The Pharmacogenomics Journal
https://doi.org/10.1038/s41397-021-00213-z

ARTICLE

Investigating the influence of relevant pharmacogenetic variants on the pharmacokinetics and pharmacodynamics of orally administered docetaxel combined with ritonavir

Maarten van Eijk[1] · Dick Pluim[1] · Thomas P. C. Dorlo[1] · Serena Marchetti[2] · Alwin D. R. Huitema[1,3,4] · Jos H. Beijnen[1,5]

Received: 14 August 2020 / Revised: 12 January 2021 / Accepted: 20 January 2021

Abstract

The anticancer drug docetaxel exhibits large interpatient pharmacokinetic and pharmacodynamic variability. In this study, we aimed to assess the functional significance of 14 polymorphisms in the CYP3A, CYP1B1, ABCB1, ABCC2, and SLCO1B3 genes for the pharmacokinetics and pharmacodynamics of oral docetaxel, co-administered with ritonavir. None of the tested CYP3A, ABCB1, ABCC2, and SLCO1B3 genotypes and diplotypes showed a significant relation with an altered bioavailability or clearance of either docetaxel or ritonavir. Similarly, no clear effect of CYP1B1 genotype on clinical outcomes was observed in a subgroup of non-small cell lung cancer (NSCLC) patients. Our post hoc power analysis indicated that our pharmacogenetic–pharmacokinetic analysis was only powered for relatively high effect sizes, which were to be expected given the high interpatient variability. This makes it unlikely that future studies will explain the high observed interpatient variability in oral docetaxel pharmacokinetics as a result of any of these separate polymorphisms and diplotypes.

Introduction

Docetaxel is a widely used anticancer agent that triggers cell death by stabilizing microtubules. It has shown to be effective in the treatment of breast, prostate, gastric, head and neck, and non-small cell lung cancer (NSCLC) [1]. In an attempt to improve patient burden and treatment related costs, an oral formulation of docetaxel, co-administered with the boosting agent ritonavir, has been developed. However, as with intravenous (IV) administration, oral administration of docetaxel has shown wide interpatient variability in systemic exposure which may ultimately lead to variation in clinical outcomes [2, 3].

Docetaxel is principally metabolized in hepatocytes by cytochrome P450 (CYP) 3A isoenzymes into inactive metabolites [3]. Uptake of docetaxel from the systemic circulation is mainly facilitated by the solute carriers OATP1B1 and OATP1B3 expressed on the basolateral side of hepatocytes, although other studies have also shown a role for the OATP1A2 transporter [4–6]. In addition, docetaxel and its metabolites are a substrate for the membrane-localized ATP-binding cassette (ABC) efflux transporters p-glycoprotein (ABCB1) and MRP2 (ABCC2) expressed in the apical side of hepatocytes and the epithelium of the small intestine [7, 8]. Ritonavir is similarly metabolized by CYP3A while it is also a substrate for CYP2D6. However, its role as a substrate for MRP2 and ABCB1 is not well established [9–11]. The genes encoding these enzymes and transporters are polymorphic and several of these single-nucleotide polymorphisms (SNPs) have been associated with altered functionality regarding the pharmacokinetics of

Supplementary information The online version contains supplementary material available at https://doi.org/10.1038/s41397-021-00213-z.

✉ Maarten van Eijk
maa.v.eijk@nki.nl

[1] Department of Pharmacy & Pharmacology, The Netherlands Cancer Institute—Antoni van Leeuwenhoek, Amsterdam, The Netherlands

[2] Division of Clinical Pharmacology, The Netherlands Cancer Institute—Antoni van Leeuwenhoek, Amsterdam, The Netherlands

[3] Department of Clinical Pharmacy, University Medical Center Utrecht, Utrecht University, Utrecht, The Netherlands

[4] Department of Pharmacology, Princess Máxima Center for Pediatric Oncology, Utrecht, The Netherlands

[5] Utrecht Institute for Pharmaceutical Sciences, Utrecht University, Utrecht, The Netherlands

Published online: 01 March 2021

Table 1 Allele frequencies of polymorphisms in 112 patients treated with oral docetaxel and ritonavir.

Gene	Polymorphism	Allele	Rs number	Effect	Function[a]	Allele frequency[b]		Literature q^c (Caucasian)
						p	*q*	
CYP3A4	−392A>G	*1B	*2740574*	–	increased	0.964	0.036	0.035
CYP3A4	15389C>T	*22	*35599367*	possible splicing defect	decreased	0.964	0.036	0.052
CYP3A7	−232A>C	*1C	*45446698*	–	increased	0.955	0.045	0.035
CYP3A5	6986A>G	*3C	*776746*	frameshift	decreased	0.058	0.942	0.930
CYP1B1	4326C>G	*3	*1056836*	Leu432Val	unknown	0.585	0.415	0.443
SLCO1B3	334T>G	*2	*4149117*	Ser112Ala	unknown	0.161	0.839	0.858
SLCO1B3	699G>A	*4	*7311358*	Met233Ile	unknown	0.161	0.839	0.859
SLCO1B3	767G>C	*5	*60140950*	Gly256Ala	unknown	0.866	0.134	0.172
ABCB1	1236C>T	*8	*1128503*	Gly411Gly	unknown	0.464	0.536	0.427
ABCB1	2677G>T/A	*7	*2032582*	Ala893Ser/Thr	unknown	0.451	0.531/0.018	0.445/0.0011
ABCB1	3435C>T	*6	*1045642*	Ile1145Ile	unknown	0.384	0.616	0.524
ABCC2	−24C>T	–	*717620*	–	unknown	0.799	0.201	0.198
ABCC2	1249G>A	–	*2273697*	Val417Ile	unknown	0.808	0.192	0.202
ABCC2	3972C>T	–	*3740066*	Ile1324Ile	unknown	0.638	0.362	0.362

[a]Expected functionality of the variant protein compared to the reference protein.

[b]Hardy-Weinberg notation for allele frequencies with p the frequency for the reference allele and q for the variant allele.

[c]Variant allele frequency as reported in dbSNP [27].

docetaxel [3]. For example; carriers of the 1236C>T (*8) polymorphism in the ABCB1 gene were found to have a significantly decreased clearance of docetaxel [12]. While patients carrying both the CYP3A4*1B and CYP3A5*1A alleles, or the CYP3A4/5*2 haplotype, showed a 64% higher clearance compared to noncarriers [13].

Furthermore, the 4326C>G (*3) polymorphism in the CYP1B1 gene has been associated with inferior survival and response to taxanes in patients carrying the variant genotype [14–16]. CYP1B1 is expressed in some tumors and while the taxanes are not among its substrates, it has been suggested that the protein encoded by the CYP1B1*3 allele reduces the efficacy of taxane treatment [17–19]. Possibly, this decreased efficacy is caused by the increased production of estradiol-3,4-quinone, an oxidation product of 4-hydroxyestradiol, which inhibits tubulin polymerization and may additionally covalently bind to docetaxel to form the inactive 4-OHE2-docetaxel adduct, thereby reducing potency [15].

To investigate whether genetic differences influence the pharmacokinetics and pharmacodynamics of oral docetaxel co-administered with ritonavir, genotyping of patients in two phase I clinical trials has been performed as a secondary endpoint [2, 20]. In this analysis, we aimed to elucidate the relationship between polymorphisms in the candidate genes CYP3A, ABCB1, ABCC2, and SLCO1B3 and the pharmacokinetics of oral docetaxel and ritonavir using a pharmacometric approach. Moreover, we investigated the relation between CYP1B1 genotype and the clinical efficacy of oral docetaxel co-administered with ritonavir. Lastly, we performed a power analysis of two simulation scenarios in order to gain perspective on the power of the performed population pharmacogenetic study.

Methods

Clinical studies and genotyping

Docetaxel, either as a drinking solution, capsule, or tablet formulation, and co-administered with ritonavir was given to patients with various tumor types in two dose-finding phase I clinical trials (NCT01173913, ISCRTN32770468) [2, 20]. These trials investigated either a weekly once daily or a weekly twice daily schedule of administration. Both study protocols were approved by the Medical Ethics Committee of the Netherlands Cancer Institute (Amsterdam, the Netherlands). All patients had provided written informed consent prior to the start of treatment. Patients included in these studies were predominantly of Caucasian descent. We selected a panel of 14 SNPs with potential mechanistic relevance for the pharmacokinetics and/or pharmacodynamics of either ritonavir or docetaxel from literature (Table 1). In total, peripheral blood samples from 124 patients were collected in a 4 mL ethylenediaminetetraacetic acid tube and stored at −20 °C. DNA was extracted using the QIAamp DNA Mini Kit (Qiagen, Hilden, Germany) according to manufacturer's protocol and subsequently stored at −20 °C. DNA concentrations were measured at 260 nm using a NanoDrop ND-1000 UV–Vis

spectrophotometer (Thermo Fisher Scientific, Ashville, NC, USA).

Genotyping was performed using TaqMan SNP Genotyping Assays (Applied Biosystems, Carlsbad, CA, USA) according to manufacturer's protocol. The TaqMan Assay IDs and probe context sequences are shown in Supplementary Table S1. Reactions were performed using the Applied Biosystems StepOne. Quality control of the TaqMan assays was incorporated by re-measurement of two positive control samples per plate in addition to two negative controls. Positive control samples for each SNP were previously measured heterozygous and homozygous samples. These were blindly selected from a list based on their genotyping outcome from a previously performed Taqman assay. Distilled water (B. Braun Melsungen AG, Germany) was used for the negative controls.

Genotype frequencies were calculated for all polymorphisms and their distribution was tested for deviations from Hardy–Weinberg Equilibrium using the chi-square test. We performed pairwise linkage disequilibrium analysis for SNPs located on the same chromosome. In the case of significant linkage between polymorphisms an analysis of observed diplotypes could be performed.

Pharmacokinetic model

Previously we have developed an integrated semi-physiological pharmacokinetic model for oral docetaxel and oral ritonavir using nonlinear mixed effects modeling. This integrated model consists of separate pharmacokinetic models for ritonavir and docetaxel and incorporates the inhibition of docetaxel clearance, and enhancement of oral docetaxel bioavailability, by ritonavir. It was built using pharmacokinetic data from patients treated with both IV and oral docetaxel co-administered with ritonavir in several phase I studies, including the phase I studies used in the current pharmacogenetic analysis [21].

Population pharmacokinetic-pharmacogenetic analysis

To screen for potential covariates we used a systematic, two-step approach. Initially we established a list of relevant and plausible parameter-covariate relationships based on the pharmacokinetic models of oral docetaxel and ritonavir and previous knowledge of the impact of the polymorphism on protein function. Table 2 shows the parameter–covariate relationships that were considered relevant. We assumed included covariates to have an impact on the bioavailability and clearance of either docetaxel or docetaxel and ritonavir.

For 12 of the 124 genotyped patients, no corresponding pharmacokinetic data was available. We therefore matched the established genotypes with the corresponding pharmacokinetic data for 112 out of 173 patients in our population pharmacokinetic model. Since the availability of genotyping data was dependent on the clinical studies in which patients were included, we assumed that this data was

Table 2 Overview of potential parameter–covariate relationships.

Candidate polymorphism /Covariate	Observed in population ($n = 112$)		PK parameter of interest	Direct relation with clinical efficacy of docetaxel treatment
CYP3A4 *1B	Wild type	104	CL_{RTV}, F_{RTV}, CL_{DOC}, F_{DOC}	None
	Heterozygous	8		
	Homozygous	0		
CYP3A4 *22	Wild type	104		
	Heterozygous	8		
	Homozygous	0		
CYP3A7 *1C	Wild type	102	CL_{RTV}, F_{RTV}, CL_{DOC}, F_{DOC}	None
	Heterozygous	10		
	Homozygous	0		
CYP3A5 *3C	Wild type	2	CL_{RTV}, F_{RTV}, CL_{DOC}, F_{DOC}	None
	Heterozygous	9		
	Homozygous	101		
CYP1B1 *3	Wild type	37	*none*	*ToS, Response*
	Heterozygous	57		
	Homozygous	18		
SLCO1B3 *2/*4 diplotype	TG/TG	0	CL_{DOC}, F_{DOC}	None
	Non-GA/GA-TG/TG	36		
	GA/GA	76		
SLCO1B3 *5	Wild type	83		
	Heterozygous	28		
	Homozygous	1		
ABCB1 *8	Wild type	16	CL_{DOC}, F_{DOC}	None
	Heterozygous	72		
	Homozygous	24		
ABCB1 *7	Wild type	13		
	Heterozygous	73		
	Homozygous	22		
	Heterozygous	2		
	Heterozygous	2		
ABCB1 *6	Wild type	15		
	Heterozygous	56		
	Homozygous	41		
ABCB1 diplotype	CGC/CGC	8	CL_{DOC}, F_{DOC}	None
	Non-CGC/CGC-TTT/TTT	91		
	TTT/TTT	25		
ABCC2 −24C>T	Wild type	70	CL_{DOC}, F_{DOC}	None
	Heterozygous	39		
	Homozygous	3		
ABCC2 1249G>A	Wild type	73		
	Heterozygous	35		
	Homozygous	4		
ABCC2 3972C>T	Wild type	46		
	Heterozygous	51		
	Homozygous	15		

PK pharmacokinetic, *CL* clearance, *F* bioavailability, *ToS* time on study, *DOC* docetaxel, *RTV* ritonavir.

missing completely at random for the patients who were not genotyped ($n = 61$). Missing data were handled by estimating an extra parameter for the effect of a missing covariate in this population, as described previously [22]. All model estimations were repeated after the inclusion of this extra parameter.

Subsequently, the distribution of individual Bayesian estimates of the pharmacokinetic parameter of interest (eta (η) distribution) versus the genotype were visually explored. These were related to the population parameter estimates according to either Eq. (1) or Eq. (2).

$$P_i = P \cdot exp(\eta_{i,BSV}) \quad (1)$$

$$P_i = P \cdot exp(\eta_{i,BSV} + \eta_{i,BOV}) \quad (2)$$

With P_i the individual parameter estimate for individual i, P the population parameter estimate, and η_i either between-subject variability or between-occasion variability effect distributed following N (0, $\omega 2$). Potentially relevant genotypes were added to the list of parameter–covariate relationships to be formally tested.

We used an automated stepwise covariate modeling (SCM) algorithm to perform this population pharmacokinetic-pharmacogenetic analysis [23, 24]. In SCM, each potential parameter-covariate relation as defined in the first step was tested univariately on the original pharmacokinetic model for a significant improvement of the model fit to the data. As quantified by the difference in objective function value (dOFV). Statistical evaluation was performed using the log-likelihood ratio test. For forward inclusion and backward elimination significance levels of $p < 0.05$ (degrees of freedom (df) = 1, dOFV = −3.84; df = 2, dOFV = −5.991) and $p < 0.001$ (df = 1, dOFV = −10.83; df = 2, dOFV = −13.82) were used, respectively. The effect of different genotypes on parameter P was coded proportionally to the respective pharmacokinetic parameter. For example,

$$P = P_{pop} \times (1 + \theta_1^{heterozygous} \times \theta_2^{homozygous}) \quad (3)$$

where P_{pop} is the population parameter estimate for the wild type, and *homozygous* and *heterozygous* can be either 0 or 1, therefore θ_1 and θ_2 are the separate fixed effects for either the heterozygous or the homozygous variant genotype. For genotypes with low minor allele frequencies resulting in <3 patients with a variant genotype, such as CYP3A5*1/*1 ($n = 2$), the homozygous covariate effect was assumed to be two times the effect of the heterozygous genotype (Eq. (4)) [12], provided that this was mechanistically plausible and correlated with the observed differences in interpatient variability.

$$P = P_{pop} \times (1 + \theta_3 \times genotype) \quad (4)$$

With θ_3 the effect of the variant allele and genotype either 0 (wild-type), 1 (heterozygous), or 2 (homozygous variant).

Genotype related to time on study and response

We performed a separate pharmacodynamic analysis in which we investigated the relationship between genotype and clinical efficacy of oral docetaxel treatment. The majority of patients in both phase I trials were diagnosed with NSCLC. In order to attain a substantial population with similar clinical prognosis our pharmacodynamic analysis was performed only on this subgroup. SNPs which were thought to have relevant pharmagenetic-pharmacodynamic relations are displayed in Table 2. Clinical outcome of patients by genotype was visualized using swimmer plots displaying time on study and response to treatment for each individual patient. Time on study was defined as the time in months from start of treatment until either progression or cessation of treatment due to other causes. Tumor response was evaluated in accordance with RECIST version 1.0 [25].

Stochastic simulation and estimation with parametric power estimation

We conducted trial simulations and power estimations for two different hypothetical scenarios using stochastic simulation and estimation in order to aid the interpretation of the results of our pharmacogenetic–pharmacokinetic analysis. For this, we simulated a data frame that included a virtual polymorphism as a covariate. The data frame was similar to the dataset used in SCM. It contained pharmacokinetic data from 173 patients of which 112 were genotyped. The assumption was made that the allele frequency of this variant allele in the population was 0.45, which results in 25% (28/112 patients) of the population having the homozygous variant genotype. For both scenarios we assumed only an effect of the homozygous variant genotype on docetaxel clearance. For the first scenario we created a full pharmacokinetic model in which carriers of this homozygous variant genotype had a fixed 25% decreased clearance of docetaxel ($\theta_{virtualSNP} = -0.25$). In the second scenario, we assumed the effect of the homozygous variant genotype to be 40% ($\theta_{virtualSNP} = -0.40$). Similar to our covariate analysis, missing genotype was assumed to be missing completely at random in this simulation study. Thus, for the group of patients for whom genotype was missing ($n = 61$), the SNP effect was

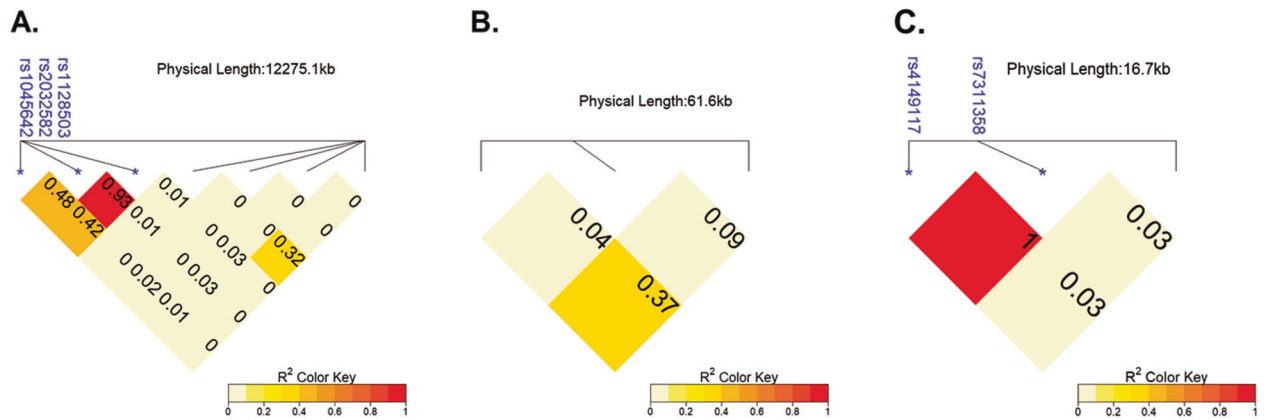

Fig. 1 Linkage disequilibrium plots. A. Heatmap of pairwise linkage disequilibrium for SNPs in the CYP3A and ABCB1 genes on chromosome 7, **B.** ABCC2 polymorphisms on chromosome 10, and **C.** SLCO1B3 polymorphisms on chromosome 12.

imputed as follows:

$$P = P_{pop} \times \left(1 + \left(\theta_{virtualSNP} \times f_{prevalence}\right)\right) \quad (5)$$

With $\theta_{virtualSNP}$ the effect of the polymorphism on the population parameter estimate, and f the prevalence of the polymorphism. We created a nested reduced version of both models by fixing the effect of the covariate to zero. The full model and simulated data frame were then used to stochastically simulate 100 datasets which were subsequently used in the parameter estimation of both the full and reduced model. A previously described parametric power estimation algorithm (PPE) was used to generate a power versus study size curve [26].

Software

Model estimations and simulations were performed using nonlinear mixed effects modeling software (NONMEM, version 7.3, ICON Development Solutions, Ellicott City, MD, USA) together with a gfortran compiler. Pirana (version 2.9.9, Certara, Princeton, New Jersey) was used as a graphical interface. The model estimations used first-order conditional estimation with interaction. We used the SCM and SSE tools from the Perl-speaks-NONMEM toolkit (PsN, version 4.7.0) [24, 26]. R (version 3.6.2) and RStudio (version 1.2.5042; Boston, MA, USA) were used for data processing, genetic analysis, plotting, and implementation of the PPE algorithm.

Results

Genotyping

Minor allele frequencies for the SNPs analyzed corresponded well with those observed in the Caucasian population as shown in Table 1 [27]. Average call rates were >99% for each SNP. The frequency distributions of most SNPs were in Hardy-Weinberg equilibrium. However, deviations from Hardy-Weinberg equilibrium were observed for the ABCB1*7 ($p = 0.0027$) and ABCB1*8 ($p = 0.002$) polymorphisms (calculations not shown). For ABCB1, linkage of the SNPs at the 1236, 2677, and 3435 loci was observed (Fig. 1A) which is in line with previous reports [28]. And as expected, we also observed a strong genetic linkage of the SLCO1B3*2 and SLCO1B3*4 SNPs ($r^2 = 1$)(Fig. 1C) [29]. Due to the complete linkage of the SLCO1B3 *2 and *4, these SNPs were only analyzed as a diplotype. For the ABCB1 SNPs an analysis of the diplotype was performed in addition to the analysis of the separate SNPs.

Population pharmacokinetic–pharmacogenetic analysis

The distributions of the between-subject variability for each patient (η) (Supplementary Figs. S1 and S2.) were visually examined and formed the basis for the selection of the parameter–covariate relationships to be investigated using SCM.

The effects of including the polymorphisms and diplotypes as covariates on both pharmacokinetic models and the corresponding effect sizes are shown in Table 3. We identified no statistically significant associations between variants of the candidate genes and any of the relevant pharmacokinetic parameters in either the oral docetaxel or the ritonavir model. Furthermore, the estimated (non-significant) effect sizes were relatively low in comparison with the clinically observed inter-patient variability in pharmacokinetics and had a high imprecision.

Table 3 Estimates of the effect of inclusion of relevant covariates in the docetaxel and ritonavir pharmacokinetic models.

PK parameter	Parameter estimate (RSE (%))	Unit	Covariate	df	Genotype	Covariate effect size (RSE (%))	*P* value	Missing parameter effect size (RSE (%))
Ritonavir model								
Clearance (CL_{RTV})	8.27 (10)	L/h						
			CYP3A4 *1B[b]	1	Heterozygous	0.18	0.49	1.25 (17)
			CYP3A4 *22[b]	1	Heterozygous	−0.18	0.39	
Population relative gut bioavailability of ritonavir after first dose ($F_{RTV,\ intrinsic}$)	1[a]	–						
			CYP3A5 *3C[c]	1	homozygous wild-type/ heterozygous	−0.02	0.93	1.49 (19)
Docetaxel model								
Population uninhibited intrinsic clearance (CL_{int0})	1980[a]	L/h						
			ABCC2 3972C>T	2	Heterozygous	0.03 (492)	0.69	1.02 (13)
					Homozygous variant	−0.11 (127)		
			CYP3A7 *1C[b]	1	Heterozygous	−0.26 [60]	0.08	
			ABCB1 *8	2	Heterozygous	0.10 (194)	0.63	
					Homozygous variant	−0.01 (2462)		
			ABCB1 *7[c]	2	Heterozygous (GT/GA)	0.08 (240)	0.29	
					TT/TA genotypes	−0.10 (142)		
			SLCO1B3 *2/*4 diplotype[b]	1	non-GA/GA-TG/TG	−0.20 (46)	0.04	
Population gut bioavailability in combination with ritonavir relative to without ($F_{ritonavir}$)	3.75 (28)	–						
			CYP3A4 *1B[b]	1	Heterozygous	−0.31 (60)	0.06	0.92 (12)
			CYP3A4 *22[b]	1	Heterozygous	0.18 (187)	0.37	
			ABCC2 1249G>A	2	Heterozygous	0.18 (70)	0.06	
					Homozygous	−0.34 (67)		
			ABCC2 −24C>T	2	Heterozygous	−0.08 (112)	0.16	
					Homozygous	0.63 (65)		
			CYP3A5 *3[c]	1	Combined homozygous wild-type/heterozygous	−0.04 (703)	0.72	
			ABCB1 diplotype	2	Non-CGC/CGC-TTT/TTT	0.18 (182)	0.46	
					TTT/TTT	0.29 (119)		

PK pharmacokinetic, *df* degrees of freedom, *RSE* relative standard error

[a]Fixed population parameter estimate.

[b]Low minor allele frequency, resulting in the absence of a homozygous variant/wild type genotype in this population.

[c]Low minor allele frequency, effect of the covariate therefore estimated using Eq. (4).

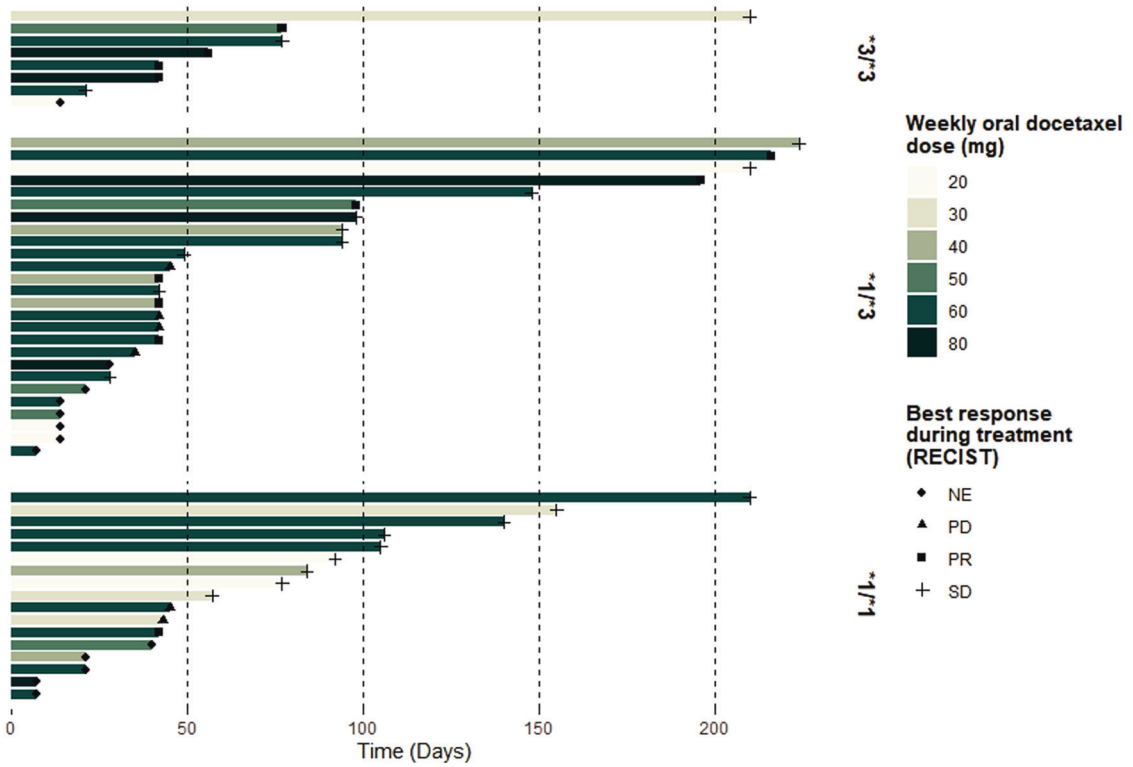

Fig. 2 Swimmer plot visualizing time on study and best outcome of treatment with oral docetaxel for 51 NSCLC patients grouped by CYP1B1 genotype. NE not evaluable; PD, progressive disease; PR partial response, SD stable disease. *1, wild-type allele; *3, variant allele.

Genotype related to time on study and response

We identified a subgroup of 51 patients with locally advanced or metastatic NSCLC from both phase I trials for which corresponding genotype data was available. We visualized the time on study and outcome per patient, received daily dose, and CYP1B1 genotype using a swimmers plot as shown in Fig. 2. We observed no distinct differences in the time on study between patients with the *3/*3 genotype and patients with either the *1/*3 and *1/*1 genotype. We did observe a higher objective response rate in patients with the *3/*3 genotype compared to the *1/*3 and *1/*1 genotypes (50% vs 16%) while the rate of patients who achieved stable disease as best response to treatment was higher in the *1/*3 and *1/*1 genotypes (49% vs 38%). The median weekly dose administered in both groups was 60 mg.

Stochastic simulation and estimation with parametric power estimation

The power versus study size for the two scenarios is shown in Fig. 3. Figure 3A illustrates that using our current analysis, a sample size of 220 patients would be required to achieve 80% power to detect a significant 25% decreased clearance of oral docetaxel as a result of an SNP with a prevalence of 25%. To achieve 80% power to detect an effect of a polymorphism that causes a 40% decrease in clearance and a prevalence of 25%, a sample size of 85 patients would be required. This indicates that our current analysis would have been sufficiently powered in the latter scenario.

Discussion

We performed a pharmacogenetic analysis of the effect of several polymorphisms in genes generally considered relevant for the pharmacokinetics and pharmacodynamics of docetaxel and ritonavir in a cohort of cancer patients, in an attempt to unravel the causes for differences in outcomes and the extensive interpatient variability in exposure to oral docetaxel.

This is not the first study investigating the influence of genetic differences on the pharmacokinetics of docetaxel [3]. We are, however, the first to examine the influence of genetic polymorphisms in a population of patients treated with oral docetaxel formulations in combination with the boosting agent ritonavir. From a pharmacokinetic point of view, orally administered docetaxel is subject to more interactions with these enzymes and transporters due to its uptake from the gastrointestinal tract and subsequent first-pass metabolism [21].

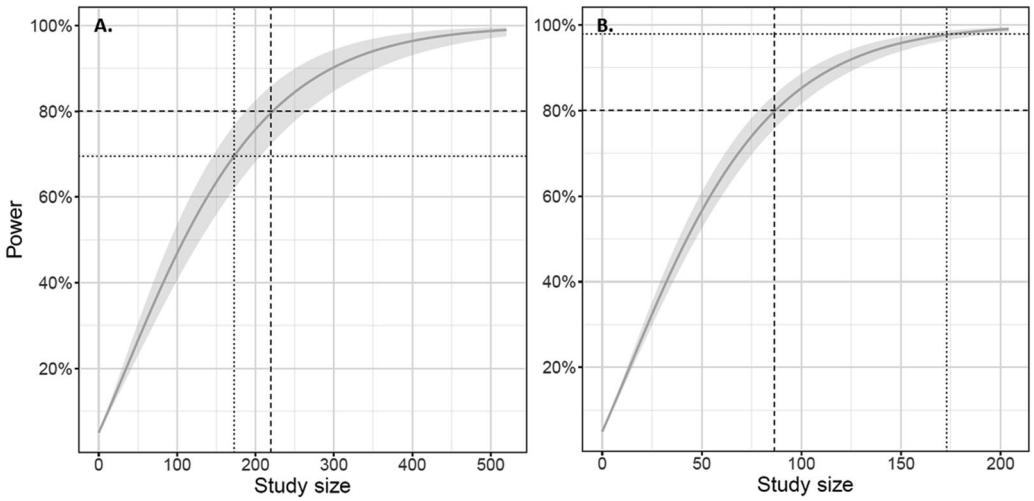

Fig. 3 PPE power curves. Curves were generated for a full model (containing either a covariate effect θvirtualSNP = −0.25 (**A.**) or a covariate effect of θvirtualSNP = −0.40 (**B.**)), both with a prevalence of 25%, versus a reduced model estimated on 100 stochastically simulated datasets. The PPE curves indicate that a study size of ~220 patients would be required to achieve 80% power in the case of an effect size of −0.25 whilst with an effect size of −0.40 a population of ~85 patients would be required to detect a significant effect of this polymorphism. Dashed line, number of patients to achieve 80% power threshold; dotted line, actual power using a study size of 173 patients; shaded region, uncertainty in the power estimate.

Our population displayed variant allele frequencies that corresponded reasonably with those observed in the Caucasian population. However, some of the allele frequencies of the investigated SNPs showed deviations from Hardy–Weinberg equilibrium. Most likely this observation is caused by genetic drift as a result of the limited number of patients included in this analysis. As expected, we observed strong linkage of the 334T>G and 699G>A polymorphisms in the SLCO1B3 gene and polymorphisms in the ABCB1 gene at positions 1236, 2677, and 3435 [28, 29]. Conversely, no linkage was observed for the CYP3A4*1B and CYP3A5*1 polymorphisms, that are known to bring about a known haplotype in the CYP3A gene [13, 30].

We did not identify a significant effect of any of the polymorphisms and diplotypes in the CYP3A, ABCB1, ABCC2, and SLCO1B3 genes on the pharmacokinetics of oral docetaxel and ritonavir.

Nevertheless, some of the investigated polymorphisms appeared to show mechanistically plausible trends towards significance when univariately tested as a covariate to the pharmacokinetic model for oral docetaxel. Patients who were heterozygous carriers of the CYP3A4*1B polymorphism displayed a trend towards a decreased bioavailability of docetaxel (31% decrease, $p = 0.06$). A trend that was also observed for homozygous carriers of the ABCC2 1249G>A polymorphism (34% decrease, $p = 0.06$). Both these effects are in accordance with the previously reported effect of these SNPs on taxane pharmacokinetics [31, 32]. Despite not resulting in a significant improvement of the models, the CYP3A4*22 polymorphism displayed estimated effect sizes consistent with the previously reported functionality (21% reduced CYP3A4 activity) of the polymorphism when added to the ritonavir (18% decreased clearance) and docetaxel model (18% increased bioavailability) [33].

In part, the absence of an observed effect may be explained by the co-administration of ritonavir. Ritonavir is a strong and irreversible inhibitor of CYP3A4 (IC50 = 0.014 μM) and CYP3A5 (IC50 = 0.09 μM) and a weak inhibitor of CYP3A7 (IC50 = 0.6 μM) [34, 35]. Additionally, it is a strong inhibitor of ABCB1 (IC50 = 0.2 μM) and a general OATP inhibitor (IC50 = 1.3–6.1 μM) [36, 37]. While some studies also suggest ritonavir may inhibit ABCC2 [38]. As a result, inherited differences in the functionality of these enzymes and transporters may have been masked in this study due to strong inhibition. Moreover, the effect of deficiencies in for example the OATP1B3 transporter may have been indiscernible due to compensatory mechanisms such as OAT2 for which docetaxel is also a substrate [39].

We also investigated the potential relation between polymorphisms in the CYP1B1 gene and clinical outcomes of oral docetaxel treatment. We selected a group of NSCLC patients who composed the largest subgroup of the various tumor types included in the original phase I trials. As opposed to previous studies we observed no detrimental effect of the CYP1B1*3/*3 genotype on clinical outcomes after treatment with oral docetaxel [14–16]. Contrary to our expectations, we even observed a higher response rate in patients with the *3/*3 genotype as opposed to the *1/*3 and *1/*1 genotype.

The previously reported effect of this variant polymorphism was more pronounced in two clinical trials in

prostate cancer patients [15, 16] as compared to the trial in NSCLC patients [14], which may suggest a more dominant role of the CYP1B1*3 polymorphism in the former tumor type.

We should note that the dismal prognosis of NSCLC patients after first line therapy, in addition to the large variation in administered treatments and limited sample size, may have obscured any effect of the CYP1B1 genotype and complicate the drawing of firm conclusions based on our analysis.

We performed a post hoc power study to aid the interpretation of the results of our pharmacokinetic–pharmacogenetic analysis and ultimately to investigate the feasibility of studying these polymorphisms in larger cohorts. We reasoned that in order to be clinically relevant, the effect of a polymorphism would have to be sufficiently large to explain the aforementioned variability in docetaxel pharmacokinetics [2, 20]. We therefore simulated two separate scenarios incorporating a virtual polymorphism with either an effect of a 25% or a 40% decreased docetaxel clearance and both with a clinically relevant prevalence of 25%.

The first simulation scenario demonstrated that a study size of about 220 patients would be required in order to achieve 80% power, indicating that our current analysis was not sufficiently powered in the event of a polymorphism with such an effect size and prevalence. In relation to the observed effect sizes in our own pharmacokinetic–pharmacogenetic analysis (Table 3) we can see that some of the polymorphisms indeed exhibit effect sizes with a similar magnitude. With the second scenario we sought to demonstrate the required study size to achieve 80% power in the event of a highly clinically relevant polymorphism with an effect size of 40%. In this case a study size of 85 patients would result in sufficient power. Meaning our study would have been sufficiently powered to detect these larger differences in pharmacokinetics as a result of these polymorphisms.

We should note that in both simulation studies we handled the missing covariate data for 61 patients by imputing the population mean of the effect of the covariate times the prevalence. Thereby using a study set up similar to our original pharmacokinetic–pharmacogenetic analysis and assuming that the missingness of the data occurred completely at random.

In conclusion, we found no significant effect of the candidate CYP3A, CYP1B1, ABCB1, ABCC2, and SLCO1B3 polymorphisms and diplotypes on the pharmacokinetics and pharmacodynamics of oral docetaxel and ritonavir in our analysis of clinical phase I data. A post hoc power analysis indicated that our pharmacokinetic–pharmacogenetic analysis was probably underpowered, given the encountered polymorphism prevalences and moderate effect sizes. Thus, based on our current study, we cannot discard the hypothesis that polymorphisms in the investigated genes contribute to some extent to the clinically observed interpatient variability in oral docetaxel pharmacokinetics. However, it can be concluded that it is unlikely that future studies will explain the high observed interpatient variability in oral docetaxel pharmacokinetics as a result of any of these separate polymorphisms and diplotypes. This finding may support feasibility assessment of larger prospective clinical trials investigating the influence of genetic polymorphisms on the pharmacokinetics of oral docetaxel combined with ritonavir.

Code availability

The NONMEM control streams for the oral docetaxel and ritonavir models that were used in our pharmacokinetic–pharmacogenetic analysis and in our trial simulations are available in the supplementary material.

Acknowledgements JHB has received a grant for translational research (ZonMw code 40-41200-98-004).

Compliance with ethical standards

Conflict of interest JHB holds a patent on oral taxane formulations and is a (part-time) employee and (indirect) shareholder of Modra Pharmaceuticals, a spin-off company that develops oral taxane formulations. The other authors declare that they have no conflict of interest.

References

1. Sanofi-Aventis. Summary of Product Characteristics (Taxotere). 2018. https://www.ema.europa.eu/en/medicines/human/EPAR/taxotere
2. De Weger VA, Stuurman FE, JJMA Hendrikx, Moes JJ, Sawicki E, ADR Huitema, et al. A dose-escalation study of bi-daily once weekly oral docetaxel either as ModraDoc001 or ModraDoc006 combined with ritonavir. Eur J Cancer. 2017;86:217–25.
3. Nieuweboer AJM, de Morrée ES, de Graan AJM, Sparreboom A, de Wit R, Mathijssen RHJ. Inter-patient variability in docetaxel pharmacokinetics: a review. Cancer Treat Rev. 2015;41:605–13.
4. Oshiro C, Marsh S, McLeod H, Carrillo MW, Klein T, Altman R. Taxane pathway. Pharmacogenet Genom. 2009;19:979–83.
5. Iusuf D, JJMA Hendrikx, Van Esch A, Van De Steeg E, Wagenaar E, Rosing H, et al. Human OATP1B1, OATP1B3 and OATP1A2 can mediate the in vivo uptake and clearance of docetaxel. Int J Cancer. 2015;136:225–33.
6. De Graan AJM, Lancaster CS, Obaidat A, Hagenbuch B, Elens L, Friberg LE, et al. Influence of polymorphic OATP1B-type carriers on the disposition of docetaxel. Clin Cancer Res. 2012;18:4433–40.
7. Baker SD, Sparreboom A, Verweij J. Clinical pharmacokinetics of docetaxel. Clin Pharmacokinet. 2006;45:235–52.
8. Van Waterschoot RAB, Lagas JS, Wagenaar E, Rosing H, Beijnen JH, Schinkel AH. Individual and combined roles of CYP3A, P-glycoprotein (MDR1/ABCB1) and MRP2 (ABCC2) in the pharmacokinetics of docetaxel. Int J Cancer. 2010;127:2959–64.

9. Hsu A, Granneman GR, Bertz RJ. Ritonavir: clinical pharmacokinetics and interactions with other anti-HIV agents. Clin Pharmacokinet. 1998;35:275–91.
10. Huisman MT, Smit JW, Crommentuyn KML, Zelcer N, Wiltshire HR, Beijnen JH, et al. Multidrug resistance protein 2 (MRP2) transports HIV protease inhibitors, and transport can be enhanced by other drugs. Aids 2002;16:2295–301.
11. Cerveny L, Ptackova Z, Durisova M, Staud F. Interactions of protease inhibitors atazanavir and ritonavir with ABCB1, ABCG2, and ABCC2 transporters: Effect on transplacental disposition in rats. Reprod Toxicol. 2018;79:57–65.
12. Bosch TM, Huitema ADR, Doodeman VD, Jansen R, Witteveen E, Smit WM, et al. Pharmacogenetic screening of CYP3A and ABCB1 in relation to population pharmacokinetics of docetaxel. Clin Cancer Res. 2006;12:5786–93.
13. Baker SD, Verweij J, Cusatis GA, Van Schaik RH, Marsh S, Orwick SJ, et al. Pharmacogenetic pathway analysis of docetaxel elimination. Clin Pharm Ther. 2009;85:155–63.
14. Vasile E, Tibaldi C, Leon GL, D'Incecco A, Giovannetti E. Cytochrome P450 1B1 (CYP1B1) polymorphisms are associated with clinical outcome of docetaxel in non-small cell lung cancer (NSCLC) patients. J Cancer Res Clin Oncol. 2015;141:1189–94.
15. Sissung TM, Danesi R, Price DK, Steinberg SM, De Wit R, Zahid M, et al. Association of the CYP1B1*3 allele with survival in patients with prostate cancer receiving docetaxel. Mol Cancer Ther. 2008;7:19–26.
16. Pastina I, Giovannetti E, Chioni A, Sissung TM, Crea F, Orlandini C, et al. Cytochrome 450 1B1 (CYP1B1) polymorphisms associated with response to docetaxel in Castration-Resistant Prostate Cancer (CRPC) patients. BMC Cancer. 2010;10.
17. Shimada T, Watanabe J, Kawajiri K, Sutter TR, Guengerich FP, Gillam EMJ, et al. Catalytic properties of polymorphic human cytochrome P450 1B1 variants. Carcinogenesis. 1999;20:1607–13.
18. Hanna IH, Dawling S, Roodi N, Guengerich FP, Parl FF. Cytochrome P450 1B1 (CYP1B1) pharmacogenetics association of polymorphisms with functional differences in estrogen hydroxylation activity. Cancer Res. 2000;60:3440–4.
19. Bournique B, Lemarié A. Docetaxel (Taxotere) is not metabolized by recombinant human CYP1B1 in vitro, but acts as an effector of this isozyme. Drug Metab Dispos. 2002;30:1149–52.
20. De Weger VA, Stuurman FE, Koolen SLW, Moes JJ, JJMA Hendrikx, Sawicki E, et al. A phase I dose escalation study of once-weekly oral administration of docetaxel as ModraDoc001 capsule or ModraDoc006 tablet in combination with ritonavir. Clin Cancer Res. 2019;25:5466–74.
21. Yu H, Janssen JM, Sawicki E, van Hasselt JGC, de Weger VA, Nuijen B, et al. A population pharmacokinetic model of oral docetaxel coadministered with ritonavir to support early clinical development. J Clin Pharm. 2020;60:340–50.
22. Keizer RJ, Zandvliet AS, Beijnen JH, Schellens JHM, Huitema ADR. Performance of methods for handling missing categorical covariate data in population pharmacokinetic analyses. AAPS J. 2012;14:601–11.
23. Jonsson EN, Karlsson MO. Automated covariate model building within NONMEM. Pharm Res. 1998;15:1463–8.
24. Lindbom L, Pihlgren P, Jonsson N. PsN-Toolkit - A collection of computer intensive statistical methods for non-linear mixed effect modeling using NONMEM. Comput Methods Prog Biomed. 2005;79:241–57.
25. Therasse P, Arbuck SG, Eisenhauer EA, Wanders J, Kaplan RS, Rubinstein L, et al. New guidelines to evaluate the response to treatment in solid tumors. European Organization for Research and Treatment of Cancer, National Cancer Institute of the United States, National Cancer Institute of Canada. J Natl Cancer Inst. 2000;92:205–16.
26. Ueckert S, Karlsson MO, Hooker AC. Accelerating Monte Carlo power studies through parametric power estimation. J Pharmacokinet Pharmacodyn. 2016;43:223–34.
27. Sherry ST, Ward M, Sirotkin K. dbSNP—database for single nucleotide polymorphisms and other classes of minor genetic variation. Genome Res. 1999;9:677–9.
28. Fung KL, Gottesman MM. A synonymous polymorphism in a common MDR1 (ABCB1) haplotype shapes protein function. Biochim Biophys Acta—Proteins Proteom. 2009;1794:860–71.
29. Smith NF, Marsh S, Scott-Horton TJ, Hamada A, Mielke S, Mross K, et al. Variants in the SLCO1B3 gene: interethnic distribution and association with paclitaxel pharmacokinetics. Clin Pharm Ther. 2007;81:76–82.
30. Lepper ER, Baker SD, Permenter M, Ries N, Van Schaik RHN, 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;11:7398–404.
31. Lian G, Yuan J, Gao Y. In vitro transport ability of ABCC2 (G1249A) polymorphic variant towards anticancer drugs. Onco Targets Ther. 2020;13:1413–9.
32. Daly AK. Significance of the minor cytochrome P450 3A isoforms. Clin Pharmacokinet. 2006;45:13–31.
33. Elens L, Van Gelder T, Hesselink DA, Haufroid V, Van Schaik RHN. CYP3A4*22: promising newly identified CYP3A4 variant allele for personalizing pharmacotherapy. Pharmacogenomics. 2013;14:47–62.
34. Granfors MT, Wang JS, Kajosaari LI, Laitila J, Neuvonen PJ, Backman JT. Differential inhibition of cytochrome P450 3A4, 3A5 and 3A7 by five human immunodeficiency virus (HIV) protease inhibitors in vitro. Basic Clin Pharm Toxicol. 2006;98:79–85.
35. Hossain MA, Tran T, Chen T, Mikus G, Greenblatt DJ. Inhibition of human cytochromes P450 in vitro by ritonavir and cobicistat. J Pharm Pharm. 2017;69:1786–93.
36. Karlgren M, Vildhede A, Norinder U, Wisniewski JR, Kimoto E, Lai Y, et al. Classification of inhibitors of hepatic organic anion transporting polypeptides (OATPs): Influence of protein expression on drug-drug interactions. J Med Chem. 2012;55:4740–63.
37. Drewe J, Gutmann H, Fricker G, Török M, Beglinger C. HIV protease inhibitor ritonavir: a more potent inhibitor of P-glycoprotein than the cyclosporine analog SDZ PSC 833. Biochem Pharm. 1999;57:1147–52.
38. Ye ZW, Camus S, Augustijns P, Annaert P. Interaction of eight HIV protease inhibitors with the canalicular efflux transporter ABCC2 (MRP2) in sandwich-cultured rat and human hepatocytes. Biopharm Drug Dispos. 2010;31:178–88.
39. Kobayashi Y, Ohshiro N, Sakai R, Ohbayashi M, Kohyama N, Yamamoto T. Transport mechanism and substrate specificity of human organic anion transporter 2 (hOat2 [SLC22A7]). J Pharm Pharm. 2005;57:573–8.