

# ABCB1, SLCO1B1 and UGT1A1 Gene Polymorphisms Are Associated with Toxicity in Metastatic Colorectal Cancer Patients Treated with First-Line Irinotecan

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**Abstract:** We tested specific gene polymorphisms known to be involved in the irinotecan (CPT-11) metabolic pathway. The combination of at least one SLCO1B1 521 T allele, one ABCB1 1236 C allele and one UGT1A1\*28 variant 7 repeat demonstrated a statistically significant association with Grade 3/4 toxicities in metastatic colorectal cancer patients.

**Key Words:** Polymorphisms, colorectal cancer, toxicity, irinotecan.

## INTRODUCTION

Currently, colorectal cancer (CRC) is the second leading cause of death by cancer in the United States. Studies show that the incidence of CRC is highest in industrialized regions, where the consumption of meats, fats, and refined carbohydrates is highest [1]. During 2006, alone, the American Cancer Society expects 55,170 deaths due to colorectal cancer, as well as 106,680 new cases of colon cancer and 41,930 new cases of rectal cancer [2]. Survival rates, however, have been increasing with the introduction of new chemotherapeutics to the clinical standard of 5-fluorouracil (5-FU) treatment.

CPT-11 [7-ethyl-10-[4-(1-piperidino)-1-piperidino] carbonyloxy-camptothecin, which was first approved in the US in 1996, plays a central role in the treatment of advanced CRC. The source of this quinoline alkaloid is a tree, *Camptotheca acuminata*, native only to China and Tibet [3]. CPT-11 functions as an inhibitor of topoisomerase I (Topo1), an enzyme involved in the unraveling of the DNA helix during replication [4]. More specifically, CPT-11 traps cellular Topo1 on chromosomal DNA in the form of drug-enzyme-DNA cleavable complexes. As a result, DNA synthesis is inhibited and typically S-phase cells are killed [5]. Preclinical and clinical studies have shown that prolonged exposure to CPT-11 and its analogs, by way of continuous intravenous infusion or oral administration, results in optimal therapeutic activity [6-8].

The metabolism of CPT-11 is complex (see Fig. (1)). Once the drug enters the body, the ester bond is cleaved by either carboxylesterase1 (CES1) or carboxylesterase2 (CES2), which are both expressed in gastrointestinal tissue, more

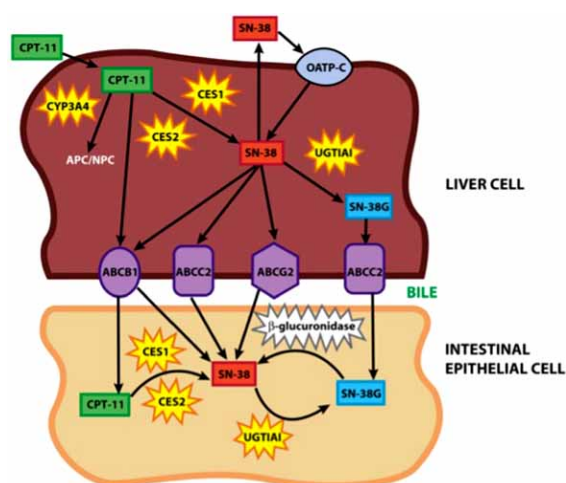


Fig. (1). CPT-11 Metabolism and Transport Schematic.

specifically, within the enterocytes, as well as in the liver [9]. It has been shown that CES2 has a 12.5- to 26-fold higher affinity for CPT-11 than that of CES1 [10,11]. Once hydrolysis is complete, CPT-11 has been fully converted to its pharmacologically active metabolite, SN-38 [7-ethyl-10-hydroxycamptothecin] [12], a topoisomerase I poison (see Fig. (2)).

Three elimination pathways exist for SN-38. First, it can be further conjugated and detoxified by the UDP-glucuronosyltransferase (UGT) 1A1 enzyme to the  $\beta$ -glucuronic acid conjugate (SN-38G) [13]. This SN-38G form is then excreted into the bile. Secondly, SN-38 can be eliminated by membrane-localized, energy-dependent outward drug pumps, which facilitate cellular efflux mechanisms. Some of these pumps include proteins belonging to the superfamily of ABC transporters [14]. Important members in this particular path-

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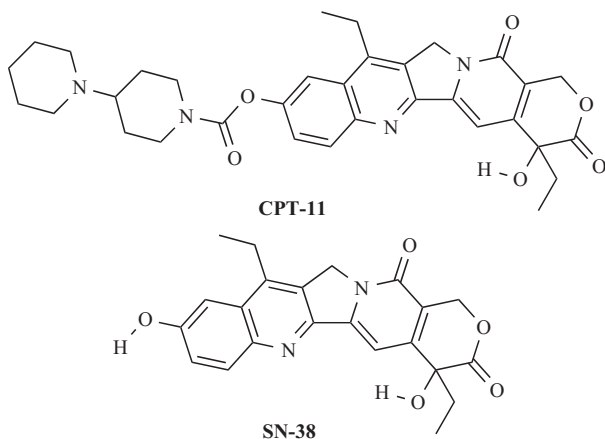


Fig. (2). Structure of CPT-11 and Its Active Metabolite, SN-38.

way include MDR1 P-glycoprotein (ABCB1), multi-drug resistance associated proteins 1 and 2 (ABCC1 and ABCC2), and the breast cancer resistance protein (ABCG2/BCRP). These protein transporters are responsible for the biliary and intestinal excretion of CPT-11, and to a lesser degree, of SN-38, and SN-38G [15-17]. One other important transporter involved is the hepatic organic anion transporter (SLCO1B1). SLCO1B1 is found at the basolateral membrane of the hepatocyte and is responsible for the uptake of differently charged amphipathic compounds [18]. It has been shown that this cellular pump is unable to transport CPT-11 or SN-38G, yet able to transport SN-38 from blood into the liver or in the opposite direction as well [19]. Thirdly, CPT-11 may be eliminated before hydrolysis to its active form by means of an oxidation reaction of the piperidine side chain, which is attached to the core chain [20,21]. The CYP3A4 enzyme, a member of the cytochrome P450 superfamily, mediates this elimination process.

Though CPT-11 has been a source of significant clinical improvement in the treatment of advanced colorectal cancer patients, the variation seen in patient response and tolerability remains a clinical challenge. The aim of this study is to find reliable genetic markers for predicting clinical outcome and toxicity in patients with metastatic CRC treated with CPT-11 chemotherapy. In search for new prognostic markers for CPT-11 treatment, we analyzed 11 polymorphisms in 7 genes of reputed importance in CPT-11 and its metabolites disposition and transport pathway: the CES1 (A1525C), CES2 (G-140C), UGT1A1 (UGT1A1\*28 TA insertion/deletion, UGT1A1 G-3156A), ABCB1 (C1236T, G2677T/A, C3435T), ABCG2 (C-421A), SLCO1B1 (T521C, A388G) and CYP3A4 (A-392G).

Our reasons for choosing these specific polymorphisms are all based on their functionality and/or their allele frequency within the population. We used the GeneEnsemble database for all of our gene references. *In vitro* and *in vivo* studies have presented controversial findings regarding the functionality of the ABCB1 3435 polymorphism. Some studies describe altered expression levels while others present altered transport efficiency [22-26]. However, it is consistently associated with human cancers, therefore we found it

necessary to include. The ABCB1 2677T variant, as suggested by *in vivo* research, demonstrates a higher substrate plasma level or higher drug dose requirements [24,27,28]. Furthermore, the ABCB1 1236 polymorphism, the third of these three most frequent ABCB1 polymorphisms, has been associated *in vivo* with higher CPT-11 drug exposure in a pharmacokinetic study [29]. The three ABCB1 polymorphisms have also been found to be in strong linkage disequilibrium [30]. In both *in vitro* and *in vivo* studies, it has been suggested that the ABCG2-421A variant results in lower protein expression and higher drug exposure [31-34]. It has been described *in vitro* that the longer repeats ((TA)<sub>7</sub> TAA allele) of the UGT1A1 promoter (TA)<sub>5,8</sub>TAA (UGT1A1\*28) repeat polymorphism cause a decrease in the transcriptional activity [35,36]. Clinical studies as well pharmacokinetic and pharmacodynamic studies have further delineated the importance of this polymorphism and another UGT1A1 -3156 polymorphism in CPT-11 toxicity, particularly neutropenic sepsis [37-39]. The hepatic organic anion transporter polymorphisms (SLCO1B1 T521C and A388G) were chosen because of previous study findings, both *in vitro* and *in vivo*, which reported markedly reduced substrate uptake [19,40]. Interestingly, the most common polymorphism in the CYP3A4 gene (A-392G) has been associated with various disease states [41,42]. Lastly the CES1 and CES2 polymorphisms were chosen based on their allele frequencies. As of yet, there has been no functional data reported regarding either polymorphism. We are aware of the functional data reported on CES2 polymorphisms, however because the variant allele frequency is so low we excluded them from our small study [43].

## PATIENTS AND METHODS

### Eligible Subjects

Fifty-four patients with metastatic colorectal cancer (UICC stage IV) who were treated with first-line 5-FU/Leucovorin and CPT-11 chemotherapy at the University of Southern California/Norris Comprehensive Cancer Center, Los Angeles, or at the Los Angeles County / University of Southern California Medical Center (LAC/USC) between 1999 and 2003 were eligible for the present study. A portion of these 54 patients was enrolled in the 3C-00-4 (12 patients) and 3C-01-4 (19 patients) clinical trials. The remaining 23 patients were not included in a clinical trial, though they were all treated following the same protocol at the University of Southern California/Norris Comprehensive Cancer Center, Los Angeles, or at the Los Angeles County / University of Southern California Medical Center (LAC/USC). There were two different CPT-11 infusion regimens used for all patients: a modified Saltz based regimen (IFL) with 125mg/m<sup>2</sup> at day 1 and day 8, 2 weeks on 1 week off and a FOLFIRI based regimen with 180mg/m<sup>2</sup> on day 1 and day 15. When comparing the 31 patients who were enrolled in these two clinical trials to the 23 patients who were not enrolled, there were no statistically significant differences in demographic characteristics (age, sex, and race) and clinical outcome variables (tumor response, toxicity, progression-free survival, and overall survival). This study was investigated at the USC/Norris Comprehensive Cancer Center and was approved by the Institutional Review Board of the University of Southern

California for Medical Sciences. Patient data was collected prospectively for the two clinical trials and retrospectively for those not on a clinical trial. All patients involved in the study signed informed consents.

**Clinical Evaluation and Toxicity Criteria**

Those patients classified as responders (R) to therapy demonstrated a decrease in tumor burden by 50% or more for at least six weeks. CT imaging for response was performed every six weeks. Patients with evaluable but non-measurable disease were classified as demonstrating complete response (CR) only if the tumor and all evidence of disease had disappeared. Progressive disease (PD) was defined as a 25% or more increase in tumor burden (compared to the smallest measurement) or the appearance of new lesions. Patients, who did not experience a response and did not progress within the first 12 weeks following the start of CPT-11/5-FU-based chemotherapy, were classified as having stable disease (SD).

**Toxicity**

All toxicity measurements were graded in accordance with the National Cancer Institute Common Toxicity Criteria 2.0. Grade 0, 1, 2, 3, and 4 represents none, mild, moderate, severe, and life-threatening respectively. Toxicity data was collected prospectively for all patients on a protocol. Toxicity data was collected retrospectively from patient progress notes for all those not included in a clinical trial.

**Polymorphism Investigation**

Blood samples were collected from each patient, and genomic DNA was extracted using the QiaAmp kit (Qiagen, Valencia, CA). Polymorphisms were determined using the PCR-RFLP assay. Each assay was repeated twice for every

individual polymorphism. Table 1 lists the forward and reverse primers, the annealing temperature, and the specific restriction enzyme (New England Biolabs, Maryland, USA) used for each polymorphism studied under PCR amplification.

The PCR products were incubated with the corresponding restriction enzyme and run on 4% Nusieve ethidium bromide-stained agarose gels. Those PCR products that could not be defined using restriction enzymes were sequenced at the USC core lab facility.

**STATISTICAL ANALYSIS**

Objective tumor response, toxicity and overall survival were the primary endpoints. Patients with complete response or partial response (tumor burden decreased by ≥50% for at least six weeks) were classified as responders, while patients with stable disease or progressive disease were classified as non-responders. Patients who experienced grade 3 or 4 toxicity were grouped together. The overall survival time was calculated as the time from the first day of CPT-11 based treatment until death from any cause, or until the date of the last follow-up.

The associations of each polymorphism with tumor response, toxicity, and demographic variables were examined using contingency tables and the Fisher’s exact test.

All reported *P* values were two-sided and no adjustments were made to account for multiple hypothesis testing. All analyses were performed using the SAS statistical package version 9.1 [SAS/STAT® SAS Institute Inc., Cary NC].

**RESULTS**

Of the eligible study participants, results from the assays for the above mentioned polymorphisms were obtained as

**Table 1. Primers and Restriction Enzymes**

Polymorphism	Forward Primer	Reverse Primer	Annealing Temp.	Rest. Enzyme
ABCBI 3435	GAGCCATCCTGTTTACTG	GCATGTATGTTGGCCTCCTT	60	Hyp188 III
ABCBI 2677	ATGGTTGGCAACTAACACTGTTA	AGCAGTAGGGAGTAACAAAATAACA	50	BseYI
ABCBI 1236	TGTGTCTGTGAATTGCCTGAAG	CCTCTGCATCAGCTGGACTGT	60	HaeIII
ABCC2 3972	CTGAGCTGGATCTGGTCCTC	CTTCCCTCCATCCAAATGA	60	sequenced
ABCG2 -421	CCTTAGTTATGTATCTTTGTG	GAAACTTCTGAATCAGAGTCAT	59	sequenced
CES1 1525	CCCAAGACGGTGATAGGAGA	CCAGGAGAGGACAAAATTGC	60	MseI
CES2 -140	GAAGGGGCTGTGCCATTC	CGTGCACATCCTCAGAGAAG	60	sequenced
CYP3A4 -392	AGGACAGCCCATAGAGACAAGG	ATCAATGTTACTGGGGAGTCC	58	MseI
SLCO1B1 521	wt:CATACATGTGGATATATGT mt:CATACATGTGGATATATGC	TGTAAGAAAGCCCCAATGGT	59	Sequenced
SLCO1B1 388	GCAAAATGTTTAATTCAGTGATGTTT	TCCCACTATCTCAGGTGATGC	55	sequenced
UGT1A1 -3156	CAAAGGAAGTTTGGGGAACA	CACCACCACTTCTGGAACCTT	60	DdeI
UGT1A1*28 (TA) <sub>n</sub>	GTCACGTGACACAGTCAAAC	TTTGCTCCTGCCAGAGGTT	55	sequenced

**Table 2. Demographic and Clinical Parameters of Patients with Metastatic CRC Treated with First-Line 5-FU/Leucovorin/CPT-11**

Characteristics	Number of Patients	Frequency %
Median age, years (range)	56 (34-77)	
Gender		
Female	23	43%
Male	31	57%
Race		
Asian	12	22%
Black	3	6%
Caucasian	29	54%
Hispanic	10	19%
Response		
Complete response	3	6%
Partial response	20	38%
Stable disease	24	45%
Progressive disease	6	11%
Inevaluable/early off study	1	
Toxicity		
Grade 0-2	25	47%
Grade 3-4	28	53%
Inevaluable/early off study	1	

follows: CES1 1525: 54 patients, CES2 -140: 54 patients, ABCB1 1236: 49 patients, ABCB1 2677: 52 patients, ABCB1 3435: 53 patients, UGT1A1\*28: 51 patients, UGT1A1 -3156: 53 patients, SLCO1B1 521: 52 patients, SLCO1B1 388: 54 patients and CYP3A4 -392: 54 patients. The genotype data from some patients was not available due to the quantity of the genomic DNA available.

Please refer to Tables 2 and 3 for baseline demographics and a comprehensive review of all polymorphisms and their association with toxicity and response. Polymorphisms may be broken up into three genotype groups (wild type/wild type, wild type/variant and variant/variant) or grouped together into two if the variant/variant frequency was less than 5%. Types of toxicity seen in patients included diarrhea, vomiting, neutropenia, and dyspnea. One patient experienced septic shock. Please refer to Table 4 for a comprehensive list of the percentages of patients seen with each type of grade 3/4 toxicity. We also examined each polymorphism and their associations with race. Only one polymorphism, CES2 G-140C, had a significant association with race. Furthermore, UGT1A1\*28 and UGT1A1 -3156 polymorphisms were strongly linked ( $P < 0.0001$ , Fisher's Exact Test).

#### **SLCO1B1 Polymorphisms and Toxicity**

This study detected no statistically significant association between the SLCO1B1 A388G polymorphism and clinical

outcome. However, we found that the patients homozygous for the variant C allele at SLCO1B1 521, showed a statistically significant ( $P = 0.029$  Fisher's Exact Test; Table 3) lower incidence of Grade 3-4 toxicity than those patients with the other two possible genotypes.

#### **Combined Analysis of SLCO1B1, ABCB1, and UGT1A1 Polymorphisms for Toxicity**

When we considered the genes involved in the hepatic uptake and elimination of SN-38, significant associations between SLCO1B1 T521C, ABCB1 C1236T, and UGT1A1\*28 and A-3156G polymorphisms and toxicity were found (Table 5). Patients who carried the wild-type T allele of the hepatic organic anion transporter (SLCO1B1) T521C and the wild-type C allele of the MDR1 P-glycoprotein (ABCB1) C1236T were more likely to experience grade 3-4 toxicity ( $P$  value = 0.005, Fisher's exact test) and grade 3-4 neutropenia ( $P$  value = 0.008, Fisher's exact test) than those with the variant type C/C of SLCO1B1 T521C and the variant T/T of ABCB1 C1236T. When the drug detoxifier, UGT1A1\*28, was assessed along with the drug transporter combination (SLCO1B1 T521C and ABCB1 C1236T), we found that patients with the wild-type SLCO1B1 521 T allele, the wild-type ABCB1 1236 C allele and the variant allele 7 had an increased chance of having grade 3-4 toxicity and grade 3-4 neutropenia when compared with any of the other genotype combinations ( $P$  value = 0.002,  $P$  value = 0.032 respectively,

Table 3. Genomic Polymorphisms and Clinical Outcome

Polymorphism	n	Response			Toxicity		
		Yes	No	P*value	Grade 0-2	Grade 3-4	P*value
CES1 (A1525C)				0.22			0.71
A/A	46	18 (39%)	28 (61%)		22 (49%)	23 (51%)	
A/C, C/C	8	5 (71%)	2 (29%)		3 (38%)	5 (63%)	
CES2 (G-140C)				0.74			1.00
G/G	43	19 (45%)	23 (55%)		20 (47%)	23 (53%)	
G/C	11	4 (36%)	7 (64%)		5 (50%)	5 (50%)	
UGT1A1*28				0.34			0.12
6/6	29	15 (54%)	13 (46%)		17 (59%)	12 (41%)	
6/7	18	6 (33%)	12 (67%)		5 (29%)	12 (71%)	
7/7	4	1 (25%)	3 (75%)		3 (75%)	1 (25%)	
UGT1A1 (G-3156A)				0.39			0.40
G/G	32	16 (50%)	16 (50%)		17 (53%)	15 (47%)	
G/A, A/A	21	7 (35%)	13 (65%)		8 (40%)	12 (60%)	
ABCB1 (C1236T)				0.33			0.29
C/C	18	5 (28%)	13 (72%)		8 (47%)	9 (53%)	
C/T	21	10 (48%)	11 (52%)		8 (38%)	13 (62%)	
T/T	10	5 (56%)	4 (44%)		7 (70%)	3 (30%)	
ABCB1 (G2677T)				0.31			0.76
G/G	13	3 (23%)	10 (77%)		6 (50%)	6 (50%)	
G/T	26	11 (44%)	14 (56%)		11 (42%)	15 (58%)	
T/T	13	7 (54%)	6 (46%)		7 (54%)	6 (46%)	
ABCB1 (C3435T)				0.18			0.41
C/C	15	4 (29%)	10 (71%)		6 (43%)	8 (57%)	
C/T	31	14 (45%)	17 (55%)		13 (42%)	18 (58%)	
T/T	7	5 (71%)	2 (29%)		5 (71%)	2 (29%)	
ABCG2 (C-421A)				0.25			0.56
C/C	33	16 (50%)	16 (50%)		17 (52%)	16 (48%)	
C/A, A/A	19	6 (32%)	13 (68%)		7 (39%)	11 (61%)	
SLCO1B1 (T521C)				0.68			0.029
T/T	37	16 (44%)	20 (56%)		12 (33%)	24 (67%)	
T/C	6	2 (33%)	4 (67%)		4 (67%)	2 (33%)	
C/C	9	5 (56%)	4 (44%)		7 (78%)	2 (22%)	
SLCO1B1 (A388G)				0.59			0.71
A/A	13	6 (46%)	7 (54%)		7 (58%)	5 (42%)	
A/G	28	13 (48%)	14 (52%)		12 (43%)	16 (57%)	
G/G	13	4 (31%)	9 (69%)		6 (46%)	7 (54%)	

\* Based on the Fisher's exact tests.

**Table 4. Frequency of Various Toxicities**

Type of Toxicity	Percent of Patients
Diarrhea (grade 3)	19%
Nausea and vomiting (grade 3)	9%
Neutropenia (grade 3)	28%
Neutropenic sepsis (grade 4)	1.90%
Dyspnea (grade 3)	1.90%
deep vein thrombosis (grade 3)	1.90%
fatigue (grade 3)	1.90%

Fisher's exact test; Table 5 and Fig. (3)). Furthermore, the data suggests that those patients who are homozygous wild type for both transporters and carry two copies of the variant allele 6 are at a reduced risk of acquiring grade 3/4 overall toxicity and grade 3/4 neutropenia (Table 5). A similar pattern was also observed for UGT1A1 G-3156A (P value = 0.022, P value = 0.030 respectively, Fisher's exact test; Table 5), where risk of toxicity was increased with the wild type allele of both transporters and at least one variant A allele. It is also important to note that UGT1A1\*28 is strongly linked to UGT1A1 -3156 (p<.0001, Fisher's Exact Test).

#### Other Gene Polymorphisms and Response, Overall Survival and Toxicity

There was no significant data implicated between the polymorphisms of CES1, CES2, ABCC2, ABCG2, UGT1A1

and CYP3A4 and response, overall survival and toxicity. Table 3 lists each individual polymorphism, response, toxicity and their associated p-values.

#### DISCUSSION

The increasing number of therapeutic options available to treat metastatic colorectal cancer patients is raising awareness in the field for the importance of identifying individual genetic differences in key enzymes involved in the metabolism, and influx/efflux of anticancer drugs. Many studies have successfully described molecular predictors of 5-FU and Oxaliplatin treatment modalities, though few have yet to successfully characterize any molecular predictors for determining the clinical outcome of CPT-11 therapy [19-25,37,38].

This small pilot study shows an unprecedented significant relationship between the ABCB1, SLCO1B1 and UGT1A1 gene polymorphisms and toxicity. These preliminary findings provide further encouragement to include both drug metabolism and drug transport in pharmacogenetic analyses when attempting to understand patient-drug variability.

#### SLCO1B1 T521C, ABCB1 C1236T and UGT1A1\*28 and G-3156A in Combination with Toxicity

SLCO1B1 is found exclusively in the liver. Its primary function is to move a variety of endogenous and xenobiotic substances from the blood to the liver and/or from liver to blood [19,44]. Interestingly, Tirona *et al.* found that by using site directed mutagenesis in HeLa cells, both the SLCO1B1 388 and 521 variants had a lower Vmax and Km when transporting [<sup>3</sup>H] esterone sulfate as a substrate. This suggests

**Table 5. A Combined Analysis of Polymorphisms and Toxicity**

Combination†			n	Overall Toxicity			Neutropenia		
				Grade 0-2	Grade 3-4	P value*	Grade 0-2	Grade 3-4	P value*
SLCO1B1 (T521C)	ABCB1 (C1236T)								
any T	any C		31	9 (29%)	22 (71%)		17 (55%)	14 (45%)	
C/C	T/T		16	12 (75%)	4 (25%)	0.005	15 (94%)	1 (6%)	
SLCO1B1 (T521C)	ABCB1 (C1236T)	UGT1A1 *28							
any T	any C	6/6	14	6 (43%)	8 (57%)		8 (57%)	6 (43%)	
any T	any C	any 7	15	3 (20%)	12 (80%)	0.002	8 (53%)	7 (47%)	
C/C	T/T	6/6	11	10 (91%)	1 (9%)		11 (100%)	0 (0%)	
C/C	T/T	any 7	4	2 (50%)	2 (50%)		3 (75%)	1 (25%)	
SLCO1B1 (T521C)	ABCB1 (C1236T)	UGT1A1 (G-3156A)							
any T	any C	G/G	18	6 (33%)	12 (67%)		11 (61%)	7 (39%)	
any T	any C	any A	12	3 (25%)	9 (75%)		6 (50%)	6 (50%)	
C/C	T/T	G/G	11	9 (82%)	2 (18%)		11 (100%)	0 (0%)	
C/C	T/T	any A	5	3 (60%)	2 (40%)	0.022	4 (80%)	1 (20%)	

\* Based on the Fisher's exact tests.

† Any T of OATP-C T521C and any C of ABCB1 C1236T as a group vs. C/C of OATP-C T521C or T/T of ABCB1 C1236T as a group

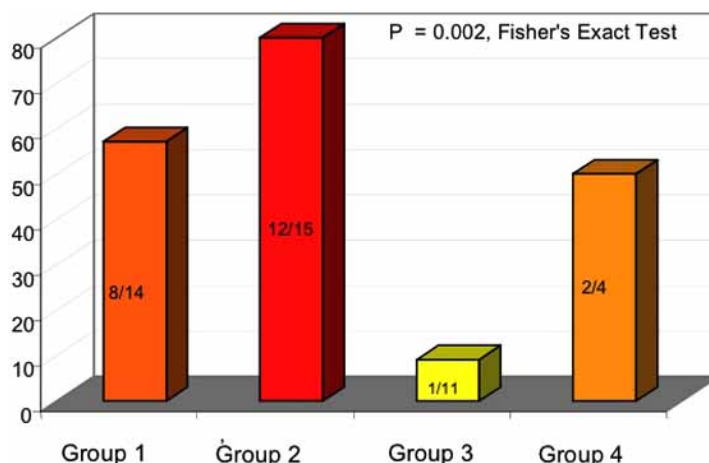


Fig. (3). Percent Chance of Grade 3/4 Toxicity.

that the Asn130Asp and Val174Ala may alter cell surface expression and transport activity, but has not been confirmed *in vivo* [40]. Nozawa *et al.* did not find similar altered transport function and cell surface expression when studying the same polymorphisms in HEK293 cells [45]. Later however, this same group showed that the SLCO1B1 transporter specifically transports only SN-38 and not CPT-11 or SN-38G, and that the T521C polymorphism does decrease transport function but did not reach statistical significance.

Our patients homozygous for the wild-type T allele at base pair 521 were more likely to exhibit Grade 3-4 toxicity. At first, these results appear counterintuitive, but by examining more genes responsible for SN-38 hepatic elimination, a more comprehensive picture develops (see Fig. (1)). When combining two genes responsible for SN-38 influx and efflux in order to elucidate any possible correlation between drug transport and toxicity, we found a stronger statistical association. Our decision to look at the ABCB1 C1236T polymorphism in combination with SLCO1B1 was not random. We have seen that the ABCB1 1236 variant allele favors a longer time to tumor progression in patients treated with CPT-11 (P=0.06 Fisher's Exact Test, data not published) and this, like the SLCO1B1 polymorphism, is also counterintuitive. Therefore, we found these results warranted further investigation, a systems biology approach. Our data indicates that the function of both genes (ABCB1 and SLCO1B1) is important when considering CPT-11 toxicity. The patients with both wild-type alleles for each transport gene (SLCO1B1 521T and ABCB1 1236C) had a much greater probability of getting grade 3/4 toxicity, including grade 3/4 neutropenia. A recent paper on hepatic clearance describes vectorial transport of different substrates in double-transfected Madrin-Darby canine kidney II cells expressing both SLCO1B1 and ABCB1 [46]. This could be a model system for understanding SN-38 pharmacokinetics *in vitro*.

Ando *et al.*, and later Innocenti *et al.*, found that a (TA)<sub>7</sub> insertion/deletion and the variant allele -3156G>A in the promoter region of the UGT1A1 gene correlated highly with Grade 4 neutropenia in CPT-11 treated patients[37,47]. They suggested that these variants have a lower level of functionality, associated with higher blood levels of SN-38 resulting

in neutropenia. Based on these previous studies and our significant findings with SLCO1B1, ABCB1 and toxicity, we wanted to further determine the impact of analyzing drug transport and metabolism collectively. We chose to include the UGT1A1 polymorphisms in our combination study because of the vast amount of data demonstrating their association with CPT-11 toxicity specifically. It is important to note, there was no association between UGT1A1 polymorphisms and toxicity in our patient cohort, which may be due to our small sample size. Using the same pathway approach, SLCO1B1 polymorphism, ABCB1 1236 polymorphism and UGT1A1\*28 repeat showed a statistically significant association (P=.002, Fisher's exact test).

We suggest that the transport activity of both the influx and efflux transporters modulates the effect the UGT1A1 polymorphisms have on toxicity. A possible explanation for these results may be that those patients who have the highest chance of grade 3-4 toxicity are transporting the SN-38 from the blood, (*via* SLCO1B1) through the liver into the bile (*via* ABCB1) so efficiently that the weaker UGT1A1 variant enzyme is unable to detoxify the available SN-38 fast enough. The SN-38 may then either cause diarrhea and vomiting or, neutropenia by re-entering the circulation *via* another OATP transporter in the intestine [48]. These transporters could possibly explain the different degrees of toxicity seen within the patient population carrying the UGT1A1 variant alleles. Analyzing these three gene polymorphisms together as a system has never been done. However, although they represent key players in the hepatic elimination of SN-38, these polymorphisms cannot possibly explain the physiological mechanism of this complex drug metabolism in its entirety. We are suggesting this simple schematic to better understand our clinical observations.

This pilot study demonstrates a systems biology approach to help explain patient variability in CPT-11 toxicity by combining both drug transport and metabolism gene polymorphisms. Further *in vitro* and *in vivo* studies examining the pharmacokinetics and pharmacodynamics of these three genes and their respective polymorphisms are necessary to elucidate the functional mechanism behind each combination of polymorphisms.

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