementary Methods.

### Patients and treatment

Patients ($n = 120$) from 5 clinical trials involving sorafenib treatment were used for subsequent pharmacogenetic analysis, consisting of 2 phase I trials and 3 phase II trials. The phase I trials were BAY-BEV (200 mg twice daily sorafenib with bevacizumab; $n = 27$; ref. 3), and BAY-KS (200 mg qd or 200–400 mg twice daily sorafenib with ritonavir in Kaposi's sarcoma; $n = 8$; unpublished data), AUC data from patients on the BAY-KS trial who received ritonavir were not available; thus potential AUC-influencing drug–drug interactions between ritonavir and sorafenib were not accounted for in future analyses. The phase II trials were BAY-CRPC (400 mg twice daily sorafenib in castration resistant prostate cancer; $n = 46$; refs. 2, 4), BAY-NSCLC (400 mg twice daily sorafenib in non–small cell lung cancer; $n = 22$) (20, 21), and BAY-CRC (400 mg twice daily sorafenib with cetuximab in colorectal cancer; $n = 17$; unpublished data). Written informed consent was obtained from all patients before enrollment on the trials and genotyping was approved by the Institutional Review Board of the National Cancer Institute. All inclusion/exclusion criteria and genotyping methods are detailed in the Supplementary Methods.

### Sorafenib exposure

Exposure (AUC) data were represented as day 1 dose-normalized $AUC_{0-12h}$ (ng × h/mL/mg), as previously reported (22). Steady-state exposures were not available for all 120 patients. Furthermore, exposure values were dose normalized to compare AUC from the 5 different trials with patients administered different doses. Linear pharmacokinetics were not assumed from first-dose AUC values, rather individual patient exposures were correlated to physiologic changes [i.e., HFSR, progression-free survival (PFS), and plasma bilirubin concentration].

### Statistical considerations

Hardy–Weinberg equilibrium was tested by the $\chi^2$ test. Genetic linkage statistics were obtained with Haploview (Broad Institute). Comparisons of genotype versus demographics, preclinical measures, and pharmacokinetics were conducted with nonparametric statistical tests including the Wilcoxon rank-sum test or Kruskal Wallis ANOVA. Kruskal–Wallis ANOVA was also used to compare sorafenib AUC with the clinical grade of HFSRs. The Fisher Exact Test was used to compare between different AUC percentiles and genotype. Linear regression was conducted to compare the CrCl and serum glutamic oxaloacetic transaminase (SGOT) to sorafenib AUC and was also used in comparisons between AUC and the bilirubin change from baseline in the different genotype groupings. Cox model analysis was conducted to compare genotypes versus PFS. Statistical significance was assigned if $P < 0.05$ as this study was conducted in an exploratory mode for potential confirmation in independent data.

## Results

### Case report

The patient was a 12-year-old boy who enrolled on a phase I trial of sorafenib for children with neurofibromatosis type 1 and inoperable plexiform neurofibromas (23). Before treatment, he had a total elevated bilirubin of 2.0 mg/dL with a direct of 0.3 mg/dL, normal serum alanine aminotransferase and aspartate aminotransferase, and was clinically diagnosed with Gilbert's syndrome. This was later confirmed with genetic testing as he was found to be homozygous for the $A(TA)_7TAA$ allele of the *UGT1A1* gene (i.e., *UGT1A1*28/*28*). The protocol required a bilirubin concentration within normal limits for study entry, except for patients with Gilbert's syndrome. He was treated with a dose of 115 mg/m$^2$ twice daily [approximately 50% of the adult maximum tolerated dose (MTD) based on an average adult body surface area of 1.8 m$^2$].

The patient had day 1 pharmacokinetics conducted, and his $AUC_{0-24h}$ was noted to be 81 μg·h/mL, which is greater than the average $AUC_{0-24h}$ of 28 ± 17 μg·h/mL observed in children treated at the MTD of 200 mg/m$^2$ on the refractory solid tumor phase I trial (24). The patient came off-treatment after 9 days due to dose limiting grade 3 tumor pain. The protocol was subsequently amended to exclude patients with known Gilbert's syndrome from trial participation.

On the basis of this case observation and the published case reports that sorafenib induced hyperbilirubinemia in a small number of patients who carry a *UGT1A1*28* allele (14, 15), we hypothesized that genetic variation in *UGT1A1* was a potential source of alterations in sorafenib exposure and sorafenib-induced hyperbilirubinemia. Moreover, because UGT1A9 is known to primarily glucuronidate sorafenib (8), we hypothesized that *UGT1A9* alleles might also contribute to both endpoints; thus, we studied sorafenib and sorafenib-N-oxide glucuronidation by UGT1A1 and UGT1A9 *in vitro* (Supplementary Methods and Results), and ascertained *UGT1A1* and *UGT1A9* genotypes in patients treated with sorafenib for comparison with pharmacokinetics and sorafenib-induced hyperbilirubinemia endpoints. Patient characteristics are reported in Table 1.

### *In vitro* sorafenib glucuronidation

Sorafenib glucuronidation by UGT1A9 was confirmed via *in vitro* metabolism experiments with recombinant *UGT1A9* via liquid chromatography-mass spectrometry, whereas a similar experiment with UGT1A1 did not metabolize sorafenib (see Supplementary Results). This is in agreement with literature (7, 8). On the basis of exploratory studies, neither UGT1A1 not UGT1A9 glucuronidated the CYP3A4-mediated sorafenib-N-oxide (M-2; data not shown).

On the basis of previous reports (14, 15, 17), we hypothesized that UGT1A1 could bind sorafenib and that this binding event could inhibit bilirubin glucuronidation. Increasing amounts of sorafenib were added to an *in vitro*



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**Table 1.** Patient demographics and baseline characteristics

| Characteristics | Values *n* (%) or median (95% CI) |
|---|---|
| Total | 120 (100.0) |
| Male | 86 (71.7) |
| Female | 34 (28.3) |
| Age | 63.2 (60.9–65.4) |
| Race | |
| Caucasian | 99 (82.5) |
| African American | 11 (9.2) |
| Hispanic | 4 (3.3) |
| Asian | 5 (4.2) |
| Unknown | 1 (0.8) |
| BSA ($m^2$) | 2.0 (1.9–2.0) |
| Study/disease | |
| BAY-BEV/solid tumors | 27 (22.5) |
| BAY-KS/Kaposi's sarcoma | 8 (6.7) |
| BAY-CRPC/prostate | 46 (38.3) |
| BAY-NSCLC/lung | 22 (18.3) |
| BAY-CRC/colorectal | 17 (14.2) |
| Albumin (g/dL) | 3.6 (3.5–3.7) |
| Alkaline phosphatase (U/L) | 81.0 (74.0–89.0) |
| Total bilirubin (mg/dL) | 0.6 (0.6–0.7) |
| SGOT (U/L) | 26 (24–28) |
| CrCl (mL/min) | 90.9 (84.8–97.7) |
| CrCl (<60 mL/min) | 17 (14.2) |

UGT1A1-catalyzed bilirubin glucuronidation enzyme activity assay to determine the extent and mechanism of inhibition by sorafenib. Bilirubin concentrations used were based on literature reports of its $K_m$ for UGT1A1-mediated glucuronidation, ranging between 0.2 and 26 μmol/L (25–28). Graphical modeling suggested sorafenib best fits a mixed-mode (mixed-type) inhibitor of UGT1A1 (model correlation $r = 0.96$), which shows properties of both a competitive and noncompetitive inhibitor (Supplementary Results). Based on model correlations (noncompetitive $r = 0.93$; competitive $r = 0.88$), it was suggested that although sorafenib is a mixed-type inhibitor, it exhibits more noncompetitive-type inhibitor characteristics than competitive.

A bilirubin $K_m$ of 5.9 μmol/L and an inhibitor constant ($K_i$) of 11.8 μmol/L were determined empirically through the mixed-type model. The $IC_{50}$ of sorafenib for UGT1A1-mediated bilirubin glucuronidation was determined to be 18 μmol/L following a separate experiment (see Supplementary Methods). These values were slightly higher than the literature values obtained by studying the mixed-type sorafenib-mediated inhibition of SN38 glucuronidation by UGT1A1, in which $K_i$ was found to be 2.7 μmol/L (17). The most likely reason for this discrepancy from literature is due to the different substrates used in the experiment (SN38 vs. bilirubin). Because sorafenib shows a competitive inhibition factor (model correlation 0.88), the substrate likely has an affect on inhibitor $K_i$.

**Genotype versus sorafenib exposure**

On the basis of the case report presented above, data indicating that sorafenib is glucuronidated by UGT1A9 (Supplementary Results), and the sorafenib-mediated inhibition of bilirubin glucuronidation through UGT1A1 (Supplementary Results), we next hypothesized that genetic variants in *UGT1A1* and *UGT1A9* would affect sorafenib pharmacokinetics and bilirubin metabolism in patients with solid tumors who received sorafenib.

We excluded patients with low CrCl (<60 mL/min) and a single patient with abnormally high SGOT (90 U/L) given the importance of hepatic and renal function in sorafenib pharmacokinetics (Supplementary Results). Carriers of *UGT1A1*28/*28* tended to be younger and have higher median total bilirubin. Patients with wild-type *CYP3A4* and *CYP3A5* alleles had lower median SGOT and median CrCl than variant allele carriers, respectively. A more detailed summary can be found in the Supplementary Results section. None of the above associations are likely to have altered the results presented in later sections.

Initial analysis of *UGT1A1* $A(TA)_nTAA$ and *UGT1A9*3* genotypes versus day 1 dose-normalized sorafenib $AUC_{0-12h}$ revealed 5 patients carrying *UGT1A1*28/*28* ($n = 4$) and *UGT1A9*3/*3* ($n = 1$) have higher AUCs than those patients corresponding to any other genotypes with normal CrCl and SGOT (i.e., all > 93rd percentile; range = 109.9–198.6 ng·h/mL/mg), and 1 patient carrying *UGT1A1*28/*28* had an AUC in the 76th percentile (AUC = 72.5 ng·h/mL/mg). Interestingly, of the 3 remaining patients carrying *UGT1A1*28/*28*, 2 had the lowest AUCs (i.e. less than 3rd percentile; range = 2.7–6.3 ng·h/mL/mg) while one had an AUC in the 21st percentile (AUC = 18.0 ng·h/mL/mg). For this reason, *UGT1A1*28* status was considered to confer different phenotypes: those having an abnormally high exposure, those having exposures matching the rest of the cohort, and those having low exposure (Fig. 1). Analysis of *UGT1A1* and *UGT1A9* genotypes versus sorafenib AUC in the different genotype groupings did not lead to a statistically significant result due to the wide variability in phenotype in the *UGT1A1*28/*28* genotype grouping ($P = 0.32$; Kruskal-Wallis ANOVA; Fig. 1); however, there were strongly significant differences in the odds of having AUCs greater than 93rd percentile (i.e., ≥107 ng·h/mL/mg) and also carrying *UGT1A1*28/*28* ($n = 4$) or *UGT1A9*3/*3* [$n = 1$; OR (95% CI) = 179.7 (8.5–3787)]; $P < 0.0001$; Fisher exact test). In addition, there was a strongly significant difference in the odds of carrying *UGT1A1*28/*28* and also having AUCs of 3rd or less percentile [OR (95%CI) = 57.3 (2.5–1326); $P = 0.0084$; Fisher exact test], further justifying consideration of *UGT1A1*28* alleles as conferring different phenotypes. Although *UGT1A9* IVS+1 (399C>T) and $-118dT_{9/10}$ were in linkage disequilibrium with *UGT1A1* $A(TA)_nTAA$ (Supplementary Results), these polymorphisms were not associated with alterations in AUC ($P \geq 0.39$; data not shown). Neither *CYP3A4*1B* ($P = 0.42$) nor *CYP3A5*3C* ($P = 0.52$) status was associated with increased sorafenib exposure. Therefore, *UGT1A1*28* and possibly



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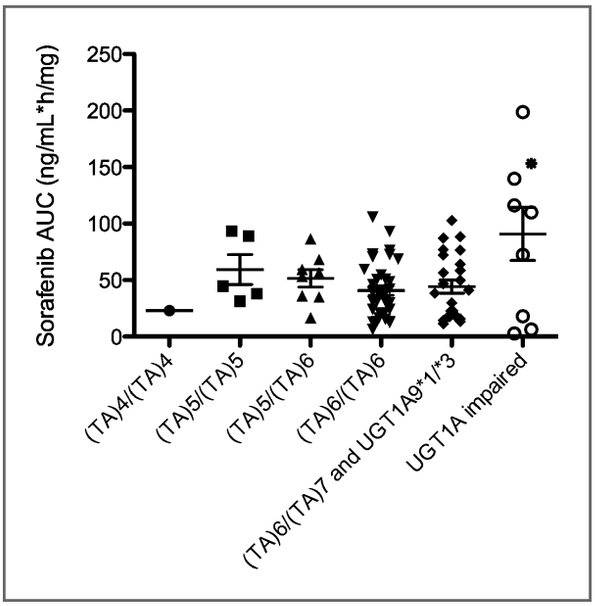


**Figure 1.** Dose-normalized sorafenib AUC versus *UGT1A* genotype. Each *UGT1A9*1/*3* carrier also carried *UGT1A1* $(TA)_6/(TA)_7$ (AUC = 17.3 and 20.2 ng/mL·h/mg) while the single *UGT1A9*3/*3* carrier also carried *UGT1A1* $(TA)_6/(TA)_6$ (AUC = 153.3 ng/mL·h/mg, as indicated by *). *UGT1A*-impaired implies patients with deficient UGT1A1 or UGT1A9 metabolism based on genetics. Excluded patients ($n = 38$) are described in the Supplementary Results section and $n = 82$ individuals were included in this analysis. Of these, AUC data for $n = 8$ patients participating on BAY-KS were not available; thus potential drug–drug interactions between ritonavir and sorafenib were not accounted for. There was no association between *UGT1A1* and *UGT1A9* genotype status when compared with sorafenib AUC ($P = 0.20$; Kruskal–Wallis ANOVA).

the *UGT1A9*3* single-nucleotide polymorphism (SNP) seemed to be the major predictive alleles associated with phenotype.

To further study the apparently different phenotypes for patients carrying only *UGT1A1*28* alleles ($n = 8$), we genotyped 1,936 polymorphisms in 225 genes involved in clinical pharmacology with the Drug Metabolizing Enzymes and Transporters (DMET) Plus panel (Coriell Institute). DMET genotyping was only successful (≥90% call rate) in a total of 6 patients with the following AUCs (ng·h/mL/mg): 2.7, 6.3, 18.0, 109.9, 116.2, 139.6. DMET analysis revealed that only the *ABCC2* -24C>T SNP cosegregated with sorafenib metabolism phenotype. After sequencing the *ABCC2* -24C>T SNP in all patients carrying *UGT1A1*28/*28*, it was determined that the patient with the lowest observed AUC (i.e., 2.7 ng·h/mL/mg) was double variant, whereas patients with the next lowest AUCs (6.3, 18.0, and 72.5 ng·h/mL/mg) were heterozygous followed by those with the highest AUCs (109.9, 116.2, and 139.6) who carried homozygous wild-type alleles. A single patient with AUC = 198.6 ng·h/mL/mg was not ascertainable as *ABCC2* genotyping by direct sequencing was not successful. Upon sequencing the whole population for *ABCC2* -24C>T, it was determined that individuals carrying only variant alleles in this SNP tended to have lower median AUC (29.8 versus 40.5 ng·h/mL/mg) than individuals carrying 1 or 2 copies of wild-type allele, but this was not statistically significant ($P = 0.21$). Therefore, the *ABCC2* -24C>T SNP only seems to modify AUC phenotype in those carrying only *UGT1A1*28* alleles.

### Genotype versus bilirubin change following sorafenib

Because previous case-report data indicated that sorafenib might induce bilirubin changes in patients based on *UGT1A1* allele status (14, 15), we hypothesized that sorafenib exposure would correspond to greater increases in postsorafenib bilirubin concentration in those patients with low functioning *UGT1A1* alleles (i.e., *UGT1A1*28*). Analysis of bilirubin versus genotype was only conducted in men with prostate cancer receiving sorafenib as comprehensive bilirubin data were not obtained in other trials. The median change in bilirubin plasma concentration was 0 mg/dL (range = −0.3 to 0.5 mg/dL; $n = 45$). A total of 3 patients with normal CrCL developed hyperbilirubinemia (i.e., bilirubin concentration ≥1.0 mg/dL) following sorafenib (*UGT1A1* $(TA)_5/(TA)_6$ $n = 1$, $(TA)_6/(TA)_7$ $n = 2$), and 2 patients that presented with hyperbilirubinemia before the sorafenib dose had a further rise in bilirubin concentration following sorafenib (*UGT1A1* $(TA)_6/(TA)_7$ $n = 1$, $(TA)_7/(TA)_7$ $n = 1$; the latter patient had a 0.4 mg/dL increase).

*UGT1A1* $A(TA)_nTAA$ status was not related to change in bilirubin from baseline ($P = 0.39$; Fig. 2A). However, regression analysis indicated that sorafenib exposure was related to bilirubin serum concentration in patients with normal CrCl ($R^2 = 0.29$; $P = 0.0005$; Fig. 2B). When regression analyses were stratified on the basis of *UGT1A1* $A(TA)_nTAA$ genotype status, this analysis revealed that sorafenib exposure was not related to bilirubin increases in patients carrying either *UGT1A1* $(TA)_{5/5}$ or $(TA)_{5/6}$, or *UGT1A1* $(TA)_{6/6}$ genotypes ($R^2 = 0.43$ and 0.030 respectively; $P = 0.35$ and 0.51, respectively; Fig. 2C and D). However, only 4 individuals carried a copy of *UGT1A1* $(TA)_5$, and there is an apparent (albeit nonsignificant) proportional increase in both AUC and sorafenib-induced bilirubin changes consistent with the rather high $R^2$ for this genotype grouping. When the data were stratified by *UGT1A1* $(TA)_{6/7}$ and *UGT1A1* $(TA)_{7/7}$ genotypes, a significant (or marginally nonsignificant) relationship was observed in both cases with a relatively high correlation ($R^2 = 0.38$ and 0.77 respectively; $P = 0.032$ 0.051, respectively; Fig. 2E and F). For patients with the *UGT1A1* $(TA)_{6/7}$ genotype, the data indicate that bilirubin increased by 0.1 mg/dL for every 25.7 (ng/mL·h/mg) unit increase in sorafenib AUC. Carriers of *UGT1A1* $(TA)_{7/7}$ had a similar relationship between bilirubin and AUC (i.e., a 30.9 ng/mL·h/mg unit increase in AUC corresponded to a 0.1 mg/dL increase in bilirubin). These data are consistent with previous case reports in which *UGT1A1* $(TA)_{6/7}$ carriers developed jaundice following sorafenib treatment and are also consistent with our results that sorafenib is a mixed inhibitor of UGT1A1.



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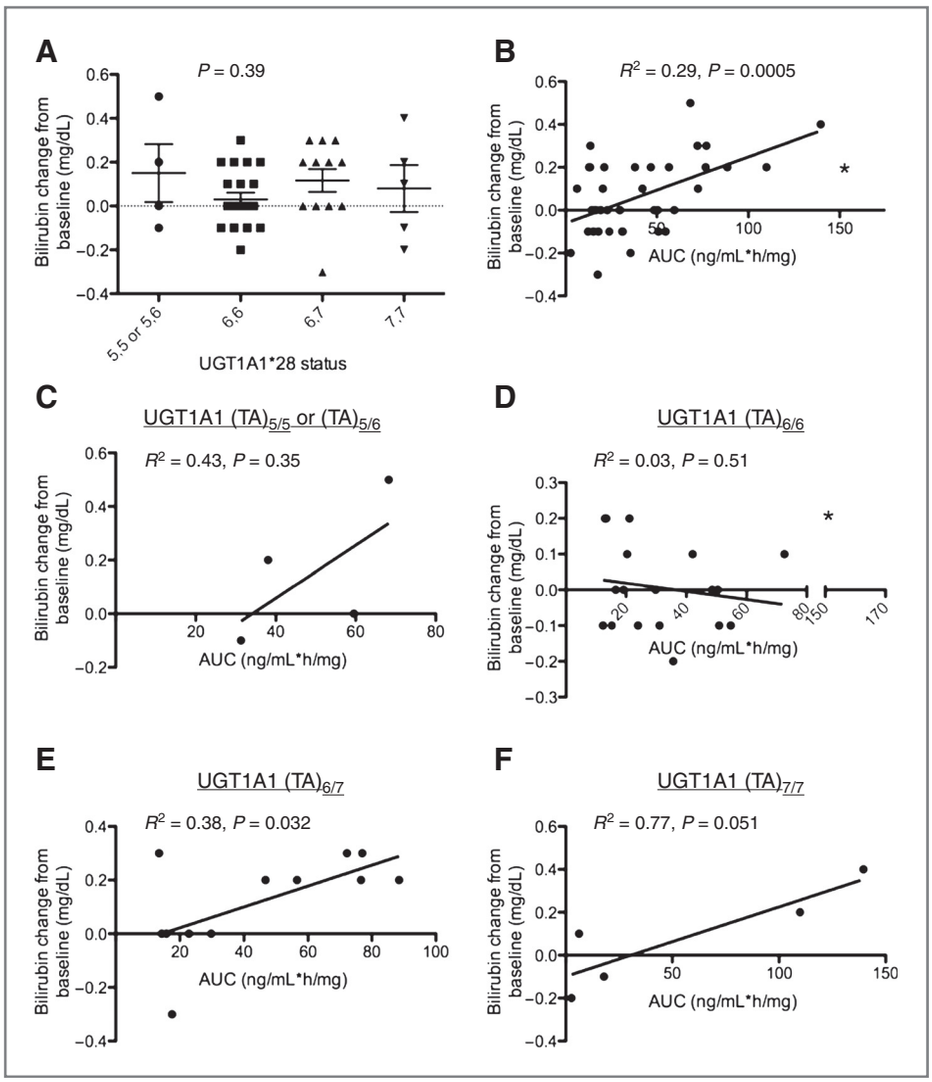


Figure 2. *UGT1A1* A(TA)$_n$TAA status versus bilirubin change from baseline following sorafenib (mg/dL; A); and sorafenib AUC (ng/mL·h/mg) versus bilirubin change from baseline (mg/dL; B) in patients with *UGT1A1* (TA)$_{5/5}$ or *UGT1A1* (TA)$_{5/6}$ (C), *UGT1A1* (TA)$_{6/6}$ (D), *UGT1A1* (TA)$_{6/7}$ (E), or *UGT1A1* (TA)$_{7/7}$ (F). *, a patient who carried *UGT1A1* (TA)$_{6/6}$ that also carried 2 variants at UGT1A9*3 and was thus excluded from analysis in the *UGT1A1* (TA)$_{6/6}$ cohort.

**Genotype versus PFS and toxicity**

The *CYP3A4*1B* allele was weakly associated with PFS according to a Cox model analysis accounting for the multiple clinical trials in which the present patient cohort was ascertained (data not shown). Those patients carrying variant alleles at *CYP3A4*1B* ($n = 19$) tended to have shorter PFS than those patients carrying homozygous wild-type alleles ($n = 99$; $P = 0.034$). None of the other alleles studied herein were related to PFS ($P > 0.05$); however, the small numbers of variants within each trial led to wide CIs for the individual HR estimates and the present results with respect to PFS should be interpreted with caution.

Consistent with previous literature (22), the incidence of HFSR was associated with increases in sorafenib AUC ($P = 0.0054$; Fig. 3). However, all patients who had AUC more than 100 ng·h/mL/mg ($n = 7$) developed HFSR regardless of genotype ($P = 0.0085$); thus, although *UGT1A* genotypes were not associated with HFSR in this study (due to heterogeneity of phenotype and low allele frequency), it is likely that *UGT1A1*28/*28* and *UGT1A9*3* carriers are subject to increased incidence of HFSR as they likely have higher exposure to sorafenib, and this should be confirmed by future studies.

## Discussion

The case report presented here involved a child with Gilbert's syndrome (*UGT1A1*28/*28*) who upon receiving sorafenib for treatment of a neurofibroma had higher than expected sorafenib exposure. On the basis of this and previously published observations (14–17), we hypothesized and assessed whether *UGT1A1* genotype was associated with sorafenib systemic exposure and serum bilirubin concentrations in patients treated with sorafenib. We confirmed previous hypotheses and findings (14–17) that sorafenib is an inhibitor of, but is not metabolized by, UGT1A1; rather, UGT1A9 is involved in sorafenib glucuronidation, which also confirms another report (8). It is



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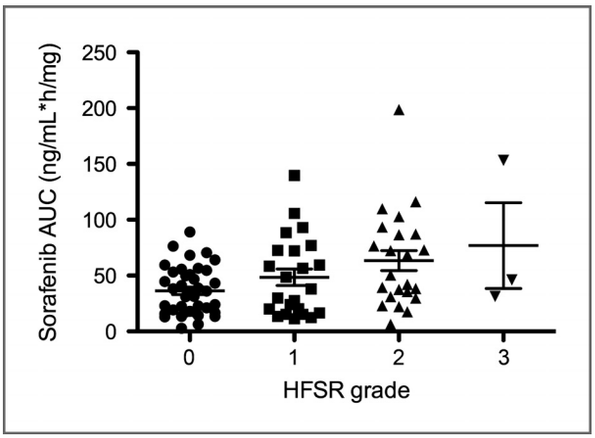


Figure 3. Sorafenib exposure is related to HFSR. HFSR was associated with increases in sorafenib AUC ($P = 0.0054$).

unclear as to the exact reason for the approximately 4- to 10-fold difference in $K_i$ values, however there is one plausible reason. Both this study and literature described sorafenib as a mixed-type inhibitor of UGT1A1, and although sorafenib shows more noncompetitive characteristics (based on higher model correlation), there is a competitive inhibitor portion to the $K_i$ calculation that is potentially altered based on the substrate used. The UGT1A1 substrate used in the literature was SN38, whereas bilirubin was used in this study, and the affinity of that substrate may affect the $K_i$ of sorafenib. SN38 has a $K_m$ of 11 μmol/L for UGT1A1 (29), whereas bilirubin has a reported $K_m$ range of 0.2 to 26 μmol/L (25–28) due to the numerous stereoisomers present. Furthermore, the $K_i$ of 11 μmol/L and $IC_{50}$ of 18 μmol/L are clinically relevant plasma concentrations following 400 mg twice daily dosing, which typically result in maximum plasma concentrations between 11 and 21 μmol/L after a cycle lasting either 7 or 28 days (30). Although sorafenib is more than 99% plasma protein bound, there is a high enough intrahepatic sorafenib concentration in patients to inhibit the UGT1A1-mediated bilirubin glucuronidation. From our data, patients carrying at least one allele of *UGT1A1*28* and having sorafenib AUC more than 60 ng·h/mL, or 130 nmol/L·h, were related to increases in serum bilirubin following sorafenib treatment (Fig. 2E and F). This provides clinical evidence that intrahepatic sorafenib concentrations can be achieved in high enough levels to inhibit UGT1A1.

These results suggest that patients carrying only *UGT1A1*28*, and possibly *UGT1A9*3*, alleles are at an increased risk of elevated sorafenib concentrations, as well as a greater incidence of HFSR. However, there remained a group of patients carrying *UGT1A1*28/*28* and *ABCC2* -24C>T that had abnormally low sorafenib AUC, thereby complicating this analysis. Nonetheless, sorafenib AUC was well correlated with change in bilirubin concentrations in patients carrying at least a single *UGT1A1*28* allele; conversely, it was not correlated with AUC in patients who carried only wild-type genotypes at this site.

The mechanism underlying the observation that individuals carrying *UGT1A1*28/*28* also have high AUC, a phenotype that seems to be modified by *ABCC2* -24C>T, is currently unclear. We tested the hypothesis that *UGT1A1*28* alleles were merely in linkage disequilibrium with *UGT1A9* alleles [i.e., UGT1A9*3, *UGT1A9* IVS+1 (399C>T), and -118$dT_{9/10}$) that were truly responsible for the observed differences in AUC. Although linkage was observed, the current *UGT1A9* alleles did not explain the association between *UGT1A1*28/*28* and AUC. We also tested whether or not *ABCC2* -24C>T was itself related to AUC regardless of *UGT1A1* genotype; this also revealed no apparent association outside of those patients carrying *UGT1A1*28/*28*. Only parent sorafenib pharmacokinetics were analyzed, however, no bias was expected through analysis of metabolites, because it has previously been shown that altering one metabolic pathway of sorafenib does not alter parent drug pharmacokinetics (6).

These results show that UGT1A1 binds sorafenib; others showed that sorafenib binds to ABCC2 and inhibits transport of other substrates but is not transported by ABCC2 (31). Elimination of sorafenib glucuronides through ABCC2 may also be involved, however it is unclear whether sorafenib glucuronides are actually transported by ABCC2. One previous report has also shown that the liver contains binding sites for drugs that act as "sinks" to slowly dissociate bound drug that is subsequently metabolized by liver enzymes (32). It is therefore possible that given the extensive enterohepatic circulation of sorafenib (10, 11), individuals expressing relatively high levels of liver UGT1A1 (i.e., those not carrying *UGT1A1*28*) and possibly other proteins that bind sorafenib have a greater propensity to extrude sorafenib from the serum and hold sorafenib in the liver where it can be metabolized more extensively before hepatobiliary elimination. This may explain the significantly higher sorafenib AUCs of individuals who have reduced expression of UGT1A1 due to genetic polymorphisms. Still, our hypothesis does not explain why individuals carrying both *UGT1A1*28* and *ABCC2* -24C>T variants have some of the lowest AUCs. As both proteins are involved in bilirubin elimination (19), we expected that patients carrying both *UGT1A1*28* and *ABCC2* -24C>T would have less ability to glucuronidate and eliminate bilirubin due to decreased sorafenib-mediated UGT1A1 inhibition; however, this was not the case in this patient cohort (data not shown). Therefore, the fact that both proteins regulate bilirubin concentrations that could in turn influence binding of sorafenib to UGT1A1 in the liver is not likely to be the cause of *ABCC2* -24C>T modifying the sorafenib exposure phenotype of patients carrying *UGT1A1*28/*28*.

Although our data did not point to a specific mechanism underlying associations between *UGT1A1*28/*28* and AUC, we observed a clear relationship between sorafenib exposure and bilirubin concentration. We showed that sorafenib inhibits bilirubin glucuronidation as a mixed inhibitor *in vitro*. Based on this observation and the aforementioned case reports, we assessed whether or not sorafenib inhibited bilirubin glucuronidation in patients and



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whether or not this depended on *UGT1A1* A(TA)$_n$TAA status. The results indicated that sorafenib exposure was not correlated with total bilirubin concentration in patients that do not carry *UGT1A1*28*; however, there was an increasingly strong correlation between sorafenib AUC and total bilirubin in patients carrying a single copy or 2 copies of *UGT1A1*28*. Therefore, hyperbilirubinemia seems to be a marker of high sorafenib exposure in patients expressing low levels of UGT1A1 and care should be taken in monitoring patients carrying *UGT1A1*28* that are known to have high sorafenib exposure. Nonetheless, none of the genes studied in this investigation were related to the PFS of the various sorafenib studies with the exception of the weak association with the *CYP3A4*1B* allele. The results of the present Cox model should be interpreted with caution.

To our knowledge, this pilot study represents the first exploration of sorafenib AUC in patients based on *UGT1A1* and *UGT1A9* genotype status and suggests that future studies should focus on the *UGT1A1* A(TA)$_n$TAA and *UGT1A9*3* alleles and bilirubin increases in relation to sorafenib exposure, and HFSR.

## Disclosure of Potential Conflicts of Interest

The content of this publication does not necessarily reflect the views or policies of the Department of Health and Human Services, nor does mention of trade names, commercial products, or organization imply endorsement by the U.S. Government. No potential conflicts of interest were disclosed.

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# Sorafenib Is an Inhibitor of UGT1A1 but Is Metabolized by UGT1A9: Implications of Genetic Variants on Pharmacokinetics and Hyperbilirubinemia

Cody J. Peer, Tristan M. Sissung, AeRang Kim, et al.

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