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Pharmacogenetic Pathway Analysis of Docetaxel Elimination

SD Baker¹, J Verweij², GA Cusatis³, RH van Schaik⁴, S Marsh⁵, SJ Orwick¹, RM Franke¹, S Hu¹, EG Schuetz¹, V Lamba¹, WA Messersmith³, AC Wolff³, MA Carducci³, and A Sparreboom¹

¹Department of Pharmaceutical Sciences, St. Jude Children's Research Hospital, Memphis, Tennessee, USA ²Department of Medical Oncology, Erasmus MC–Daniel den Hoed Cancer Center, Rotterdam, The Netherlands ³Department of Oncology, The Sidney Kimmel Comprehensive Cancer Center at Johns Hopkins, Baltimore, Maryland, USA ⁴Department of Clinical Chemistry, Erasmus MC, Rotterdam, The Netherlands ⁵Department of Medicine, Washington University School of Medicine, St. Louis, Missouri, USA

Abstract

The purpose of this study was to evaluate the affinity of docetaxel for 14 transporter proteins and assess the functional significance of 17 variants in five genes involved in drug elimination. Among the transfected models investigated, OATP1B3 (*SLCO1B3*) was identified as the most efficient influx transporter for docetaxel. None of the observed genotypes (*SLCO1B3*, *ABCB1*, and *ABCC2*) was related with docetaxel clearance in 92 white patients ($P > 0.17$). However, the simultaneous presence of the *CYP3A4*1B* and *CYP3A5*1A* alleles was associated with a 64% increase in docetaxel clearance ($P = 0.0015$), independent of both sex and CYP3A activity (as determined using the erythromycin breath test). This haplotype was also associated with increased midazolam clearance in another population ($P = 0.0198$). An analysis of the *CYP3A* locus among CEPH-HapMap samples revealed that *CYP3A4*1B* is present exclusively among a subset of CYP3A5 expressors. Therefore, future studies should first stratify the population on the basis of *CYP3A5* genotype and then compare CYP3A activity between individuals with and without the *CYP3A4*1B* allele.

Docetaxel, a tubulin-interactive agent, is one of the most widely used chemotherapeutic agents and has been approved for the treatment of breast, lung, ovarian, prostate, gastric, and head and neck cancers. The pharmacokinetic profile of docetaxel is characterized by substantial interindividual variability, with an up to tenfold difference in drug clearance, even in patients with normal hepatic function.¹ This degree of variability has important toxicological and therapeutic ramifications. Previously, it has been demonstrated that a 50% decrease in docetaxel clearance increases the odds of developing grade 4 neutropenia by 430%.² Moreover, systemic exposure to docetaxel is a known predictor of time to tumor progression in patients with non-small-cell-lung cancer.³ In these patients, a smaller

Correspondence: SD Baker (sharyn.baker@stjude.org).

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docetaxel area under the curve has been associated with a shorter time to progression and time to death.⁴

The erratic or unpredictable response of individuals to docetaxel remains a major challenge for modern chemotherapy. Recent population pharmacokinetic analyses have attempted to identify demographic and physiological factors that may influence the clearance of docetaxel.⁵ However, the magnitude of interindividual pharmacokinetic variability of this agent is still largely unexplained. A critical determinant of this variability is associated with the differential expressions of polymorphic drug-metabolizing enzymes and/or transporters at the sites of elimination. Previous *in vitro* studies have established that the elimination of docetaxel is dictated mainly by the hepatic enzyme CYP3A4 and, to a lesser extent, by CYP3A5.⁶ The importance of these enzymes has recently been confirmed in mice with a deletion of the entire *CYP3A* gene cluster. In these mice, the clearance of docetaxel was decreased sevenfold when compared with that in wild-type controls.⁷

In the past decade, important new insights have also been obtained on polymorphic transporters involved in docetaxel elimination. Specifically, there is compelling evidence suggesting that hepatocellular uptake of taxanes from sinusoidal blood is regulated, at least in part, by the solute carrier OATP1B3 (*SLCO1B3*; OATP8),⁸ whereas the ATP-binding cassette (ABC) transporters ABCB1 (P-glycoprotein)⁹ and ABCC2 (MRP2; cMOAT),¹⁰ are involved in the secretion of taxanes from the liver into the bile. In this study, we tested the hypothesis that an inherited variation in the genes involved in the docetaxel elimination pathway contributes to interindividual differences in the clearance of docetaxel in cancer patients.

Results

Interaction of docetaxel with transporters

Influx transport of docetaxel was assessed for the solute carrier superfamily members OATP1A2 (OATP-A), OATP1B1 (OATP-C, OATP2), OATP1B3, rOatp1b2 (*oatp4*), OAT1 (NKT), OAT2 (NLT), OAT3, OCT1, OCT2, OCT3, and SLC10A1 (NTCP) (Figure 1a). Of these transporters, only the hepatocellular-localized proteins OATP1B3 (2.62-fold vs. control), its rodent ortholog rOatp1b2 (2.23-fold), and OAT2 (2.27-fold) were capable of increasing the intracellular concentrations of docetaxel to a significant extent. Docetaxel was also shown to be transported by the ABC transporter ABCC2 (but not by ABCC4 (MRP4) or ABCG2 (BCRP)), causing a significant decrease in the intracellular concentration relative to control cells (Figure 1b).

Patients

Complete pharmacologic data were available for 92 adult white patients with cancer (of whom 41 were women) with a median age of 63 years (range, 24–83 years) (Table 1). The most frequent primary tumor types were breast cancer ($n = 25$), prostate cancer ($n = 24$), and lung cancer ($n = 11$).

Docetaxel pharmacokinetics

The overall mean clearance of docetaxel was 23.4 l/h, and the observed range in clearance was 7.6-fold between the fastest and the slowest clearances. Because clearance was not significantly dependent on the total dose ($P = 0.12$), pharmacokinetic information from all patients was pooled in subsequent analyses without further correction. The clearance of docetaxel was unrelated to performance status (0 vs. 1 vs. 2; $P = 0.13$), age ($P = 0.86$), α 1-acid glycoprotein ($P = 0.46$), bilirubin ($P = 0.72$), or body surface area ($P = 0.10$). Weak but statistically significant relationships were found between clearance and the erythromycin breath test (ERMBT) parameter C20 ($R^2 = 0.072$; $P = 0.036$) as well as sex ($P = 0.0042$), with women having, on average, a 35% lower clearance rate than men. During multiple regression analysis, both ERMBT ($P = 0.0066$) and sex ($P = 0.0026$) were retained as significant independent variables in the final model for docetaxel clearance ($R^2 = 0.13$).

Results of genotyping

Seventeen single-nucleotide polymorphisms (SNPs) were analyzed in five genes of putative relevance to docetaxel elimination (Table 2), including *SLCO1B3*, *CYP3A4*, *CYP3A5*, *ABCB1*, and *ABCC2*. The relative frequencies of the variant alleles were comparable with previously reported estimates.^{11–14} The OAT2 gene (*SLC22A7*) was not considered in this analysis because nonsynonymous coding region SNPs have been reported at a low frequency (<1%) only in certain ethnic groups but not in subjects of European ancestry.¹⁵ The frequency distributions of all SNPs were in Hardy–Weinberg equilibrium (data not shown).

Twelve haplotypes were observed in *SLCO1B3*, the most common of which (GAAGAT at positions 334, 439, 699, 767, 1559, and 1679; haplotype *SLCO1B3*1*) had a frequency of 64%. As predicted, significant linkage was observed between *SLCO1B3* 334T>G and the SNP at the 699 locus ($D' = 0.86$; $P > 0.001$).¹⁶ Similarly, the *CYP3A4* –392A>G (*CYP3A4*1B*) and *CYP3A5* 6986A>G (*CYP3A5*3C*) SNPs were found to be in linkage ($D' = 0.79$; $P < 0.001$), as described previously.^{17,18} For these two SNPs, four haplotypes were observed, with frequencies of 84.3% (*CYP3A4*1A/CYP3A5*3C*; haplotype *CYP3A4/5*1*), 7.99% (*CYP3A4*1B/CYP3A5*1A*; haplotype *CYP3A4/5*2*), 6.00% (*CYP3A4*1A/CYP3A5*1A*; haplotype *CYP3A4/5*3*), and 1.76% (*CYP3A4*1B/CYP3A5*3C*; haplotype *CYP3A4/5*4*). A total of 8 and 14 haplotypes, respectively, were observed for *ABCB1* (frequency of CGC at positions 1236, 2677, and 3435, 43%; haplotype *ABCB1*1*) and *ABCC2* (frequency of ACGTCG at positions –1019, –24, 1249, IVS26 –34, 3972, and 4544, 35%; haplotype *ABCC2*1*). The extent of linkage between individual SNPs in these ABC transporter genes was similar to that reported previously¹¹ (data not shown).

Genotype–phenotype relationships

The presence of the *CYP3A4*1B* and *CYP3A5*1A* alleles was associated with a 62% ($P = 0.055$) (Figure 2a) and 49% ($P = 0.020$) (Figure 2b) increase in docetaxel clearance, respectively. In the study population consisting of only white patients, the effect of these two minor *CYP3A* alleles was particularly evident when they were assessed simultaneously. The six individuals who were carrying haplotype *CYP3A4/5*2* (that is, those having at least one *CYP3A4*1B* allele and at least one *CYP3A5*1A* allele) showed a 64% higher clearance

of docetaxel than the others ($P = 0.0015$) (Figure 2c). This genotypic information was retained in the multiple regression model as a statistically significant independent predictor of clearance after concurrent incorporation of ERMBT and sex ($P = 0.0044$; $R^2 = 0.21$). Interestingly, in a separate population of white patients studied previously, haplotype *CYP3A4/5*2* was also associated with a 46% increase in the clearance of another CYP3A substrate drug, midazolam ($P = 0.0198$) (Figure 3a),¹⁷ but not with parameters obtained from the ERMBT (Figure 3b).¹⁹

None of the investigated SNPs in transporter genes was found to be significantly associated with the clearance of docetaxel (Table 3). Likewise, none of the common haplotypes in *SLCO1B3*, *ABCB1*, or *ABCC2* was a predictor of clearance (Figure 4). Similar results were obtained when the study population was stratified by sex and also when the analysis was restricted to patients with the *CYP3A5*3C/*3C* genotype (i.e., CYP3A5 nonexpressors²⁰) (data not shown).

Visual genotyping of the *CYP3A* locus

We next used the Centre d'Etude du Polymorphisme Humain (CEPH) HapMap samples (<http://www.cephb.fr/cephdb/>) comprising Utah residents of northern and western European ancestry (<http://www.hapmap.org/downloads/encode1.html.en>) to carry out a visual evaluation of the *CYP3A5*1A* and *CYP3A4*1B* genotypes relative to other SNPs across the *CYP3A* locus. Although there are many programs that will designate haplotypes, an alternative approach is to visually stratify by genotypes that are known to be informative (e.g., CYP3A5 expressor or non-expressor) and use this information to drive haplotype selection. This visual genotyping of the *CYP3A* locus was carried out for the CEPH HapMap samples, and it was immediately apparent that, among white individuals, carriers of the *CYP3A4*1B* allele were found only as a subset of CYP3A5 expressors (Figure 5). Although we observed the presence of the *CYP3A4*1B* genotype in four (1.76%) of the CYP3A5 non-expressor patients, it was still rare compared to the *CYP3A4/5*2* haplotype (7.99%).

Discussion

In this study, we assessed the pharmacogenetics of known elimination pathways for docetaxel in a cohort of adult cancer patients in an attempt to identify inherited variability as a factor contributing to the extensive interindividual pharmacokinetic variability associated with this drug. The results of this analysis suggest that the clearance of docetaxel is not significantly dependent on a series of known reduced-function alleles in the *SLCO1B3*, *ABCB1*, and *ABCC2* genes. In contrast, the simultaneous presence of two known functional alleles in the *CYP3A4* and *CYP3A5* genes was associated with enhanced elimination of docetaxel.

The OATP1B3 transporter, encoded by the *SLCO1B3* gene, belongs to the class of organic anion transporting polypeptides and is particularly highly expressed at the basolateral (sinusoidal) plasma membrane of hepatocytes.²¹ Little is known about the functional role of *SLCO1B3* variants, although two frequent [334T>G (*2) and 699G>A (*4)] and one rare [1559A>C (*7)] nonsynonymous SNPs have been reported to result in dramatically altered transport of prototypical substrates and/or cellular localization *in vitro*.^{22,23} In this analysis,

none of the studied *SLCO1B3* variants was significantly associated with the pharmacokinetics of docetaxel. However, it is possible that individuals carrying the reduced-function alleles in *SLCO1B3* can nevertheless efficiently accumulate docetaxel into hepatocytes through compensatory mechanisms such as the organic anion transporter OAT2. This possibility is supported by our observation that docetaxel is a substrate for this transporter, as is the related drug paclitaxel.²⁴ In contrast to our data, obtained in *Xenopus laevis* oocytes, Yamaguchi *et al.* did not observe substantial transport of docetaxel by OATP1B3 in an overexpressed HEK293 cell model.²⁵ This discrepancy likely results from differential expression of other transporters relevant to docetaxel transport in the two model systems.

In this study, an association was noted between the *CYP3A4*1B* and *CYP3A5*3C* variants and the clearance of docetaxel, particularly when these SNPs were assessed in combination. Specifically, the simultaneous presence of these two alleles was associated with a 64% increase in clearance. Unfortunately, in most other studies involving patients undergoing treatment with docetaxel such haplotype analyses were not performed.^{26–28} However, the current genotype–phenotype relationship is remarkably similar to data reported in a previous study involving a white population of cancer patients. In that study, four patients who were simultaneously carrying the *CYP3A4*1B* and *CYP3A5*1A* alleles showed a 75% increase in docetaxel clearance as compared to the 43 other patients.²⁹ Previous investigations have shown that the *CYP3A4*1B* allele is most frequently associated with an increase in function.³⁰ In addition, it has been reported that there is no significant difference in CYP3A4 content between human livers belonging to the *CYP3A5*3C/*3C* genotype and those that contain at least one *CYP3A5*1A* allele.³¹ However, the content of CYP3A4 and CYP3A5 combined was more than twofold higher in livers belonging to genotypes with at least one *CYP3A5*1A* allele.³¹ Theoretically, the predicted effect of the combined presence of the *CYP3A4*1B* and *CYP3A5*1A* alleles would be a more rapid hepatic metabolism of CYP3A substrates, which is consistent with the observed increase in clearance of both docetaxel and midazolam in carriers of the *CYP3A4/5*2* haplotype. The lack of a similar association for erythromycin is possibly attributable to confounding genetic factors associated with variable expression and function of hepatocellular-localized solute carriers.³²

Interestingly, an analysis of the *CYP3A* locus among the CEPH-HapMap samples revealed that the *CYP3A4*1B* allele is exclusively present among a subset of CYP3A5 expressors. Therefore the visual genotyping results, coupled with our *in vivo* observations, suggest that individuals with the *CYP3A4/5*2* haplotype may have a higher level of CYP3A4. It is possible that the *CYP3A4*1B* SNP—either alone or in combination with other *CYP3A4* promoter SNPs in linkage disequilibrium (including rs1851426, rs2140129, rs2140130, rs2177179, rs2687091, and rs2740566)—could affect CYP3A4 expression. A number of studies have reported that the *CYP3A4*1B* allele engineered into reporter plasmids is associated with increased transcriptional activity.^{33,34} However, the association of *CYP3A4*1B* with altered clearance of CYP3A substrates *in vivo* has been less reproducible.^{17,19,35,36} Our results suggest one crucial reason for this discrepancy, namely, that previous studies examining the effect of *CYP3A4*1B* genotypes on traits in white

subjects have not carried out an initial segregation on the basis of *CYP3A5* genotypes. Future studies involving white patients should first stratify the subjects on the basis of *CYP3A5* genotype and then compare CYP3A activity between expressors and non-expressors of *CYP3A5* with and without the *CYP3A4*1B* allele. Importantly, the current results cannot be extrapolated directly to other populations because the haplotype structure of the *CYP3A* locus is significantly different in populations of other ethnicities. For example, Asians completely lack the *CYP3A4*1B* allele; among African Americans the *CYP3A4*1B* allele is found on multiple haplotypes in both expressors and non-expressors of *CYP3A5*; and in Tanzanians it has been associated with lower CYP3A4 activity.³⁷

The extent to which CYP3A4 metabolizes docetaxel is also likely to be determined, at least in part, by the intracellular residence time within hepatocytes. This process, in turn, is partly dependent on the efflux transporters ABCB1 and ABCC2, which are localized on the biliary surface of hepatocytes and are involved in secretion of docetaxel into the bile. In recent years, various genetic variants in *ABCB1* and *ABCC2* have been described that may affect transporter expression or function.¹² In a previous study, Bosch *et al.* reported that the clearance of docetaxel was reduced by 25% in patients with the *ABCB1* 1236TT genotype.²⁶ These authors went on to recommend that the dose of docetaxel should accordingly be reduced by 25% in such patients. In our study, none of the variant *ABCB1* genotypes (including the 1236C>T SNP) or haplotypes was associated with docetaxel clearance, a finding that is in line with results reported by other investigators.^{27–29} Likewise, no associations were noted between the pharmacokinetics of docetaxel and *ABCC2* genotype or haplotype variants. It should be noted that the absence of any association between gene variants and docetaxel clearance is consistent with preclinical observations. In mice, even the complete absence of either of these two transporters did not have any substantial influence on the systemic exposure to taxanes after intravenous administration.^{9,38} This finding supports the notion that metabolism is the predominant elimination mechanism for docetaxel and that (unlike for anticancer drugs such as irinotecan¹¹ or methotrexate)³⁹ the involvement of the ABC transporters in this process is relatively unimportant regardless of *ABCB1* or *ABCC2* genotype status.

In contrast to the negative data obtained with studies evaluating ABC transporter genotypes in relation to substrate pharmacokinetics, some recent studies have suggested that common allelic variants in the *ABCB1* gene may influence the susceptibility to toxicity and treatment outcome independent of an effect on clearance.¹² This paradox might be explained by the possibility that the high, genotype-dependent expression of ABCB1 in various target tissues such as bone marrow could directly control local intracellular drug levels, and that this might contribute to the altered pharmacodynamic effects of docetaxel without affecting measures of systemic exposure, as has been observed in some^{29,40} but not all studies.⁴¹

An interesting observation in our study was that, besides phenotypic CYP3A activity as determined using the ERMBT, sex was a significant independent variable affecting docetaxel clearance, with women having a 35% lower clearance than men. Sex-dependent clearance has also been reported recently for paclitaxel,⁴² adding to a growing body of evidence that the pharmacokinetics and toxicity of various anticancer drugs exhibit significant sex-related dimorphism.⁴³ The current observation is also consistent with a

previous study indicating that female patients treated with docetaxel are at increased risk for severe neutropenia.⁴⁴

Collectively, this study indicates that the simultaneous presence of the *CYP3A4*1B* and *CYP3A5*1A* alleles is associated with a dramatically altered clearance of docetaxel in a white population. Incorporation of this genotype-related information should be explored further as a potential tool for identifying individuals who might be at increased risk for treatment failure following chemotherapy with docetaxel because of undertreatment.

Methods

In vitro transport studies

Generally labeled [³H]docetaxel was obtained from Moravek Biochemicals (Brea, CA). In all *in vitro* experiments, radiolabeled docetaxel was mixed with unlabeled drug (Sigma-Aldrich, St. Louis, MO) to the desired concentration. *Xenopus laevis* oocytes injected with OATP1A2, OATP1B1, OATP1B3, rOatp1b2 (Oatp4), OAT1, OAT3, OCT1, or SLC10A1 (NTCP) cRNA, along with water-injected controls, were obtained from BD Biosciences (Woburn, MA). Uptake experiments were performed as described previously.⁸

HEK293 cells stably transfected with OCT2 or OCT3 were obtained from Dr Heinz Bönisch (Bonn, Germany), and cells expressing OAT2 were provided by Dr Yuichi Sugiyama (Tokyo, Japan). All HEK293 transfectants, along with pcDNA vector-transfected controls, were cultured in Dulbecco's modified Eagle's medium (Invitrogen, Carlsbad, CA) supplemented with 10% fetal bovine serum, penicillin (100 U/ml), streptomycin (100 µg/ml), and G418 sulfate (400–800 µg/ml) at 37 °C under 5% CO₂ and 95% humidity. Prior to the transport experiments, HEK293 cells were seeded in six-well plates at a density of 5×10^6 cells per well in 2 ml medium. When cells reached 95% confluence, sodium butyrate (5 µmol/l) was added to the medium for 24 h to induce expression of the respective transporter genes. Next, the transport experiment was initiated by adding serum-free medium containing [³H]docetaxel (total concentration, 0.2–5 µmol/l) to OCT- or OAT-expressing cells. At a predetermined time interval, the experiment was terminated by adding ice-cold phosphate-buffered saline after the removal of the incubation buffer. After being washed with ice-cold phosphate-buffered saline, the cells were mixed with 900 µl sodium hydroxide (1 N) for 20 min. An aliquot of 25 µl lysate was used for protein estimation using a bicinchoninic acid protein-assay kit (Pierce Biotechnology, Rockford, IL), and 600 µl were used for analysis of total radioactivity using an LS 6500 scintillation counter (Beckman Coulter, Fullerton, CA).

MDCKII parent cells, along with their ABCC2-transfected counterparts, and Saos-2 cells containing pcDNA empty vector, ABCG2, or ABCC4 were provided by Dr John Schuetz (Memphis, TN). These cells were maintained in DMEM containing 10% fetal bovine serum, penicillin (100 U/ml), streptomycin (100 µg/ml), and G418 sulfate (500 µg/ml; Saos-2 cells only) and were seeded in six-well plates at a density of 5×10^5 per well. At 70% confluence, the cells were washed with phosphate-buffered saline, and the medium was replaced with fresh, serum-free medium containing [³H]docetaxel (total concentration, 5 µmol/l) for 4 h. At the end of drug incubation, the medium was removed and the cells were washed two

times with ice-cold phosphate-buffered saline. The cells were then handled as described earlier for HEK293 cells.

Patients

Detailed eligibility criteria for selection of patients have been defined previously.^{5,45–47} Each patient had a pathologically confirmed diagnosis of a solid tumor for which docetaxel, given alone or in combination, was a reasonable therapeutic option. Additional common eligibility criteria included: age >18 years; adequate bone marrow function (absolute neutrophil count > $1.5 \times 10^9/l$; platelet count > $100 \times 10^9/l$); adequate renal function [serum creatinine $\leq 2.0 \times$ the upper limit of normal]; and adequate hepatic function (serum bilirubin $\leq 1.5 \times$ upper limit of normal). The use of phenytoin, carbamazepine, rifampin, phenobarbital, St. John's wort, ketoconazole, or other compounds known to affect the pharmacokinetics of docetaxel was not allowed. The study protocols were approved by the local ethical review boards, and all patients provided written informed consent before they were enrolled in the study. Toxicity and efficacy data were not considered as pharmacodynamic end points in the study because different doses of docetaxel and schedules were used in the studied population.

Sample collection and analysis

Within 1 week before docetaxel administration, baseline CYP3A activity was assessed using the ERMBT as described previously.¹⁹ During the first course of treatment with docetaxel, blood samples were collected in tubes containing lithium heparin as anticoagulant and immediately centrifuged (at least 2,000g at 4 °C for 10 min) to separate plasma, which was then stored at or below –20 °C until analysis. These blood samples were obtained at the following time points: immediately before the infusion; 30 min after the start of the infusion; immediately before the end of the infusion; and 10 and 30 min, 1, 3, 7, 24, and 48 h, and on days 8, 15, and 22 after the end of the infusion. Docetaxel concentrations in plasma were determined using high-performance liquid chromatography with tandem mass-spectrometric detection in accordance with a validated procedure.⁴⁸ The lower limit of quantification using 1,000 μ l plasma samples was 0.5 nmol/l (~0.4 ng/ml).

Noncompartmental analysis

Individual plasma concentrations of docetaxel were analyzed by noncompartmental methods using WinNonlin version 5.0 (Pharsight, Mountain View, CA). The primary pharmacokinetic parameter, clearance, was calculated as dose divided by the area under the plasma concentration-time curve, extrapolated to infinity.

Genotyping procedures

Genomic DNA was extracted from 1 ml of whole blood using the UltraSens Virus kit (Qiagen, Valencia, CA), and the REPLI-g mini/midi kit (Qiagen) was used for amplifying genomic DNA in case of limited sample availability. SNPs in *SLCO1B3* were identified from the literature²³ and SNPs of interest in the *CYP3A4*, *CYP3A5*, *ABCB1*, and *ABCC2* genes were selected on the basis of the predicted frequency of occurrence and/or the anticipated phenotypic change.^{11,12,30} Variations in *CYP3A4* (*CYP3A4*1B*) and *CYP3A5*

(*CYP3A5*3C*) were initially analyzed using restriction fragment length polymorphism–based techniques, as described previously.^{13,14} Direct nucleotide sequencing was used on samples for which no reliable genotype was obtained from the restriction fragment length polymorphism assay. Primers for all genetic variants were designed using Primer version 5.0 (Table 4), and PCRs were performed using the HotStart Taq Master Mix (Qiagen, Valencia, CA). The efficiency and quality of PCR for all genes were confirmed by running the PCR products on a 1.5%-agarose gel. After cleanup using ExoSAP-IT (USB, Cleveland, OH), the PCR products were subjected to direct sequencing. Sequence analysis was performed with Sequencher Version 4.5 (Gene Codes, Ann Arbor, MI). The genotype was termed “variant” if it differed from the Refseq consensus sequence for the SNP position (<http://www.ncbi.nlm.nih.gov/RefSeq/>). Genotype-frequency analyses of Hardy–Weinberg equilibrium, linkage disequilibrium (*D'*), and haplotypes were carried out using the Polymorphism and Haplotype Analysis Suite version 0.9.⁴⁹

Statistical considerations

Pharmacokinetic data are presented as a median value along with the range of values, unless stated otherwise. The association of variant genotypes or haplotypes with the clearance of docetaxel was analyzed using a two-tailed Mann–Whitney *U*-test (two-group comparisons) or a Kruskal–Wallis test (multiple-group comparisons). For the purpose of relating continuous variables with clearance, a correlation test was performed. Relationships between clearance and two or more independent variables were assessed using multiple regression analysis. *P* < 0.05 was considered statistically significant. All calculations were performed using the software package NCSS v2005 (J. Hintze, Kaysville, UT).

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Figure 1.

Accumulation of docetaxel by (a) *Xenopus laevis* oocytes (hatched bars) or mammalian cells (black bars) expressing solute carriers, and (b) mammalian cells expressing ATP-binding cassette transporters. The data represent the mean of 6–33 observations, and are expressed as a percentage of the control (white bars). For clarity, only a single control bar is shown for the solute carriers. Error bars represent the standard error. The asterisk (*) denotes significant difference from the control value ($P < 0.05$).

Figure 2.

Docetaxel clearance as a function of (a) *CYP3A4*1B* genotype, (b) *CYP3A5*3C* genotype, and (c) combined presence of the *CYP3A4*1B* and *CYP3A5*1A* alleles (haplotype *CYP3A4/5*2*). Each symbol represents an individual patient, and horizontal lines indicate median values. In b, the black circle represents the only individual with two copies of the *CYP3A5*1A* allele, and the black triangles in the *CYP3A5* non-expressors represent carriers of the *CYP3A4*1B* allele. In c, all individuals in the group of *CYP3A4/5*2* carriers had the *CYP3A4*1A*1B/CYP3A5*1A*3C* diplotype, and the black triangles in the group of noncarriers represent *CYP3A5* expressors.

Figure 3. Association of the *CYP3A4/5*2* haplotype with (a) midazolam clearance and (b) erythromycin breath test concentration at 20 min (ERMBT C20). Each symbol represents an individual patient, and horizontal lines indicate median values. The black triangles in the group of noncarriers represent *CYP3A5* expressors.

Figure 4.

Docetaxel clearance as a function of the most common variant haplotypes in (a) *SLCO1B3* (haplotype GAAGAT at positions 334, 439, 699, 767, 1559, and 1679; *SLCO1B3*1*), (b) *ABCB1* (haplotype CGC at positions 1236, 2677, and 3435; *ABCB1*1*), and (c) *ABCC2* (haplotype ACGTCG at positions -1019, -24, 1249, IVS26 -34, 3972, and 4544; *ABCC2*1*). Each symbol represents an individual patient, and horizontal lines indicate median values.

Figure 5.

Visual genotypes of the *CYP3A* locus in CEPH-HapMap samples stratified on the basis of informative *CYP3A* genotypes. Each sample (identified on the *x*-axis) is arranged by *CYP3A* locus SNPs (on the *y*-axis). Genotypes are color-coded as follows: gray, homozygous wild type; orange, heterozygous; red, homozygous variant. Groups labeled 1 and 2 represent *CYP3A5* expressors carrying *CYP3A5*1A* (rs776746) stratified by the presence or absence of the *CYP3A4*1B* (rs2740574) allele, respectively. Among *CYP3A5* non-expressors, groups labeled 3, 4, 5, and 6 were further segregated by *CYP3A7* SNPs, the SNP 3' of *CYP3A4* (rs1233983), and the *CYP3A4* intron 7 SNP (rs2246709), respectively.

Table 1
Baseline patient characteristics^a

Characteristic	Value
Baseline screening	
Total evaluable	92
Age (years)	63 (24–83)
Sex (male/female)	51 (55)/41 (45)
Body surface area (m ²)	1.95 (1.40–2.72)
ECOG performance status	1 (0–1)
Primary tumor site	
Breast	25 (27)
Prostate	24 (26)
Lung	11 (12)
Head and neck	7 (8)
Other	25 (27)
Pretherapy chemistry and hematology	
Total bilirubin (× ULN)	0.42 (0.17–1.25)
Aspartate aminotransferase (× ULN)	0.78 (0.30–4.70)
Alanine aminotransferase (× ULN)	0.60 (0.10–6.61)
Alkaline phosphatase (× ULN)	0.82 (0.39–8.69)
α1-Acid glycoprotein (mg/dl)	126 (60–257)
Erythromycin breath test C20 (%dose/min)	0.047 (0.0015–0.124)

ECOG, Eastern Cooperative Oncology Group; N/A, not available; ULN, upper limit of institutional normal.

^a Continuous data are given as median with range in parentheses, and categorical data as number of patients with percentage of the total population in parentheses.

Table 2

Description and allele frequencies of the studied variants

Gene (allele) ^b	dbSNP ID	Region	Effect ^c	Allele frequency ^d	
				p	q
SLCO1B3					
334T>G (*2)	rs4149117	Exon 3	S112A	0.147	0.853
439A>G (*3)	N/A	Exon 4	T147A	0.995	0.005
699G>A (*4)	rs7311358	Exon 6	M233I	0.159	0.841
767G>C (*5)	N/A	Exon 7	G256A	0.811	0.189
1559A>C (*6)	N/A	Exon 11	H520P	1.00	0
1679T>C (*7)	rs12299012	Exon 11	V560A	0.984	0.016
CYP3A4					
−392A>G (*1B)	rs2740574	5′-Flanking	—	0.951	0.049
CYP3A5					
6986A>G (*3C)	rs776746	Intron 3	Frameshift	0.076	0.924
ABCB1					
1236C>T (*8)	rs1128503	Exon 12	G412G	0.539	0.461
2677G>T/A (*7)	rs2032582	Exon 21	A893S/T	0.556	0.422/0.022
3435C>T (*6)	rs1045642	Exon 26	I1145I	0.478	0.522
ABCC2					
−1019A>G	rs2804402	5′-Flanking	—	0.614	0.386
−24C>T	rs717620	5′-UTR	—	0.815	0.185
1249G>A	rs2273697	Exon 10	V417I	0.789	0.211
IVS26 −34T>C	rs8187698	Intron 26	Exon 26	0.946	0.054
3972C>T	rs3740066	Exon 28	I1324I	0.647	0.353
4544G>A ^d	rs8187710	Exon 32	C1515Y	0.967	0.033

UTR, untranslated region; dbSNP, single-nucleotide polymorphism database.

^a Hardy–Weinberg notation for allele frequencies; *p*, frequency for reference allele and *q*, frequency for variant allele.

^b Number represents position in nucleotide sequence with star nomenclature in parentheses where available.

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^cNumber represents amino acid codon;

^dIn complete linkage disequilibrium with the polymorphisms 3844-73A>G, 4146^d+11G>C, 4290G>T (V1430V), 4488C>T (H1469H), and 4508+12G>A.

Table 3

Clearance of docetaxel as a function of the observed genotypes

Gene (allele)	Reference genotype		Heterozygote genotype		Variant genotype	
	Value ^a	N	Value ^a	N	Value ^a	N
<i>SLCO1B3</i>						
334T>G	32.1	1	27.0 (15.8–36.3)	25	23.2 (20.7–27.5)	65
439A>G	23.3 (20.8–27.4)	90	29.7	1	N/A	0
699G>A	40.1	2	27.0 (15.8–34.2)	25	22.2 (19.8–26.8)	63
767G>C	22.7 (18.6–28.3)	60	26.8 (21.1–31.0)	25	16.0	4
1559A>C	23.4 (20.8–27.5)	91	N/A	0	N/A	0
1679T>C	23.2 (20.8–27.4)	89	28.5	1	63.4	1
<i>CYP3A4</i>						
–392A>G	22.9 (20.7–27.0)	82	37.0 (15.0–50.4)	9	N/A	0
<i>CYP3A5</i>						
6986A>G	34.2	1	33.6 (18.6–42.6)	12	22.9 (19.8–26.8)	78
<i>ABCB1</i>						
1236C>T	21.7 (15.3–29.6)	24	24.7 (18.8–28.5)	48	22.6 (16.4–31.0)	17
2677G>T/A ^c	23.2 (18.1–29.7)	25	25.2 (20.8–28.5)	50	20.7 (15.0–48.5)	15
3435C>T	21.1 (14.5–29.6)	19	26.4 (18.8–28.9)	48	24.7 (18.6–28.4)	23
<i>ABCC2</i>						
–1019A>G	27.1 (18.8–29.7)	34	21.8 (18.6–28.5)	43	21.0 (14.5–27.0)	14
–24C>T	24.7 (21.1–28.5)	61	21.7 (16.9–28.1)	26	20.3	4
1249G>A	23.6 (18.6–27.4)	54	23.2 (17.3–29.2)	33	35.8	2
1VS26–34T>C	23.3 (20.8–27.4)	82	24.8 (9.70–34.2)	8	28.3	1
3972C>T	24.0 (15.8–28.4)	38	21.8 (18.1–27.5)	41	27.6 (21.5–42.6)	12
4544G>A	23.4 (20.8–27.5)	83	20.5 (9.70–73.9)	6	N/A	0

N, number of patients; N/A, not available.

^aData represent median clearance of docetaxel in units of l/h with range in the parentheses.^bP values were obtained from a two-tailed Mann–Whitney U-test (two-group comparisons) or a Kruskal–Wallis test (multiple-group comparisons).

c. In the heterozygote group, 49 patients had the GT genotype and 1 had the GA genotype; in the variant group, 12 patients had the TT genotype and 3 had the TA genotype.

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Table 4

PcR primer sequences

Polymorphism	Forward 5'-3'	Reverse 5'-3'
<i>SLCO1B3</i> 334T>G	CATGGTCTTTG AGGGAAGGT	AGGAAATATACTC CATTTCTCTATGC
<i>SLCO1B3</i> 439A>G	CAGCGGTTCAA ATAAAGGAGA	TGTGTTGTTTA AGAATCGACTGC
<i>SLCO1B3</i> 699G>A	AAAAGCATGTTA AATGAAAACCA	GGAAGAATGG TGTCTGCAC
<i>SLCO1B3</i> 767G>C	ACATTTGGTTTA CTTTCTTCATCTAT	AATCCTCTT CCCCTTTTAT
<i>SLCO1B3</i> 1559A>	GGGAGCTATTT TGCCTTCAC	TGTCAATCAGT GTGTGCCTTA
<i>SLCO1B3</i> 1679T>C	GGGAGCTATT TGCCTTCAC	TGTCAATCAGT GTGTGCCTTA
<i>CYP3A4</i> -392A>G	TGGGATGAATT TCAAGTATTTTG	CAGATAAGGG AAAGAGAGGC
<i>CYP3A5</i> 6986A>G	CCTGCCTTCAA TTTTTCACTG	GCAATGTAGGA AGGAGGGCT
<i>ABCB1</i> 1236C>T	TGAACAGTCAG TTCCTATATCCTG	TGTGCAATGT GACTGCTGAT
<i>ABCB1</i> 2677G>T/A	GCAGGCTATAG GTTCCAGGCT	TGAGGAATGG TTATAAACACAT
<i>ABCB1</i> 3435C>T	TCACAGTAACT TGGCAGTTTCAG	ACTATAGGCC AGAGAGGCTG
<i>ABCC2</i> -1019A>G	TCCTTTGACTC CTTTCTTCTTT	GCCTCAGCTT CCTGAGTAGC
	*GGATTTTGACA ATTGCAT TCC	*ACTACAGGC ACATGCCAACA
<i>ABCC2</i> -24C>T	TGTCCTTAGGAAA ATGAAAGACTG	TTGCACATCTA ACATTCTGGTT
	*ATTGTTGGCC AGCTCTGTT	*TCTTGTGG TGACCAACCTA
<i>ABCC2</i> 1249G>A	GGGCAAAGAA GTGTGTGGAT	CAGCAGAGCA AGATGGAAGA
	*GGAGCACAT CCTTCCATTGT	*TTGCCCAAAC TCCCATTAAG
<i>ABCC2</i> IVS26 -34T>C	CATGATTCCG TCCTCTGCTT	ACCTCATTTTC CACTTTTGTGT
	*TTCCGTCCTC TGCTTCTGT	*CAACAGCCAC AATGTTGGTC
<i>ABCC2</i> 3972C>T	GGAACCTTCCA AAAGTGCAAG	GTGTGATCC CTGGCTGCTAT
	*TAGCTGGGAC ACTGCTACCC	*CTTCCCTC CATCCAAATGA
<i>ABCC2</i> 4544G>A	GGCACCTT TGTAACAACAT	TGCACCTATTT GCATCACCA
	*GATGTGTGTA GCTGTGGCTCA	*CAGAAATGAA AGGCAAAGGAA

* Nested.

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