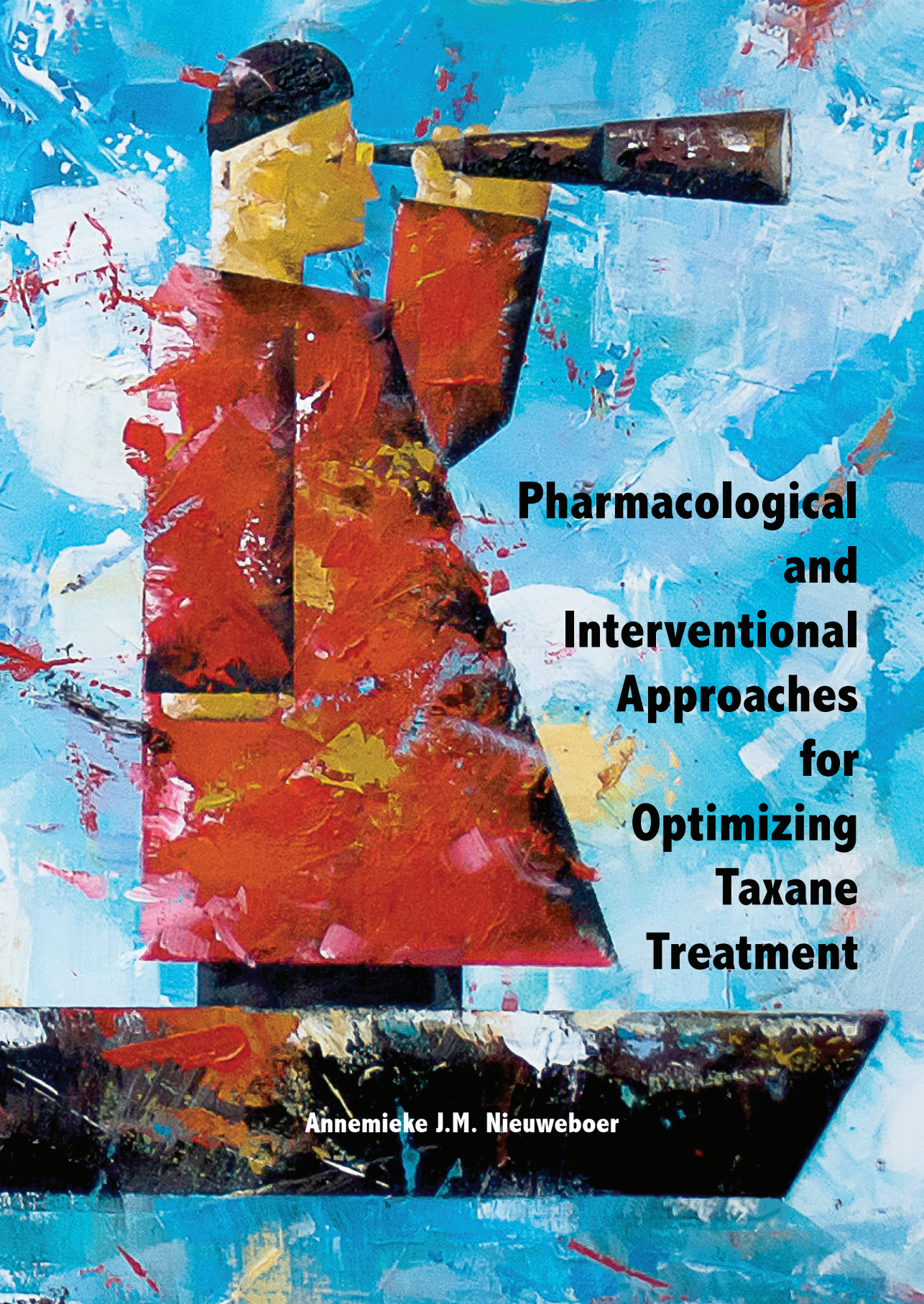


Pharmacological and Interventional Approaches for Optimizing Taxane Treatment

Annemieke J.M. Nieuweboer

Pharmacological and Interventional Approaches for Optimizing Taxane Treatment

Annemieke J.M. Nieuweboer

Colofon

The painting on the cover is made by: Dorus Brekelmans
Lay-out by: Roderick van Klink
Printing by: Ridderprint B.V.
ISBN: 978-94-6299-366-2

De uitgave van dit proefschrift is mede tot stand gekomen met een financiële bijdrage van: Sanofi Genzyme, Teva Nederland, Bayer B.V. en Pfizer.

Pharmacological and Interventional Approaches for Optimizing Taxane Treatment

Farmacologische en interventionele methoden voor het optimaliseren van anti-kanker behandeling met taxanen

Proefschrift

ter verkrijging van de graad van doctor aan de
Erasmus Universiteit Rotterdam
op gezag van de
rector magnificus

prof.dr. H.A.P. Pols

en volgens besluit van het College voor Promoties.
De openbare verdediging zal plaatsvinden op

vrijdag 9 september 2016 om 13.30 uur

door

Annemieke J.M. Nieuweboer

geboren te Eindhoven

Erasmus University Rotterdam

PROMOTIECOMMISSIE

Promotoren:	Prof.dr. A.H.J. Mathijssen
	Prof.dr. R. de Wit
Overige leden:	Prof.dr. T. van Gelder
	Prof.dr. R.H.N. van Schaik
	Prof.dr. A. Sparreboom

“Talent is universally divided, but opportunity is not.”
Hillary Clinton

Voor mijn ouders

CONTENTS

Chapter 1	Introduction	9
Part I: Paclitaxel		
Chapter 2	Influence of drug formulation on OATP1B-mediated transport of paclitaxel.	15
Chapter 3	Predicting paclitaxel-induced neutropenia using the DMET platform.	35
Part II: Docetaxel		
Chapter 4	Role of genetic variation in docetaxel-induced neutropenia and pharmacokinetics.	53
Chapter 5	Inter-patient variability in docetaxel pharmacokinetics: A review.	71
Part III: Cabazitaxel		
Chapter 6	The influence of prior novel androgen receptor targeted therapy on the efficacy of cabazitaxel in men with metastatic castration-resistant prostate cancer.	95
Chapter 7	Quantification of cabazitaxel in human plasma by liquid chromatography/triple-quadrupole mass spectrometry: a practical solution for non-specific binding.	111
Chapter 8	Effects of budesonide on cabazitaxel-induced diarrhea in a randomized open-label multicenter phase II study: CABARESC	125
Chapter 9	Summary	143
Appendices		149
Appendix I.	Nederlandse samenvatting	
Appendix II.	Dankwoord	
Appendix III.	Curriculum Vitae	
Appendix IV.	Publications	
Appendix V.	PhD Portfolio	

Chapter 1

Introduction

INTRODUCTION

Cancer and taxanes

Globally, 14.1 million people received a cancer diagnosis in 2012 (1). Cancer of the breast, prostate, lung, stomach and colorectal region are the most common malignancies in the Western world (1). For the treatment of these tumor types, the antineoplastic agents paclitaxel, docetaxel and, more recently, cabazitaxel are used. The collective term of these drugs is 'Taxanes', as paclitaxel and docetaxel were derived from different species of the pacific yew tree (*Taxus brevifolia* and *Taxus baccata*, respectively). Cabazitaxel is a semisynthetic dimethyloxy offshoot of docetaxel, with a change in the capacity to cross the blood-brain barrier and in the affinity for p-glycoprotein (2). Paclitaxel, docetaxel and cabazitaxel thus have common ground, despite their differences in chemical structure and their solvent.

Despite acceptable response rates, the use of taxanes has its downsides. Currently, the dosages of these drugs are determined using body surface area (3). However, taxanes have a small therapeutic window and a large inter-patient variability (IIV) in pharmacokinetics (PK) and toxicity (4). Patients are consequently at risk of receiving a higher or lower dose than optimally required. Because the PK of taxanes are associated with the toxicity and efficacy of these anti-cancer drugs, patients are at risk of insufficient efficacy and disproportionate toxicity (4,5). Toxicities as neutropenia, diarrhea, neurotoxicity, nausea and fatigue are common in taxane-treated patients. Hence, it is clinically relevant to be able to predict the exposure, efficacy and toxicity of taxanes, and to understand the corresponding mechanisms of factors influencing these endpoints. To improve current abilities in the management of taxane therapy, this thesis focuses on this topic.

Effects of drug solvents on taxane pharmacokinetics

Because solubilizing taxanes in water is problematic, these drugs are formulated using the solubilizing agents Kolliphor EL (KEL; or Cremophor EL (CrEL)) and Polysorbate 80 (PS80). Though, both solvents were seen to affect the drug disposition of paclitaxel and docetaxel, respectively (6). This results in a great increase in systemic exposure to mainly paclitaxel. It was already shown that KEL inhibited the hepatic elimination of paclitaxel (7). As it is known that Organic Anion Transporting Polypeptide (OATP) 1B-type transporters are responsible for the hepatic uptake of taxanes (8), we hypothesized that CrEL and PS80 affected the uptake of taxanes by these transporters. These experiments and results are demonstrated in **Chapter 2** of this thesis.

Effects of genetic variation on taxane pharmacokinetics and toxicity

Genetic variation has also been studied as a potential factor influencing taxane PK and pharmacodynamics (PD) (9). This was most often done using a candidate gene approach. However, published results were contradictory. Genes involved in drug transport and metabolism could potentially affect taxane PK and PD. In this thesis, we used the Drug Metabolizing Enzymes and Transporters platform, containing 1936 single nucleotide polymorphisms (SNPs) in 225 genes involved

in drug metabolism and transport to test this hypothesis. Using this platform, we aimed for the development of a genetic model predictive of paclitaxel and docetaxel-induced neutropenia and docetaxel clearance. This execution is described in **Chapters 3** and **4**.

Factors influencing docetaxel pharmacokinetics

As mentioned, a correlation exists between docetaxel exposure and efficacy. However, multiple factors hypothetically influence the pharmacokinetics of docetaxel considering inter-individual variability is high. In **Chapter 5**, we reviewed the literature and focused on factors influencing the pharmacokinetics of docetaxel such as gender, hormonal status and co-medication.

Effects of other drugs on cabazitaxel pharmacokinetics and toxicity

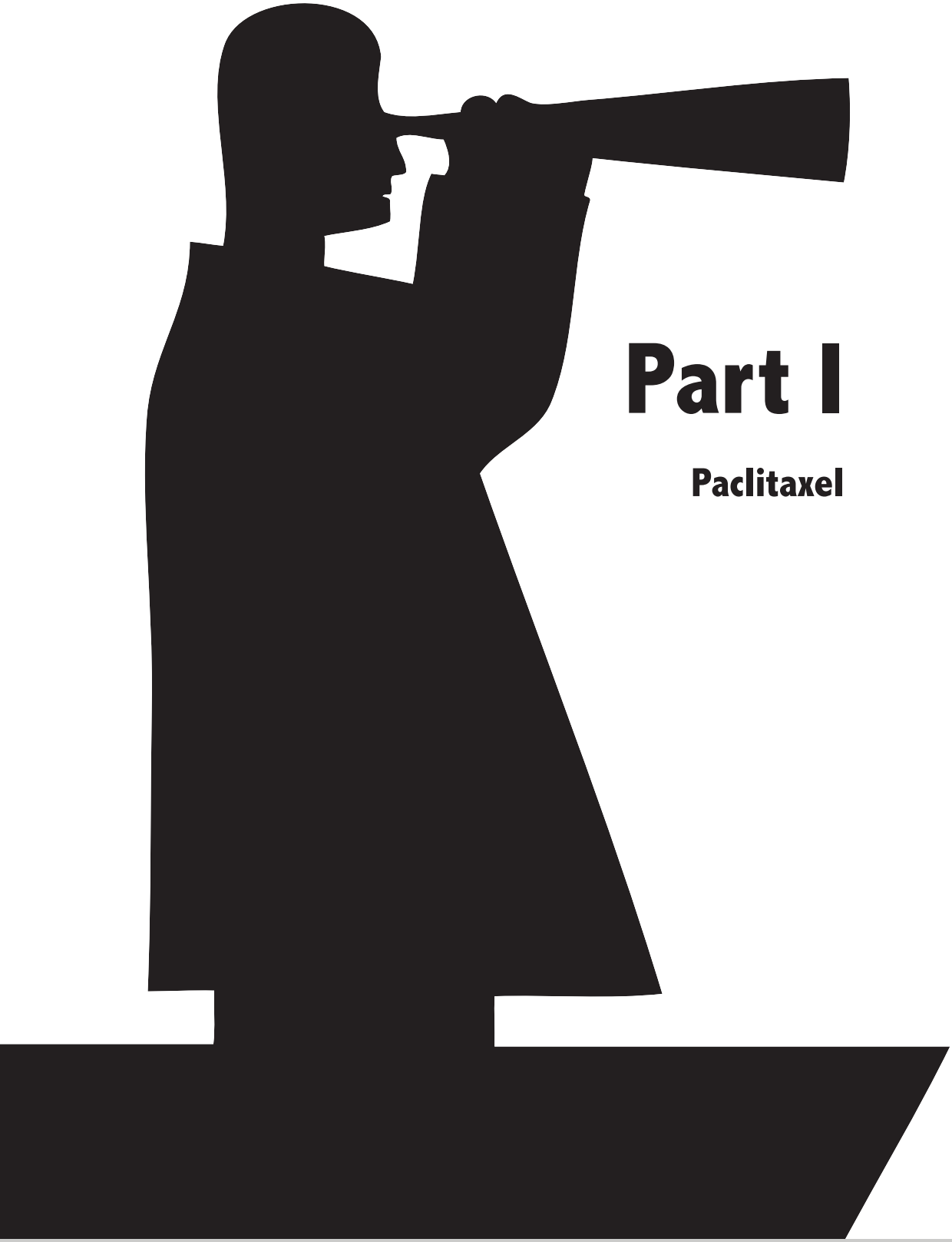
Docetaxel is among other indications used for the first line treatment of patients with metastatic castration resistant prostate cancer (mCRPC). A second line substitute that prolonged overall survival had been lacking for many years until cabazitaxel was registered in 2011 (10). Soon after its registration, novel androgen receptor targeted drugs (ART) abiraterone and enzalutamide also became available for the treatment of mCRPC. These drugs were demonstrated to be effective in both the pre-docetaxel and post-docetaxel setting (11-14). However, the efficacy of docetaxel in mCRPC patients was diminished after treatment with abiraterone (15). Preclinical studies demonstrated cross-resistance between enzalutamide and docetaxel in vivo, possibly explaining the reduced effect of docetaxel after abiraterone. We have investigated the effects of preceding ART on the efficacy of cabazitaxel in **Chapter 6**.

As mentioned, cabazitaxel is able to prolong survival of patients suffering from metastatic castration resistance prostate cancer. Unfortunately, in the registration study, half of the cabazitaxel-treated patients suffered from cabazitaxel-induced diarrhea (10). Six percent of these patients suffered from severe diarrhea, resulting in hospital admittance (10). To decrease the incidence and severity of cabazitaxel-induced diarrhea we developed a large randomized controlled trial looking into the hypothesis that the anti-inflammatory drug budesonide would be able to decrease the incidence and severity of cabazitaxel-induced diarrhea (**Chapter 8**). To investigate whether budesonide would influence the pharmacokinetics of cabazitaxel, we first performed a pharmacokinetic study preceding the clinical trial as described in Chapter 8. In **Chapter 7**, we demonstrate a validated assay that is able to determine cabazitaxel concentrations in human plasma.

The goal of this thesis was to improve current abilities in the management of taxane therapy using interventional and pharmacological approaches. Thereby aiming for providing patients with an optimally effective therapy, while reducing toxicity. This thesis sheds new light on factors influencing the pharmacokinetics and pharmacodynamics of the taxanes paclitaxel, docetaxel and cabazitaxel, further optimizing taxane therapy.

REFERENCES

1. J. Ferlay, I. Soerjomataram, R. Dikshit et al. Cancer incidence and mortality worldwide: sources, methods and major patterns in GLOBOCAN 2012. Int J Cancer. 2015;136:E359-86.
2. J.A. Yared, K.H. Tkaczuk et al. Update on taxane development: new analogs and new formulations. Drug Des Devel Ther. 2012;6:371-84.
3. H.Gurney. Dose calculation of anticancer drugs: a review of the current practice and introduction of an alternative. J Clin Oncol. 1996;14:2590-611.
4. P. Gerritsen-van Schieveen, B. Royer et al. Level of evidence for therapeutic drug monitoring of taxanes. Fundamental & Clinical Pharmacology 2011;25:414–424.
5. R. Bruno, N. Vivier, C. Veyrat-Follet, et al. Population pharmacokinetics and pharmacokinetic-pharmacodynamic relationships for docetaxel. Invest New Drugs. 2001;19:163-9.
6. K.L. Hennenfent, R. Govindan et al. Novel formulations of taxanes: a review. Old wine in a new bottle? Ann Oncol 2006;17:735–49.
7. A. Sparreboom, O. van Tellingen, W.J. Nooijen et al. Nonlinear pharmacokinetics of paclitaxel in mice results from the pharmaceutical vehicle Cremophor EL. Cancer Res 1996;56:2112–5.
8. N.F. Smith, M.R. Acharya, N. Desai et al. Identification of OATP1B3 as a high-affinity hepatocellular transporter of paclitaxel. Cancer Biol Ther 2005;4(8):815-818.
9. R.S. Jabir, R. Naidu, M.A. Annuar et al. Pharmacogenetics of taxanes: impact of gene polymorphisms of drug transporters on pharmacokinetics and toxicity. Pharmacogenomics 2012;13(16):1979–1988.
10. J.S. de Bono, S. Oudard, M. Ozguroglu et al. Prenisone plus cabazitaxel or mitoxantrone for metastatic castration-resistant prostate cancer progressing after docetaxel treatment: a randomised open-label trial. Lancet. 2010;376(9747):1147-1154.
11. J.S. de Bono, C.J. Logothetis, A. Molina et al. Abiraterone and increased survival in metastatic prostate cancer. N Engl J Med. 2011;364:1995-2005.
12. H.I. Scher, K. Fizazi, F. Saad et al. Increased survival with enzalutamide in prostate cancer after chemotherapy. N Engl J Med. 2012;367:1187-97.
13. C.J. Ryan, M.R. Smith, J.S. de Bono et al. Abiraterone in metastatic prostate cancer without previous chemotherapy. N Engl J Med. 2013;368:138-48.
14. T.M. Beer, A.J. Armstrong, D.E. Rathkopf et al. Enzalutamide in Metastatic Prostate Cancer before Chemotherapy. N Engl J Med. 2014;371(5):424-33.
15. J. Mezynski, C. Pezaro, D. Bianchini et al. Antitumour activity of docetaxel following treatment with the CYP17A1 inhibitor abiraterone: clinical evidence for cross-resistance? Ann Oncol. 2012;23:2943-7.



Part I

Paclitaxel

Chapter 2

Influence of Drug Formulation on OATP1B-Mediated Transport of Paclitaxel

A.J.M. Nieuweboer, S. Hu, C. Gui, B. Hagenbuch, I.M. Ghobadi Moghaddam-Helmantel, A.A. Gibson, P. de Bruijn, A.H.J. Mathijssen and A. Sparreboom.

Cancer Research. 2014 Jun 1;74(11):3137-45.

ABSTRACT

Taxane antineoplastic agents are extensively taken up into hepatocytes by OATP1B-type transporters before metabolism and excretion. Because the biodistributional properties imposed upon these agents by different solubilizers drive clinically important pharmacodynamic endpoints, we tested the hypothesis that the *in vitro* and *in vivo* interaction of taxanes with OATP1B transporters is affected by the choice of drug delivery system. Transport of paclitaxel, docetaxel, and cabazitaxel was studied *in vitro* using various cell lines transfected with OATP1B1, OATP1B3, or the rodent equivalent OATP1B2. Pharmacokinetic studies were done in wild-type and OATP1B2-knockout mice in the presence or absence of polysorbate 80 (PS80) or Kolliphor EL (formerly Cremophor EL; CrEL). Paclitaxel and docetaxel, but not cabazitaxel, were transported substrates of OATP1B1, OATP1B3, and OATP1B2, and these *in vitro* transport processes were strongly reduced in the presence of clinically relevant concentrations of PS80 and CrEL. When paclitaxel was administered without any solubilizers, deficiency of OATP1B2 in mice was associated with a significantly decreased systemic clearance because of a liver distribution defect ($P = 0.000484$). However, this genotype dependence of paclitaxel clearance was masked in the presence of PS80 or CrEL because of significant inhibition of OATP1B2-mediated hepatocellular uptake of the drug ($P < 0.05$). Our findings confirm the importance of OATP1B-type transporters in the hepatic elimination of taxanes and indicate that this process can be inhibited by PS80 and CrEL. These results suggest that the likelihood of drug-drug interactions mediated by these transporters is strongly dependent on the selected taxane solubilizer.

INTRODUCTION

The nonionic surfactants Kolliphor EL (formerly Cremophor EL; CrEL) and polysorbate 80 (Tween 80; PS80) are widely used to solubilize drugs, including the taxane-based antineoplastic agents paclitaxel (1), docetaxel (2), and cabazitaxel (3). A wealth of experimental data has indicated that these solubilizers are biologically and pharmacologically active compounds, and their use as drug formulation vehicles has been implicated in clinically important toxic side effects such as acute hypersensitivity reactions (4). CrEL and PS80 have also been found to influence the disposition of solubilized drugs administered intravenously (5). This is particularly striking in the case of paclitaxel formulated in CrEL, where the overall resulting effect is a highly increased systemic exposure to paclitaxel (6), which is dependent on the dose and time-varying blood concentrations of the solubilizer (7). Kinetic experiments (8, 9) and model-based predictions (10, 11) have revealed that paclitaxel undergoes reversible partitioning into a circulating surfactant microemulsion that acts as a nano-sink and reduces the fraction of free drug available for extravascular distribution.

In line with these predictions, it was demonstrated that CrEL can inhibit the hepatic elimination of paclitaxel in the isolated perfused rat liver, the main organ of elimination (12), by preventing the drug from reaching sites of metabolism and excretion (13). This process is believed to be primarily mediated by the organic anion-transporting polypeptides OATP1B3 (in humans; refs 14 and 15) and OATP1B2 (in rodents; refs 16 and 17), which are uptake transporters localized to the basolateral membrane of hepatocytes (18). To add further to the complexity of the carrier-mediated disposition properties of paclitaxel, CrEL was found to strongly inhibit the uptake of OATP1B3 substrates *in vitro* into cells overexpressing the transporter (19). However, the mechanistic basis underlying this observation, as well as its *in vivo* relevance, remains unclear.

Because the biodistributional properties imposed upon taxanes by different solubilizers drive clinically-important pharmacodynamic endpoints that further depend, at least in part, on whether or not the pharmacokinetics of carrier-released (free) drug is formulation-dependent (10), we here tested the hypothesis that the *in vitro* and *in vivo* interaction of paclitaxel with OATP1B-type transporters is affected by the choice of a particular drug delivery system.

MATERIALS AND METHODS

In vitro transport studies

Xenopus laevis oocytes injected with OATP1B1, OATP1B3, or rat OATP1B2 cRNA along with water-injected controls were obtained from BD Biosciences. The transporter nomenclature used throughout is based on recent recommendations proposed by Hagenbuch and Stieger (20). The transporter-expressing oocytes were functionally characterized by assessing the uptake of estrone-3-sulfate (2 µmol/L) by OATP1B1 and OATP1B2, and of estradiol-17β-D-glucuronide (2 µmol/L) by OATP1B3. Human embryonal kidney (HEK293) cells overexpressing OATP1B1, OATP1B3, or OATP1B2 have been described previously (21). The Chinese hamster ovary (CHO) cells expressing OATP1B1 or OATP1B3 were generated as follows. The open reading frames of the two transporters (22) were PCR amplified in order to introduce a 6-His tag at the C-terminal end. The amplicons were ligated into pcDNA5/FRT, and after verifying the sequences, Flp-In-CHO cells were transfected with the plasmids in the presence of pOG44 following the manufacturer's protocols (Life Technologies). Cells were selected with hygromycin B (600 µg/mL) and single clones were isolated by limited dilution. Overexpression of transporters was confirmed using TaqMan probes (Applied Biosystems). The cell culture conditions and details of accumulation experiments for [^{3}H]paclitaxel (specific activity, 25.6 Ci/mmol; Vitrax) and [^{3}H]docetaxel (specific activity, 60.0 Ci/mmol; American RadioChemicals) were described earlier (21). Radioactivity was quantified by liquid scintillation counting using a LS 6500 Counter (Beckman).

Intracellular concentrations of cabazitaxel were measured by liquid chromatography-tandem mass spectrometry (LC/MS-MS), as described (23). Total protein was measured using a Pierce BCA Protein Assay Kit (Thermo Scientific) and quantified using a Biotek µQuant microplate spectrophotometer. Drug uptake results were normalized to total protein content and then to data obtained in cells carrying an empty vector plasmid, which was set to 100%.

Animal experiments

Wild-type and OATP1B2 knockout [OATP1B2(-/-)] mice, both on a DBA/1lacJ background, between 8 and 12 weeks of age, were housed in a temperature-controlled environment with a 12-hour light cycle. Experiments were approved by the Institutional Animal Care and Use Committee of St. Jude Children's Research Hospital. All mice received a standard diet and water *ad libitum* and were fasted 3 hours before drug administration. Paclitaxel was formulated in CrEL-ethanol (1:1, v/v; Taxol; Bristol-Myers Squibb), PS80-ethanol (1:1, v/v) or as an albumin-bound nanoparticle (nab-paclitaxel; ABI-007; Abraxane; Celgene) without either CrEL or PS80. These solutions were diluted in normal saline and administered by intravenous bolus in the tail vain at a dose of 10 mg/kg. Docetaxel (Taxotere; Sanofi-Aventis) and cabazitaxel (Jevtana; Sanofi-Aventis) were formulated in PS80-ethanol (1:1, v/v), diluted in normal saline (docetaxel) or 5% dextrose in water (cabazitaxel), and then administered by tail vein injection at a dose of 10 mg/kg.

In all experiments, at select time points after drug administration, blood samples (30 µL each) were taken from individual mice at 3.5, 7.5, and 15 min from the submandibular vein using a lancet, and at 30 and 60 min from the retro-orbital venous plexus using a capillary. A final blood

draw was obtained at 120 minutes by a cardiac puncture using a syringe and needle. Isoflurane was used as an anesthetic. The total blood volume collected during the procedure from each mouse was 150 µL. All blood samples were centrifuged at 1,500 × g for 5 minutes, and plasma was separated and stored at -80°C until analysis. Livers, kidneys, and small intestines were collected from the same animals at 120 min. The lumen of small intestines were purged using saline to remove remaining content. A separate group of mice was euthanized by CO_2 asphyxiation at 5 min and the same tissues were immediately collected and flash-frozen on dry ice. To prevent continuing metabolic activity, liver tissues were snap-frozen using liquid nitrogen. All tissue specimens were stored at -80°C until further processing, as described (21). Samples were analyzed by LC/MS-MS (see **Supplementary Methods** for details; ref 24), and noncompartmental parameters were calculated using WinNonlin 6.2 software (Pharsight). Concentrations in tissue were corrected for contaminating plasma (25).

Statistical considerations

All data are presented as mean ± SD. Statistical analyses were done using SPSS version 20 (SPSS), and $P < 0.05$ was regarded as statistically significant. Student's t-test (2 groups) or one-way ANOVA (>2 groups) was used for statistical analysis on *in vitro* uptake data, plasma pharmacokinetic parameters, and tissue concentrations.

RESULTS

Paclitaxel transport *in vitro*.

Although previous *in vitro* studies have consistently identified paclitaxel as a potent inhibitor of OATP1B1- (22, 26) and OATP1B3-mediated transport (22, 27, 28), the actual transport of paclitaxel itself by these transporters remains controversial (14, 28-30). The presented methodological details were selected on the basis of existing literature in order to clarify the reported discrepancies in taxane transport by OATPS. In line with these conflicting data, we found that the interaction of paclitaxel with human OATP1B1 and OATP1B3 was dependent on cell context, with both proteins being able to take up paclitaxel when expressed in HEK293 cells or CHO cells, but no noticeable transport occurring by OATP1B1 expressed in *X. laevis* oocytes (Fig. 1A). Paclitaxel was also found to be transported into cells expressing mouse OATP1B2 or rat OATP1B2 (Fig. 1B), as predicted from studies done in isolated rat hepatocytes (31).

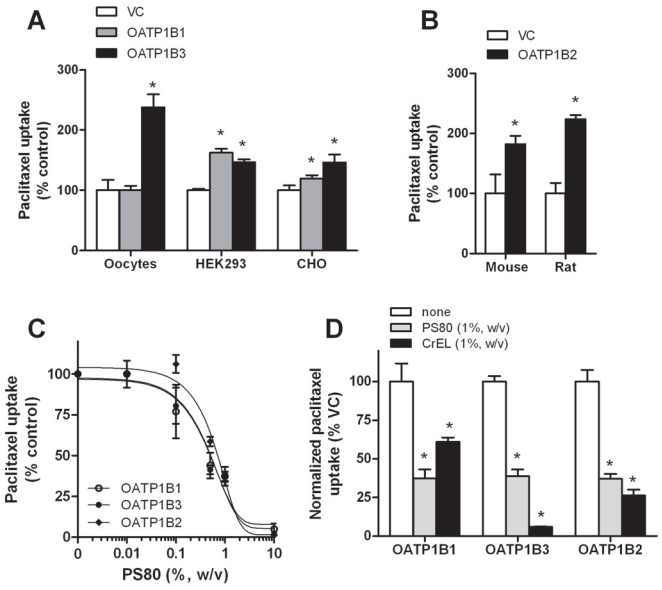


Figure 1. *In vitro* transport studies of paclitaxel. **A**, the uptake of paclitaxel by human OATP1B1 and human OATP1B3 was assessed in OATP1B1 and OATP1B3-injected *X. laevis* oocytes (paclitaxel concentration, 2 µmol/L; 30 minute incubations), HEK293 cells (2 µmol/L; 30 minutes) and CHO cells (1 µmol/L; 2 minutes) and their respective vector controls (VC). **B**, the uptake of paclitaxel by mouse OATP1B2 transfected in HEK293 cells (0.1 µmol/L; 15 minutes) or rat OATP1B2 transfected in *Xenopus laevis* oocytes (2 µmol/L; 30 minutes). **C**, the uptake of paclitaxel by human OATP1B1, human OATP1B3 in CHO cells (1 µmol; 2 minutes), or mouse OATP1B2 in HEK293 cells (0.1 µmol/L; 15 minutes) was evaluated in the presence of different concentrations of PS80. **D**, the uptake of paclitaxel by human OATP1B1, human OATP1B3 in CHO cells (1 µmol/L; 2 minutes), or mouse OATP1B2 in HEK293 cells (0.1 µmol/L; 15 minutes) was evaluated in the presence of either CrEL (1%, w/v) or PS80 (1%, w/v), with results normalized to transporter-specific uptake. Data are presented as the mean percentage of uptake values in the VC cells (bars) for 4 to 16 observations per condition, along with SD (error bars). *, a statistically significant difference compared with VC ($P < 0.05$).

In the absence of solubilizers, the transport of paclitaxel into CHO cells transfected with OATP1B1 or OATP1B3 was found to be time-dependent and saturable (Fig. 2) with a Michaelis-Menten constant (*K*m) of 0.408 ± 0.190 µmol/L and 2.36 ± 1.40 µmol/L, respectively, and a maximum velocity (*V*max) of 22.1 ± 3.20 pmol/mg/min and 14.2 ± 5.26 pmol/mg/min, respectively, which values are similar to those reported previously for docetaxel (21).

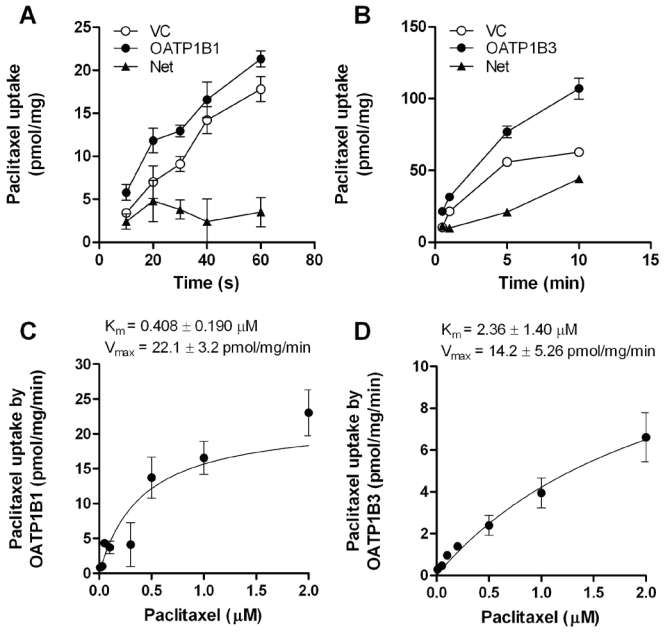


Figure 2. *In vitro* transport of paclitaxel by OATP1B1 and OATP1B3. (**A**) Time-dependence of transport by OATP1B1 (**A**) and OATP1B3 (**B**) and concentration-dependence of transport by OATP1B1 (**C**) and OATP1B3 (**D**) was evaluated in transfected CHO cells, where data represent the mean of 2 to 4 independent experiments in cells stably expressing OATP1B1, OATP1B3, or in control cells (VC), and the net difference. In panels (**A**) and (**B**), the paclitaxel concentration was 0.5 μmol. In panels (**C**) and (**D**), the incubation time was 1 min. Km denotes the Michaelis-Menten constant, and Vmax the maximum velocity. Error bars represent the standard error. Data in panels (**C**) and (**D**) were previously reported in De Graan et al. (21).

Next, we evaluated the ability of CrEL and PS80 to inhibit the intracellular accumulation of paclitaxel into CHO cells overexpressing human OATP1B1, human OATP1B3, or mouse OATP1B2. Recently, it was reported that the effect of CrEL on the uptake of several substrates by OATP1B2, OATP1B1, or OATP1B3 was dose dependent (19). Similar to these findings, we found that PS80 also inhibited paclitaxel uptake in a concentration-dependent manner (Fig. 1C), which is in line with our observation that PS80 also affects the transporter-mediated uptake of docetaxel (21). The mechanism by which CrEL and PS80 inhibit OATP1B-type transporters is unclear and requires additional investigation. After correcting for non-specific inhibition of paclitaxel uptake occurring in cells transfected with an empty expression plasmid because of drug trapping in solubilizer micelles (Fig. 3), CrEL directly inhibited the transporters in decreasing order of potency OATP1B3 > OATP1B2 > OATP1B1, whereas PS80 inhibited all transporters to a similar extent (Fig. 1D).

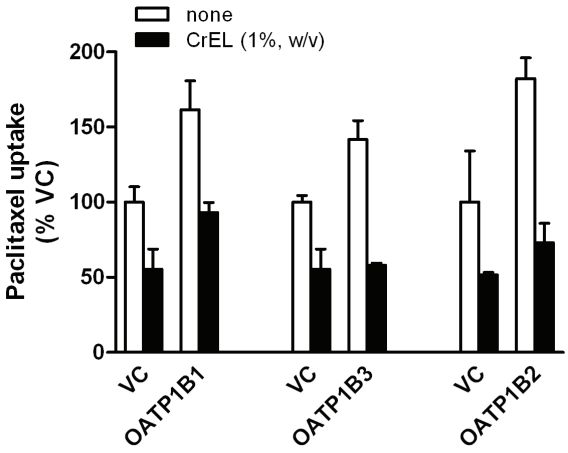


Figure 3. The uptake of paclitaxel by human OATP1B1, human OATP1B3 in CHO cells (1 µmol; 2-min), or mouse OATP1B2 in HEK293 cells (0.1 µmol; 15-min) evaluated in the absence (none) or presence of 1% (w/v) Cremophor EL (CrEL). Data are presented relative to the mean percentage of uptake values in the vector control (VC) cells (bars) in the absence of CrEL for 4-16 observations per condition, along with SD (error bars).

Paclitaxel tissue distribution studies *in vivo.*

We next evaluated the possible importance of these transporters for paclitaxel disposition in mice with a genetic deletion of OATP1B2 [OATP1B2(-/-) mice]. Early after administration (5 minutes) of paclitaxel as an albumin-bound nanoparticle (nab-paclitaxel) in the absence of a solubilizer, uptake into the liver was dramatically decreased in OATP1B2(-/-) mice (Fig. 4A). This finding suggests that immediately after infusion paclitaxel uptake into the liver is mainly transporter-mediated, and is consistent with the notion that the amorphous nab-paclitaxel nanoparticles rapidly dissolve into soluble albumin-paclitaxel complexes with a size similar to that of native albumin, with no nanoparticles detected at any time point post infusion (32). Therefore, phagocytosis-based uptake mechanisms in the liver involving the reticulo-endothelial system, which are relevant to the biodistribution of paclitaxel nanoparticles that remain stable in the circulation (33), are not contributing in the case of nab-paclitaxel.

As predicted based on the *in vitro* inhibition data, the differences in uptake of paclitaxel into the liver between wild-type and OATP1B2(-/-) mice were much less pronounced in the presence of PS80 or CrEL. In particular, the liver uptake in wild-type mice receiving paclitaxel formulated in PS80 was similar to that observed in OATP1B2(-/-) mice receiving the drug without solubilizers, whereas uptake was further reduced by about two-fold in the presence of CrEL (Fig. 4A). Over time, the formulation-dependent differences in liver uptake normalized to control levels for the PS80 group (Fig. 4B), but remained noticeably reduced in the presence of CrEL. This is consistent with the fact that PS80 is very rapidly cleared, with plasma levels becoming undetectable within 15 minutes after intravenous administration (34), whereas the half-life of CrEL amounts to >17 hours (6).

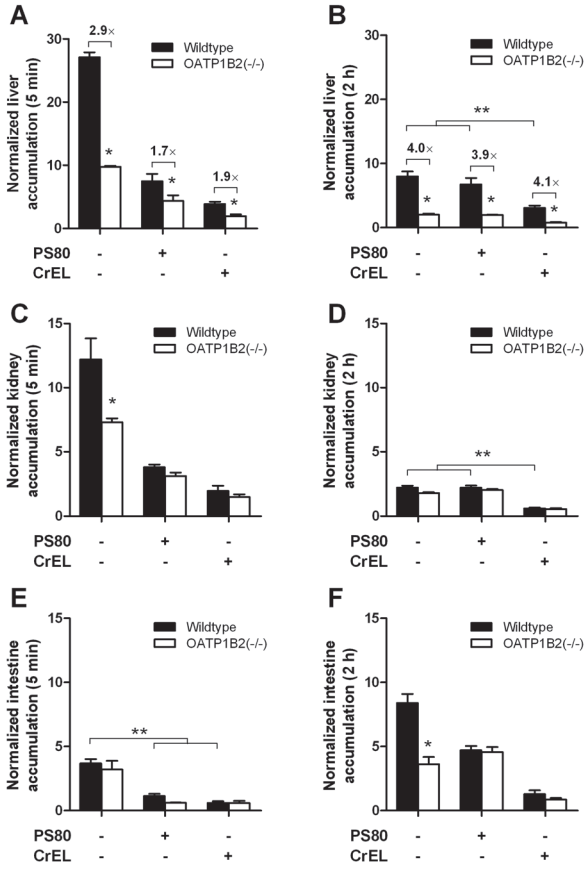


Figure 4. Influence of paclitaxel formulation and OATP1B2-deficiency on paclitaxel distribution. Paclitaxel was formulated as nab-paclitaxel in the absence of PS80 or CrEL, PS80, or CrEL (see Materials and Methods for details), and total paclitaxel levels were determined in wild-type and OATP1B2(-/-) mice at 5 minutes and 2 hours after intravenous injection in liver (**A and B**), kidney (**C and D**), and intestine (**E and F**). Tissue levels were normalized to the corresponding plasma concentration (5 minute data) or the AUC from time 0 to 2 hours (2 hour data). Data are presented as the mean (bars) of four observations per condition per time point, along with SD (error bars). *, a statistically significant difference compared with the corresponding wild-type group ($P < 0.05$); **, a statistically significant difference compared with the other formulations ($P < 0.05$).

Interestingly, a similar solubilizer-, time-, and genotype-dependent distribution of paclitaxel was observed for uptake into kidney (Fig. 4C and D) and intestine (Fig. 4E and F). The significant reduction of paclitaxel administered without a solubilizer (i.e., nab-paclitaxel) in the kidneys of OATP1B2(-/-) mice at 5 minutes after drug administration is possibly the result of low expression of OATP1B2 in renal cells of mice (35), and a similar phenotype has been reported previously for the OATP1B2 substrate, hydroxyurea (36). The concentrations of paclitaxel in the intestine after the administration of nab-paclitaxel were not dependent on OATP1B2 genotype at the early time point, where appearance of the drug likely reflects direct intestinal secretion (37). At the 2-hour time point, the higher levels in the intestine are presumably the result of hepatobiliary secretion becoming an increasingly dominant contributor to the elimination of paclitaxel.

Effects of formulation and transport on taxane clearance *in vivo.*

As anticipated from the tissue distribution findings, the plasma concentration-time profiles of paclitaxel were inversely related to corresponding drug levels in liver, and concentrations in plasma were consistently higher by 5-to 7-fold for the CrEL-containing formulation compared with the other groups (Fig. 5A and B). The notion that the slow clearance of paclitaxel administered in CrEL is due to a distribution defect rather than an event occurring in the terminal elimination phase is also consistent with the observed terminal half-lives of paclitaxel that were not significantly dependent on the formulation or genotype (Table 1).

Table 1. Plasma pharmacokinetic parameters of paclitaxel (10 mg/kg) in mice using different formulations.*

Solubilizer	Mouse genotype	Cmax (μg/ml)	AUC (μg×h/ml)	CL (L/h/kg)	T1/2 (h)
None	OATP1B2(-/-)	9.37 ± 3.46	8.51 ± 4.32	1.36 ± 0.493	1.64 ± 0.668
None	Wildtype	4.61 ± 0.608	3.44 ± 0.394	2.38 ± 1.26	1.70 ± 0.427
PS80	OATP1B2(-/-)	14.0 ± 3.12	6.93 ± 0.832	1.26 ± 0.454	1.06 ± 0.265
PS80	Wildtype	12.4 ± 3.53	4.83 ± 0.761	1.94 ± 0.479	1.07 ± 0.230
CrEL	OATP1B2(-/-)	36.9 ± 10.9	25.6 ± 5.23	0.406 ± 0.0962	1.39 ± 0.294
CrEL	Wildtype	35.8 ± 8.60	18.8 ± 4.90	0.565 ± 0.174	1.30 ± 0.365

* Data represent mean ± SD. Abbreviations: PS80, polysorbate 80; CrEL, Kolliphor EL (formerly Cremophor EL); AUC, area under the curve; CL, systemic clearance; T1/2, half-life of the terminal phase; Cmax, peak plasma concentration. Note: 'None' refers to nab-paclitaxel.

After the administration of nab-paclitaxel, the peak plasma concentration of paclitaxel was significantly increased in OATP1B2(-/-) mice, and this genotype dependence was nullified in the presence of PS80 or CrEL (Fig. 5C). Based on total area under the curve (AUC), a significant but blunted influence of genotype was still noted for PS80-based formulation, but not for CrEL (Fig. 5D). Because the same PS80 formulation is used in the clinical preparation of docetaxel and cabazitaxel, we also evaluated the comparative plasma pharmacokinetic properties of the two other approved taxanes in the same mouse model. Studies performed in transfected cells confirmed that, like paclitaxel, docetaxel is a transported substrate of OATP1B2 (21), but this was not noted under the experimental conditions applied for cabazitaxel (Fig. 6A). The lack of transport of cabazitaxel by OATP1B2 is somewhat surprising considering its structural similarity with docetaxel, both having a 10-deacetylbaccatin III backbone (3), and because cabazitaxel has been reported to inhibit OATP1B1 and OATP1B3, albeit at relatively high concentrations (Jevtana prescribing information, see: http:// products .sanofi.us/jevtana/ jevtana.pdf). As expected based on the *in vitro* studies, the AUC (Fig. 6B) and plasma levels at the time points evaluated were significantly increased in the absence of OATP1B2 for paclitaxel (formulated in PS80; Fig. 6C) and docetaxel (Fig. 6D), but not for cabazitaxel (Fig. 6E).

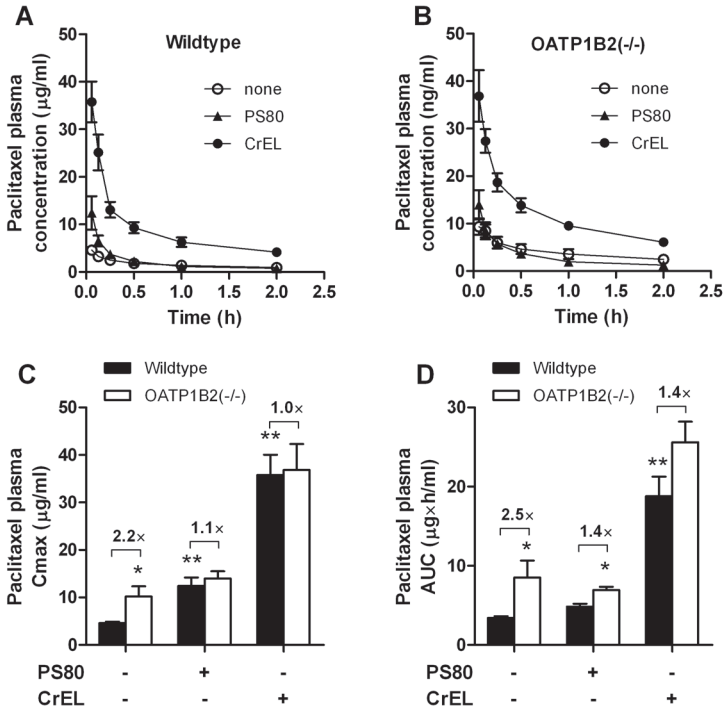


Figure 5. Influence of formulation and OATP1B2-deficiency on paclitaxel plasma pharmacokinetics. Paclitaxel was formulated as nab-paclitaxel in the absence of PS80 or CrEL, in PS80, or in CrEL (see Materials and Methods for details), and total paclitaxel levels were determined in wildtype (**A**) and OATP1B2(-/-) mice (**B**) at in plasma samples taken at serial time points after dosing (5-120 minutes). The resulting concentration-time profiles were used to derived peak plasma concentrations (*Cmax*) (**C**) and area under the curve (AUC) (**D**). Data are presented as the mean (symbols or bars) of four observations per condition per time point, along with SD (error bars). *, a statistically significant difference compared with the corresponding wild-type group ($P < 0.05$); **, a statistically significant difference compared with the other formulations ($P < 0.05$).

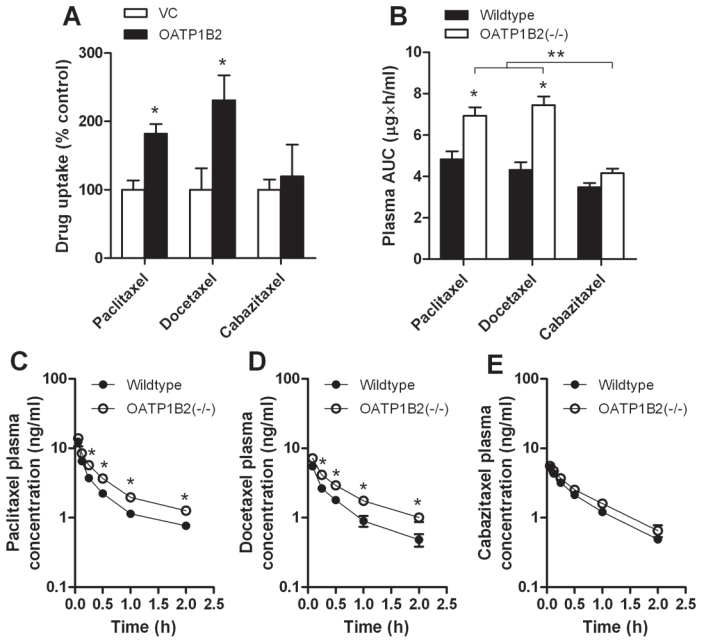


Figure 6. Comparative effects of formulation and OATP1B2-deficiency on transport of paclitaxel, docetaxel, and cabazitaxel. **A**, the uptake of paclitaxel, docetaxel, and cabazitaxel by mouse OATP1B2 was evaluated in transfected HEK293 cells (0.1 µmol/L; 15 minutes). Data are presented as the mean percentage of uptake values in the VC cells (bars) for 4 to 11 observations per condition, along with SD (error bars). *, a statistically significant difference compared with VC ($P < 0.05$). **B**, AUC of paclitaxel in wild-type and OATP1B2(-/-) mice following administration of paclitaxel, docetaxel, or cabazitaxel, all formulated in PS80 (see Materials and Methods for details), along with the corresponding plasma concentration-time profiles for paclitaxel (**C**), docetaxel (**D**), and cabazitaxel (**E**). Data are presented as the mean (bars or symbols) of four observations per condition per time point, along with SD (error bars). *, a statistically significant difference compared with the corresponding wildtype group ($P < 0.05$); **, a statistically significant difference compared with the other formulations ($P < 0.05$).

DISCUSSION

This study adds to a growing body of knowledge that solute carriers belonging to the OATP1B family can have a dramatic impact on the hepatocellular accumulation and systemic clearance of structurally diverse anticancer drugs. Using an array of *in vitro* transport assays, including intracellular accumulation studies in multiple-transfected model systems, paclitaxel was confirmed to be a high-affinity substrate for both OATP1B1 and OATP1B3. We found that the interaction of paclitaxel with OATP1B1 and OATP1B3 was strongly dependent on cell context, and this has obvious implications for future screening strategies aimed at identifying novel substrates for these transporters. The relatively low *K*m observed for paclitaxel transport by OATP1B1 suggests that this route of entry into hepatocytes may be saturated first, and this is consistent with results obtained in humanized transgenic mice indicating that OATP1B1 does not substantially contribute to paclitaxel transport *in vivo* (15), when administered at an intravenous dose of 10 mg/kg. Indeed, the *K*m for OATP1B1 of 0.408 µmol/L is substantially lower than the peak plasma concentration of unbound paclitaxel in

patients receiving nab-paclitaxel at the recommended dose of 260 mg/m^2 (on average, 1.50 μmol/L), but much higher than that observed for paclitaxel in CrEL at 175 mg/m^2 (on average, 0.143 μmol/L; ref. 38). This suggests that the contribution of OATP1B1 to the disposition of paclitaxel in patients is likely to be dependent on the prescribed product, in addition to the total dose and duration of infusion.

Our *in vitro* studies also confirmed that paclitaxel and docetaxel, but not cabazitaxel, are transported substrates of mouse OATP1B2 and, for paclitaxel, rat OATP1B2, the amino acid sequence of which is 81% identical to that of the murine transporter. Moreover, the rodent OATP1B2 transporters share more than 60% amino acid sequence homology to the two human isoforms, and on the basis of their shared basolateral localization in hepatocytes and overlapping substrate specificity (39), it is possible that, in the context of paclitaxel, the rodent OATP1B2 fulfills the same function in the liver as OATP1B1 and OATP1B3 in humans. Based on this premise, we evaluated the pharmacokinetic properties of paclitaxel in a mouse model with a genetic deletion of OATP1B2. One possible limitation of this model is the fact that, unlike in humans, mouse hepatocytes express multiple members of OATP1A subfamily, related transporters that can potentially provide compensatory restoration of function when OATP1B2 is lost (40). Despite this limitation, compared to wild-type mice, the systemic exposure to nab-paclitaxel, administered without PS80 or CrEL, in the OATP1B2(-/-) mice was increased by 2.5-fold. Our previously reported gene expression profiling and enzyme activity measurements in liver samples exclude alterations in alternate transport mechanisms or metabolic pathways as a likely cause of the delayed clearance phenotype in OATP1B2(-/-) mice (20). Thus, these findings suggest that OATP1B2-mediated transport of paclitaxel is an important process in the elimination of this drug in mice, depending on the solubilizer used for drug formulation.

We previously reported that the presence of PS80, the pharmaceutical vehicle used to solubilize docetaxel in clinical preparations, even in relatively low amounts, can completely nullify the genotype-dependent transport of docetaxel by OATP1B1 (21), and similar findings have been reported for CrEL, used in one of the clinical preparations of paclitaxel (19). Based on our current *in vitro* and subsequent confirmatory *in vivo* studies, it appears that the interaction of paclitaxel with OATP1B2 is strongly diminished in the presence of PS80 and CrEL in a fashion that is consistent with the known disposition properties of these respective solubilizers. It is interesting to note that a previous study demonstrated that mice deficient in all Oatp1a and Oatp1b genes display a rather modest increase (<2-fold) in concentrations of paclitaxel in plasma following intravenous administration of a PS80-based formulation that is very similar to our present findings (17). These authors speculated that the lack of differences in plasma levels of paclitaxel early after its administration (up to 3.5-minutes) may be because of saturation of Oatp1a/Oatp1b-mediated liver uptake, and that this distribution process is predominantly dependent on other uptake mechanisms. Our current findings now provide an alternative explanation, where the initially high levels of PS80 in plasma can cause both temporary partitioning into a circulating PS80 microemulsion as well as directly inhibit the transporters required for hepatocellular uptake. These two mechanisms combined likely also explain the results obtained for paclitaxel in the presence of CrEL, although here the former mechanism clearly remains the dominant contributor to the overall disposition phenotypes.

The present observation that PS80 and CrEL can directly inhibit OATP1B-type transporters suggests that the pharmacokinetic profile of carrier-released (free) paclitaxel is not formulation

independent. This finding contrasts previously made assumptions (10), and may have important ramification for a proper interpretation of the clinical pharmacology of paclitaxel. Firstly, we previously found that several common, naturally occurring genetic variants in OATP1B3 with impaired function were not associated with the pharmacokinetics of paclitaxel in a cohort of 90 patients with cancer receiving the drug in a CrEL-based formulation (16). This somewhat unexpected observation is consistent with our current findings in that the interaction of paclitaxel with OATP1B3 may be masked by CrEL irrespective of an individual's genotypic constitution. It also suggests that the impact of reduced function variants of OATP1B1 and/or OATP1B3 on the clearance of paclitaxel may be much more pronounced for CrEL-free formulations of the drug, such as nab-paclitaxel.

Secondly, it can be postulated that intrinsic physiologic and environmental variables influencing OATP1B1- or OATP1B3-mediated uptake of paclitaxel into hepatocytes may have a more profound influence on drug clearance for formulations lacking solubilizers. For example, substrates of OATP1B1 and OATP1B3 for which the liver is the main organ of elimination are highly liable to drug interactions associated with these transporters (41, 42). Although formal drug interaction studies have not been performed to date with nab-paclitaxel, our present findings strongly suggest that interactions at the level of hepatocellular uptake mechanisms would be exacerbated with a formulation like nab-paclitaxel. Conversely, CrEL may act as a perpetrator in known pharmacokinetic interactions involving other OATP1B substrates coadministered with paclitaxel in Phase I clinical trials (reviewed in ref. 4), such as etoposide (43), docetaxel (44), oxaliplatin (45), and SN-38 (46).

Thirdly, OATP1B1 is expressed at relatively high levels in tumors of the colon, endometrium, oesophagus, lung, prostate, stomach, testis, and bladder, and both OATP1B1 and OATP1B3 contribute to the *in vitro* cytotoxicity of paclitaxel in ovarian cancer cells (29). Although systemic exposure to free paclitaxel is believed to be the dominant driver of drug-induced cytotoxicity at tumor sites (10), it is conceivable that these transporters can contribute directly to tumoral uptake and that this process can be inhibited by solubilizers such as PS80 and CrEL, leading to diminished antitumor activity. This possibility would be consistent with available clinical data on the comparative efficacy of the various paclitaxel formulations (reviewed in ref.10), and with preclinical findings suggesting that (i) the absorption rate constant of paclitaxel uptake into tumors is dramatically decreased in the presence of CrEL compared with nab-paclitaxel (47), and (ii) a tumor-delivery mechanism exists for nab-paclitaxel that is independent of SPARC (48), a matricellular glycoprotein produced by tumors and/or neighbouring stroma that facilitates the intracellular accumulation of intact albumin nanoparticles (49).

Collectively, our findings demonstrate the importance of OATP1B-type solute carriers in the hepatic elimination of paclitaxel, and indicate that solubilizers used in clinical preparations of this agent can inhibit this process in a time-dependent fashion. These results offer a mechanistic basis for previously reported interrelationships of taxane disposition with PS80 and CrEL, and suggest that the likelihood of drug-drug interactions mediated by these transporters is strongly dependent on the selected paclitaxel formulation.

REFERENCES

1. Zhang Z, Mei L, Feng SS. Paclitaxel drug delivery systems. Expert Opin Drug Deliv. 2013;10:325-40.
2. Tan Q, Liu X, Fu X, Li Q, Dou J, Zhai G. Current development in nanoformulations of docetaxel. Expert Opin Drug Deliv. 2012;9:975-90.
3. Vrignaud P, Semiond D, Lejeune P, Bouchard H, Calvet L, Combeau C, et al. Preclinical antitumor activity of cabazitaxel, a semisynthetic taxane active in taxane-resistant tumors. Clin Cancer Res 2013;19:2973-83.
4. ten Tije AJ, Verweij J, Loos WJ, Sparreboom A. Pharmacological effects of formulation vehicles : implications for cancer chemotherapy. Clin Pharmacokinet. 2003;42:665-85.
5. Hennenfent KL, Govindan R. Novel formulations of taxanes: a review. Old wine in a new bottle? Ann Oncol. 2006;17:735-49.
6. Sparreboom A, van Tellingen O, Nooijen WJ, Beijnen JH. Nonlinear pharmacokinetics of paclitaxel in mice results from the pharmaceutical vehicle Cremophor EL. Cancer Res. 1996;56:2112-5.
7. van Zuylen L, Karlsson MO, Verweij J, Brouwer E, de Bruijn P, Nooter K, et al. Pharmacokinetic modeling of paclitaxel encapsulation in Cremophor EL micelles. Cancer Chemother Pharmacol. 2001;47:309-18.
8. Sparreboom A, van Zuylen L, Brouwer E, Loos WJ, de Bruijn P, Gelderblom H, et al. Cremophor EL-mediated alteration of paclitaxel distribution in human blood: clinical pharmacokinetic implications. Cancer Res. 1999;59:1454-7.
9. van Tellingen O, Huizing MT, Panday VR, Schellens JH, Nooijen WJ, Beijnen JH. Cremophor EL causes (pseudo-) non-linear pharmacokinetics of paclitaxel in patients. Br J Cancer. 1999;81:330-5.
10. Ait-Oudhia S, Straubinger RM, Mager DE. Meta-analysis of nanoparticulate paclitaxel delivery system pharmacokinetics and model prediction of associated neutropenia. Pharm Res. 2012;29:2833-44.
11. Henningsson A, Karlsson MO, Vigano L, Gianni L, Verweij J, Sparreboom A. Mechanism-based pharmacokinetic model for paclitaxel. J Clin Oncol. 2001;19:4065-73.
12. Rochat B. Role of cytochrome P450 activity in the fate of anticancer agents and in drug resistance: focus on tamoxifen, paclitaxel and imatinib metabolism. Clin Pharmacokinet. 2005;44:349-66.
13. Ellis AG, Webster LK. Inhibition of paclitaxel elimination in the isolated perfused rat liver by Cremophor EL. Cancer Chemother Pharmacol. 1999;43:13-8.
14. Smith NF, Acharya MR, Desai N, Figg WD, Sparreboom A. Identification of OATP1B3 as a high-affinity hepatocellular transporter of paclitaxel. Cancer Biol Ther. 2005;4:815-8.
15. van de Steeg E, van Esch A, Wagenaar E, Kenworthy KE, Schinkel AH. Influence of human OATP1B1, OATP1B3, and OATP1A2 on the pharmacokinetics of methotrexate and paclitaxel in humanized transgenic mice. Clin Cancer Res. 2013;19:821-32.
16. 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 Pharmacol Ther. 2007;81:76-82.
17. van de Steeg E, van Esch A, Wagenaar E, van der Kruijssen CM, van Tellingen O, Kenworthy KE, et al. High impact of Oatp1a/1b transporters on in vivo disposition of the hydrophobic anticancer drug paclitaxel. Clin Cancer Res. 2011;17:294-301.
18. Konig J, Muller F, Fromm MF. Transporters and drug-drug interactions: important determinants of drug disposition and effects. Pharmacol Rev. 2013;65:944-66.
19. Engel A, Oswald S, Siegmund W, Keiser M. Pharmaceutical excipients influence the function of human uptake transporting proteins. Mol Pharm. 2012;9:2577-81.
20. Hagenbuch B, Stieger B. The SLCO (former SLC21) superfamily of transporters. Mol Aspects Med. 2013;34:396-412.

21. de Graan AJ, 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.

22. Gui C, Miao Y, Thompson L, Wahlgren B, Mock M, Stieger B, et al. Effect of pregnane X receptor ligands on transport mediated by human OATP1B1 and OATP1B3. Eur J Pharmacol. 2008;584:57-65.

23. de Bruijn P, de Graan AJ, Nieuweboer A, Mathijssen RH, Lam MH, de Wit R, et al. Quantification of cabazitaxel in human plasma by liquid chromatography/triple-quadrupole mass spectrometry: a practical solution for non-specific binding. Journal of pharmaceutical and biomedical analysis. 2012;59:117-22.

24. Engels FK, Mathot RA, Loos WJ, van Schaik RH, Verweij J. Influence of high-dose ketoconazole on the pharmacokinetics of docetaxel. Cancer Biol Ther. 2006;5:833-9.

25. Kaliss N, Pressman D. Plasma and blood volumes of mouse organs, as determined with radioactive iodoproteins. Proc Soc Exp Biol Med. 1950;75:16-20.

26. Gui C, Wahlgren B, Lushington GH, Hagenbuch B. Identification, Ki determination and CoMFA analysis of nuclear receptor ligands as competitive inhibitors of OATP1B1-mediated estradiol-17beta-glucuronide transport. Pharmacol Res. 2009;60:50-6.

27. Letschert K, Faulstich H, Keller D, Keppler D. Molecular characterization and inhibition of amanitin uptake into human hepatocytes. Toxicol Sci. 2006;91:140-9.

28. Yamaguchi H, Kobayashi M, Okada M, Takeuchi T, Unno M, Abe T, et al. Rapid screening of antineoplastic candidates for the human organic anion transporter OATP1B3 substrates using fluorescent probes. Cancer Lett. 2008;260:163-9.

29. Svoboda M, Wlcek K, Taferner B, Hering S, Stieger B, Tong D, et al. Expression of organic anion-transporting polypeptides 1B1 and 1B3 in ovarian cancer cells: relevance for paclitaxel transport. Biomed Pharmacother. 2011;65:417-26.

30. Takano M, Otani Y, Tanda M, Kawami M, Nagai J, Yumoto R. Paclitaxel-resistance conferred by altered expression of efflux and influx transporters for paclitaxel in the human hepatoma cell line, HepG2. Drug Metab Pharmacokinet. 2009;24:418-27.

31. Tanino T, Nawa A, Nakao M, Noda M, Fujiwara S, Iwaki M. Organic anion transporting polypeptide 2-mediated uptake of paclitaxel and 2'-ethylcarbonate-linked paclitaxel in freshly isolated rat hepatocytes. J Pharm Pharmacol. 2009;61:1029-35.

32. Desai N, De T, Ci S, Louie L, Trieu V. Characterization and in vitro/in vivo dissolution of nab-paclitaxel nanoparticles. AACR Meeting Abstracts. 2008;2008:5624-.

33. Yeh TK, Lu Z, Wientjes MG, Au JL. Formulating paclitaxel in nanoparticles alters its disposition. Pharm Res. 2005;22:867-74.

34. van Tellingen O, Beijnen JH, Verweij J, Scherrenburg EJ, Nooijen WJ, Sparreboom A. Rapid esterase-sensitive breakdown of polysorbate 80 and its impact on the plasma pharmacokinetics of docetaxel and metabolites in mice. Clin Cancer Res 1999;5:2918-24.

35. Zaher H, Meyer zu Schwabedissen HE, Tirona RG, Cox ML, Obert LA, Agrawal N, et al. Targeted disruption of murine organic anion-transporting polypeptide 1b2 (Oatp1b2/Slco1b2) significantly alters disposition of prototypical drug substrates pravastatin and rifampin. Mol Pharmacol. 2008;74:320-9.

36. Walker AL, Lancaster CS, Finkelstein D, Ware RE, Sparreboom A. Organic anion transporting polypeptide 1B (OATP1B) transporters modulate hydroxyurea pharmacokinetics. Am J Physiol Cell Physiol. 2013;305(12):C1223-9

37. Sparreboom A, van Asperen J, Mayer U, Schinkel AH, Smit JW, Meijer DK, et al. Limited oral bioavailability and active epithelial excretion of paclitaxel (Taxol) caused by P-glycoprotein in the intestine. Proc Natl Acad Sci U

S A. 1997;94:2031-5.

38. Gardner ER, Dahut WL, Scripture CD, Jones J, Aragon-Ching JB, Desai N, et al. Randomized crossover pharmacokinetic study of solvent-based paclitaxel and nab-paclitaxel. Clin Cancer Res 2008;14:4200-5.

39. Roth M, Obaidat A, Hagenbuch B. OATPs, OATs and OCTs: the organic anion and cation transporters of the SLCO and SLC22A gene superfamilies. Br J Pharmacol. 2012;165:1260-87.

40. Iusuf D, van de Steeg E, Schinkel AH. Functions of OATP1A and 1B transporters in vivo: insights from mouse models. Trends Pharmacol Sci. 2012;33:100-8.

41. Muller F, Fromm MF. Transporter-mediated drug-drug interactions. Pharmacogenomics. 2011;12:1017-37.

42. Zolk O, Fromm MF. Transporter-mediated drug uptake and efflux: important determinants of adverse drug reactions. Clin Pharmacol Ther. 2011;89:798-805.

43. Fahrmayr C, Konig J, Auge D, Mieth M, Fromm MF. Identification of drugs and drug metabolites as substrates of multidrug resistance protein 2 (MRP2) using triple-transfected MDCK-OATP1B1-UGT1A1-MRP2 cells. Br J Pharmacol. 2012;165:1836-47.

44. Izquierdo MA, Garcia M, Ponton JL, Martinez M, Valenti V, Navarro M, et al. A phase I clinical and pharmacokinetic study of paclitaxel and docetaxel given in combination in patients with solid tumours. Eur J Cancer. 2006;42:1789-96.

45. Lancaster CS, Sprowl JA, Walker AL, Hu S, Gibson AA, Sparreboom A. Modulation of OATP1B-type transporter function alters cellular uptake and disposition of platinum chemotherapeutics. Mol Cancer Ther. 2013;12:1537-44.

46. Fujita KI, Sugiura T, Okumura H, Umeda S, Nakamichi N, Watanabe Y, et al. Direct Inhibition and Down-regulation by Uremic Plasma Components of Hepatic Uptake Transporter for SN-38, an Active Metabolite of Irinotecan, in Humans. Pharm Res. 2013;31(1):204-15.

47. Desai N, Trieu V, Yao Z, Louie L, Ci S, Yang A, et al. Increased antitumor activity, intratumor paclitaxel concentrations, and endothelial cell transport of cremophor-free, albumin-bound paclitaxel, ABI-007, compared with cremophor-based paclitaxel. Clin Cancer Res 2006;12:1317-24.

48. Neesse A, Frese KK, Chan DS, Bapiro TE, Howat WJ, Richards FM, et al. SPARC independent drug delivery and antitumour effects of nab-paclitaxel in genetically engineered mice. Gut. 2014 Jun;63(6):974-83

49. Yardley DA. nab-Paclitaxel mechanisms of action and delivery. J Control Release. 2013;170:365-72.

SUPPLEMENTARY METHODS

Determination of paclitaxel and cabazitaxel concentrations

Paclitaxel and cabazitaxel were quantified using validated methods involving reversed-phase liquid chromatography coupled to tandem mass-spectrometric detection (LC-MS/MS). Sample extracts were injected onto an Alltima HP C18 HL 3-µm column (50×2.1mm internal diameter, Alltech Applied Science) by a Waters 2795 Separation Module. The mobile phase for determination of paclitaxel was composed of acetonitrile and water containing formic acid (0.1% v/v), and was delivered using linear gradient settings at a flow rate of 0.2 ml/min. Detection was performed with a MicroMass Quatro Micro triple-quadrupole mass spectrometer (Waters) in the positive ion mode. The electrospray ionization was set at 3.8 kV and the cone voltage at 18 V. The dwell times were set at 150 ms and the inter-channel delay at 50 ms. A multiple-reaction monitoring (MRM) mode was applied for the quantitation with the following parameters: *m/z* 854>286, collision energy at 20 eV for paclitaxel and *m/z* 859>291, collision energy at 20 eV for the internal standard, paclitaxel-d5. The collision cell pressure was set at ~4×10^{-3} mbar (argon). Samples for the quantitation of paclitaxel were prepared by extraction of 100-µl aliquots with 200-µl of an acetonitrile solution of the internal standard, and 1 ml of n-butylchloride. After vigorously mixing and centrifugation for 10 min at 18,000 *g*, the clear supernatant was evaporated at a temperature of 70°C. The residue was dissolved in 150 µl of a mixture of acetonitrile-water-formic acid (40:60:0.1, v/v/v), from which aliquots of 5 µL were injected into the LC-MS/MS-system. The lower limit of quantitation (LLQ) for paclitaxel was determined to be 20.0 ng/mL, and the calibration curve ranged from 20.0 to 1,000 ng/ml. The within-run and between-run precisions were within 7.14%, while the accuracy ranged from 88.5 to 94.1%.

The mobile phase for determination of cabazitaxel was composed of acetonitrile and water containing ammonium formate (0.2% v/v), and was delivered using linear gradient settings at a flow rate of 0.2 ml/min. Detection was performed with a MicroMass Quatro Micro triple-quadrupole mass spectrometer (Waters) in the positive ion mode. The electrospray ionization was set at 3.5 kV and the cone voltage at 20V. The dwell times were set at 150 ms and the inter-channel delay at 50 ms. The MRM mode was applied for the quantitation with the following parameters: *m/z* 836>555, collision energy at 10 eV for cabazitaxel and *m/z* 842>561, collision energy at 10 eV for the internal standard, cabazitaxel-$^{2}H_{6}$. The collision cell pressure was set at ~4×10^{-3} mbar (argon). Samples for the quantitation of cabazitaxel were prepared by extraction of 100-µl aliquots with 20 µl of ammonium hydroxide (4%), 100 µl of an internal standard solution in acetonitrile and 1 ml of n-butylchloride. After vigorously mixing and centrifugation for 10 min at 18,000 *g*, the clear supernatant was evaporated at a temperature of 70°C under a stream of nitrogen. The residue was dissolved in 100 µl of a mixture of acetonitrile-water-ammonium formate (40:60:0.2, v/v/v), from which aliquots of 10 µl were injected into the LC-MS/MS-system. The LLQ for cabazitaxel was determined to be 1.00 ng/ml, and two separate calibration curves were used for drug quantitation, one in the concentration range of 1.00 to 100 ng/mL, and another in the concentration range of 40.0 to 4,000 ng/ml. For the former, the within-run and between-run precisions were within 8.75%, while the accuracy ranged from 88.5 to 94.1%. For the latter, the within-run and between-run precisions were within 4.99%, while the accuracy ranged from 95.8 to 100.3%.

Disruption of mouse tissues

Tissue samples were kept frozen at -70ºC until the time of processing. All organis were diluted in blank (drug-free) human plasma (1:4, w/v) in a 2-ml Eppendorf vial. Next, a 5-mm stainless steel bead (Qiagen) was added, and the samples were homogenized with a Tissuelyser (Qiagen), and processed for 4 min at 40 Hz. Finally, the beads were removed and the homogenized samples were stored at -70ºC until analysis by LC-MS/MS as described above for plasma samples.

Chapter 3

Predicting paclitaxel-induced neutropenia using the DMET platform

A.J.M. Nieuweboer, M. Smid, A.M. de Graan, S. Elbouazzaoui, P. de Bruijn, J.W.M. Martens, A.H.J. Mathijssen and R.H.N. van Schaik

Pharmacogenomics 2015;16(11):1231-1241

ABSTRACT

The use of paclitaxel in cancer treatment is limited by paclitaxel-induced neutropenia. We investigated the ability of genetic variation in drug-metabolizing enzymes and transporters to predict hematological toxicity. Using a discovery and validation approach, we identified a pharmacogenetic predictive model for neutropenia. For this, a drug-metabolizing enzymes and transporters plus DNA chip was used, which contains 1936 SNPs in 225 metabolic enzyme and drug-transporter genes. Our 10-SNP model in 279 paclitaxel-dosed patients reached 43% sensitivity in the validation cohort. Analysis in 3-weekly treated patients only resulted in improved sensitivity of 79%, with a specificity of 33%. None of our models reached statistical significance. Our drug-metabolizing enzymes and transporter-based SNP-models are currently of limited value for predicting paclitaxel-induced neutropenia in clinical practice.

INTRODUCTION

Paclitaxel is an antineoplastic drug, used for the treatment of several types of cancer such as non-small-cell lung cancer and ovarian cancer (1, 2). Regarding its metabolism, it is known that the uptake of paclitaxel in the liver occurs through 1B-type organic anion transporters (3, 4). Hepatic metabolism is thereafter performed by cytochrome (CYP) P450 enzymes CYP3A4 and CYP2C8 (5, 6). Subsequently, paclitaxel is effluxed out of the hepatocytes by the transporters ABCB1 and ABCC2 (7, 8). Besides for hepatobiliary secretion, ABCB1 and ABCC2 are also responsible for the intestinal secretion of paclitaxel (5, 9).

The pharmacokinetics and thus the exposure to paclitaxel has a high inter-individual variability, which is reflected in the incidence and severity of paclitaxel-induced toxicities (10). A correlation exists between these highly variable pharmacokinetics and paclitaxel-induced toxicities (11, 12). Neurotoxicity and neutropenia are paclitaxel's main dose limiting toxicities. In this, neutropenia reduces the effectiveness of the immune system and increases the risk of severe bacterial infections (12).

It is clinically relevant to predict which patients are predisposed for severe paclitaxel-induced toxicity. One aspect is predicting paclitaxel pharmacokinetics. The role of 'pharmaco'-genetics however is less broadly explored. Using a candidate gene approach, a limited number of SNPs has been identified to be associated with pharmacokinetics and paclitaxel-induced-toxicity (13, 14). For a multi-gene approach, the Affymetrix Drug Metabolizing Enzymes and Transporters (DMET) Plus Premier Pack (Affymetrix, CA, USA) was used previously. Using the DMET approach, genetic variation correlated with specific chemotherapy-induced toxicity such as gastrointestinal toxicity in colorectal cancer patients and osteonecrosis of the jaw in multiple myeloma patients could be identified (15-17). This suggests that the DMET platform has a great potential in explaining chemotherapy-induced toxicity.

We hypothesize that variation in genes not thought to be primarily involved in paclitaxel pharmacokinetics but still related to drug metabolism could be helpful for predicting neutropenia (defined as an absolute neutrophil count (ANC) $<1x10^9$/L) and leukopenia (defined as an absolute white blood cell count (WBC) $<2x10^9$/L). By using the Affymetrix DMET Plus Platform, we investigated whether polymorphisms in genes that are involved in drug metabolism and transport could predict neutropenia.

PATIENTS AND METHODS

Patients

Patients treated with paclitaxel for solid tumors (Table 1) were included in a prospective pharmacological study (enumerated as NTR2311 at www.trialregister.nl). Patients were above the age of 18 and had a confirmed diagnosis of malignancy (cytologically or histologically proven). In addition, patients' WHO Performance Score had to be 0 or 1 and hematopoietic, renal and hepatic functions should be adequate according to the product information of paclitaxel (18). Strong inducers and inhibitors of CYP2C8 and CYP3A4 were prohibited (19). Patients were treated in a combination regimen in which they started with weekly courses and then switched to a 3-weekly schedule, a weekly regimen, or a 3-weekly regimen. Throughout the whole period of paclitaxel treatment, neutrophil and leukocyte count values were scored. ANC and WBC values have been determined following the hospitals' diagnostic protocols. These data have been collected from patient charts retrospectively. The lowest ANC or WBC score was determined throughout all paclitaxel cycles. Methods of the obtaining of pharmacokinetic data as well as the calculation of pharmacokinetic parameters were previously described by de Graan *et al.* (20). The Medical Ethical Committee of the Erasmus University Medical Center in Rotterdam, The Netherlands, approved this trial (MEC2003.264) and all procedures were done according to the declaration of Helsinki.

SNP analysis

Genetic variant analysis was performed as described previously by de Graan *et al. (*20). Summarizing, whole blood was drawn from all patients and from this blood, genomic DNA was isolated using MagnaPure LC (Roche Diagnostics GmbH) according to the instructions of the manufacturer. Genotyping was done using the Affymetrix DMET Plus Premier Pack according to the descriptions of Dumaual *et al.* (21). As a measure of quality and reliable readability, the genotypes of all studied SNPs were stated as either 'call' or 'no call'. A sample showing a call rate below 90% was removed from the analysis. Also, patients with missing clinical data (e.g. ANC values) were excluded.

Predictive model design

Genotypes were disqualified from the data analysis when the same in all patients or not in Hardy Weinberg Equilibrium (HWE) (P>0.05). All 1936 genetic variants on 225 genes involved in metabolism or transport were statistically tested as described previously (20). Patients receiving treatment every 3-weeks (n=76) were grouped into a high and low risk group based on their nadir ANC. Patients were labelled as high risk for neutropenia if the ANC nadir was below the value of $1x10^9$/L, otherwise as low risk. Patients with a nadir WBC $<2x10^9$/L were considered as high risk for leukopenia, otherwise as low risk. For both groups separately, patients were randomly divided into a discovery and a validation cohort. Because of the explorative character of this analysis, the total cohort was split into a discovery and a validation cohort, despite the knowledge that paclitaxel-induced neutropenia is schedule dependent.

Table 1. Patient characteristics of the total cohort

	Total cohort	Discovery cohort ANC<1x10^9/L	Validation cohort ANC<1x10^9/L	Discovery cohort WBC<2x10^9/L	Validation cohort WBC<2x10^9/L
Number of patients (n)	279	140	139	140	139
Male	146	69	77	72	74
Female	133	71	62	68	65
Mean age, years (range)	60.7 (19-82)	60.6 (19-82)	60.6 (26-82)	61.5 (27-82)	59.7 (18-79)
Cancer Type					
Oesophagus (%)	152 (54.5)	78 (55.7)	73 (52.5)	76 (54.3)	76 (54.7)
Ovarian (%)	42 (15.1)	24 (17.1)	19 (13.7)	22 (15.7)	20 (14.4)
Cervix (%)	19 (6.8)	11 (7.9)	8 (5.8)	10 (7.1)	9 (6.5)
Endometrium (%)	18 (6.5)	9 (6.4)	9 (6.5)	10(7.1)	8 (5.8)
Mamma (%)	12 (4.3)	6 (4.3)	6 (4.3)	6 (4.3)	6 (4.3)
Rest (%)	36 (12.9)	12 (8.6)	24 (17.3)	16 (11.4)	20 (14.4)
Nadir endpoint*, mean (range)	1.59 (0.05-10.0)	1.59 (0.05-7.3)	1.58 (0.1-10.0)	2.89 (0.05-10.2)	2.70 (0.34-7.7)
Cytotoxic comedication (n)					
cisplatin (%)	37 (13.2)	17 (12.1)	20 (14.2)	16 (11.4)	21 (14.9)
carboplatin (%)	228 (81.1)	116 (82.9)	112 (79.4)	121 (86.4)	109 (77.3)
bleomycin (%)	2 (0.7)	1 (0.7)	1 (0.7)	0 (0)	2 (1.4)
ifosfamide (%)	2 (0.7)	0 (0)	2 (1.4)	0 (0)	2 (1.4)
sorafenib (%)	1 (0.4)	0 (0)	1 (0.7)	0 (0)	1 (0.7)
trastuzumab (%)	4 (1.4)	2 (0.7)	2 (1.4)	2 (0.7)	2 (1.4)
none (%)	7 (2.5)	4 (2.9)	3 (2.2)	1 (0.7)	4 (2.8)

Data are represented as absolute numbers unless stated otherwise. By 'endpoint'* is meant ANC (value x109/L) or WBC (value x109/L) , depending on the analysis. Abbreviations: absolute neutrophil count (ANC), white blood cell count (WBC). One patient was treated with the combination cisplatin & ifosfamide and one patient was treated with the combination carboplatin & sorafenib. These patients were separately mentioned for both drugs in the combination.

In the discovery samples, the conditional probability for each SNP was calculated for each variant using this formula: P (A|B)= P (A&B) / P (B). In the case of ANC nadir being the endpoint: *A* indicates an ANC nadir below the value of $1x10^9$/L (high risk) and *B* stands for the genotype. This formula gives the probability of a patient having an ANC nadir below the value of $1x10^9$/L, given the manifestation of a certain genotype (wild-type (WT); heterozygous (HTZ), homozygous (HOZ)). This gives a probability for each possible genotype of each SNP, which is incorporated in the prediction analysis as a weight.

SNPs were selected if the following selection criteria were met: WT, HTZ and HOZ genotype had to be represented at least five times, the difference in probability of WT and HOZ > 0.2, and both the probability for WT and HOZ should be at least 10% higher than the 'standard risk'. This risk was derived from the clinical data of all patients; for example, considering ANC, 45 out of 76 patients show a nadir ANC <$1x10^9$/L, thus a 'standard risk' of 59.2%. To obtain a final selection of SNPs, all SNPs showing the exact same genotype in all patients in the discovery cohort were excluded. Deciding which of the SNPs had to be included was based on the highest probability in WT/HOZ genotypes. The probabilities of the final list of SNPs were used as weights in the analysis to predict the samples in the validation cohort.

Prediction of the validation samples

A total probability weighted score was determined for every individual patient by adding up all probabilities of the SNPs in the model. In case the DMET chip was unable to call the genotype for a SNP in a particular patient, the standard risk was used. In a ROC-curve, the scores of the patients in the discovery cohort were associated with the risk for neutropenia. Here, a threshold was selected at which all or most patients having a nadir ANC<$1x10^9$/L were included. In the validation cohort, the probability-weighted scores were also calculated and then compared with the threshold chosen in the discovery cohort. The validation cohort samples were predicted as high or low risk. Finally, these predictions were matched with the actual nadir ANC to make apparent how many of these samples were predicted correctly.

Statistics

All analyses were done using STATA version 13 (StataCorp LP, TX, USA). Data are presented as means with ranges, unless stated otherwise. P-values are two-sided and statistically significant was defined as p-value <0.05. Dissimilarities amongst both patient cohorts were studied using the Chi-square test or the Mann-Whitney test, depending on whether variables were categorical of continuous. The influence of covariables on the association between the prediction model and a patient being at risk for an ANC count below $1x10^9$/L was tested by logistic regression. To associate ANC as continuous variable with a SNP, a test for trend across ordered groups was used, which is an extension of the Wilcoxon rank-sum test (22). Because these are exploratory analyses, no correction for false discovery rate was made.

RESULTS

Correlation between neutropenia and clearance of paclitaxel

Pharmacokinetic data of 251 patients was available. Patients with an ANC nadir <1x10^9/L had a significantly lower clearance of paclitaxel than patients with normal or high ANC nadirs (*P*<0.0001, Figure 1).

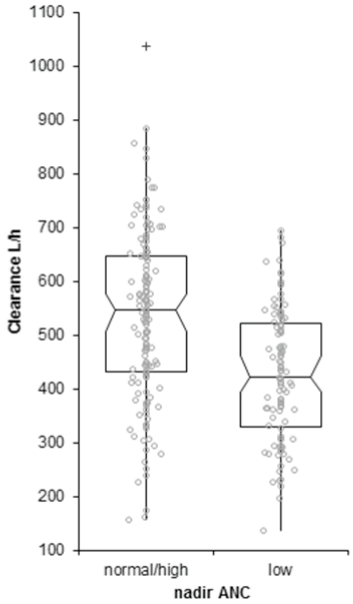


Figure 1. Clearance (L/h) of patients with ANC nadirs <1x10^9/L and patients with ANC nadirs ≥1x10^9/L. Outliers >1.5 and <3 times the Inter Quartile Range (IQR) are represented by a plus sign (+), outliers >3 IQR are shown as a cross sign (x). ANC: absolute neutrophil count.

Trend test

ANC levels (treated as a continuous variable) were correlated with the SNPs that were in HWE, using a test for trend across ordered groups in the total cohort of patients. In total, 23 SNPs were found to be associated with ANC levels (Table 2).

The same analysis was done after dividing the patients into two groups according to their treatment regimen (weekly and 3-weekly treated patients). The SNP rs9285726 in *MAT1A* (Figure 2) was the only SNP leading to a significant difference in ANC nadirs between SNP variant and wildtype (WT) patients in both weekly and 3-weekly treated patients (*P*<0.03). Patients with the variant SNP had higher ANC nadirs than patients with the wild-type genotype.

Table 2. 23 SNPs significantly associated (P<0.05) with ANC<1*10^9/L in the total cohort

Common Name	SNP ID RS	Change	P-value
SLC7A7_39780A>G(K509K)	rs1061040	A/G	0.011
SLC13A1_1911>(rs2204295)	rs2204295	G/C	0.012
SLC5A6_>(R94R)	rs56970590	A/G	0.013
CYP4F11_78A>G(G26G)	rs2305801	G/A	0.020
MAT1A_14030>(rs9285726)	rs9285726	T/A	0.020
SLC15A1_66322G>A(S616S)	rs8187840	G/A	0.020
ABCG1_60972>(rs3788007)	rs3788007	G/A	0.022
CHST2_2082>(rs6664)	rs6664	C/T	0.023
SLC10A1_225G>A(T75T)	rs4646285	G/A	0.023
SLC5A6_7914>(rs7081)	rs7081	G/A	0.024
CHST13_6702>(rs4305381)	rs4305381	A/C	0.029
GSTP1_2265C>T(A114V)	rs1138272	C/T	0.031
CYP39A1_56503T>A(N324K)	rs7761731	A/T	0.033
CYP51A1_>(S437P)	rs59683852	C/T	0.033
SLC16A1_>(rs12727968)	rs12727968	C/A	0.033
SULT2B1_23737C>T(P40P)	rs2544794	C/T	0.034
PPARD_-21172>(rs3798343)	rs3798343	C/G	0.036
CHST5_-386>(rs2550915)	rs2550915	C/G	0.037
SULT1A2_A3_>(rs11150564)	rs11150564	C/T	0.037
SLC15A1_28672G>A(S117N)	rs2297322	A/G	0.041
CYP4F11_10505T>C(C276R)	rs8104361	C/T	0.042
ABCC6_65693G>A(R1268Q)	rs2238472	G/A	0.044
ADH5_-159>(rs1154400)	rs1154400	G/A	0.044

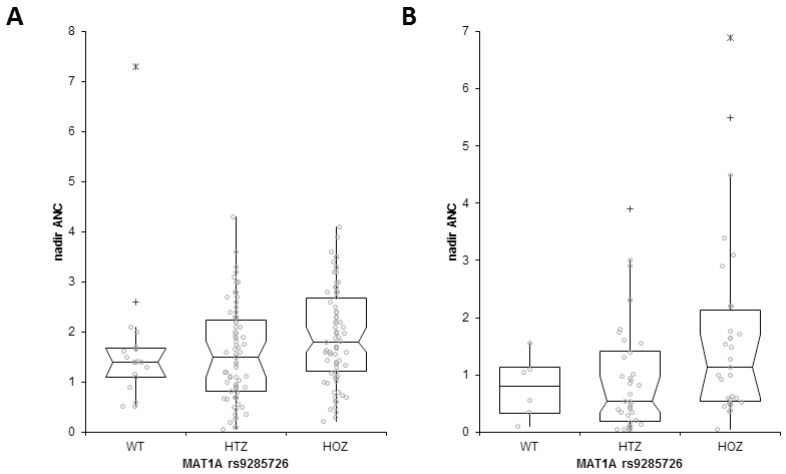


Figure 2. Nadir absolute neutrophil count per genotype of the SNP rs9285726 on the gene MAT1A. On the x-axis, wild-type genotype is shown as WT, the heterozygous variant genotype as HTZ and the homozygous variant genotype as HOZ. (A) The nadir ANC per genotype is shown for the patients treated in a weekly regimen. (B) The nadir ANC per genotype is shown for the patients treated in a 3-weekly regimen. Outliers >1.5 and <3 times the Inter Quartile Range (IQR) are represented by a plus sign (+), outliers >3 Inter Quartile Range are shown as a cross sign (x). ANC: absolute neutrophil count.

Identification of patients at high risk for an absolute neutrophil count <1x10^9/L in the total cohort

Using the cut-off value of ANC <1x10^9/L (decision limit for antibiotic treatment), a total of 279 patients were included in the discovery analysis (146 males and 133 females). The mean ANC nadir (lowest value during treatment) was 1.5x10^9/L (range 0.05 – 7.3x10^9/L) with 116 patients (42%) showing a nadir ANC <1x10^9/L. Age was not significantly associated with ANC nadir <1x10^9/L (Mann-Whitney *P*=0.09), but in females the incidence of an ANC nadir <1x10^9/L was higher than in men (48% vs. 36% respectively; *P*=0.03). Subsequently, the total group of patients was randomly split into a discovery (n=140) and a validation cohort (n=139). Both cohorts were checked for bias in age and gender. Patient characteristics were comparable in both cohorts (Table 1). Treatment with cytotoxic comedication was not significantly different between the discovery and validation cohorts for each endpoint (*P*≥0.4).

In the discovery and the validation cohort, a comparable proportion of patients (58/140 (21%) and 58/139 (21%) respectively) had an ANC nadir <1x10^9/L (defined as high risk patients). In the discovery cohort, SNPs associated with high risk were identified and combined in a genetic profile containing 10 SNPs: *ABCC4* rs2274405, *ABCC6* rs2238472, *ABP1* rs4725373, *CHST10* rs1530030, *CYP11B1* rs5303, *CYP2B6* rs2279344, *GSTP1* rs1695, *SLC13A1* rs2204295, *SLC6A6* rs9036 and *VKORC1* rs8050894. A ROC curve of the discovery cohort was generated and 90% sensitivity was defined as the cut-off point (Figure 3). This cut-off was chosen because it gave the most optimal balance between specificity and sensitivity. Patients with a probability score above the cut-off value were defined as high-risk patients. Hereafter, we validated the predictive SNP-model using the validation cohort, consisting

of both 1-weekly and 3-weekly dosing regimen patients. Here, 25 out of 58 patients having a true ANC nadir<1x10^9/L were identified, resulting in a sensitivity of 43%. Specificity was 68%, identifying 55 out of 81 patients with an ANC nadir >1x10^9/L; the positive predictive value (PPV) was 49%. The Chi-square test of the 2*x*2 table containing predicted ANC nadirs versus the true nadir level was not statistically significant (*P*=0.18).

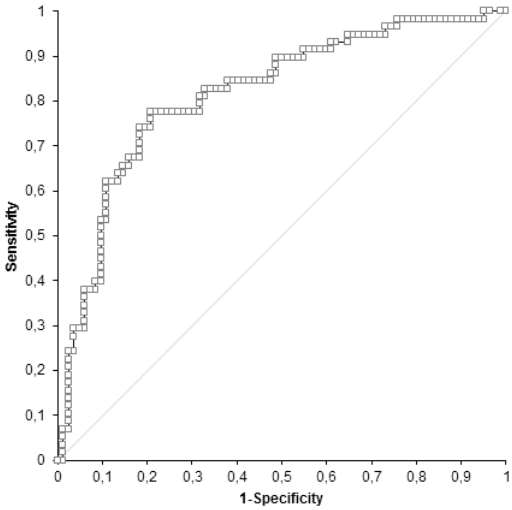


Figure 3. Receiver operation curve of the predictive model for absolute neutrophil count <1x10^9/L in the discovery samples of the total cohort of patients. This is a representation of the ability of the predictive model to distinguish between patients with nadir absolute neutrophil counts (ANCs) below and above the value of <1x10^9/L. Here, the true positives (sensitivity, y-axis) are set out against the false positives (1-sensitivity, x-axis) at several different cut-off points. The single thin line represents an example in which no distinguishing ability is seen for a test. The boxes (score sum) represent the developed predictive model for predicting ANC <1x10^9/L.

Identification of patients at high risk for a white blood cell count <2x10^9/L in the total cohort

Next to neutropenia, leukopenia is often seen in paclitaxel-treated cancer patients. Therefore, a similar approach was used to predict which patients would be at high risk for WBC values below 2x10^9/L after paclitaxel infusion. Again, the cohort was split into a discovery (n=140) and a validation cohort (n=139). In the discovery set, 37 patients had a WBC nadir <2x10^9/L and in the validation set this number of patients was 36. SNPs included in the WBC predictive genetic model are*:* *CHST1* rs750398, *SLC22A11* rs2078267, *SLCO3A1* rs2283458, *SULT1A1* rs9282861, *CYP4F8* rs4646523, *SULT2A1* rs296365, *ABCC5* rs7636910, *SLC22A7* rs2270860, *SLC22A7* rs2242416. None of the identified SNPs were similar to the SNPs found in the analysis for ANC nadirs. In this case, the closest data-point on the ROC-curve to 90% sensitivity was chosen (identification of 33/37 low nadir WBC patients) to be the cut-off point in the discovery cohort. Patients with a probability score above this cut-off point were predicted as patients with a WBC <2x10^9/L. Sensitivity of this model was 56%, specificity was 38% and PPV was 24%. This model was however not statistically significant.

Identification of 3-weekly treated patients at high risk for an absolute neutrophil count <1x10^9/L

Patients treated with a 3-weekly paclitaxel regimen received higher doses of paclitaxel than patients treated with a weekly regimen. These patients were therefore seen to drop below the ANC value of 1x10^9/L more often than the weekly treated patients. Therefore, this subgroup (n=76) was tested separately and split in a discovery cohort (n=38) and a validation cohort (n=38). In the discovery cohort, 22/38 (58%) had an ANC nadir <1x10^9/L and in the validation cohort, 23/38 (60%) patients had an ANC nadir <1x10^9/L (Table 3). The following 19 SNPs were identified for inclusion in the genetic model for predicting neutropenia in 3-weekly treated patients: *ABCB11* rs2287622, *ABCC6* rs2238472, *ALB* rs3756067, *CBR3* rs1056892, *CYP2B6* rs4803418, *CYP2D6 rs1800716*/rs3892097, *CYP2F1* rs305968, *CYP4F1* rs3765070, *FMO3* rs2266782, *GSTA4* rs405729, *GSTM5* rs11807, *GSTZ1* rs3177427, *NAT2* rs1799930, *SLC16A1* rs1049434, *SLC22A11* rs2078267, *SLC22A2* rs624249, *SLC5A6* rs1395, *SLC7A7* rs2281677, and *SULT1A2* rs11150564. The ROC-curve cut-off point was set at 100% sensitivity because of the shape of this particular ROC-curve; in other words, the 90% sensitivity point in the ROC-curve had the same specificity as at 100% sensitivity, and thus the highest sensitivity was chosen. Patients with a probability score above this value were defined as patients with an ANC <1x10^9/L. Sensitivity of this model was 79%: 18/23 true neutropenic patients had been identified as patients with ANC <1x10^9/L. Specificity was 33% and the PPV was 64%. The Odds Ratio (OR) in the logistic regression on the 'predicted ANC<1x10^9/L' versus 'true ANC<1x10^9/L' was 1.8 (*P*=0.43), although this difference did not reach statistical significance.

Table 3. Patient characteristics of patients treated in a 3-weekly schedule

	Discovery cohort ANC<1x10^9/L	Validation cohort ANC<1x10^9/L
Number of patients (n)	38	38
Male	8	8
Female	30	30
Mean age, years (range)	58.2 (27-82)	57.2 (18-76)
Cancer Type		
Oesophagus (%)	1 (0.7)	0 (0)
Ovarian (%)	15 (39.5)	17 (44.7)
Cervix (%)	3 (7.9)	1 (2.6)
Endometrium (%)	7 (18.4)	8 (21.1)
Mamma (%)	1 (2.6)	1 (2.6)
Rest (%)	11 (28.9)	11 (28.9)
Nadir ANC, mean (range)	1.5 (0.05-10.0)	1.6 (0.05-5.5)
Cytotoxic comedication (n)		
cisplatin (%)	10 (25.6)	9 (23.1)
carboplatin (%)	26 (66.7)	27 (69.2)
bleomycin (%)	0 (0)	2 (5.1)
ifosfamide (%)	1 (2.6)	1 (2.6)
sorafenib (%)	1 (2.6)	0 (0)
none (%)	1 (2.6)	0 (0)

Data are represented as absolute numbers unless stated otherwise. Abbreviations: Absolute neutrophil count (ANC). One patient was treated with the combination cisplatin & ifosfamide and one patient was treated with the combination carboplatin & sorafenib. These patients were separately mentioned for both drugs in the combination.

DISCUSSION

Out of the total number of SNPs in HWE, we created a list of SNPs statistically significantly associated (P<0.05) with severe neutropenia (ANC <1x10^9/L). Our final list consisted out of 23 SNPs (Table 2). SNPs in the SLC gene family were best associated with neutropenia. The SNP rs776173 (*CYP39A1*) also came forward in this list (Figure 4), and was previously shown to be associated with the incidence of docetaxel induced grade 4 neutropenia, tested in 42 Japanese patients (P=0.049, OR=9.0)

(23). In that study, 28 SNPs were associated with docetaxel AUC (P<0.05). The association between these 28 SNPs and neutrophil counts were validated in the remaining 32 patients (23). Only rs776173 (*CYP39A1*) was significantly associated with grade 4 neutropenia. The validation of this particular SNP in our analysis shows that this SNP might be of clinical relevance, despite the fact this SNP was not found to be in any of our predicted models. The exclusion of this particular SNP in an early phase of the analyses could have been based on the fact this SNP had a lower p-value in our population than another SNP in the same gene. The other 22 SNPs in our list (Table 2) were not previously associated with toxicities of taxanes.

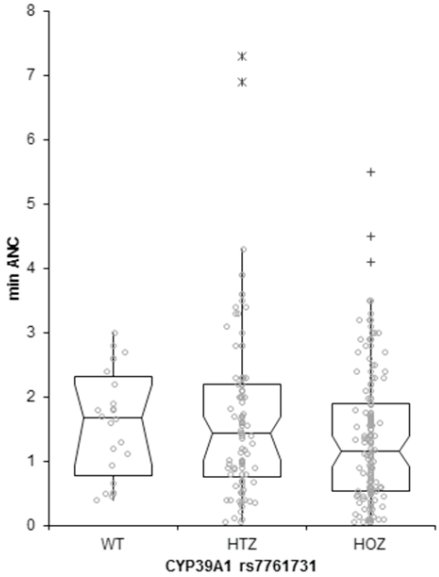


Figure 4. Total cohort nadir absolute neutrophil count per genotype of the SNP 7761731 on the gene *CYP39A1*. On the x-axis, wild-type genotype is shown as WT, the heterozygous variant genotype as HTZ and the homozygous variant genotype as HOZ. Outliers >1.5 and <3 times the Inter Quartile Range (IQR) are represented by a plus sign (+),outliers >3 IQR are shown as a cross sign (x). ANC: absolute neutrophil count.

With the use of the Affymetrix DMETPlus Premier Pack, we have also identified three predictive models that could either predict leukopenia or neutropenia. Using the total cohort of patients treated with paclitaxel in different regimen, leukopenia was predicted in the validation cohort by a nine SNP profile with a sensitivity of 56%, and neutropenia was predicted by a 10 SNP profile with a sensitivity of 43%. Additionally, a 19 SNP model was developed for predicting neutropenia in the 3-weekly treated patients only. One of these 19 SNPs was located in the *ABCB11* gene (BSEP/PFIC2/SPGP), a gene encoding a drug transporter previously found to play a role in paclitaxel resistance (24). This model had an improved sensitivity of 79%, but was not statistically significant (P<0.05). One SNP, rs9285726 in *MAT1A,* was significantly associated with neutropenia in both 3-weekly treated patients and weekly-treated patients.

SNPs in some of the same genes as found in the previously published DMET paper were again identified in our analyses (20). For example, in our predictive model for ANC in the total cohort of patients, SNPs in the genes *SLC6A6* (rs9036) and *VKORC1* (rs8050894) were identified. These genes, however not the same SNPs, were also described in our model predicting paclitaxel clearance (20).

This paper by de Graan *et al.* has also described the following genes that have also been identified in this analysis: SLC22A11 (rs2078267) and *GSTZ1* (rs3177427). In our model, rs2078267 in *SLC22A11* was identified to predict leukopenia in all patients and neutropenia in 3-weekly treated patients. rs3177427 in *GSTZ1* was selected to predicted neutropenia in the 3-weekly treated patients. To sum, SNPs in SLC22A11, GSTZ1, SLC6A6 and VKORC1 were found in the analysis on predicting paclitaxel clearance as well as toxicity. However, not all selected SNPs are identical in both analyses. The exclusion of these SNPs because of neutral criteria such as a lower P-value for this SNP than another SNP in the same gene or a larger amount of missing data could have caused this. Interestingly, also SNPs in OATP1B1 (SLCO1B1) and OATP1B3 (SLCO1B3), drug transporters that were described to be involved in hepatic uptake of paclitaxel (3, 4), did not show up in the top genes in our analyses, for which we do not have a clear explanation. The hypothesis free analysis using an exploratory and a validation cohort seems to be a more open approach than candidate gene analysis, but may suffer from chance findings, which is intended to be counteracted by using exploratory and validation cohorts. The fact that also only few SNPs from our recent models do overlap the models focusing on paclitaxel pharmacokinetics that have previously been developed by de Graan *et al.* (20), strengthens the idea that factors influencing pharmacodynamics and pharmacokinetics do not always overlap. For gaining an adequate predictive model with high clinical impact, the model would ideally have a high sensitivity as well as a high specificity, aiming for the correct identification of all patients at high risk for neutropenia (ANC $<1x10^9$/L). A wrongful recognition of a low risk patient as a high-risk patient would not have immediate serious medical consequences. On the other hand, addressing a high-risk patient as a low risk patient could have more serious consequences, but is analogous to the current standard of practice; that is, patients are hospitalized upon showing neutropenia and/or neutropenic fever. Being able to identify high-risk patients would mean that all of these patients would receive proper follow-up in the weeks after paclitaxel administration and could hypothetically receive a lower dose of paclitaxel than their genetically different equals. Before this could be clinical practice, the correlation between a lower dose and treatment outcome has to be addressed. Patients at low risk for neutropenia could be send home for a longer period, with less frequent outpatient visits. This would decrease patient burden and would also decrease health care costs.

CONCLUSIONS

We found a 10-SNP model in 279 paclitaxel-dosed patients reaching 43% sensitivity in the validation cohort for predicting hematological toxicity. Analysis in 3-weekly-treated patients resulted in improved sensitivity of 79%, with a specificity of 33%, although both approaches did not lead to statistically significant models. Therefore, we assume that variation in genes that are involved in drug metabolism and transport only partly covers the inter-individual variability in hematological toxicity (25). SNPs in genes not involved in drug metabolism or transport or additional factors as the environment could also contribute in developing paclitaxel-induced hematological toxicity.

FUTURE PERSPECTIVE

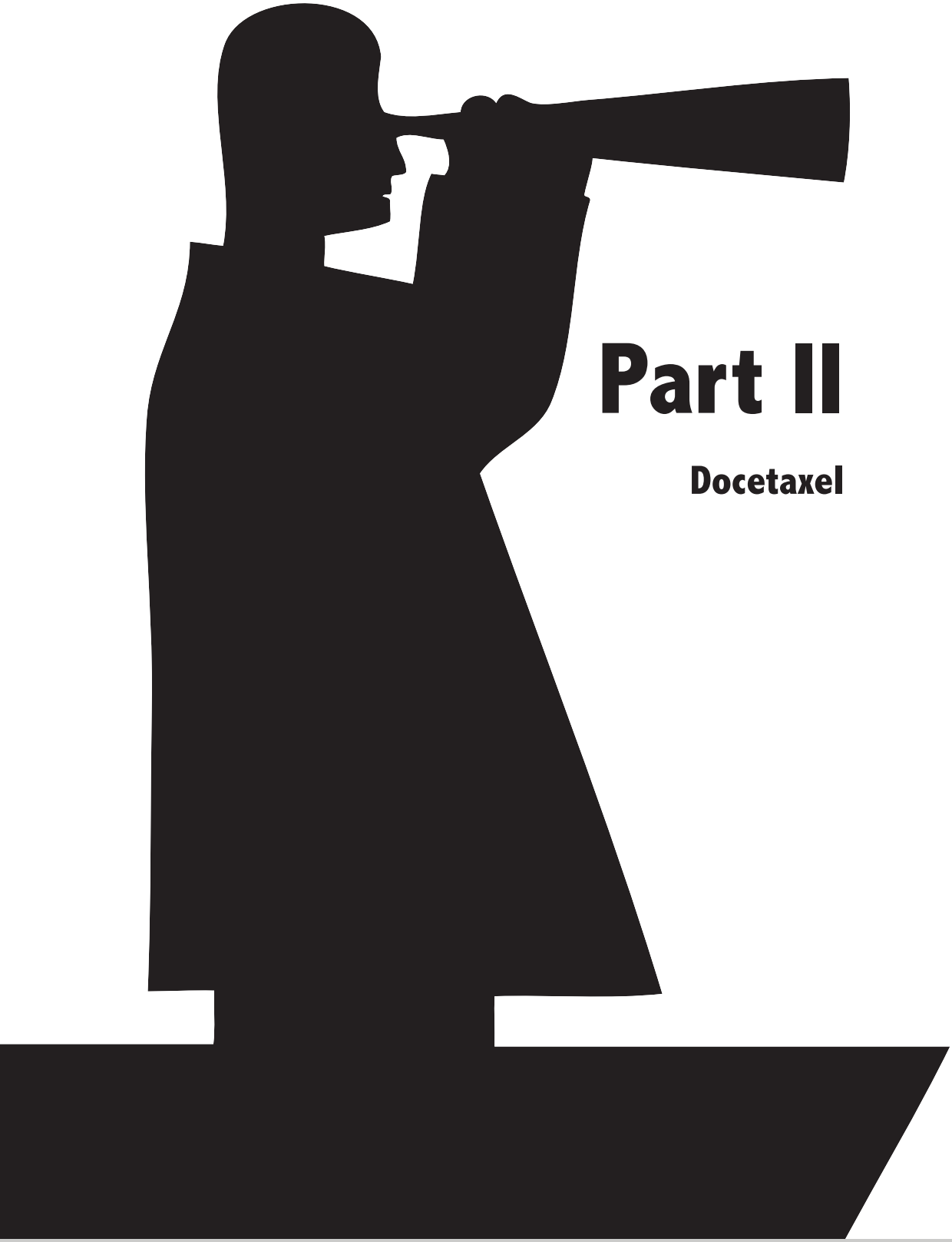
To reduce patient burden and health care costs, it is crucial to be able to predict which patients will suffer from neutropenia and associated complications. Our predictive genetic models reached acceptable sensitivity, but unfortunately did not reach statistical significance. In future studies aiming for similar purposes, large homogeneous subgroups of patients should be used for gaining optimal sensitivity. As it seems that variation in genes involved in drug metabolism and transport only partially explains the inter-individual variability in hematological toxicity, additional factors such as environmental factors (i.e., co-medication) should be investigated and potentially included in future models. To add, the SNPs rs9285726 in *MAT1A* and rs776173 in *CYP39A1* deserve additional investigation since these were shown to be associated with neutropenia and need further validation. In particular, this last mentioned SNP in *CYP39A1* might be clinically relevant, as this SNP had already been found to be associated with taxane-induced neutropenia.

REFERENCES

1. Wani MC, Taylor HL, Wall ME, Coggon P, Mcphail AT: Plant antitumor agents. VI. The isolation and structure of taxol, a novel antileukemic and antitumor agent from Taxus brevifolia. J Am Chem Soc 1971;93(9):2325-2327.
2. Huizing MT, Sewberath Misser VH, Pieters RC et al.: Taxanes: A new class of antitumor agents. Cancer Invest. 1995;13(4):381-404.
3. Nieuweboer AJM, Hu S, Gui C et al.: Influence of drug formulation on OATP1B-mediated transport of paclitaxel. Cancer Res. 2014;74(11):3137-3145.
4. Smith NF, Acharya MR, Desai N, Figg WD, Sparreboom A: Identification of OATP1B3 as a high-affinity hepatocellular transporter of paclitaxel. Cancer Biol Ther. 2005;4(8);815-818.
5. Sonnichsen DS, Liu Q, Schuetz EG, Schuetz JD, Pappo A, Relling MV: Variability in human cytochrome P450 paclitaxel metabolism. J Pharmacol Exp Ther. 1995;275(2);566-575.
6. Mielke S: Individualized pharmacotherapy with paclitaxel. Curr Opin Oncol. 2007;19(6);586-589.
7. Huisman MT, Chhatta AA, Van Tellingen O, Beijnen JH, Schinkel AH: MRP2 (ABCC2) transports taxanes and confers paclitaxel resistance and both processes are stimulated by probenecid. Int J Cancer. 2005;116(5):824-829.
8. Lagas JS, Vlaming ML, Van Tellingen O et al.: Multidrug resistance protein 2 is an important determinant of paclitaxel pharmacokinetics. Clin Cancer Res. 2006;12(20 Pt 1):6125-6132.
9. Huisman MT, Chhatta AA, van Tellingen O et al.: MRP2 (ABCC2) transports taxanes and confers paclitaxel resistance and both processes are stimulated by probenecid. Int J Cancer. 2005;116(5):824-9.
10. Henningsson A, Marsh S, Loos WJ et al.: Association of CYP2C8, CYP3A4, CYP3A5, and ABCB1 polymorphisms with the pharmacokinetics of paclitaxel. Clin Cancer Res. 2005;11(22):8097-8104.
11. Mielke S, Sparreboom A, Steinberg SM et al.: Association of Paclitaxel pharmacokinetics with the development of peripheral neuropathy in patients with advanced cancer. Clin Cancer Res. 12005;1(13):4843-4850.
12. Markman M: Taxol: an important new drug in the management of epithelial ovarian cancer. Yale J Biol Med. 1991;64(6):583-590.
13. Hertz DL: Germline pharmacogenetics of paclitaxel for cancer treatment. Pharmacogenomics. 2013;14(9):1065-1084.
14. De Graan AJ, Elens L, Sprowl JA et al.: CYP3A4*22 genotype and systemic exposure affect paclitaxel-induced neurotoxicity. Clin Cancer Res. 2013;19(12):3316-3324.
15. Di Martino MT, Arbitrio M, Guzzi PH et al.: A peroxisome proliferator-activated receptor gamma (PPARG) polymorphism is associated with zoledronic acid-related osteonecrosis of the jaw in multiple myeloma patients: analysis by DMET microarray profiling. Br J Haematol. 2011;154(4):529–533.
16. Di Martino MT1, Arbitrio M, Leone E et al.: Single nucleotide polymorphisms of ABCC5 and ABCG1 transporter genes correlate to irinotecan-associated gastrointestinal toxicity in colorectal cancer patients: a DMET microarray profiling study. Cancer Biol Ther. 2011;12(9):780-7.
17. Deeken JF, Cormier T, Price DK et al.: A pharmacogenetic study of docetaxel and thalidomide in patients with castration-resistant prostate cancer using the DMET genotyping platform. Pharmacogenomics J. 2010;10(3):191-9.
18. Spc_Paclitaxel.: http://packageinserts.bms.com/pi/pi_taxol.pdf.
19. P450 Drug Interaction Table: Sustrates
20. http://medicine.iupui.edu/clinpharm/ddis/main-table/.
21. De Graan AJ, Elens L, Smid M et al.: A pharmacogenetic predictive model for paclitaxel clearance based on the

DMET platform. Clin Cancer Res. 2013;19(18):5210-5217.

22. Dumaual C, Miao X, Daly TM et al.: Comprehensive assessment of metabolic enzyme and transporter genes using the Affymetrix Targeted Genotyping System. Pharmacogenomics. 2007;8(3);293-305.
23. Cuzick J: A Wilcoxon-type test for trend. Stat Med. 1985;4(1):87-90.
24. Uchiyama T, Kanno H, Ishitani K et al.: An SNP in CYP39A1 is associated with severe neutropenia induced by docetaxel. Cancer Chemother Pharmacol. 2012;69(6):1617-1624.
25. Le XF, Bast RC Jr.: Src family kinases and paclitaxel sensitivity. Cancer Biol Ther. 2011;12(4):260-9.
26. Mathijssen RH, Sparreboom A, Verweij J: Determining the optimal dose in the development of anticancer agents. Nat Rev Clin Oncol. 2014;11(5):272-281.



Part II

Docetaxel

Chapter 4

Role of genetic variation in docetaxel-induced neutropenia and pharmacokinetics

A.J.M. Nieuweboer, M. Smid, A.M. de Graan, S. Elbouazzaoui, P. de Bruijn, F.A.L.M. Eskens, P. Hamberg, J.W.M. Martens, A. Sparreboom, R. de Wit, R.H.N. van Schaik and A.H.J. Mathijssen

The Pharmacogenomics Journal. 2015 Sep 8. Epub ahead of print.

ABSTRACT

Docetaxel is used for treatment of several solid malignancies. In this study, we aimed for predicting docetaxel clearance and docetaxel-induced neutropenia by developing several genetic models. Therefore, pharmacokinetic data and absolute neutrophil counts (ANCs) of 213 docetaxel-treated cancer patients were collected. Next, patients were genotyped for 1936 Single Nucleotide Polymorphisms (SNPs) in 225 genes using the Drug-Metabolizing Enzymes and Transporters (DMET) platform and thereafter split into two cohorts. The combination of SNPs that best predicted severe neutropenia or low clearance was selected in one cohort and validated in the other. Patients with severe neutropenia had lower docetaxel clearance than patients with ANCs in the normal range (P=0.01). Severe neutropenia was predicted with 70% sensitivity. True low clearance (1SD<mean clearance) was identified in 80% of cases. These models however did not reach statistical significance. To improve the predictive value of these models, the addition of non-genetic influencing factors is needed.

INTRODUCTION

Neutropenia is one of docetaxels' main dose limiting toxicities, which may cause neutropenic fever, subsequent dose reductions and early discontinuation of treatment. Dose calculation of docetaxel is currently solely based on a patients weight and length (body surface area) (1). Unfortunately, due to a wide variation in docetaxel exposure between patients this dosing strategy does not give much, if any, information on the actual systemic exposure to docetaxel in an individual patient (2). It is known that the risk of drug-related toxicities is correlated with the exposure to docetaxel, but is also highly variable (3). Therefore, it is still difficult to predict which patients will suffer from severe toxicity.

It is highly relevant to determine which factors are associated with the variability in docetaxel exposure and toxicity. Multiple environmental factors (that is, smoking behaviour, co-medication and adenoviruses), intrinsic factors (that is, gender and age) have been explored for their effects on the variation between patients in pharmacokinetics or pharmacodynamics (4-14). Besides these factors, a limited amount of genetic variants was studied for their role in this pharmacologic variability and differences in patients' predisposition for toxicities (15). However, the results of these studies have often been conflicting.

If it would be possible to better understand and predict the inter-patient variability in exposure and toxicity of docetaxel, dosing docetaxel could be individualized. As a result, physicians may attempt to administer a higher dose, within the dose-range of the therapeutic window, to individual patients with a potentially higher chance of obtaining anti-tumor efficacy.

In the current project, we studied the correlations between the risk of severe neutropenia (absolute neutrophil count (ANC) nadir $<1x10^9$/L or grade 3 neutropenia according to the Common Terminology Criteria for Adverse Events v.4.0) (http://www.eortc.be/services/doc/ ctc/ CTCAE_4.03_2010-06-14_ QuickReference_5x7.pdf) and the exposure to docetaxel in a large set of docetaxel-treated cancer patients, of which the majority was treated in a 3-weekly regimen. In addition, we investigated the effects of pharmacogenetic variability on the inter-patient variability in docetaxel exposure and docetaxel-induced severe neutropenia. Because previous candidate gene approaches did not give a final answer on this question, we here used the Drug-Metabolizing Enzymes and Transporters (DMET) platform, which enables us to simultaneously study 1936 single nucleotide polymorphisms (SNPs) on 225 genes involved in drug transport and metabolism. Subsequently, we aimed for developing genetic models to predict the risk of severe neutropenia and a low docetaxel clearance during docetaxel therapy.

METHODS

Patient inclusion

All 240 Caucasian cancer patients who have given written informed consent (between June 2004 and January 2014) for a pharmacokinetic study (Dutch Trial Registry number NTR2311 at www.trialregister.nl) were included in the current study. This study was carried out according to the declaration of Helsinki and had been approved by the Medical Ethical Board of the Erasmus Medical Center, Rotterdam, the Netherlands. All patients were receiving docetaxel for solid malignancies. Patients were treated in different treatment regimens. As shown in Table 1, the majority of patients were treated with 75mg/m^2 or 100mg/m^2 docetaxel every three weeks. Patients with a World Health Organization performance status ≥2 and the intake of strong CYP3A4 inducers or inhibitors were excluded (http://medicine.iup ui.edu/clinpharm/ddis/main-table/).

Renal and hepatic function had to be sufficient according to hospital protocol. Neutrophil counts had to be >1.5 x 10^9/L for docetaxel administration according to the product information of docetaxel (16). Neutrophil blood counts were drawn according to the physician's assessment.

Table 1. Patient characteristics

	Docetaxel-induced neutropenia		Low docetaxel clearance	
	Identification cohort	**Validation cohort**	**Identification cohort**	**Validation cohort**
Number of patients	98	99	96	98
Male (n, %)	37 (38%)	38 (38%)	41 (44%)	37 (38%)
Female (n, %)	61 (62%)	61 (62%)	55 (56%)	61(62%)
Premenopausal	19 (31%)	20 (33%)	18 (33%)	18 (29%)
Postmenopausal	36 (59%)	35 (57%)	31 (58%)	39 (64%)
Unknown	6 (10%)	6 (10%)	5 (9%)	4 (7%)
Mean age, years (range)	56 (17-80)	54 (24-79)	58 (17-80)	56 (24-79)
Cancer Type (n, %)				
Breast	56 (57%)	53 (54%)	45 (47%)	56 (57%)
Prostate	24 (25%)	21 (21%)	29 (30%)	6 (7%)
Melanoma	3 (3%)	6 (6%)	5(5%)	21 (21%)
Head/neck	3 (3%)	3 (3%)	1 (1%)	3 (3%)
Sarcoma	1 (1%)	6 (6%)	4(4%)	4 (4%)
Lung	1 (1%)	4 (4%)	0 (0%)	3 (3%)
Rest	10 (10%)	6 (6%)	12 (13%)	5 (5%)

Regimen (n, %)				
Weekly	1 (1%)	4 (4%)	1 (1%)	3 (3%)
3 weekly	82 (84%)	85 (86%)	84 (87%)	78 (80%)
Unknown or only 1 course	15 (15%)	10 (10%)	11 (12%)	17 (17%)
Median dose (n, %)				
30 mg/m^2	0 (0%)	5 (5%)	2 (2%)	3 (3%)
60 mg/m^2	0 (0%)	2 (2%)	1 (1%)	1 (1%)
75 mg/m^2	45 (46%)	43 (43%)	50 (52%)	40 (41%)
100 mg/m^2	46 (47%)	46 (47%)	42 (44%)	45 (46%)
Unknown	1 (1%)	0 (0%)	0 (0%)	0 (0%)
Rest	6 (6%)	3 (3%)	1 (1%)	9 (9%)
ANC data				
Patients with ANC <1x10^9/L	46 (47%)	46 (46%)	43 (45%)	42 (43%)
Patients with ANC ≥1x10^9/L	52 (53%)	53 (54%)	45 (47%)	48 (49%)
Unknown	0 (0%)	0 (0%)	8 (8%)	8 (8%)
Mean clearance (range)	45.2 (16.2-95.9)	44.7 (13.0-76.9)	45.6 (16.2-95.9)	44.1 (13.0-76.9)

Abbreviations: ANC: absolute neutrophil count

Pharmacogenetic and pharmacokinetic analyses

Pharmacogenetic and pharmacokinetic analyses were performed as described previously (17). For pharmacokinetic purposes, patients were sampled using a validated limited sampling strategy during at least one of the cycles with docetaxel (18). As an anti-coagulant, lithium heparin was used in all collected tubes. Pharmacokinetic measurements and sample handling was done as described before by de Graan *et al*. (19).

For pharmacogenetic purposes, patients were genotyped using whole blood. The MagnaPure LC (Roche Diagnostics GmbH, Mannheim Germany) was used for DNA isolation and the DMET Plus Premier Pack (DMET, Affymetrix, Santa Clara, CA, USA) was used for genotyping (17, 20). All 1936 SNPs were run simultaneously on this chip. The quality and trustworthiness of these genotypes was indicated by the "call-rate". When the call-rate for a sample was below 90%, this sample was removed from the analysis. Patients with missing neutrophil counts, pharmacokinetic data, or neurotoxicity data were excluded from the analysis of that particular topic.

Associations between docetaxel clearance and severe docetaxel-induced neutropenia

Neutrophil counts and neurotoxicity data were collected from patients' charts. Treating physicians had scored the neurotoxicity grade according to the common terminology criteria for adverse events (CTCAE) versions 2 to 4 (http://www.eortc.be/services/doc/ctc/CTCAE _4.03_ 2010-06-14_QuickReference_5x7.pfd).

Genetic model for predicting low absolute neutrophil counts

Patients were clustered in a 'high risk' and 'low risk' group according to their nadir ANC; with a nadir $<1.0x10^9$/L used to label patients as 'high risk'. All 197 patients for whom ANC (nadir) data were available were included in this analysis and randomly separated in an identification cohort (n=98) and a validation cohort (n=99). To exclude as much patient bound bias as possible, the cohorts were checked for bias in age, gender, and smoking status using the Chi-square test. For all patients, genotyping was done for 1936 SNPs in 225 genes that are involved in drug metabolism and/or drug transport using the DMET Plus Premier Pack (Affymetrix) according to former descriptions (17). SNPs of which only one genotype was observed in all patients (n= 985) and SNPs that were not in Hardy-Weinberg Equilibrium ($P<0.05$) (n=131) were excluded from the analysis. This resulted in 815 remaining SNPs. The genotypes of the SNPs were analysed in the identification cohort using conditional probability as previously described (17). This means that the probability of a patient of having severe neutropenia is calculated, given the manifestation of a particular genotype for a certain SNP. Thus, for each SNP, three probabilities (wildtype, heterozygous and homozygous genotype) were calculated. To select SNPs for inclusion in the profile, the following criteria were used: the probabilities of the wildtype and homozygous genotype had to be 15% higher than the standard risk of developing severe neutropenia. This standard risk was defined as the proportion of patients in the identification set showing a risk of severe neutropenia and was calculated to be 47% (46 out of 98 patients). We were aiming for finding SNPs that predicted the risk for a particular patient of severe neutropenia better than the overall risk of 47%. Next, the difference in probability score between WT and HOZ had to be >20%. SNPs reaching these criteria were checked for collinearity, i.e. all patients in the identification set having the same genotype for that SNP. If collinearity existed, one of the SNPs was randomly selected to be used, or in case of missing calls in one of the SNPs, the SNP with the most observations was kept.

The probabilities were subsequently used as weight in the analysis for predicting severe neutropenia. A score for each sample was calculated as the sum of probabilities of the SNPs in the profile. This score was associated with the 'high risk' or 'low risk' status of the patients in the identification set by using a receiver operation curve. In this curve, the capability of the predictive model for predicting severe neutropenia was made visual. A threshold was selected, based on the desired sensitivity in the identification set. Next, the probability scores for the patients in the validation cohort were calculated and compared with this threshold. Patients with a value above this threshold were predicted as 'high risk' patients, thus at risk for severe neutropenia. The predictions were matched with the ANC status and the sensitivity, specificity and positive predictive value were then calculated.

A genetic model for predicting low docetaxel clearance

A similar process as described for the nadir ANC was used to generate a model for the prediction of low docetaxel clearance. Here, low clearance was defined as clearance 1 standard deviation below the mean. This applied to 30 patients. Of all 194 patients with available data, patients were again split in an identification cohort and a validation cohort. Both cohorts were checked for bias in age, gender and smoking status. Both the discovery and validation cohort comprised 15 patients with low clearance. Identifying relevant SNPs associated with low clearance, calculation of the probability score, selecting the threshold and predicting the samples in the validation cohort were done as described for the nadir ANC model. Conditional probability was again used to select the relevant SNPs in the identification cohort as earlier described.

Statistics

SPSS version 21 (SPSS Inc.) and STATA version 13 (StatCorp LP, TX, USA) were used to perform the statistical analyses. A P-value <0.05 was defined to be statistically significant and data are shown as a mean with a range. The predictive value of genetic profiling on severe docetaxel-related neutropenia was considered as the primary endpoint. Secondary endpoints were the associations between docetaxel pharmacokinetics and neurotoxicity and severe docetaxel-induced neutropenia. In univariate analysis of SNP versus endpoints, no adjustments for multiple testing were made in view of the exploratory nature of the analysis. Comparisons between a category and continuous variable where tested using the Mann-Whitney test in case of 2 groups, in case of multiple groups the Kruskal-Wallis rank test was used. For comparisons between groups the Chi-square test was used. If the number of observations was too low to use the Chi-square test, a Fisher's exact test was used. To associate ANC as continuous variable with a SNP, a test for trend across ordered groups was used, which is an extension of the Wilcoxon rank-sum test (21).

RESULTS

Association between docetaxel clearance and severe docetaxel-induced neutropenia

A total of 213 patients had been genotyped and had demographic data available. A trend was seen between low clearance and severe neutropenia in the total population of which these data were available (n=202, *P*=0.056) when using the definition of low clearance being 1 standard deviation below the mean clearance (Table 2). However, in females only (n=73) it was seen that low clearance was associated with severe neutropenia (*P*=0.003), possibly indicating that females are more sensitive to higher docetaxel concentrations than male patients. Pharmacokinetic data were available for 194 of the genotyped patients**.** Of these 194 patients, 30 patients (15%) had a defined low clearance (1 SD < mean CL). For 197 of the total 213 patients, nadir ANC data were available. Of these 197 patients, 92 patients (47%) had severe neutropenia. Patients with severe neutropenia had a lower docetaxel clearance than patients with an ANC ≥1.0x10^9/L (mean difference=5.0 L/h, *P*=0.01). Neurotoxicity was

not significantly associated with docetaxel clearance (*P*=0.6) (Table 2). Neurotoxicity was seen in 66 out of these 125 patients (53%) of whom 15 had grade 2/3 neurotoxicity. Neurotoxicity was also not significantly associated with the cumulative dose of docetaxel (P=0.5).

Table 2. Correlations between clearance and docetaxel-induced toxicities

ANC	**ANC Count (*10^9/L)**	**Patients (n)**	**High CL [*1] (n)**	**Low CL [*1] (n)**	**P-value**
	≥ 1	106	96	10	
	< 1	96	78	18	*0.056*
Neurotoxicity	**Grade**	**Patients (n)**	**Mean CL (range)**		
	0	59	46.5 (16.2-84.0)		
	1	51	46.8 (20.3-95.9)		
	2	15	47.7 (22.0-62.7)		*0.627*

ANC: absolute neutrophil count, [*1] High CL: CL ≠1SD < mean CL, [*1] Low CL: CL 1SD, Mean CL: in L/h

Association between docetaxel exposure and severe docetaxel-induced neutropenia

For analyzing the association between the area under the curve (AUC) of docetaxel and severe neutropenia and neurotoxicity, patients receiving 30mg/m^2 docetaxel were excluded. We found that patients with severe neutropenia had a significantly higher docetaxel AUC than patients without severe neutropenia (n=196, *P*=0.008). Neurotoxicity was not correlated with the AUC of docetaxel (n=122, *P*=0.9).

Model for predicting severe neutropenia

In both the identification cohort (n=98) and the validation cohort (n=99), 46 patients (47%) had severe neutropenia. Patient characteristics in the identification cohort were similar to those in the validation cohort (n=99) (Table 1). There were no significant differences between the numbers of patients that smoked cigarettes in both cohorts. No differences in age (*P*=0.2) and gender (*P*=0.9) were seen in the two cohorts. In the identification cohort, 24 SNPs have been selected for inclusion in the predictive profile according to the criteria described in the method section (Table 3).

A probability score was calculated for all patients in the identification cohort, and the value for the prediction threshold was chosen where 90% of patients in the identification cohort with severe neutropenia showed scores above that particular threshold (Figure 1). Thus, the threshold was chosen to result in 90% sensitivity in the identification cohort (Figure 1). Next, the probability score of the patients in the validation cohort were calculated and – using the selected threshold – patients were predicted as high or low risk of severe neutropenia. In the validation cohort, the profile showed a

sensitivity of 70%, specificity of 21% and a positive predictive value of 43% (*P*=0.27). In total, 32 out of 46 patients with severe neutropenia were correctly predicted.

Table 3. SNP model for predicting docetaxel-induced neutropenia

rs SNP ID	Gene	Common name	Allele change
rs12248560	CYP2C19	CYP2C19_-806>(rs12248560)	C/T
rs2852425	NNMT	NNMT_15229>(rs2852425)	T/C
rs818202	CDA	CDA_1169>(rs818202)	G/A
rs2020861	FMO2	FMO2_13733A>G(S195S)	C/T
rs7512785	FMO2	FMO2_24435>(rs7512785)	C/T
rs7515157	FMO2	FMO2_24625>(rs7515157)	T/C
rs131713	ARSA	ARSA_>(rs131713)	C/T
rs3770602	ABCB11	ABCB11_-7056>(rs3770602)	T/C
rs3832043	UGT1A9	UGT1A9_>(rs3832043)	-/T
rs6759892	UGT1A9	UGT1A9_>(rs6759892)	G/T
rs7586110	UGTA1A1	UGT1A1_>(rs7586110)	T/G
rs13197674	GSTA4	GSTA4_-1366>(rs13197674)	G/A
rs8192879	CYP7A1	CYP7A1_9082>(rs8192879)	G/A
rs1138541	SLCO5A1	SLCO5A1_160281>(rs1138541)	A/G
rs4149057	SLCO1B1	SLCO1B1_37091T>C(L191L)	T/C
rs1339067	SLC15A1	SLC15A1_48241T>C(A449A)	C/T
rs8187758	SLC28A1	SLC28A1_17884C>A(Q237K)	C/A
rs2305367	SLC28A1	SLC28A1_45450G>A(K383K)	A/G
rs1060463	CYP4F11	CYP4F11_20043G>A(D446N)	G/A
rs2228099	ARNT	ARNT_40155G>C(V189V)	C/G
rs1051740	EPHX1	EPHX1_3203T>C(Y113H)	C/T
rs3749442	ABCC5	ABCC5_71596C>T(L1208L)	C/T
rs272893	SLC22A4	SLC22A4_32753T>C(I306T)	T/C
rs1050152	SLC22A4	SLC22A4_46011C>T(L503F)	C/T

SNPs: single nucleotide polymorphisms

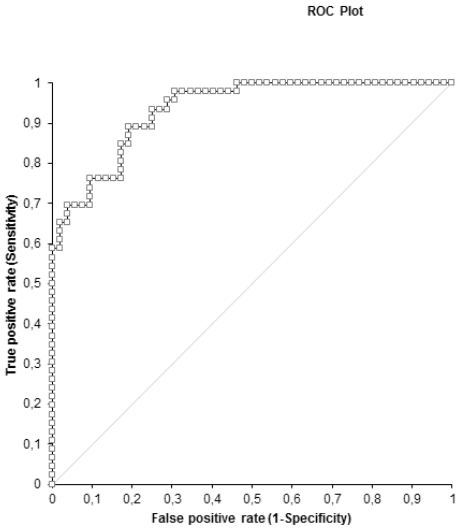


Figure 1. The ROC-curve for the genetic model for predicting neutropenia. This curve represents the true positives and the false positives at different cut-offs. The line from the left lower corner to the right upper corner is an example of a test with no distinctive ability. The curve of boxes (score sum) represents the ROC-curve of the genetic model predictive for ANCs <1.0x10^9/L.

A genetic model for predicting low docetaxel clearance

For this analysis, the total cohort of 194 patients for whom clearance data were available was split into an identification cohort (n=96) and a validation cohort (n=98). The cohorts were found similar regarding age, gender and smoking status. Both groups contained 15 patients (15% and 16% respectively) with a defined low clearance (1 SD < mean). By using conditional probability, 14 SNPs were selected in the patients from the identification cohort (Table 4). A cut-off point closest to reach a sensitivity of 90% to predict low clearance in the identification cohort was chosen, and similarly as described for neutropenia, the selected SNPs were subsequently validated in the validation cohort. The patients with a probability score above the cut-off point were predicted to have low docetaxel clearance. For this SNP-based profile, the sensitivity was 80%, the specificity was 35% and the positive predictive value was 18% (*P*=0.26).

Significant SNPs associated with neutropenia or low docetaxel clearance

When analysing the genotypes from all patients of whom ANC nadir data were available, 31 SNPs were statistically significantly associated with severe neutropenia (P<0.05, test for trend, Table 5). Thirty-three SNPs were associated with low docetaxel clearance (*P*<0.05, test for trend) in the patients for whom pharmacokinetic data were available (Table 6). Only rs1881668 in *SULT1E1* was found associated with both severe neutropenia and low docetaxel clearance.

Table 4. SNP model for predicting low docetaxel clearance

rs ID SNP	Gene	Common Name	Allele Change
rs2242046	SLC28A1	SLC28A1_47738G>A(D521N)	G/A
rs3743369	SLCO3A1	SLCO3A1_>(rs3743369)	A/G
rs11859842	SULT1A2	SULT1A2_A3_>(rs11859842)	A/G
rs2479390	GSTM5	GSTM5_1141>(rs2479390)	A/G
rs3788010	ABCG1	ABCG1_76650>(rs3788010)	A/G
rs1044317	ABCG1	ABCG1_40560>(rs1044317)	A/G
rs1541290	ABCG1	ABCG1_>(rs1541290)	A/G
rs6759892	UGT1A9	UGT1A9_>(rs6759892)	G/T
rs3814055	NR1I12	NR1I2_-26063>(rs3814055)	C/T
rs7662029	UGT2B7	UGT2B7_-327>(rs7662029)	A/G
rs3756067	ALB	ALB_-400>(rs3756067)	A/G
rs405729	GSTA4	GSTA4_16261>(rs405729)	A/G
rs2292334	SLC22A3	SLC22A3_88737G>A(A411A)	G/A
rs1208	NAT2	NAT2_803G>A(R268K)	A/G

SNPs: single nucleotide polymorphisms

Table 5. Significant SNPs associated with severe neutropenia

rs ID SNP	Gene	Common Name	Allele Change	p-value
rs1050152	SLC22A4	SLC22A4_46011C>T(L503F)	C/T	0.001
rs1800822	FMO3	FMO3_15136C>T(S147S)	C/T	0.004
rs1799814	CYP1A1	CYP1A1_2452C>A(T461N)	C/A	0.014
rs2305367	SLC28A1	SLC28A1_45450G>A(K383K)	A/G	0.016
rs1202283	ABCB4	ABCB4_22490C>T(N168N)	T/C	0.016
rs1060253	SLC7A5	SLC7A5_36891>(rs1060253)	C/G	0.017
rs6915115	PPARD	PPARD_-19920>(rs6915115)	C/T	0.018
rs3765070	CYP4F11	CYP4F11_4927T>C(I106I)	C/T	0.019
rs4148271	UGT2B15	UGT2B15_328324>(rs4148271)	T/A	0.021
rs6755571	UGT1A4	UGT1A4_>(rs6755571)	C/A	0.022
rs272879	SLC22A4	SLC22A4_40237C>G(T394T)	G/C	0.023
rs909530	FMO3	FMO3_21375C>T(N285N)	C/T	0.024
rs1060463	CYP4F11	CYP4F11_20043G>A(D446N)	G/A	0.026
rs3786362	TYMS	TYMS_4505A>G(E127E)	A/G	0.027
rs272893	SCL22A4	SLC22A4_32753T>C(I306T)	T/C	0.027
rs7483	GSTM3	GSTM3_3209G>A(V224I)	A/G	0.030
rs2274407	ABCC4	ABCC4_94534G>T(R304S)	G/T	0.033
s1881668	SULT1E1	SULT1E1_-2094>(rs1881668)	C/G	0.034
rs854560	PON	PON1_7704T>G(L55V)	T/A	0.037
rs2140516	SLC13A1	SLC13A1_30767A>G(N174S)	A/G	0.037
rs17102596	MAT1A	MAT1A_14007>(rs17102596)	A/G	0.040
rs6577	GSTA2	GSTA2_7331A>C(E210A)	A/C	0.040
rs1803684	GSTA2	GSTA2_-10>(rs1803684)	C/G	0.040
rs7748890	GSTA5	GSTA5_-3506>(rs7748890)	C/T	0.040
rs1736565	FMO6	FMO6_>(rs1736565)	C/T	0.042
rs818202	CDA	CDA_1169>(rs818202)	G/A	0.043
rs82693844	CSHT9	CHST9_-66>(rs28693844)	T/C	0.046
rs16947	CYP2D6	CYP2D6_2851T>C(C296R)	T/C	0.047
rs17596954	GSTM5	GSTM5_2656T>C(L128L)	C/T	0.048
rs7087728	MAT1A	MAT1A_15710>(rs7087728)	T/C	0.050
rs8187832	SLC15A1	SLC15A1_64082C>T(N509N)	C/T	0.050

SNPs: single nucleotide polymorphisms

Table 6. Significant SNPs associated with low docetaxel clearance

rs ID SNP	Gene	Common Name	Allele Change	p-value
rs8192868	TBXAS1	TBXAS1_186455G>A(E450K)	A/G	0.001
rs3822172	SULT1E1	SULT1E1_-478>(rs3822172)	A/G	0.002
rs7512785	FMO2	FMO2_24435>(rs7512785)	C/T	0.005
rs7515157	FMO2	FMO2_24625>(rs7515157)	T/C	0.005
rs3736599	SULT1E1	SULT1E1_-2459>(rs3736599)	G/A	0.007
rs2835286	CBR3	CBR3_10872>(rs2835286)	A/G	0.011
rs2020869	FMO2	FMO2_23300>(rs2020869)	A/G	0.011
rs1801282	PPARG	PPARG_-28080>(rs1801282)	C/G	0.011
rs7853758	SLC28A3	SLC28A3_54623C>T(L461L)	T/C	0.011
rs13197674	GSTA4	GSTA4_-1366>(rs13197674)	G/A	0.012
rs12208357	SLC22A1	SLC22A1_181C>T(R61C)	C/T	0.012
rs683369	SLC22A1	SLC22A1_8237G>C(L160F)	C/G	0.012
rs2231142	ABCG2	ABCG2_8825C>A(Q141K)	C/A	0.014
rs2297322	SLC15A1	SLC15A1_28672G>A(S117N)	A/G	0.014
rs6196	NR3C1	NR3C1_118915T>C(N766N)	T/C	0.015
rs1883322	PPARD	PPARD_-9059>(rs1883322)	T/C	0.016
rs2214102	ABCB1	ABCB1_-1>(rs2214102)	A/G	0.018
rs207440	XDH	XDH_75121G>A(E1239E)	A/G	0.021
rs2237667	PPARD	PPARD_-6341>(rs2267667)	G/C	0.023
rs3322	RALBP1	RALBP1_23648>(rs3322)	A/G	0.024
rs7751481	PPARD	PPARD_-7112>(rs7751481)	G/A	0.025
rs506008	GSTM4	GSTM4_2688T>C(F178F)	T/C	0.026
rs2072671	CDA	CDA_79A>C(K27Q)	C/A	0.029
rs3749442	ABCC5	ABCC5_71596C>T(L1208L)	C/T	0.031
rs305968	CYP2F1	CYP2F1_96G>A(P32P)	G/A	0.032
rs6906237	PPARD	PPARD_-3339>(rs6906237)	A/C	0.032
rs1881668	SULT1E1	SULT1E1_-2094>(rs1881668)	C/G	0.033
rs41507953	EPHX2	EPHX2_9780A>G(K55R)	A/G	0.034
rs909921	TPSG1	TPSG1_1450>(rs909921)	G/A	0.038
rs17863783	UGT1A6	UGT1A6_>(rs17863783)	G/T	0.046
unknown	CYP2C8	CYP2C8_2189A>-(unk1)	-/A	0.047
rs1049434	SLC16A1	SLC16A1_15385T>A(D490E)	A/T	0.048
rs7867504	SCL28A3	SLC28A3_35313A>G(T89T)	G/A	0.050

SNPs: single nucleotide polymorphisms

DISCUSSION

In this study, almost 50% of the included patients had severe neutropenia, confirming that neutropenia is indeed a frequently occurring side effect of docetaxel therapy, potentially leading to neutropenic fever, which is of great clinical relevance. As expected, patients with severe neutropenia had a significantly lower docetaxel clearance than patients with ANCs ≥1.0x10^9/L. For predicting severe neutropenia, we developed a DMET-based 24-SNP model with a sensitivity of 70%. Unfortunately, this model did not reach the significance level. Also, a 14-SNP model was developed for predicting low docetaxel clearance with 80% sensitivity. Again however, this model did not reach statistical significance. This indicates that the 1936 SNPs in the 225 genes on the DMET chip are probably not (fully) representative for explaining inter-patient variation in docetaxel clearance and neutropenia in our identification and validation sets. Despite the large cohort of patients studied, a lack of power due to sample size cannot be excluded as a reason for not reaching statistical significance. Another reason for this might be the impact of non-genetic variables such as gender, castration status, menopausal status and drug-drug interactions on inter-individual variability in pharmacokinetics as well as toxicity (22-24). For neutropenia, previous cytotoxic treatment might also influence the severity of toxicity, as it theoretically changes the bone marrow 'reserve'.

A trend was seen for the association between low clearance and severe neutropenia in the total population when defining low clearance as clearance that is 1 standard deviation below the mean clearance in this population. When looking at absolute numbers, we found that 18 out of 28 low clearance patients had neutropenia (64%), which is more than the standard risk for severe neutropenia that we observed in our total patient population (47%). Nonetheless, patients who do not easily clear docetaxel are not always prone for neutropenia, suggesting that other mechanisms may also be involved in the development of this dose-limiting side effect (25). When separately analyzing only female patients, low clearance was significantly associated with severe neutropenia. This may suggest that female patients might be more sensitive to higher docetaxel concentrations than male patients. However, this difference may also be caused by the fact that female breast cancer patients tend to get higher doses than most male patients. It yet remains unclear if rs1881668 in *SULT1E1,* a protein that plays a role in the levels of oestrogens, which was found associated with both neutropenia and low docetaxel clearance, may partly be responsible for this gender based difference (26).

Interestingly, neurotoxicity was not associated with docetaxel clearance, contrary to the related taxane paclitaxel (27). A SNP recently correlated to paclitaxel-induced neurotoxicity (*CYP3A4*22*) was not available on the DMET chip, so a potential relationship is not excluded. Also, the incidence of docetaxel-induced neuropathy seems to be lower than that of paclitaxel, although the cohorts of patients were not fully comparable (28, 29). The effects of co-prescribed anti-cancer agents on neurotoxicity seem limited because the majority of patients received docetaxel mono-therapy. The fact that neurotoxicity was not significantly correlated with the cumulative dose of docetaxel might be due to the fact that only 18 out of 137 patients that this data was available for suffered from grade 2/3 neurotoxicity.

Some of the identified SNPs in the developed models have also been previously described in the context of toxicity, clearance and taxane therapy. In the gene *FMO3* for example, the SNP rs909530

is previously identified to be associated with docetaxel-induced febrile neutropenia and a reduction in neutrophil count cycle (from cycle 1 to cycle 2) in a group of 100 Lebanese cancer patients (30). In that study, also rs1065852 in *CYP2D6* was identified to be associated with febrile neutropenia. We found another variation (rs16947) in *CYP2D6* to be associated with severe neutropenia, suggesting a possible role of this phase I enzyme in the existence of docetaxel-related neutropenia. Other SNPs identified in that study did not come up in our analysis and vice versa (30). The different ethnic background of the included patients in these studies may have played a role.

We found that rs13197674 in the glutathione S-transferase gene (*GSTA4)* was associated with low docetaxel clearance. This SNP had already been described to be associated with low paclitaxel clearance (17). The *GSTA4* gene is involved in the defence against by-products of oxidative metabolism (31). Two other SNPs on *GSTA4* have also been described to be associated with docetaxel clearance (32). Next to the SNP in GSTA4, SNPs in the genes *CDA* (cytadine transaminase), *EPHX2* (epoxide hydroxylase 2) and SLC22A1 (solute carrier family 22 member A1) *w*ere also previously found to be associated with low paclitaxel clearance (17). In addition, variation in the *CDA* gene had been correlated with haematological toxicity in gemcitabine patients (33). We however, did not find the same SNPs. Differences in the incidences of these genotypes make it possible that SNPs in this analysis have been excluded based on a lack of variety or for example not being in Hardy-Weinberg Equilibrium. However, different SNPs could have similar effects on the function of a gene. The value of these findings thus lies in the apparent importance of these particular genes in taxane pharmacokinetics.

The limited effects of genetic variation on docetaxel clearance are, most likely, attributed to masking effects of other factors influencing docetaxel PK. Dividing the cohorts based on patients' gender could possibly improve results (9, 23). However, limited amounts of patients do not allow such subset analyses. Endocrine factors, among other factors, such as menopausal status and castration status might also influence docetaxel PK, and therefore mask underlying genetic effects (23, 25).

In conclusion, the majority of patients at a higher risk for severe neutropenia and low docetaxel clearance could be identified using models based on genetic variation. However, in this study these models did not reach statistically significance, indicating that variation in the DMET genes is (at most) of limited value for explaining inter-patient variability in severe docetaxel-induced neutropenia and docetaxel clearance. Our findings indicate that new predictive models need to be developed combining genetic, environmental and intrinsic factors in sufficiently sized and homogeneous patient cohorts.

REFERENCES

1. Mosteller RD. Simplified calculation of body-surface area. N Engl J Med 1987;317(17):1098.
2. Bins S, Ratain MJ, Mathijssen RH. Conventional dosing of anticancer agents: precisely wrong or just inaccurate? Clin Pharmacol Ther. 2014;95(4):361-364.
3. Baker SD, Li J, ten Tije AJ, Figg WD, Graveland W, Verweij J, et al. Relationship of systemic exposure to unbound docetaxel and neutropenia. Clin Pharmacol Ther. 2005;77(1):43-53.
4. de Graan AJ, Loos WJ, Friberg LE, Baker SD, van der Bol JM, van Doorn L, et al. Influence of smoking on the pharmacokinetics and toxicity profiles of taxane therapy. Clinical Cancer Res. 2012;18(16):4425-4432.
5. Wonganan P, Zamboni WC, Strychor S, Dekker JD, Croyle MA. Drug-virus interaction: effect of administration of recombinant adenoviruses on the pharmacokinetics of docetaxel in a rat model. Cancer Gene Ther. 2009;16(5):405-414.
6. Puisset F, Alexandre J, Treluyer JM, Raoul V, Roche H, Goldwasser F, et al. Clinical pharmacodynamic factors in docetaxel toxicity. Br J Cancer. 2007;97(3):290-296.
7. Bruno R, Vivier N, Veyrat-Follet C, Montay G, Rhodes GR. Population pharmacokinetics and pharmacokinetic-pharmacodynamic relationships for docetaxel. Invest New Drugs. 2001;19(2):163-169.
8. Chew SC, Sandanaraj E, Singh O, Chen X, Tan EH, Lim WT, et al. Influence of SLCO1B3 haplotype-tag SNPs on docetaxel disposition in Chinese nasopharyngeal cancer patients. Br J Clin Pharmacol . 2012;73(4):606-618.
9. Baker SD, Verweij J, Cusatis GA, van Schaik RH, Marsh S, Orwick SJ, et al. Pharmacogenetic pathway analysis of docetaxel elimination. Clin Pharmacol Ther. 2009;85(2):155-163.
10. Mathijssen RH, Sparreboom A, Verweij J. Determining the optimal dose in the development of anticancer agents. Nat Rev Clin Oncol. 2014;11(5):272-281.
11. Kraut EH, Rhoades C, Zhang Y, Cheng H, Aimiumu J, Chen P, et al. Phase I and pharmacokinetic study of erlotinib (OSI-774) in combination with docetaxel in squamous cell carcinoma of the head and neck (SSCHN). Cancer Chem and Pharm. 2011;67(3):579-586.
12. Moulder S, Gladish G, Ensor J, Gonzalez-Angulo AM, Cristofanilli M, Murray JL, et al. A phase 1 study of weekly everolimus (RAD001) in combination with docetaxel in patients with metastatic breast cancer. Cancer. 2012;118(9):2378-2384.
13. Itoh K, Sasaki Y, Fujii H, Minami H, Ohtsu T, Wakita H, et al. Study of dose escalation and sequence switching of administration of the combination of docetaxel and doxorubicin in advanced breast cancer. Clin Cancer Res. 2000;6(10):4082-4090.
14. Hamberg P, Mathijssen RH, de Bruijn P, Leonowens C, van der Biessen D, Eskens FA, et al. Impact of pazopanib on docetaxel exposure: results of a phase I combination study with two different docetaxel schedules. Cancer Chemother and Pharmacol. 2014;75(2):365-71.
15. Krens SD, McLeod HL, Hertz DL. Pharmacogenetics, enzyme probes and therapeutic drug monitoring as potential tools for individualizing taxane therapy. Pharmacogenomics. 2013;14(5):555-574.
16. SPC_docetaxel. Taxotere 160mg/8ml concentrate for solution for infusion. Available at:
17. http://www.medicines.org.uk/emc/medicine/25464/spc.
18. de Graan AJ, Elens L, Smid M, Martens JW, Sparreboom A, Nieuweboer AJ, et al. A pharmacogenetic predictive model for paclitaxel clearance based on the DMET platform. Clin Cancer Res. 2013;19(18):5210-5217.
19. Lustig V, Rosing H, Warmerdam LJC, Huizing MT, Bokkel Huinink WW, Dubbelman AC, et al. Limited Sampling Models for the Pharmacokinetics of Docetaxel. Clin Drug Invest. 1997;13(5):247-254.
20. de Graan AJ, 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(16):4433-4440.

21. Dumaual C, Miao X, Daly TM, Bruckner C, Njau R, Fu DJ, et al. Comprehensive assessment of metabolic enzyme and transporter genes using the Affymetrix Targeted Genotyping System. Pharmacogenomics. 2007;8(3):293-305.
22. Cuzick J. A Wilcoxon-type test for trend. Stat Med. 1985;4(1):87-90.
23. Nieuweboer AJ, de Morrée ES, de Graan AJ, Sparreboom A, de Wit R, Mathijssen RH. Inter-patient variability in docetaxel pharmacokinetics: A review. Cancer Treat Rev. 2015;41(7):605-13
24. Franke RM, Carducci MA, Rudek MA, Baker SD, Sparreboom A. Castration-dependent pharmacokinetics of docetaxel in patients with prostate cancer. J Clin Oncol. 2010;28(30):4562-4567.
25. Fajac A, Gligorov J, Rezai K, Levy P, Levy E, Selle F, et al. Effect of ABCB1 C3435T polymorphism on docetaxel pharmacokinetics according to menopausal status in breast cancer patients. Br J Cancer. 2010;103(4):560-566.
26. Iusuf D, Hendrikx JJ, 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(1):225-233.
27. Ihunnah CA, Wada T, Philips BJ, Ravuri SK, Gibbs RB, Kirisci L, et al. Estrogen sulfotransferase/SULT1E1 promotes human adipogenesis. Mol Cell Biol. 2014;34(9):1682-1694.
28. de Graan AJ, Elens L, Sprowl JA, Sparreboom A, Friberg LE, van der Holt B, et al. CYP3A4*22 genotype and systemic exposure affect paclitaxel-induced neurotoxicity. Clin Cancer Res. 2013;19(12):3316-3324.
29. Argyriou AA, Kyritsis AP, Makatsoris T, Kalofonos HP. Chemotherapy-induced peripheral neuropathy in adults: a comprehensive update of the literature. Cancer Manag Res. 2014;6:135-147.
30. Lee JJ, Swain SM. Peripheral neuropathy induced by microtubule-stabilizing agents. J Clin Oncol. 2006;24(10):1633-1642.
31. Awada Z, Haider S, Tfayli A, Bazarbachi A, El-Saghir NS, Salem Z, et al. Pharmacogenomics variation in drug metabolizing enzymes and transporters in relation to docetaxel toxicity in Lebanese breast cancer patients: paving the way for OMICs in low and middle income countries. OMICS 2013;17(7):353-367.
32. Liu S, Stoesz SP, Pickett CB. Identification of a novel human glutathione S-transferase using bioinformatics. Arch Biochem Biophys. 1998;352(2):306-313.
33. Edvardsen H, Brunsvig PF, Solvang H, Tsalenko A, Andersen A, Syvanen AC, et al. SNPs in genes coding for ROS metabolism and signalling in association with docetaxel clearance. Pharmacogenomics J. 2010;10(6):513-523.
34. Li H, Wang X, Wang X. The impact of CDA A79C gene polymorphisms on the response and hematologic toxicity in gemcitabine-treated patients: A meta-analysis. Int J Biol Markers. 2014;29(3): e224-32.

Chapter 5

Inter-patient variability in docetaxel pharmacokinetics: a review

A.J.M. Nieuweboer*, E.S. de Morrée*, A.M. de Graan, A. Sparreboom, R. de Wit, A.H.J. Mathijssen

Cancer Treatment Reviews. 2015 Jul;41(7):605-13

* these authors contributed equally

ABSTRACT

Docetaxel is a frequently used chemotherapeutic agent in the treatment of solid cancers. Because of the large inter-individual variability (IIV) in the pharmacokinetics (PK) of docetaxel, it is challenging to determine the optimal dose in individual patients in order to achieve optimal efficacy and acceptable toxicity. Despite the established correlation between systemic docetaxel exposure and efficacy, the precise factors influencing docetaxel PK are not yet completely understood. This review article highlights currently known factors that influence docetaxel PK, and focuses on those that are clinically relevant. For example, liver impairment should be taken into account when calculating docetaxel dosages as this may decrease docetaxel clearance. In addition, drug-drug interactions may be of distinct clinical importance when using docetaxel. Particularly, drugs strongly inhibiting CYP3A4 such as ketoconazole should not be concurrently administered without dose modification, as they may decrease the clearance of docetaxel. Gender, castration status, and menopausal status might be of importance as potential factors influencing docetaxel PK. The role of pharmacogenetics in predicting docetaxel PK is still limited, since no polymorphisms of clinical importance have yet been established.

INTRODUCTION

Docetaxel is approved for the treatment of several solid malignancies, including non-small cell lung cancer (NSCLC), metastatic castration resistant prostate cancer (mCRPC), breast cancer and head and neck cancer (1,2). Most of these cancers typically occur in elderly people, who may have comorbidities, organ dysfunction and are using various medications. The pharmacokinetics (PK) of docetaxel are highly variable, ranging from 30-45% (3). Therefore, it is challenging to predict toxicity and antitumor activity of docetaxel in individual patients. Ideally, patients would be individually dosed to prevent toxicity and improve the efficacy of docetaxel.

It is believed that the systemic exposure to a drug like docetaxel is related to its efficacy (4). This was also shown by Bruno et al., who found that the area under the plasma-concentration time curve (AUC) of the initial course of docetaxel was a predictor of time to progression in NSCLC patients (5). Also, a decreased clearance (CL) increased the risk of grade 4 and febrile neutropenia (6). Knowledge of factors that are of importance for PK variability could therefore lead to the optimization of docetaxel therapy.

Several studies have focused on determining factors that may influence docetaxel PK, aiming for better prediction of toxicity and exposure to docetaxel (see Figure 1). This excessive sum of studies makes it difficult to extract clinically relevant findings for usage in daily clinical practice. Hence, no label changes for docetaxel dosing have been made in the last decade although the current dosing strategy using body surface area (BSA) has been criticized, as this dosing strategy does not reduce the inter-individual variability (IIV) in docetaxel PK to an absolute minimum, since it does not account for other factors influencing docetaxel PK. This review article gives a comprehensive summary on the currently available and clinically relevant factors influencing docetaxel PK that can aid in individualizing docetaxel therapy in current clinical practice.

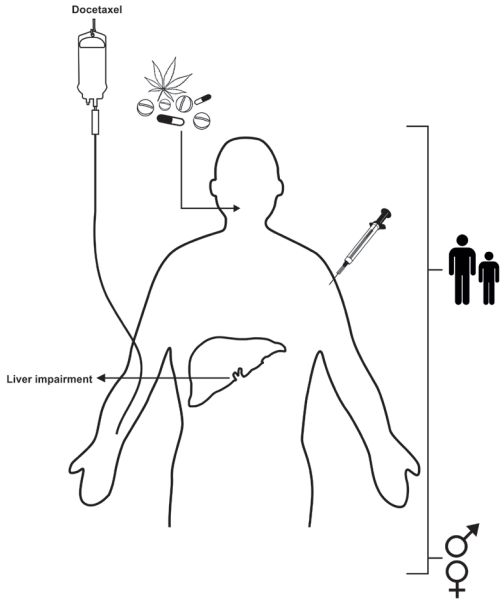


Figure 1. Factors influencing docetaxel pharmacokinetics. Co-medication and the use of complementary alternative medicines (CAMs) impact docetaxel PK in a clinically relevant way and should be taken into account when optimizing docetaxel treatment. In addition, patient related factors such as liver impairment, gender and hormonal status could potentially influence docetaxel PK.

DRUG TRANSPORTERS INVOLVED IN DOCETAXEL PHARMACOLOGY

Drug transporters and docetaxel pharmacokinetics

The activity of docetaxel-transporters could be altered due to drug-drug interactions, which potentially influences the PK of docetaxel. The largest family of drug transporters consists out of passive transporters: the solute carriers (SLCs), which cover 48% of the total amount of transporters. Docetaxel is a known substrate of SLC22A7 (7), SLCO1B1 (8), SLCO1B3 (9), SLC22A7 (7) and possibly SLCO1A2 (7,10). Besides SLCs, members of the ATP-binding cassette (ABC) transporters are extensively studied with regard to multidrug resistance and the PK of several anticancer drugs. Docetaxel is known to be transported by ABCB1 (ATP-binding cassette transporter B1, p-glycoprotein (p-gp)) [11]{Manzo, 2012 #2255}, ABCC2 (canalicular multispecific organic anion transporter 1 (*cMOAT*), MRP2) (12) and ABCC10 (multidrug resistance-associated protein 7 (MRP7)) (13).

Absorption

The gastro-intestinal absorption of docetaxel is limited. This is because ABCB1 directly excretes docetaxel into the intestinal lumen or bile (14). Moreover, docetaxel‘ s bioavailability is greatly reduced by the liver‘s first pass effect (15). Docetaxel is currently only being administered intravenously. As oral administrations of docetaxel could be more patient friendly, research is ongoing to improve the bioavailability of docetaxel (16,17).

Tissue distribution and accumulation

Over ninety percent of docetaxel is bound to plasma proteins (1). Because of its lipophilic properties, docetaxel has a large distribution volume, indicating accumulation in several tissues (1). Based on a bio-distribution study in cancer patients, a high uptake of [^{11}C]-docetaxel in the liver and gall bladder was seen, while there w as fewer uptake in the small intestines, kidney, bone marrow, lungs and bladder (18). Uptake of docetaxel in the brain was limited, resulting from an effective blood brain barrier containing efflux transporters like ABCB1 and ABCC2 (19).

Docetaxel metabolism and excretion

Hepatic uptake

Docetaxel is metabolized in the liver (Figure 2). Uptake is facilitated via uptake transporters such as Organic Anion Transporting Peptides (OATP) 1B1 and OATP1B3, which belong to the SLC family. These transporters mediate the uptake of docetaxel from sinusoidal blood into the hepatocytes (8-10). Iusuf and colleagues recently found that OATP1A2 was also involved in the *in vivo* uptake of docetaxel (10). Animal studies with the OATP1B3/OATP1B1 orthologue OATP1B2 showed that the CL of docetaxel is substantially decreased in OATP1B2 knockout mice (8, 10, 20, 21) in a manner that resembles drug phenotypes observed in mice with a deficiency of metabolic Cyp3a activity (22). Therefore, co-medication that inhibits both OATP1B1 and 1B3 should only be used with caution in combination with docetaxel. Also, we previously found that docetaxel's formulation vehicle polysorbate 80 could inhibit the uptake of docetaxel via interaction with OATP1B1 and OATP1B3 (8, 21).

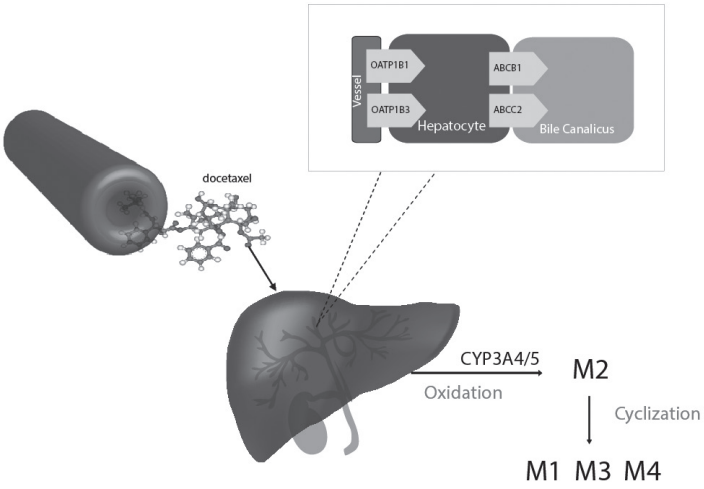


Figure 2. A schematic overview of docetaxel metabolism. Docetaxel is transported from the blood into the hepatocytes by OATP1B1 and OATP1B3. CYP3A4, and to a lesser extend CYP3A5, are responsible for the metabolism of docetaxel. ABCB1 and ABCC2 are accountable for the transport from hepatocyte into the bile canalicus.

Metabolism of docetaxel

Docetaxel is mainly metabolized via CYP3A4 and to a lesser extent by CYP3A5, and is processed into four metabolites (Figure 2) (23). A methyl group of docetaxel is oxidized into a primary alcohol forming metabolite M2. Further oxidation of M2 leads to formation of unstable metabolites of the alcohol that will lead to diastereoisomers (M1/M3) and a ketone metabolite (M4) (24). There are no indications that docetaxel-metabolites undergo phase II metabolism. All four metabolites showed limited anti-tumor activity (25), which suggests that the metabolism of docetaxel is the main contributor in the inactivation of the drug.

Excretion of docetaxel

Docetaxel and its metabolites are mainly excreted into bile via ABCB1 and ABCC2 mediated transport (11,12). Tumor cells can also express ABCB1 what will cause efflux of docetaxel and thus possible docetaxel-resistance. Therefore, clinical studies were designed to combine ABCB1-inhibitors in combination with docetaxel therapy. The first studies focused on PK interactions between ABCB1 inhibitors and docetaxel but did not show any interactions on PK level (26-28), indicating that the role of the ABCB1 transporter in the elimination of docetaxel is probably not the most dominant.

PHARMACOKINETIC DRUG INTERACTIONS

Cancer patients use numerous drugs for the treatment of chemotherapy related side effects, comorbidities, and the management of cancer related pain. Therefore, studying pharmacokinetic drug-drug interactions is highly clinically important. As docetaxel has a narrow therapeutic window, pharmacokinetic interactions with drugs for supportive care as well as with complementary alternative medicines (CAMs) are of great clinical relevance for its pharmacodynamics. According to FDA guidelines, drug-drug interactions are generally considered clinically relevant when the difference in exposure after the addition of the co-medication of subject is 25% or more (29). As the IIV of docetaxel already ranges from 30-45%, it is therefore challenging to identify clinically relevant drug-drug interactions.

Interactions with anti-cancer agents

The concurrent use of anti-cancer drugs is common in the treatment of many tumor types. Current regulations regarding the clinical implementation of new anti-cancer regimens oblige extensive Phase I studies looking into synergistic effects and pharmacokinetic interactions. Here, we will not focus on possible synergistic effect of combination strategies, but only review pharmacokinetic effects and adverse events. In Table 1, an overview of studied anti-cancer drug combinations is given (30-51).

Docetaxel CL decreased with 50% when topotecan was administered on day 1-4 preceding the administration of docetaxel (42). This resulted in increased neutropenia. The combination docetaxel and everolimus was associated with severe neutropenia and wide variation in the CL of both drugs (48). Authors state that concomitant treatment with these drugs is unpredictable due to a large variability in the CL of both drugs (48).

Table 1. Drug-drug interactions with anti-cancer drugs

Drugs	Interaction*	Effect	Ref
Cytostatics			
Cisplatin	no		[30]
Estramustine	no		[31]
5-FU	no		[32]
Capecitabine	no		[33]
Irinotecan	no		[34]
Carboplatin	no		[35]
Gemcitabine	no		[36]
Methotrexate	no		[37]
Cisplatin and 5-FU	no		[38]
Vinorelbine	no		[39]
Doxorubicin	yes	DTX followed by doxorubicin: duration grade 4 neutropenia	[40]
Ifosfamide	yes	DTX preceding ifosfamide: ↓ AUC ifosfamide	[41]
		On DTX AUC: no effect	
Topotecan	yes	Topotecan 1-4 days before DTX: DTX CL 50% ↓	[42]
Paclitaxel	yes	No effect DTX on paclitaxel	[43]
		DTX before paclitaxel: nadir ANC ↓	
Protein kinase inhibitors			
Lapatinib	no		[44]
Sunitinib	no		[45]
Imatinib	no	Inhibits CYP3A4, no effect on DTX CL	[46]
Erlotinib	yes	Substantial toxicity, not related to PK	[47]
Everolimus	yes	Substantial neutropenia and highly variable CL	[48]
Monoclonal antibodies			
Pertuzumab	no		[49]
Other			
Amifostine	no		[50]
Bortezomib	no		[51]

*clinically relevant interaction, DTX=docetaxel, AUC= area under the curve, CL=clearance, ANC= absolute neutrophil count, PK=pharmacokinetics, Ref=reference

The combination docetaxel-erlotinib was associated with severe toxicity, without a significant change in PK (47). In contrast, in a phase I and PK study on the combination of docetaxel and pazopanib, a lower docetaxel CL was found due to pazopanib co-treatment (52). This probably results from OATP1B1 and CYP3A4 inhibition. For doxorubicin holds that when given before the administration of docetaxel instead of after the administration, a longer duration of grade 4 neutropenia was seen (40). The AUC of ifosfamide decreased when the administration was preceded by docetaxel (41).

Interactions with supportive medication

Patients may receive co-medication for treatment-associated side-effects such as nausea and vomiting. These toxicities can often be well treated with anti-emetic prophylaxis. Aprepitant was shown to inhibit CYP3A4 and induce CYP2C9 (53). Therefore, this drug could hypothetically decrease docetaxel CL (54). However, neither aprepitant nor other studied antiemetic drugs showed a clinically relevant interaction with docetaxel so far (see Table 2) and can therefore be safely used in docetaxel-treated patients (55-57). Besides regular drugs for the management of nausea and vomiting, cannabis was demonstrated to be effective and was approved by the FDA (58). No effects on docetaxel PK were demonstrated (59).

Interactions with Complementary Alternative Medicines (CAMs)

It is estimated that 40% of cancer patients seek relieve from anticancer therapy related adverse events by using complementary alternative medicine (CAMs) (60). Herbal and dietary supplements mostly influence the PK of docetaxel via CYP3A4, drug transporters and other metabolic pathways, thereby again potentially influencing toxicity and therapeutic efficacy (61). Patients should thus be well counseled if preference to CAMs is given in supportive care. Here, we discuss frequently used CAMs in cancer patients with regard to docetaxel PK (Table 2).

In breast cancer patients, a trend towards reduced docetaxel CL was found for patients using 600 mg of garlic twice daily for 12 consecutive days (62). In addition, St. John's wort was found to decrease docetaxel AUC from 3,035 ± 756 to 2,682 ± 717 ng h/mL, indicating that concomitant use of docetaxel and St. John's wort could diminish clinical efficacy and should thus be avoided (63). *Echinacea purpurea* also induces CYP3A4 activity, but does not influence docetaxel PK (64). This is consistent with earlier observations that the administration of other CYP3A4-inducing medications, such as dexamethasone, does not substantially alter the clearance of docetaxel (14). These collective observations are congruent with the supposition that, since >90% of the docetaxel dose is already metabolized by CYP3A enzymes in a normal (uninduced) state, induction of this route is unlikely to result in a further substantial increase in the extent to which the drug undergoes metabolic inactivation.

Preclinical studies suggested that components in grape seed, green tea and milk thistle potentially inhibit CYP3A4 activity, which could alter docetaxel PK. This however needs further validation in clinical setting (29). To note, caution is warranted when interpreting the results of studies on CAMs, as various (non-standardized) formulations with different concentrations of the active compound are available and used (65). Concentrations of the active compound in these varying formulations could differ and similarities in study outcome could be masked.

Table 2. Drug-drug interactions with co-administered medication

Subject	Co-administration	Endpoint	Interaction*	Effect	Ref
Co-medication	Dexamethason *premedication*	CL	no		[6]
	Ketoconazole	CL	yes	50% CL ↓	[66]
		PK	yes	40% CL ↓, no difference in AUC	[3]
	Polysorbate 80	CL and Fu	yes	Fu P80 treated samples > Fu pretreatment samples	[81]
		CL	yes	↑ P80 AUC associated with ↓ unboud DTX CL	[79]
Supportive therapy	Aprepitant	PK	no		[55]
	Granisetron	PK	no		[56]
	Cannabis	PK	no		[59]
	Casopitant	PK	no		[57]
CAMs	Echinacea purpurea	PK	no		[64]
	St. John's worth	PK	yes	increases AUC and decrease CL	[63]
	Garlic	CL	no	trend towards decreased CL	[62]

*clinically relevant interaction, CL=clearance, PK=pharmacokinetics, AUC=area under the curve, Fu=unbound docetaxel, P80=polysorbate 80, DTX=docetaxel, Ref=reference

Interactions with co-medication

Ketoconazole, used for the treatment of fungal infections, is a strong inhibitor of CYP3A4. Co-administration of ketoconazole decreased docetaxel CL with 40-50% (Table 2) (3, 66). Also, ketoconazole co-administration increased the IIV of docetaxel CL around 8% (3) and should therefore be avoided. Pre-medication with dexamethasone did not show an association with docetaxel PK (6).

To sum, for safe and optimal care, clinicians should be aware of drug-drug interactions and take these into account when administering docetaxel to patients as these interactions influence both PK and pharmacodynamics.

PATIENT FACTORS

In addition to drug-drug interactions, patient related factors might play a role in the large pharmacokinetic IIV of docetaxel. Some factors have been studied extensively and are discussed below.

Gender, age and ethnicity

The effects of gender on docetaxel metabolism have been investigated in multiple studies and the results are indistinct (8, 9, 67). A previous study found that females had a 35% lower docetaxel CL than males and a gender effect on docetaxel metabolism was suggested (9) while others observed no clear effect of gender on docetaxel PK (8, 67). This might be due to underlying and masking factors, as hormonal factors such as menopausal status and castration status may play a role in the discrepancy regarding the effect of gender, masking potential clinically relevant effects.

Age is of insignificant influence on docetaxel PK (9, 67-71). Docetaxel CL and its variability was not altered in elderly patients compared to younger patients (70).

Regarding ethnicity, Japanese patients are usually treated with a lower dose than patients in Western countries. This resulted from different recommended phase II doses during early drug development, due to differences in (dose-limiting) toxicity between Asian and non-Asian patients (72). However, no statistically significant differences in docetaxel CL were seen when races were compared, suggesting that ethnicity does not substantially contribute in explaining the large docetaxel IIV (73, 74).

Hormonal status

The influence of castration status on docetaxel PK was investigated in 30 men with mCRPC (Table 3) (7). It was shown that castration status did not modify CYP3A levels, confirming earlier findings (75). However, castrated males showed increased docetaxel CL and a 2-fold decrease in AUC compared to non-castrated patients. These findings were further supported by studies in rodents, where castrated rats had reduced docetaxel peak concentrations (7). The increased expression of hepatic rOatp2 (slc22a7) was reported as a potential explanation for this finding (7). This increase in rOatp2 expression was hypothesized to result in increased hepatic docetaxel uptake and thus in increased metabolism.

Table 3. Patient and environmental factors influencing pharmacokinetics

Subject	Factor	Endpoint	Effect PK	Effect description	Ref
Patient factors	Liver impairment	CL	yes	Moderate and severe liver impairment ↓ CL	[78-80]
	α1-acid glycoprotein	CL	yes	↑ AAG leads to ↓ DTX CL	[82]
			no		[54,79,81]
	Menopausal status	PK	yes	AUC: premenopausal < postmenopausal woman with genotype C3435T (CC)	[76]
	Castration status	CL, AUC	yes	100% ↑CL and 2-fold ↓ AUC in castrated vs. non-castrated patients	[7]
	Ethnicity	CL	no		[73, 74]
	Gender	CL	yes	Woman 35% ↓ CL than men	[9]
			no		[8,67]
	Age	CL	no		[9,67,68,69,70,71]
	BSA >2.0 m^2	CL	yes	33% CL ↑	[68]
	BMI ≥30 kh/m^2	CL	no		[68]
Environmental factors	Smoking	PK	no	↓ grade 4 neutropenia in smokers	[85]

PK=pharmacokinetics, CL=clearance, AAG=α1-acid glycoprotein, AUC=area under the curve, DTX=docetaxel, Ref=reference

Menopausal status was shown to affect docetaxel PK with premenopausal woman having a lower AUC (4124 µg h/l, n=53) than postmenopausal woman (4598 µg h/l, n=33) [76]. This study also showed that docetaxel AUC was significantly different in 40 pre-menopausal and post-menopausal women carrying the same C3435T genotype (CC), with a lower AUC in premenopausal woman (76). No effect was seen in woman with other genotypes. Castration status and menopausal status could thus potentially be part of the underlying mechanisms explaining the discrepancy in the influence of gender on docetaxel PK. At this point, the influence of menopausal and castration status has to be validated in larger cohorts and is not yet usable in a clinical setting.

Obesity

When separating patients into quartiles based on their BSA, the mean docetaxel CL was highest in the highest BSA quadrant and lowest in the lowest BSA quadrant (69). In patients with a BSA > $2m^2$, a 33% increase in docetaxel CL was seen compared to patients with a BSA ≤ $2m^2$ (68). A BMI of ≥ 30 kg/m^2 was not associated with higher docetaxel CL (68, 77). Thus, no dose adaptations need to be made for obesity. However, extensively obese patients with a BSA > 2 m^2 had an increased docetaxel CL and may need a higher dose than patients with a BSA ≤ 2 m^2. This hypothesis however needs validation in larger cohorts.

Liver impairment

Liver impairment was shown to decrease docetaxel CL (Table 3) (78-80). Minami and colleagues demonstrated that patients with grade 2 and 3 elevations of transaminases at baseline together with alkaline phosphatase elevation had around a 30% decrease of docetaxel CL (78). Their advice was to consider a 20-40% dose reduction for patients with a grade 2 and 3 transaminase increase in combination with alkaline phosphatase elevation.

Plasma proteins

Plasma proteins are seen as possible determinants for docetaxel PK, as docetaxel is highly bound to proteins. Some studies looked into the relation between α 1-acid glycoprotein (AAG) and docetaxel PK (54, 79, 81, 82) (see Table 3). Ambiguous results were found. This discrepancy could possibly be caused by the fact that unbound docetaxel CL was used to test a possible correlation with AAG concentrations, which eliminates the effect of protein binding as a confounder (79). Also, AAG is an acute phase reactant, which could mask a potential effect. It is also known that in critically ill patients, albumin levels are low due to altered distribution between intravascular and extra vascular compartments (83). Decreased albumin levels thus might make up for the increase of AAG, explaining why no effect is seen in some of the studies that focus on AAG only. As also the expression of CYP3A4 is decreased during inflammatory response, the question rises whether AAG is mechanistically responsible for changes in PK or that increased levels of AAG are only a sign of ongoing inflammatory response, decreasing CYP3A4 activity (84). Currently, no definite clinical actions can be taken based on baseline plasma protein values.

ENVIRONMENTAL FACTORS

In addition to patient related factors, environmental factors may play a role in docetaxel PK. Smoking has been studied as such and demonstrated to have no effect on docetaxel PK (85). However, the incidence of grade 4 neutropenia was lower in smokers who were treated with docetaxel (35%) than in non-smokers (52%) (85). One of the supposed mechanisms for this effect is that patients inhale small particles when smoking, which could result in IL-6 and granulocyte macrophage colony stimulating factor release, that encourages the proliferation of pre-cursors in the bone marrow (86-88). Thus, the effect of smoking on docetaxel PK seems to be limited and at this point, the advantages of quitting smoking still seem to offset the possible protective effect on hematological toxicity.

CURRENT ALTERNATIVES FOR BSA-BASED DOSING

The BSA-based formula does not account for the factors described in the previous paragraph that potentially influence the PK of docetaxel, such as obesity, gender, hormonal status and liver impairment. To improve individualized dosing of docetaxel, other strategies have been studied and will be discussed here.

Therapeutic drug monitoring

A priori therapeutic drug monitoring (TDM) is a tool for calculating the optimal dose of a drug (4). Generally, drugs with a narrow therapeutic window and an existing correlation between toxicity and exposure may be suited for such an approach. Since docetaxel matches these criteria, docetaxel dosing could hypothetically be individualized by using TDM. To investigate this hypothesis, a TDM strategy was developed using a validated limited sampling model based on Bayesian analysis. Using TDM, the IIV in PK decreased significantly with 39% (89). Unfortunately, the incidence of hematological toxicity was not different in TDM dosed patients from patients that had been dosed using BSA. Despite the fact that relatively cheap immuno-assays for determining docetaxel plasma concentrations are currently (commercially) available, a problem of TDM is that it is still time-consuming for both patients and professionals.

BSA dose banding

To improve the current BSA strategy, it was recently suggested that dose-banding could be an alternative (90). A limited amount of predefined BSA ranges was used to determine an initial docetaxel dose and for adaptation of the dose in patients with extreme BSA values. This strategy was feasible, since the difference in the calculated docetaxel dosage was marginal compared to regular BSA dosing. This strategy has the potential to simplify pharmacy processes and to improve patient safety (90).

Probe-drug phenotyping

As an alternative for accounting for individual factors influencing docetaxel PK, researchers tried to predict CYP3A4 activity as a measure for docetaxel CL with the use of probes, such as

antipyrine, midazolam and erythromycin (9, 54, 67, 82, 91). The erythromycin breath test, antipyrine CL, dexamethasone CL and midazolam exposure tests were demonstrated to be successful in predicting CYP3A4 activity and thereby docetaxel PK (9, 54, 67, 92, 93). To add, urinary 6-beta-hydroxy cortisol was used in a formula for the estimation of docetaxel CL (91). Compared with BSA-based dosing, using this method these researchers were able to reduce docetaxel's IIV significantly. However, the complexity of such methods, the interaction with several other mechanisms such as docetaxel transport and for example the interference with polysorbate 80, currently obstruct clinical application of these strategies (8, 79, 81, 94).

Pharmacogenetics

The effect of genetic variation on docetaxel PK has been studied extensively (see Table 4, refs. 8, 9, 62, 67, 73, 75, 95-100). Some of the studied single nucleotide polymorphisms (SNPs) have been associated with docetaxel PK alteration. For example, the SNP rs12762549 in *ABCC2* resulted in a significantly decreased docetaxel CL (98). A 50% increase in docetaxel CL was seen in patients carrying one *1A allele (rs776746) in *CYP3A5* (9). When carrying both *CYP3A4* *1B and *CYP3A5* *1/*3 alleles an increase in docetaxel CL was seen, as well as for carrying both *CYP3A4* *1B and *CYP3A5* *1A alleles (9, 95). Contradictory results have been shown for SNPs in *ABCB1* (rs1128563, 1236C>T) and *SLCO1B3* (rs11045585, IVS12-5676A>G) (8, 9, 97-99). For clinical applicability, these SNPs have to be validated in larger cohorts, possibly using genome wide association studies next to the usual candidate gene approach.

RECOMMENDATIONS

The high IIV in the PK of docetaxel renders it difficult to accurately choose an individual dose resulting in optimal docetaxel exposure, leading to efficacy at the cost of acceptable toxicity. Today, only BSA is used for calculating docetaxel dosages. However, this method does not fully reduce the high IIV of docetaxel PK to an absolute minimum. Unfortunately, no superior alternatives for the current dosing strategy are presently available. A new dosing strategy could therefore use some additional, and clinically applicable, tools for decreasing IIV and individualizing docetaxel treatment. Tools for such a strategy could thus be demographic factors partly explaining docetaxel' s high IIV in PK.

From current knowledge, several recommendations can be given on factors influencing docetaxel PK in order to optimize docetaxel dosing. Liver impairment may decrease docetaxel CL, and should be taken into account. Also, hormonal status and gender may be of clinical relevance in future dosing strategies for docetaxel.

Drug-drug interactions have been established and some are distinctly relevant. These interactions are most probably mediated by drug transporters and cytochrome P450 iso-enzymes. Therefore, notice should be taken when using CYP3A4 inhibiting drugs in combination with docetaxel. Interactions at the level of uptake transporters may also be of relevance, as these are likely influencing hepatic uptake of docetaxel and thus drug elimination. The role of pharmacogenetics is currently still limited, and special recommendations on preemptive genotyping cannot be given.

Table 4. SNPs associated with docetaxel pharmacokinetics

Gene	SNP	rs number	Endpoint	Effect PK	Effect	Ref
ABCB1	3435C>T, CC genotype only	rs2032582	AUC	yes	↓ AUC in premenopausal vs. postmenopausal woman	[76]
	3435C>T		CL	no		[9,67,95-97]
	1236C>T	rs1128503	CL	yes	25% ↑ CL	[97]
			CL	no		[9]
	2677G>T/A	rs2032582	CL/AUC	no		[9,95-97]
ABCC2	101620771C>G, 52425235C>G	rs12762549	CL	yes	↓ CL	[98]
	-1019A>G	rs2804402	CL	no		[9]
	-24C>T	rs717620	CL	no		[9]
	1249G>A	rs2273697	CL	no		[9]
	IVS26G>A	rs8187698	CL	no		[9]
	3972C>T	rs3740066	CL	no		[9]
	4544G>A	rs8187710	CL	no		[9]

Gene	SNP	rs number	Endpoint	Effect PK	Effect	Ref
SLCO1B1	-1187G>A	rs4149015	CL	no		[8]
	c.3386G>A	rs2306283	CL	no		[8]
	c.521T>C	rs4149056	CL	no		[8]
SLCO1B3	IVS12-5676A>G	rs11045585	AUC	yes	↑AUC if genotype GG	[99]
			CL	no		[8,98]
	334T>G	rs4149117	CL	no		[8,9]
	439A>G	rs57585902	CL	no		[9]
	699G>A	rs7311358	CL	no		[8,9]
	767G>A	rs60140950	CL	no		[9]
	1559A>C	N/A	CL	no		[9]
	1679T>C	rs12299012	CL	no		[9]
CYP3A4	-392A>G>G (*1B)	rs2740574	CL	trend	62% ↑CL with one *1B allele	[9]
				no		[62,73,97]
	878T>C (*18 allele)	rs28371759	Vmax	yes	Vmax ↓	[100]

Gene	SNP	rs number	Endpoint	Effect PK	Effect	Ref
CYP3A5	6986A>G	rs776746	CL	yes	49% ↑ CL with one *1A allele	[9]
	22893G>A (*3)	rs776746	PK	no		[67,74,96,97]
	27289C>A (*2)	rs28365083	PK	no		[97]
	CYP3A4*1B and CYP3A5*1/*3		PK	yes	↑CL and ↓AUC	[95]
	CYP3A4*1B and CYP3A5 *1A		CL	yes	64% ↑CL	[9]

PK=pharmacokinetics, CL=clearance, AUC=area under the curve, Vmax=maximum velocity, Ref=reference

REFERENCES

1. Clarke SJ, Rivory LP. Clinical pharmacokinetics of docetaxel. Clin Pharmacokinet. 1999;36:99-114.
2. Tannock IF, de Wit R, Berry WR, Horti J, Pluzanska A, Chi KN, et al. Docetaxel plus prednisone or mitoxantrone plus prednisone for advanced prostate cancer. N Engl J Med. 2004;351:1502-12.
3. Engels FK, Mathot RA, Loos WJ, van Schaik RH, Verweij J. Influence of high-dose ketoconazole on the pharmacokinetics of docetaxel. Cancer Biol Ther. 2006;5:833-9.
4. Mathijssen RH, Sparreboom A, Verweij J. Determining the optimal dose in the development of anticancer agents. Nat Rev Clin Oncol. 2014;11:272-81.
5. Bruno R, Hille D, Riva A, Vivier N, ten Bokkel Huinnink WW, van Oosterom AT, et al. Population pharmacokinetics/pharmacodynamics of docetaxel in phase II studies in patients with cancer. J Clin Oncol. 1998;16:187-96.
6. Bruno R, Vivier N, Veyrat-Follet C, Montay G, Rhodes GR. Population pharmacokinetics and pharmacokinetic-pharmacodynamic relationships for docetaxel. Invest New Drugs. 2001;19:163-9.
7. Franke RM, Carducci MA, Rudek MA, Baker SD, Sparreboom A. Castration-dependent pharmacokinetics of docetaxel in patients with prostate cancer. J Clin Oncol. 2010;28:4562-7.
8. de Graan AJ, 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.
9. Baker SD, Verweij J, Cusatis GA, van Schaik RH, Marsh S, Orwick SJ, et al. Pharmacogenetic pathway analysis of docetaxel elimination. Clin Pharmacol Ther. 2009;85:155-63.
10. Iusuf D, Hendrikx JJ, 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.
11. Wils P, Phung-Ba V, Warnery A, Lechardeur D, Raeissi S, Hidalgo IJ, et al. Polarized transport of docetaxel and vinblastine mediated by P-glycoprotein in human intestinal epithelial cell monolayers. Biochem Pharmacol. 1994;48:1528-30.
12. Huisman MT, Chhatta AA, van Tellingen O, Beijnen JH, Schinkel AH. MRP2 (ABCC2) transports taxanes and confers paclitaxel resistance and both processes are stimulated by probenecid. Int J Cancer. 2005;116:824-9.
13. Hopper-Borge E, Chen ZS, Shchaveleva I, Belinsky MG, Kruh GD. Analysis of the drug resistance profile of multidrug resistance protein 7 (ABCC10): resistance to docetaxel. Cancer Res. 2004;64:4927-30.
14. Baker SD, Sparreboom A, Verweij J. Clinical pharmacokinetics of docetaxel: recent developments. Clin Pharmacokinet. 2006;45:235-52.
15. Bardelmeijer HA, Ouwehand M, Buckle T, Huisman MT, Schellens JH, Beijnen JH, et al. Low systemic exposure of oral docetaxel in mice resulting from extensive first-pass metabolism is boosted by ritonavir. Cancer Res. 2002;62:6158-64.
16. Jibodh RA, Lagas JS, Nuijen B, Beijnen JH, Schellens JH. Taxanes: old drugs, new oral formulations. Eur J Pharmacol. 2013;717:40-6.
17. Stuurman FE, Nuijen B, Beijnen JH, Schellens JH. Oral anticancer drugs: mechanisms of low bioavailability and strategies for improvement. Clin Pharmacokinet. 2013;52:399-414.
18. van der Veldt AA, Hendrikse NH, Smit EF, Mooijer MP, Rijnders AY, Gerritsen WR, et al. Biodistribution and radiation dosimetry of 11C-labelled docetaxel in cancer patients. Eur J Nucl Med Mol Imaging. 2010;37:1950-8.
19. Deeken JF, Loscher W. The blood-brain barrier and cancer: transporters, treatment, and Trojan horses. Clin Cancer Res. 2007;13:1663-74.
20. Lee HH, Leake BF, Teft W, Tirona RG, Kim RB, Ho RH. Contribution of hepatic organic anion transporting polpeptides (OATPs) to docetaxel uptake and clearance. Mol Cancer Ther. 2015; 14(4):994-1003

21. Nieuweboer AJM, Hu S, Gui C, Hagenbuch B, Ghobadi Moghaddam-Helmantel I, Gibson AA, et al. Influence of Drug Formulation on OATP1B-Mediated Transport of Paclitaxel. Cancer Res. 2014;74:3137-45.

22. van Herwaarden AE, Wagenaar E, van der Kruijssen CM, van Waterschoot RA, Smit JW, Song JY, et al. Knockout of cytochrome P450 3A yields new mouse models for understanding xenobiotic metabolism. J Clin Invest. 2007;117:3583-92.

23. 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.

24. Monegier B, Gaillard C, Sablé S, Vuilhorgne M. Structures of the major human metabolites of docetaxel (RP 56976 - Taxotere®). Tetrahedron Letters. 1994;35:3715-8.

25. Sparreboom A, Van Tellingen O, Scherrenburg EJ, Boesen JJ, Huizing MT, Nooijen WJ, et al. Isolation, purification and biological activity of major docetaxel metabolites from human feces. Drug Metab Dispos. 1996;24:655-8.

26. van Zuylen L, Sparreboom A, van der Gaast A, van der Burg ME, van Beurden V, Bol CJ, et al. The orally administered P-glycoprotein inhibitor R101933 does not alter the plasma pharmacokinetics of docetaxel. Clin Cancer Res. 2000;6:1365-71.

27. Fracasso PM, Goldstein LJ, de Alwis DP, Rader JS, Arquette MA, Goodner SA, et al. Phase I study of docetaxel in combination with the P-glycoprotein inhibitor, zosuquidar, in resistant malignancies. Clin Cancer Res. 2004;10:7220-8.

28. Dieras V, Bonneterre J, Laurence V, Degardin M, Pierga JY, Bonneterre ME, et al. Phase I combining a P-glycoprotein inhibitor, MS209, in combination with docetaxel in patients with advanced malignancies. Clin Cancer Res. 2005;11:6256-60.

29. Mooiman KD, Maas-Bakker RF, Hendrikx JJ, Bank PC, Rosing H, Beijnen JH, et al. The effect of complementary and alternative medicines on CYP3A4-mediated metabolism of three different substrates: 7-benzyloxy-4-trifluoromethyl-coumarin, midazolam and docetaxel. J Pharm Pharmacol. 2014;66:865-74.

30. Millward MJ, Zalcberg J, Bishop JF, Webster LK, Zimet A, Rischin D, et al. Phase I trial of docetaxel and cisplatin in previously untreated patients with advanced non-small-cell lung cancer. J Clin Oncol. 1997;15:750-8.

31. Petrylak DP, Macarthur RB, O'Connor J, Shelton G, Judge T, Balog J, et al. Phase I trial of docetaxel with estramustine in androgen-independent prostate cancer. J Clin Oncol. 1999;17:958-67.

32. Van Den Neste E, de Valeriola D, Kerger J, Bleiberg H, Kusenda Z, Brassinne C, et al. A phase I and pharmacokinetic study of docetaxel administered in combination with continuous intravenous infusion of 5-fluorouracil in patients with advanced solid tumors. Clin Cancer Res. 2000;6:64-71.

33. Pronk LC, Vasey P, Sparreboom A, Reigner B, Planting AS, Gordon RJ, et al. A phase I and pharmacokinetic study of the combination of capecitabine and docetaxel in patients with advanced solid tumours. Br J Cancer. 2000;83:22-9.

34. Adjei AA, Klein CE, Kastrissios H, Goldberg RM, Alberts SR, Pitot HC, et al. Phase I and pharmacokinetic study of irinotecan and docetaxel in patients with advanced solid tumors: preliminary evidence of clinical activity. J Clin Oncol. 2000;18:1116-23.

35. Oka M, Fukuda M, Nagashima S, Fukuda M, Kinoshita A, Soda H, et al. Phase I study of second-line chemotherapy with docetaxel and carboplatin in advanced non-small-cell lung cancer. Cancer Chemother Pharmacol. 2001;48:446-50.

36. Dumez H, Louwerens M, Pawinsky A, Planting AS, de Jonge MJ, Van Oosterom AT, et al. The impact of drug administration sequence and pharmacokinetic interaction in a phase I study of the combination of docetaxel and gemcitabine in patients with advanced solid tumors. Anticancer Drugs. 2002;13:583-93.

37. Louwerens M, Smorenburg C, Sparreboom A, Loos WJ, Verweij J, de Wit R. Phase I pharmacokinetic and sequence finding study of the combination of docetaxel and methotrexate in patients with solid tumours. Eur J Cancer. 2002;38:497-504.

38. Felici A, Loos WJ, Verweij J, Cirillo I, de Bruijn P, Nooter K, et al. A pharmacokinetic interaction study of docetaxel and cisplatin plus or minus 5-fluorouracil in the treatment of patients with recurrent or metastatic solid tumors. Cancer Chemother Pharmacol. 2006;58:673-80.

39. Bonneterre J, Campone M, Koralewski P, Blasinska-Morawiec M, Deporte-Fety R, Chatelut E, et al. Vinorelbine/docetaxel combination treatment of metastatic breast cancer: a phase I study. Cancer Chemother Pharmacol. 2007;60:365-73.

40. Itoh K, Sasaki Y, Fujii H, Minami H, Ohtsu T, Wakita H, et al. Study of dose escalation and sequence switching of administration of the combination of docetaxel and doxorubicin in advanced breast cancer. Clin Cancer Res. 2000;6:4082-90.

41. Schrijvers D, Pronk L, Highley M, Bruno R, Locci-Tonelli D, De Bruijn E, et al. Pharmacokinetics of ifosfamide are changed by combination with docetaxel: results of a phase I pharmacologic study. Am J Clin Oncol. 2000;23:358-63.

42. Zamboni WC, Egorin MJ, Van Echo DA, Day RS, Meisenberg BR, Brooks SE, et al. Pharmacokinetic and pharmacodynamic study of the combination of docetaxel and topotecan in patients with solid tumors. J Clin Oncol. 2000;18:3288-94.

43. Izquierdo MA, Garcia M, Ponton JL, Martinez M, Valenti V, Navarro M, et al. A phase I clinical and pharmacokinetic study of paclitaxel and docetaxel given in combination in patients with solid tumours. Eur J Cancer. 2006;42:1789-96.

44. LoRusso PM, Jones SF, Koch KM, Arya N, Fleming RA, Loftiss J, et al. Phase I and pharmacokinetic study of lapatinib and docetaxel in patients with advanced cancer. J Clin Oncol. 2008;26:3051-6.

45. Robert F, Sandler A, Schiller JH, Liu G, Harper K, Verkh L, et al. Sunitinib in combination with docetaxel in patients with advanced solid tumors: a phase I dose-escalation study. Cancer Chemother Pharmacol. 2010;66:669-80.

46. Connolly RM, Rudek MA, Garrett-Mayer E, Jeter SC, Donehower MG, Wright LA, et al. Docetaxel metabolism is not altered by imatinib: findings from an early phase study in metastatic breast cancer. Breast Cancer Res Treat. 2011;127:153-62.

47. Kraut EH, Rhoades C, Zhang Y, Cheng H, Aimiumu J, Chen P, et al. Phase I and pharmacokinetic study of erlotinib (OSI-774) in combination with docetaxel in squamous cell carcinoma of the head and neck (SSCHN). Cancer Chemother Pharmacol. 2011;67:579-86.

48. Moulder S, Gladish G, Ensor J, Gonzalez-Angulo AM, Cristofanilli M, Murray JL, et al. A phase 1 study of weekly everolimus (RAD001) in combination with docetaxel in patients with metastatic breast cancer. Cancer. 2012;118:2378-84.

49. Attard G, Kitzen J, Blagden SP, Fong PC, Pronk LC, Zhi J, et al. A phase Ib study of pertuzumab, a recombinant humanised antibody to HER2, and docetaxel in patients with advanced solid tumours. Br J Cancer. 2007;97:1338-43.

50. Freyer G, Hennebert P, Awada A, Gil T, Kerger J, Selleslags J, et al. Influence of amifostine on the toxicity and pharmacokinetics of docetaxel in metastatic breast cancer patients: a pilot study. Clin Cancer Res. 2002;8:95-102.

51. Messersmith WA, Baker SD, Lassiter L, Sullivan RA, Dinh K, Almuete VI, et al. Phase I trial of bortezomib in combination with docetaxel in patients with advanced solid tumors. Clin Cancer Res. 2006;12:1270-5.

52. Hamberg P, Mathijssen RH, de Bruijn P, Leonowens C, van der Biessen D, Eskens FA, et al. Impact of pazopanib

on docetaxel exposure: results of a phase I combination study with two different docetaxel schedules. Cancer Chemother Pharmacol. 2015;75:365-71.

53. Shadle CR, Lee Y, Majumdar AK, Petty KJ, Gargano C, Bradstreet TE, et al. Evaluation of potential inductive effects of aprepitant on cytochrome P450 3A4 and 2C9 activity. J Clin Pharmacol. 2004;44:215-23.
54. 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;6:1255-8.
55. Kaneta T, Fujita K, Akiyama Y, Kawara K, Sunakawa Y, Kawachi A, et al. No pharmacokinetic alteration of docetaxel following coadministration of aprepitant 3 h before docetaxel infusion. Cancer Chemother Pharmacol. 2014;74:539-47.
56. Miyata M, Yasuda K, Burioka N, Takane H, Suyama H, Shigeoka Y, et al. The influence of granisetron on the pharmacokinetics and pharmacodynamics of docetaxel in Asian lung cancer patients. Cancer J. 2006;12:69-72.
57. Dandamudi UB, Adams LM, Johnson B, Bauman J, Morris S, Murray S, et al. Lack of effect of casopitant on the pharmacokinetics of docetaxel in patients with cancer. Cancer Chemother Pharmacol. 2011;67:783-90.
58. Tramer MR, Carroll D, Campbell FA, Reynolds DJ, Moore RA, McQuay HJ. Cannabinoids for control of chemotherapy induced nausea and vomiting: quantitative systematic review. BMJ. 2001;323:16-21.
59. Engels FK, de Jong FA, Sparreboom A, Mathot RA, Loos WJ, Kitzen JJ, et al. Medicinal cannabis does not influence the clinical pharmacokinetics of irinotecan and docetaxel. Oncologist. 2007;12:291-300.
60. Horneber M, Bueschel G, Dennert G, Less D, Ritter E, Zwahlen M. How many cancer patients use complementary and alternative medicine: a systematic review and metaanalysis. Integr Cancer Ther. 2012;11:187-203.
61. Werneke U, Earl J, Seydel C, Horn O, Crichton P, Fannon D. Potential health risks of complementary alternative medicines in cancer patients. Br J Cancer. 2004;90:408-13.
62. Cox MC, Low J, Lee J, Walshe J, Denduluri N, Berman A, et al. Influence of garlic (Allium sativum) on the pharmacokinetics of docetaxel. Clin Cancer Res. 2006;12:4636-40.
63. Goey AK, Meijerman I, Rosing H, Marchetti S, Mergui-Roelvink M, Keessen M, et al. The Effect of St John's Wort on the Pharmacokinetics of Docetaxel. Clin Pharmacokinet. 2014;53(1):103-10.
64. Goey AK, Meijerman I, Rosing H, Burgers JA, Mergui-Roelvink M, Keessen M, et al. The effect of Echinacea purpurea on the pharmacokinetics of docetaxel. Br J Clin Pharmacol. 2013;76:467-74.
65. Tascilar M, de Jong FA, Verweij J, Mathijssen RH. Complementary and alternative medicine during cancer treatment: beyond innocence. Oncologist. 2006;11:732-41.
66. Engels FK, Ten Tije AJ, Baker SD, Lee CK, Loos WJ, Vulto AG, et al. Effect of cytochrome P450 3A4 inhibition on the pharmacokinetics of docetaxel. Clin Pharmacol Ther. 2004;75:448-54.
67. 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;20:3683-90.
68. Rudek MA, Sparreboom A, Garrett-Mayer ES, Armstrong DK, Wolff AC, Verweij J, et al. Factors affecting pharmacokinetic variability following doxorubicin and docetaxel-based therapy. Eur J Cancer. 2004;40:1170-8.
69. Slaviero KA, Clarke SJ, McLachlan AJ, Blair EY, Rivory LP. Population pharmacokinetics of weekly docetaxel in patients with advanced cancer. Br J Clin Pharmacol. 2004;57:44-53.
70. ten Tije AJ, Verweij J, Carducci MA, Graveland W, Rogers T, Pronk T, et al. Prospective evaluation of the pharmacokinetics and toxicity profile of docetaxel in the elderly. J Clin Oncol. 2005;23:1070-7.
71. Hurria A, Fleming MT, Baker SD, Kelly WK, Cutchall K, Panageas K, et al. Pharmacokinetics and toxicity of weekly docetaxel in older patients. Clin Cancer Res. 2006;12:6100-5.

72. Yano R, Konno A, Watanabe K, Tsukamoto H, Kayano Y, Ohnaka H et al. Pharmacoethnicity of docetaxel-induced severe neutropenia: integrated analysis of published phase II and III trials. Int J Clin Oncol. 2013 Feb;18(1):96-104.

73. Lewis LD, Miller AA, Rosner GL, Dowell JE, Valdivieso M, Relling MV, et al. A comparison of the pharmacokinetics and pharmacodynamics of docetaxel between African-American and Caucasian cancer patients: CALGB 9871. Clin Cancer Res. 2007;13:3302-11.

74. Hor SY, Lee SC, Wong CI, Lim YW, Lim RC, Wang LZ, et al. PXR, CAR and HNF4alpha genotypes and their association with pharmacokinetics and pharmacodynamics of docetaxel and doxorubicin in Asian patients. Pharmacogenomics J. 2008 Apr;8(2):139-46.

75. Hutson PR, Oettel K, Douglas J, Ritter M, Messing E, Staab MJ, et al. Effect of medical castration on CYP3A4 enzyme activity using the erythromycin breath test. Cancer Chemother Pharmacol. 2008;62:373-7.

76. Fajac A, Gligorov J, Rezai K, Levy P, Levy E, Selle F, et al. Effect of ABCB1 C3435T polymorphism on docetaxel pharmacokinetics according to menopausal status in breast cancer patients. Br J Cancer. 2010;103:560-6.

77. Sparreboom A, Wolff AC, Mathijssen RH, Chatelut E, Rowinsky EK, Verweij J, et al. Evaluation of alternate size descriptors for dose calculation of anticancer drugs in the obese. J Clin Oncol. 2007;25:4707-13.

78. Minami H, Kawada K, Sasaki Y, Tahara M, Igarashi T, Itoh K, et al. Population pharmacokinetics of docetaxel in patients with hepatic dysfunction treated in an oncology practice. Cancer Sci. 2009;100:144-9.

79. Baker SD, Li J, ten Tije AJ, Figg WD, Graveland W, Verweij J, et al. Relationship of systemic exposure to unbound docetaxel and neutropenia. Clin Pharmacol Ther. 2005;77:43-53.

80. Hooker AC, Ten Tije AJ, Carducci MA, Weber J, Garrett-Mayer E, Gelderblom H, et al. Population pharmacokinetic model for docetaxel in patients with varying degrees of liver function: incorporating cytochrome P4503A activity measurements. Clin Pharmacol Ther. 2008;84:111-8.

81. Loos WJ, Baker SD, Verweij J, Boonstra JG, Sparreboom A. Clinical pharmacokinetics of unbound docetaxel: role of polysorbate 80 and serum proteins. Clin Pharmacol Ther. 2003;74:364-71.

82. Puisset F, Alexandre J, Treluyer JM, Raoul V, Roche H, Goldwasser F, et al. Clinical pharmacodynamic factors in docetaxel toxicity. Br J Cancer. 2007;97:290-6.

83. Nicholson JP, Wolmarans MR, Park GR. The role of albumin in critical illness. Br J Anaesth. 2000;85:599-610.

84. Kacevska M, Robertson GR, Clarke SJ, Liddle C. Inflammation and CYP3A4-mediated drug metabolism in advanced cancer: impact and implications for chemotherapeutic drug dosing. Expert Opin Drug Metab Toxicol. 2008;4:137-49.

85. de Graan AJ, Loos WJ, Friberg LE, Baker SD, van der Bol JM, van Doorn L, et al. Influence of smoking on the pharmacokinetics and toxicity profiles of taxane therapy. Clin Cancer Res. 2012;18:4425-32.

86. Suwa T, Hogg JC, Vincent R, Mukae H, Fujii T, van Eeden SF. Ambient air particulates stimulate alveolar macrophages of smokers to promote differentiation of myeloid precursor cells. Exp Lung Res. 2002;28:1-18.

87. Fujii T, Hayashi S, Hogg JC, Mukae H, Suwa T, Goto Y, et al. Interaction of alveolar macrophages and airway epithelial cells following exposure to particulate matter produces mediators that stimulate the bone marrow. Am J Respir Cell Mol Biol. 2002;27:34-41.

88. Lewis LD, Ratain MJ. Might cigarettes be a “smoking gun” to reduce taxane myelotoxicity? Clin Cancer Res. 2012;18:4219-21.

89. Engels FK, Loos WJ, van der Bol JM, de Bruijn P, Mathijssen RH, Verweij J, et al. Therapeutic drug monitoring for the individualization of docetaxel dosing: a randomized pharmacokinetic study. Clin Cancer Res. 2011;17:353-62.

90. Chatelut E, White-Koning ML, Mathijssen RH, Puisset F, Baker SD, Sparreboom A. Dose banding as an alternative to body surface area-based dosing of chemotherapeutic agents. Br J Cancer. 2012;107:1100-6.

91. 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;23:1061-9.

92. Michael M, Cullinane C, Hatzimihalis A, O'Kane C, Milner A, Booth R, et al. Docetaxel pharmacokinetics and its correlation with two in vivo probes for cytochrome P450 enzymes: the C(14)-erythromycin breath test and the antipyrine clearance test. Cancer Chemother Pharmacol. 2012;69:125-35.

93. Puisset F, Chatelut E, Sparreboom A, Delord JP, Berchery D, Lochon I, et al. Dexamethasone as a probe for CYP3A4 metabolism: evidence of gender effect. Cancer Chemother Pharmacol. 2007;60:305-8.

94. Krens SD, McLeod HL, Hertz DL. Pharmacogenetics, enzyme probes and therapeutic drug monitoring as potential tools for individualizing taxane therapy. Pharmacogenomics. 2013;14:555-74.

95. Tran A, Jullien V, Alexandre J, Rey E, Rabillon F, Girre V, et al. Pharmacokinetics and toxicity of docetaxel: role of CYP3A, MDR1, and GST polymorphisms. Clin Pharmacol Ther. 2006;79:570-80.

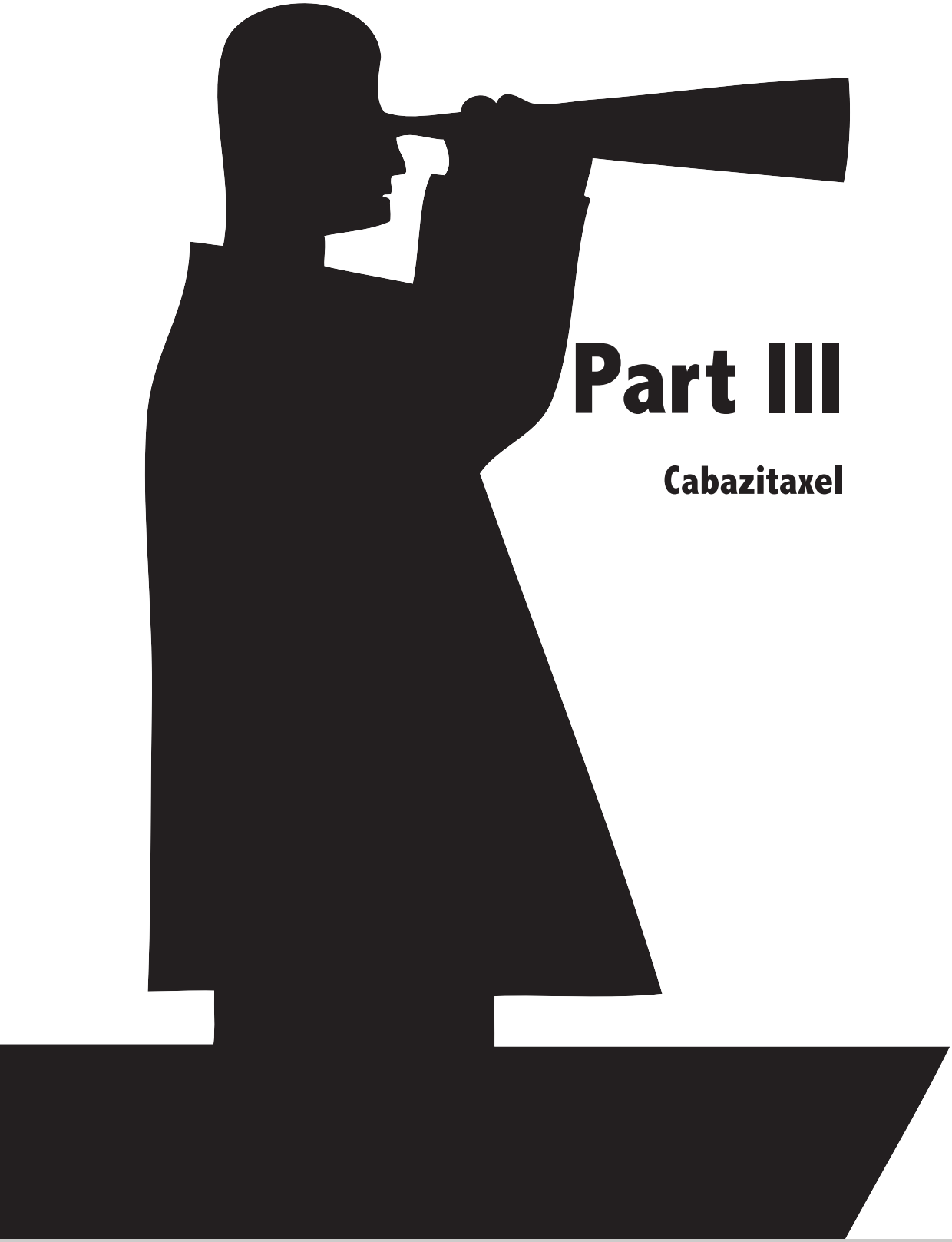
96. Puisset F, Chatelut E, Dalenc F, Busi F, Cresteil T, Azema J, et al. Dexamethasone as a probe for docetaxel clearance. Cancer Chemother Pharmacol. 2004;54:265-72.

97. Bosch TM, Huitema AD, 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.

98. Lewis LD, Miller AA, Owzar K, Bies RR, Markova S, Jiang C, et al. The relationship of polymorphisms in ABCC2 and SLCO1B3 with docetaxel pharmacokinetics and neutropenia: CALGB 60805 (Alliance). Pharmacogenet Genomics. 2013;23:29-33.

99. Chew SC, Singh O, Chen X, Ramasamy RD, Kulkarni T, Lee EJ, et al. The effects of CYP3A4, CYP3A5, ABCB1, ABCC2, ABCG2 and SLCO1B3 single nucleotide polymorphisms on the pharmacokinetics and pharmacodynamics of docetaxel in nasopharyngeal carcinoma patients. Cancer Chemother Pharmacol. 2011;67:1471-8.

100. Maekawa K, Harakawa N, Yoshimura T, Kim SR, Fujimura Y, Aohara F, et al. CYP3A4*16 and CYP3A4*18 alleles found in East Asians exhibit differential catalytic activities for seven CYP3A4 substrate drugs. Drug Metab Dispos. 2010;38:2100-4.



Part III

Cabazitaxel

Chapter 6

The influence of prior novel androgen receptor targeted therapy on the efficacy of cabazitaxel in men with metastatic castration-resistant prostate cancer

A.J.M. Nieuweboer*, R.J. van Soest*, E.S. de Morrée, D. Chitu, A.M. Bergman, S.H. Goey, M.M. Bos, N. van der Meer, P. Hamberg, R. de Wit, A.H.J. Mathijssen, on behalf of the Dutch Uro-Oncology Studygroup (DUOS)

European Journal of Cancer. 2015 Nov;51(17):2562-9 .

* these authors contributed equally

ABSTRACT

The treatment armamentarium for metastatic castration-resistant prostate cancer (mCRPC) has expanded with the introduction of several new therapies. In this treatment continuum, it is unclear whether the efficacy of cabazitaxel is affected by prior novel androgen receptor targeted therapies (ART) such as abiraterone and enzalutamide. In this study, we investigated the influence of prior ART on the efficacy of cabazitaxel in men with mCRPC. Data from an ongoing multicenter, phase II trial were used comprising 114 men with mCRPC treated with cabazitaxel in the post-docetaxel setting. The primary endpoints of the current analysis were prostate-specific antigen (PSA) response (≥50%), and overall survival (OS). Univariate and multivariable analyses were conducted to investigate the influence of prior ART on the efficacy of cabazitaxel. From the 114 patients included in this analysis, 44 men received prior ART and 70 men did not receive prior ART before treatment with cabazitaxel. PSA response rates while on cabazitaxel treatment were similar in patients with and without prior ART (34% versus 40%, respectively, *P*=0.53). Likewise, median OS was not significantly different between men with and without prior ART (13.0 versus 14.0 months, respectively, logrank *P*=0.65). In multivariable analysis, the only variables significantly associated with OS were performance status, serum albumin and alkaline phosphatase. Our study showed that prior treatment with ART may not influence the efficacy of cabazitaxel in men with mCRPC. With emerging evidence of cross-resistance in the treatment of mCRPC, cabazitaxel provides a good treatment option irrespective of prior ART.

INTRODUCTION

The treatment armamentarium for metastatic castration-resistant prostate cancer (mCRPC) has changed considerably over the past few years, with the introduction of several new drugs that provide substantial survival benefits (1-6). Cabazitaxel, abiraterone, enzalutamide and radium-223 all demonstrated to prolong life in the post-docetaxel setting and subsequently became approved for the treatment of this disease. Moreover, the novel androgen receptor (AR)-targeted therapies abiraterone and enzalutamide have shown survival improvement when used in chemotherapy-naïve mCRPC (5, 6). These advances also come with new challenges. Although there are emerging biomarkers such as the androgen receptor splice variant AR-V7 (7), no established biomarkers for treatment selection exist at the current time and the optimal treatment sequence in mCRPC is still undetermined.

Retrospective series studies suggested that overall survival benefit obtained by the new therapies cannot be added up, as cross-resistance between docetaxel and AR-targeted agents has been observed (8-10). Reduced efficacy of docetaxel was observed in men with mCRPC who had previously been treated with abiraterone, suggesting clinical cross-resistance (8-10). In a post-hoc analysis of the COU-AA-302 study, a prostate-specific antigen (PSA) response rate of 45% was observed in patients treated with taxane chemotherapy after abiraterone (11). This was slightly higher as compared to previous reports of docetaxel in this setting, with PSA response rates ranging from 26 to 40% (8-10). However, the observed response rates were lower as compared with a contemporary cohort of abiraterone-naïve patients treated with docetaxel (PSA response rate ≥50%: 64%), which might support the hypothesis of cross-resistance (12).

Preclinical studies revealed that the AR may confer cross-resistance between enzalutamide and docetaxel *in vivo*, which is induced by an overlapping working mechanism on AR nuclear translocation (13, 14). These findings are confirmed in clinical studies (15), and raise concern whether prior treatment with abiraterone or enzalutamide may affect the efficacy of subsequent cabazitaxel treatment. Emerging preclinical and retrospective clinical data suggested that cabazitaxel, in contrast to docetaxel, has sustained efficacy in men with mCRPC after prior abiraterone treatment (16, 17). In two retrospective studies, cabazitaxel efficacy after abiraterone treatment was investigated and compared to the TROPIC trial of cabazitaxel in abiraterone-naïve patients as an historical control group (2, 16, 17). These studies suggested retained efficacy of cabazitaxel after prior abiraterone, as the observed PSA response rates were similar when compared to the TROPIC trial. However, to date, the efficacy of cabazitaxel has never been directly compared between patients with and without prior abiraterone or enzalutamide, which limits clinical conclusions.

In the current study, we aimed to investigate the influence of prior novel AR-targeted therapy (ART) on the efficacy of cabazitaxel. For this purpose, we used data from a randomized phase II trial to directly compare clinical outcome and response to cabazitaxel in men with and without prior ART.

PATIENTS AND METHODS

Study population and data collection

CABARESC (Dutch Trial Registry number: NTR 2991, EudraCT number: 2011-003346-40) is an ongoing randomized, open-label, multicenter, phase II trial that was designed to investigate the effects of budesonide on cabazitaxel-induced diarrhea. The primary endpoint of the original study was the incidence of grade 2-4 diarrhea. In order to detect a reduction of 15% in grade 2-4 diarrhea a total sample size of 250 patients was planned for this study. Eligible men were randomized to either cabazitaxel (25 mg/m^2) and prednisone (10 mg daily) plus oral budesonide (9 mg daily during 44 days), or standard cabazitaxel 25 mg/m^2 plus prednisone (10 mg daily). It has been shown previously that budesonide does not affect the pharmacokinectics of cabazitaxel (18).

Full inclusion and exclusion criteria of the CABARESC trial are shown in the Supplementary materials and methods. In brief, patients were eligible if they had mCRPC with documented disease progression during or after treatment with docetaxel, as defined by rising PSA levels, appearance of new lesions or documented disease progression based on CT scan or bone scan. Cabazitaxel treatment was continued until disease progression, unacceptable toxicity, or until 10 cycles have been administered. Administration of G-CSF was allowed during the study. Patients were randomly assigned to the treatment groups through a centralized stratified randomization process using the following stratification factors: center, age (≥65 versus <65 years) and previous radiotherapy (yes versus no). In this study, data were prospectively collected at baseline and for every cycle including: hematology and biochemistry laboratory values, performance status, age, prior treatment with ART, duration of treatment with ART, PSA values (every 3 weeks), and survival status.

For the current unplanned analysis, we included patients who were randomized and went off-study between December 2011 and May 2014. As the CABARESC study is still recruiting, the primary endpoint of the original study (incidence of grade 2-4 diarrhea) was not reported, and no data per arm were analysed. The CABARESC study was approved by the institutional review board at each participating center. Written informed consent was obtained from all participants. The study protocol allowed for secondary analyses that do not involve the primary endpoint of the CABARESC study to be conducted before reporting of the original study.

Data collection and definitions

The primary objective of the current analysis was to explore the influence of prior ART on the efficacy of cabazitaxel in men with mCRPC. Primary endpoints of this analysis were the proportion of patients with a ≥50% PSA response, and overall survival (OS). As a secondary endpoint, we investigated PSA progression-free survival (PSA-PFS). For the definition of PSA response and PSA-PFS, Prostate Cancer Working Group 2 (PCWG2) criteria were used (19). As recommended by the PCWG2, PSA response was defined as ≥50% decline from baseline, and PSA progression as a 25% increase (and a minimum of 2 ng/ml) from baseline or nadir. In most cases this was confirmed by a second measurement; however confirmation was not routinely performed for all patients. A taxane induced PSA flare during the first 12 weeks of treatment was ignored (20). OS was defined by time

from randomization to death from any cause. Since bone scans and CT-scans were not performed according to regular intervals in the study protocol, we did not include radiological PFS in our analyses.

Descriptive statistics were used to compare baseline characteristics in the ART pretreated versus non-pretreated patients, with statistical evaluation using Fisher's exact test for categorical variables and Kruskal-Wallis test for continuous variables. OS and PSA-PFS were calculated using the Kaplan-Meier method with statistical evaluation by the logrank test.

Model building and statistical considerations

We conducted univariate and multivariable Cox regression analyses including prior treatment with ART (yes/no) and duration of prior ART to investigate its effect on PSA response and OS of men treated with cabazitaxel. For this purpose, Cox proportional hazards models were constructed including established prognostic factors from the Halabi nomogram (21): baseline serum PSA, lactate dehydrogenase (LDH), albumin, alkaline phosphatase, hemoglobin, and performance status. The multivariable model was constructed using backward elimination at the 5% level. A log transformation was applied to variables with a non-normal distribution.

RESULTS

Baseline characteristics

In the CABARESC trial, 141 patients were randomized and went off-study between December 2011 and May 2014. Of these 141 patients, 27 men were excluded from analysis for the following reasons: five patients had missing PSA values at baseline, nine patients were randomized but never received cabazitaxel treatment due to rapid worsening of performance status or death, and 13 patients had received previous study treatment with orteronel (Figure 1). Patients who received prior orteronel were excluded from this analysis since this is not a clinically approved regimen in the treatment of mCRPC. All patients had received prior docetaxel chemotherapy. Forty-four out of 114 patients (39%) had received prior ART in the post-docetaxel setting, of whom 39 had received abiraterone, three had received enzalutamide, and two had received both abiraterone and enzalutamide. The remaining 70 patients had received no prior ART before study treatment with cabazitaxel.

Baseline characteristics of men with and without prior ART are shown in Table 1. Known prognostic variables were evenly distributed among subgroups, except for a significantly lower albumin level in men with prior ART (Table 1). The median number of cabazitaxel cycles received was six in the ART group, and five for men without prior ART.

Table 1. Baseline characteristics of patients with and without prior novel AR targeted therapy.

Characteristic	Prior abiraterone or enzalutamide	No prior abiraterone or enzalutamide	*P*-value
Number of patients	44	70	
Age, years, median (range)	69 (53-83)	68 (49-82)	0.093
WHO performance score n (%)			
0	18 (41)	25 (36)	
1	25 (57)	44 (63)	0.56
Missing	1	1	
PSA, ng/ml, median (range)	210 (15-5000)	154 (12.5-4172)	0.25
LDH at baseline, median (range)	287 (90-724)	273 (38-1843)	0.83
Hemoglobin at baseline, mmol/L, median (range)	8 (6-10)	8 (5-9)	0.96
Alkaline phosphatase at baseline, IU/L, median (range)	124 (50-907)	126 (43-1023)	0.83
Albumin at baseline, g/L, median (range)	37 (26-46)	41 (25-49)	0.013
Duration of treatment with abiraterone/enzalutamide, months, median (range)	6.1 (0.9-22)	-	

PSA - prostate-specific antigen; LDH - Lactate Dehydrogenase

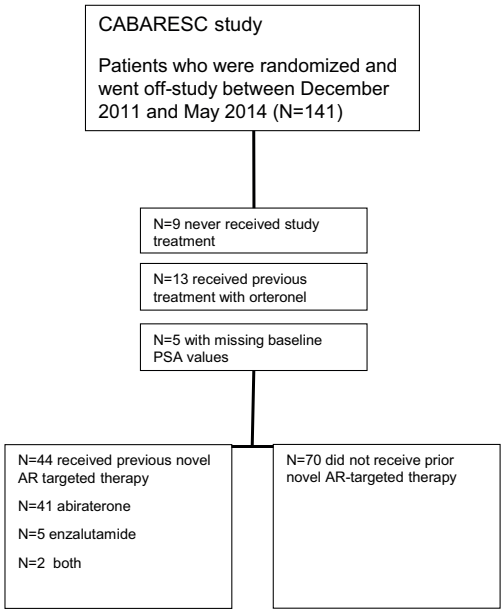


Figure 1. CONSORT diagram.

The influence of prior novel AR-targeted therapy on the efficacy of cabazitaxel

PSA response rates (≥50%) while on cabazitaxel treatment were similar in patients with and without prior ART (34% versus 40% respectively, *P*=0.53). Waterfall plots of the maximum PSA change while on cabazitaxel treatment for men with and without prior ART are shown in Figure 2. Likewise, median PSA-PFS was not significantly different between the two groups. Men who received prior ART had a median PSA-PFS of 4.8 months, versus 6.5 months for men without prior ART (logrank *P*=0.32) (Figure 3A). Median OS was similar for patients previously treated with ART versus patients who were not previously treated with ART, with a median OS of 13.0 versus 14.0 months respectively (logrank *P*=0.65) (Figure 3B).

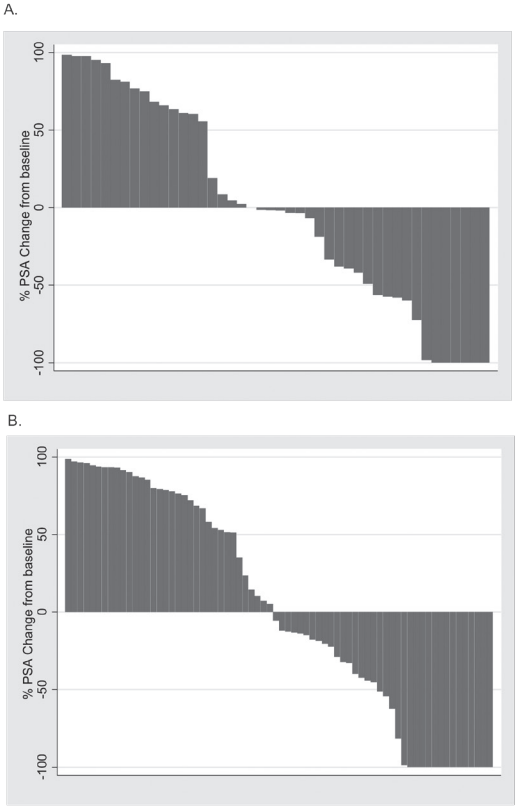


Figure 2. Waterfall plots of the maximum prostate-specific antigen (PSA) change from baseline during treatment with cabazitaxel in men with (A) and without (B) prior novel androgen receptor (AR)-targeted therapy.

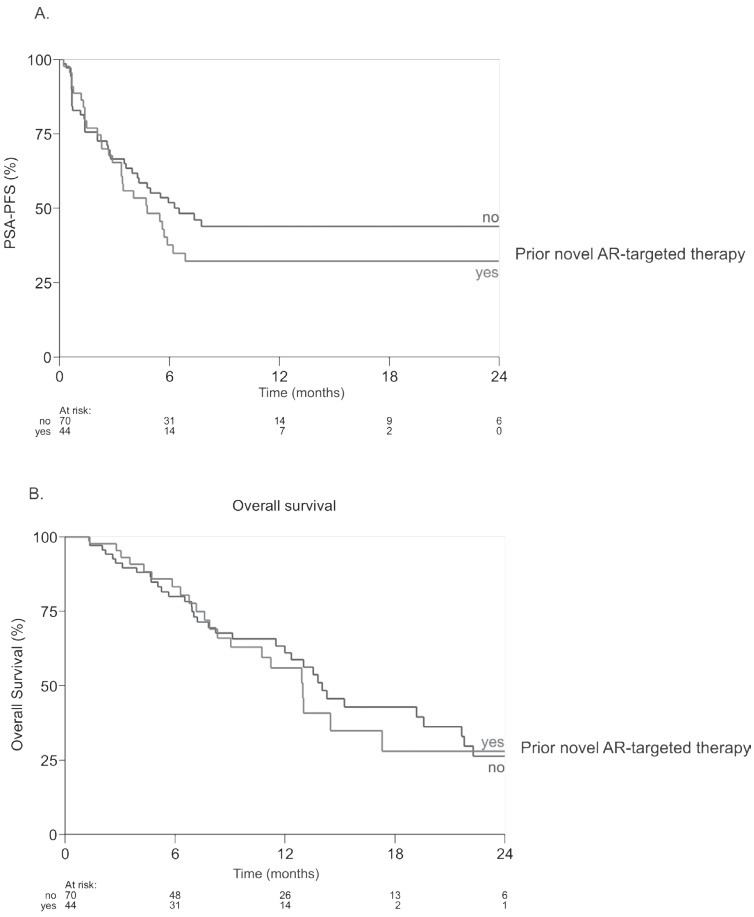


Figure 3. Kaplan–Meier estimates of PSA progression-free survival (PSA-PFS) (A) and overall survival (OS) (B) in men treated with cabazitaxel with and without prior novel androgen receptor (AR)-targeted therapy.

Univariable and multivariable analyses for OS and PSA response.

Factors significantly associated with OS in univariate analysis are shown in Table 2 and included performance status, alkaline phosphatase, albumin, hemoglobin and LDH at baseline. Prior ART and the duration of prior ART were not significantly associated with OS (hazard ratio (HR)=1.14; 95% confidence interval (CI): 0.66-1.97, *P*=0.65 and HR=1.00; 95%CI: 0.92-1.09, *P*=0.98, respectively). From the significant variables in univariate analysis, a multivariate model for OS was constructed (Table 2). The only variables significantly associated with OS in multivariable analysis were performance status, alkaline phosphatase and albumin at baseline. Univariate logistic regression analyses for PSA response (≥50%) are shown in Table 3. Prior ART or the duration of prior ART were not significantly associated with PSA response (OR=0.78; 95%CI: 0.35-1.70, *P*=0.53 and OR=1.00; 95%CI: 0.88-1.13, *P*=0.98, respectively). Baseline hemoglobin was the only variable that was significantly associated with PSA response.

Table 2. Univariate and multivariable analyses for OS

	Univariate		Multivariable	
Variable	**Hazard Ratio (95% CI)**	***P*-Value**	**Hazard Ratio (95% CI)**	***P*-Value**
Age (≥ median)	1.01 (0.97-1.05)	0.71		
WHO performance score (1 vs. 0)	1.83 (1.01-3.32)	0.039	2.23 (1.06-4.69)	0.035
Hemoglobin at baseline	0.68 (0.53-0.88)	0.005	0.88 (0.63-1.24)	0.47
PSA at baseline	1.12 (0.92-1.37)	0.26		
Alkaline phosphatase at baseline	1.84 (1.31-2.60)	<0.001	1.65 (1.06-2.57)	0.026
LDH at baseline	1.69 (1.02-2.81)	0.049	0.74 (0.42-1.29)	0.29
Albumin at baseline	0.90 (0.86-0.95)	<0.001	0.87 (0.81-0.92)	<0.001
Prior novel AR-targeted therapy (yes/no)	1.14 (0.66-1.97)	0.65		
Duration of prior AR-targeted therapy	1.00 (0.92-1.09)	0.98		

PSA - prostate-specific antigen; LDH - Lactate Dehydrogenase; AR – Androgen receptor

Table 3. Univariate logistic regression analysis for PSA response (≥50%)

	Univariate	
Variable	**Odds ratio (95% CI)**	***P*-Value**
Age	1.03 (0.98-1.09)	0.26
WHO performance score (1 vs. 0)	0.58 (0.26-1.25)	0.17
Hemoglobin at baseline	1.63 (1.06-2.50)	0.026
PSA at baseline	1.17 (0.88-1.56)	0.29
Alkaline phosphatase at baseline	0.64 (0.37-1.09)	0.11
LDH at baseline	0.68 (0.34-1.35)	0.27
Albumin at baseline	1.06 (0.99-1.14)	0.074
Prior novel AR-targeted therapy (yes/no)	0.78 (0.35-1.70)	0.53
Duration of prior AR-targeted therapy	1.00 (0.88-1.13)	0.98

PSA - prostate-specific antigen; LDH – Lactate Dehydrogenase; AR – Androgen receptor

DISCUSSION

In this study, prior ART did not influence the efficacy of cabazitaxel in patients with mCRPC. PSA response rates, OS and PSA-PFS were similar in patients with and without prior ART. The only variables significantly associated with OS were performance status, alkaline phosphatase and albumin at baseline.

We used data from a prospective phase II trial to directly compare the efficacy of cabazitaxel in men who received prior ART versus men who did not receive prior ART. To date, only two retrospective studies have been published that investigated the response of patients treated with cabazitaxel after abiraterone, which were limited by the use of an historical control group (16, 17). In our study, we aimed to overcome this limitation by directly comparing clinical outcome and response to treatment with cabazitaxel between patients with and without prior ART, within the same study population. Our results strengthen the conclusions of the two previous studies (17, 18) and confirm activity and lack of cross-resistance for cabazitaxel after prior ART in men with mCRPC.

Our observed PSA response rates in ART pretreated men (36%) are in line with those reported by Pezaro et al. and Al Nakouzi et al., which ranged from 40 to 45% (16, 17). These findings are concordant with the TROPIC trial of cabazitaxel in ART-naïve men and with the ART-naïve patients in the current analysis, demonstrating PSA response to cabazitaxel in both second- and third line treatment for mCRPC (2). The only variable that was significantly associated with lower PSA response rates was haemoglobin (<median), which might reflect the worse prognostic features of this patient subgroup.

Taken together, these findings suggest that there might be no cross-resistance between ART and cabazitaxel. This is especially of interest, since an increasing number of reports have suggested impaired efficacy of docetaxel after abiraterone, suggesting cross-resistance with ART for this taxane (8-10). Clinical cross-resistance could be explained by preclinical data from our group that showed an overlapping working mechanism on AR-nuclear translocation for both abiraterone and enzalutamide, as well as docetaxel (13, 14). Docetaxel inhibited tumor growth and AR signalling in enzalutamide-naïve tumors, but did not in enzalutamide-resistant tumors, demonstrating cross-resistance between enzalutamide and docetaxel *in vivo*. Interestingly, in this preclinical model, cross-resistance was not observed for cabazitaxel, that demonstrated sustained antitumor activity even in tumors previously treated with enzalutamide (13). In the current analysis we confirmed these preclinical findings, showing similar activity of cabazitaxel either when delivered before or after ART. An explanation for the lack of cross-resistance for cabazitaxel could be that cabazitaxel, in contrast to docetaxel, is less dependent on the AR for exerting its antitumor activity (11). Moreover, it has been shown that cabazitaxel supresses microtubule dynamics more potently as compared with docetaxel, with higher intratumoral concentrations, and stronger cytotoxic effects (22, 23).

The main strength of our study is the use of prospective trial data to directly compare the efficacy of cabazitaxel in patients who did and did not receive prior ART within the same study population. Thus far, this is the only study that directly compared the influence of prior ART (post-docetaxel) on the efficacy of cabazitaxel. As an inherent limitation, the original CABARESC study was not designed for the aim of the current unplanned analysis and had a different primary endpoint. As a

result, this study might be underpowered to detect a potential significant difference between patients with and without prior ART. In addition, some parameters such as Gleason score and the duration of response to LHRH agonists/antagonists have not been captured in our database. However, it has been shown previously that tumor differentiation and the duration of response to prior LHRH agonists/antagonists do not affect clinical outcome of men treated with cabazitaxel (24).

In conclusion, our study showed that prior treatment with ART may not influence the efficacy of cabazitaxel in men with mCRPC. With emerging evidence of cross-resistance between the currently available therapies in mCRPC, cabazitaxel provides a good treatment option both before and after novel AR-targeted therapies in the post-docetaxel setting.

REFERENCES

1. de Bono JS, Logothetis CJ, Molina A, et al. Abiraterone and increased survival in metastatic prostate cancer. N Engl J Med. 2011;364:1995-2005.
2. de Bono JS, Oudard S, Ozguroglu M, et al. Prednisone plus cabazitaxel or mitoxantrone for metastatic castration-resistant prostate cancer progressing after docetaxel treatment: a randomised open-label trial. Lancet. 2010;376:1147-54.
3. Parker C, Nilsson S, Heinrich D, et al. Alpha emitter radium-223 and survival in metastatic prostate cancer. N Engl J Med. 2013;369:213-23.
4. Scher HI, Fizazi K, Saad F, et al. Increased Survival with Enzalutamide in Prostate Cancer after Chemotherapy. N Engl J Med. 2012;367:1187-97.
5. Ryan CJ, Smith MR, Fizazi K, et al. Abiraterone acetate plus prednisone versus placebo plus prednisone in chemotherapy-naive men with metastatic castration-resistant prostate cancer (COU-AA-302): final overall survival analysis of a randomised, double-blind, placebo-controlled phase 3 study. Lancet Oncol. 2015;16:152-60.
6. Beer TM, Armstrong AJ, Rathkopf DE, et al. Enzalutamide in Metastatic Prostate Cancer before Chemotherapy. N Engl J Med. 2014;371:424-33.
7. Antonarakis ES, Lu C, Wang H, et al. AR-V7 and resistance to enzalutamide and abiraterone in prostate cancer. N Engl J Med. 2014;371:1028-38.
8. Suzman DL, Luber B, Schweizer MT, Nadal R, Antonarakis ES**.** Clinical activity of enzalutamide versus docetaxel in men with castration-resistant prostate cancer progressing after abiraterone. Prostate. 2014;74:1278-85.
9. Mezynski J, Pezaro C, Bianchini D, et al. Antitumour activity of docetaxel following treatment with the CYP17A1 inhibitor abiraterone: clinical evidence for cross-resistance? Ann Oncol. 2012;23:2943-7.
10. Schweizer MT, Zhou XC, Wang H, et al. The Influence of Prior Abiraterone Treatment on the Clinical Activity of Docetaxel in Men with Metastatic Castration-resistant Prostate Cancer. Eur Urol. 2014;66:646-52.
11. de Bono JS, Smith MR, Saad F, et al. Response to taxane chemotherapy as first subsequent therapy after abiraterone acetate (AA) in patients (pts) with metastatic castration-resistant prostate cancer (mCRPC): Post-hoc analysis of COU-AA-302. J Clin Oncol 33, 2015 (suppl 7; abstr 184).
12. Tannock IF, Fizazi K, Ivanov S, et al. Aflibercept versus placebo in combination with docetaxel and prednisone for treatment of men with metastatic castration-resistant prostate cancer (VENICE): a phase 3, double-blind randomised trial. Lancet Oncol. 2013;14:760-86.
13. van Soest RJ, de Morree ES, Kweldam CF, et al. Targeting the Androgen Receptor Confers In Vivo Cross-resistance Between Enzalutamide and Docetaxel, But Not Cabazitaxel, in Castration-resistant Prostate Cancer. Eur Urol. 2015;67:981-5.
14. van Soest RJ, van Royen ME, de Morree ES, et al. Cross-resistance between taxanes and new hormonal agents abiraterone and enzalutamide may affect drug sequence choices in metastatic castration-resistant prostate cancer. Eur J Cancer. 2013;49:3821-30.
15. Nadal R, Zhang Z, Rahman H, et al. Clinical activity of enzalutamide in Docetaxel-naive and Docetaxel-pretreated patients with metastatic castration-resistant prostate cancer. Prostate. 2014;74:1560-8.
16. Al Nakouzi N, Le Moulec S, Albiges L, et al. Cabazitaxel Remains Active in Patients Progressing After Docetaxel Followed by Novel Androgen Receptor Pathway Targeted Therapies. Eur Urol 2015;68:228-35
17. Pezaro CJ, Omlin AG, Altavilla A, et al. Activity of Cabazitaxel in Castration-resistant Prostate Cancer Progressing After Docetaxel and Next-generation Endocrine Agents. Eur Urol. 2014;66:459-65.

18. De Graan AJ, Nieuweboer A, Loos WJ, et al. Effect of budesonide on the plasma exposure of cabazitaxel (Jevtana®) in castrate resistant prostate cancer patients. ASCPT 2012, PIII-41, 2012.

19. Scher HI, Halabi S, Tannock I, et al. Design and end points of clinical trials for patients with progressive prostate cancer and castrate levels of testosterone: Recommendations of the prostate cancer clinical trials working group. J Clin Oncol. 2008;26:1148-59.

20. Angelergues A, Maillet D, Flechon A, et al. Prostate-specific antigen flare induced by cabazitaxel-based chemotherapy in patients with metastatic castration-resistant prostate cancer. Eur J Cancer. 2014;50:1602-9.

21. Halabi S, Lin CY, Small EJ, et al. Prognostic Model Predicting Metastatic Castration-Resistant Prostate Cancer Survival in Men Treated With Second-Line Chemotherapy. J Natl Cancer Inst. 2013;105:1729-37.

22. Azarenko O, Smiyun G, Mah J, Wilson L, Jordan MA**.** Antiproliferative Mechanism of Action of the Novel Taxane Cabazitaxel as Compared with the Parent Compound Docetaxel in MCF7 Breast Cancer Cells. Mol Cancer Ther. 2014;13:2092-103.

23. de Leeuw R, Berman-Booty LD, Schiewer MJ, et al. Novel actions of next-generation taxanes benefit advanced stages of prostate cancer. Clin Cancer Res. 2015;21:795-807.

24. Oudard S, de Bono JS, Ozguroglu M, et al. Impact of cabazitaxel + prednisone on overall survival at 2 years and in patients with aggressive disease: post-hoc analyses of TROPIC trial. Ann Oncol. 2012 (23 Suppl 9; abstr 933).

SUPPLEMENTARY MATERIALS AND METHODS

Inclusion and exclusion criteria CABARESC study

Inclusion criteria:

- Metastatic castrate resistant prostate cancer (mCRPC) patients with documented disease progression, defined as: documented rising PSA levels (at least 2 consecutive rises in PSA over a reference value taken at least 1 week apart, or a PSA rise of ≥ 2.0 µg/l), appearance of new lesions or documented disease progression based on CT scan or bone scan.
- Previous treatment with a docetaxel-containing regimen
- Age ³ 18 years;
- WHO performance status £ 1
- Adequate renal function (within 21 days before randomization) defined as serum creatinin ≤ 1.5 x ULN and/or calculated creatinin clearance ≥ 50ml/min, according to MDRD formula.
- Adequate hepatic functions (within 21 days before randomization) defined as: total bilirubin ≤ 1.0 x ULN; alanine aminotransferase (ALAT) and aspartate aminotransferase (ASAT) ≤2.5x ULN, in case of liver metastasis < 5 ULN; alkaline phosphatase (AF) < 5x ULN) In case of bone metastasis, AF < 10x ULN is accepted.
- Adequate hematological blood counts (within 21 days before randomization) defined as (absolute neutrophil count (ANC) ≥1.5 x 109/L and platelets ≥ 100 x 109/L);
- Castration, either surgically or by continued LHRH agonist therapy
- Written informed consent according to ICH-GCP

Exclusion criteria:

- Impossibility or unwillingness to take oral drugs
- Serious illness or medical unstable condition requiring treatment, brain metastases or history of psychiatric disorder that would prohibit the understanding and giving of informed consent.
- Use of medications or dietary supplements known to induce or inhibit CYP3A
- Known hypersensitivity to corticosteroids
- Any active systemic or local bacterial, viral, fungal - or yeast infection.
- Ulcerative colitis, Crohn's disease or celiac disease (active or in medical history)
- Ostomy
- Planned/active simultaneous yellow fever vaccine
- Geographical, psychological or other non-medical conditions interfering with follow-up

Chapter 7

Quantification of cabazitaxel in human plasma by liquid chromatography / triple-quadrupole mass spectrometry: a practical solution for non-specific binding

P. de Bruijn, A.M. de Graan, A.J.M. Nieuweboer, A.H.J. Mathijssen, M. Lam, R. de Wit, E.A.C. Wiemer and W. J. Loos

Journal of Pharmaceutical and Biomedical Analysis. 2012 Feb 5;59:117-22.

ABSTRACT

A rapid and sensitive liquid chromatography/tandem mass spectrometry (LC-MS/MS) method has been developed and validated for the quantitative determination of cabazitaxel, a novel tubulin-binding taxane, in 100 µl aliquots of human lithium heparinized plasma with deuterated cabazitaxel as internal standard. The sample extraction and cleaning-up involved a simple liquid-liquid extraction with 20 µL aliquots of 4% ammonium hydroxide, 100 µl aliquots of acetonitrile and 1 ml aliquots of n-butylchloride. Chromatographic separations were achieved on a reversed phase C_{18} column eluted at a flow-rate of 0.200 ml/min on a gradient of acetonitrile. The overall cycle time of the method was 5 min, with cabazitaxel eluting at 3.0 min. The multiple reaction monitoring transitions were set at 836>555 (*m/z*), and 842>561 (*m/z*) for cabazitaxel and the internal standard, respectively. The calibration curves were linear over the range of 1.00 to 100 ng/mL with the lower limit of quantitation validated at 1.00 ng/ml. The within-run and between-run precisions, also at the level of the LLQ, were within 8.75%, while the accuracy ranged from 88.5 to 94.1%. As dilution of samples prior to extraction resulted in a loss of cabazitaxel of approximately 6.5% per dilution step, a second calibration curve ranging 40.0 to 4000 ng/ml was validated and was also linear. The within-run and between-run precisions in this range were within 4.99%, while the accuracy ranged from 95.8 to 100.3%. The method was successfully applied to samples derived from a clinical study.

INTRODUCTION

Acquired and intrinsic resistance to docetaxel and paclitaxel (i.e., the two approved first generation taxanes) is still an important concern in daily clinical practice. Therefore, the intravenously available semi-synthetic taxanes, cabazitaxel (Jevtana®; XRP6258; TXD258; RPR116258A) and larotaxel (RPR109881A) were selected for clinical development as a result of their efficacy in a broad range of cell-lines and tumor models of mouse and human origin. Also, both compounds showed greater potency than docetaxel in cell lines expressing the drug transporter p-glycoprotein (reviewed in (1-3)).

While larotaxel is currently still under clinical evaluation, cabazitaxel has been approved in the US by the Food and Drug Administration (FDA) in June 2010 (3) and in Europe by the European Medicines Agency (EMA) in January 2011 (4) in combination with prednisone for the treatment of patients with castration-resistant metastatic prostate cancer whose disease progresses after docetaxel treatment, based on the results of the TROPIC trail investigating cabazitaxel plus prednisone versus mitoxantrone plus prednisone following docetaxel failure (5). Cabazitaxel is currently being investigated in the setting of metastatic breast cancer progressing after taxane or anthracycline based chemotherapeutic regimens (6-7).

A population pharmacokinetic model was developed using pharmacokinetic data from five different studies (4), from which two currently have been published as peer-reviewed manuscripts (7-8). The pharmacokinetics of cabazitaxel are linear in the studied dose-range of 10-30 mg/m^2 given as 1 h infusions and are consistent with a three-compartment pharmacokinetic model with half lives in the initial, intermediate and terminal phase of approximately 4.4 minutes, 1.6 hours, and 95 hours respectively. The drug has a fast plasma clearance estimated to be 48.5 l/h in the studied population and has a large volume of distribution of 4870 l. The *ex-vivo* protein binding was 91.6%, mainly to albumin and lipoproteins, while the drug displays low binding to α1-acid glycoprotein. Cabazitaxel is extensively metabolized by cytochrome P450 iso-enzymes CYP3A4 and CYP3A5, with CYP2C8 playing a minor role in its metabolism. Cabazitaxel and its metabolites are mainly excreted via the feces (76% of the dose) and to a lesser extent through the urinary pathway (3.7% of the dose).

Neutropenia is the principle dose-limiting and most commonly observed toxicity in cabazitaxel treatment when administered as 1h infusion every 3 weeks (8). In the phase III trial comparing the efficacy of prednisone plus cabazitaxel to mitoxantrone, grade ≥3 neutropenia was observed in 82% of the patients in the cabazitaxel arm, with 8% of the patients experiencing febrile neutropenia (5). Of the non-hematological toxicities diarrhea is the most commonly observed side effect seen in this regimen (5, 8). Overall, diarrhea occurred in 47% of the patients with 6% experiencing grade ≥3 diarrhea (5). In a weekly schedule, diarrhea was even more pronounced and considered a dose-limited toxicity (9).

As cabazitaxel is a promising new anticancer agent for taxanes-resistant tumors, it is expected that numerous subsequent clinical studies investigating both single agent and cabazitaxel–based combinations will be initiated. For this purpose, quantitation of cabazitaxel is imperative.

To the best of our knowledge, no reports have been published describing a validated bioanalytical method for the quantitation of cabazitaxel. We have developed and validated a sensitive and selective liquid chromatography-tandem mass spectrometry (LC-MS/MS) assay for cabazitaxel in human plasma, according to the Guidance for Industry, Bioanalytical Method

Validation, as specified by the FDA. In addition, we discuss the non-specific binding of cabazitaxel observed during sample preparation and provide a simple and practical solution to deal with this phenomenon during pharmacokinetic analysis.

EXPERIMENTAL

Chemicals

Cabazitaxel (purity 92.9%) and the deuterated internal standard, $^{2}H_{6}$-cabazitaxel (purity 97.4%), were kindly supplied by Sanofi-Aventis (Frankfurt am Main, Germany). All chemicals were of analytical grade or higher. Acetonitrile, methanol and water were purchased from Biosolve BV (Valkenswaard, The Netherlands). Dimethyl sulfoxide, ammonium formate and n-butylchloride were from Sigma-Aldrich (Zwijndrecht, The Netherlands). Formic acid and ammonium hydroxide were obtained from J.T. Baker (Deventer, The Netherlands) and 2-propanol from Merck (Darmstadt, Germany). Blank lithium heparinized plasma was purchased from Biological Specialty Corporation (BSC, Colmar, PA, USA).

Preparation of stock solutions, calibration standards and quality control samples

Cabazitaxel stock solutions were prepared at 1 mg/ml free base in dimethyl sulfoxide. Stock solutions were aliquotted and stored at T<-70°C. Separate stock solutions were prepared for the construction of the calibration curve standards and the pools of quality control samples. The internal standard stock solution was prepared at 1 mg/mL in dimethyl sulfoxide, which subsequently was aliquotted and stored at T<-70°C. Internal standard working solutions were prepared at concentrations of 100 and 1000 ng/ml in acetonitrile, which were stored at T<8°C for a maximum of 3 months.

Calibration standards were prepared in duplicate on the day of analysis, by addition of 10 µL aliquots of appropriate dilutions of cabazitaxel stock solution in acetonitrile/DMSO (1:1, v/v) to 190 µl aliquots of human lithium heparinized plasma with concentrations of 1.00, 2.50, 10.0, 25.0, 50.0, 75.0, 90.0 and 100 ng/ml for quantitation of cabazitaxel in the concentration range of 1.00 to 100 ng/mL and 40.0, 120, 500, 1000, 2500, 3600 and 4000 ng/ml for concentrations of cabazitaxel in the range of 40.0 to 4000 ng/mL.

Pools of QC samples were prepared in human lithium heparinized plasma at concentrations of 1.00 ng/ml (lower limit of quantitation, LLQ), 3.00 ng/ml (QC Low), 40.0 ng/ml (QC Middle) and 80.0 ng/ml (QC High) for calibration standard curve in the range of 1.00 to 100 ng/ml and at 40.0 ng/ml (LLQ; i.e., QC Middle above), 120 ng/ml (QC Low), 1500 ng/ml (QC Middle) and 3000 ng/ml (QC High) for calibration standard curve in the range of 40.0 to 4000 ng/ml. Pools of QC samples were aliquotted and stored at T<-70 and T<-20°C upon processing.

Sample preparation

For both calibration ranges, aliquots of 20 µl 4% ammonium hydroxide and 100 µl of internal standard working solution (100 ng/ml for the range of 1.00 - 100 ng/mL and 1000 ng/ml for the range of 40.0 – 4000 ng/ml) were added to 100 µl of plasma samples in 2 ml microcentrifuge tubes followed by 1 ml aliquots of n-butylchloride. Hereafter, the samples were vigorously mixed for 10 minutes and then centrifuged at 18000 x *g* at ambient temperature for 10 minutes. Aliquots of 1 ml of the organic phase were transferred into 4.5 ml glass tubes and evaporated under nitrogen at T=70°C. The residues were resuspended in 100 µL aliquots of acetonitrile/water/ammonium formate (40:60:0.2, v/v/v). After 5 minutes of centrifugation at 3000 x *g*, the supernatants were transferred into 350 µl 96-well plates, which were placed into the chilled (T=10°C) autosampler, from which aliquots of 50 µl were injected onto the HPLC column for the low-range method (1.00 – 100 ng/ml) and 10 µl for the high-range method (40.0 – 4000 ng/ml).

Equipment

The LC-MS/MS system was purchased from Waters Chromatography B.V. (Etten-Leur, The Netherlands) and consisted of a Waters 2795 Separation Module coupled to a Quatro micro API Mass Spectrometer. The MassLynx V4.1 SCN627 software package was used for the acquisition and processing of data. Quantification was performed using QuanLynx as implemented in the MassLynx software.

Chromatographic conditions

Analytes were separated on an Alltima HP C_{18} HL column 3 µm, 50 mm x 2.1 mm, (Grace, Breda, The Netherlands) thermostatted at T=40°C. A gradient at a flow-rate of 0.20 ml/min was achieved with mobile phase A, composed of 2 mmol/L ammonium formate and mobile phase B, composed of acetonitrile. A linear gradient was used, with 50% to 15% mobile phase A, from 0 to 1.5 minutes, followed by holding on 15% mobile phase A (i.e., 85% mobile phase B) for 1.5 minutes. This was succeeded by a linear gradient back to 50% mobile phase A from 3.0 to 3.1 minutes, which was held for 1.9 minute to re-equilibrate. The overall run time of the assay was 5 minutes. A pre-column volume of 300 µl was applied and a parallel injection was enabled. The needle wash solvent was composed of acetonitrile/methanol/water/2-propanol/formic acid (25:25:25:25:0.1, v/v/v/v/v).

Mass spectrometry

Tandem mass spectrometry was performed in the positive ion electrospray ionization mode. Mass transitions of *m/z* were optimized for cabazitaxel and $^{2}H_{6}$-cabazitaxel (internal standard) by infusion of the respective analytes in acetonitrile/water/formic acid (40:60:0.1, v/v/v) via a T-union. Optimal MS settings were manually adjusted. The desolvation gas was set at 800 L/hour and the cone gas at 25 l/h (nitrogen). The ionspray voltage was kept at 3.50 kV and the cone voltage at 20 V for cabazitaxel and 19 V for the IS, with a source temperature of T=120°C and desolvation temperature of

T=350°C. The dwell times were set at 150 ms and the inter-channel delay at 50 ms. Multiple reaction monitoring (MRM) mode was applied for the quantitation with the following parameters: *m/z* 836 > 555, collision energy at 10 V for cabazitaxel and *m/z* 842 > 561, collision energy at 10 V for the internal standard (Figure 1). The collision cell pirani pressure was set at ~$5e^{-3}$ mbar (argon). The column effluent was passed through the mass spectrometer and monitored between 2 and 4 minutes after start of MS method, 0-2 minutes and 4-5 minutes sent to waste.

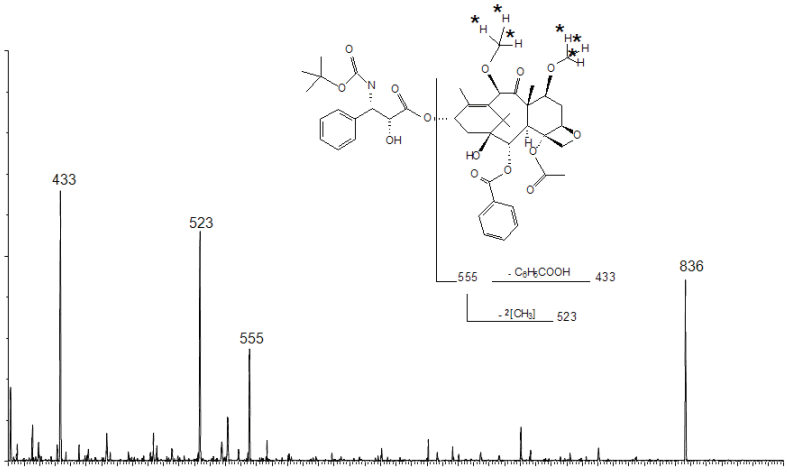


Figure 1. Mass spectrum and chemical structure of cabazitaxel. The asterisks represent the deuterium atoms in the stable labeled internal standard $^{2}H_{6}$-cabazitaxel

Quantitation

Calibration curves were generated using peak area ratios of cabazitaxel to internal standard $^{2}H_{6}$-cabazitaxel versus the known cabazitaxel concentrations with a linear regression analysis with a weighting scheme of 1/concentration for the low curve and 1/concentration^{2} for the high curve.

Method validation

The quantitative LC-MS/MS method was validated in accordance with the Guidance for Industry, Bioanalytical Method Validation, as specified by the FDA (http://www.fda.gov/downloads/Drugs/GuidanceComplianceRegulatoryInformation/Guidances/ucm070107.pdf).

Potential presence of endogenous contaminating compounds that may interfere with the analytical assay was determined by analyzing blank human lithium heparinized plasma samples of ten different lots. The following substances were investigated for interference with the analytical method: aprepitant, budesonide, dexamethasone, domperidon, granisetron, lorazepam, oxazepam, paracetamol and metoclopramide. All drugs have been dissolved/diluted in water to a concentration of 1 mg/ml followed by a 500-fold dilution in blank human lithium heparinized plasma. Aliquots have subsequently been diluted in plasma containing the different drugs to yield final cabazitaxel

concentrations of 8.00 and 50.0 ng/ml, which have been processed and compared to equal concentrations in blank human lithium heparinized plasma.

Accuracy (ACC), within-run precision (WRP) and the between-run precision (BRP) for both calibration curve ranges were determined by analyzing 5 replicates of pools of LLQ and QC samples independently over a three-run period, with the calibration curve standards processed in duplicate. The ACC, WRP and BRP at the level of the LLQ and QC samples were calculated by one-way analysis of variance, using the run as the variable as earlier described (10-11).

For the validation of the LLQ, besides the validation of the pools as described above, blank human lithium heparinized plasma of 10 different donors were spiked at a concentration of 1.00 ng/ml and quantitated in a separate run.

The evaluation of the matrix effect was tested by comparing the MS/MS response of cabazitaxel at a concentration of 3.00 and 80.0 ng/ml (for the low curve) and at 3000 ng/ml (for the high curve) spiked in triplicate in acetonitrile/water/formic acid (40:60:0.1, v/v/v) to the MS/MS responses of the analytes spiked in triplicate into extracts of blank human lithium heparinized plasma, as described recently (10, 12).

Extraction recovery was determined by comparing the MS/MS response of cabazitaxel at a concentration of 3.00 and 80.0 ng/ml (for the low curve) and at 3,000 ng/mL (for the high curve) spiked in triplicate into six different lots of blank lithium heparinized plasma before extraction, to the MS/MS responses of the analytes spiked in triplicate into extracts of blank human lithium heparinized plasma after extraction, corrected for the evaporated volume of organic phase (10, 12).

The stability of cabazitaxel in human lithium heparinized plasma was tested with QC low (3.00 ng/ml) and both QC high (80.0 and 3000 ng/ml) at ambient temperature for a period of 18 hours as well as following 3 freeze-thaw cycles, in which the samples were thawed for at least 30 minutes followed by refreezing for at least 18 hours. Long-term stability at T<-20°C and T<-70°C in human lithium heparinized plasma has been investigated using the same QC samples as described above. Also the storage stability of processed samples in the autosampler was tested in triplicate at the concentration of QC low and both QC high. QC samples were processed in triplicate and repeatedly injected on different time points.

Non-specific binding

As during the initial method validation non-specific binding of cabazitaxel was observed (see results section), an experiment was conducted to establish the potential loss of cabazitaxel during sample dilution. QC high (80.0 ng/ml) was serially diluted (in triplicate) to 40.0, 20.0, 10.0 and 5.00 ng/ml in blank plasma, subsequently processed and the individual accuracies estimated.

Application of method to clinical samples

To demonstrate the applicability of the validated bioanalytical method, blood samples were collected from seven patients enrolled in a clinical study in which cabazitaxel was administered intravenously over 60 minutes at a dose of 25mg/m^2 (see www.trialregister.nl; NTR study number 2840). Twelve blood samples in the presence of lithium heparin as anticoagulant were obtained during the

first 24 h after administration. A last blood sample was drawn between day 7 and 9. Samples were processed within 15 minutes of collection to isolate the plasma, which was stored at T<-70°C before analysis as described. All patients provided written informed consent and the local institutional review boards approved the clinical protocol (MEC 2011-091).

Individual pharmacokinetic parameters were estimated using non-compartmental analysis using the software program Phoenix WinNonLin 6.1 (Pharsight, Mountain View, CA).

RESULTS AND DISCUSSION

LC-MS/MS conditions

Cabazitaxel belongs to the group of taxanes with a single protonation site located on the secondary amine of the side-chain. Optimal sensitivity was achieved by the addition of ammonium formate, presumably by generation of an intermediate ion [M + $NH4^+$] which may dissociate into [M + H^+] and ammonia during the electrospray process. Therefore, for optimal signals the mobile phase was composed of 2 mM aqueous ammonium formate and acetonitrile.

The cabazitaxel product ion spectra (Figure 1) yielded abundant product ions suitable for use in multiple reaction monitoring. Main fragment *m/z* 555 resulted from the baccatin III core while *m/z* 523 is generated by loss of the methyl groups of the baccatin III core and *m/z* 433 was generated by loss of the benzoic acid from the baccatin III core. The product ion at *m/z* 555 was selected as the MRM ion for quantitation of cabazitaxel and the product ion at *m/z* 561 for its stable isotope labeled internal standard $^2H_6$-cabazitaxel.

Cabazitaxel was separated from early eluting hydrophilic, potentially response-suppressing, matrix components by applying a step gradient. A relative short analysis time of 5 minutes, with cabazitaxel eluting at 3.0 minutes (Figure 2) was maintained.

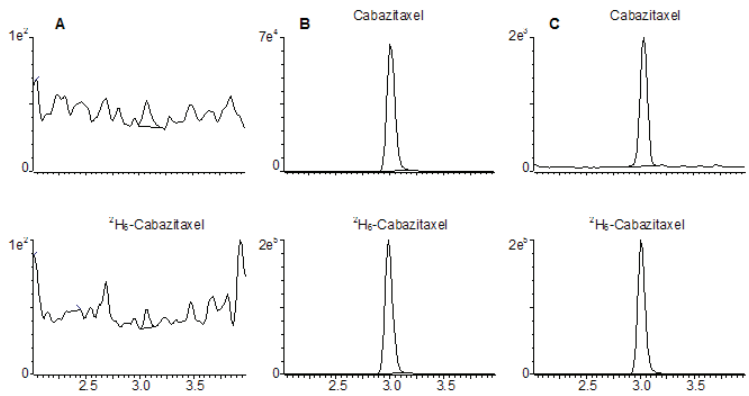


Figure 2. Representative chromatograms of (A) double blank processed plasma sample collected prior to the administration of 25 mg/m^2 cabazitaxel, (B) plasma sample taken prior to the end of infusion containing 267 ng/ml cabazitaxel and (C) plasma sample collected on day 7 containing 1.03 ng/ml cabazitaxel. Samples A and C are quantitated on the calibration curve in the range of 1.00 – 100 ng/ml cabazitaxel and sample B on the calibration curve in the range of 40.0 – 4000 ng/ml.

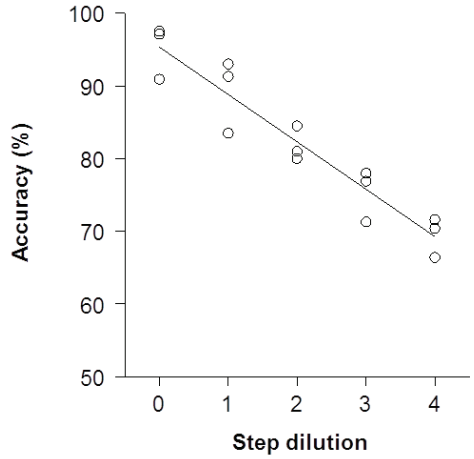


Figure 3. Linear correlation between the number of dilution steps and the observed accuracy of QC sample high spiked with 80.0 ng/ml cabazitaxel. Dilution "0" represents the accuracy following no dilution, "1" following 2-fold dilution in one step (i.e. 40.0 ng/ml), "2" following 4-fold dilution in 2 steps (i.e. 20.0 ng/ml), "3" following 8-fold dilution in 3 steps (i.e. 40.0 ng/ml) and "4" following 16-fold dilution in 4 steps (i.e. 5.00 ng/ml). The equation of the linear curve is: y = -6.5 * x + 95.3, in which the -6.5 represents the loss of 6.5% of cabazitaxel per dilution step.

Non-specific binding

During the initial method validation a QC diluted spiked at a concentration of 4000 ng/ml was included on the calibration curve standards in the range of 1.00 – 100 ng/ml. This QC sample was 50-fold diluted in blank plasma in 2-steps prior to extraction. The accuracy was below 85% during the first two validation runs. Therefore a new pool was prepared, which also failed for the accuracy. Non-specific binding is a well-known drawback of taxanes (13), therefore, non-specific binding during sample dilution was most likely the underlying explanation of the low accuracy of the diluted QC sample. This was investigated by serial dilution of the QC sample with a spiked concentration of 80.0 ng/mL. As shown in Figure 3, the accuracy decreases linearly by 6.5% following each sequential dilution step. Addition of polysorbate 80 or Cremophor EL up to concentrations of 1% to overcome non-specific binding (13), did not result in increased accuracies. Also, accuracy could not be improved by using pipette tips and/or laboratory tubes of different materials and brands (data not shown).

Eventually, validation of the method at a higher concentration range, to avoid additional steps in sample processing for samples in which the concentration exceeds the 100 ng/mL, was performed. The range of 40.0 to 4000 ng/ml was selected based on the available data of maximum concentrations of 535 ± 305 ng/mL following the administration of 25 mg/m^2 cabazitaxel (8).

Assay performance

The results of the method were linear ($r \geq 0.9987$) in the concentration range of 1.00 to 100 ng/ml as well as in the range of 40.0 to 4000 ng/ml ($r \geq 0.9950$) in human lithium heparinized plasma. None of the potentially co-administrated drugs interfered with the quantitation of cabazitaxel.

The LLQ was validated at 1.00 ng/ml with the measured concentrations of cabazitaxel in nine out of ten independently spiked plasma samples falling within the acceptable range of accuracy of 80-100%. The average measured concentration in all ten samples was 0.940 ± 0.087 ng/mL. The within-run and between-run precisions and the accuracies at seven tested concentrations in human lithium heparinized plasma, including at the level of the LLQ, are summarized in Table 1. All fall within the accepted ranges as specified by the Food and Drug Administration.

The mean measured extraction efficiencies for cabazitaxel were 92%, 100% and 106% at the concentrations of 3.00, 80.0 and 3000 ng/ml, respectively. The values for the matrix effect were 66%, 72% and 86% at 3.00, 80.0 and 3000 ng/ml, respectively.

Cabazitaxel was stable in lithium heparinized plasma (*i*) at ambient temperature for at least 18 hours, (*ii*) during three consecutive freeze-thaw cycles and (*iii*) at least 6 months when stored at T<–20°C and T<–70°C. As processed samples, cabazitaxel was stable for at least 20 hours in the chilled (T=10°C) autosampler (Table 2).

Table 1. Calculations of the between-run and within-run precisions and the average accuracy of the LLQ and QC samples[1]

Sample	Spiked (ng/mL)	GM (ng/mL)	ACC (%)	WRP (%)	BRP (%)	n[2]
Range 1.00 – 100 ng/mL						
LLQ	1.00	0.885	88.5	8.75	5.98	13 of 15
Low	3.00	2.81	93.7	8.12	#[3]	13 of 15
Middle	40.0	37.3	93.3	2.94	#[3]	15 of 15
High	80.0	75.3	94.1	3.03	0.67	15 of 15
Range 40.0 – 4,000 ng/mL						
LLQ	40.0	39.7	99.3	4.75	3.72	15 of 15
Low	120	119	99.2	4.15	3.29	15 of 15
Middle	1500	1504	100.3	4.99	#[3]	15 of 15
High	3000	2873	95.8	4.11	#[3]	15 of 15

Abbreviations: GM, grand mean; ACC, average accuracy; WRP, within-run precision; BRP, between-run precision

[1], n=5 in 3 separate runs

[2], number of individual samples falling within acceptable range of accuracy of 85-115% (80-120% at LLQ)

[3], no additional variation observed by performing the assay in different runs.

Table 2. Stability of cabazitaxel

Condition	(% to concentration at the initial time point)		
	3.00 ng/mL	80.0 ng/mL	3,000 ng/mL
Ambient temp (18h)	105	95.5	99.8
3 freeze/thaw cycles	100	103	100
Processed sample (T=10°C, 20h)	112	109	105
T<-20°C (6 months)	90.5	94.9	111
T<-70°C (6 months)	90.3	93.7	107

Clinical application

As shown in Figure 4, plasma concentration versus time curves could be readily determined. The data indicate that the lower limit of quantitation of 1.00 ng/ml is sufficient for monitoring drug-plasma levels in samples obtained from patients treated with cabazitaxel at a dose of 25 mg/m^2. Preliminary pharmacokinetic data revealed maximum concentrations of 189 ± 14.7 ng/ml (n=7) and $AUC_{(0\text{-}24h)}$ values of 248 ± 31.9 ng*h/ml (n=7). We were able to quantitate concentrations up to 7-9 days after administration with concentrations in the range of 1.03 to 1.25 ng/ml. The method is thus sensitive enough for pharmacokinetic analysis in clinical pharmacokinetic studies beyond 24h. Representative chromatograms are shown in Figure 2.

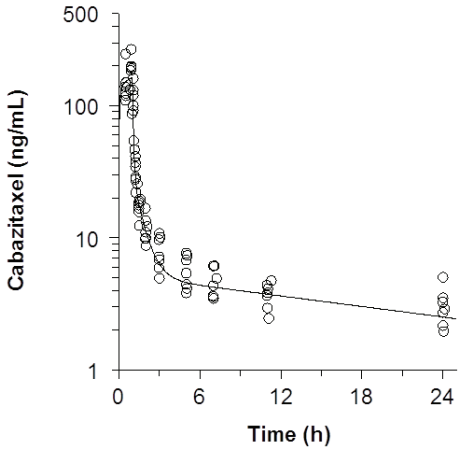


Figure 4. Average plasma concentration-time profile of cabazitaxel in 7 patients following the administration of 25 mg/m^2 as a 1 h infusion in the first course. Open symbols represent individual concentration data. The solid line represents the fit according to a three-compartmental pharmacokinetic model (model 19 with $1/y^2$ weighting; Phoenix WinNonLin version 6.1; Pharsight, Mountain View, CA).

CONCLUSION

A sensitive, selective, accurate and precise LC-MS/MS method has been developed and validated for the analysis of cabazitaxel in human heparinized plasma, which meets the current requirements of bioanalytical method validation. As sample dilution prior to extraction results in the loss of cabazitaxel, a secondary calibration range has been validated to avoid sample dilution. This method will prove to be a valuable tool for pharmacokinetic (interaction) studies with cabazitaxel.

REFERENCES

1. O. Metzger-Filho, C. Moulin, E. de Azambuja, A. Ahmad. Larotaxel: broadening the road with new taxanes, Expert Opin Investig Drugs. 2009;18: 1183-1189.
2. D.P. Michielsen, J.G. Braeckman, L. Denis. Cabazitaxel for the treatment of prostate cancer. Expert Opin Pharmacother. 2011;12:977-982.
3. S. Oudard. TROPIC: Phase III trial of cabazitaxel for the treatment of metastatic castration-resistant prostate cancer. Future Oncol. 2011;7:497-506.
4. Assessment Report for Jevtana (cabazitaxel). Procedure No.: EMEA/H/C/002018, European Medicines Agency, London, 2011.
5. J.S. de Bono, S. Oudard, M. Ozguroglu, S. Hansen, J.P. Machiels, I. Kocak, G. Gravis, I. Bodrogi, M.J. Mackenzie, L. Shen, M. Roessner, S. Gupta, A.O. Sartor. Prednisone plus cabazitaxel or mitoxantrone for metastatic castration-resistant prostate cancer progressing after docetaxel treatment: a randomised open-label trial. Lancet. 2010;376:1147-1154.
6. X. Pivot, P. Koralewski, J.L. Hidalgo, A. Chan, A. Goncalves, G. Schwartsmann, S. Assadourian, J.P. Lotz. A multicenter phase II study of XRP6258 administered as a 1-h i.v. infusion every 3 weeks in taxane-resistant metastatic breast cancer patients. Ann Oncol. 2008;19:1547-1552.
7. C. Villanueva, A. Awada, M. Campone, J.P. Machiels, T. Besse, E. Magherini, F. Dubin, D. Semiond, X. Pivot. A multicentre dose-escalating study of cabazitaxel (XRP6258) in combination with capecitabine in patients with metastatic breast cancer progressing after anthracycline and taxane treatment: A phase I/II study. Eur J Cancer. 2011;47:1037-1045.
8. A.C. Mita, L.J. Denis, E.K. Rowinsky, J.S. Debono, A.D. Goetz, L. Ochoa, B. Forouzesh, M. Beeram, A. Patnaik, K. Molpus, D. Semiond, M. Besenval, A.W. Tolcher. Phase I and pharmacokinetic study of XRP6258 (RPR 116258A), a novel taxane, administered as a 1-hour infusion every 3 weeks in patients with advanced solid tumor. Clin Cancer Res. 2009;15:723-730.
9. K.A. Gelmon, J. Latreille, A. Tolcher, L. Genier, B. Fisher, D. Forand, S. D'Aloisio, L. Vernillet, L. Daigneault, A. Lebecq, M. Besenval, E. Eisenhauer. Phase I dose-finding study of a new taxane, RPR 109881A, administered as a one-hour intravenous infusion days 1 and 8 to patients with advanced solid tumors. J Clin Oncol. 2000;18:4098-4108.
10. P. de Bruijn, I.M. Moghaddam-Helmantel, M.J. de Jonge, T. Meyer, M.H. Lam, J. Verweij, E.A. Wiemer, W.J. Loos. Validated bioanalytical method for the quantification of RGB-286638, a novel multi-targeted protein kinase inhibitor, in human plasma and urine by liquid chromatography/tandem triple-quadrupole mass spectrometry. J Pharm Biomed Anal. 2009;50:977-982.
11. H. Rosing, W.Y. Man, E. Doyle, A. Bult, J.H. Beijnen, Bioanalytical liquid chromatographic method validation. A review of current practices and procedures. J Liq Chromatogr R T. 2000;23:329-354.
12. B.K. Matuszewski, M.L. Constanzer, C.M. Chavez-Eng. Strategies for the assessment of matrix effect in quantitative bioanalytical methods based on HPLC-MS/MS. Anal Chem. 2003;75:3019-3030.
13. W.J. Loos, W.C. Zamboni, F.K. Engels, P. de Bruijn, M.H. Lam, R. de Wit, J. Verweij, E.A. Wiemer. Pitfalls of the application of microdialysis in clinical oncology: controversial findings with docetaxel. J Pharm Biomed Anal. 2007;45:288-294.

Chapter 8

Effects of budesonide on cabazitaxel-induced diarrhea in a randomized open-label multicenter phase II study: CABARESC

A.J.M. Nieuweboer, A.M. de Graan, P. Hamberg, S. Bins, R. van Soest, R.J. van Alphen, A.M. Bergman, A. Beeker, H. van Halteren, A.J. ten Tije, H. Zuetenhorst, N. van der Meer, D. Chitu, R. de Wit, A.H.J. Mathijssen

Submitted for publication.

ABSTRACT

Forty-seven percent of patients in the registration trial of cabazitaxel reported diarrhea of any grade (TROPIC, De Bono et al., Lancet 2010). The objective of this study was to investigate the effect of budesonide on the incidence and severity of cabazitaxel-induced diarrhea. An open-label, randomized, phase II multi-center trial was performed in metastatic castration resistant prostate cancer patients after docetaxel treatment between December 2011 and October 2015. Two hundred and forty-six metastatic castration resistant prostate cancer patients were randomized to receive standard of care cabazitaxel 25 mg/m^2 Q3W plus prednisone 10 mg (group CABA) or same dose/ schedule of cabazitaxel with concomitant budesonide 9 mg QD during the first two treatment cycles (group BUD). The incidence and severity of diarrhea during the first two cycles of cabazitaxel was the primary outcome measure. An intention-to-treat principle was used. Chi-squared tests were used to compare incidence numbers. In the phase II trial, 227 patients were evaluable. Grade 2-3 diarrhea occurred in 35 patients (15%) and grade 4 diarrhea was not reported. The incidence of grade 2-3 diarrhea was comparable in both treatment groups: 14 of 113 patients in group CABA (12%) versus 21 of 114 patients in group BUD (18%) (P=0.21). Seven patients were admitted to the hospital with diarrhea (n=5 group CABA vs. n=2 group BUD). PSA response was seen in 30% of patients and not affected by budesonide co-administration (P=0.29). Other toxicities were also not affected by budesonide co-administration. Conclusive, the incidence of cabazitaxel-induced diarrhea was notably less than reported in the TROPIC trial and and appears manageable in routine practice. Budesonide co-administration did not influence the incidence of cabazitaxel-induced diarrhea.

INTRODUCTION

Several therapeutic options are available for the treatment of metastatic castration resistant prostate cancer (mCRPC). Cabazitaxel is currently approved for second line treatment of mCRPC after progression on docetaxel treatment. In the registration trial of cabazitaxel, diarrhea was reported in 47% of patients (1). Six percent of patients had grade 3-4 diarrhea, requiring hospital admissions (1).

In order to avoid dose reductions of an effective regimen and to preserve the patients' quality of life, we aimed to search for measures to reduce the incidence and severity of cabazitaxel-induced diarrhea. It has previously been shown that co-administration of budesonide is able to reduce the incidence of diarrhea in irinotecan as well as in 5-FU-treated patients (2,3) Budesonide, a locally active corticosteroid, reduced diarrhea with ≥2 grades in 86% of irinotecan and 57% of 5-FU-treated patients that suffered from grade 3-4 diarrhea and did not respond to loperamide treatment (2). In the second study, budesonide co-administration in patients treated with irinotecan resulted in shorter and fewer periods of diarrhea compared to placebo (3). Budesonide has a 90% first pass effect and thus a low systemic availability, so little to none systemic toxicity can be expected from budesonide co-administration. Based on these data, we assumed budesonide to be a safe and potent drug to prevent cabazitaxel-induced diarrhea. Since the co-administration of budesonide and cabazitaxel has never been tested, our study was conducted in two parts. First, we investigated the safety of budesonide on the pharmacokinetics (PK) of cabazitaxel in a limited-size cross-over trial, to exclude a negative effect of budesonide on cabazitaxel exposure. Next, a sufficiently powered randomized phase II setting was chosen to explore the effects of budesonide on cabazitaxel-induced diarrhea.

PATIENTS AND METHODS

Phase II Trial

The randomized open-label phase II multicenter study was registered in the European Clinical Trials Database (no. 2011-003346-40) and in the Dutch Trial Registry (no. NTR2991). Only mCRPC patients with documented disease progression during or after docetaxel therapy were eligible for study participation. Disease progression was defined as at least two consecutive rises in PSA (taken ≥1 week apart) or progression according to RECIST (version 1.1). Patients had to be ≥18 years of age. A World Health Organisation (WHO) performance status of ≥1 and adequate renal, hepatic and hematopoietic functions were required for study inclusion. Patients had to be able to take oral drugs. The use of strong CYP3A4 inhibitors or inducers was prohibited.

Patients with an ostomy, ulcerative colitis, Crohn's disease or celiac disease were excluded. For the stratification of patients, the following factors were used: age (≥65 versus <65 years), center of enrolment, and previous radiotherapy on back, abdomen or pelvis (yes versus no).

Treatment

Patients were randomized to receive either standard of care cabazitaxel 25 mg/m^2 3-weekly plus prednisone 10 mg (group CABA) or cabazitaxel with concomitant budesonide 9 mg daily added for the first two courses (group BUD) as diarrhea was expected to occur in the first two treatment cycles (1). Prednisone had to be started at least one week before the first cycle of cabazitaxel. Patients started budesonide two days before their first cabazitaxel administration and took budesonide one hour before breakfast. Cabazitaxel was continued for a maximum of 10 cycles for evaluation of toxicity, but could be continued beyond 10 cycles if in the interest of the patient, unless progression or unacceptable toxicity occurred. Standard premedication was given (1). In case of severe toxicity, one dose reduction of 20% (to 20 mg/m^2) was allowed.

Objectives and enpoints

Primary objective of this trial was to study the effects of budesonide co-treatment on the grade of cabazitaxel-induced diarrhea during the first two treatment cycles. Primary endpoint was the difference in the incidence of grade 2-4 diarrhea in the budesonide co-treated arm compared to the arm without budesonide. Secondary objectives were to study the effects of budesonide on other cabazitaxel-induced adverse events and the effects of cabazitaxel and budesonide on PSA response. PSA response was defined as a >50% change in PSA after start of treatment with cabazitaxel. PSA at baseline was considered the last measured PSA value before start of cabazitaxel treatment.

Data acquisition

Patients kept a diary on their stools, co-medication and adverse events during the first two cabazitaxel cycles. Loperamide use in case of diarrhea was accepted according to regular protocol.

Prior to each treatment cycle PSA was measured, and adverse events were monitored. Common Terminology Criteria for Adverse Events (CTCAE) versions 2-4 were used.

Statistics for the Phase II trial analyses

The incidence of grade 2-4 diarrhea in the CABA group was estimated to be 25%. A reduction of 15% in the BUD group was considered clinically relevant and a two-sided significance level of $\alpha = 0.05$ and a power of 80% was defined. Power calculation showed that 113 evaluable patients per treatment arm were required. After the first 57 evaluable patients in each arm were included, an interim analysis was performed. If the percentage of patients with grade 3-4 diarrhea in the budesonide group would exceed the incidence observed in the control group by 15% the study would end for reasons of futility. Since the PK study had shown no drug interaction and the cabazitaxel dose and regimen was identical in the two treatment arms the study was not powered to test for differences in anti-tumor efficacy (4).

The results of the primary endpoint of the phase II study were based on the information reported by physicians on the adverse event forms. Analyses were done according an 'intention-to-treat' principle. The incidences of adverse events were compared between both treatment arms using a Chi-squared test.

Pharmacokinetic Study

In a randomized cross-over PK study, we investigated the effects of budesonide on the exposure to cabazitaxel. It was registered in the Dutch Trial Registry (no. NTR2840). Inclusion criteria were similar as those described above. Chemotherapy or radiotherapy <4 weeks before start with cabazitaxel was prohibited.

Patients were randomized to receive standard of care cabazitaxel 25mg/m^2 Q3W plus prednisone 10 mg without budesonide in course 1 and with daily 9 mg budesonide (3 capsules of 3 mg) in course 2. In group B, budesonide was added to the cycles of cabazitaxel in the opposite order. Patients as well as their treating physicians, documented diarrhea and other (serious) adverse events in a diary or patient record.

Pharmacokinetic sampling and analysis

Cabazitaxel PK sampling was performed prior to infusion, 30 minutes after start and 5 minutes before stop of the infusion. Post-infusion, samples were drawn at 5 minutes, 15 minutes, 30 minutes, 1 hour (h), 2h, 4h, 6h, 11h and 23h. Samples were collected in the presence of lithium heparin and processed to plasma which were thereafter added into polypropylene tubes and stored at <-70°C until the time of analysis. Analyses were done using a validated LC-MS/MS method as described by de Bruijn *et al.* (5). Area under the curves from 0 to 24 hours (AUC 0-24h) were estimated doing a non-compartmental analysis using Phoenix v 6.1 (Pharsight, Mountain View, CA).

Statistics for pharmacokinetic analyses

Patients were considered evaluable for analysis when two cabazitaxel courses had been administered and PK sampling was performed adequately. A difference in PK was defined as a difference in cabazitaxel area under the curve (AUC) of >20%. Based on a power analysis assuming an inter-individual variability in cabazitaxel PK of 20% and a power of 80%, with a 2-sided P-value of ≤ 0.05, a sample size of 18 patients was calculated. A Student's *t*-test was used for statistical testing. Both the PK study and the randomized phase II study were approved by the ethics committee of the Erasmus University Medical Center as well as approved by the local committees of the participating hospitals and performed according to the values of the Declaration of Helsinki. Informed consent was obtained in all patients.

RESULTS

Patient and treatment characteristics in the Phase II trial

Between December 2011 and October 2015, 246 patients were included in 22 different hospitals in the Netherlands (see Table 1). All patients were randomized to either one of both treatment arms. Patient characteristics are shown in Table 2. Nineteen patients were considered ineligible (11 from arm CABA and 8 from arm BUD, see Table 3). For the primary endpoint, 227 patients were considered eligible (CABA n=113, BUD n=114).

The median number of cabazitaxel cycles was six in both treatment arms. Reasons for discontinuing the study are shown in Table 4. In three patients, diarrhea was one of the reasons for study discontinuation. Three patients in arm B received no budesonide, 12 patients (11%) only in one cycle and 96 patients (84%) received two cycles of cabazitaxel with concomitant budesonide. Three patients received >3 cycles with concomitant budesonide. For twenty-five patients (12%), a dose reduction was made in cycle 2. In one of these 25 patients, diarrhea was the reason for dose modification.

Table 1. Patient inclusion per participating medical center

City	Hospital	Number of included patients
Alkmaar	Medical Center of Alkmaar	2
Amsterdam	Antoni van Leeuwenhoek Hospital/ Dutch Cancer Institute	15
Breda	Amphia Hospital	11
Delft	Reinier de Graaf Hospital	9
Delfzijl	Ommelander Hospital Delfzicht	1
Deventer	Deventer Hospital	3
Dordrecht	Albert Schweitzer Hospital	6
Eindhoven	Catharina Hospital	8
Geldrop	St. Anna Hospital	5
Groningen	Martini Hospital	2
Haarlem	Kennemer Gasthuis	5
Hoofddorp	Spaarne Hospital	14
Leeuwarden	Medical Center of Leeuwarden	6
Leiden	Leiden University Medical Center	2
Leidschendam	Medical Center Haaglanden Antoniushove	5
Maastricht	Maastricht University Medical Center	5
Rotterdam	Erasmus University Medical Center	50
Rotterdam	Sint Franciscus Gasthuis	52
Tilburg	Tweesteden Hospital	17
Vlissingen	Admiraal de Ruyter Hospital	13
Zutphen	Gelre Hospital	7
Zwolle	Isala Hospital	8

Table 2. Patient characteristics

	Group CABA	Group BUD	Total
Total	113 (100%)	114(100%)	227 (100%)
Median age at baseline	69	68	68
Range	49-85	51-83	49-85
Age >64 years	84 (74%)	84 (74%)	168 (74%)
Age ≤64 years	29 (26%)	30 (26%)	59 (26%)
WHO performance status [0-5]			
WHO 0	49 (43%)	43 (38%)	92 (41%)
WHO 1	64 (57%)	69 (61%)	133 (59%)
Not reported	-	2 (2%)	2 (1%)
Average number of stools baseline			
≤1	67 (59%)	55 (48%)	122 (54%)
1-2	21 (19%)	34 (30%)	55 (24%)
2-3	7 (6%)	6 (5%)	13 (6%)
3-4	3 (3%)	3 (3%)	6 (3%)
>4	1 (1%)	2 (2%)	3 (1%)
unknown	14 (12%)	14 (12%)	28 (12%)
Type of castration			
Surgical	12 (11%)	19 (17%)	31 (14%)
Chemical	101 (98%)	95 (83%)	196 (86%)
Number of prior chemotherapeutic regimens*			
1	102 (90%)	105(92%)	207 (91%)
2	11 (10%)	7 (6%)	18 (8%)
3	-	1 (1%)	1 (0%)
4	-	1 (1%)	1 (0%)
Median	1	1	1
Range	1-2	2-3	3-4

	Group CABA	Group BUD	Total
Prior therapy with abiraterone			
No	82 (73%)	74 (65%)	156 (69%)
Yes	31 (27%)	40 (35%)	71 (31%)
Prior therapy with orteronel (TAK 700)			
no	106 (94%)	106 (93%)	212 (93%)
yes	7 (6%)	8 (7%)	15 (7%)
Chemotherapy within the last 4 weeks before randomization			
no	78 (69%)	81(71%)	159 (70%)
yes	3 (3%)	3 (3%)	6 (3%)
missing	32 (28%)	30 (26%)	62 (27%)
Prior irradiation back, abdomen and/or pelvis			
no	53 (47%)	55 (48%)	108 (48%)
yes	60 (53%)	59 (52%)	119 (52%)

*Experimental chemotherapeutical therapies included

Table 3. Reasons for Ineligibility

Reason for ineligibility	Group CABA	Group BUD	Total
No cabazitaxel in study context*	3	7	10
Initial cabazitaxel dose <25mg/m^2	5	1	6
Treatment started before randomization	1	0	1
Death before start therapy	1	0	1
Long treatment delay after randomization[¤]	1	0	1
Total	11	8	19

*due to disease progression and worsening of patient conditions. [¤]due to ASAT and ALAT > 2 upper limit of normal without liver metastases there was a time of two months between randomisation and start of cabazitaxel therapy.

Table 4. Reasons for patients going off-protocol

Primary reason	Group CABA	Group BUD	Total
Withdrawal of consent	0	1	1
Progression of disease	47	34	81
Toxicity	16	22	38
Death	5	5	10
Other	9	16	25
Unknown	14	17	31

Effects of budesonide on the incidence and severity of diarrhea

In group CABA, 14 out of 113 patients (12%) had grade 2-3 diarrhea in one of the first two cycles of cabazitaxel treatment (see Table 5). In group BUD, 21 out of 114 patients (18%) had grade 2-3 diarrhea in either one or both (n=2) of the first two cycles of cabazitaxel treatment (P = 0.21). Seven patients (3%) (CABA n=5, BUD n=2) were hospitalized for diarrhea during cabazitaxel treatment. Grade 3 diarrhea occurred in 4% of patients. The incidence of grade 3 diarrhea was not different between group CABA (n=5) and group BUD (n=4) (*P*=0.72). Interestingly, no grade 4 diarrhea was observed. Data from patients' diarries is shown in Table 6.

Table 5. Effect of budesonide on diarrhea

	Group CABA	**Group BUD**	**Total**	**P-value**
Total	113 (100%)	114 (100%)	227 (100%)	.
Grade 2-3 diarrhea	14 (12%)	21 (18%)	35 (15%)	0.21
Grade 3 diarrhea	5 (4%)	4 (4%)	9 (4%)	0.27
Hospitalization with diarrhea	5 (4%)	2 (2%)	7 (3%)	0.25

Table 6. Patient diaries' information

Minimum number of stools per day	**Group CABA**	**Group BUD**	**Total**
0	79 (70%)	75 (66%)	154 (68%)
1	17 (15%)	19 (17%)	36 (16%)
2	2 (2%)	4(4%)	6 (3%)
3	-	1(1%)	1 (1%)
unknown	15 (13%)	15 (13%)	30 (12%)
Maximum number of stools per day			
Number of patients	98	99	197
Median (range)	3.00 (1.00-13.0)	3.00 (1.00-40.00)*	3.00(1.00-40.00)*
1	4 (4%)	3 (3%)	7 (3%)
2	27 (24%)	27 (24%)	54 (24%)
3	31 (27%)	25 (22%)	56 (25%)

Minimum number of stools per day	Group CABA	Group BUD	Total
4	15 (13%)	19 (17%)	34 (15%)
5	10 (9%)	9 (8%)	19 (8%)
6	3 (3%)	9 (8%)	12 (5%)
7	2 (2%)	4 (4%)	6 (3%)
8	1 (1%)	2 (2%)	3 (1%)
9	2 (2%)	-	2 (1%)
10	2 (2%)	-	2 (1%)
13	1 (1%)	-	1 (0%)
40	-	1 (1%)	1 (0%)
unknown	15 (13%)	15 (13%)	30 (13%)
Average number of stools per day			
Number of patients	98	99	197
Median (range)	1.32 (0.40-3.98)	1.50 (0.39-4.31)	1.37 (0.39-4.31)

* One patient reported a maximum of 40 stools.

Effects of cabazitaxel and/or budesonide on PSA response

PSA decrease of at least 50% was seen in 30% of all patients (38/113 patients in arm A versus 31/114 in arm B). Concomitant use of budesonide had no effect on PSA response (P=0.29).

Effects of budesonide on other cabazitaxel-induced serious adverse events

Budesonide had no effect on neutropenia, leukopenia, thrombocytopenia, nausea, vomiting, alopecia and motor and sensory neuropathy during the first two cycles (see Table 7). No correlation was seen between the occurrence of neutropenia and grade 2-4 diarrhea (P=0.32).

Effects of budesonide on cabazitaxel pharmacokinetics

Between May 2011 and August 2011, 21 Caucasian patients were included in the pharmacokinetic study. Two patients were excluded after inclusion due to a decline in clinical performance status. One patient received only one course of cabazitaxel. The other 18 patients were evaluable for PK analyses. The median age at study entry was 66 (range 46-75) and 53% of patients received radiotherapy prior to cabazitaxel treatment. No statistically significant difference in mean dose-corrected AUC (0-24h) was found between the courses with concomitant budesonide (4.6 ng*h/mL, SD 1.2) and without concomitant budesonide (4.2 ng*h/mL, SD 1.1, *P* = 0.21) (see Table 8). Therefore, budesonide co-administration had no was considered to be safe for investigation in the phase II study.

Table 7. Effect of budesonide on adverse events

	Group CABA	Group BUD	Total	P-value
Total	113 (100%)	114 (100%)	227 (100%)	.
Neutropenia	55 (49%)	57 (50%)	112 (49%)	0.84
Leukopenia	47 (42%)	49 (43%)	96 (42%)	0.83
Thrombocytopenia	4 (4%)	4 (4%)	8 (4%)	0.99
Neuropathy, motor	5 (4%)	6 (5%)	11 (5%)	0.77
Neuropathy, sensory	29 (26%)	36 (32%)	65 (29%)	0.32
Alopecia	10 (9%)	13 (11%)	23 (10%)	0.52
Nausea	36 (32%)	36 (32%)	72 (32%)	0.96
Vomiting	20 (18%)	17 (15%)	37 (16%)	0.57

Table 8. Pharmacokinetic effect of budesonide on cabazitaxel exposure

	Course without Budesonide	Course with budesonide	P-value
AUC mean (SD)	4.16 (1.11)	4.6 (1.24)	0.21
AUC median (range)	4.48 (2.46-6.2)	4.43 (2.15-6.93)	

All units are: ng*h/mL. AUC: area under the curve, SD: standard deviation.

DISCUSSION

The registration trial of cabazitaxel (TROPIC) reported all grades of diarrhea in 47% of cabazitaxel-treated patients (1). We thus aimed to find methods to reduce the incidence and severity of cabazitaxel-induced diarrhea in order to preserve patients' quality of life as well as to avoid toxicity related dose-reductions and premature discontinuation of a potentially highly effective chemotherapy regimen. Our initial PK-study showed that budesonide had no negative effects on the exposure of cabazitaxel, and therefore the open-label multicentre phase II trial (CABARESC) was initiated.

In this phase II trial, the incidence of diarrhea was markedly lower than expected, based on the data from the TROPIC trial (1). Diarrhea also appeared quite manageable, since only 7 patients (3%, versus 6% in TROPIC) required hospitalization for diarrhea and moreover only 2 patients reported diarrhea grade 2-3 in both cycles. Diarrhea was the reason of dose-reduction in only one case and thus not dose limiting. Moreover, diarrhea was never the sole reason for stopping treatment.

As mentioned, grade 3 diarrhea was reported in only 4% of patients and grade 4 was not reported at all, compared to 6% grade 3-4 diarrhea reported in the TROPIC trial (1). In the UK early access program and in the Spanish expanded access program similar grades were reported (6, 7, 8). These data, including those for our trial, add to the accumulating evidence that real-world toxicity of cabazitaxel is less than that experienced in the TROPIC trial (1).

The effects of budesonide on cabazitaxel-induced diarrhea were smaller than anticipated. This may partly be due to the lower incidence of grade 2-4 diarrhea in the control group than expected (15% versus 25% respectively). To avoid underestimation of the incidence and severity of diarrhea, patients also reported their stools in a diary. However, most patients report a maximum of <4 stools, maximally defined as grade I diarrhea.

Chemotherapy-induced diarrhea is thought to be predominantly secretory with an exudative component and to be due to drug-toxicity on rapidly dividing cells of the intestinal epithelium (9-11). It is possible that cabazitaxel-induced diarrhea has no inflammatory foundation and that budesonide did therefore not result in a clinically meaningful effect.

CONCLUSIONS

In this study, the incidence of cabazitaxel-induced diarrhea is notably lower than anticipated, not dose limiting, and appears manageable in routine clinical practise. The addition of budesonide to cabazitaxel therapy however had no influence on the incidence of diarrhea.

ACKNOWLEDGEMENTS

We would like to thank all participating patients and study managers at the sites for their participation and cooperation. We also would like to thank the following participating physicians for their work in this study: M.P. Hendriks MD, M.M.E.M. Bos MD Phd, Z. Erjavec MD, W.I. Vervenne MD, J. van der Bosch MD, G.J.M. Creemers MD PhD, R.E.H. Smeets MD, R.S. de Jong MD PhD, Ph. Kuijer, MD PhD, M.B. Polée MD PhD, Prof. A.J. Gelderblom MD PhD, M. Los, MD PhD, M. Aarts MD PhD, H. Meulenbeld MD PhD, J.L.L.M. Coenen MD.

REFERENCES

1. de Bono JS, Oudard S, Ozguroglu M, et al. Prednisone plus cabazitaxel or mitoxantrone for metastatic castration-resistant prostate cancer progressing after docetaxel treatment: a randomised open-label trial. Lancet 2010;376:1147-54.
2. Lenfers BH, Loeffler TM, Droege CM, Hausamen TU. Substantial activity of budesonide in patients with irinotecan (CPT-11) and 5-fluorouracil induced diarrhea and failure of loperamide treatment. Ann Oncol 1999;10:1251-3.
3. Karthaus M, Ballo H, Abenhardt W, et al. Prospective, double-blind, placebo-controlled, multicenter, randomized phase III study with orally administered budesonide for prevention of irinotecan (CPT-11)-induced diarrhea in patients with advanced colorectal cancer. Oncology 2005;68:326-32.
4. De Graan AJ, Nieuweboer AJM, Loos WJ, et al. Effect of budesonide on the plasma exposure of cabazitaxel (Jevtana®) in castrate resistant prostate cancer patients. ASCPT 2012, PIII-41, 2012.
5. de Bruijn P, de Graan AJ, Nieuweboer AJM, et al. Quantification of cabazitaxel in human plasma by liquid chromatography/triple-quadrupole mass spectrometry: a practical solution for non-specific binding. J Pharm Biomed Anal 2012;59:117-22.
6. Bahl A, Masson S, Malik Z et al. Final quality of life and safety data for patients with metastatic castration-resistant prostate cancer treated with cabazitaxel in the UK Early Access Programme (EAP) (NCT01254277). BJU Int. 2015;116(6):880-7.
7. Castellano D, Antón Aparicio LM, Esteban E et al. Cabazitaxel for metastatic castration-resistant prostate cancer: safety data from the Spanish expanded access program. Expert Opin Drug Saf. 2014;13(9):1165-73.
8. Heidenreich A, Bracarda S, Mason M, et al. Safety of cabazitaxel in senior adults with metastatic castration-resistant prostate cancer: results of the European compassionate-use programme. Eur J Cancer. 2014;50(6):1090-9
9. Richardson G, Dobish R. Chemotherapy induced diarrhea. J Oncol Pharm Pract. 2007;13(4):181-98.
10. Saliba F, Hagipantelli R, et al. Pathophysiology and therapy of irinotecan-induced delayed-onset diarrhea in patients with advanced colorectal cancer: a prospective assessment.
11. J Clin Oncol. 1998;16(8):2745-51.
12. Shaw C, Taylor L. Treatment-related diarrhea in patients with cancer. Clin J Oncol Nurs. 2012;16(4):413-7.

Chapter 9

Summary

SUMMARY

The taxanes paclitaxel, docetaxel and cabazitaxel are used in the treatment of patients with solid tumors. These compounds are known for their anti-cancer effect, but also for their impact on quality of life, as taxanes are notorious for causing toxicity. For example, taxanes may cause neutropenia, neurotoxicity, fatigue, nausea, and vomiting. These toxicities can be dose limiting or cause premature therapy determination. It would therefore be convenient to be able to predict which patients will suffer from taxane-induced toxicity. Understanding the pharmacokinetics and pharmacodynamics of taxanes would therefore aid in the individualization of taxane therapy as the exposure is linked to efficacy as well as toxicity. However, multiple factors may be able to influence the pharmacokinetics and pharmacodynamics of a drug. Identifying these factors would allow us to act on possible consequences. In this thesis, we looked into different factors influencing the pharmacokinetics as well as the pharmacodynamics and toxicity of taxanes. We aimed for a more individualized approach of taxane therapy, reducing toxicity while optimizing efficacy.

In **Chapter 2**, we looked into the role of solvents in the pharmacokinetics of taxanes. Because taxanes are known to be poorly soluble in water, solvents Kolliphor EL (KEL; or Cremophor EL (CrEL)) and Polysorbate 80 (PS80) are used for the formulations of these drugs. Previous research has showed that solvents influence the distribution of taxanes and that the hepatic elimination of paclitaxel was diminished when the formulation of this drug contained KEL (1,2). Previous literature also showed that the Organic Anion Transporting Polypeptide (OATP) 1B-type transporters take up taxanes for hepatic elimination (3). Using this knowledge, we hypothesized that the presence of solvents in taxane formulation would also affect the uptake of taxanes by OATP1-type transporters. Cell lines as well as wild-type and OATP1B2-knock out mice were used for these pharmacokinetic experiments. Studies were done in absence or presence of the solvents CrEL and PS80. Results show that cabazitaxel is not transported by OATP1B1, OATP1B2, and OATP1B3. The uptake of paclitaxel and docetaxel is reduced in the presence of solvents CrEL and PS80. As a result, mice with an OATP1B2 deficiency had significantly lower clearance of paclitaxel in absence of solvents only. In other words, the effect of solvents on the uptake of paclitaxel by OATP1B2 masked the effect of OATP1B2 deficiency. This study confirms our hypothesis that solvents affect taxane pharmacokinetics, the hepatocellular uptake of taxanes by OATP1B2 in specific.

Patients treated with paclitaxel are at risk for neutropenia and subsequently for severe (opportunistic) infections and sepsis. Knowing which patients are prone to suffer from neutropenia could help in anticipating on this problem (for example resulting in close monitoring of these patients). In **Chapter 3**, we investigated whether genetic variation in drug metabolizing enzymes and transporters would be able to identify patients prone for hematological toxicity. Instead of using a candidate-gene approach, we used the drug metabolizing enzymes and transporters (DMET) platform, a chip containing 1936 single nucleotide polymorphisms (SNPs) in 225 genes encoding for enzymes and transporters involved in drug metabolism and transport. We identified a 10-SNP predictive model for neutropenia in 279 paclitaxel-treated patients. The sensitivity of this model was limited (43%).

We aimed for improved sensitivity by developing a genetic model predictive of neutropenia in the 3-weekly treated patients only. This resulted in a model with an ability to identify 79% of patients with paclitaxel-induced neutropenia.

Also for docetaxel neutropenia is a frequent clinical problem and a dose-limiting toxicity. In **Chapter 4** we described that in our population of 213 docetaxel-treated patients, we saw that patients with severe neutropenia had a significantly lower docetaxel clearance than patients with normal neutrophil counts. We developed a model that could predict neutropenia with 70% sensitivity. Since the exposure to docetaxel correlates with its toxicity, we also developed a model predicting low docetaxel clearance (and thus high exposure) (4). Patients with low clearance (defined as 1 standard deviation below the mean clearance) could be correctly identified in 80% of cases. These models, as also the models for paclitaxel, did not reach statistical significance. Raising the assumption that genetic variation within patients only is not enough to accurately predict taxane-induced toxicity and to do so, the effects of non-genetic factors on the pharmacokinetics and pharmacodynamics of taxanes must be taken into account.

To look into all known and clinically relevant factors influencing docetaxel pharmacokinetics, an extensive literature search was performed. **Chapter 5** summarizes current knowledge on factors influencing docetaxel pharmacokinetics and therefore (potentially) also efficacy. To sum, liver impairment and hormonal factors should be taken into account in docetaxel therapy. Liver impairment decreases docetaxel clearance. Hormonal factors such as castration status and menopausal status also influence docetaxel pharmacokinetics. This review confirmed that the value of pharmacogenetics in predicting docetaxel pharmacokinetics is still limited. Drug-drug interactions on the other hand are of clinical relevance, especially with drugs inhibiting Cytochrome P450 3A4.

Since the first introduction of cabazitaxel, multiple new drugs for the treatment of castration resistant prostate cancer (mCRPC) were brought onto the market for the treatment of these patients. Both androgen receptor targeted drugs abiraterone as well as enzalutamide are frequently used in the treatment of mCRPC. However, with the introduction of these new therapies, it remained unclear what the most effective treatment continuum would be. For a long time, it was indistinct whether the efficacy of cabazitaxel was affected by preceding therapy with androgen receptor targeted drugs. In **Chapter 6**, we studied the effects of preceding abiraterone and/or enzalutamide therapy in patients with mCRPC on cabazitaxel efficacy. In this study, data was collected from 114 mCRPC patients that had been treated with cabazitaxel (in the prospective CABARESC trial). All were treated after a regimen of docetaxel therapy. Looking at prostate-specific antigen response and overall survival as a measure for cabazitaxel efficacy, we showed that preceding therapy with abiraterone or enzalutamide does not affect cabazitaxel efficacy. Based on these data, cabazitaxel seems to be useful at any stage of post-docetaxel mCRPC treatment.

In **Chapter 7**, a method for the quantitative measurement of cabazitaxel has been developed using liquid chromatography/tandem mass spectrometry (LC-MS/MS). Using this method, cabazitaxel

levels can be determined in human lithium heparinized plasma samples. This method was then used in the clinical trial determining the effect of the corticosteroid budesonide on the pharmacokinetics of cabazitaxel, preceding the CABARESC study (**Chapter 8**). In accordance with the hypothesis this particular study showed no clinically relevant effects of budesonide on the pharmacokinetics of cabazitaxel. Subsequently, we used budesonide to test the hypothesis that co-administrating this corticosteroid with cabazitaxel would diminish the incidence and severity of cabazitaxel-induced diarrhea.

Budesonide was chosen for this purpose because of its low systemic availability and thus fortunate safety profile. This drug was previously shown to decrease the severity of irinotecan-induced diarrhea (5, 6). We hypothesized that the locally anti-inflammatory effect of budesonide would prevent the occurrence of cabazitaxel-induced diarrhea. We included 227 evaluable mCRPC patients in our study, of which half received budesonide for two courses, starting 2 days before the first cabazitaxel administration. Analyses of the data showed that the incidence of cabazitaxel-induced diarrhea was lower than reported previously. Diarrhea was no dose limiting toxicity and well manageable in routine clinical practise. Budesonide had no significant effect on the incidence and severity of cabazitaxel.

To conclude, multiple factors may influence the pharmacokinetics and pharmacodynamics of taxanes. Some of these factors are clinically relevant and should be taken into account when administering a taxane to a patient with cancer. Factors such as hormonal status deserve more thorough investigation, as its effects seems to be under-exposed but potentially important. The combination of patient-bound and environmental factors with genetic factors could potentially increase the value of predictive models in a clinical setting. The ultimate goal should be to increase quality of life by diminishing taxane-induced toxicity, as well as to optimize taxane-efficacy.

REFERENCES

1. K.L. Hennenfent, R. Govindan et al. Novel formulations of taxanes: a review. Old wine in a new bottle? Ann Oncol 2006;17:735–49.
2. A. Sparreboom, O. van Tellingen, W.J. Nooijen et al. Nonlinear pharmacokinetics of paclitaxel in mice results from the pharmaceutical vehicle Cremophor EL. Cancer Res 1996;56:2112–5.
3. N.F. Smith, M.R. Acharya, N. Desai et al. Identification of OATP1B3 as a high-affinity hepatocellular transporter of paclitaxel. Cancer Biol Ther 2005;4(8):815-818.
4. Baker SD, Li J, ten Tije AJ, Figg WD, Graveland W, Verweij J, et al. Relationship of systemic exposure to unbound docetaxel and neutropenia. Clin Pharmacol Ther. 2005;77(1):43-53.
5. Lenfers BH, Loeffler TM, Droege CM, Hausamen TU. Substantial activity of budesonide in patients with irinotecan (CPT-11) and 5-fluorouracil induced diarrhea and failure of loperamide treatment. Ann Oncol 1999;10:1251-3.
6. Karthaus M, Ballo H, Abenhardt W, et al. Prospective, double-blind, placebo-controlled, multicenter, randomized phase III study with orally administered budesonide for prevention of irinotecan (CPT-11)-induced diarrhea in patients with advanced colorectal cancer. Oncology 2005;68:326-32.

Appendix I

Nederlandse Samenvatting

NEDERLANDSE SAMENVATTING

De afgelopen jaren zijn de anti-kanker middelen paclitaxel, docetaxel en cabazitaxel veel gebruikt voor de behandeling van verschillende soorten kanker. Deze drie chemotherapeutica noemen we ook wel 'Taxanen'. Taxanen kunnen overlevingswinst bieden aan patiënten met onder andere borstkanker, longkanker en prostaatkanker. Aan de behandeling met deze middelen zitten echter ook nadelen.

De toediening van taxanen resulteert in sommige gevallen in ernstige bijwerkingen. Voorbeelden daarvan zijn gevoelsstoornissen aan handen en voeten, vermoeidheid, haarverlies, misselijkheid en diarree. Van tevoren valt echter niet goed te voorspellen welke patiënten last zullen krijgen van deze bijwerkingen. Ook valt niet goed te voorspellen hoe groot de blootstelling aan het medicijn precies zal zijn. Dit valt te wijten aan een grote variatie in de farmacokinetiek van taxanen. Farmacokinetiek is de verzamelnaam voor de opname, verdeling, omzetting en uitscheiding van een medicijn door het lichaam. Voor taxanen is aangetoond dat er een verband bestaat tussen de blootstelling aan het geneesmiddel en zijn werkzaamheid en bijwerkingen. Taxanen staan daarnaast bekend om hun kleine 'therapeutische breedte', wat zoveel wil zeggen als: er is maar een klein verschil tussen de meest effectieve dosering en een dosering die te hoog of te laag is, wat kan resulteren in veel bijwerkingen of een suboptimaal effect van de therapie. Als patiënten een te hoge blootstelling hebben aan een taxaan doordat ze het middel langzaam uit het lichaam verwerken en uitscheiden, is het voorstelbaar dat deze mensen meer last zullen hebben van ernstige bijwerkingen dan patiënten die het middel snel verwerken en uitscheiden. Andersom geldt dat mensen die het geneesmiddel heel snel kwijt zijn, een groter risico lopen op een suboptimaal effect van de therapie. Het is daarom nuttig om de blootstelling van patiënten aan taxanen te kunnen voorspellen om zo een optimale dosering te kunnen berekenen. Op dit moment worden patiënten gedoseerd op basis van hun lengte en gewicht. Gebleken is dat deze strategie de variatie in de farmacokinetiek tussen patiënten niet reduceert.

Naast farmacokinetische variatie is er ook een grote variatie in de 'farmacodynamiek' van taxanen. Met farmacodynamiek bedoelt men de werking van het geneesmiddel op het lichaam. Hieronder vallen de bijwerkingen van geneesmiddelen, maar ook zijn werkzaamheid. Deze variatie tussen patiënten hangt deels samen met de variatie in farmacokinetiek. Om bijwerkingen te verminderen en de effectiviteit van de therapie te verbeteren zou het dus ook relevant zijn om de farmacodynamiek voor individuele patiënten te kunnen voorspellen.

Naar alle waarschijnlijkheid zijn er meerdere factoren die de farmacokinetiek en farmacodynamiek van taxanen beïnvloeden. In dit proefschrift wordt een multifactoriële benadering besproken welke als doel heeft inzicht te creëren met betrekking tot factoren die de farmacokinetiek en farmacodynamiek van taxanen beïnvloeden. Met andere woorden, dit proefschrift beschrijft onderzoek naar factoren die zowel de bijwerkingen van taxanen als de blootstelling aan taxanen en daarmee mogelijk de effectiviteit beïnvloeden.

Zoals genoemd zijn de taxanen ondanks hun gemeenschappelijke noemer wel degelijk verschillend van elkaar. Neem bijvoorbeeld alleen al het middel waarin zij zijn opgelost omdat de taxanen zelf heel lastig in water op te lossen zijn. Paclitaxel is oplosbaar in water omdat Cremophor, een oplosmiddel, is toegevoegd aan de formulering van paclitaxel. In een andere variant van de

paclitaxel formulering is paclitaxel gebonden aan een eiwit. Docetaxel en cabazitaxel zijn beide wateroplosbaar gemaakt door aan de formulering het oplosmiddel Polysorbaat 80 toe te voegen. Deze oplosmiddelen blijken echter de farmacokinetiek en daarmee de blootstelling aan taxanen te beïnvloeden. Omdat taxanen met name worden afgebroken door de lever en de opname van taxanen in de lever geschiedt middels specifieke 'transport-' eiwitten is de invloed van deze oplosmiddelen op transport-eiwitten in de lever onderzocht in **Hoofdstuk 2**. Dit onderzoek bevestigt de rol van transport-eiwitten in de afbraak van paclitaxel en docetaxel en laat zien dat het toevoegen van oplosmiddelen aan deze middelen resulteert in een verminderde werking van de transporter eiwitten in de lever. Dit betekent dat de oplosmiddelen dus leiden tot een verhoogde blootstelling aan paclitaxel en docetaxel.

Naast de invloed van de oplosmiddelen van taxanen lijkt de variatie in farmacokinetiek en -dynamiek tussen patiënten toch een multifactoriële grondslag te hebben. Men heeft zich afgevraagd of ons genetisch materiaal (DNA) en de verschillen tussen mensen mogelijk ook een rol spelen in de verschillen tussen patiënten in farmacokinetiek. Voor zowel docetaxel als paclitaxel zijn eerder studies gedaan naar enkele stukjes van DNA (genen). Echter, geen van deze genen verklaarde de verschillen in farmacokinetiek en –dynamiek volledig. In dit proefschrift staan meerdere onderzoeken beschreven (**Hoofdstuk 3 en 4**) die zich hebben gericht op de invloed van genetische variatie op de farmacokinetiek en –dynamiek van taxanen. In deze studies is geprobeerd om grote aantallen genen in kaart te brengen en de variatie tussen mensen in verband te brengen met de blootstelling aan taxanen en het risico op bijwerkingen. Zo is geprobeerd de bijwerkingen en de blootstelling aan paclitaxel en docetaxel te voorspellen middels genetische modellen. In deze modellen zijn genetische variaties geïncludeerd die mogelijk verklarend zouden zijn voor de verschillen in kinetiek en dynamiek in patiënten. Gebruik makend van deze modellen konden de meeste patiënten met een hoog risico op een laag aantal witte bloedcellen en een lage klaring en dus een hoge blootstelling geïdentificeerd worden. Echter waren deze modellen niet voorspellend genoeg, wat betekent dat ze (nog) niet in de kliniek gebruikt kunnen worden.

Naast genetische factoren en het feit dat farmacokinetiek ook voorspellend kan zijn voor de toxiciteit van taxanen is in dit proefschrift ook gekeken naar andere factoren die van invloed zijn op de farmacokinetiek van docetaxel (**Hoofdstuk 5**). Dit is gedaan door middels bestaande literatuur in kaart te brengen wat we weten van factoren die de farmacokinetiek van docetaxel beïnvloeden. Uit dit onderzoek kwam naar voren dat met name leverenzym-stoornissen ervoor zorgen dat docetaxel minder goed afgebroken kan worden, en dat daarmee de blootstelling aan docetaxel hoog is. Ook het geslacht en de lichaamsbouw van de patiënt bleek van invloed op de farmacokinetiek. Daarnaast is naar aanleiding van dit onderzoek het advies gegeven goed op te letten met het gebruik van andere medicatie en alternatieve geneesmiddelen. Deze middelen kunnen namelijk de afbraak van docetaxel beïnvloeden en daarmee de blootstelling aan deze anti-kanker therapie en potentieel dus ook de effectiviteit en toxiciteit.

Voor cabazitaxel is ook onderzocht of andere medicijnen de effectiviteit van deze chemotherapie beïnvloeden. Cabazitaxel wordt op dit moment gebruikt in de behandeling van uitgezaaide prostaatkanker. Voor deze groep patiënten zijn er de afgelopen jaren vele medicijnen bijgekomen die

hun overleving kan verlengen. Voorbeelden van deze medicijnen zijn abiraterone en enzalutamide; beide middelen welke zijn gericht op een eiwit waaraan mannelijke hormonen zich kunnen binden. Mannelijke hormonen kunnen de groei van een prostaattumor bevorderen. Het was echter nog onduidelijk of de volgorde van het toedienen van deze verschillende medicijnen van invloed is op hun effect. In **Hoofdstuk 6** is een studie beschreven waarin gekeken is of de behandeling met abiraterone of enzalutamide voorafgaand aan de behandeling met cabazitaxel van invloed is op het effect van cabazitaxel op de ziekte. Dat onderzoek is gedaan door mannen met prostaatkanker die behandeld waren met abiraterone of enzalutamide, en daarna cabazitaxel toegediend kregen, te vergelijken met mannen welke direct cabazitaxel kregen. Ze werden vergeleken in tijd van overleving en daarnaast werd gekeken naar het Prostaat Specifiek Antigeen (PSA), een maat voor activiteit van de ziekte. Beide parameters waren niet verschillend in beide groepen, wat betekent dat voorafgaande behandeling met abiraterone of enzalutamide de effectiviteit van cabazitaxel niet vermindert.

Bij de registratiestudie van cabazitaxel viel op dat bijna de helft van de mannen last had van diarree. Een klein deel van deze groep moest daarbij zelfs in het ziekenhuis opgenomen worden. Om de ernst en het voorkomen van diarree te verminderen is in de studie beschreven in **Hoofdstuk 8** een ontstekingsremmer toegevoegd aan de behandeling met cabazitaxel. Patiënten slikten elke dag een corticosteroïd (budesonide). Voorafgaand aan deze grote studie bleek uit een kleiner door ons uitgevoerd onderzoek dat budesonide geen (negatief) effect heeft op de farmacokinetiek van cabazitaxel. In **Hoofdstuk 7** staat beschreven hoe de concentraties van cabazitaxel bepaald kunnen worden in het plasma van patiënten behandeld met dit middel. In de studie naar de effecten van budesonide op het vóórkomen en de ernst van diarree in patiënten die met cabazitaxel behandeld werden, bleek dat budesonide geen duidelijk positief effect had op het voorkomen van diarree en dat de diarree goed onder controle te houden was.

Toekomst visie

De inzichten met betrekking tot de farmacokinetiek en farmacodynamiek van taxanen welke in dit proefschrift naar voren zijn gekomen kunnen in de toekomst gebruikt worden om deze kennis verder uit te breiden. Het uiteindelijke doel zal zijn elke patiënt een individuele dosis toe te kennen van het taxaan waarmee zij behandeld worden, om zo een optimale balans tussen effectiviteit en bijwerkingen te kunnen bewerkstelligen. Meerdere factoren blijken een invloed te hebben op de farmacokinetiek en farmacodynamiek van taxanen. Sommige van deze factoren zijn klinisch relevant en moeten meegenomen worden in de besluitvorming bij therapie met taxanen. Factoren als hormonale status verdienen meer diepgaand onderzoek, omdat zij onderbelicht zijn maar potentieel belangrijk. De combinatie van patient- en omgevingsgebonden factoren met genetische factoren zouden potentieel de waarde van voorspellende modellen in de klinische setting kunnen vergroten. Het uiteindelijk doel zal zijn de kwaliteit van leven van met taxanen behandelde patiënten te vergroten door de bijwerkingen van taxanen te verminderen en het effect te optimaliseren.

Appendix II

Dankwoord

DANKWOORD

Ik ben trots dat u vandaag dit boekje in handen heeft. Echter zonder de hulp, steun en input van velen was dit proefschrift er nooit geweest. Op deze laatste paar bladzijden van dit boekje wil ik dan ook een aantal mensen bedanken en vereeuwigen. Omdat het afronden van mijn proefschrift nagenoeg samenviel met het afronden van mijn studie maak ik van de gelegenheid gebruik ook aan (de mensen in) die periode te memoreren.

Allereerst wil ik alle patiënten bedanken die geheel belangeloos hebben deelgenomen aan de studies. Velen van u reageerden vrijwel hetzelfde: “Ik doe het voor de mensen die er straks misschien wel wat aan hebben”. Ik heb bewondering voor het feit dat u in die fase van uw leven nog ruimte had voor andermans belangen. Ik ben u daarvoor dan ook zeer erkentelijk. Zonder mensen als u bestaat er geen vooruitgang in de oncologie.

Dan wil ik uiteraard mijn promotoren bedanken. Prof. dr. A.H.J. Mathijssen, beste Ron, ik wil je bedanken voor het vertrouwen dat je mij als groentje gaf om met dit onderzoek te starten. Dank dat je altijd zo laagdrempelig te benaderen was om over praktische en wetenschappelijke zaken van gedachten te wisselen. Maar ook dat je met me mee dacht toen ik tussentijds aan mijn coschappen wilde beginnen en ik je er van verzekerde dat het proefschrift ook dan af zou komen, ook al zag je dat in eerste instantie niet zo zitten. Ik bewonder je gedrevenheid en deskundigheid. Prof. dr. R. de Wit, beste Ronald, dankjewel voor de mogelijkheid aan dit promotietraject te beginnen. Bedankt dat ook jij met me mee wilde denken toen ik toch eerder de kliniek in wilde dan gepland, voor je visie en uiteraard voor je bijdrage aan dit proefschrift. Ook bedank ik je voor de politieke lessen die je me hebt meegegeven en de verschillende podia die je me geboden hebt om dit onderzoek te profileren.

Prof. dr. R.H.N. van Schaik, beste Ron, dankjewel voor de prettige samenwerking. Toen ik er eenmaal achter was dat ik gewoon met het manuscript naar je kantoor moest komen om zo de rode inkt te laten vloeien hadden we binnen no-time twee papers gepubliceerd. Ik heb veel gehad aan je feedback en je kennis, waarvoor dank.

Prof. dr. A. Sparreboom, beste Alex, met name in de korte periode dat ik op het lab in Memphis mocht zijn viel het tempo waarin gewerkt wordt en de kennis over farmacologie die zich daar bevindt mij het meeste op. Je liet me tijdens de revisies van mijn stukken ook elke keer weer een nieuwe invalshoek zien en schroomde niet te benoemen dat een stuk soms gewoonweg nog niet goed genoeg was. Je hebt me daarmee gestimuleerd mijn schrijfvaardigheid maar zeker ook mijn kennis en diepgang verder te verbeteren. Ik voel mij dan ook vereerd dat je de plas bent over gestoken om aanwezig te zijn op mijn promotiedag.

Prof. dr. T. van Gelder, beste Teun, bij onze eerste ontmoeting vond ik je stem zo bekend voorkomen maar ik wist niet goed waar ik je van zou kunnen kennen. Ik kwam er later achter dat ik daarvoor gewoon wat verder vooraan had moeten gaan zitten in de collegezaal. Ik wil je bedanken voor het feit dat je vandaag front row zal zitten als opponent in de kleine commissie.

Beste dr. A. Huitema, ik wil u bedanken voor het zitting nemen in de grote commissie. Beste dr. R. van Soest, beste Robert, dank voor je inzet voor de Cabaresc studie en voor het zitting nemen in de grote commissie. Dear professor Sharyn Baker, thank you for attending my PhD defence as part of the dissertation committee. I am honored that you travelled so far to be here on this day.

Ook wil ik een aantal personen bedanken die mij geïnspireerd hebben onderzoek te willen doen. Prof. dr. E. Zwarthoff, beste Ellen, je wist me te enthousiasmeren voor wetenschappelijk onderzoek. Ik ben je dankbaar voor de begeleiding die ik van je heb mogen ontvangen en je oprechte interesse in hoe het mij verging. Het cirkeltje is voor mij rond nu je zitting hebt genomen in de grote commissie, waarvoor veel dank.

Beste dr. M. Bontenbal, de onderwijsprijzen die u in de afgelopen jaren hebt mogen ontvangen zijn er het bewijs van dat uw benadering van de oncologie door velen wordt gewaardeerd. Zo ook door mij. Dank voor de eerste adviezen en de lijntjes die u voor mij heeft gelegd destijds. Het is het begin geweest van wat nu voor u ligt.

Beste dr. T. Zuiverloon, beste Tahlita, jij stimuleerde me in het onderzoek verder te gaan en pikte het als ik je weer eens aan het opjagen was. Ik zal je wijze lessen over het waarom van promoveren niet snel vergeten. Onze review was mijn eerste paper en ik ben er nog steeds trots op.

Het laboratorium Translationele Farmacologie was jaren mijn 'thuisbasis'. Zonder mijn collega's was er geen proefschrift geweest en was het met mijn geestelijke gesteldheid mogelijk ook anders gesteld geweest.

Sander, naast mijn waardering voor je inzet voor de Cabaresc studie ben ik je ook een woord van dank verschuldigd voor je relativerende droge humor en de vele hilarische filmpjes die mijn leven rijker is geworden. Ook je IT deskundigheid kwam vaak zeer goed van pas als ik weer eens vast zat. Jacqueline, dankjewel voor je gezelligheid en je reistips! "Nee geen hakken in je backpack". Peter, dankjewel voor je geduld en je kennis, alsmede voor al je werk. Je bescheidenheid maakt dat het misschien niet altijd opvalt hoe groot jouw toegevoegde waarde is, maar laat het bij deze dan zwart op wit staan. Inge, als vanzelfsprekend heb ik je werk maar ook je gezelligheid en hoe attent je altijd bent zeer gewaardeerd. Mei, ook jij bedankt voor je werk en de lekkere ei-vrije hapjes en de moeite die je daar elke keer weer voor deed. Patricia, als kamergenoot wil ik je graag bedanken voor je behulpzaamheid, jij staat altijd voor een ander klaar. Anne-Joy, dank voor je begeleiding en alle inside info die ik als student van je kreeg. Karel, voor jou geldt hetzelfde. Sorry dat je erfenis (lees: pieper) meteen in mijn eerste week in het riool belandde. Walter, Carolien, Marijn, Erik, Herman, Xander, Ton, Roelof, Leni en Bimla dank jullie wel voor jullie input en gezelligheid. Maaike en José, dankjewel voor alles, wat moeten ze/we zonder jullie!

Alle coauteurs wil ik bedanken voor hun bijdrage aan het onderzoek in dit proefschrift. Alle artsen en (research) verpleegkundigen in de Daniel, op de Centrumlocatie en in het gehele land, bedankt voor al jullie hulp en deelname in de 03-264 en de Cabaresc studie. Een aantal van de co-auteurs wil ik nog in het bijzonder bedanken. Beste Marcel, je was de drijvende kracht achter de DMET papers en daarvoor ben ik je zeer erkentelijk alsmede voor je geduld met mij. Samira, wat was het een gedoe om alle

samples gerund te krijgen, zonder jou was het niet gelukt! Beste Linda, Nelly, Tessa, René, Dana en alle andere leden van het Cabaresc studie team, heel erg veel dank voor alle uren die jullie in de Cabaresc hebben gestoken zonder te mopperen (in ieder geval niet waar ik bij was). Het was altijd een fijn idee te weten dat ik op jullie deskundigheid kon rekenen en dat aan alles werd gedacht. Nelly, als jij een stuk leest weet je zeker dat niets over het hoofd gezien wordt, hulde aan jouw kritische blik, het heeft de Cabaresc en ook mijn werk beter gemaakt. Beste dr. P. Hamberg, beste Paul, dankjewel voor alle energie en moeite ten aanzien van de Cabaresc studie. Zonder jullie en de patiënten uit het SFG was het einde van de studie nog niet eens in zicht geweest. Dear Shuiying and Alice, thank you for all the work in the OATP project. I hope you are both doing well and wish you all the best both in your career as well as personally.

Lieve Ellen, ik denk dat ik wel kan zeggen dat we de meeste hoogte- en dieptepunten van de promotie rollercoaster samen hebben beleefd. Ook al zaten we niet op hetzelfde lab, het klikte vanaf moment één en na een paar weken deelden we al een kamer in Dublin. Je humor, opbeurende woorden, de nodige bakkies pleu-ahr, en een enkel frietje hebben me door de promotie-dipjes geholpen. “Wij komen er wel”! En zo is het (zelfs in dezelfde week)!
Lieve Lisette, als kamergenoot was je er altijd om even te sparren en ook om de dagelijkse beslommeringen mee te bespreken. Hoogtepunt was uiteraard om jouw promotie als paranimf mee te mogen maken. Goede tweede is uiteraard jullie muisexperiment waar ik een nieuwe kant van mezelf ontdekte. In de afgelopen jaren ben je voor mij een vriendin geworden, een waarop je kunt bouwen. Dankjewel voor al jullie adviezen en steun meiden, zonder jullie was het niks geworden!

Lieve meiden van jaarclub Santé: Anouk, Charlotte, Cleo, Danielle, Els, Ingeborg, Linda, Marjolein, Marije, Yvette en Sil, jullie hebben ervoor gezorgd dat Rotterdam mijn thuis werd. Ik hoop dat we nog heel vaak met elkaar op mooie dingen mogen proosten: Santé!

Lieve Anouk, geen poespas bij jou. Je oprechte interesse en nuchterheid waardeer ik zeer. What you see is what you get, dankjewel! Het maakt niet uit in welke steden onze huizen staan!

Lieve Inge, al vanaf de clubvorming hadden we een klik en vele verenigingen verder is die er gelukkig nog steeds. De Zeil, Skadi, op elk weekendje waren we er, al moest men er niet gek van opkijken als we onze eigen planning hadden. Toch vonden we op het water niet altijd waar we naar op zoek waren. Dankjewel voor alle hilarische momenten die we hebben gedeeld en de steun en vriendschap die ik over de jaren heb mogen ervaren.

Lieve Marije, dankjewel voor je vriendschap. We kunnen het tijdens onze etentjes altijd zo goed hebben over alle dingen die ons bezig houden, ook al is dat soms in totaal verschillende werelden. Het blijft altijd waardevol en heerlijk verfrissend!

Lieve Sil, als medico’s van de club mochten we al snel ervaren hoe het was om uit je (stapel) bed gehaald te worden voor een medisch noodgeval. Groene klompen (hoe ironisch) aan de kant: met een stabiele zijligging en een lepel kregen wij iedereen weer op de been. Zowel op de velden, in de kroeg en op het water zijn wij een goed team. Dankjewel voor je vriendschap!

Lieve Katrien en Judith, dank jullie wel dat jullie mij als groentje in Hotel de Botel hebben opgenomen en Rotterdam hebben laten zien. Daarbij kwamen later ook Saar, Naomi en Iris. Lieve meiden, ik heb genoten van de gezelligheid, de verhalen, het gezonde koken (ook dank namens mijn moeder) en dat jullie er altijd waren om even bij binnen te lopen. We lopen tegenwoordig wat minder makkelijk bij elkaar binnen, maar mijn deur staat nog altijd voor jullie open!

Lieve Yvonne, we gaan al jaren terug. Van brugwuppen met te grote rugzakken naar nu. De afstand maakt dat we elkaar soms een tijdje minder zien, maar het is altijd weer als vanouds. Je vriendschap is me heel dierbaar. Ik heb je altijd bewonderd om je discipline en het feit dat de onfortuinlijke loting onze vriendschap nooit een seconde in de weg heeft gestaan. Ik hoop dat we nog vele jaren vriendschap mogen delen!

Lieve Mus, dank voor alle keren dat je er voor me was, naar me luisterde alsof je het niet al tien keer eerder had gehoord, en het nog onthield ook. Dankjewel voor je humor en je vriendschap. Ik zal nooit meer vergeten hoe we in ons eerste jaar bijna de collegezaal uitgezet zijn omdat we niet meer bijkwamen van de lach, hoe we in Bulgarije op de OK stonden en vele avonden in onze stamkroeg sleten waar ze op de vraag of ze Engels spraken gewoon, “Houston, we’ve got a problem!” antwoordden. Ik ben dan ook heel blij dat je naast me wil staan op mijn promotiedag.

Last but not least wil ik mijn familie bedanken. Zonder jullie was alles anders geweest.

Lieve familie Nieuweboer, zonder jullie aanwezigheid zijn de feestjes niet compleet, dank voor jullie interesse in mij, ik hoop dat we elkaar nog vele jaren mogen volgen en zien. Lieve Monique, Carlien en Joris, elke keer als we elkaar zagen was het weer wat anders wat ik aan het doen was, dankjewel dat jullie het probeerden bij te houden en met me mee hebben geleefd.

Lieve tante Danielle en oom Jaap, dank voor alles wat we tot nu toe met elkaar hebben mogen delen en dat jullie er altijd (voor me) zijn! Ik waardeer jullie warmte en interesse in mij en vind het altijd een feestje om elkaar weer te zien.

Lieve Oma, dankjewel voor uw niet aflatende interesse en trots. U heeft heel wat kaarsjes opgebrand de afgelopen jaren en ons vanaf kleins af aan gestimuleerd onze hersenen te gebruiken met memory en kruiswoordpuzzels maar toch was de ‘bolletjes’ kant van het rapport altijd het belangrijkst. Ik ken geen andere oma’s die hun kleinkinderen achter op de fiets het woord ‘anticiperen’ bij brengen, uiteraard voorafgaand en gevolgd door heel hard vals zingen.

Lieve Frederieke, dankjewel voor de fijne zus die je bent en dat je mijn paranimf wilde zijn. We hebben al zoveel mooie dingen mogen beleven. Je weet ook telkens weer een feitje of een herinnering op te vissen die ik dan al lang weer vergeten ben. Ik hoop dat we de komende jaren nog veel meer herinneringen mogen maken en delen!

Lieve Sander, jij hoort er voor mij bij alsof het nooit anders is geweest. Toch is dat wel zo, want het staat me nog heel helder voor de geest dat je moeder in groep acht uitleg kwam geven over het beroep van apotheker en in plaats van capsules salmiak snoepjes gebruikte. Het heeft ongetwijfeld wat in mijn getriggerd. Wie had dat gedacht, van schoppend schooljoch naar zwager?!

Lieve Frank, ik ben blij dat ik geneeskunde heb kunnen afronden, want mijn tweede studiekeuze was toch op de second best business school geweest (als ik jou mag geloven althans). Ik bewonder je doorzettingsvermogen en je loyaliteit en wil je bedanken voor het zijn van de fijne broer die je voor mij bent. Als jij er niet bent wordt er echt minder gelachen aan tafel (behalve door mij dan, want ik lach ook om mijn eigen grappen natuurlijk..).

Lieve Mam, je hebt me geleerd hard te werken, maar je hebt me ook laten zien dat alles relatief is. Dankjewel dat je altijd naar me luistert. Je bent er altijd op de belangrijke momenten, maar ook op de doodgewone momenten heb jij de gave om er altijd wat feestelijks van te maken. Ook na een drukke werkweek trek jij alles uit de (koel)kast als we weer met elkaar aan tafel zitten. Ik ken niemand anders die altijd de champagne koud heeft staan voor een onverwacht feestelijk of anderszins memorabel moment! Je hebt me geleerd het leven te vieren. Dankjewel voor al je adviezen en je goede voorbeeld.

Lieve Pap, dokter Spock is inmiddels dokter Nieuweboer geworden maar zal voor jou waarschijnlijk altijd dokter Spock blijven. Jouw nuchtere kijk op het leven heeft me geleerd wat belangrijk is en om dicht bij mezelf te blijven. Je weet me regelmatig een andere kijk op dingen te geven. Dankjewel dat ik altijd op je kan bouwen.

Lieve Pap en Mam, ik draag dit boekje aan jullie op. Laat het symbool staan voor het feit dat jullie me hebben geleerd door te zetten en af te maken waar ik aan begonnen ben. “Kan ik niet ligt op het kerkhof”. Jullie hebben me het vertrouwen gegeven dat als ik ergens voor ging ik het dan ook kon bereiken. Het is een fijn gevoel te weten dat ik altijd op jullie steun en liefde kan rekenen. Dankjewel voor alles wat jullie me hebben (mee)gegeven.

Lieve Bart-Jan, dankjewel voor je niet aflatende steun en liefde. Ik ben je dankbaar voor wie je bent en voor alles wat je voor mij betekent. In het bijzonder voor je stabiliteit en rust, je relativeringsvermogen, je geduld, je humor en je onverflauwde vertrouwen in mij, zelfs als ik dat zelf (even) niet had (all of the above...). Ik hoop dat we nog heel veel samen mogen vieren!

Appendix III

Curriculum Vitae

CURRICULUM VITAE

Annemieke Johanna Maria Nieuweboer werd geboren op 21 mei 1989 te Eindhoven. In 2007 behaalde zij haar Atheneum-diploma aan het Pleincollege Bisschop Bekkers te Eindhoven. Van september 2007 tot september 2011 studeerde zij Geneeskunde aan de Erasmus Universiteit Rotterdam. In het derde jaar van haar studie heeft zij stage gelopen op de afdeling kinderoncologie in het St. George University Hospital Plovdiv, Bulgarije. Ook heeft zij zich tijdens haar studie ingezet op het laboratorium Experimentele Pathologie in het Erasmus MC Rotterdam, onder leiding van Prof. dr. E. Zwarthoff. Van 2009 tot 2011 was zij werkzaam in het studententeam op de afdeling Oncologische Chirurgie van de Daniel den Hoek Kliniek, waar zij het laatste jaar ook teamleider was. Ter afsluiting van de doctoraal fase van de studie geneeskunde heeft zij in 2011 onderzoek gedaan op de afdeling Interne Oncologie in het Erasmus MC, onder leiding van Prof. dr. A.H.J. Mathijssen. In deze periode zijn meerdere studies uitgevoerd naar de toxiciteit en farmacologie van taxanen. Direct aansluitend op deze stage is zij in november 2011 haar promotieonderzoek gestart onder supervisie van Prof. dr. A.H.J. Mathijssen en Prof. dr. R. de Wit. Tijdens haar promotieonderzoek heeft Annemieke voor 1.5 jaar zitting genomen in het bestuur van de landelijke belangenorganisatie voor promovendi Promovendi Netwerk Nederland en was ze tevens reviewer voor het op medisch studenten gerichte tijdschrift 'Erasmus Journal of Medicine'. In 2013 heeft zij onder begeleiding van Prof. dr. A. Sparreboom stage gelopen op de afdeling Pharmaceutical Sciences in het St. Jude Children's Research Hospital te Memphis, Verenigde Staten. In juni 2014 hervatte zij haar medische opleiding. Gelijktijdig is zij doorgegaan met het werk aan dit proefschrift. Zij heeft haar opleiding tot arts afgesloten met een coschap 'Emergency Medicine' in het Prince of Wales Hospital te Sydney, Australië. In juli 2016 behaalde zij haar artsendiploma.

Appendix IV

Publications

PUBLICATIONS

A.J.M. Nieuweboer*, T.C.M. Zuiverloon*, H. Vékony, W.J. Kirkels, C.H. Bangma, E.C. Zwarthoff. Markers Predicting Response to Bacillus Calmette-Guérin Immunotherapy in High-Risk Bladder Cancer Patients: a Systematic Review. *European Urology, 2012 Jan;61(1):128-45*.

P. Bruijn, A.M. de Graan, A.J.M. Nieuweboer, A.H.J. Mathijssen, M. Lam, R. de Wit, E.A.C. Wiemer, W.J. Loos. Quantification of cabazitaxel in human plasma by liquid chromatography/triple-quadrupole mass spectrometry: a practical solution for non-specific binding. *Journal of Pharmaceutical and Biomedical Analysis, 2012 Feb 5;59:117-22.*

L. Elens, A.J.M. Nieuweboer, S.J. Clarke, K.A. Charles, A.M. de Graan, V. Haufroid, A.H.J. Mathijssen, R.H.N. van Schaik. CYP3A4 intron 6 C>T SNP (CYP3A4*22) encodes lower CYP3A4 activity in cancer patients, as measured with probes midazolam and erythromycin. *Pharmacogenomics, 2013 Jan;14(2):137-49.*

L. Elens, A.J.M Nieuweboer, S.J. Clarke, K.A. Charles, A.M. de Graan, V. Haufroid, T. van Gelder, A.H.J. Mathijssen, R.H.N. van Schaik. Impact of POR*28 on the clinical pharmacokinetics of CYP3A phenotyping probes midazolam and erythromycin. *Pharmacogenetics and Genomics, 2013 Mar; 23(3):148-55.*

T.C.M. Zuiverloon, W.Beukers, K.A. van der Keur, A.J.M. Nieuweboer, T. Reinert, L. Dyrskjot, T.F. Orntoft, E.C. Zwarthoff. Combinations of urinary biomarkers for surveillance of patients with incident non-muscle invasive bladder cancer: The European FP7 UROMOL Project. *Journal of Urology, 2013 May;189(5):1945-51.*

A.M. de Graan, L. Elens, M. Smid, J.W.M. Martens, A. Sparreboom, A.J.M. Nieuweboer, L.E. Friberg, S. Elbouazzaoui, E.A.C. Wiemer, B. van der Holt, J. Verweij, R.H.N. van Schaik, A.H.J. Mathijssen. A pharmacogenetic predictive model for paclitaxel clearance based on the DMET platform. *Clinical Cancer Research, 2013 Sep 15;19(18):5210-7.*

A.J.M. Nieuweboer, S. Hu, C. Gui, B. Hagenbuch, I.M. Ghobadi Moghaddam-Helmantel, A.A. Gibson, P. de Bruijn, A.H.J. Mathijssen, A. Sparreboom. Influence of drug formulation on OATP1B-mediated transport of paclitaxel. *Cancer Research, 2014 Jun 1;74(11):3137-45.*

S. Kraff , A.J.M. Nieuweboer, A.H.J. Mathijssen, F. Baty, A.M. de Graan, R.H.N. van Schaik, U. Jaehde, M. Joerger. Pharmacokinetically based dosing of weekly paclitaxel to reduce drug-related neurotoxicity based on a single sample strategy. *Cancer Chemotherapy and Pharmacology, 2015 May;75(5):975-83.*

A.J.M. Nieuweboer*, E.S. de Morrée*, A.M. de Graan, A. Sparreboom, R. de Wit, A.H.J. Mathijssen. Inter-patient variability in docetaxel pharmacokinetics: A review. *Cancer Treatment Reviews, 2015 Jul;41(7):605-13.*

A.J.M. Nieuweboer, M. Smid, A.M. de Graan, S. Elbouazzaoui, P. de Bruijn, J.W.M. Martens, A.H.J. Mathijssen, R.H.N. van Schaik. Predicting paclitaxel-induced neutropenia using the DMET platform. *Pharmacogenomics. 2015;16(11):1231-41.*

A.J.M. Nieuweboer, M. Smid, A.M. de Graan, S. Elbouazzaoui, P. de Bruijn, F.A. Eskens, P. Hamberg, J.W.M. Martens, A. Sparreboom, R. de Wit, R.H.N. van Schaik, A.H.J. Mathijssen. Role of genetic variation in docetaxel-induced neutropenia and pharmacokinetics. *The Pharmacogenomics Journal, 2015 Sep 8. Epub ahead of print.*

A.J.M. Nieuweboer *, R.J. van Soest*, E.S. de Morrée, D. Chitu, A.M. Bergman, S.H. Goey, M.M. Bos, N. van der Meer, P. Hamberg, R. de Wit, A.H.J. Mathijssen; Dutch Uro-Oncology Studygroup (DUOS). The influence of prior novel androgen receptor targeted therapy on the efficacy of cabazitaxel in men with metastatic castration-resistant prostate cancer. *European Journal of Cancer, 2015 Nov;51(17):2562-9.*

W. Onstenk, A.M. Sieuwerts, J. Kraan, M. Van, A.J.M. Nieuweboer, A.H.J. Mathijssen, P. Hamberg, H.J. Meulenbeld, B. De Laere, L.Y. Dirix, R.J. van Soest, M.P. Lolkema, J.W.M. Martens, W.M. van Weerden, G.W. Jenster, J.A. Foekens, R. de Wit, S. Sleijfer. Efficacy of Cabazitaxel in Castration-resistant Prostate Cancer Is Independent of the Presence of AR-V7 in Circulating Tumor Cells. *European Urology, 2015 Dec;68(6):939-45.*

A.J.M. Nieuweboer, A.M. de Graan, P.Hamberg, S. Bins, R.J. van Soest, R.J. van Alphen, A.M. Bergman, A. Beeker, H. van Halteren, A.T. ten Tije, H. Zuetenhorst, N. van der Meer, D. Chitu, R. de Wit, A.H.J. Mathijssen, also on behalf of the Dutch Uro-Oncology Studygroup (DUOS). Effects of budesonide on cabazitaxel-induced diarrhea in a randomized open-label multicenter phase II study: CABARESC. *Submitted for publication.*

B.Gao, Y. Lu, A.J.M. Nieuweboer, H. Xu, J. Beesley, I. Boere, A.M. de Graan, P.de Bruijn, H. Gurney, C. Kennedy, Y. Chiew, S.E. Johnatty, P.Beale, M. Harrison, C. Luccarini, A.M. Dunning, A.H.J. Mathijssen, P. Harnett, R.L. Balleine, G. Chenevix-Trench, S. MacGregor and A. DeFazio. Genome-wide association study of paclitaxel and carboplatin disposition in epithelial ovarian cancer. *Submitted for publication.*

** authors attributed equally to this paper*

Appendix V

PhD Portfolio

PhD PORTFOLIO

Summary of PhD training

General Courses		
Certificate 'Good Clinical Practice'	2011	0.5 ECTS
Certificate 'Role and responsibilities of Facility Management and Study Director'	2011	0.5 ECTS
Certificate BROK (Basic course Regulatory processes and Organization of Clinical Research with Human Subjects) (Erasmus MC)	2011	1.0 ECTS
Specific Courses		
Certificate Course Presenting Skills for Scientists (MolMed)	2011	1.0 ECTS
Certificate Course Principles of Clinical Pharmacology (NIHCC)	2012	1.0 ECTS
Certificate Course on Biostatistical Methods, (NIHES)	2013	5.7 ECTS
Certificate SNP Course: SNPs and Human Diseases (MolMed)	2013	1.1 ECTS
Certificate Biomedical English Writing and Communication (MolMed)	2014	4.0 ECTS
Presentations		
Tour d'Europe Rotterdam	2011	0.5 ECTS
Dutch Uro-Oncology Studygroup Openingsymposium	2011	0.5 ECTS
Reference meeting Urology Erasmus University Medical Center	2012	0.5 ECTS
Poster Presentation EORTC-NCI-AACR Symposium	2012	0.5 ECTS
Dutch Uro-Oncology Studygroup Yearsymposium	2012	0.5 ECTS
Pharmacology Meeting Sophia Children's Hospital Rotterdam	2012	0.5 ECTS
IKNL Netwerking days	2013	0.5 ECTS
Scientific Meeting Internal Oncology Erasmus University Medical Center	2013	0.5 ECTS
Poster Presentation ASCO	2013	0.5 ECTS
OIO meetings	2011-2014	1 ECTS
Conferences		
24th EORTC-NCI-AACR Symposium on 'Molecular Targets and Cancer Therapeutics', Dublin, Ireland	2012	0.4 ECTS
ASCO Annual Meeting, Chicago, USA	2013	1.0 ECTS
NVKF&B Scientific Day	2012-2013	0.2 ECTS
Dutch Uro-Oncology Studygroup Yearsymposium	2013	0.2 ECTS
Scientific Meeting Internal Oncology Erasmus University Medical Center	2011-2014	0.2 ECTS
PhD Day Erasmus PhD Association Rotterdam	2011-2013	0.2 ECTS

Other

OIO Meetings	2010-2014	3 ECTS
Farmacogenetics Meetings	2011-2014	2 ECTS
Board Promovendi Netwerk Nederland	2011-2013	5 ECTS
Internship department of Pharmaceutical Sciences, St Jude Children's Research Hospital, Memphis, TN, USA	2013	2 ECTS