Expert Opinion



# Ethnic differences in the metabolism, toxicology and efficacy of three anticancer drugs

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***Introduction:*** Large inter-individual and inter-ethnic differences are observed in efficacies and toxicities of medical drugs. To improve the predictability of these differences, pharmacogenetic information has been applied to clinical situations. Expanding pharmacogenetic information would be a valuable tool to the medical community as well as the patient to fulfill the promise of personalized anticancer drug therapy.

***Areas covered:*** This review highlights genetic polymorphisms and ethnic differences of genes, *UGT1As, CYP3A4, CES1As, ABCB1, ABCC2, ABCG2, SLCO1B1, CDA* and *CYP2D6,* involved in metabolism and disposition of three anticancer drugs: irinotecan, gemcitabine and tamoxifen.

***Expert opinion:*** Recent pharmacogenetic studies have successfully identified distinct ethnic differences in genetic polymorphisms that are potentially involved in efficacies and toxicities of anticancer drugs. This achievement has led to personalized irinotecan therapy, reflecting ethnic differences in *UGT1A1* genotypes, and possible benefits of genetic testing have also been suggested for gemcitabine and tamoxifen therapy, which still requires further validation. The ultimate goal for patients is a high rate or even perfect prediction of efficacies and toxicities of anticancer drugs in each ethnic population. For this challenge, more clinical studies combined with comprehensive omics approaches are necessary to further advance the field.

**Keywords:** ethnic difference, gemcitabine, genetic polymorphism, haplotype, irinotecan, pharmacogenomics, tamoxifen

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## 1. Introduction

The term ethnic group refers to a group of people who share a common heritage, often consisting of a common language, social background and culture. Ethnicity is an important factor for drug metabolism, disposition and response including efficacy and toxicity. Ethnic differences in drug responses are now recognized to be determined by genetic and environmental factors to a varying extent, depending on the drug. 'Pharmacogenomics', defined as an academic field studying the influence of genetic factors on drug responses, mainly targets genetic polymorphisms such as single nucleotide polymorphisms (SNPs). In recent pharmacogenetic research for personalized therapy based on genetic testing, issues of ethnic differences in genetic polymorphisms have been an area of focus. The current review describes three representative anticancer drugs of which the distinct ethnic differences in genetic polymorphisms of their metabolic enzymes have been demonstrated and recently validated to some extent. Irinotecan is one of the most extensively studied drugs and ethnic-dependent genotyping of a detoxifying enzyme has been clinically applied for predicting severe neutropenia. The detoxifying enzyme of gemcitabine has also been the subject of study due to its large ethnic difference in

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**Article highlights.**

- Recent progress in pharmacogenomic research on anticancer drugs has successfully identified several useful genetic markers of drug metabolism/disposition, which potentially affect toxicity and efficacy. These genetic markers have distinct ethnic frequencies.
- Cumulative evidence of the association of *UGT1A1* genotypes with severe toxicities, especially neutropenia, after irinotecan therapy, has led to the clinical application of *UGT1A1* genetic testing in the US (for *UGT1A1*28*) and in Japan (for *UGT1A1*28* and **6*), reflecting ethnic differences in high risk groups.
- Gemcitabine toxicities, especially neutropenia, can be predicted by reduced cytidine deaminase activity, and *CDA*3* (208G>A, A70T) is a responsible factor for reduced enzyme activity in East Asians and some African populations. Effects of *CDA*2* (79A>C, K27Q) vary depending on the study.
- Efficacy of tamoxifen could be predicted by *CYP2D6* genotypes but these predictions still need validation. Larger prospective randomized clinical trials that compare outcomes between genotype-guided and non-guided dosing of tamoxifen are necessary for concluding whether *CYP2D6* genotyping is really useful for tamoxifen therapy.
- To improve more precise predictions of toxicity/efficacy and identify responsible factors for their ethnic differences, further comprehensive omics approaches are needed for each ethnic group.

This box summarizes key points contained in the article.

functional SNP frequencies and their possible relation to toxicity. An activating enzyme of tamoxifen has emerged to be highly polymorphic among ethnic groups and the relation of its genetic polymorphisms to efficacy is discussed. Based on cumulative clinical evidence, the main themes in this review are toxicities for irinotecan and gemcitabine, and efficacy for tamoxifen.

## 2. Irinotecan

For the past decade, extensive irinotecan pharmacogenetic studies have been conducted in different ethnic populations and demonstrated the significant relevance of *UGT1A1* polymorphisms to irinotecan toxicities in an ethnic-dependent manner. These efforts have brought about the realization of personalized irinotecan therapy in the US (August 2005) and in Japan (March 2009). From these studies, the importance of considering ethnic differences in haplotype structures covering the *UGT1A* gene complex has also emerged. This section overviews the cumulative evidence of ethnic-dependent genetic polymorphisms/haplotypes that are related to the risk of irinotecan toxicities, particularly focusing on uridine diphosphate glucuronosyltransferase (UGT)1A enzymes and also recent findings on transporters and other enzymes.

### 2.1 Irinotecan metabolism/disposition and toxicity

Irinotecan is widely applied to the treatment of a broad range of carcinomas, including colorectal and lung cancers. The active metabolite SN-38 (7-ethyl-10-hydroxycamptothecin), a topoisomerase I inhibitor, is generated by hydrolysis of the parent compound by carboxylesterases (CESs) in the liver, and is subsequently glucuronidated by UGT1A, such as 1A1, 1A7 and 1A9, to form an inactive metabolite, SN-38 glucuronide (SN-38G) (Figure 1) [1-4]. Irinotecan is also inactivated by CYP3A4 to produce 7-ethyl-10-[4-N-(5-aminopentanoicacid)-1-piperidino] carbonyloxycamptothecin (APC) and 7-ethyl-10-(4-amino-1-piperidino)carbonyloxycamptothecin [5]. Irinotecan and its metabolites are excreted into bile and urine via the action of ATP-binding cassette (ABC) transporters, such as P-glycoprotein (P-gp/ABCB1), multi-drug resistance-associated protein 2 (MRP2/ABCC2) and breast cancer resistance protein (BCRP/ABCG2) [6]. Transport of SN-38 from plasma into the liver is mediated by the organic anion transporting polypeptide C (OATP-C/SLCO1B1) [7]. Multi-drug resistance-associated protein 1 (MRP1/ABCC1) is also known to contribute to acquisition of irinotecan resistance in cancer cells [8].

Dose-limiting toxicities in irinotecan therapy are severe diarrhea and leucopenia, in which increased plasma SN-38 levels mainly via lowered UGT activity are thought to be involved [9]. Because biliary SN-38G excreted into the small intestine is cleaved by bacterial glucuronidases in the colon to re-regenerate SN-38, this process is also assumed to be one of the mechanisms of late-onset diarrhea [10].

### 2.2 Genetic polymorphisms and ethnic differences of *UGT1As*

#### 2.2.1 *Structure of UGT1A genes*

UGTs catalyze the transfer of glucuronoic acid to a variety of endogenous and exogenous compounds and facilitate their detoxification and excretion into the bile or urine. Among four human UGT subfamilies (i.e., UGT1, UGT2, UGT3 and UGT8), UGT1A isozymes (especially, 1A1, 1A7, 1A9 and 1A10) are demonstrated to glucuronidate SN-38 [1-4,11]. UGT1A1 and 1A9 are expressed in the liver and the gastrointestinal tract, while UGT1A7 and 1A10 are detected in extrahepatic tissues [12]. The human *UGT1A* gene complex spans ~ 200 kb on chromosome 2q37, and consists of nine active (*1A8, 1A10, 1A9, 1A7, 1A6, 1A5, 1A4, 1A3* and *1A1*) and four inactive (*1A12P, 1A11P, 1A13P* and *1A2P*) exon 1 segments and common exons 2 – 5 (Block C) (Figure 2). Each *UGT1A* gene transcript is formed by splicing one exon 1 (encoding N-terminal substrate-binding domain) with the common exons 2 – 5 (encoding C-terminal UDP-glucuronoic acid-binding domain) [13].

#### 2.2.2 *Major polymorphisms of UGT1As affecting SN-38 glucuronidation*

UGT1A1 is abundantly expressed in the liver and is responsible for bilirubin glucuronidation in humans. Presently, > 100

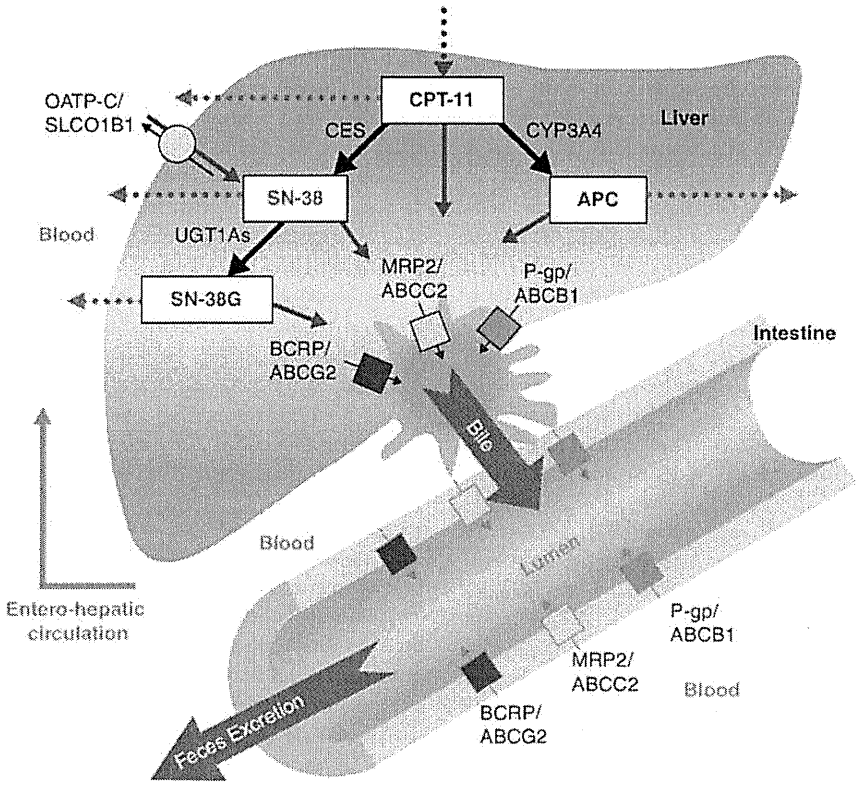


**Figure 1. Metabolism and excretion pathways of irinotecan.**

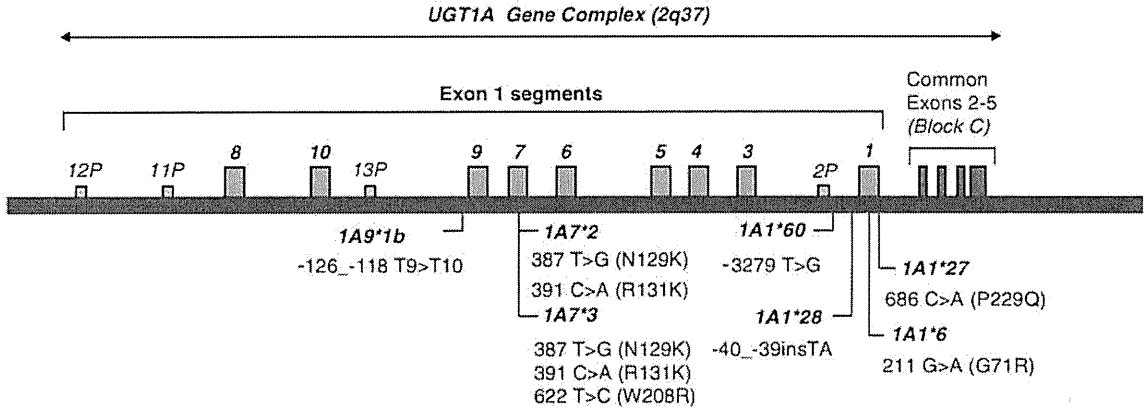


**Figure 2. Structure of the *UGT1A* gene complex and the major *UGT1A1, 1A7* and *1A9* polymorphisms.**

genetic variations of *UGT1A1* have been identified, which include causative variations for hyperbilirubinemia, such as Gilbert's syndrome and Crigler-Najjar syndrome types I and II (http://www.pharmacogenomics.pha.ulaval.ca/sgc/ugt).

The most typical *UGT1A1* polymorphism is a variation of the number of TA repeats ($A(TA)_nTAA$, n = 5 – 8) in the promoter region. The wild-type allele contains six TA repeats (n = 6) that are located -54 to -39 from the translational start codon. *UGT1A1*28* (n = 7), a common variation in Gilbert's syndrome [14], has reduced transcriptional activity (63% of the wild-type allele) [15] and rare variations **36* (n = 5) and **37* (n = 8) have enhanced (130%) and reduced (50%)

transcriptional activity, respectively (Table 1). *UGT1A1*28* shows high frequencies in Africans (35 – 56%) and Caucasians (26 – 39%) but lower frequencies in East Asians (7 – 16%) [16-20]. *UGT1A1*36* and **37* are detected in Africans (4 – 10% and 1 – 12%) and Caucasians (0.5 – 1.7% and 0 – 0.9%), but not in Asians [18]. *UGT1A1*6* [211G>A (G71R)] was originally found in Japanese patients with Crigler-Najjar type II and Gilbert's syndrome and has shown reduced SN-38 glucuronidation activity [3,21]. *UGT1A1*6* is commonly found in East Asians (13 – 24%) but rarely detected in Caucasians and Africans [17-19,22]. The *UGT1A1*60* allele (-3279T>G), located in the distal enhancer region (phenobarbital-responsive enhancer module; PBREM), has reduced transcriptional activity and was associated with increased plasma bilirubin levels [23]. The **60* are commonly found in Africans (63 – 85%) and Caucasians (35 – 55%) and to a lesser extent in East Asians (17 – 33%) [16,18,20,22]. A rare variation *UGT1A1*27* [686C>A (P229Q)] in exon 1 is mostly found in Asians with Gilbert's syndrome [18,22,24-26] and was shown to have lower or marginal SN-38 glucuronidation activity [3,21]. Another rare variation *1A1*7* [1456T>G (Y486D)] in exon 5 detected in Asians with Crigler-Najjar type II [18,26] showed reduced SN-38 glucuronidation activity [3,21]. It is also recognized that the frequencies of *UGT1A1* polymorphisms of South and Middle East Asians are almost the same as those of Caucasians [18].

Among *1A7* variations, *1A7*3* [387T>G, 391C>A, 622C>T (N129K, R131K, W208R)] and *1A7*4* [622C>T (W208R)] were shown to cause reduced SN-38G formation *in vitro* [3,27]. The frequencies of *1A7*3* are 22 – 27% in Africans, 37 – 41% in Caucasians and 17 – 26% in Asians [20,22,25,27-30].

As for *1A9* variations, *1A9*1b* (originally named **22*) [-118 (T)9>10)] is commonly detected in all ethnic groups surveyed (36 – 67%) [16,19,20,25,28,31-35]. This *1A9* polymorphism was reported to enhance transcriptional activity *in vitro* [31], but evidence of clinical significance was not obtained (see Section 2.3.2.1).

### 2.2.3 Haplotype structures and ethnic differences of *UGT1A*

#### 2.2.3.1 *UGT1A1*

The first haplotype analysis of the *UGT1A1* enhancer (PBREM)/promoter region was conducted in Caucasians and African-Americans and revealed a close linkage between the (TA)7 polymorphism (**28*), -3279T>G (**60*) and -3156G>A (**93*) [36]. Subsequently, distinct *UGT1A1* haplotypes extending to exon 1 were identified in Japanese [37]. This study revealed that the **6* allele is in complete linkage disequilibrium (LD) ($|D'| = 1$) with the **28* allele [(TA)7)], and haplotype analysis estimated that the **6* and **28* alleles [(TA)7] are on distinct chromosomes, and thereby haplotypes **6* and **28* were defined according to functional allele names. The **27* allele [686C>A (P229Q)] was also found to be in complete LD with the **28* allele [(TA)7], and the haplotype harboring the **27* allele was estimated as a subtype of the **28* haplotype group (**28c*) because of the existence of the **28* allele on the same chromosome. The haplotypes in this study were defined as follows: **1* haplotype as the wild type without minor alleles of the four polymorphisms (**60, *28, *6* and **27*), **6* haplotype with only the **6* allele, **28* haplotype as having both **60* and **28* alleles, and **60* haplotype as bearing only the **60* allele.

Further studies have also confirmed the ethnic specific *UGT1A1* haplotype structures, that is, **6* for East Asians (frequencies of 14 – 24%), **36b* (including (TA)5) (1 – 2% and 4%) and **37b* (including (TA)8) for Caucasians and Africans (1 and 7 – 12%, respectively), and the higher frequency of **28* (including (TA)7) in Caucasians (36 – 39%) and Africans (35 – 45%) than in the East Asians (6 – 13%) [24,28,36-38].

#### 2.2.3.2 *1A9-1A7-1A1*

The combinatorial haplotypes among *1A9, 1A7* and *1A1* were first estimated in Japanese subjects [25]. This study revealed close linkages between *1A9*1b* [-118(T)10] and *1A7*1*, between *1A7*2* and the *1A1*60* haplotype [-3279T>G without (TA)7] and between *1A7*3* and *1A1*6* or *1A1*28*. The five major combinatorial haplotypes (*1A9-1A7-1A1*) consisted of **1b-*1-*1, *1-*3-*6, *1-*2-*60, *1b-*1-*28* and **1-*3-*28* at frequencies of 58, 13, 11, 5.8 and 5.6%, respectively (Table 2). Comparable haplotype structures covering *1A9-1A7-1A1* were also found in Korean cancer patients (Table 2) [28], but the frequency of **1-*3-*6* (*1A9-1A7-1A1*) (20.4%) was much higher than **1-*3-*28* (0.6%).

The close linkages between the *UGT1A1*28* and *UGT1A7*3* [29], *1A7*2* or **3* and *1A9*1* [-118(T)9] [39], or the distinct haplotype structures of *1A9-1A1* between Caucasians and Asians were also demonstrated [32]. Combinatorial haplotype analysis through *1A9-1A7-1A1* segments was also conducted in Caucasians [20]. The five major combinatorial haplotypes (*1A9-1A7-1A1*) were **1b-*1-*1, *1-*3-*28, *1-*2-*1, *1-*3-*60* and **1-*2-*60* at frequencies of 33, 27, 14, 5.1 and 4.6%, respectively (Table 2). This study showed close linkage of *1A7*3* with *1A1*28* as previously reported, but this linkage was weaker in Caucasians than in East Asians, in which linkage of *1A7*3* with **28* or **6* was observed.

These comprehensive haplotype analyses on *UGT1A* genes have revealed linkages among functional polymorphisms of *1A9, 1A7* and *1A1*, and that their combinatorial haplotype structures vary among ethnicity.

## 2.3 Association and ethnic dependency of *UGT1A* polymorphisms with PK and adverse reactions of irinotecan

### 2.3.1 Contribution of *UGT1A1* genotypes

Based on cumulative knowledge of *UGT1A1* polymorphisms, initial irinotecan pharmacogenetic studies had mainly focused on *UGT1A1* polymorphisms. The first retrospective study was reported by Ando *et al.* [40], showing significant

**Table 1. A list of genetic variations for irinotecan pharmacogenetic studies referenced in this paper.**

| Allele (haplotype) | | rs | Region | Nucleotide change | Amino-acid change | *In vitro* (or cell line) | | Allele frequency | | Ref. | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | | | Activity/ expression | Africans | Caucasians | East Asians | *In vitro* Enzymatic activity | Frequency |
| *UGT1A1*[a] | *6 | rs4148323 | Exon 1 | 211G>A | G71R | Reduced | 0 | 0 - 0.007 | 0.130 - 0.241 | [3,21] | [17-19,22] |
| | *7 | rs34993780 | Exon 5 | 1456T>G | Y486D | Reduced | 0 | 0 | 0 - 0.014 | [3,21] | [24-26] |
| | *27 | rs35350960 | Exon 1 | 686C>A | P229Q | Reduced or no change | 0 | 0 | 0.003 - 0.01 | [3,21] | [22,24-25] |
| | *28 | rs8175347 | Promoter A(TA)nTAA | (TA)6 >(TA)7 | | Reduced | 0.346 - 0.560 | 0.257 - 0.388 | 0.068 - 0.160 | [15] | [16-20] |
| | *36 | | Promoter A(TA)nTAA | (TA)6 >(TA)5 | | Increased | 0.036 - 0.10 | 0.005 - 0.017 | 0 | [15] | [18] |
| | *37 | | Promoter A(TA)nTAA | (TA)6 >(TA)8 | | Reduced | 0.009 - 0.115 | 0. - 0.009 | 0 | [15] | [18] |
| | *60 | rs3755319 | PBREM | -3279T>G | | Reduced | 0.632 - 0.851 | 0.351 - 0.550 | 0.167 - 0.327 | [23] | [16,18,20,22] |
| | *93 | rs10929302 | PBREM | -3156G>A | | | 0.28 - 0.37 | 0.27 - 0.31 | 0.09 - 0.13 | | [16,20,22,25,36] |
| *UGT1A7*[a] | (*2, *3) | rs17868323 | Exon 1 | 387T>G | N129K | Reduced or no change | NA | 0.60 - 0.65 | 0.35 - 0.42 | [3,27] | [20,25,28-30] |
| | (*2, *3) | rs17863778 | Exon 1 | 391C>A | R131K | Reduced | NA | 0.60 - 0.65 | 0.35 - 0.42 | [3,27] | [25,28-30] |
| | (*3, *4) | rs11692021 | Exon 1 | 622T>C | W208R | Reduced | 0.22 - 0.27 | 0.37 - 0.41 | 0.17 - 0.26 | [3,27] | [20,22,25,27-30] |
| *UGT1A9*[a] | *1b or *22[b] | rs35426722 | Promoter118(T)n | (T)9 >(T)10 | | Increased or no change | 0.44 | 0.36 - 0.42 | 0.44 - 0.67 | [16,31] | [16,19,20,25,28, 31-35] |
| *ABCB1* | (*2) | rs1128503 | Exon 12 | 1236C>T | G412G | | 0.124 - 0.215 | 0.340 - 0.451 | 0.572 - 0.720 | | [22,66,68,69,76] |
| | (*2) | rs2032582 | Exon 21 | 2677G>T | A893S | Conflicting | 0.087 - 0.150 | 0.380 - 0.469 | 0.360 - 0.589 | [6,66] | [22,66,68,69,76] |
| | (*10) | | Exon 21 | 2677G>A | A893T | | 0.005 | 0.02 - 0.10 | 0.120 - 0.220 | | [66,68-69] |
| | (*2) | rs1045642 | Exon 26 | 3435C>T | I1145I | Conflicting | 0.100 - 0.270 | 0.460 - 0.590 | 0.369 - 0.540 | [6,66] | [22,66,68,69,76] |
| | *2 | | | 1236T/2677T/ 3435T | A893S | | 0.075 | 0.410 | 0.365 - 0.386 | | [68,69] |
| *ABCC1* | | rs3765129 | Intron 11 | IVS11-48C>T | | | 0.004 - 0.028 | 0.168 - 0.172 | 0.087 - 0.125 | | [22,76] |
| *ABCC2* | | | 5'-Flank | -1774delG | | Reduced | - | - | 0.34 - 0.343 | [72] | [72,73] |
| | | rs717020 | Exon 1 | -24C>T | | Reduced | 0.22 | 0.179 - 0.195 | 0.17 - 0.248 | [72,77] | [22,72-76] |
| | | rs3740066 | Exon 28 | 3972C>T | I1324I | No change | 0.275 | 0.336 - 0.0365 | 0.216 - 0.299 | [72] | [22,72-76] |
| *ABCG2* | | rs2622604 | Intron 1 | T>C | | | 0.080 - 0.135 | 0.252 | 0.106 - 0.229 | | [22] |
| | | rs2231137 | Exon 2 | 34G>A | V12M | No change | 0.05 | 0.017 - 0.065 | 0.192 - 0.289 | [80] | [22,76,82-83] |
| | | rs2231142 | Exon 5 | 421C>A | Q141K | Reduced | 0.004 - 0.038 | 0.075 - 0.115 | 0.292 - 0.343 | [80,81] | [22,76,82-83] |
| *SLCO1B1* | (*1b, *15,*17) | rs2306283 | Exon 5 | 388A>G | N130D | No change | 0.74 - 0.829 | 0.3 - 0.456 | 0.651 - 0.827 | [86] | [22,76,86,88-89] |
| | (*5, *15, *17) | rs4149056 | Exon 6 | 521T>C | V174A | Reduced | 0.009 - 0.115 | 0.14 - 0.189 | 0.07 - 0.175 | [7] | [22,76,86,88-89] |
| *CES1A3* | Pseudogene | | | | | No function | 0.949 | 0.856 | 0.675 - 0.687 | [106] | [106,107] |
| *CYP3A4* | *1B | rs2740574 | 5'-flank | -392A>G | | Conflicting | 0.530 - 0.866 | 0.025 - 0.096 | 0 | [18] | [18,22] |
| | *3 | rs4986910 | Exon 12 | 1334T>C | M445T | No change | - | 0.013 - 0.042 | 0 | [99] | [18,22] |
| | *16 | rs12721627 | Exon 7 | 554C>G | T185S | Reduced | - | - | 0.014 - 0.050 | [97,98] | [18] |
| | *18 | rs28371759 | Exon 10 | 878T>C | L293P | Increased/ no change/ decreased | - | - | 0.017 - 0.028 | [97,98,96] | [18,22] |

[a]UGT alleles nomenclature (http://www.pharmacogenomics.pha.ulaval.ca/sgc/ugt_alleles as of 1 October 2010).

[b]Original haplotype name.

PBREM: Phenobarbital-responsive enhancer module.

**Table 2. Comparison of combinatorial haplotypes of *UGT1A1, 1A7* and *1A9* between Caucasians and Asians.**

| Combinatorial haplotype[a] | | | | | Haplotype frequency[b] | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| *1A9* | - | *1A7* | - | *1A1* | Caucasians[c] (N = 250) | | Japanese[d] (N = 196) | | Koreans[e] (N = 81) | |
| **1b* | - | **1* | - | **1* | 33.4 | 54.4 | 58.3 | 59.5 | 47.5 | 51.8 |
| **1* | - | **2* | - | **1* | 14.4 | | 0.5 | | 0 | |
| **1* | - | **3* | - | **1* | 3.6 | | 0.5 | | 3.1 | |
| *Other combinations* | | | | **1* | 3 | | 0.2 | | 1.2 | |
| **1* | - | **2* | - | **60* | 4.6 | 11.8 | 11.3 | 12.7 | 14.8 | 17.2 |
| **1* | - | **3* | - | **60* | 5.1 | | 0.5 | | 0.6 | |
| *Other combinations* | | | | **60* | 2.1 | | 0.9 | | 1.8 | |
| **1* | - | **3* | - | **28* | 26.6 | 28.9 | 5.8 | 12.5 | 0.6 | 7.3 |
| **1b* | - | **1* | - | **28* | 1.6 | | 5.6 | | 4.9 | |
| *Other combinations* | | | | **28* | 0.7 | | 1.1 | | 1.8 | |
| **1* | - | **3* | - | **6* | | ND | 13.1 | 15.3 | 20.4 | 23.5 |
| *Other combinations* | | | | **6* | | | 2.2 | | 3.1 | |

[a]*UGT1A9*1b* is closely linked with *UGT1A7*1* and *UGT1A1*1* in both Caucasians and East Asians. *UGT1A7*3* is closely linked with *UGT1A1*28* or *6 in East Asians but weakly linked with *UGT1A1*28* in Caucasians.

[b]Data from the literature were arranged according to the haplotype definition by Saeki *et al.* (2006) [25].

[c]Cecchin *et al.* (2009) [20]. Only haplotypes with a frequency greater than 0.5% were included.

[d]Saeki *et al.* (2006) [25].

[e]Han *et al.* (2006) [28].

N: The number of subjects is listed in parentheses.

association of *UGT1A1*28* with irinotecan toxicities (grade 4 leucopenia and/or grade 3 or 4 diarrhea) in Japanese patients treated with various irinotecan-containing regimens. Although the effects of *1A1*6* and **27* were not significant in this study, their additive contributions to the toxicities were also suggested. Further prospective studies have also shown the significant relevance of *UGT1A1*28* to the reduced rate of SN-38G formation (AUC ratio of SN-38G: SN-38) and to grade 3 or 4 diarrhea [41], or grade 4 neutropenia [42] in caucasian populations after irinotecan monotherapy. The clinical importance of **28* to severe diarrhea or neutropenia after combination therapy with 5-fluorouracil (5-FU), raltitrexed, capecitabin or oxaliplatin was also shown in other caucasian populations (Table 3) [30,43-53].

In parallel with these findings on *UGT1A1*28*, clinical studies on the roles of other *UGT1A1* polymorphisms specific for East Asians, such as **6*, have also progressed. Additive and comparative effects of **6* to **28* on reduced SN-38G formation and severe neutropenia (grade 3/4) were shown in Japanese cancer patients after irinotecan monotherapy and combination therapy with cisplatin, which suggested the clinical importance of **6* as well as **28* [37,54-56]. The significant contribution of *1A1*6*, but not **28*, to reduced SN-38G formation and grade 4 neutropenia was reported in Korean patients after combination therapy of irinotecan plus cisplatin [28], where the **28* frequency (7.3%) was much lower than *1A1*6* (23.5%) and no **28* homozygous patients were found (Table 2). Significant associations of **6* or **28* (or **27*) [57-61] to severe toxicities (neutropenia) were also found in Chinese or Japanese populations (Table 3). These findings suggested that both **6* and **28* would be suitable markers to predict severe irinotecan toxicities for East Asians, although the frequencies of **6* and **28* vary among populations, which may affect statistical significance.

Regarding the **60* haplotype [-3279T>G without (TA)7], trends of **60* haplotype-dependent decrease in SN-38G formation [37,54] were observed in Japanese, but contribution of **60* to irinotecan severe toxicities was not demonstrated [28,54,62].

The **93* allele (-3156G>A), a variation closely linked to the **28* allele, was suggested as a better predictor for UGT1A1 status than the **28* allele in Caucasians [42], and recent studies showed significant relevance to severe neutropenia (Table 3) [49,63].

### 2.3.2 Relevance of 1A7 and 1A9 genotypes considering comprehensive UGT1A haplotypes

Because UGT1A7, 1A9 and 1A10, which are expressed in the gastrointestinal tract, also have SN-38G formation activity [2-4,11], their polymorphisms are assumed to contribute to irinotecan pharmacokinetics (PK)/pharmacodynamics (PD). Because recent comprehensive haplotype analyses have revealed ethnic-dependent linkages among *UGT1A* polymorphisms (see section 2.2.3.2), the possible relevance of *UGT1A7* and *1A9* polymorphisms was evaluated considering the combinatorial haplotypes (*1A9-1A7-1A1*).

#### 2.3.2.1 UGT1A9

Although *1A9*1b* [-118(T)10] was reported to enhance *in vitro* transcription [31], associations of *1A9*1b* with hepatic 1A9 protein levels or SN-38G formation were inconsistent

**Table 3. Association of genetic polymorphisms with severe irinotecan toxicities.**

| Ethnic group | N[a] | Responsible polymorphisms | Toxicity | Tumor type | Regimen | Ref. |
|---|---|---|---|---|---|---|
| Caucasians | 18 (2) | *UGT1A1*28* | Neutropenia, diarrhea (G3/4) | Various | Monotherapy | [41] |
| | 50(16) | *UGT1A1*28* | Neutropenia (G4) | Various | Monotherapy | [42] |
| | 95 | *UGT1A1*28* | Diarrhea (G3/4) | CRC | Monotherapy and combination (5-FU or raltitrexed) | [43] |
| | 75 | *UGT1A1*28* | Neutropenia (G3/4) | CRC | Combination (5-FU) | [44] |
| | 49 | *UGT1A1*28* | Neutropenia (G3/4) | CRC | Combination (5-FU) | [45] |
| | 103[b] | *UGT1A1*28* | Neutropenia (G4) | CRC | Combination (oxaliplatin) | [46] |
| | 250 | *UGT1A1*28* | Neutropenia (G3/4) (first cycle) | CRC | Combination (FOLFIRI) | [47] |
| | 56 | *UGT1A1*28* | Diarrhea (G3/4) | CRC | Combination (raltitrexed) | [48] |
| | 105 | *UGT1A1*28* *UGT1A7*3* | Hematologic toxicity (G3/4), diarrhea (G3) | CRC | Combination (5-FU/folic acid, oxaliplatin) | [30] |
| | 55 (11) | *UGT1A7(*2, *3) - 1A9*1*[-118(T)9] | Diarrhea (G3/4) | CRC | Combination (capecitabine) | [39] |
| | 250 | *UGT1A7*3* | Hematologic toxicity (G3/4) (first cycle) | CRC | Combination (FOLFIRI) | [20] |
| | 89 | *UGT1A1*28* *UGT1A1*93* | Neutropenia (G4) | CRC | Combination (LV5FU2) | [49] |
| | 25 (25) | *UGT1A1*28* *ABCB1*1236CC (wild) *SLCO1B1*521CC (wild) | Diarrhea (G3/4) | CRC | Combination (5-FU + LV) | [50] |
| | 208 | *UGT1A1*28* | Neutropenia (G3/4) | CRC | Monotherapy and combination (capecitabin) | [51] |
| | 136 | *UGT1A1*28* *ABCB1*3435C>T | Neutropenia (G3/4) | CRC | Combination (FUIRI or Lv5FU2-IRI) | [52] |
| | 149 | *UGT1A1*28* | Hematologic toxicity (G3/4) | CRC | Combination (FUIRI or FOLFOX) | [53] |
| | | *UGT1A1*28* *UGT1A7*3* | Non-hematologic toxicity (G3/4) | | | |
| | 67 (19) | *UGT1A1*93* *ABCC1* IVS11-48C>T, *SLCO1B1*1b* | Neutropenia (ANC nadir) | | Monotherapy | [76] |
| | 1[c] | *UGT1A1 *28* *SLCO1B1*15* | Neutropenia (G4) | Nasopharynx | Combination (vincristin) | [92] |
| Japanese | 118 | *UGT1A1*28* | Leucopenia (G4) and/or diarrhea (G3/4) | Various | Monotherapy and combination (various) | [40] |
| | 55 | *UGT1A1*6 and *28* *ABCC2-1774delG* *ABCG2*IIB SLCO1B1*15* *ABCB1*2* | Neutropenia (G3/4) Diarrhea (G3) | Various | Monotherapy | [54,71] |
| | 30 | *UGT1A1*6 and *28* | Neutropenia (G3/4) | Ovarian Cervical | Combination (cisplatin) | [56] |
| | 49 | *UGT1A1*6* | Neutropenia (G3/4) | Various | Monotherapy | [58] |
| | 133 | *UGT1A1*6* | Neutropenia (G3/4) | Various | Monotherapy and combination (various) | [59] |
| | 78 | *UGT1A1*6,* *UGT1A1*27* | Neutropenia (G4) | NSCLC | Combination (palitaxel or gemcitaine) | [61] |
| | 87 | *UGT1A1*93,* *ABCB1*3435C>T | Neutropenia (≧ G3) Diarrhea (≧ G3) | SCLC | Combination (cisplatin) | [63] |
| | 108 | *ABCG2* rs2622604 | Myelosuppression (G3/4) | Various | Monotherapy and combination | [85] |
| | 11 | *SLCO1B1*15* | Neutropenia (G4) | | | [100] |

[a]Number of subjects. Number of other ethnicities is described in parentheses.

[b]A subgroup consisting of 87% Caucasians.

[c]Case report.

[d]*UGT1A1*6* was not determined.

CRC: Colorectal cancer; SCLC: Small cell lung cancer.

**Table 3. Association of genetic polymorphisms with severe irinotecan toxicities (continued).**

| Ethnic group | N[a] | Responsible polymorphisms | Toxicity | Tumor type | Regimen | Ref. |
|---|---|---|---|---|---|---|
| | | | | | Monotherapy and combination (cisplatin) | |
| | 1[c] | *UGT1A1 *6 and *28 SLCO1B1*15* | Diarrhea (G4) Neutropenia (G4) | Pharyngeal | Combination (docetaxel) | [91] |
| Koreans | 81 | *UGT1A1*6* | Neutropenia (G4) | NSCLC | Combination (cisplatin) | [28] |
| | 107 | *UGT1A1*6* | Neutropenia (G4) | NSCLC | Combination (cisplatin) | [79] |
| | | *UGT1A9*1 and *1/*1b* | | | | |
| | | *SLCO1B1* 521T>C | | | | |
| | | *UGT1A9*1* | Diarrhea (G3) | | | |
| | | *ABCC23972CC(wild)* | | | | |
| | | *ABCG2* 34G>A (V12M) | | | | |
| Chinese | 36 (19) | *UGT1A1*6* | Neutropenia (G4) | Various | Monotherapy | [57] |
| | 128 | *UGT1A1*28*[d] | Neutropenia (G3/4), diarrhea (G3/4) | CRC | Combination (5-FU + LV) | [60] |

[a]Number of subjects. Number of other ethnicities is described in parentheses.
[b]A subgroup consisting of 87% Caucasians.
[c]Case report.
[d]*UGT1A1*6* was not determined.
CRC: Colorectal cancer; SCLC: Small cell lung cancer.

among studies [16,32-33]. Considering the combinatorial haplotypes of *1A9-1A1*, *1A9*1b*-dependent increase in SN-38G AUC and reduction in severe toxicities were related to its close linkage with *1A1*1*, and thus the clinical significance of *1A9*1b* was not demonstrated [20,54].

#### 2.3.2.2 *UGT1A7*

Regarding *1A7* polymorphisms, no associations of *1A7*2* and **3* with severe toxicities (grade 4 leukopenia or grade 3 diarrhea) were observed in Japanese patients [64]. Another study in Japanese patients considering the combinatorial haplotypes (*1A9-1A7-1A1*) revealed that alterations in the AUC ratio (SN-38G:SN-38) and the incidence of neutropenia were independent of the *1A9* or *1A7* haplotypes but *UGT1A1*6 or *28* were the most suitable markers to predict severe neutropenia [54]. In Korean patients, the association between homozygous *1A9*1- 1A7*3 - 1A1*6* and lowered AUC ratios (SN-38G:SN-38) was observed as well as that between homozygous *1A1*6* and grade 4 neutropenia [28]. Because of the close linkage of *1A7*3* to *1A1*6* and/or *1A9*1* [-118(T)9], the benefit of genotyping *1A1*6* was suggested for Korean patients [28].

Meanwhile, linkage of *UGT1A7*3* to **28* is incomplete, and the possible clinical contribution of *1A7*3* in addition to **28* was suggested in Caucasians. Association of *1A7*2* or **3* subtype, which was linked to *1A9*1* [-118(T)9] but not to *UGT1A1*28*, with reduced incidence of grade 3 or 4 diarrhea was observed in colorectal cancer (CRC) patients (including 83% Caucasians) after capecitabine/irinotecan therapy (**Table 3**) [39]. This observation was interpreted to suggest that *UGT1A7* low-activity alleles protected against severe diarrhea by reduced SN-38G excretion into gut, resulting in reduced regeneration of SN-38 from SN-38G by bacterial β-glucuronidase. A significant association of *UGT1A7*3* with hematologic toxicities was observed in caucasian patients treated with irinotecan and 5-FU-containing regimens, and combination of *UGT1A1*28* and *UGT1A7*3* was indicated to be a superior predictor of hematological or non-hematological toxicities [30,53] and clinical outcome (**Table 3**) [20].

### 2.4 Contributions of transporter polymorphisms to irinotecan-PK-PD

Because there are still substantial numbers of patients with *UGT1A* wild type who suffer from severe irinotecan toxicities, the clinical significance of drug transporter genetic polymorphisms has been investigated in various ethnic groups, and these studies have also revealed distinct ethnic differences in transporter polymorphisms/haplotypes.

#### 2.4.1 *Ethnic differences in major polymorphisms of transporter genes*

For *ABCB1*, as association of common polymorphism 3435C>T with reduced P-gp expression/function has been reported [65], this SNP and haplotypes harboring 3435C>T and its linked 1236C>T and 2677G>T (A893S) have been an area of focus [66]. The frequency of *ABCB1*2* containing these three SNPs [67] is high in Caucasians and Asians (37 – 41%) but low in Africans (8%) (**Table 1**) [68,69]. Association of *ABCB1*2* with lower renal clearance of irinotecan and its metabolites [70] and with higher AUC of SN-38 and grade 3 diarrhea after monotherapy was observed in Japanese cancer patients [71]. Other studies have also shown association of *ABCB1* 3435TT with severe diarrhea after combination with cisplatin in Japanese [63] and with severe neutropenia in Caucasians after combination with 5-FU-containing regimens

(Table 3) [52]. Lower excretion from intestinal cells or bone marrow cells by *ABCB1*2* may be involved in these adverse reactions.

*ABCC2*-1774delG is commonly found in East Asians (34%) (Table 1) [72,73] and was associated with reduced MRP2 expression and chemical-induced hepatitis [72]. Patients with homozygous -1774delG together with *ABCB1*2 and ABCG2*IIB* (see below) showed higher SN-38 AUC and neutropenia after irinotecan monotherapy [71]. *ABCC2*-24C>T and its linked 3972C>T (Ile1324Ile) are common in all ethnic groups (18 – 25% and 21 – 34%, respectively) (Table 1) [22,72-76]. *ABCC2*-24C>T caused reduced promoter activity [72,77], but no *in vitro* effect of 3972C>T (I1324I) was shown [72]. Effects of *ABCC2* 3972C>T (I1324I) are conflicting among studies; higher AUC of irinotecan and its metabolites in Caucasians [78], lower incidence of grade 3 diarrhea in Koreans [79] and no effects of irinotecan-PK/PD in Japanese (Table 3) [71].

For *ABCG2*, 34G>A (V12M), which has no influence on BCRP expression or activity [80], and 421C>A (Q141K), which has reduced protein expression [80,81], are commonly detected in East Asians (19 – 29% and 29 – 34%, respectively) but less in Africans and Caucasians (5 – 7% and 0.4 – 12%, respectively) (Table 1) [22,76,82-83]. Association of *ABCG2* 34G>A (V12M) with a higher incidence of grade 3 diarrhea was shown in Korean patients after a combination therapy of irinotecan and cisplatin [79], but a decreasing trend in grade 3/4 neutropenia was observed in Japanese patients [71]. Haplotype **IIB* (including 421C>A (Q141K)) showed a moderate association with neutropenia in Japanese patients (Table 3) [71], but other studies did not show its clinical relevance [79,84-85].

For *SLCO1B1*, 388A>G (N130D) (tagging SNP of **1b*), which has no alteration in transporter activity [86] but is associated with altered PK of statins [87], is commonly observed in Asians and Africans (> 65%) and Caucasians (30 – 46%) [22,76,86,88-89]. *SLCO1B1* 521T>C (V174A) (tagging SNP of **5*, **15* and **17*), which reduced *in vitro* SN-38 influx [7], was commonly detected in all ethnic groups (7 – 19%) (Table 1). Association of 521T>C (V174A) with higher SN-38 AUC level and severe neutropenia after irinotecan-containing therapy was observed in East Asians (Table 3) [71,79,90-92].

A recent comprehensive analysis of 42 SNPs in African-American and Caucasian patients showed significant association of *ABCC1* IVS11-48C>T, *SLCO1B1*1b* as well as *UGT1A1*93* to neutropenia after irinotecan monotherapy [76]. Another study covering 170 SNPs in Japanese cancer patients revealed the association of *ABCG2* intron SNP with severe myelosuppression (Table 3) [85]. The clinical significance of these SNPs should be further investigated.

All these findings on the clinical relevance of transporter polymorphisms still vary among studies, which might be partly attributed to ethnic differences in combination with transporter genotypes and the regimens used. However, as additive effects of transporter polymorphisms to the incidence of severe toxicities were suggested [71], evaluation of total candidate polymorphisms rather than each individual SNP should be considered for each regimen.

## 2.5 Relevance of enzyme polymorphisms for another metabolic pathway (CYP3A4) and for activation (CESs)

### 2.5.1 *CYP3A4 polymorphisms*

Because correlations between *in vivo* CYP3A4 activity and irinotecan PK parameters have been shown [93], the clinical impact of *CYP3A4* polymorphisms on irinotecan therapy has been investigated. *CYP3A4*1B* (-392A>G), a SNP in the 5'-flanking region, is commonly found in African-Americans (53 – 87%) and to a lesser extent in Caucasians (3 – 10%), but not in Asians (Table 1) [22,18]. Clinical studies showed no significant impact of *CYP3A4*1B* on irinotecan-PK/PD in caucasian patients [94,95]. *CYP3A4*3*, found in Caucasians (1.3 – 4.2%) (Table 1) [18,22], showed insignificant association with reduced clearance of irinotecan lactone [94]. *CYP3A4*16* [554C>G (Thr185Ser), which was shown to have reduced enzymatic activity [94,95], is detected in East Asians (1.4 – 5%) and also in Mexicans (5%) but not in Africans or Caucasians (Table 1) [18]. Patients bearing *CYP3A4*16* showed significantly decreased AUC ratios (APC:irinotecan) after irinotecan treatment but no significant association with total clearance of irinotecan or toxicities [96]. *CYP3A4*18* [878T>C (L293P)] is also observed in East Asians specifically (1.7 – 2.8%) (Table 1) [18,22]. *In vitro* function of this allele has revealed variability among substrates [97-99], and no association with irinotecan-PK/PD was shown in Japanese patients [96]. Because CYP3A4 activity can be largely influenced by non-genetic factors, a benefit of CYP3A4-phenotyping reflecting non-genetic factors was suggested for irinotecan dosing as well as *UGT1A1* genotyping [100].

### 2.5.2 *CES genotype*

Both CES1 (expressed in liver and lung) and CES2 (expressed in intestine and kidney) are involved in producing SN-38. Hydrolytic activity of CES2 for irinotecan was reported to be much higher than that of CES1 [101]. Detected *CES2* variations were all rare [102,103] and no clinical relevance of major *CES2* polymorphisms has been demonstrated so far [45,76,85,94,103]. Recent studies have revealed the precise gene structure of human *CES1* and identified two functional genes, *CES1A1* (*1A1*) and *CES1A2* (*1A2*), which have 98% homology and are inversely located (tail-to-tail) on chromosome 16q13-q22 (*1A2-1A1*) [104]. Further studies have identified *CES1A1* variants (*var1A1*) in which exon 1 was replaced with exon 1 of *CES1A2*, and a pseudogene *CES1A3* (*1A3*) replaced *CES1A2* [105,106]. Four *CES1A* haplotypes (*1A2-1A1*, *1A2-var1A1*, *1A3-1A1* and *1A3-var1A1*) were defined and the *CES1A* genotypes were also categorized by the number of functional *CES1A* genes (*1A1*, *1A2* and

*varIA1*) into three groups (two, three or four functional genes). Frequencies of the pseudogene (*1A3*) are higher in Africans (95%) and Caucasians (86%) than in Asians (68 - 69%) (Table 1) [106,107]. Japanese patients with three or four active *CES1A* genes showed increased *in vivo* CES activity (PK parameter) compared with those with two functional genes, but the influence of the *CES* genotypes on SN-38 formation was relatively small and their clinical impact on irinotecan-PD has not been elucidated [107].

### 2.6 Relevance to efficacy of irinotecan therapy

The relevance of *UGT1A* genotypes to irinotecan efficacy is still controversial. One initial study showed a trend, although insignificant, of poorer overall survival (OS) in CRC patients with *UGT1A1*28* after irinotecan monotherapy or combination therapy with 5-FU (FOLFIRI) [43]. This poorer response was interpreted to be due to reduced irinotecan dosages because of severe diarrhea. Other studies also showed no significant relationship of *UGT1A1*28* or the haplotype including *UGT1A1*28* and *UGT1A7*3* to response rate (complete response (CR) + partial response (PR)) and time to progression or OS in the CRC patients after combination therapy with 5-FU, whereas their association with severe toxicities was significant [45,53]. No relationship of *UGT1A*6* and **27* genotypes to tumor response was reported in Japanese NSCLC patients after combination therapy with paclitaxel or gemcitabine [61]. On the other hand, a recent study with a larger number of CRC patients receiving FOLFIRI therapy has shown a significant relevance of *UGT1A1*28* and the haplotype including *UGT1A1*28, *93, *60* and *UGT1A7*3* to increased response rate (CR + PR), and also the relation of **28* to the prolongation of time to progression [20]. Furthermore, in a dose-escalation study of irinotecan in FOLFIRI therapy in CRC patients without *UGT1A1*28/*28*, the response rate (CR + PR) after receiving maximum tolerated dose was higher in patients with **28/*1* genotype compared with those with **1/*1* [108]. This study indicates that an appropriate dose setting based on *UGT1A* genotypes could achieve better efficacy by careful management for minimizing toxicity. However, there are several issues to be considered for obtaining better responses because efficacy could be influenced by several factors, such as function of pharmacodynamic genes, properties of target tumors (tumor stage, acquisition of resistance, etc.) and other patient background factors (hepatic/renal dysfunction, inflammatory status, etc.) in addition to the exposure level of SN-38 in the tissues, which would be a major causal factor for severe toxicities. The influence of genotypes of pharmacodynamic genes for irinotecan has also been investigated in caucasian patients with irinotecan monotherapy and a significant relevance of the haplotype of *XRCC1* harboring -1449del GGCC and 1196G>A (R399Q) to the reduced response (CR + PR) was observed [109]. Further study in Turkish CRC patients with irinotecan-containing regimens has also revealed a significant association of the *XRCC1* 1196G>A (R399Q) genotype with short OS although no influence of this SNP on tumor response was observed [110]. Taken together, further challenges include optimizing irinotecan dosage to achieve better responses as well as to minimize toxicities considering both genotypes and background factors in each regimen.

### 2.7 Regulatory status and current issues of irinotecan therapy

Based on cumulative evidence supporting the clinical significance of *UGT1A1*28*, the FDA in the US approved a label amendment for irinotecan (Camptosar) (NDA 20-571/S-024/S-027/S-028), adding a recommendation of a reduction in the starting dose by at least one level of irinotecan for *UGT1A1*28* homozygous patients (July 2005), and clinical use of a genetic diagnostic kit for the **28* allele (August 2005). Subsequently, in Japan, considering the clinical relevance of **6* in addition to **28* to irinotecan toxicities in East Asians, the Ministry of Health, Labor and Welfare of Japan approved changes of irinotecan labels (Campto® and Topotecin®) by adding a caution for the risk of severe toxicities in patients either homozygous or compound heterozygous for *UGT1A1*28* and **6* (**28/*28, *6/*6, *28/*6*) (June 2008) and the clinical use of a diagnostic kit for *UGT1A1*28* and **6* (March 2009). Furthermore, in Singapore where three Asian ethnic groups (i.e., Indians, Malays and Chinese) comprise the population, the Health Sciences Authority Pharmacogenetic Advisory Committee in Singapore has recommended updating the label for irinotecan considering the increased risk for serious adverse reaction associated with homozygotes and compound heterozygotes of *UGT1A1*28* and **6*, as in the case in Japan (http://www.hsa.gov.sg/publish/hsaportal/en/health_products_regulation/safety_information/product_safety_alerts/safety_alerts_2010/association_between.html). This introduction of the label change is based on the studies originated by Chowbay and co-workers [57] showing that genotype distributions of *UGT1A1* in Indians are similar to Caucasians (**28* is a dominant defective allele), those of Chinese are similar to Japanese (**6* as well as **28* are defective alleles) and those of Malays are in between. Thus, both **28* and **6* have been taken into account to cover all ethnic groups in Singapore.

Because of limited scales of retrospective investigations and moderate predictability, the genetic test is not mandatory. A recent meta-analysis on Caucasians (10 sets of patients) to evaluate the relation of irinotecan dosage and risk of toxicities [111] showed that the risk of hematologic toxicities was higher in patients with **28* homozygous who received middle and high doses of irinotecan (> 150 mg/m$^2$) but not low doses (< 150 mg/m$^2$), suggesting the benefit of **28*-genotyping only with regimens of higher doses of irinotecan. However, another meta-analysis on 15 clinical studies showed a significant association between **28* homozygotes and increased risk of neutropenia even at low doses of irinotecan regimens (< 150 mg/m$^2$) [112]. It should be noted that these studies did not include clinical studies in East Asians, where

*UGT1A1*6* and regimens containing co-administered drugs such as cisplatin or paclitaxel caused severe toxicities at low doses of irinotecan [26,36,52,61]. Further prospective studies are ongoing for appropriate irinotecan dose setting in Japan, based on the *UGT1A1* genotype **6 or *28*.

## 3. Gemcitabine

Gemcitabine (2′, 2′-difluorodeoxycytidine) is a deoxycytidine analog with antineoplastic activity against solid tumors including pancreatic and NSCLCs [113]. This section reviews the current pharmacogenomic status of gemcitabine-induced myelotoxicities as well as efficacy, and its ethnic differences.

### 3.1 Gemcitabine metabolism/disposition and toxicity

After infusion, gemcitabine is transported into cells by equilibrative and concentrative nucleoside transporters, and then phosphorylated initially by deoxycytidine kinase to 2′, 2′-difluorodeoxycytidine monophosphate (Figure 3) [114]. The drug is further phosphorylated to its active diphosphorylated and triphosphorylated forms, dFdCDP and dFdCTP. dFdCDP inhibits ribonucleotide reductase to deplete cellular deoxynucleotides pools, especially dCTP, and this is a self-potentiating mechanism of gemcitabine that leads to increased formation of active gemcitabine di- and triphosphates. Also, dFdCTP is incorporated into DNA and inhibits DNA synthesis. Most gemcitabine is rapidly metabolized by cytidine deaminase (CDA) to an inactive form 2′, 2′-difluorodeoxyuridine and excreted into urine.

Toxicities by gemcitabine are generally mild, but severe toxicities such as myelosuppression and interstitial pneumonia are occasionally observed [115]. The frequencies of grade 4 neutropenia were 13 and 6%, as described in the package inserts of Japan and the US, respectively.

### 3.2 Association of *CDA* polymorphisms and gemcitabine-induced toxicities

Because CDA is the enzyme responsible for detoxifying gemcitabine, its decrease in activity increases AUC of gemcitabine leading to enhanced metabolic activation and inhibition of DNA synthesis, resulting in high damage in tissues with active cell proliferation such as bone marrow. Until now, two non-synonymous SNPs 79A>C (K27Q) (**2*) and 208G>A (A70T) (**3*) and their functional significance have been reported [114].

Regarding 79A>C (K27Q) (**2*), minor (C) allele frequencies are 0.30 – 0.36 in Caucasians, 0.04 – 0.11 in Africans and 0.20 in Asians (Table 4) [116-119]. The recombinant enzyme with Gln27 showed reduced activity by 34% with an increase in $K_m$ for gemcitabine as compared to the wild-type enzyme with Lys27 [116]. However, the minor allele of this SNP has been shown to be associated with < 1.7-fold higher enzymatic activity for gemcitabine by catalytic tests using lysates of red blood cells taken from caucasian cancer patients [120,121]. In accordance with this, the minor allele (C) is associated with lower frequencies of grade 3 and 4 neutropenia in 65 caucasian NSCLC patients treated with gemcitabine and cisplatin [121]. On the other hand, another study in the US reported the association of the C allele with increased grade 3 and 4 toxicities in 55 gemcitabine- and cisplatin-treated patients with metastatic breast cancer [122]. Moreover, a recent caucasian study reported no significant effect of 79A>C on PK of gemcitabine [123]. Another recent study performed in France also showed no impact of 79A>C genotypes on serum CDA activity [124]. Similarly, no impact of 79A>C was observed for CDA activity, PK and toxicities of gemcitabine in 256 Japanese cancer patients [125]. The reason for these discrepancies is unknown. Difference in activities between recombinant Gln27 enzymes (reduced activity) and lysates of red blood cells taken from caucasian cancer patients (increased activity) may be caused by population-specific linkage of 79A>C with polymorphism(s) that can induce higher expression of CDA proteins. Another possible factor is regimen including concomitant drugs for treatment [126]. Because the effect of 79A>C on the CDA catalytic activities is small, precise and well-planned clinical studies using large sample sizes are necessary for evaluation of the actual impact of 79A>C polymorphism.

As for 208G>A (A70T) (**3*), the mutant enzyme expressed in yeast has reduced activity for both ara-C and cytidine [114]. Ethnic differences have been reported in the minor allelic frequency of 208G>A (Table 4) [116-119]. This polymorphism was detected in East Asian populations such as Japanese (0.022 – 0.043), Korean (0.005) and Chinese (0.005) and some Africans (Ghanaian + Kenyan, 0.131), but was not detected in Caucasians and African-Americans. Plasma of Japanese patients with the minor allele had reduced activity for gemcitabine and cytidine in an allele number-dependent manner [125]. Furthermore, an allele was associated with reduced clearance of gemcitabine, that is, ~ 80 and 20% of residual clearance values in heterozygotes and homozygotes for **3*, respectively, compared to that in the wild type. A constructed population pharmacokinetic model in Japanese showed that heterozygous and homozygous 208A alleles contribute to the 17.1 and 63.9% reduction in clearance [127]. In addition, 1 and 3 patients from 256 and 242 Japanese cancer patients administered gemcitabine, respectively, suffered from prolonged severe grade 4 myelosuppression, and 3 out of the 4 patients had homozygous A/A allele, suggesting that in Japanese, 208G>A is a major determinant for severe myelosuppression by gemcitabine [128,129]. In contrast to the Japanese studies, although > 10% show grade 3 and 4 myelotoxicities, 208G>A polymorphism has not been found in French studies including a case that ended in death [124,130]. The patients with toxicities had indeed > 70% lower CDA activity in serum [124]. Thus, no useful genetic markers have been found for non-East Asian patients, and other factors rather than 208G>A are probably involved in reducing enzymatic activities. Intensive re-sequencing of *CDA*

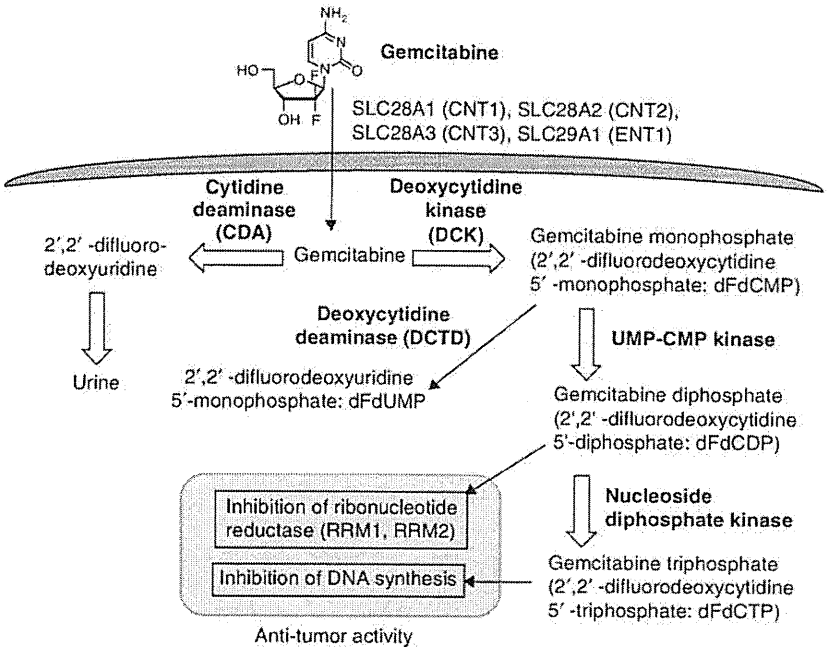


**Figure 3. Activation and inactivation pathways of gemcitabine.**

**Table 4. Ethnic differences in allele frequencies of *CDA* polymorphisms.**

| | *2<br>79A>C<br>Lys27Gln<br>rs2072671 | *3<br>208G>A<br>Ala70Thr<br>rs60369023 | Ref. |
|---|---|---|---|
| Japanese | 0.20 – 0.21 | 0.02 – 0.04 | [119,125] |
| Korean | 0.15 | 0.01 | [119] |
| Chinese | 0.12 | 0.01 | [118] |
| Chinese-Americans | 0.16 | 0 | [119] |
| Caucasian-Americans | 0.30 – 0.33 | 0 | [116,119] |
| Europeans (British) | 0.36 | 0 | [117] |
| African-Americans | 0.09 – 0.11 | 0 | [116,119] |
| Africans (Ghanaian + Kenyans) | 0.04 | 0.13 | [117] |

including transcriptional regulatory and all intronic regions is necessary to identify causative variation(s).

For other gemcitabine-related genes, a study in Asians has shown the significant association of *SLC28A1* 1561G>A (D521N) with decreased neutrophil and platelet nadir counts [131]. However, the variant protein was found to retain transporting function *in vitro*. This SNP is frequent in European Americans (minor allele frequency: 0.51) compared to African-Americans (0.10) and Asians (0.034) [132]. In addition, combinatorial effects of the SNPs on grade 3/4 neutropenia were also investigated for 17 SNPs of 8 genes for gemcitabine metabolism and disposition in 154 potentially resectable and 149 locally advanced pancreatic cancer patients (mostly caucasian patients) treated with gemcitabine-based chemotherapy [133,134]. Significantly associated SNPs were *CDA* 435C>T (T145T, rs1048977), *DCK* IVS6-1205C>T (rs4694362), *DCK* IVS2+9846A>G (rs12648166) and *CNT3* (*SLC28A3*) 267A>G (T89T, rs7867504) for the potentially resectable patients and *CDA**2 [79A>C (K27Q)], *DCK* IVS6-1205C>T, *RRM1* 2223A>G (T741T, rs3177016) and *ENT1* (*SCL29A1*) IVS2+913C>T (rs9394992) for the locally advanced patients. Thus, only an intronic SNP *DCK* IVS6-1205C>T overlapped the two patient groups, of which the functional significance is unclear. The influence of polymorphisms rather than *CDA**3 seems to be a lack of evidence on their reproducibility and clinical importance for gemcitabine toxicity.

### 3.3 Relevance of genetic polymorphisms to gemcitabine efficacy

The relevance of pharmacogenomic information on the efficacy of gemcitabine is still controversial. The minor allele (C) of *CDA*2* [79A>C(K27Q)] was reported associated with decreased response, shorter time to progression and OS in 65 caucasian NSCLC patients treated with gemcitabine and cisplatin [121]. Additive effects of SNPs in genes related to transport and metabolism of gemcitabine were significant in the potentially resectable pancreatic cancer patients with OS for *CDA*2* [79A>C (K27Q)], *DCK* IVS6-1205C>T, *DCTD* 348T>C (V116V, rs7663494), *SLC28A3* 1381C>T (I.461L, rs7853758), *SCL29A1* IVS2-549T>C (rs324148) and IVS2+913C>T, and in the locally advanced pancreatic cancer patients with progression-free survival for *CDA*79A>C (K27Q), *RRM1* 2223A>G (T741T) and 850C>A (R284R, rs183484) and *SCL29A1* IVS12-201A>G (rs760370) [133,134]. Thus, only *CDA*2* overlapped between the two patient groups, but the influence of *CDA*2* directed opposite between these studies. As for *RRM1* SNPs, several studies also reported a significant association with gemcitabine efficacies but the tendencies and corresponding SNPs were also variable [135-137]. Further studies are still needed to clarify these issues in a larger number of patients.

## 4. Tamoxifen

Ethnic differences in genetic variations also contribute to the efficacy of anticancer drugs. In this section, we select the anti-breast cancer drug tamoxifen as many papers have been published recently showing the importance of *CYP2D6* polymorphisms.

### 4.1 Pathways for metabolic activation of tamoxifen

Tamoxifen has potent anti-estrogenic action by binding to the estrogen receptor, resulting in competition with estrogen and inhibition of growth of ER-positive breast cancers [138]. Tamoxifen is a prodrug that is metabolized by cytochrome P450s to *N*-desmethyl-tamoxifen and an active metabolite 4-hydroxy-tamoxifen, and then into endoxifen (*N*-hydroxy-desmethyl-tamoxifen). CYP2D6 and CYP3A4/5 mainly mediate 4-hydroxylation and *N*-desmethylation, respectively. Because the plasma concentration of endoxifen is about six times higher than that of 4-hydroxy-tamoxifen and their potency is equivalent, endoxifen is thought to be the most important metabolite of tamoxifen action [139]. Reduction in enzyme activities by genetic polymorphisms of *CYP2D6* and *CYP3A4/5* could result in impaired efficacy of tamoxifen. Because the majority of recent publications have focused on *CYP2D6*, and frequent polymorphisms were not reported in the major CYP3A isoform *CYP3A4*, the effects of *CYP2D6* genotypes are discussed in this section.

### 4.2 *CYP2D6* polymorphisms and their influences on tamoxifen efficacy

Though its hepatic expression levels are relatively low, CYP2D6 is responsible for about 25% of the metabolism of known drugs [140]. *CYP2D6* is a polymorphic gene and 80 haplotypes are now published in the CYP allele nomenclature committee homepage (http://www.cypalleles.ki.se/cyp2d6.htm). Several haplotypes (e.g., **9, *10, *17, *29* and **41*) are associated with reduced enzyme activity and some (**3, *4, *5* and **6*) are with null activity. Large inter-ethnic differences are observed in the frequencies of *CYP2D6* haplotypes (Table 5) [140-146]. The frequencies for null haplotypes are about 24% in Caucasians, 7% in East Asians and 9% in Africans, and those for reduced activity are 10% in Caucasians, 53% in East Asians and 47% in Africans. On the basis of metabolizing capacity, the subjects can be divided into four phenotypes by combinations of these defective haplotypes and gene duplications. Typical classification is as follows: ultrarapid metabolizers (UMs) have three or more functional *CYP2D6* gene(s) due to gene duplication, resulting in expressing excessive amounts of CYP2D6 proteins. Extensive metabolizers (EMs) carry two alleles with normal enzyme activities. Subjects bearing one allele with normal and one with null or decreased activity are classified as heterozygous EMs, and subjects having two alleles with decreased activities or one allele with decreased and one with null activity are classified as intermediate metabolizers (IMs). Heterozygous EMs and IMs are often combined and designated as 'IMs'. Poor metabolizers (PMs) have two non-functional alleles lacking CYP2D6 activities. Resulting PM frequencies are ~ 6% in Caucasians, and 0.5 - 1% in East Asians and Africans. Mean plasma endoxifen concentration was reported to increase according to the number of functional haplotypes [147]. Two subsequent reports supported this phenomenon [148,149].

The majority of reports showed that patients with one or two deficient *CYP2D6* haplotypes were associated with a worse clinical outcome such as shorter recurrence-free survival [149-152]. A retrospective analysis of 1325 German and US cohorts of patients treated with adjuvant tamoxifen for early stage breast cancer showed that IMs (including heterozygous EMs) and PMs had a hazard ratio (HR) of 1.40 and 1.90, respectively, for time to recurrence when compared with EMs (including UMs) [150]. In Japanese, *CYP2D6* deficient haplotypes were significantly associated with shorter recurrence-free survival (HR = 4.44 and 9.52 for heterozygous and homozygous deficient haplotypes vs EMs) [149]. They included both reduced and null activity haplotypes as well as deficient haplotypes. However, the influence of *CYP2D6* genotypes on tamoxifen efficacy is still controversial. From 2005 to 2007, three reports showed that patients with *CYP2D6*4*, a major null haplotype in Caucasians, showed insignificant or even better clinical outcome [153-155]. The discrepancies may be caused by several factors such as genotyped *CYP2D6* haplotype numbers, co-medication of CYP2D6 inhibitors, tamoxifen adherence (compliance), tamoxifen doses and treatment length, and node status. CYP2D6 inhibitors may reduce the plasma endoxifen concentrations and the strong inhibitors, paroxetine and fluoxetine, are commonly used for treatment of tamoxifen-induced hot flashes and depression from cancer [147].

Table 5. Ethnic differences in major *CYP2D6* haplotypes with reduced or null activities or duplications.

| | Null (%) | | | | Reduced (%) | | | | Duplication (%) | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | *3 | *4 | *5 | *6 | *9 | *10[a] | *17 | *29 | *41 | *1 × N | *2 × N | *4 × N | *10 × N |
| Caucasians | 0 – 2 | 14 – 21 | 2 – 7 | 0 – 1 | 0 – 3 | 1 – 2 | 0 | 0 | 4 – 10 | 0 – 1 | 0 – 2 | 0 – 1 | 0 – 4 |
| East Asians | 0 – 1 | 0 – 1 | 5 – 7 | 0 | 0 | 36 – 64 | 0 | 0 | 0 – 6 | 0 – 2 | 0 – 1 | 0 | 0 – 1 |
| Africans | 0 – 1 | 1 – 8 | 1 – 7 | 0 | 0 | 3 – 9 | 18 – 34 | 3 – 17 | 1 – 10 | 0 – 3 | 0 – 3 | 0 – 1 | 0 |

Summarized from references [128-134].

[a]In Japanese and probably East Asians, most of the **10* allele exist as **36-*10*. Because *CYP2D6.36* had only slight enzymatic activity, additional **36* render negligible activity.

Recent reports assessed these confounding factors. Schroth *et al.* [156] demonstrated that comprehensive genotyping of *CYP2D6* (full haplotype coverage) is important for increased predictability of tamoxifen clinical outcome. Results from full haplotype coverage showed a significant association of PMs with higher relapse-free survival (Cox proportional HR = 2.77, vs EMs). On the other hand, only a **4* analysis resulted in misclassification of a third of patients and its HR did not reach significance. Kiyotani *et al.* [157] showed that significant effects of *CYP2D6* low or null activity haplotypes were observed for shorter recurrence-free survival only in tamoxifen monotherapy ($p = 0.000036$ for trend) but not in combination chemotherapy ($p = 0.53$). Their review of previous publications also supports this notion. Also, they showed that node status did not affect outcome. Another paper with two archival cohorts reported that comprehensive *CYP2D6* genotyping and adherence to tamoxifen are suggested to be useful for identification of breast cancer patients likely to benefit from adjuvant tamoxifen [158]. In their study, inclusion of co-medication status of strong CYP2D6 inhibitors, paroxetine and fluoxetine, did not influence the HR. In line with this, poor adherence of tamoxifen, not concomitant usage of CYP2D6 inhibitors, led to lower event-free survival in another multivariate analysis, though its HR was small (0.987) [159].

Although many studies support the usefulness of *CYP2D6* genotyping, larger prospective randomized clinical trials that compare outcomes between genotype-guided and non-guided dosing of tamoxifen would be necessary for concluding whether *CYP2D6* genotyping is really useful for tamoxifen therapy. In addition, differences in effect sizes of IM and PM haplotypes should be precisely analyzed because their frequencies are ethnic-specific. Because effects of genetic polymorphisms on catalytic activities are known to be substrate-dependent [98], the influence of reduced-activity haplotypes on tamoxifen metabolism should be assessed *in vitro*.

### 4.3 Relevance of *CYP2D6* polymorphisms in tamoxifen-induced hot flashes

Hot flashes are the most common side effect of tamoxifen, occurring in > 50% of women receiving tamoxifen [160], and the onset of hot flashes can result in patient noncompliance. As for the association with *CYP2D6* genotype, Goetz *et al.* first reported that patients with *CYP2D6*4/*4* genotype did not experience moderate to severe (grade 2 – 3) hot flashes relative to EM and IM patients [151], suggesting that metabolic activation of tamoxifen by CYP2D6 could be involved in the onset of hot flashes. In line with this, patients with hot flashes showed longer recurrence-free survival than those who did not report hot flashes [161]. A recent paper also reported a trend toward fewer severe hot flashes in PMs compared to IMs + EMs [162]. However, one report contradicted this phenomenon, although the sample size was very small [163].

## 5. Conclusions

Recent pharmacogenomic progress was summarized for irinotecan, gemcitabine and tamoxifen focusing on ethnic differences of metabolizing enzymes and transporters. For irinotecan, studies with *UGT1A* haplotypes have collectively revealed the primary importance of *UGT1A1* genotypes/haplotypes for prediction of severe irinotecan toxicities in an ethnic-specific manner (**28* for Caucasians, and **6* and/or **28* for Asians). All these efforts have brought personalized irinotecan therapy to the US (for **28*) and Japan (for **28* and **6*), and also to Singapore (for **28* and **6*). Further prospective studies considering ethnic-specific genotypes, such as *UGT1A7*3* or *UGT1A1*93* and/or transporter genetic markers, and regimens would provide safer and more effective dosing strategies for irinotecan therapy. Regarding gemcitabine, *CDA*3* is a useful marker for severe myelotoxicities by gemcitabine in Japanese patients, but not in Caucasians. For tamoxifen, the majority of recent evidence suggested that *CYP2D6* variant haplotypes with low or null activities, which showed different frequencies among ethnic populations, are associated with worse clinical outcomes compared to EM haplotypes. Prospective clinical studies using specific ethnic populations are necessary for opening an era of selection and dose-adjustment of anticancer drugs based on pharmacogenomic information.

## 6. Expert opinion

Recent pharmacogenomic studies have successfully identified several useful genetic markers that are related to alteration of drug metabolism and disposition, and ethnic differences in their frequencies. In other words, ethnic differences in drug metabolism and disposition are, at least partly, attributable to ethnic differences in frequencies of functional genetic variations. All of this effort has practically led to the implementation of personalized irinotecan therapy in the US, Japan and now in Singapore, reflecting ethnic differences in genotype distributions. It is worth noting that a label change of irinotecan in Singapore has been considered for both *UGT1A1*28* and **6* to encompass the risk groups of three ethnic populations with distinct genotypes. It would be reasonable to consider further revision of the irinotecan label in other countries, considering the frequencies of *UGT1A1*28* and **6* in various countries. Global collaborative research that includes sharing knowledge and clinical data from different ethnic groups may facilitate personalized therapy in other countries.

Regarding ethnic differences in response to irinotecan therapy, it has been recognized that there is higher susceptibility (toxicity and efficacy) in Japanese than Caucasians after irinotecan/cispaltin regimens [164], and lower response (diarrhea and response rate) in Africans than Caucasians after combination of irinotecan with 5-FU or oxaliplatin [165]. These susceptibility differences among ethnic groups cannot be explained by frequencies of candidate high risk markers identified so far, such as *UGT1A1*28 or *6*. Thus, prediction of efficacies and toxicities by already established genetic markers such as *UGT1A1*28* or **6* for irinotecan is still insufficient. Recent integrated pharmacogenetic studies on multiple genes have detected novel genetic markers [76,86] or additive effects of transporter polymorphisms [71], but further validation studies are still needed to avoid possible false positive markers in a larger population. On-going prospective studies on irinotecan may provide the appropriate doses for each genotype. In addition and of primary importance is the optimization of the dose of irinotecan for high risk groups to avoid severe toxicities with a sufficient efficacy. Further challenges of dose-escalations for non-high risk groups [108] should also be taken into consideration for efficacy improvement.

For gemcitabine, *CDA*3* was not found in caucasian patients with toxicities, whose CDA activities were very low, suggesting that undefined polymorphisms associated with reduced enzyme activity may exist in caucasian populations. Therefore, comprehensive genetic screening is necessary including 5′- and 3′-flanking regions as well as entire intronic regions with the next generation sequencers. The impact of other genes related to gemcitabine metabolism and disposition has been analyzed and some were reported to significantly influence gemcitabine toxicities. However, further validation studies are still necessary prior to clinical application.

For tamoxifen, increasing evidence supports the usefulness of *CYP2D6* genotyping to predict tamoxifen efficacy. Larger prospective randomized clinical trials that compare outcomes between genotype-guided and non-guided dosing of tamoxifen are necessary for concluding whether *CYP2D6* genotyping is sufficiently useful for tamoxifen therapy. In addition to *CYP2D6*, studies on the effects of polymorphisms in other related genes such as *CYP3A4* are warranted. Recently, an intronic SNP rs3740065A>G in *ABCC2*, which was in linkage disequilibrium with -1774delG, was reported to be significantly associated with longer recurrence-free survival in tamoxifen treatment [149,166]. This report shows the new direction of pharmacogenomic studies on tamoxifen.

Because the extent of adverse reactions may depend on the adopted regimens, further comprehensive pharmacogenetic studies should be conducted on other enzymes involved in the metabolism of co-administered drugs. This analysis is particularly important for cancer therapy because multiple anticancer drugs are commonly used in most regimens. In addition, environmental factors, such as food, smoking or disease status, would be involved in susceptibility to anticancer drugs. Other diagnostic approaches including proteomics and metabolomics, which can reflect these environmental factors, would provide additional effective biomarkers to more precisely explain ethnic differences. Another emerging issue is the lack of knowledge of molecular markers related to very rare but life-threatening adverse events after anticancer

chemotherapies. For streamlining research on such rare cases, a network should be established among hospitals, academic and regulatory agencies to manage all aspects of research, starting with sample collection and ending with multiple analyses. These rare cases require a comprehensive and interdisciplinary approach.

The goal is the high-rate or even perfect prediction of efficacies and toxicities of anticancer drugs in each ethnic population. Further extensive efforts in all cancer research fields and global collaboration would facilitate reaching this goal.

## Declaration of interest

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