Background Document on the UGT1A1 Polymorphisms and Irinotecan Toxicity: ACPS November 3, 2004 Advisory Committee Meeting

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1. Introduction

Pfizer is strongly committed to fully investigate and understand the potential value of genotyping in improving the safety and efficacy of irinotecan.

Several recent publications suggest an association between the UGT1A1 7/7 genotype and irinotecan toxicities. This background document is a review of the published literature examining the clinical impact of UGT1A1 polymorphisms. Clinical studies currently underway that contain a pharmacogenomics component examining UGT1A1 and other factors involved in the metabolism, distribution, and transport of irinotecan and its active metabolite, SN-38, are summarized. In the near future, as studies better define the population at risk and diagnostic tests become readily available, healthcare providers and patients can be provided important information that will allow for better benefit/risk evaluation in the use of irinotecan as a chemotherapeutic agent.

2. Summary

The disposition of irinotecan is quite complex and involves numerous metabolic enzymes and transport proteins. SN-38, the active metabolite of irinotecan, is principally eliminated via UGT1A1-mediated metabolism to SN-38G, a biologically inactive glucuronide conjugate, which is then cleared via biliary excretion.

The natural function of UGT1A1 is the catalysis of bilirubin glucuronidation. A genetic polymorphism in the UGT1A1 promoter (UGT1A1*28) results in enzyme underexpression, causing an impairment of bilirubin metabolism (reduced glucuronidation), clinically recognized as Gilbert's syndrome (UGT1A1 7/7 genotype). Case reports describing severe neutropenia in Gilbert's patients receiving standard starting doses of irinotecan suggested a link between this UGT1A1 polymorphism and irinotecan toxicity. The current Camptosar label indicates that patients with abnormal glucoronidation of bilirubin, such as those with Gilbert's syndrome, may also be at greater risk of myelosuppression when receiving therapy with Camptosar (PRECAUTIONS: *Patients at Particular Risk*).

Results from several recently published trials suggest that patients who are homozygous for the UGT1A1*28 allele (known as the "7/7" genotype) are at greater risk for irinotecan-induced severe diarrhea or neutropenia. A trend for lower ratios of [SN-38G plasma AUC/SN-38 plasma AUC] has been observed in patients who are homozygous for UGT1A1*28. These findings are consistent with the hypothesis of reduced SN-38 to SN-38G metabolism in patients with this UGT1A1 polymorphism.

Although these results are intriguing, it is important to note that the individual trials were small (sample sizes ranging from 20-118), with only 3 to 20% of the patients having the 7/7 genotype. The studies utilized a variety of irinotecan dosing schedules and combination regimens that are known to have an impact on the degree and severity of diarrhea and/or neutropenia. The small sample sizes and trial design issues make estimation of the risk to 7/7 genotype patients difficult. For example, some trials found a significant association between UGT1A1 genotype and neutropenia but not for diarrhea. Other trials reported a genotype association with diarrhea but found no association with neutropenia. In all studies, a substantial proportion of 7/7 patients did not experience severe toxicity. Thus the precise, quantitative implications of UGT1A1 genotype on the safety, efficacy, and development of individualized patient dosing of irinotecan are not yet clear.

Based on the pharmacology of irinotecan, it might be expected that pre-treatment serum bilirubin concentrations could guide irinotecan starting doses. In fact, reduced, safe starting doses have been defined for hepatically compromised patients based on bilirubin and/or AST/ALT values exceeding institutional upper normal limit (hepatic dysfunction label supplement submitted June 25, 2004). However, in patients with serum bilirubin values within the normal range, the association between baseline bilirubin and toxicity, while statistically significant, is not strong enough to guide starting-dose selection.

Reports from the initial irinotecan pharmacogenomic trials prompted the pharmacogenomic component of NCCTG's N9741, phase III, metastatic colorectal cancer study of several irinotecan- and oxaliplatin-based regimens. Pharmacogenomic and clinical data from N9741 are currently being analyzed collaboratively with investigators and Pfizer clinicians and scientists. In addition, Pfizer is conducting pharmacogenomic correlative studies in a companion study to its sponsored, phase III, metastatic colorectal cancer trial (known as the BICC-C trial) as well as several other company-sponsored and large cooperative-group trials. These trials will look for associations between toxicity, efficacy, and genotype. These activities underscore Pfizer's strong commitment to fully investigate and understand the potential value of genotyping in improving the safety and efficacy of irinotecan.

3. Clinical and Regulatory Overview

Irinotecan hydrochloride injection (CPT-11, CAMPTOSAR® Injection) is an antineoplastic topoisomerase-I inhibitor with broad activity in colorectal cancer and other tumors. Irinotecan was originally developed in Japan by the Yakult Honsha Company. Licensing rights for clinical development in the US were granted to Pharmacia, whereas similar rights in Europe were granted to Aventis.

Irinotecan was first approved in the US for the treatment of metastatic colorectal cancer after failure of first-line treatment with 5-FU. This initial approval was based on tumor response rate data from phase II, uncontrolled studies. Conditional marketing authorization in the US was granted in 1996 under FDA regulations designed to accelerate approval of new and promising drugs for serious or life-threatening illnesses.

Subsequently, Aventis completed two European randomized, phase III studies comparing second-line irinotecan therapy with best supportive care or with infusional 5-FU-based therapy and provided the data from these trials to Pharmacia. The survival advantages associated with irinotecan use in each of these trials were the basis for full FDA approval for irinotecan as second-line therapy for patients with metastatic colorectal cancer in September 1998.

In the first-line therapy of colorectal cancer, two phase III, randomized, controlled, multicenter, multinational, clinical trials were conducted to evaluate whether the combination of irinotecan with 5-FU/LV would improve tumor control and survival relative to standard 5-FU/LV alone in patients with previously untreated metastatic colorectal cancer. The results of these trials were the basis for approval of irinotecan in combination with 5-FU/LV as first-line therapy of metastatic colorectal cancer in April 2000.

4. Human Safety Overview

Virtually all studies of irinotecan have reported neutropenia and/or delayed diarrhea (diarrhea generally occurring more than 8 hours after irinotecan administration) as the dose-limiting toxicities. The frequency of neutropenic fever has been low (usually 3-8%). Clinically significant thrombocytopenia or severe anemia is uncommon. Occurrences of ileus and/or colitis (sometimes with gastrointestinal bleeding) have been observed, but have been rare.

Patients may have transient cholinergic symptoms of rhinitis, increased salvation, miosis, lacrimation, diaphoresis, flushing, and intestinal hyperperistalsis that can cause abdominal cramping and diarrhea (early diarrhea). If they occur, cholinergic symptoms manifest during or shortly after drug infusion and are most commonly mild or moderate in severity.

Other adverse events have included nausea/vomiting, anorexia, delayed abdominal cramping, alopecia, and asthenia. Elevations in serum creatinine have sometimes occurred in association with dehydration as a consequence of diarrhea or severe vomiting, or due to occasional tumor lysis syndrome. Elevations in hepatic enzymes have been noted, but almost all of these patients have had progressive liver involvement with tumor and a relationship to irinotecan has not clearly been established.

Based on this toxicity profile, recommendations for supportive care include immediate initiation of loperamide therapy for delayed diarrhea, IV or subcutaneous atropine as prophylaxis or therapy of cholinergic symptoms, and antiemetics for prevention of nausea and vomiting. Consistent with American Society of Clinical Oncology guidelines, routine prophylactic use of a colony-stimulating factor is not advised, given the low rate of neutropenic fever generally associated with irinotecan use.

Subsequent to the NDA filing for irinotecan as a single agent, further analysis of baseline variables that might predict neutropenia was performed. Univariate and multiple regression analyses showed that in addition to prior pelvic/abdominal irradiation, mild elevations in bilirubin above the normal range can result in variable but significant increases in the likelihood of grade 3+ neutropenia during the first cycle of treatment. This led to filing a label revision stating, in PRECAUTIONS: *Patients at Particular Risk*, the following text:

"In clinical trials of the weekly dosage schedule, it has been noted that patients with modestly elevated baseline serum total bilirubin levels (1.0 to 2.0 mg/dL) have had a significantly greater likelihood of experiencing first-course grade 3 or 4 neutropenia than those with bilirubin levels that were less than 1.0 mg/dL (50.0% [19/38] versus 17.7% [47/226]; p<0.001). Patients with abnormal glucuronidation of bilirubin, such as those with Gilbert's syndrome, may also be at greater risk of myelosuppression when receiving therapy with CAMPTOSAR. An association between baseline bilirubin elevations and an increased risk of late diarrhea has not been observed in studies of the weekly dosage schedule."

The predictive value of baseline serum bilirubin levels up to 1.5 mg/dl for chemotherapy-induced toxicity or efficacy in patients receiving single-agent irinotecan for metastatic colorectal cancer was recently evaluated [Meyerhardt 2004]. The median follow-up of 287 patients was 15.8 months. It was concluded that "*baseline serum bilirubin levels does not reliably predict overall irinotecan-related toxicity or efficacy.*"

5. Irinotecan Pharmacokinetic/Pharmacodynamic and Pharmacogenomic Overview

The complex disposition pathways and plasma pharmacokinetics (PK) of irinotecan have been well-characterized [Slatter 2000; reviewed in Mathijssen 2001]. In aqueous environments, camptothecin and its derivatives exist as two interconvertible species: a biologically active, lactone form in a pH-dependent equilibrium with a biologically inactive, carboxylate (or hydroxyacid anion) form. Lower pH promotes the formation of the lactone while higher pH favors the carboxylate. In the case of SN-38 circulating in the bloodstream, this equilibrium is also affected by preferential binding of SN-38 lactone to serum albumin. While only the lactone form of camptothecin and its derivatives is cytotoxic, "total" (lactone + carboxylate) concentrations showed a strong correlation within individual patients between the AUC values for lactone and total species [e.g., Rivory 1994]. Thus, PK parameters computed from analysis of total (lactone + carboxylate) concentrations accurately reflect the PK of the bioactive lactone species.

Key features of irinotecan metabolism include carboxylesterase cleavage of the water-solubilizing dipiperidino moiety to yield the potent topoisomerase-I inhibitor SN-38 and CYP3A4-mediated oxidation of irinotecan to the biologically inactive APC metabolite. SN-38 is excreted in bile but its principal elimination route appears to be via glucuronidation carried out by UDP-glucuronosyltransferases, principally UGT1A1 and UGT1A7 [Tukey 2002]. SN-38 also may be formed within the gastrointestinal lumen via β-glucuronidase-mediated hydrolysis of SN-38G excreted in bile. In cancer patients with relatively normal organ function, irinotecan and metabolite PK parameters exhibit marked interpatient variability but fall within reproducible ranges and, in most trials, appear to be dose-proportional [Rowinsky 1994; de Forni 1994]. Relative SN-38 exposure is greater in patients with hepatic dysfunction, correlating with a lower MTD in such patients [Raymond 2002].

Based on the pharmacology of irinotecan, it might be expected that pre-treatment serum bilirubin concentrations could guide irinotecan starting doses. In fact, reduced, safe starting doses have been defined for hepatically compromised patients based on bilirubin and/or AST/ALT values exceeding institutional upper normal limit (proposed label revision has been submitted to the agency). However, in patients with serum bilirubin values within the normal range, the association between baseline bilirubin and toxicity, while statistically significant, is not strong enough to guide starting dose selection.

With regard to PK/pharmacodynamic relationships, weak yet statistically significant associations have been found between PK parameters and toxicities (neutropenia and diarrhea) in patients with uncompromised organ function. For example, in a pivotal phase II trial supporting registration of single-agent irinotecan in 2nd-line colorectal cancer [Study M/6475/0006], the variability in the severity of neutropenia or diarrhea was poorly associated with SN-38 exposure (Figues 1-3 and Table 1). Several published studies, which analyzed the association between PK parameters and neutropenia, or diarrhea reached conflicting conclusions [Mathijssen 2001]. Promising initial results suggested that a "biliary index" computed from the plasma AUCs of irinotecan, SN-38, and SN-38G might be predictive of severe diarrhea [Gupta 1994] but this finding has not been confirmed in several subsequent trials [Mathijssen 2001]. These results may be indicative of a poor correlation between plasma irinotecan and SN-38 levels and those at the relevant sites of action for efficacy and toxicity (bone marrow, gastrointestinal epithelium, tumors). This theoretical disconnect in plasma and local CPT-11, SN-38, and SN-38G concentrations could be due to variable expression of the enzyme systems responsible for irinotecan activation (carboxylesterases) and those that facilitate SN-38 detoxification/elimination (i.e., UGT1A and the ABC transporter family) [Mathijssen 2001]. There is currently an incomplete understanding of the complex relationships between the PK of the various systems that control irinotecan and metabolite disposition and the probability of toxicity in an individual patient. Thus, the use of traditional dose modification approaches based on the achievement of target plasma exposures of irinotecan or its metabolites is not likely to succeed as an approach to optimize dosing.

Results obtained in several recently conducted trials (sample sizes ranging from 20-118) suggest that genetic factors may contribute to interpatient variability in irinotecan disposition and toxicity. In particular, the homozygous "7/7" promoter genotype causing UGT1A1 underexpression has been associated with an increased severity of irinotecan-induced diarrhea or leucopenia/neutropenia and decreased conversion of SN-38 to SN-38G [Ando 2000; Iyer 2002; Innocenti 2004; Mathijssen 2003; Sai 2004]. It is interesting to note that the association between the adverse event severity (neutropenia and diarrhea) and genotype appears to be stronger than between systemic exposure (CPT-11, SN-38, and SN-38G) and genotype. These findings may be consequences of the complex factors, discussed above, that potentially affect the disposition of these compounds.

There is considerable interest in further exploring the proposed associations between irinotecan-induced toxicities and polymorphisms in genes that affect target tissue exposures to irinotecan and SN-38 in clinical trials that are larger than those conducted to date. In addition to UGT1A1, other genes of interest

include UGT1A7 as well as several members of the ABC transport protein family that are reported to play key roles in cellular efflux of irinotecan [e.g., BCRP; Zamber 2003]. The findings from such studies may lead to a better understanding of factors that predict toxicity and efficacy and may ultimately play some role in the development of genotype-based dosing recommendations.

Figure 1. Scatter plots of Cycle-1 diarrhea severity vs Cycle-1, Day-1 irinotecan AUC_{0-24} (upper) and SN-38 AUC_{0-24} (lower) in Study M/6475/0006

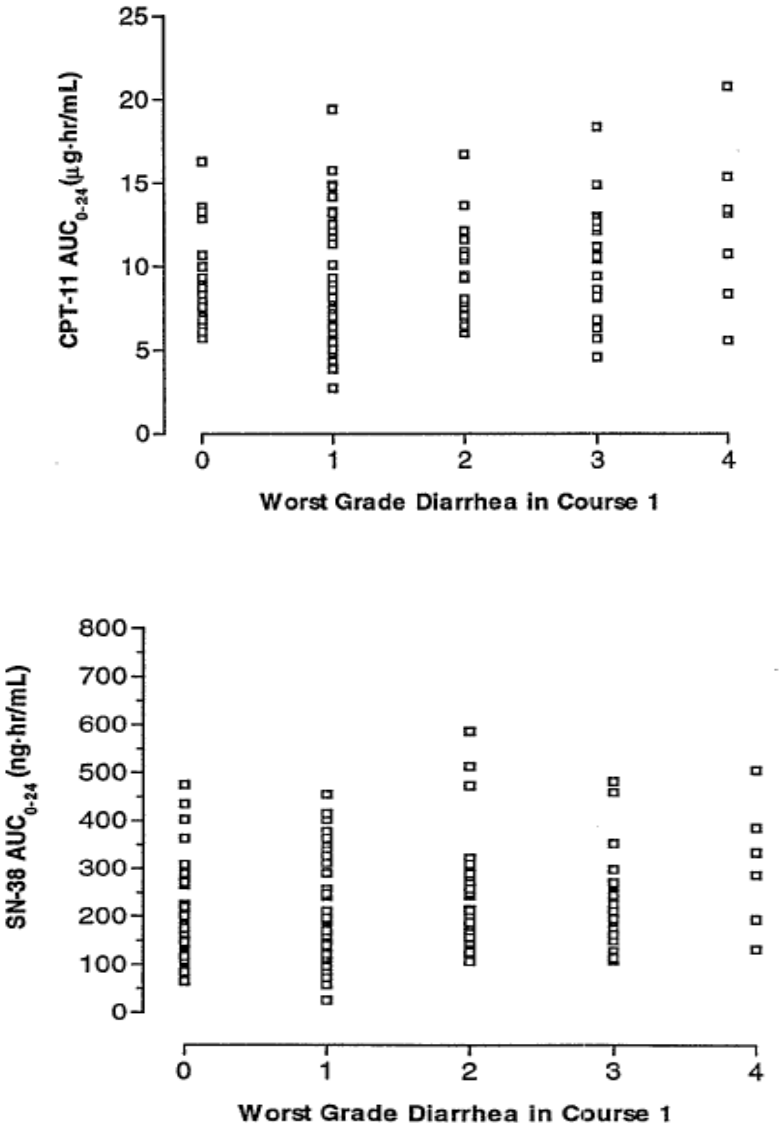


Figure 2. Scatter plots of Cycle-1 neutropenia severity vs Cycle-1, Day-1 irinotecan AUC_{0-24} (upper) and SN-38 AUC_{0-24} (lower) in Study M/6475/0006

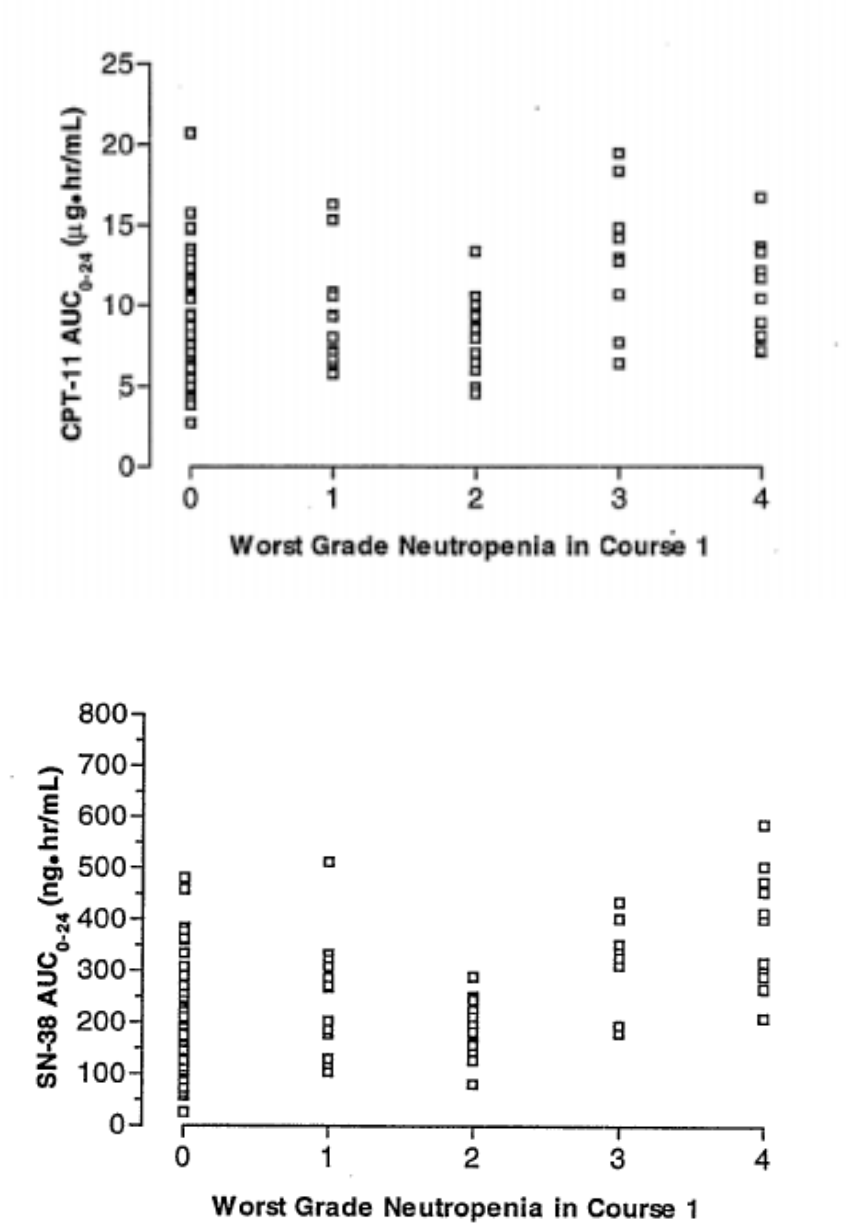
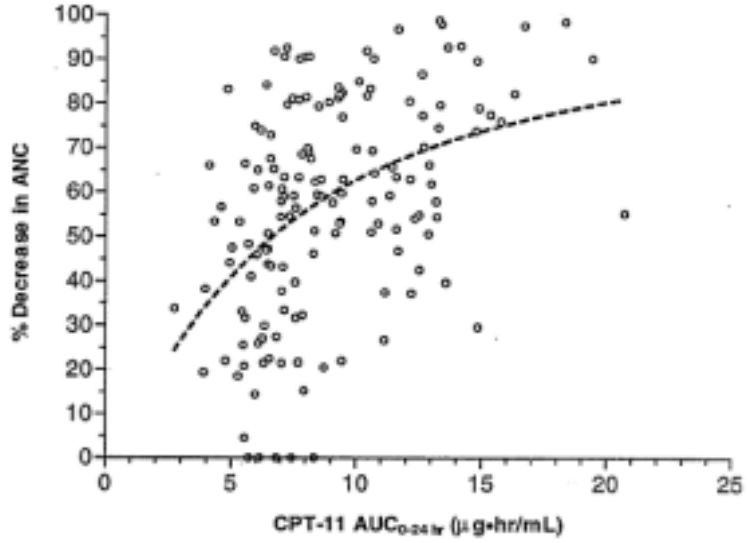


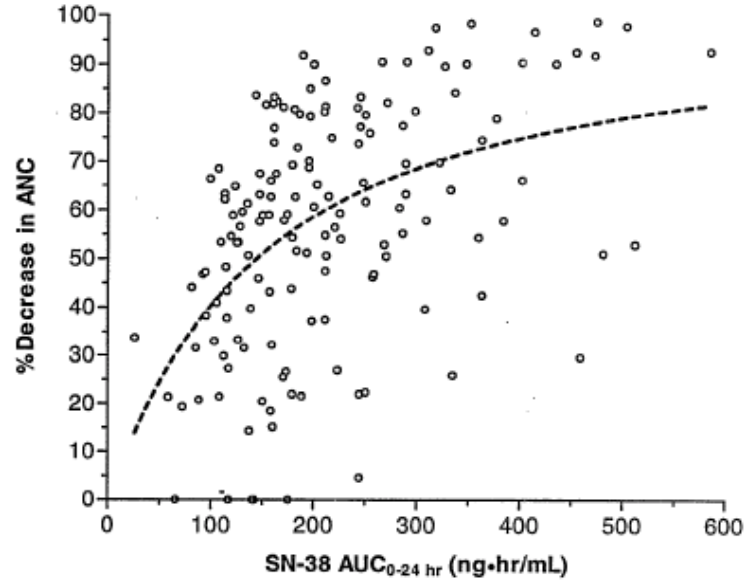
Table 1. Relationships between irinotecan and SN-38 PK parameters and diarrhea or neutropenia in Study M/6475/0006

PK Parameter	Late Diarrhea		Neutropenia	
	r[a]	p value[b]	r[a]	p value[b]
Irinotecan C_{max}, μg/mL	0.09	0.2501	0.12	0.1436
Irinotecan AUC_{0-24}, μg·h/mL	0.21	0.0086	0.26	0.0013
SN-38 C_{max}, ng/mL	0.13	0.1069	0.38	0.0001
SN-38 AUC_{0-24}, ng·h/mL	0.14	0.0799	0.43	0.0001

[a] Spearman correlation coeffecient.
[b] Spearman rank order correlation test.

Figure 3. Relationship between Cycle-1, Day-1 irinotecan (upper) and SN-38 (lower) AUC_{0-24} and % ANC decrease in Study M/6475/0006. Dashed lines represent the fit to an E_{max} model





6. The UGT1 Gene and UGT1A1 Isoform

The UGT1 gene is located on chromosome 2 and contains at least 13 different promoters/first exons which are spliced to common exons 2 thorough 5, resulting in separate isoforms with unique N-termini and conserved C-terminal domains [Ritter 1992; Gong 2001]. In terms of nucleotide sequence, exons 1 for UGT1A1 and UGT1A6 are unique, sharing only ~50% of identity with all other exon 1 regions. The other exon 1s can be clustered in two groups of high sequence identity. Each exon 1 determines substrate specificity while the N-terminal interacts with UDP-glucuronic acid.

Although there is some selectivity in substrate specificity between different UGTs, there is also remarkable redundancy to accept similar compounds as substrates [Tukey 2000]. The number of compounds that can serve as substrates for UGTs range in the thousands, and since few glucuronides retain biological activity, glucuronidation is a major detoxification mechanism [Dutton 1975].

It is not surprising that the UGTs are specifically regulated and distributed in critical tissues which come into xenobiotic contact [Strassburg 1998; Strassburg 2000]. Although the liver is a major site for glucuronidation, there is also a significant contribution from extrahepatic tissues, primarily the gastrointestinal tract. The mRNAs for UGT1A isoforms are differentially expressed in hepatic and extrahepatic tissues [Tukey 2001] with different tissues showing different levels of expression. The glucuronidation activity in the most proximal (esophagus) and most distal (colon) gastrointestinal tract is clearly reduced compared to the jejunum and liver. Interindividual polymorphic regulation of the expression of UGTs in the small intestine is in contrast to the total absence of polymorphic variation in liver [Strassburg 2000].

UGT1A1 is the only isoform that contributes in a biologically relevant way to bilirubin glucuronidation [Bosma 2003]. Mutations in the UGT1A1 gene are responsible for severe (Crigler-Najjar syndrome) and mild (Gilbert syndrome) forms of hyperbilirubinemia [Kadakol 2000]. The reduced glucuronidating activity of these mutated forms contributes to lower biliribin excretion by the liver. In addition to these mutations, the UGT1A1 gene shows great sequence variation among individuals in the form of insertion/deletion and single nucleotide polymorphisms (SNPs). Table 2 lists some of the polymorphic alleles of the UGT1A1 gene [Tukey 2000].

Table 2. Polymorphic alleles of the UGT1A1 gene				
Allele	**Nucleotide Changes**	**Protein Changes**	**Type**	**Exon**
UGT1A1*1	Wild type	—	—	—
UGT1A1*2	879 del 13	Truncation	Deletion	2
UGT1A1*3	1124 C→T	S375F	Missense	4
UGT1A1*4	1069 C→T	Q357X	Nonsense	3
UGT1A1*5	991 C→T	Q331 del 44	132 nt deletion	2
UGT1A1*6	221G→A	G71R	Missense	1
UGT1A1*7	145 T→G	Y486D	Missense	5
UGT1A1*8	625 C→T	R209W	Missense	1
UGT1A1*9	992 A→G	Q331R	Missense	2
UGT1A1*10	1021 C→T	R341X	Nonsense	3
UGT1A1*11	923 G→A	G308E	Missense	2
UGT1A1*12	524 T→A	L175Q	Missense	1
UGT1A1*13	508 del 3	F170del	Deletion	1
UGT1A1*14	826 G→C	G276R	Missense	1
UGT1A1*15	529 T→C	C177R	Missense	1
UGT1A1*16	1070 A→G	O357R	Missense	3
UGT1A1*17	1143 C→G	S381R	Missense	4
UGT1A1*18	1201 G→C	A401P	Missense	4
UGT1A1*19	1005 G→A	W335X	Missense	3
UGT1A1*20	1102 G→A	A368T	Missense	4
UGT1A1*21	1223 ins G	Frameshift	Frameshift	4
UGT1A1*22	875 C→T	A292V	Missense	2
UGT1A1*23	1282 A→G	K426E	Missense	4
UGT1A1*24	1309 A→T	K437X	Missense	5
UGT1A1*25	840 C→A	C280X	Missense	1
UGT1A1*26	973 del G	Frameshift	Frameshift	2
UGT1A1*27	686 C→A	P229Q	Missense	1
UGT1A1*28	***TAATA7***	***Transcription***	***Insertion***	***Promotor***
UGT1A1*29	1099 C→G	R367G	Missense	4
UGT1A1*30	44 T→G	L15R	Missense	1
UGT1A1*31	11609 CC→GT	P387R	2nt miss.	4
UGT1A1*32	1006 C→T	R336W	Missense	3
UGT1A1*33	881 T→C	I294T	Missense	2

Of the 33 reported variable positions, the most extensively studied is a variation of the TATA box. The most common form is UGT1A1*1(wild type), which contains 6 TA repeats in the TATA box. The UGT1A1*28 variant allele contains 7 TA repeats. There are also variant alleles containing 5 or 8 TA repeats. Individuals may have any combination of two alleles. Individuals having 2 wild-type alleles (6 TA repeats) are classified as having a 6/6 homozygous genotype; 2 UGT1A1*28 alleles as a 7/7 homozygous genotype; and one wild-type allele and one UGT1A1*28 allele as a 6/7 heterozygous genotype. The number of TA repeats in the TATA box is associated with enhanced [$(TA)_5$] or reduced [$(TA)_{7,8}$] UGT1A1 expression [Beutler 1998]. There are additional SNPs causing reduced enzymatic activity (-3279T>G, 211G>A, and 686C>A) [Jinno 2003].

Multiple polymorphic positions along the gene can be inherited as a single block (the haplotype). Haplotypes are a more comprehensive representation of sequence variation in the whole gene. The haplotype structure of the promoter region of UGT1A1 gene has been studied in Caucasian and African-American populations [Innocenti 2002]. In addition, the haplotype structure for the whole gene was recently reported for the Japanese population [Sai 2004]. The Japanese study demonstrated that there are two independent haplotype blocks and that variants in the 3′-terminal end of the gene (3′-UTR) may modulate mRNA stability contributing to the modulation of UGT1A1 expression.

Recent discoveries have contributed to a greater understanding of the extent and significance of variation in the UGT1A1 gene. Ultimately, the field will need to define the frequency of haplotypes in the different populations, their biological function, and their potential association with clinical phenotypes.

7. UGT1A1 and Irinotecan Toxicity

The association of UGT1A1 with the glucuronidation of bilirubin and SN-38 [Iyer 1998], along with the knowledge of polymorphic variation affecting the activity of UGT1A1**,** suggested the investigation of the association of these variants with pharmacokinetic values (SN38/SN38 AUC) and/or toxicity endpoints (primarily diarrhea and neutropenia). Most studies have focused on the UGT1A1*28 variant (a common polymorphism in the UGT1A1 promoter TATA box). A list of published papers and meeting abstracts reports is presented in Table 3.

Results from these trials suggest that patients who are homozygous for the UGT1A1*28 allele (known as the "7/7" genotype) are at greater risk for irinotecan-induced severe diarrhea or neutropenia. A trend for lower ratios of [SN-38G plasma AUC/SN-38 plasma AUC] has been observed in patients who are homozygous for UGT1A1*28. These findings are consistent with the hypothesis of reduced SN-38 to SN-38G metabolism in patients with this UGT1A1 polymorphism.

Although these results are intriguing, it is important to note that the individual trials were small (sample sizes ranging from 20-118), with only 3 to 20% of the patients having the 7/7 genotype. The studies utilized a variety of irinotecan dosing schedules and combination regimens that are known to have an impact on the degree and severity of diarrhea and/or neutropenia. The small sample sizes and trial design issues make estimation of the risk to 7/7 genotype patients difficult. For example, some trials found a significant association between UGT1A1 genotype and neutropenia but not for diarrhea [e.g., Innocenti 2004]. Other trials reported a genotype association with diarrhea but found no association with neutropenia [e.g., Marcuello 2004]. In all studies, a substantial proportion of 7/7 patients did not experience severe toxicity, and in one study there was no significant association between the polymorphism and toxicity whatsoever [Carlini 2004]. In addition, some studies were based on Japanese populations, which differ from the Caucasian population in haplotypes and varying genotype frequencies. Thus, the precise implications of UGT1A1 genotype on the safety, efficacy, and development of individualized patient dosing of irinotecan are not yet clear.

Table 3. Summary of Published Studies on Association between UGT1A1*28 Genotypes and Irinotecan Toxicity and PK					
Reference	**Irinotecan Dosage & Schedule**	**Study Design**	**Sample Size**	**PK Relationship**	**Toxicity Relationship**
Full Papers					
Ando, Cancer Res 2000	Variety of doses, schedules, and combos	Retrospective	118 Japanese pts; 7/118 pts were 7/7	Not evaluated	7/7 genotype had 5.2-fold risk of gr 4 leukopenia and/or diarrhea (P<0.001) compared to pooled 6/7 and 6/6
Ando, Ther Drug Monitor 2002	Variety of doses, schedules, and combos	Retrospective	100 Japanese pts for PK; 14/100 were genotyped for UGT1A1 and 4/14 were 7/7 or 6/7	7/7 or 6/7 genotype SN-38G/SN-38 AUC ratio <25th percentile of 100 PK patients	Not evaluated
Iyer, Pharmacogenom J 2002	300 mg/m^2, single dose every 3-weeks	Prospective	20; 9/20 were 6/6, 7/20 were 6/7, and 4 were 7/7	7/7 genotype SN-38G/SN-38 AUC ratio was 3.9-fold lower than 6/6 genotype (P=0.001)	7/7 genotype had 2.5-fold lower ANC nadir than 6/6 pts (P=0.04)
Font, Invest New Drugs 2003	Irino, 70 mg/m^2/day + docetaxel, 25 mg/m^2/ day, days 1, 8 & 15 every 28 days	Prospective	51 2nd-line NSCLC patients; 25/51 were 6/6, 19/51 were 6/7, and 7/51 were 7/7	Not evaluated	No genotype-dependent differences in toxicity. Non-significant trend to improved time-to-tumor progression and survival in 6/7 and 7/7 relative to 6/6.
Mathijssen, Clin Cancer Res 2003	200-350 mg/m^2, single dose every 3-weeks	Prospective	58 genotyped (majority EU Caucasian); 2/58 were 7/7, 22/58 were 6/7, and 34/58 were 6/6	Non-significant trend for genotype-dependent decrease in SN-38G/SN-38 AUC ratio (6/6 vs 6/7 vs 7/7)	Not evaluated
Innocenti, JCO 2004	350 mg/m^2, single dose every 3-weeks	Prospective	66; 30/66 were 6/6, 25/66 were 6/7, and 6/66 were 7/7; remainder were 6/8, 5/6, or 7/8	7/7 genotype SN-38G/SN-38 AUC ratio was 1.8-fold lower than 6/6 genotype (P=0.03)	7/7 genotype had 9.3-fold higher risk of gr 4 leukopenia (P=0.001)

Table 3 (Continued)					
Reference	**Irinotecan Dosage & Schedule**	**Study Design**	**Sample Size**	**PK Relationship**	**Toxicity Relationship**
Full Papers					
Sai, Clin Pharmacol Ther 2004		Retrospective	41 Japanese pts genotyped; 15/41 were 6/7 and 3/41 were 7/7	Significant genotype-dependent decrease in SN-38G/SN-38 AUC ratio (6/6 vs 6/7 vs 7/7; P=0.0014) Significant genotype-dependent pre-treatment bilirubin (6/6 vs 6/7 vs 7/7; P=0.0007)	Not evaluated
Marcuello, Brit J Cancer 2004	Variety of doses, schedules, and combos	Unspecified	95; 45/95 were 6/7 and 10/95 were 7/7	Not evaluated	Severe diarrhea in 7/10 7/7, 15/45 6/7, and 7/40 6/6 (P=0.005). Severe myelosuppression increased in 6/7 and 7/7 but did not reach statistical significance.
Rouits, Clin Cancer Res 2004	Two irinotecan/FU regimens	Retrospective	75; 7/95 7/7, 35/95 6/7, 31/95 6/6, and 2/95 5/6 or 5/7	Not evaluated	7/7 group at greater risk of severe neutropenia compared to 6/7 and 6/6 (P=0.02 and 0.003, respect). No significant difference for severe diarrhea.

Table 3 (Continued)					
Reference	**Irinotecan Dosage & Schedule**	**Study Design**	**Sample Size**	**PK Relationship**	**Toxicity Relationship**
Abstracts/Presentations					
Ando, Proc ASCO 2003	Unspecified	Unspecified	119 Japanese genotyped for UGT1A1*28 and UGT1A1 T3263G	Not evaluated	Severe tox 6.2-fold more likely in pts with both UGT1A1*28 and T3263G than in pts with wild-type UGT1A1
Chowbay, Proc ASCO 2003	100 mg/m^2, weekly	Prospective	20 Chinese pts genotyped; 12 6/6, 6 6/7, and 2 7/7	No significant genotype-dependent differences in irino, SN-38, or SN-38G AUC	Not evaluated
Carlini, Proc ASCO, 2004	Irino, 100-125 mg/m^2/day, days 1 & 8 every 21 days + capecitabine, 900-1000 mg/m^2 bid, days 2-25 every 21 days	Prospective	67 chemonaive metastatic CRC genotyped for UGT1A1*28 as well as UGT1A6 & UGT1A7 polymorphisms	Not evaluated	No significant associations between UGT1A1 genotypes and toxicity or efficacy. UGT1A7 genotypes conferring lower activity were significantly associated with higher response rate and lack of toxicity.
Grem, Proc ASCO 2004	Irino, 70-140 mg/m^2/24 h + LV, 500 mg/m^2/30 min + FU 2000-3900 mg/m^2/48 h, days 1 & 15 every 4 weeks	Prospective	30 GI cancer pts genotyped; 9/30 were 6/6 & 21/30 were 6/7 or 7/7	End-of-infusion SN-38G/SN-38 plasma level ratio was lower in 6/6 than in 6/7 or 7/7 genotypes (P=0.037)	Not reported
Massacesi, Proc ASCO 2004	Irino, 80 mg/m^2/day, days 1, 8, 15, 22 every 5 weeks + raltitrexed, 3 mg/m^2, single dose every 3 weeks	Prospective	56 pre-treated CRC patients genotyped; genotype frequencies not reported	Not evaluated	6/6 protective for diarrhea (P<0.00005), emesis (P<0.0001, and asthenia (P=0.006)
Singh, Proc ASCO 2004	Irino, 600 mg fixed dose	Prospective	86 adult pts genotyped; 44/86 were 6/6, 37 were 6/7, and 5 were 7/7	Significant genotype-dependent trend to SN-38G/SN-38 AUC ratio (P=0.022)	Not evaluated

8. Ongoing Studies

Preliminary reports from these trials prompted the pharmacogenomic component of NCCTG's N9741, a phase III metastatic colorectal cancer study of several irinotecan- and oxaliplatin-based regimens. Pharmacogenomic and clinical data from N9741 are currently being analyzed collaboratively by investigators and Pfizer clinicians and scientists. In addition, Pfizer is conducting pharmacogenomic correlative studies in a companion study to its sponsored, phase III metastatic colorectal cancer trial (known as the BICC-C trial) as well as several other company sponsored and large cooperative group trials (Table 4). These trials will look for associations between toxicity, efficacy, and genotype for numerous genes in addition to UGT1A1. These activities underscore Pfizer's strong commitment to fully

investigate and understand the potential value of genotyping in improving the safety and efficacy of irinotecan.

Table 4. Irinotecan Clinical Trials with a Pharmacogenomic Component[1]

Protocol	Enrollment Goal	Correlative Studies
Aventis V307 (PETACC3)	>1000 (1 of 2 arms with irinotecan)	LOH, MSI, SMAD-4 Immunopath: TS, DPD, TP, telomerase,
NCCTG N9741[2]	520[3]	ABCB1, ABCG2, CYP3A4/5, DYPD, ERCC2, GSTM1/GSTP1, MTHFR, TS, UGTA1A, XRCC1
SWOG 0124	620 (1 of 2 arms with irinotecan)	UGT1A1, ERCC-1, XRCC1
NCCTG N0147	4800 (2 of 3 arms with irinotecan)	UGT1A1, TS, MTHFR, excision repair cross complementation Immunopath: TS, DYPD, carboylesterase-2, EGFR, topo I MSI
440E-ONC-0020-366 (Pfizer)	>250	Carboxylesterase family, UGT1A family, c-MOAT, CYP3A4/5, DYPD, topo I, BCRP/ABCG2, TS, β-glucuronidase family, COX-2
CALGB 80203[4]	2200 (1 of 2 arms with irinotecan)	TYMS, DYPD, MTHFR, UGT1A1, CYP3A4/5, ABCB1, GSTP1, XRCC1, ERCC2, EGFR
E3301[4]	72	UGT1A1 Immunopath: VEGF, COX-2

[1] Trials are in progress unless otherwise specified.
[2] Trial completed; pharmacogenomic analysis is in progress.
[3] Number of patients for whom genomic analyses were performed.
[4] Not a Pfizer-sponsored study.

The inability to replicate many results from association studies for detection of genetic variants contributing to common complex traits may be due to several factors, including confounding from population structure, variability of phenotype, and allelic heterogeneity (multiple alleles contributing to same phenotype). In addition to these factors, publication bias, failure to attribute results to chance, and inadequate sample sizes, can contribute to the lack of replication [Colhoun 2003; Cardon 2001].

In order to avoid some of the limitations of small pharmacogenomic studies investigating the association of genetic variants with safety and efficacy end points with irinotecan treatment, Pfizer sponsors and actively participates in several large ongoing trials.

It is clear that the body of evidence is growing rapidly and warrants further monitoring. Pfizer hopes to place this literature into context with data from the phase III studies in the near future and will continue to work with the medical and scientific community and the Agency to address this important question.

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