Toward individualized treatment: prediction of anticancer drug disposition and toxicity with pharmacogenetics

John F. Deeken[a], William D. Figg[b], Susan E. Bates[a] and Alex Sparreboom[b]

A great deal of effort has been spent in defining the pharmacokinetics and pharmacodynamics of investigational and registered anticancer agents. Often, there is a marked variability in drug handling between individual patients, which contributes to variability in the pharmacodynamic effects of a given dose of a drug. A combination of physiological variables, genetic characteristics (pharmacogenetics) and environmental factors is known to alter the relationship between the absolute dose and the concentration–time profile in plasma. A variety of strategies are now being evaluated in patients with cancer to improve the therapeutic index of anticancer drugs by implementation of pharmacogenetic imprinting through genotyping or phenotyping individual patients. The efforts have mainly focused on variants in genes encoding the drug-metabolizing enzymes thiopurine *S*-methyltransferase, dihydropyrimidine dehydrogenase, members of the cytochrome P450 family, including the CYP2B, 2C, 2D and 3A subfamilies, members of the UDP glucuronosyltransferase family, as well as the ATP-binding cassette transporters ABCB1 (P-glycoprotein) and ABCG2 (breast cancer resistance protein). Several of these genotyping strategies have been shown to have substantial impact on therapeutic outcome and should eventually lead to improved anticancer chemotherapy. *Anti-Cancer Drugs* 18:111–126 © 2007 Lippincott Williams & Wilkins.

Anti-Cancer Drugs 2007, **18**:111–126

Keywords: anticancer drugs, pharmacogenetics, pharmacokinetics, toxicity

[a]Medical Oncology Branch, Center for Cancer Research and [b]Clinical Pharmacology Research Core, National Cancer Institute, Bethesda, Maryland, USA.

Correspondence to A. Sparreboom, Department of Pharmaceutical Sciences, St Jude Children's Research Hospital, DTRC, Mail Stop 314, Room D1034B, 332 North Lauderdale, Memphis, TN 38105, USA
Tel: +1 901 495 5346; fax: +1 901 495 3125; e-mail: alex.sparreboom@stjude.org

Sponsorship: This work was supported, in part, by the Intramural Research Program of the National Institutes of Health, National Cancer Institute, Center for Cancer Research, Bethesda, Maryland, USA.

Disclaimer: The content of this paper does not necessarily reflect the views or policies of the Department of Health and Human Services nor does mention of trade names, commercial products or organizations imply endorsement by the US Government.

Received 16 May 2006 **Revised form accepted** 6 September 2006

Introduction

Individualizing therapy for patients being treated with pharmaceutical agents is an overarching goal of basic and clinical research in this first part of the 21st century. In no area of medicine is this goal more critical than in cancer chemotherapy. A better ability to predict which agents to use in individual patients and an improved means of dosing drugs are critical needs facing clinical researchers in oncology care.

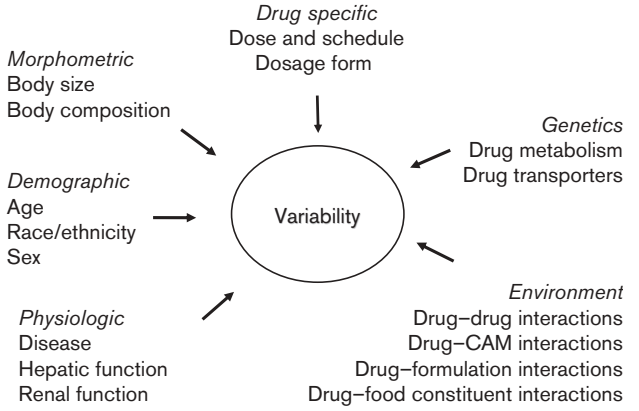
Many sources of interindividual variation exist in drug toxicity and efficacy, both pharmacokinetic and pharmacodynamic, as highlighted in Fig. 1. These include patients' body size and composition, age, ethnicity, and sex, as well as physiologic aspects, especially disease state and organ function. Environmental factors include interactions with food, drinks, environmental toxins, other drugs (prescription, over-the-counter and herbal medications), as well as interactions with drug formulation components. Finally, a patient's genetic profile is a critical – but not the only – aspect to account for and explain inter patient variability in drug response.

The pharmacogenetic differences between patients are also multifactorial, as shown in Fig. 2. One factor of growing importance as more targeted therapies are developed is polymorphisms in drug targets, including cell surface receptors and target proteins. Another is polymorphisms in hormonal-regulated enzymes. Finally, and the focus of this review, are polymorphisms in the genes involved in drug pharmacokinetics that impact drug absorption, distribution, metabolism and excretion.

A growing body of research is highlighting the role that variations in the genes encoding drug-metabolizing enzymes and drug transporters play in explaining, at least in part, the substantial interindividual variability seen in the clinical profile of several important drugs. Severe toxicity might occur in the absence of normal metabolism of active compounds, whereas the therapeutic effect of a drug could be diminished in the absence of activation of a prodrug. Diminished efficacy could be due to the induction of metabolism enzymes or transporters, an induction that may be affected by genetic variation. The importance in detecting polymorphisms for a given

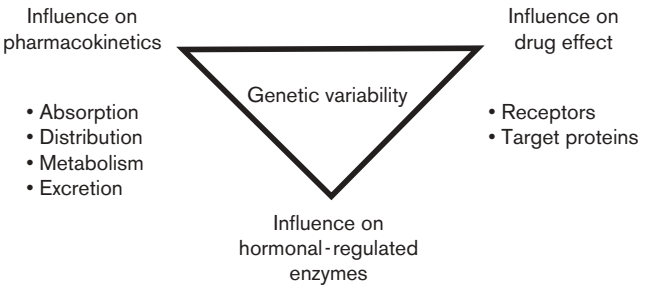
0959-4973

Fig. 1



Sources of pharmacokinetic and pharmacodynamic variability. *CAM, complementary and alternative medicine.

Fig. 2



Three different aspects of pharmacogenetics in drug therapy.

enzyme depends on the contribution of the variant gene product to pharmacological response, the availability of alternative pathways of elimination and the prevalence of the least-common variant allele. Although many substrates have been identified for the known polymorphic drug-metabolizing enzymes and transporters, few cancer chemotherapeutic agents have been associated with such genetically determined sources of variability. This review will highlight discoveries in the pharmacogenomics of drug-metabolizing enzymes and drug transporters, including the existence and frequency of single nucleotide polymorphisms (SNPs) in the genes encoding these proteins, and how they might influence the disposition of chemotherapeutic agents.

As the field of pharmacogenomics, and especially how it applies to cancer chemotherapy, is a nascent area of clinical research, many findings are incomplete. Different researchers study different drugs and often different polymorphisms, using various *in-vitro* and *in-vivo* methodologies. Information often is incomplete and at times contradictory. Thus, often the research findings on specific polymorphisms are difficult to apply to specific clinical situations. How this knowledge, can be applied towards individualizing treatment strategies, if known, will be presented as well.

Pharmacogenomics of drug-metabolizing enzymes

Thiopurine *S*-methyltransferase

Thiopurine *S*-methyltransferase (TPMT) genetic polymorphism represents a striking example of the potential clinical impact of pharmacogenetics. is a cytosolic enzyme in peripheral tissues, including erythrocytes, involved in the inactivation of the thiopurine drugs 6-mercaptopurine, azathioprine and 6-thioguanine. These prodrugs require enzymatic activation by hypoxanthine phosphoribosyltransferase to form thiopurine nucleotides. These nucleotides, in turn, exert their cytotoxic effect by being incorporated into DNA as well as inhibiting de-novo purine synthesis. The major inactivation pathway of these agents in hematopoietic cells is via the TPMT enzyme, which transfers the methyl group from *S*-adenosyl methionine to the sulfur atoms of thiopurines.

TPMT activity in normal volunteers has a trimodal distribution, with 90% of individuals having high enzymatic activity, 10% having intermediate activity and 0.3% having low or no detectable enzymatic activity. TPMT deficiency leads to high intracellular accumulation of the active drug and in turn severe – and even fatal – hematologic toxicity [1]. The level of enzymatic activity in individual patients has been found to be determined by whether they are heterozygote or homozygote for certain SNPs within the *TPMT* gene. The variants usually associated with TPMT deficiency are *TPMT*2*, *TPMT*3A* and *TPMT*3C*. These three variants together represent over 95% of the noted deficiencies in the TPMT activity [2]. The frequencies of allelic variants in different ethnic populations are listed in Table 1 [3–10].

Knowing a patient's genotype before starting the therapy with 6-mercaptopurine, azathioprine and 6-thioguanine is becoming a common practice. For example, patients heterozygote for the *TPMT* gene, with one wild-type and one variant gene copy, have been found to tolerate about 65% of the standard dosing of these agents, whereas patients homozygote with two variant gene copies can tolerate only 10% of the standard dose [11]. A genetic test for *TPMT* variants is available through commercial laboratories. The US Food and Drug Administration is considering changing the labeling of 6-mercaptopurine to incorporate guidance on genotyping testing and dose modification.

Dihydropyrimidine dehydrogenase

Dihydropyrimidine dehydrogenase (DPD) is an enzyme primarily expressed in the liver, and it functions in the

Table 1 Ethnic frequency (%) of allelic variants in the *TPMT* gene

Allelic variant	SNPs	Caucasian	African-Americans	Asians	Hispanics	Africans
*TPMT*2*	G238C	0.17–0.5	0.4	0	0.7–2.2	0
*TPMT*3A*	G460A A719G	3.2–5.7	0.8	0–0.3	1.5–3.1	0
*TPMT*3C*	A719G	0.17–4.8	2.4	2.9–3.7	1.0	7.6

SNP, single nucleotide polymorphism.
Sources: Caucasians [3,4], African-Americans [5], Asians [6,7], Hispanics [8,9], Africans [3,10].

initial and rate-limiting step of pyrimidine catabolism. In addition to catabolizing uracil and thymine, DPD also metabolizes 5-fluorouracil (5-FU). Administered 5-FU can undergo two different enzymatic pathways. In target tissues, it undergoes anabolism to become cytotoxic nucleotides that are incorporated into replicating RNA and DNA. 5-FU can also inhibit thymidylate synthase in the DNA replication pathway. In the liver, DPD metabolizes the active drug into inactive dihydrofluorouracil, which then undergoes additional enzymatic steps before being secreted. More than 85% of an administered dose of 5-FU is inactivated by DPD [12].

Mutations and SNPs in the gene encoding DPD can cause deficiencies in enzymatic activity. Patients with DPD deficiency have a reduced capacity to metabolize 5-FU and are at risk of developing severe – and even life-threatening – reactions [13–15]. Thirty-nine different mutations and polymorphisms have been identified in the human DPD gene (*DYPD*) [16], and 14 of these have been identified in patients experiencing excessive 5-FU toxicity. Between 3 and 5% of cancer patients may experience this severe 5-FU-related toxicity owing to alterations in the DPD gene. The most common mutation is a point mutation in the invariant splice donor site (*IVS14 + 1G > A* mutation). Van Kuilenburg *et al.* [14] found this mutation in about one-quarter of patients experiencing severe 5-FU toxicity. This mutation has been found in patients of Dutch, German, Finnish, Turkish and Taiwanese descent, but has not been found in African-Americans or Japanese. The ethnic frequency of this polymorphism is listed in Table 2 [15].

Identifying at-risk patients before initiating therapy has proven to be difficult, and genotyping tests for *DYPD* mutations typically have low sensitivity. Laboratory-based testing for DPD activity is laborious and not conducive to wide use in clinical oncology. Several methods have been developed to screen for *DYPD* mutations [16] or to assess DPD activity before therapy [17–20]. Magne *et al.* [21] recently analyzed DPD activity in a population of patients with 5-FU-related toxicities and reported that only 28 out of 144 patients (19%) exhibited reduced enzyme activity (below 100 pmol/min/mg of protein). Remarkably, *DYPD* mutations were only detected in two of 102 patients [21]. These findings suggest that patients with normal DPD activity may develop 5-FU-related toxicities and that genotype alone does not predict which patients will develop toxicity following treatment with 5-FU. To circumvent the shortcoming of genotyping as a the sole predictive strategy to treatment optimization with drugs like 5-FU, current work is focused on the development of a rapid and noninvasive [2-^{13}C] uracil breath test to determine a-*priori* DPD activity [22]. The clinical utility and predictive power of this approach, however, still needs to be ascertained before routine implementation.

Table 2 Ethnic frequency (%) of the *IVS14 + 1G > A* variant in the *DYPD* gene

Ethnic/nationality	Allele frequency (%)
European	0.47–2.2
Turkish	0.75
Taiwanese	0.0–2.7
Japanese	0
African-Americans	0

Source: [15].

Cytochrome P450 isozymes

The cytochrome P540 (CYP) family of enzymes are expressed in many tissues, with the largest activity in the liver. They function by conducting oxidative metabolism on many endogenous substrates and over 90% of all drugs. These include many chemotherapy agents. Genetic variants, including SNPs, exist in these enzymes, and clinically significant alterations in drug metabolism, efficacy and toxicity can occur owing to these variants. The discovery of these genetic variants, and the determination of their clinical relevance, have been fruitful areas of clinical research over the past decade. Recently, the US Food and Drug Administration approved the first gene chip platform to rapidly genotype patients for SNP variations in two important members of the CYP system.

The CYP superfamily consists of over 60 enzymes that have been grouped into several families and subfamilies. Here, we will discuss only those CYP450 enzymes of most relevance to clinical oncology on the basis of our current understanding i.e. CYP2B6, CYP2C8, CYP2C9, CYP2C19, CYP2D6, CYP3A4 and CYP3A5. For each enzyme, information will be presented on: tissue expression and localization; drugs known to be substrates, as well as drugs that inhibit or induce enzyme activity; genetic variations affecting enzyme sequence; and the impact, if known, of wild-type and variant proteins on the metabolism of chemotherapeutic agents.

CYP2B6

CYP2B6 is expressed in the liver and in extrahepatic tissues. Over 70 drugs are known to be completely or partially metabolized by this enzyme [23]. These include bupropion, efavirenz and methadone. Thiotepa and ticlopidine are known to inhibit the enzyme, whereas the enzyme can be induced by exposure to phenobarbital and rifampin [24].

Enzymatic activity of CYP2B6 can vary up to 100-fold between individuals [25]. Women appear to have higher hepatic expression of the enzyme, including higher mRNA expression by a factor of 3.9, higher protein level by 1.6-fold and a 1.7-fold higher enzymatic activity level as measured by *N*-demethylation of *S*-mephenytoin [26]. In addition, more men were found to be poor metabolizers (PMs) by this *S*-mephenytoin test; 20% of men ($n = 45$) were PMs, whereas only 7% ($n = 28$) of women were PMs.

The gene encoding CYP2B6 is located on chromosome 19q13.2. Forty SNPs have been identified that lead to 37 different allelic variants, which comprise variants *CYP2B6*2* to *CYP2B6*25*. Table 3 [26–29] lists the allelic variants that have SNPs with a frequency of more than 1% in an ethnic population.

A number of these variants have been found to affect function and expression. Kirchheiner *et al.* [27] found that the *CYP2B6*4* variant, containing the A785G SNP, had increased enzymatic activity measured by bupropion hydroxylation, with heterozygote individuals (*1/*4) having 1.6 higher clearance than wild-type individuals. Both the *CYP2B6*4* and *CYP2B6*6* carry the A785G variant. The additional G516T variant in the *CYP2B6*6* allele decreases protein expression. It is not clear whether the overall effect of the *CYP2B6*6* variant will be reduced activity owing to decreased protein expression or higher activity owing to increased catalytic potential [30,31]. Lang *et al.* [28] reported that the C1459T polymorphism, found in the *CYP2B6*5* and *CYP2B6**7 allelic variants, leads to lower protein expression in both homozygote and heterozygote individuals. Lamba *et al.* [26] found a correlation between this SNP and diminished activity in women but not in men. Lang *et al.* [32] also found an in-vitro decrease in the expression or function in the *CYP2B6*11*, **12*, **13*, **14* and **15* variants, a finding that needs to be validated *in vivo*. Klein *et al.* [33] found a decreased expression of the *CYP2B6*18*, **19*, **20* and **21* variants *in vitro*. Finally, Zukunft *et al.* [34] found a natural occurring SNP in the TATA box (–C82T), which led *in vitro* to an increased expression and function of the enzyme.

Table 3 **Ethnic frequency (%) of allelic variants in *CYP2B6***

Allelic variant	SNPs	Caucasians	African-Americans	Asians	Hispanics
*CYP2B6*2*	C64T	5.3–9.0	0	4.7	
*CYP2B6*3*	C777A	0.5		0	
*CYP2B6*4*	A785G	4.0–32.6	16.7	9.3	14.3
*CYP2B6*5*	C1459T	9.5–13	9.0	1.1	0
*CYP2B6*6*	G516T A785G	25.6		16.4	
*CYP2B6*7*	G516T A785G C1459T	0–3.0		0	
*CYP2B6*8*	A415G	1.0	0	0	0

SNP, single nucleotide polymorphism.
Sources: Caucasians [26–29], African-Americans [26], Asians [29], Hispanics [26].

CYP2B6 is involved in the metabolism of chemotherapy agents, including cyclophosphamide and ifosfamide. Schmidt *et al.* [35] showed that the sex difference in CYP2B6 expression translated to sex-dependent differences in ifosfamide metabolism *in vitro*. Liver microsomes for women had a 2-fold higher *N*-dechloroethylation of ifosfamide than those from men. Xie *et al.* [36] has explored the effect of the G516T SNP found in the *CYP2B6*6* and *7 variants on cyclophosphamide metabolism. Earlier studies had shown no difference in metabolism to 4-hydroxy-cyclophosphamide as a function of total CYP protein between wild-type individuals and those homozygous for the *CYP2B6*6* allele. Later, when looking at patients with hematologic malignancies treated with cyclophosphamide, Xie *et al.* [36] explored the relative clearance of the drug by CYP enzymes CYP2B6, CYP2C9 and CYP2C19. Although total drug metabolism was relatively constant, the relative contribution of CYP2B6 in total drug metabolism was 2-fold higher in patients with the G516T variant than in those with the wild-type variant [36]. Further clinical investigation is needed to assess fully what impact sex and genetic variation in *CYP2B6* have on the efficacy and toxicity of cyclophosphamide and ifosfamide.

The CYP2C subfamily. The CYP2C subfamily has four members (CYP2C8, CYP2C9, CYP2C18 and CYP2C19) and together this subfamily metabolizes approximately 20% of all clinically used drugs. Polymorphisms in the *CYP2C8*, *CYP2C9* and *CYP2C19* genes have been shown to correlate with toxicity of certain anticancer drugs in affected individuals [37].

CYP2C8

Expression of *CYP2C8* by mRNA has been found in the liver as well as in the kidney, lung, adrenal gland, brain, uterus, mammary gland, ovary and duodenum. The enzyme metabolizes about 5% of drugs that undergo oxidative metabolism in the liver [38]. Substrates for the enzyme include paclitaxel, amiodarone, torsemide, cerivastatin and repaglimide. Inhibitors include gemfibrozil and montelukast [24]. In addition, dexamethasone, phenobarbital and cortisol are known to induce enzymatic

activity of CYP2C8. In fact, CYP2C8 appears to be the most inducible member of the 2C subfamily *in vitro* [38].

Like the other members of the 2C subfamily, the gene encoding CYP2C8 is located at 10q24. There are a number of nonsynonymous SNPs that have been identified as well as three synonymous SNPs in the 5′ untranslated region of the gene, encoding nine different allelic variants. Table 4 [38–40] lists the allelic variants with a frequency greater than 1% in an ethnic population.

Paclitaxel, which is also metabolized by the CYP enzymes 3A4 and 3A5, has been used as a probe for *in vitro* analyses on the effect of SNPs in the *CYP2C8* gene. The *CYP2C8*2* variant has only 15% of the paclitaxel hydroxylation activity seen in the wild-type variant [41]. The *CYP2C8*3* variant has only 50% of the *in vitro* 6α-hydroxylation activity as wild-type [42,43]. Of note, these *in vitro* observations did not explain the significant interpatient variability observed with paclitaxel clearance when tested in a normal Caucasian population [44]. It is possible that paclitaxel can be eliminated through compensatory pathways in individuals who carry one or more of the *CYP2C8* variant alleles.

Garcia-Martin *et al.* [45] recently found that patients with the *CYP2C8*3* allele had reduced clearance of *R*-(−)-ibuprofen, whereas patients with the *CYP2C8*3* and *CYP2C9*2* SNP variants had diminished clearance of *S*-(+)-ibuprofen. Of note, these authors and Yasar *et al.* [46] found that the *CYP2C8*3* and *CYP2C9*2* alleles are in incomplete linkage disequilibrium. The *CYP2C8*5* variant, with a frame shift at 475delA, leads to an early stop codon at residue 177, which results in an enzyme missing 64% of its structure. One report documented a Japanese patient who was homozygous for this variant and experienced rhabdomylosis while receiving the CYP2C8 substrate drug cerivastatin [47]. The *CYP2C8*8* variant, with a frequency of only 0.0025 in a Japanese population, was found to have only 10–20% of the activity *in vitro* of the wild-type enzyme [48].

In view of the apparent contradiction between *in vitro* and *in vivo* results pertaining to the importance of *CYP2C8* variants, especially in the context of paclitaxel metabolism, more rigorous assessment is urgently needed to determine the clinical significance of such polymorphisms with respect to treatment outcome with anticancer substrate drugs.

CYP2C9

CYP2C9 is the principal member of the 2C subfamily in the liver [37]. Expression is also found in the kidney, testes, adrenal glands, prostate, ovary and duodenum [38]. A large number of commonly used drugs are metabolized by CYP2C9. These include the anti-inflammatory drugs diclofenac, naproxen, ibuprofen and celecoxib, the diabetic agents glyburide, tolbutamide and glipizide, the angiotensin II receptor blockers losartan and irbesartan, as well as S-warfarin, zafirlukast, phenytoin and fluoxetine. The enzyme can be induced by rifampin and secobarbital [24]. Numerous drugs are known to inhibit CYP2C9, including amiodarone, fluconazole and isoniazid. Serious adverse reactions can occur from use of these inhibitors. For example, patients on warfarin, tolbutamide or phenytoin can experience significant overanticoagulation, hypoglycemia or neurotoxicity [49].

The gene is located on 10q24. Thirty-four SNPs are contained in 27 allelic variants, designated **2* to **24*. Variants with a frequency greater than 1% are listed in Table 5 [51–57]. Studies have generally shown decreased activity of the *CYP2C9*2* variant, both *in vitro* and *in vivo* [49]. Kirchheiner *et al.* [58] found decreased clearance of tolbutamide in patients with the *CYP2C9*2* and *CYP2C9*3* variants. Individuals with the *CYP2C9*2* variant had 75% of the clearance of the drug compared with wild-type individuals. The *CYP2C9*3* variant causes a substantial reduction in enzymatic activity. Individuals with this variant had only 16% of the clearance of wild-type individuals and should be considered PMs. Patients with the *CYP2C9*3* variant also require less warfarin dosing to sustain therapeutic anticoagulation. This variant is also associated with an increased risk for bleeding in patients on warfarin [59]. In patients with the *CYP2C9*3* variant, the mean clearance of S-warfarin, glipizide, celecoxib and fluovostatin was below 25% of the level seen in wild-type individuals, whereas patients heterozygous for this variant (*1/*3) had clearances of these drugs ranging between 40 and 70% compared with wild-type [60]. The *CYP2C9*4* polymorphism was found in a Japanese patient with an adverse reaction to phenytoin [61], whereas the rare *CYP2C9*6* variant was found in an African-American who also had an adverse

Table 4 Ethnic frequency (%) of allelic variants in *CYP2C8*

Allelic variant	SNPs	Caucasians	African-Americans	Asians	Others
*CYP2C8*2*	A805T	0.4	4.0–18	0	Malaysian: 3.5
*CYP2C8*3*	G416A A1196G	9.5–23	2.0–3.3	0.7–5.0	Malaysian: 5.3
*CYP2C8*4*	C792G	7.5–11	0		

SNP, single nucleotide polymorphism.
Sources: Caucasian [38], African-Americans [38,39], Asians [38], other [40].

Table 5 Ethnic frequency (%) of allelic variants in *CYP2C9*

Allelic variant	SNPs	Caucasians	African-Americans	Asians	Hispanics	Africans
*CYP2C9*2*	C430T	6.8–13.2	1.0–2.5	0	8.0	
*CYP2C9*3*	A1075C	4.3–15.9	0.5–1.25	0–2.2	6.0	
*CYP2C9*5*	C1080G	0	1.7			
*CYP2C9*7*	C55A					7.1
*CYP2C9*8*	G449A		6.7			7.1
*CYP2C9*9*	A752G	0.5	13.3			14.3
*CYP2C9*11*	C1003T	1				
*CYP2C9*12*	C1465T	0.5				

SNP, single nucleotide polymorphism.
Sources: Caucasians [51–54], African Americans [52,54,55], Asians [52,56], Hispanics [57], Africans [52].

Table 6 Ethnic frequency (%) of the poor metabolizing allelic variants (2* and **3*) in the *CYP2C19* gene**

Allelic variant	SNPs	Caucasians	African-Americans	Asians	Africans	Middle Eastern
*CYP2C19*2*	G681A	1.2–3.8	0–7.0	5.7–13.5	0.4–2.6	2–2.9
*CYP2C19*3*	G636A	0	0	1.6–1.9	0–3.2	0

SNP, single nucleotide polymorphism.
Source: [63].

reaction to that drug [62]. Allabi *et al.* [51] found a decreased *in vivo* enzymatic activity, as measured by the metabolism of losartan, for the *CYP2C9*5* and *CYP2C9*6* variants. Blaisdell *et al.* [51] found that the *CYP2C9*11* and *CYP2C9*12* variants were a PM phenotype, with the *CYP2C9*11* variants having a 2-fold lower clearance of tolbutamide in an *in vitro* model than the wild-type. Using an *in vitro* bacterial cDNA expression system and site-directed mutagenesis as a model, DeLozier *et al.* [52] found that the *CYP2C9*15* and *CYP2C9*18* allelic variants were likely null alleles. These authors also reported that using an *in vitro* model, the *CYP2C9*14* and *CYP2C9*16* variants had a greater than 90% reduction in clearance of tolbutamide than the wild-type, whereas the *CYP2C9*17* and *CYP2C9*19* variants had a more modest 30–40% reduction in enzymatic activity.

Cyclophosphamide is partially metabolized by CYP2C9. Using a yeast model expressing wild-type, *CYP2C9*2* and *CYP2C9*3* variants, Xie *et al.* [31] found a 3-fold reduction in drug metabolism in these recombinant variants. No difference was found, however, in human microsomes expressing these variants when compared with microsomes from patients with the wild-type variant. Hence, the *in vivo* relevance of genetic variation in the *CYP2C9* gene with respect to cyclophosphamide treatment remains unclear.

CYP2C19

Expression of CYP2C19 is limited to the liver and the duodenum [38]. Commonly used drugs that are metabolized by this enzyme include the proton pump inhibitors lansoprazole, omeprazole, pantoprazole and rabeprazole; the antiseizure medications *S*-mephenytoin and diazepam; as well as amitriptyline, citalopram, indomethacine, nelfinavir, proguanil, propronalol and teniposide. Drugs known to inhibit enzymatic activity include fluoxetine, ketoconazole, lansoprazole, omeprazole and ticlopidine [24].

The gene is also located on 10q24. Combinations of 23 different SNPs in exon coding regions comprise 25 different allelic variants, listed in Table 6 [63]. The *CYP2C19*2* and *CYP2C19*3* variants are notable for both leading to premature stop codons and premature truncation of the protein, with a resultant completely inactive enzyme. As noted in Table 6, there is a high frequency of the *CYP2C19*2* variant in African-Americans, Caucasians and Asians, whereas the *CYP2C19*3* is less common. It is estimated that the *CYP2C19*2* defective allele accounts for 75–85% of the PMs in Asians and Caucasians. The *CYP2C19*4* and *CYP2C19*5* variants also lead to translation of inactive enzymes, whereas the *CYP2C19*6* and *CYP2C19*8* variants have 2 and 9% of the relative activity of the wild-type version, respectively [37]. Cyclophosphamide and ifosfamide undergo 4-hydroxylation by CYP2C19, and both of their metabolites are active compounds [64,65]. Timm *et al.* [66] reported that for patients treated with cyclophosphamide at dosages below 1000 mg/m^2, there was a significant correlation with the *CYP2C19*2* genotype and pharmacokinetic parameters, whereas there were no correlations in polymorphisms in other genes, including *CYP2B6*, *CYP2C9*, *CYP3A5*, and *GSTA1*. This correlation was lost at dosages above 1000 mg/m^2, indicating the potential induction of a CYP or other metabolic pathway. Thalidomide, an antiangiogenesis agent being actively investigated in clinical trials, is also metabolized by CYP2C19 to three different metabolites [67]. No study has yet shown a correlation between *CYP2C19* genotype and alteration in the pharmacokinetics, efficacy or toxicity of thalidomide.

CYP2D6

While representing only a small percentage of total expressed CYP enzyme protein in the liver, CYP2D6 has a significant role in drug metabolism. It is estimated that between 20 and 25% of all clinically used drugs are metabolized by this enzyme [68]. These include commonly used antidepressants such as amitriptyline, desipramine, and paroxetine, β-blockers such as carvedilol, *S*-metoprolol, propafenone and timolol, antipsychotics including haloperidol, risperidone and thioridazin, antiarrhythmics such as flecainide and lidocaine, as well as the antiemetic ondansetron. Known inhibitors include amiodarone, buproprion, chlorpheniramine, cimetidine, clomipramine, duloxetine, fluoxetine, haloperidol, paroxetine, methadone, mibefradil, ritonavir and quinidine. Both dexamethasone and rifampin can induce enzymatic activity [24,68].

The gene encoding CYP2D6 is located on 22q13. Thus far, there have been 58 different allelic variants described. Including allelic subtypes, there are 101 known variants, including 26 null alleles. In fact, about 71% of the *CYP2D6* alleles are functional in Caucasians, whereas 26% are nonfunctional. Only about 50% of *CYP2D6* alleles are functional in Asians. This high level of variance may be due to the close proximity of the gene to two pseudogenes (*CYP2D7* and *CYP2D8*), as well as the increased relative activity of the locus compared with other CYP genes [68]. The most common of these variants are listed in Table 7 [69]. In addition to these variants, *CYP2D6* gene duplication, which causes individuals to be ultrarapid metabolizers, has been found in a number of different ethnic groups at high frequencies, including Swedish (2%), German (3.6%), Spanish (7–10%), Italian (10%), Saudi Arabians (20%) and Ethiopian (29%) [70].

Recent evidence has shown that the *CYP2D6* genotype may have a profound impact on patients treated with tamoxifen. About 90% of tamoxifen is metabolized to an inactive compound, *N*-desmethyl-tamoxifen, by CYP3A4, whereas about 10% is metabolized by CYP2D6 to 4-hydroxy-tamoxifen. This latter compound has a 50- to 100-fold higher activity level than the parent compound [71]. In a recent retrospective analysis of 223 women treated with tamoxifen, patients were genotyped for the **4* and **6* variants of *CYP2D6*. Women homozygous for the *CYP2D6*4* variant had a worse relapse-free time ($P = 0.023$) and disease free-survival ($P = 0.012$), but not overall survival ($P = 0.169$) compared with women with other variants, leading to the hypothesis that tamoxifen was being rapidly metabolized in these patients. Interestingly, and consistent with these clinical parameters, was the finding that women homozygous for the *CYP2D6*4* variant had a lower incidence of hot flashes relative to patients who were heterozygous or homozygous for the wild-type variant [72].

CYP3A4 and CYP3A5

The CYP3A subfamily of enzymes is the largest cytochrome P450 subfamily in terms of expression level in the liver, accounting for 30% or more of total CYP protein [73]. In all, there are four family members, i.e. CYP3A4, CYP3A5, CYP3A7 and CYP3A43. CYP3A7 is the predominant CYP3A member expressed in the fetus, but is present only at low levels in the adult [74]. CYP3A43 was recently discovered, but appears to have a limited role in drug metabolism [75]. This enzyme has only low expression levels in the liver, prostate and testis, and thus may be less relevant to clinical pharmacology. It does have, however, a role in metabolizing testosterone to less active forms of the hormone, which, coupled with the existence of polymorphisms in the gene encoding CYP3A43, raises the possibility that this enzyme plays a role in the risk of developing prostate cancer [76]. The predominant enzymes in this subfamily in terms of drug pharmacogenetics are CYP3A4 and CYP3A5, with the former playing the more significant role.

CYP3A4 is highly expressed in the liver. It is also expressed in the breast, small intestine and large intestine [77]. It is estimated that over 50% of all current medications as well as endogenous steroids and environmental compounds and toxins are metabolized by this enzyme [73,78,79]. Commonly used medications metabolized by CYP3A4 include the macrolide antibiotics erythromycin and clarithromycin; the benzodiazepines alprazolam, midazolam and triazolam; calcium channel blockers amlodipine, diltiazem, nifedipine and verapamil; the hyperlipidemic agents atorvastatin, lovastatin, and simvastatin; as well as dextromethorphan, propranolol,

Table 7 Ethnic frequency (%) of allelic variants in the *CYP2D6* gene

Allelic variant	SNPs	Caucasians	African-Americans	Asians	Hispanics	Africans	Middle Easterns
*CYP2D6*2*	C2850T	22–34	17.5–26.9	9.2–20	18.5–22.8	10.9–78	10–16
*CYP2D6*3*	2549A del	1.0–3.9	0.3–0.6	0–0.8	0	0–0.5	0–0.8
*CYP2D6*4*	G1846A	11.6–23.0	5.8–8.5	0.5–1.2	3.6–10.3	1.2–7.0	3.5–11.3
*CYP2D6*5*	2D6 deleted	1.6–7.3	6.0–6.9	4.5–6.2	2.3–4.2	33–6.1	0.1–1.5
*CYP2D6*6*	1797T del	0.7–1.4	0.5			0	0.7
*CYP2D6*10*	C100T	1.4–8.0	2.5–7.5	38.1–70	1.8–7.4	3.1–8.6	0.3–6.1
*CYP2D6*17*	C1023T	0.1–0.3	14.6–26	0–0.5	0	9.0–34	0.1–0.3

SNP, single nucleotide polymorphism.
Source: [69].

quinine, salmeterol, sildenafil and zolpidem, and many others. Drugs that inhibit enzyme activity include the antiretroviral agents indinavir, nelfinavir and ritonavir, as well as amiodarone, cimetidine, clarithromycin, erythromycin, diltiazem, ketoconazole, fluvoxamine, nefazodone and verapamil, and grapefruit juice. Drugs that can induce enzymatic activity include carbamazepine, phenobarbital, phenytoin, rifampin, troglitazone, rifabutin and St John's wort [24].

CYP3A4 activity shows wide interindividual variation of up to 40-fold [73]. Ozdemir *et al.* [80] initially estimated that 60–90% of the observed variation in metabolism of CYP3A4 substrates could be explained by genetic differences. Several lines of evidence have clouded this picture, as well as the findings that health status, endogenous hormone levels and age affect enzyme expression and activity [81]. Thus, for the moment it is not clear that genetic variants play a critical role in explaining interindividual variation in the metabolism of CYP3A4 substrates.

The gene encoding CYP3A4 is located at 7q21. Thus far, 39 different allelic variants have been named, arising from *22* nonsynonymous SNPs, nine synonymous SNPs and eight SNPs in the promoter region [82]. The SNP variants leading to alterations in amino acid sequences along with their ethnic frequencies are shown in Table 8 [77,83–85]. Although there are no null alleles, a number of these variants lead to proteins with reduced function. These include the variants *CYP3A4*2*, **4*, **5* and **6* [86]. The *CYP3A4*18* variant was found *in vitro* to have increased activity, but the effect of this polymorphism on *in vivo* activity has not yet been shown.

Like CYP3A4, the CYP3A5 enzyme is expressed in the liver, small intestine and large intestine [87]. Unlike CYP3A4, CYP3A5 is also significantly expressed in extrahepatic tissues, including the prostate, kidney, adrenal gland and pituitary gland [88–91]. Its expression is silenced, however, in 60–90% of Caucasians and a lower percentage of African-Americans owing to polymorphisms in the gene. These include the *CYP3A5*3C* variant, with the A6986G SNP in intron 3, which causes a complete silencing of genetic expression. Up to 90% of Caucasians and 30% of African-Americans are homozygous for this variant and thus deficient in *CYP3A5* expression [92,93]. Table 9 [29,84,94,95] shows the ethnic frequencies of these allelic variants.

Complicating our understanding of this enzyme is the significant overlap in the drugs that are substrates, inhibitors, and inducers of *CYP3A5* [94]. Thus, the role of *CYP3A5* is not always clear, and depends on the drug and site of action of the drug. Genetic variants of this enzyme, including those that lead to no enzyme expression at all *in vivo*, could have significant importance in clinical pharmacology. A further complication in this area of research is the fact that the probe drug used for investigating these enzymes, midazolam, may not be an accurate probe for pharmacogenetic studies – at least in cancer patients – and does not correlate with genotype variation in *CYP3A4* and *CYP3A5* [96].

A number of important chemotherapy agents are metabolized by both CYP3A4 and CYP3A5, including docetaxel, paclitaxel (in part), etoposide and vincristine, and the immunosuppressive agents sirolimus and cyclosporine. These two enzymes account for an estimated 64–93% of docetaxel total metabolism [97]. The clearance of docetaxel is correlated with CYP3A activity, as measured by midazolam metabolism [98], the erythromycin breath test [99], urinary excretion of cortisol [100,101] or dexamethasone pharmacokinetic parameters [102]. The role of genetic variations in CYP3A4 and CYP3A5 and docetaxel pharmacology, however, is not yet clear. Interestingly, Goh *et al.* [98] found that individuals who had the *CYP3A5* genotype causing no protein expression actually had a trend (although not statistically significant) towards a higher mean drug clearance than those patients with one or two active *CYP3A5* alleles, as if a compensatory increase in another subfamily member had resulted. For etoposide clearance, Kishi *et al.* [103] found that the *CYP3A5* PM genotype predicted lower

Table 8 Ethnic frequency (%) of allelic variants in *CYP3A4*

Allelic variant	SNPs	Caucasians	African-Americans	Asians	Hispanics	Africans
*CYP3A4*2*	T15713C	2.7				
*CYP3A4*3*	T1334C	0.6–4.2		0 0.3		0
*CYP3A4*4*	A352G			0.4–3.3		
*CYP3A4*5*	C653G			0.6–1.4		
*CYP3A4*6*	831insA	4.0	0	0.1–0.5		
*CYP3A4*7*	G167A	1.1				
*CYP3A4*10*	G520C	0.5–4.0	0	0		
*CYP3A4*15*	G485A	0	2.4–4.2	0		4.2
*CYP3A4*16*	C554G	0	0	1.3–5.0	5.0	
*CYP3A4*17*	T566C	0–2.1	0	0–5.0		0
*CYP3A4*18*	T878C	0		0.8–2.8		0
*CYP3A4*19*	C1399T			2.1		

SNP, single nucleotide polymorphism.
Sources: Caucasians [77,83,84], African-Americans [77,84], Asians [77,83–85], Hispanics [77], Africans [83].

Table 9 Ethnic frequency (%) of allelic variants in CYP3A5

Allelic variant	SNPs	Caucasians	African-Americans	Asians	Hispanics	Africans
*CYP3A5*2*	C27289A	0–1.3	0	0		
*CYP3A5*3*	A6986G	90.8–93.2	32.2	72.9–74.0	63	28.6
*CYP3A5*6*	G14690A	0.43	11.9	0	3.7	16.8
*CYP3A5*7*	27131–32insT	0	8.5–19	0		1.9
*CYP3A5*8*	C3699T	0		0		4
*CYP3A5*9*	G19386A	0	0	1–5.0		
*CYP3A5*10*	T29753C	2		1		1

SNP, single nucleotide polymorphism.
Sources: Caucasians [29,84,94], African Americans [84,94], Asians [29,84,94], Hispanics [84], Africans [94,95].

drug clearance, but only in patients of African descent and not in Caucasians. Paclitaxel is metabolized by both CYP3A4 and CYP2C8. The parent drug can be metabolized by CYP3A4 to 3-*p*-hydroxypaclitaxel and then CYP2C8 to 6α,3-*p*-dihydroxypaclitaxel, or can be metabolized first by CYP2C8 to 6α-hydroxypaclitaxel and then CYP3A4 to 6α,3-*p*-dihydroxypaclitaxel. By giving an agent that inhibits CYP3A4 (PSC-833, valspodar), levels of 6α-hydroxypaclitaxel have been shown to increase along with toxicities in patients treated with both drugs. This was, at least in part, due to the inhibition of P-glycoprotein (ABCB1) by PSC-833, but the inhibition of CYP3A4 was also a potential cause of toxicities in patients treated with this combination. This interaction required a reduction in paclitaxel dosing levels by 50% in patients treated with PSC-833 [104].

DeMichele *et al.* [105] investigated genotypes for *CYP3A4*, *CYP3A5*, as well as in the glutathione *S*-transferase gene in 90 node-positive breast cancer patients receiving anthracycline-based chemotherapy followed by high-dose, multiagent chemotherapy followed by stem cell rescue. They found that patients with the *CYP3A4*1B* and *CYP3A5*3C* variants who also did not have deletions in the gene encoding glutathione *S*-transferase (*GSTM1* or *GSTT1*) had a nearly 5-fold poorer disease-free survival rate and 4-fold poorer overall survival rate than women who did not have these variants in CYP3A but had deletions in GST [105]. Zhang *et al.* [106] found that patients homozygous for *CYP3A5*3* had higher dose-adjusted concentrations of tacrolimus at 1 week, 1 month and 3 months after kidney transplantation than those heterozygous or homozygous for the wild-type allele. Seven other investigators have confirmed this finding [107].

In terms of the effect of CYP3A4 and CYP3A5 on explaining interindividual variation in drug metabolism, much still needs to be researched and conflicting evidence needs to be reconciled. Open questions remain unanswered, including whether polymorphisms in CYP3A5 have the greater impact in explaining variability in drug disposition than polymorphisms in CYP3A4, and whether influences such as health status and age better explain the variability in CYP3A4 expression and activity than genotypic differences. Given the important substrates in clinical oncology metabolized by these enzymes, and the known wide variability in these drugs' pharmacokinetic profiles, it is important that we resolve these questions once and for all in the months and years ahead.

UDP glucuronosyltransferase

UDP glucuronosyltransferases (UGTs) are membrane-bound enzymes that serve as one of the primary pathways in phase II drug metabolism. These enzymes catalyze the conjugation of glucuronic acid from uridine diphosphoglucuronic acid to endogenous and exogenous substrates. This, in turn, increases the polarity of the conjugated molecule and facilitates the elimination of the new product in the bile or urine. A wide range of substrates are metabolized by UGT enzymes and UGTs are thought to be responsible for 35% of all drugs metabolized by conjugation reactions, or 'phase II' reactions, including drug metabolism via acetylation, glucuronidation, sulfation and methylation [78]. Although predominantly expressed in the liver, UGTs are also expressed in a wide range of tissues, including the kidneys, brain, placenta, breast, prostate, and uterus. In their putative role in metabolizing exogenous environmental toxins, UGTs are also expressed in the nasal mucosa, gastrointestinal tract, white blood cells and the skin [108].

Eighteen different functioning UGT proteins are encoded in the genome, and are classified into two families – UGT1 and UGT2. They are further classified into three subfamilies owing to their sequence homology – UGT1A, UGT2A and UGT2B. The best studied, and thus far the most critical UGT family member in terms of chemotherapy pharmacology, is UGT1A1.

UGT1A1

A critical endogenous substrate for UGT1A1 is bilirubin produced from the catabolism of heme. Several familial forms of hyperbilirubinemia, including Crigler–Najjar type 1 and the more common Gilbert's syndrome, have been found to be due to genetic polymorphisms in the *UGT1A1* gene. To date, more than 60 different genetic variations have been identified in the *UGT1A1* gene and its promoter. One of these, and the one that has been most frequently associated with Gilbert's syndrome, is based on the number of TA dinucleotide repeats in the

Table 10 Ethnic frequency (%) of TA repeat variants in *UGT1A1*

No. of TA repeats	Caucasians	African-Americans	Asians	Hispanics
5	0	4.5	0	0
6	65.7–71.1	63.6	85–100	50–62.5
7	29.0–34.3	18.2	0–15	37.5–50
8	0	13.7	0	0

Source: [109].

TATA-box region of the promoter. The wild-type has six repeated TA pairs. The *UGT1A1*28* has seven and is found in patients with Gilbert's syndrome. Two additional variants, *UGT1A1 *33* and *UGT1A1*34*, have five and eight TA repeats, respectively. Increasing the number of repeats leads to a decrease in the rate of transcription initiation and thus lowers protein expression. Thus, the mild hyperbilirubinemia of Gilbert's syndrome is due to a lower protein expression of UGT1A1. The ethnic frequency of these polymorphic alleles varies considerably (Table 10) [109] with Caucasians and African-Americans more commonly having variants in the promoter region, whereas Asians more commonly have missense mutations in the coding region [110].

The importance of genetic variation in the *UGT1A1* gene has been most extensively studied in the context of chemotherapeutic treatment with the topoisomerase I inhibitor, irinotecan. This prodrug is activated *in vivo* by carboxylesterase 2 (hCE2) to its active metabolite, 7-ethyl-10-hydroxy-camptothecin (SN-38), which is 1000-fold more potent than its parent drug. SN-38 is glucuronidated by several UGT1A isoforms, including UGT1A1 and UGT1A9, and is then subsequently eliminated mainly in the bile [111]. Numerous studies have shown that genetic polymorphisms in the *UGT1A1* gene correlate with irinotecan's pharmacokinetics and toxicity. In a retrospective study of 118 Japanese patients treated with the drug, Ando *et al.* [112] reported that patients with the *UGT1A1*28* allelic variation had a higher incidence of toxicity, including myelosuppression and severe diarrhea, than those with the wild-type allele. The frequency of the *UGT1A1*28* allele was 3.5-fold higher in those patients experiencing grade 4 leucopenia and/or grade 3 or 4 diarrhea. Almost half of all patients who experienced severe toxicity carried one or more *UGT1A1*28* alleles. Of the 92 patients without toxicity, 3 and 11% were homozygous and heterozygous, respectively, for the *UGT1A1*28* allele. Iyer *et al.* [113] then studied patients prospectively, and found that the glucuronidation rate of SN-38 was 3.9-fold lower in patients with the *UGT1A1*28* variant than those who were homozygous for the wild-type allele. In addition, patients carrying the *UGT1A1*28* allele had a 2.5-fold lower absolute neutrophil count at the nadir after treatment with irinotecan. Additional researchers have confirmed these findings [114,115].

On the basis of the confirmatory studies, in 2005 the US Food and Drug Administration changed the labeling of irinotecan to suggest that all patients be genotyped for the promoter polymorphism [116]. If patients are found to have the *UGT1A1*28* allele, it was recommended that they receive a lower dose. Unfortunately, not until late 2005 was a test licensed to perform this genotyping and clinicians remain, to this day, without clear guidance on what sort of dose reduction to perform if patients are found to be heterozygous or homozygous for the *UGT1A1*28* allele.

Pharmacogenomics of drug transporting polypeptides

In addition to the effect of metabolism enzymes on drug pharmacokinetics, transporting proteins expressed in the gastrointestinal tract, liver and kidney may affect both the absorption and elimination of substrates. Most of the transporters identified thus far have been categorized into two superfamilies: the ATP-binding cassette transporters (ABC) and the solute carrier (SLC) transporter family. The Human Genome Organization Nomenclature Committee has categorized the 49 ABC transporters into seven families (ABCA to ABCG), and the 360 SLC transporters into 46 families (SLC1 to SLC46). Some members of these families have been very well categorized and found to transport specific endogenous and exogenous substrates including drugs. Others are only beginning to be characterized and may or may not transport pharmaceutical agents.

Genetic variations in some of the genes encoding these transporters have also been identified, and found to correlate with significant variation in the expression and function of these proteins. In turn, these genetic polymorphisms help explain interpatient variability in the pharmacology and response to commonly used drugs [117,118]. Differences in protein expression and function can impact the oral absorption as well as the liver or renal elimination of agents. They can also affect cellular influx and efflux at the drug target, a fact especially relevant in cancer therapy.

Research on many of these transporters is just beginning to identify and characterize their expression, function and genetic variations. The most extensively studied over the past decade is ABCB1, also known as P-glycoprotein. Another ABC transporter, ABCG2 [also known as breast cancer resistance protein (BCRP)], has been found to be of importance clinically, and both are discussed below.

ABCB1

ABCB1 is encoded by the *MDR-1* gene and is expressed at high levels in the liver and the kidney, where it serves as an efflux transporter involved in the elimination of numerous endogenous and exogenous substrates [119]. It

is found in the epithelium of the small intestine located at the apical enterocyte membrane where it is involved in the efflux of substrates into the gut lumen from the circulation. It is expressed in the placenta serving as part of the maternal–fetal barrier. It is also expressed in the vasculature of the central nervous system, preventing entry of substrates into the central nervous system from the blood stream, where it serves a key role in forming the blood–brain barrier. Finally, it is expressed in a number of malignant cells, both *in vitro* and *in vivo*, and is a central mechanism of multidrug resistance [120].

A substantial number of approved chemotherapy agents are substrates for ABCB1, including actinomycin D, daunorubicin, docetaxel, doxorubicin, etoposide, paclitaxel, vinblastine and vincristine [121]. Agents that inhibit ABCB1 that have been tested preclinically and clinically as a means to overcome ABCB1 drug efflux and multidrug resistance include verapamil, the phenothiazines, quinidine, quinacrine, quinine, amiodarone, several neuroleptics, tamoxifen, progesterone, cyclosporine, dexverapamil, dexniguldipine, GF-902128, PSC-833 and VX-710 [120]. Hoffmeyer *et al.* [122] were the first to systematically perform sequencing analysis on human DNA of the *ABCB1* gene. They identified 15 polymorphisms, eight in exon sequences and seven in introns [122]. One SNP, a synonymous wobble mutation at C3435T in exon 26, was found to correlate with a significant reduction in protein expression along the duodenum. This finding by Hoffmeyer *et al.* [122] has been confirmed by some investigators, whereas others have found no correlation between this SNP and protein expression [123–127].

This SNP also correlated with altered oral absorption of a number of drugs thought to be substrates for the transporter [128]. Genotypic variation has been found to correlate with response to neoadjuvant chemotherapy in breast cancer [129]. Additional research has found that alterations in recommended drug dosing can be generated by knowing patients' *ABCB1* genotype for drugs such as immune modulators and antiretrovirals [130,131]. The ethnic frequency of this and other SNPs in the *ABCB1* gene are listed in Table 11 [132].

Thus, whereas earlier studies showed significant correlations between SNPs and transporter expression and function of ABCB1, subsequent studies have provided conflicting results. These contradictory findings still must be resolved. Nevertheless, a survey of the literature indicates that most common genetic variants in the *ABCB1* gene are not strongly associated with the pharmacokinetics of anticancer drugs, unless ABCB1 plays a crucial role in the oral absorption of a substrate drug.

Table 11 Ethnic frequency (%) of allelic variants in the *ABCB1* gene

Allelic variant	Caucasians	African-Americans	Asians	Africans	Middle Easterns
C1236T			62		
G2677T	42–48		37		
G2677A	2		19		
C3435T	33–65	14–16	37–47	17–27	45

Source: [132].

ABCG2

ABCG2 was initially discovered in a breast cell cancer cell line resistant to anthracyclines [133]. It is highly expressed in the placenta, and also has significant expression in the central nervous system, liver, adrenal gland, placenta, prostate, testes and uterus. Lower levels are found in the small and large intestine, stomach, lung, kidney and pancreas [133,134]. Although it was discovered in a breast cancer-resistant cell line, and was named BCRP from that discovery, its expression in nonlactating normal and cancerous breast tissue does not appear to be clinically significant.

A growing number of drugs have been identified as substrates for ABCG2. These include the antibiotics ciprofloxacin, oflaxacin and norfloxacin [135], cimetidine [136], the anti-lipid agent cerivastatin [127], as well as antiviral agents zidovudine [138], and lamivudine [139]. Agents found to inhibit the transporter include the immune suppressants tacrolimus, sirolimus and cyclosporine A [140]; the antivirals ritonavir, saquinavir and nelfinavir [141]; and the proton pump inhibitors pantoprazole and omeprazole [142]. Chemotherapy agents that are substrates for ABCG2 include mitoxantrone, methotrexate [143], flavopiridol [144], topotecan [145], irinotecan and its active metabolite SN-38 [146], UCN-01 [147], imantinib [148], and gefitinib [149].

Several SNPs have been identified in coding regions of the gene and at least four nonsynonymous SNPs have been identified. These SNPs occur at mRNA positions 34 (V12M; exon 2), 421 (Q141K, exon 16), 616 (I206L, exon 6) and 1768 (N590Y, exon 15). The ethnic frequencies of these SNPs are shown in Table 12 [150–153]. The SNPs of V12M, I206L and N590Y have not to date been found to confer an alteration in protein expression or function. The nonsynonymous substitution C421A, in which a lysine is substituted for glutamine at codon 141 (Q141K), has been shown to have a functional significance. Various

Table 12 Ethnic frequencies (%) of allelic variants in *ABCG2* gene

Allelic variant	Caucasians	African-Americans	Asians	Hispanics	Africans	Middle Easterns
V12M	2	4	20–45	40		5
Q141K	11–14	2.3–5.0	15–35	10	1.0	13
I206L	0	0	0	10		0
N590Y	1					

Sources: [150–153].

researchers have found that this SNP can lead to lower plasma membrane expression [150,154–156] reduced drug efflux [157,158], and reduced ATPase activity [156,157]. This loss-of-function polymorphism can lead to lower IC_{50} levels in cell lines exposed to cytotoxic agents that are ABCG2 substrates, including mitoxantrone, irinotecan and SN-38. Plasma concentrations of orally administered drugs, including oral topotecan, are higher in patients with the Q141K SNP, which correlates with the protein's role in drug excretion. The SNP also has been found to correlate with higher drug levels *in vivo* in patients exposed to topotecan, irinotecan and diflomotecan, coinciding with its role in biliary and intestinal elimination of xenobiotics. Further clinical testing will be needed to determine whether prospective genotyping and dose modifications on the basis of this polymorphism are warranted.

Conclusion

Over the past decade, great advances have been made in discovering and understanding the proteins involved in drug transport and metabolism, as well as in polymorphisms and allelic variants in the genes encoding these proteins. This knowledge has opened up the possibility that we may one day be able to understand how these proteins and their genetic variants alter function within individual patients. This, in turn, could allow us to choose and dose medications on the basis of each individual's drug metabolism profile.

Finding ways to individualize medical therapy, drug choice and dosage is a top priority for medical research and government regulators. The US Food and Drug Administration identified this field as a 'Key Opportunity' in its 2004 'Critical Path' for the future. The National Institutes of Health is currently in its second 5-year funding cycle, supporting the development of a multi-institutional Pharmacogenomics Research Network, as well as building a comprehensive clinical research database for investigators from around the world looking for correlations between genotype and phenotype in medical therapy. In no area is this research endeavor more relevant than in medical oncology, in which treatment with chemotherapy needs to be performed within a narrow therapeutic index, balancing drug efficacy with significant drug toxicity.

In the last few decades, a great number of genetic variants in drug-metabolizing and transporter genes have been identified, but most variants are rare and occur in less than 1% of patients. Although *in vitro* models have indicated that genetic variants may have an increased or decreased phenotype when compared with wild-type, very few clinical studies have been performed to explore how these genetic variants affect drug disposition within patients. Finally, whereas the elimination pathways for some of our current chemotherapeutic agents have been fairly well described, including those for cyclophosphamide and irinotecan, for the vast majority of the drugs used in medical oncology we still do not know which enzymes and transporters are involved in their disposition.

To bridge this gulf between what is known about genetic variants in metabolizing enzymes and transporters, and how little is known on whether and how we should alter our dosing schema of specific drugs on the basis of these genetic variants, there is a critical need to expand greatly the pharmacogenomic component of clinical trials in all phases of drug development. This applies to newer targeted therapies undergoing phase I and phase II studies, as well as to more traditional cytotoxic chemotherapies being used in large phase III trials. As the cases of drugs such as 5-FU, irinotecan and 6-mercaptopurine show, we may be putting our patients at risk of unnecessary toxicity if we ignore the pharmacogenomic aspects of drug therapy. Alternatively, we might be giving some patients subtherapeutic doses of chemotherapy if we ignore genotypic variation that causes the rapid metabolism or excretion of cancer drugs. Ignoring the pharmacogenomic aspect of cancer care under this scenario puts our patients' chances of treatment, and even cure, at risk.

References

1 McLeod HL, Krynetski EY, Relling MV, Evans WE. Genetic polymorphism of thiopurine methyltransferase and its clinical relevance for childhood acute lymphoblastic leukemia. *Leukemia* 2000; **14**:567–572.

2 McLeod HL, Siva C. The thiopurine *S*-methyltransferase gene locus: implications for clinical pharmacogenomics. *Pharmacogenomics* 2002; **3**:89–98.

3 Ameyaw MM, Collie-Duguid ES, Powrie RH, Ofori-Adjei D, McLeod HL. Thiopurine methyltransferase alleles in British and Ghanaian populations. *Hum Mol Genet* 1999; **8**:367–370.

4 Spire-Vayron de la Moureyre C, Debuysere H, Sabbagh N, Marez D, Vinner E, Chevalier ED, *et al.* Detection of known and new mutations in the thiopurine *S*-methyltransferase gene by single-strand conformation polymorphism analysis. *Hum Mutat* 1998; **12**:177–185.

5 Hon YY, Fessing MY, Pui CH, Relling MV, Krynetski EY, Evans WE. Polymorphism of the thiopurine *S*-methyltransferase gene in African-Americans. *Hum Mol Genet* 1999; **8**:371–376.

6 Kumagai K, Hiyama K, Ishioka S, Sato H, Yamanishi Y, McLeod HL, *et al.* Allelotype frequency of the thiopurine methyltransferase (TPMT) gene in Japanese. *Pharmacogenetics* 2001; **11**:275–278.

7 Zhang JP, Zhou SF, Chen X, Huang M. Determination of intra-ethnic differences in the polymorphisms of thiopurine *S*-methyltransferase in Chinese. *Clin Chim Acta* 2006; **365**:337–341.

8 Boson WL, Romano-Silva MA, Correa H, Falcao RP, Teixeira-Vidigal PV, De Marco L. Thiopurine methyltransferase polymorphisms in a Brazilian population. *Pharmacogenomics J* 2003; **3**:178–182.

9 Larovere LE, de Kremer RD, Lambooy LH, De Abreu RA. Genetic polymorphism of thiopurine *S*-methyltransferase in Argentina. *Ann Clin Biochem* 2003; **40**:388–393.

10 McLeod HL, Pritchard SC, Githang'a J, Indalo A, Ameyaw MM, Powrie RH, *et al.* Ethnic differences in thiopurine methyltransferase pharmacogenetics: evidence for allele specificity in Caucasian and Kenyan individuals. *Pharmacogenetics* 1999; **9**:773–776.

11 Relling MV, Hancock ML, Rivera GK, Sandlund JT, Ribeiro RC, Krynetski EY, *et al.* Mercaptopurine therapy intolerance and heterozygosity at the thiopurine *S*-methyltransferase gene locus. *J Natl Cancer Inst* 1999; **91**:2001–2008.

12 Heggie GD, Sommadossi JP, Cross DS, Huster WJ, Diasio RB. Clinical pharmacokinetics of 5-fluorouracil and its metabolites in plasma, urine, and bile. *Cancer Res* 1987; **47**:2203–2206.

13 Maring JG, van Kuilenburg AB, Haasjes J, Piersma H, Groen HJ, Uges DR, *et al.* Reduced 5-FU clearance in a patient with low DPD activity due to heterozygosity for a mutant allele of the DPYD gene. *Br J Cancer* 2002; **86**:1028–1033.

14 van Kuilenburg AB, Meinsma R, Zoetekouw L, van Gennip AH. High prevalence of the IVS14 + 1G > A mutation in the dihydropyrimidine dehydrogenase gene of patients with severe 5-fluorouracil-associated toxicity. *Pharmacogenetics* 2002; **12**:555–558.

15 van Kuilenburg AB. Dihydropyrimidine dehydrogenase and the efficacy and toxicity of 5-fluorouracil. *Eur J Cancer* 2004; **40**:939–950.

16 van Kuilenburg AB, Muller EW, Haasjes J, Meinsma R, Zoetekouw L, Waterham HR, *et al.* Lethal outcome of a patient with a complete dihydropyrimidine dehydrogenase (DPD) deficiency after administration of 5-fluorouracil: frequency of the common IVS14 + 1G > A mutation causing DPD deficiency. *Clin Cancer Res* 2001; **7**:1149–1153.

17 Ezzeldin H, Okamoto Y, Johnson MR, Diasio RB. A high-throughput denaturing high-performance liquid chromatography method for the identification of variant alleles associated with dihydropyrimidine dehydrogenase deficiency. *Anal Biochem* 2002; **306**:63–73.

18 Fernandez-Salguero P, Gonzalez FJ, Etienne MC, Milano G, Kimura S. Correlation between catalytic activity and protein content for the polymorphically expressed dihydropyrimidine dehydrogenase in human lymphocytes. *Biochem Pharmacol* 1995; **50**:1015–1020.

19 Johnson MR, Yan J, Shao L, Albin N, Diasio RB. Semi-automated radioassay for determination of dihydropyrimidine dehydrogenase (DPD) activity. Screening cancer patients for DPD deficiency, a condition associated with 5-fluorouracil toxicity. *J Chromatogr B Biomed Sci Appl* 1997; **696**:183–191.

20 Kuhara T, Ohdoi C, Ohse M, van Kuilenburg AB, van Gennip AH, Sumi S, *et al.* Rapid gas chromatographic-mass spectrometric diagnosis of dihydropyrimidine dehydrogenase deficiency and dihydropyrimidinase deficiency. *J Chromatogr B Anal Technol Biomed Life Sci* 2003; **792**:107–115.

21 Magne N, Renee N, Formento J, Etienne-Grimaldi M, Francoual M, Milano G. Prospective study of dihydropyrimidine dehydrogenase (DPD) activity and DPYD IVS14 + 1G > A mutation in patients developing FU-related toxicities: an updated analysis based on a ten-year recruitment across multiple French institutions. *Proc Am Soc Clin Oncol* 2005; **23**(**16S**). abstr 2003.

22 Mattison LK, Ezzeldin H, Carpenter M, Modak A, Johnson MR, Diasio RB. Rapid identification of dihydropyrimidine dehydrogenase deficiency by using a novel 2-^{13}C-uracil breath test. *Clin Cancer Res* 2004; **10**:2652–2658.

23 Ekins S, Vandenbranden M, Ring BJ, Gillespie JS, Yang TJ, Gelboin HV, *et al.* Further characterization of the expression in liver and catalytic activity of CYP2B6. *J Pharmacol Exp Ther* 1998; **286**:1253–1259.

24 Flockhart DA. http://medicine.iupui.edu/flockhart/table.htm.

25 Yamano S, Nhamburo PT, Aoyama T, Meyer UA, Inaba T, Kalow W, *et al.* cDNA cloning and sequence and cDNA-directed expression of human P450 IIB1: identification of a normal and two variant cDNAs derived from the CYP2B locus on chromosome 19 and differential expression of the IIB mRNAs in human liver. *Biochemistry* 1989; **28**:7340–7348.

26 Lamba V, Lamba J, Yasuda K, Strom S, Davila J, Hancock ML, *et al.* Hepatic CYP2B6 expression: gender and ethnic differences and relationship to CYP2B6 genotype and CAR (constitutive androstane receptor) expression. *J Pharmacol Exp Ther* 2003; **307**:906–922.

27 Kirchheiner J, Klein C, Meineke I, Sasse J, Zanger UM, Murdter TE, *et al.* Bupropion and 4-OH-bupropion pharmacokinetics in relation to genetic polymorphisms in CYP2B6. *Pharmacogenetics* 2003; **13**:619–626.

28 Lang T, Klein K, Fischer J, Nussler AK, Neuhaus P, Hofmann U, *et al.* Extensive genetic polymorphism in the human CYP2B6 gene with impact on expression and function in human liver. *Pharmacogenetics* 2001; **11**:399–415.

29 Hiratsuka M, Takekuma Y, Endo N, Narahara K, Hamdy SI, Kishikawa Y, *et al.* Allele and genotype frequencies of CYP2B6 and CYP3A5 in the Japanese population. *Eur J Clin Pharmacol* 2002; **58**:417–421.

30 Ariyoshi N, Miyazaki M, Toide K, Sawamura Y, Kamataki T. A single nucleotide polymorphism of CYP2b6 found in Japanese enhances catalytic activity by autoactivation. *Biochem Biophys Res Commun* 2001; **281**:1256–1260.

31 Xie HJ, Yasar U, Lundgren S, Griskevicius L, Terelius Y, Hassan M, *et al.* Role of polymorphic human CYP2B6 in cyclophosphamide bioactivation. *Pharmacogenomics J* 2003; **3**:53–61.

32 Lang T, Klein K, Richter T, Zibat A, Kerb R, Eichelbaum M, *et al.* Multiple novel nonsynonymous CYP2B6 gene polymorphisms in Caucasians: demonstration of phenotypic null alleles. *J Pharmacol Exp Ther* 2004; **311**:34–43.

33 Klein K, Lang T, Saussele T, Barbosa-Sicard E, Schunck WH, Eichelbaum M, *et al.* Genetic variability of CYP2B6 in populations of African and Asian origin: allele frequencies, novel functional variants, and possible implications for anti-HIV therapy with efavirenz. *Pharmacogenet Genomics* 2005; **15**:861–873.

34 Zukunft J, Lang T, Richter T, Hirsch-Ernst KI, Nussler AK, Klein K, *et al.* A natural CYP2B6 TATA box polymorphism (−82T→C) leading to enhanced transcription and relocation of the transcriptional start site. *Mol Pharmacol* 2005; **67**:1772–1782.

35 Schmidt R, Baumann F, Hanschmann H, Geissler F, Preiss R. Gender difference in ifosfamide metabolism by human liver microsomes. *Eur J Drug Metab Pharmacokinet* 2001; **26**:193–200.

36 Xie H, Griskevicius L, Stahle L, Hassan Z, Yasar U, Rane A, *et al.* Pharmacogenetics of cyclophosphamide in patients with hematological malignancies. *Eur J Pharm Sci* 2006; **27**:54–61.

37 Goldstein JA. Clinical relevance of genetic polymorphisms in the human CYP2C subfamily. *Br J Clin Pharmacol* 2001; **52**:349–355.

38 Totah RA, Rettie AE. Cytochrome P450 2C8: substrates, inhibitors, pharmacogenetics, and clinical relevance. *Clin Pharmacol Ther* 2005; **77**:341–352.

39 Dreisbach AW, Japa S, Sigel A, Parenti MB, Hess AE, Srinouanprachanh SL, *et al.* The prevalence of CYP2C8, 2C9, 2J2, and soluble epoxide hydrolase polymorphisms in African Americans with hypertension. *Am J Hypertens* 2005; **18**:1276–1281.

40 Muthiah YD, Lee WL, Teh LK, Ong CE, Ismail R. Genetic polymorphism of CYP2C8 in three Malaysian ethnics: CYP2C8*2 and CYP2C8*3 are found in Malaysian Indians. *J Clin Pharm Ther* 2005; **30**:487–490.

41 Dai D, Zeldin DC, Blaisdell JA, Chanas B, Coulter SJ, Ghanayem BI, *et al.* Polymorphisms in human CYP2C8 decrease metabolism of the anticancer drug paclitaxel and arachidonic acid. *Pharmacogenetics* 2001; **11**:597–607.

42 Bahadur N, Leathart JB, Mutch E, Steimel-Crespi D, Dunn SA, Gilissen R, *et al.* CYP2C8 polymorphisms in Caucasians and their relationship with paclitaxel 6alpha-hydroxylase activity in human liver microsomes. *Biochem Pharmacol* 2002; **64**:1579–1589.

43 Soyama A, Saito Y, Hanioka N, Murayama N, Nakajima O, Katori N, *et al.* Non-synonymous single nucleotide alterations found in the CYP2C8 gene result in reduced *in vitro* paclitaxel metabolism. *Biol Pharm Bull* 2001; **24**:1427–1430.

44 Henningsson A, Marsh S, Loos WJ, Karlsson MO, Garsa A, Mross K, *et al.* Association of CYP2C8, CYP3A4, CYP3A5, and ABCB1 polymorphisms with the pharmacokinetics of paclitaxel. *Clin Cancer Res* 2005; **11**:8097–8104.

45 Garcia-Martin E, Martinez C, Tabares B, Frias J, Agundez JA. Interindividual variability in ibuprofen pharmacokinetics is related to interaction of cytochrome P450 2C8 and 2C9 amino acid polymorphisms. *Clin Pharmacol Ther* 2004; **76**:119–127.

46 Yasar U, Lundgren S, Eliasson E, Bennet A, Wiman B, de Faire U, *et al.* Linkage between the CYP2C8 and CYP2C9 genetic polymorphisms. *Biochem Biophys Res Commun* 2002; **299**:25–28.

47 Ishikawa C, Ozaki H, Nakajima T, Ishii T, Kanai S, Anjo S, *et al.* A frameshift variant of CYP2C8 was identified in a patient who suffered from rhabdomyolysis after administration of cerivastatin. *J Hum Genet* 2004; **49**:582–585.

48 Hichiya H, Tanaka-Kagawa T, Soyama A, Jinno H, Koyano S, Katori N, *et al.* Functional characterization of five novel CYP2C8 variants, G171S, R186X, R186G, K247R, and K383N, found in a Japanese population. *Drug Metab Dispos* 2005; **33**:630–636.

49 Rettie AE, Jones JP. Clinical and toxicological relevance of CYP2C9: drug–drug interactions and pharmacogenetics. *Annu Rev Pharmacol Toxicol* 2005; **45**:477–494.

50 Allabi AC, Gala JL, Horsmans Y, Babaoglo MO, Bozkurt A, Heusterspreute M, Yasar U. Functional impact of CYPZC9*5, CYPZC9*6, CYPZC9*8 and CYPZC9*11 in vivo among black Africans. *Clin Pharmacol Ther* 2004; **76**: 113–118.

51 Blaisdell J, Jorge-Nebert LF, Coulter S, Ferguson SS, Lee SJ, Chanas B, *et al.* Discovery of new potentially defective alleles of human CYP2C9. *Pharmacogenetics* 2004; **14**:527–537.

52 DeLozier TC, Lee SC, Coulter SJ, Goh BC, Goldstein JA. Functional characterization of novel allelic variants of CYP2C9 recently discovered in southeast Asians. *J Pharmacol Exp Ther* 2005; **315**:1085–1090.

53 Tai G, Farin F, Rieder MJ, Dreisbach AW, Veenstra DL, Verlinde CL, *et al.* In-vitro and in-vivo effects of the CYP2C9*11 polymorphism on warfarin metabolism and dose. *Pharmacogenet Genomics* 2005; **15**:475–481.
54 Dickmann LJ, Rettie AE, Kneller MB, Kim RB, Wood AJ, Stein CM, *et al.* Identification and functional characterization of a new CYP2C9 variant (CYP2C9*5) expressed among African Americans. *Mol Pharmacol* 2001; **60**:382–387.
55 Sullivan-Klose TH, Ghanayem BI, Bell DA, Zhang ZY, Kaminsky LS, Shenfield GM, *et al.* The role of the CYP2C9-Leu359 allelic variant in the tolbutamide polymorphism. *Pharmacogenetics* 1996; **6**:341–349.
56 Lee CR, Goldstein JA, Pieper JA. Cytochrome P450 2C9 polymorphisms: a comprehensive review of the in-vitro and human data. *Pharmacogenetics* 2002; **12**:251–263.
57 Llerena A, Dorado P, O'Kirwan F, Jepson R, Licinio J, Wong ML. Lower frequency of CYP2C9*2 in Mexican-Americans compared to Spaniards. *Pharmacogenomics J* 2004; **4**:403–406.
58 Kirchheiner J, Bauer S, Meineke I, Rohde W, Prang V, Meisel C, *et al.* Impact of CYP2C9 and CYP2C19 polymorphisms on tolbutamide kinetics and the insulin and glucose response in healthy volunteers. *Pharmacogenetics* 2002; **12**:101–109.
59 Higashi MK, Veenstra DL, Kondo LM, Wittkowsky AK, Srinouanprachanh SL, Farin FM, *et al.* Association between CYP2C9 genetic variants and anticoagulation-related outcomes during warfarin therapy. *JAMA* 2002; **287**:1690–1698.
60 Kirchheiner J, Brockmoller J. Clinical consequences of cytochrome P450 2C9 polymorphisms. *Clin Pharmacol Ther* 2005; **77**:1–16.
61 Imai J, Ieiri I, Mamiya K, Miyahara S, Furuumi H, Nanba E, *et al.* Polymorphism of the cytochrome P450 (CYP) 2C9 gene in Japanese epileptic patients: genetic analysis of the CYP2C9 locus. *Pharmacogenetics* 2000; **10**:85–89.
62 Kidd RS, Curry TB, Gallagher S, Edeki T, Blaisdell J, Goldstein JA. Identification of a null allele of CYP2C9 in an African-American exhibiting toxicity to phenytoin. *Pharmacogenetics* 2001; **11**:803–808.
63 Desta Z, Zhao X, Shin JG, Flockhart DA. Clinical significance of the cytochrome P450 2C19 genetic polymorphism. *Clin Pharmacokinet* 2002; **41**:913–958.
64 Chang TK, Yu L, Goldstein JA, Waxman DJ. Identification of the polymorphically expressed CYP2C19 and the wild-type CYP2C9-ILE359 allele as low-K_m catalysts of cyclophosphamide and ifosfamide activation. *Pharmacogenetics* 1997; **7**:211–221.
65 Kivisto KT, Kroemer HK, Eichelbaum M. The role of human cytochrome P450 enzymes in the metabolism of anticancer agents: implications for drug interactions. *Br J Clin Pharmacol* 1995; **40**:523–530.
66 Timm R, Kaiser R, Lotsch J, Heider U, Sezer O, Weisz K, *et al.* Association of cyclophosphamide pharmacokinetics to polymorphic cytochrome P450 2C19. *Pharmacogenomics J* 2005; **5**:365–373.
67 Ando Y, Price DK, Dahut WL, Cox MC, Reed E, Figg WD. Pharmacogenetic associations of CYP2C19 genotype with *in vivo* metabolisms and pharmacological effects of thalidomide. *Cancer Biol Ther* 2002; **1**:669–673.
68 Ingelman-Sundberg M. Genetic polymorphisms of cytochrome P450 2D6 (CYP2D6): clinical consequences, evolutionary aspects and functional diversity. *Pharmacogenomics J* 2005; **5**:6–13.
69 Bradford LD. CYP2D6 allele frequency in European Caucasians, Asians, Africans and their descendants. *Pharmacogenomics* 2002; **3**:229–243.
70 Smith NF, Figg WD, Sparreboom A. Recent advances in pharmacogenetics approaches to anti-cancer drug development. *Drug Dev Res* 2004; **62**:233–253.
71 Dehal SS, Kupfer D. CYP2D6 catalyzes tamoxifen 4-hydroxylation in human liver. *Cancer Res* 1997; **57**:3402–3406.
72 Goetz MP, Rae JM, Suman VJ, Safgren SL, Ames MM, Visscher DW, *et al.* Pharmacogenetics of tamoxifen biotransformation is associated with clinical outcomes of efficacy and hot flashes. *J Clin Oncol* 2005; **23**:9312–9318.
73 Shimada T, Yamazaki H, Mimura M, Inui Y, Guengerich FP. Interindividual variations in human liver cytochrome P-450 enzymes involved in the oxidation of drugs, carcinogens and toxic chemicals: studies with liver microsomes of 30 Japanese and 30 Caucasians. *J Pharmacol Exp Ther* 1994; **270**:414–423.
74 Schuetz JD, Beach DL, Guzelian PS. Selective expression of cytochrome P450 CYP3A mRNAs in embryonic and adult human liver. *Pharmacogenetics* 1994; **4**:11–20.
75 Domanski TL, Finta C, Halpert JR, Zaphiropoulos PG. cDNA cloning and initial characterization of CYP3A43, a novel human cytochrome P450. *Mol Pharmacol* 2001; **59**:386–392.
76 Stone A, Ratnasinghe LD, Emerson GL, Modali R, Lehman T, Runnells G, *et al.* CYP3A43 Pro(340)Ala polymorphism and prostate cancer risk in African Americans and Caucasians. *Cancer Epidemiol Biomarkers Prev* 2005; **14**:1257–1261.
77 Keshava C, McCanlies EC, Weston A. CYP3A4 polymorphisms – potential risk factors for breast and prostate cancer: a HuGE review. *Am J Epidemiol* 2004; **160**:825–841.
78 Evans WE, Relling MV. Pharmacogenomics: translating functional genomics into rational therapeutics. *Science* 1999; **286**:487–491.
79 Li AP, Kaminski DL, Rasmussen A. Substrates of human hepatic cytochrome P450 3A4. *Toxicology* 1995; **104**:1–8.
80 Ozdemir V, Kalowa W, Tang BK, Paterson AD, Walker SE, Endrenyi L, *et al.* Evaluation of the genetic component of variability in CYP3A4 activity: a repeated drug administration method. *Pharmacogenetics* 2000; **10**:373–388.
81 Wojnowski L, Kamdem LK. Clinical implications of CYP3A polymorphisms. *Expert Opin Drug Metab Toxicol* 2006; **2**:171–182.
82 http://www.imm.ki.se/CYPalleles.
83 Dai D, Tang J, Rose R, Hodgson E, Bienstock RJ, Mohrenweiser HW, *et al.* Identification of variants of CYP3A4 and characterization of their abilities to metabolize testosterone and chlorpyrifos. *J Pharmacol Exp Ther* 2001; **299**:825–831.
84 Solus JF, Arietta BJ, Harris JR, Sexton DP, Steward JQ, McMunn C, *et al.* Genetic variation in eleven phase I drug metabolism genes in an ethnically diverse population. *Pharmacogenomics* 2004; **5**:895–931.
85 Liu CH, Peck K, Huang JD, Lin MS, Wang CH, Hsu WP, *et al.* Screening CYP3A single nucleotide polymorphisms in a Han Chinese population with a genotyping chip. *Pharmacogenomics* 2005; **6**:731–747.
86 Hsieh KP, Lin YY, Cheng CL, Lai ML, Lin MS, Siest JP, *et al.* Novel mutations of CYP3A4 in Chinese. *Drug Metab Dispos* 2001; **29**:268–273.
87 Lown KS, Kolars JC, Thummel KE, Barnett JL, Kunze KL, Wrighton SA, *et al.* Interpatient heterogeneity in expression of CYP3A4 and CYP3A5 in small bowel. Lack of prediction by the erythromycin breath test. *Drug Metab Dispos* 1994; **22**:947–955.
88 Finnstrom N, Bjelfman C, Soderstrom TG, Smith G, Egevad L, Norlen BJ, *et al.* Detection of cytochrome P450 mRNA transcripts in prostate samples by RT-PCR. *Eur J Clin Invest* 2001; **31**:880–886.
89 Haehner BD, Gorski JC, Vandenbranden M, Wrighton SA, Janardan SK, Watkins PB, *et al.* Bimodal distribution of renal cytochrome P450 3A activity in humans. *Mol Pharmacol* 1996; **50**:52–59.
90 Koch I, Weil R, Wolbold R, Brockmoller J, Hustert E, Burk O, *et al.* Interindividual variability and tissue-specificity in the expression of cytochrome P450 3A mRNA. *Drug Metab Dispos* 2002; **30**:1108–1114.
91 Murray GI, Pritchard S, Melvin WT, Burke MD. Cytochrome P450 CYP3A5 in the human anterior pituitary gland. *FEBS Lett* 1995; **364**:79–82.
92 Hustert E, Haberl M, Burk O, Wolbold R, He YQ, Klein K, *et al.* The genetic determinants of the CYP3A5 polymorphism. *Pharmacogenetics* 2001; **11**:773–779.
93 Kuehl P, Zhang J, Lin Y, Lamba J, Assem M, Schuetz J, *et al.* Sequence diversity in CYP3A promoters and characterization of the genetic basis of polymorphic CYP3A5 expression. *Nat Genet* 2001; **27**:383–391.
94 Xie HG, Wood AJ, Kim RB, Stein CM, Wilkinson GR. Genetic variability in CYP3A5 and its possible consequences. *Pharmacogenomics* 2004; **5**:243–272.
95 Dandara C, Ballo R, Parker MI. CYP3A5 genotypes and risk of oesophageal cancer in two South African populations. *Cancer Lett* 2005; **225**:275–282.
96 Lepper ER, Baker SD, Permenter M, Ries N, van Schaik RH, Schenk PW, *et al.* Effect of common CYP3A4 and CYP3A5 variants on the pharmacokinetics of the cytochrome P450 3A phenotyping probe midazolam in cancer patients. *Clin Cancer Res* 2005; **11**:7398–7404.
97 Shou M, Martinet M, Korzekwa KR, Krausz KW, Gonzalez FJ, Gelboin HV. Role of human cytochrome P450 3A4 and 3A5 in the metabolism of taxotere and its derivatives: enzyme specificity, interindividual distribution and metabolic contribution in human liver. *Pharmacogenetics* 1998; **8**:391–401.
98 Goh BC, Lee SC, Wang LZ, Fan L, Guo JY, Lamba J, *et al.* Explaining interindividual variability of docetaxel pharmacokinetics and pharmacodynamics in Asians through phenotyping and genotyping strategies. *J Clin Oncol* 2002; **20**:3683–3690.
99 Hirth J, Watkins PB, Strawderman M, Schott A, Bruno R, Baker LH. The effect of an individual's cytochrome CYP3A4 activity on docetaxel clearance. *Clin Cancer Res* 2000; **6**:1255–1258.
100 Yamamoto N, Tamura T, Murakami H, Shimoyama T, Nokihara H, Ueda Y, *et al.* Randomized pharmacokinetic and pharmacodynamic study of docetaxel: dosing based on body-surface area compared with

individualized dosing based on cytochrome P450 activity estimated using a urinary metabolite of exogenous cortisol. *J Clin Oncol* 2005; **23**:1061–1069.
101 Yamamoto N, Tamura T, Kamiya Y, Sekine I, Kunitoh H, Saijo N. Correlation between docetaxel clearance and estimated cytochrome P450 activity by urinary metabolite of exogenous cortisol. *J Clin Oncol* 2000; **18**:2301–2308.
102 Puisset F, Chatelut E, Dalenc F, Busi F, Cresteil T, Azema J, *et al.* Dexamethasone as a probe for docetaxel clearance. *Cancer Chemother Pharmacol* 2004; **54**:265–272.
103 Kishi S, Yang W, Boureau B, Morand S, Das S, Chen P, *et al.* Effects of prednisone and genetic polymorphisms on etoposide disposition in children with acute lymphoblastic leukemia. *Blood* 2004; **103**:67–72.
104 Kang MH, Figg WD, Ando Y, Blagosklonny MV, Liewehr D, Fojo T, *et al.* The P-glycoprotein antagonist PSC 833 increases the plasma concentrations of 6alpha-hydroxypaclitaxel, a major metabolite of paclitaxel. *Clin Cancer Res* 2001; **7**:1610–1617.
105 DeMichele A, Aplenc R, Botbyl J, Colligan T, Wray L, Klein-Cabral M, *et al.* Drug-metabolizing enzyme polymorphisms predict clinical outcome in a node-positive breast cancer cohort. *J Clin Oncol* 2005; **23**:5552–5559.
106 Zhang X, Liu ZH, Zheng JM, Chen ZH, Tang Z, Chen JS, *et al.* Influence of CYP3A5 and MDR1 polymorphisms on tacrolimus concentration in the early stage after renal transplantation. *Clin Transplant* 2005; **19**:638–643.
107 Daly AK. Significance of the minor cytochrome P450 3A isoforms. *Clin Pharmacokinet* 2006; **45**:13–31.
108 Tukey RH, Strassburg CP. Human UDP-glucuronosyltransferases: metabolism, expression, and disease. *Annu Rev Pharmacol Toxicol* 2000; **40**:581–616.
109 http://www.PharmGKB.org.
110 Desai AA, Innocenti F, Ratain MJ. Pharmacogenomics: road to anticancer therapeutics nirvana? *Oncogene* 2003; **22**:6621–6628.
111 Iyer L, King CD, Whitington PF, Green MD, Roy SK, Tephly TR, *et al.* Genetic predisposition to the metabolism of irinotecan (CPT-11). Role of uridine diphosphate glucuronosyltransferase isoform 1A1 in the glucuronidation of its active metabolite (SN-38) in human liver microsomes. *J Clin Invest* 1998; **101**:847–854.
112 Ando Y, Saka H, Ando M, Sawa T, Muro K, Ueoka H, *et al.* Polymorphisms of UDP-glucuronosyltransferase gene and irinotecan toxicity: a pharmacogenetic analysis. *Cancer Res* 2000; **60**:6921–6926.
113 Iyer L, Das S, Janisch L, Wen M, Ramirez J, Karrison T, *et al.* UGT1A1*28 polymorphism as a determinant of irinotecan disposition and toxicity. *Pharmacogenomics J* 2002; **2**:43–47.
114 Innocenti F, Undevia SD, Iyer L, Chen PX, Das S, Kocherginsky M, *et al.* Genetic variants in the UDP-glucuronosyltransferase 1A1 gene predict the risk of severe neutropenia of irinotecan. *J Clin Oncol* 2004; **22**:1382–1388.
115 Massacesi C, Terrazzino S, Marcucci F, Rocchi MB, Lippe P, Bisonni R, *et al.* Uridine diphosphate glucuronosyl transferase 1A1 promoter polymorphism predicts the risk of gastrointestinal toxicity and fatigue induced by irinotecan-based chemotherapy. *Cancer* 2006; **106**:1007–1016.
116 http://www.fda.gov/medwatch/SAFETY/2005/Jun_PI/Camptosar_PI.pdf.
117 Evans WE, McLeod HL. Pharmacogenomics: drug disposition, drug targets, and side effects. *N Engl J Med* 2003; **348**:538–549.
118 Marzolini C, Tirona RG, Kim RB. Pharmacogenomics of the OATP and OAT families. *Pharmacogenomics* 2004; **5**:273–282.
119 Marzolini C, Paus E, Buclin T, Kim RB. Polymorphisms in human MDR1 (P-glycoprotein): recent advances and clinical relevance. *Clin Pharmacol Ther* 2004; **75**:13–33.
120 Gottesman MM, Fojo T, Bates SE. Multidrug resistance in cancer: role of ATP-dependent transporters. *Nat Rev Cancer* 2002; **2**:48–58.
121 Dey S. Single nucleotide polymorphisms in human P-glycoprotein: its impact on drug delivery and disposition. *Expert Opin Drug Deliv* 2006; **3**:23–35.
122 Hoffmeyer S, Burk O, von Richter O, Arnold HP, Brockmoller J, Johne A, *et al.* Functional polymorphisms of the human multidrug-resistance gene: multiple sequence variations and correlation of one allele with P-glycoprotein expression and activity *in vivo*. *Proc Natl Acad Sci U S A* 2000; **97**:3473–3478.
123 Hitzl M, Schaeffeler E, Hocher B, Slowinski T, Halle H, Eichelbaum M, *et al.* Variable expression of P-glycoprotein in the human placenta and its association with mutations of the multidrug resistance 1 gene (MDR1, ABCB1). *Pharmacogenetics* 2004; **14**:309–318.
124 Owen A, Goldring C, Morgan P, Chadwick D, Park BK, Pirmohamed M. Relationship between the C3435T and G2677T(A) polymorphisms in the ABCB1 gene and P-glycoprotein expression in human liver. *Br J Clin Pharmacol* 2005; **59**:365–370.
125 Goto M, Masuda S, Saito H, Uemoto S, Kiuchi T, Tanaka K, *et al.* C3435T polymorphism in the MDR1 gene affects the enterocyte expression level of CYP3A4 rather than Pgp in recipients of living-donor liver transplantation. *Pharmacogenetics* 2002; **12**:451–457.
126 Tanabe M, Ieiri I, Nagata N, Inoue K, Ito S, Kanamori Y, *et al.* Expression of P-glycoprotein in human placenta: relation to genetic polymorphism of the multidrug resistance (MDR)-1 gene. *J Pharmacol Exp Ther* 2001; **297**:1137–1143.
127 Meissner K, Jedlitschky G, Meyer zu SH, Dazert P, Eckel L, Vogelgesang S, *et al.* Modulation of multidrug resistance P-glycoprotein 1 (ABCB1) expression in human heart by hereditary polymorphisms. *Pharmacogenetics* 2004; **14**:381–385.
128 Lepper ER, Nooter K, Verweij J, Acharya MR, Figg WD, Sparreboom A. Mechanisms of resistance to anticancer drugs: the role of the polymorphic ABC transporters ABCB1 and ABCG2. *Pharmacogenomics* 2005; **6**:115–138.
129 Kafka A, Sauer G, Jaeger C, Grundmann R, Kreienberg R, Zeillinger R, *et al.* Polymorphism C3435T of the MDR-1 gene predicts response to preoperative chemotherapy in locally advanced breast cancer. *Int J Oncol* 2003; **22**:1117–1121.
130 Hesselink DA, van Gelder T, van Schaik RH. The pharmacogenetics of calcineurin inhibitors: one step closer toward individualized immunosuppression? *Pharmacogenomics* 2005; **6**:323–337.
131 Colombo S, Soranzo N, Rotger M, Sprenger R, Bleiber G, Furrer H, *et al.* Influence of ABCB1, ABCC1, ABCC2, and ABCG2 haplotypes on the cellular exposure of nelfinavir *in vivo*. *Pharmacogenet Genomics* 2005; **15**:599–608.
132 Bates SE, Deeken J, Fan C, Robey R. New and revised concepts in multidrug resistance: Sestamibi, SNPs, substrates, and stem cells. In: Teicher BE, editor. *Cancer drug resistance*. Totawa: Humana Press; 2006.
133 Doyle LA, Yang W, Abruzzo LV, Krogmann T, Gao Y, Rishi AK, *et al.* A multidrug resistance transporter from human MCF-7 breast cancer cells. *Proc Natl Acad Sci U S A* 1998; **95**:15665–15670.
134 Fetsch PA, Abati A, Litman T, Morisaki K, Honjo Y, Mittal K, *et al.* Localization of the ABCG2 mitoxantrone resistance-associated protein in normal tissues. *Cancer Lett* 2006; **235**:84–92.
135 Merino G, Alvarez AI, Pulido MM, Molina AJ, Schinkel AH, Prieto JG. Breast cancer resistance protein (BCRP/ABCG2) transports fluoroquinolone antibiotics and affects their oral availability, pharmacokinetics, and milk secretion. *Drug Metab Dispos* 2006; **34**:690–695.
136 Pavek P, Merino G, Wagenaar E, Bolscher E, Novotna M, Jonker JW, *et al.* Human breast cancer resistance protein: interactions with steroid drugs, hormones, the dietary carcinogen 2-amino-1-methyl-6-phenylimidazo(4, 5-b)pyridine, and transport of cimetidine. *J Pharmacol Exp Ther* 2005; **312**:144–152.
137 Matsushima S, Maeda K, Kondo C, Hirano M, Sasaki M, Suzuki H, *et al.* Identification of the hepatic efflux transporters of organic anions using double-transfected Madin-Darby canine kidney II cells expressing human organic anion-transporting polypeptide 1B1 (OATP1B1)/multidrug resistance-associated protein 2, OATP1B1/multidrug resistance 1, and OATP1B1/breast cancer resistance protein. *J Pharmacol Exp Ther* 2005; **314**:1059–1067.
138 Wang X, Nitanda T, Shi M, Okamoto M, Furukawa T, Sugimoto Y, *et al.* Induction of cellular resistance to nucleoside reverse transcriptase inhibitors by the wild-type breast cancer resistance protein. *Biochem Pharmacol* 2004; **68**:1363–1370.
139 Wang X, Furukawa T, Nitanda T, Okamoto M, Sugimoto Y, Akiyama S, *et al.* Breast cancer resistance protein (BCRP/ABCG2) induces cellular resistance to HIV-1 nucleoside reverse transcriptase inhibitors. *Mol Pharmacol* 2003; **63**:65–72.
140 Gupta A, Dai Y, Vethanayagam RR, Hebert MF, Thummel KE, Unadkat JD, *et al.* Cyclosporin A, tacrolimus and sirolimus are potent inhibitors of the human breast cancer resistance protein (ABCG2) and reverse resistance to mitoxantrone and topotecan. *Cancer Chemother Pharmacol* 2006; **58**:374–383.
141 Gupta A, Zhang Y, Unadkat JD, Mao Q. HIV protease inhibitors are inhibitors but not substrates of the human breast cancer resistance protein (BCRP/ABCG2). *J Pharmacol Exp Ther* 2004; **310**:334–341.
142 Breedveld P, Zelcer N, Pluim D, Sonmezer O, Tibben MM, Beijnen JH, *et al.* Mechanism of the pharmacokinetic interaction between methotrexate and benzimidazoles: potential role for breast cancer resistance protein in clinical drug-drug interactions. *Cancer Res* 2004; **64**:5804–5811.

143 Volk EL, Farley KM, Wu Y, Li F, Robey RW, Schneider E. Overexpression of wild-type breast cancer resistance protein mediates methotrexate resistance. *Cancer Res* 2002; **62**:5035–5040.
144 Robey RW, Medina-Perez WY, Nishiyama K, Lahusen T, Miyake K, Litman T, *et al.* Overexpression of the ATP-binding cassette half-transporter, ABCG2 (Mxr/BCrp/ABCP1), in flavopiridol-resistant human breast cancer cells. *Clin Cancer Res* 2001; **7**:145–152.
145 Litman T, Brangi M, Hudson E, Fetsch P, Abati A, Ross DD, *et al.* The multidrug-resistant phenotype associated with overexpression of the new ABC half-transporter, MXR (ABCG2). *J Cell Sci* 2000; **113**:2011–2021.
146 Maliepaard M, van Gastelen MA, Tohgo A, Hausheer FH, van Waardenburg RC, de Jong LA, *et al.* Circumvention of breast cancer resistance protein (BCRP)-mediated resistance to camptothecins *in vitro* using non-substrate drugs or the BCRP inhibitor GF120918. *Clin Cancer Res* 2001; **7**:935–941.
147 Robey RW, Steadman K, Polgar O, Morisaki K, Blayney M, Mistry P, *et al.* Pheophorbide a is a specific probe for ABCG2 function and inhibition. *Cancer Res* 2004; **64**:1242–1246.
148 Ozvegy-Laczka C, Hegedus T, Varady G, Ujhelly O, Schuetz JD, Varadi A, *et al.* High-affinity interaction of tyrosine kinase inhibitors with the ABCG2 multidrug transporter. *Mol Pharmacol* 2004; **65**:1485–1495.
149 Yanase K, Tsukahara S, Asada S, Ishikawa E, Imai Y, Sugimoto Y. Gefitinib reverses breast cancer resistance protein-mediated drug resistance. *Mol Cancer Ther* 2004; **3**:1119–1125.
150 Imai Y, Nakane M, Kage K, Tsukahara S, Ishikawa E, Tsuruo T, *et al.* C421A polymorphism in the human breast cancer resistance protein gene is associated with low expression of Q141 K protein and low-level drug resistance. *Mol Cancer Ther* 2002; **1**:611–616.
151 Backstrom G, Taipalensuu J, Melhus H, Brandstrom H, Svensson AC, Artursson P, *et al.* Genetic variation in the ATP-binding cassette transporter gene ABCG2 (BCRP) in a Swedish population. *Eur J Pharm Sci* 2003; **18**:359–364.
152 de Jong FA, Marsh S, Mathijssen RH, King C, Verweij J, Sparreboom A, *et al.* ABCG2 pharmacogenetics: ethnic differences in allele frequency and assessment of influence on irinotecan disposition. *Clin Cancer Res* 2004; **10**:5889–5894.
153 Zamber CP, Lamba JK, Yasuda K, Farnum J, Thummel K, Schuetz JD, *et al.* Natural allelic variants of breast cancer resistance protein (BCRP) and their relationship to BCRP expression in human intestine. *Pharmacogenetics* 2003; **13**:19–28.
154 Kondo C, Suzuki H, Itoda M, Ozawa S, Sawada J, Kobayashi D, *et al.* Functional analysis of SNPs variants of BCRP/ABCG2. *Pharm Res* 2004; **21**:1895–1903.
155 Kobayashi D, Ieiri I, Hirota T, Takane H, Maegawa S, Kigawa J, *et al.* Functional assessment of ABCG2 (BCRP) gene polymorphisms to protein expression in human placenta. *Drug Metab Dispos* 2005; **33**:94–101.
156 Morisaki K, Robey RW, Ozvegy-Laczka C, Honjo Y, Polgar O, Steadman K, *et al.* Single nucleotide polymorphisms modify the transporter activity of ABCG2. *Cancer Chemother Pharmacol* 2005; **56**:161–172.
157 Mizuarai S, Aozasa N, Kotani H. Single nucleotide polymorphisms result in impaired membrane localization and reduced atpase activity in multidrug transporter ABCG2. *Int J Cancer* 2004; **109**:238–246.
158 Sparreboom A, Gelderblom H, Marsh S, Ahluwalia R, Obach R, Principe P, *et al.* Diflomotecan pharmacokinetics in relation to ABCG2 421C>A genotype. *Clin Pharmacol Ther* 2004; **76**:38–44.