Intravenous-to-oral switch in anticancer chemotherapy
focus on taxanes and gemcitabine

ISBN/EAN: 978-90-393-55077

Printed by: Gildeprint drukkerijen, Enschede, the Netherlands

Intravenous-to-oral switch in anticancer chemotherapy
focus on taxanes and gemcitabine

Switch van intraveneuze naar orale antikanker chemotherapie
focus op taxanen en gemcitabine
(met een samenvatting in het Nederlands)

PROEFSCHRIFT

ter verkrijging van de graad van doctor aan de Universiteit Utrecht
op gezag van de rector magnificus, prof.dr. J.C. Stoof, ingevolge het besluit
van het college voor promoties in het openbaar te verdedigen
op woensdag 16 februari 2011 des middags te 4.15 uur

door

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geboren op 14 september 1980 te Weert

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The research described in this thesis was performed at the Department of Pharmacy & Pharmacology of The Netherlands Cancer Institute / Slotervaart Hospital and the Department of Medical Oncology of the Netherlands Cancer Institute / Antoni van Leeuwenhoek Hospital, Amsterdam , The Netherlands.

Publication of this thesis was financially supported by:

J.E. Jurriaanse Stichting, Rotterdam, The Netherlands
NLADF, Amsterdam, The Netherlands
NKI-AVL, Amsterdam, The Netherlands
UIPS, Universiteit Utrecht, Utrecht, The Netherlands
Sanofi-aventis Netherlands B.V., Gouda, The Netherlands

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Preface

Most anticancer chemotherapeutic drugs are given intravenously. However, there is a growing interest in developing anticancer drugs for oral application. Different classes of anticancer drugs are already orally available and widely applied such as the tyrosine kinase inhibitors (imatinib, erlotinib, gefitinib, sorafenib and sunitinib), alkylating agents (temozolamide and cyclophosphamide) and the 5FU prodrug, capecitabine, a drug for which the intravenous-to-oral switch has already been successfully implemented in clinical practice.

Oral therapy has many advantages for both patient and healthcare since it is more practical and less invasive for patients, it is associated with lower costs because administration of drugs is less expensive at home than in a hospital. In addition, more chronic, daily or bi-daily regimens can be explored. Furthermore, medications that can be taken at home is generally preferred by patients.

The demand for more drugs that can be taken orally is therefore high. However many cytostatic drugs cannot be applied orally due to a low and highly variable bioavailability. This also accounts for the taxanes and gemcitabine.

The taxanes have a poor water solubility which limits the amount of drug available for absorption after oral intake. Of the fraction that is dissolved, only a minimal amount reaches the systemic circulation due to affinity for drug transporters and metabolizing enzymes present in high levels in the gut wall and the liver. Preclinical and clinical proof of concept studies demonstrated that by inhibiting these drug transporters and metabolizing enzymes, oral treatment becomes possible. Enhancing the systemic exposure to a low-bioavailability drug with a “booster-drug” is already widely applied in anti-HIV treatment.

Gemcitabine is a nucleoside analogue. During DNA replication gemcitabine-triphosphate replaces one of the DNA building blocks, cytidine. This arrests DNA replication and results in apoptosis. Oral administration of gemcitabine, however, is not possible due to extensive pre-systemic metabolism. Hence, a gemcitabine prodrug was developed, LY2334737, in which the unstable amine group was covalently bound to valproic acid thereby protecting gemcitabine from deamination.

The aim of this thesis is to investigate the oral application of docetaxel, paclitaxel and gemcitabine. For this aim we needed to define the optimal booster, booster-dose and timing of the booster to administer in combination with docetaxel and paclitaxel.

Furthermore we needed to determine the pharmacokinetics and safety of newly developed solid pharmaceutical dosage forms of paclitaxel and docetaxel, ModraDoc001 capsules and ModraPac001 capsules, respectively. Finally we determined the pharmacokinetics and safety of the oral gemcitabine prodrug, LY2334737.

Outline of this thesis

The first chapter of this thesis gives an overview of the preclinical and clinical proof-of concept studies of orally administered docetaxel and paclitaxel. In chapter 2 the development of oral paclitaxel is discussed. The results of preclinical mouse data are translated to patients (chapter 2.1), the pharmacokinetics and pharmacology of orally administered docetaxel in patients is described (chapter 2.2) and the safety, pharmacokinetics and anti-tumor activity of a novel solid dosage form (ModraDoc001 capsules) are discussed in chapter 2.3. Furthermore, the best booster drug (chapter 2.4) and timing of the booster drug (chapter 2.5) are determined.
Chapter 3 continues with the clinical development of oral paclitaxel. The optimal booster to enhance the systemic exposure to paclitaxel is investigated (chapter 3.1) and the pharmaceutical and clinical development of a new paclitaxel dosage form is discussed (chapter 3.2).

Pharmacological studies on gemcitabine are presented in chapter 4. The results of a first-in-man study of a gemcitabine produg, LY2334737, are presented in chapter 4.1 and the pharmacokinetics of gemcitabine in a patient without renal function are discussed in chapter 4.2.

1

Introduction

Chapter 1.1

Intravenous-to-oral switch in anticancer chemotherapy: A focus on docetaxel and paclitaxel

S.L.W. Koolen, J.H. Beijnen, J.H.M. Schellens

Clinical Pharmacology & Therapeutics, 2010; 87(1): 126-9

Introduction

Oral administration of the taxanes docetaxel and paclitaxel is hampered by their affinity for drug transporters, especially ABCB1 (P-glycoprotein, P-gp), extensive first pass metabolism by cytochrome P450 (CYP3A) and poor drug solubility. Pre-clinical studies in P-gp deficient and wild type mice demonstrated that modulation of either P-gp, or CYP3A resulted in high systemic exposure to docetaxel or paclitaxel. This concept could successfully be translated to clinical trials.

Docetaxel and paclitaxel are important antineoplastic agents that are widely used in the treatment of various malignancies.[1] Docetaxel and paclitaxel are typically used in 3-weekly i.v. schedules. However, weekly i.v. schedules are increasingly being used because they cause less hematologic toxicity and equal efficacy for docetaxel in the treatment of advanced non small cell lung cancer (NSCLC) and better efficacy for paclitaxel in the treatment of metastatic breast cancer.[2,3] For dose dense scheduling of taxanes, an oral formulation would be more practical and convenient for patients and more cost effective. Furthermore, it would enable clinical investigation of the concept of metronomic therapy, which is the frequent dosing of low dose chemotherapeutic agents without prolonged drug-free breaks. These strategies demonstrated anti-angiogenic effects *in vivo* and *in vitro* experiments for various chemotherapeutic agents including docetaxel and paclitaxel.[4,5,6]

Oral paclitaxel

P-gp, a member of the ATP binding cassette (ABC) superfamily, is a transmembrane efflux pump capable to confer multidrug resistance in cancer cells.[7] P-gp mediates the efflux of various anticancer agents including the vinca alkaloids, anthracyclines and taxanes. [8] Furthermore, P-gp has an important excretory function in liver and kidney and barrier function in brain, testis, placenta and intestinal epithelium.[9,8] In mice, P-gp is encoded by two genes *mdr1a* and *mdr1b*, which together possess the same functionality as the human *MDR1* gene. The mdr1a gene is predominantly expressed in intestine and brain capillaries, whereas the mdr1b gene is predominantly expressed in the placenta and ovaries. Both genes are expressed in liver and kidney.[9,10] The development of mice lacking the *mdr1a* gene (*mdr1a*$^{-/-}$ mice) provided a good model to investigate the role of P-gp in drug disposition.[10] One of the first drugs investigated with this model was vinblastine. It was shown that *mdr1a*$^{-/-}$ mice had a decreased clearance and decreased fecal excretion of vinblastine, indicating that P-gp plays a role in drug elimination by biliary excretion and possible reuptake in the intestinal lumen.[11] These observations resulted in the hypothesis that the low oral bioavailability of paclitaxel could be due to affinity of paclitaxel for P-gp. This was confirmed in a study in which paclitaxel was administered intravenously and orally to wild type and *mdr1a*$^{-/-}$ mice. It was

demonstrated that P-gp limits the oral bioavailability (10% in wild type and 33% in *mdr1a*$^{-/-}$ mice) and increases the biliary excretion of paclitaxel.[12] Hence, mice studies were initiated to investigate whether the oral bioavailability of paclitaxel could be modulated by co-administration of a P-gp inhibitor.[13,14,15] The tested inhibitors included, among others, cyclosporin, verapamil and elacridar. These studies concluded that inhibition of P-gp enabled oral treatment of paclitaxel in mice. A proof of concept trial was initiated in 14 patients with solid tumors.[16] Patients received one course of oral paclitaxel with or without 15 mg/kg cyclosporin followed by intravenous paclitaxel in subsequent cycles. It was shown that co-administration of cyclosporin resulted in an 8-fold increase in exposure to paclitaxel and that therapeutic plasma concentrations were reached.

Phase I studies investigated the safety and tolerability of a once-daily or twice daily dose of oral paclitaxel / cyclosporin.[17,18,19] The highest once-daily oral dose investigated was 360 mg/m^2 This dose was found to be inappropriate due to non-linear pharmacokinetics, the exposure to paclitaxel did not relevantly increase compared to lower doses, and poor tolerability (acute nausea and vomiting) caused by the formulation vehicles cremophor EL and ethanol. Twice daily dosing was found to be better tolerated. The optimal dose for phase II evaluation was found to be 90 mg/m^2 paclitaxel twice daily in combination with 10 mg/kg cyclosporin. The average paclitaxel AUC was 4.57 (± 2.43) µM/L*h. The safety and efficacy was more thoroughly examined in three phase II studies in patients with metastatic breast, gastric, and advanced non small cell lung cancer (NSCLC).[20,21,22] The results are summarized in table 1. In total, 80 patients were included in these studies. The observed toxicity was generally mild and manageable and consisted of myelosuppression, peripheral neurotoxicity and gastrointestinal toxicity, central neurotoxicity was not observed. The formulation used was Paxoral (IVAX Research, Inc, Miami, Florida), an aqueous solution developed for oral administration. The current status of development of this formulation is unknown.

Cremophor EL, used as formulation vehicle, can cause severe hypersensitivity reactions and non-linear pharmacokinetics of intravenously administered paclitaxel. The influence of cremophor EL after oral administration is, however, limited to the gastrointestinal tract since it is not able to reach the circulation after oral intake.[23] Therefore, oral paclitaxel can be safely administered without anti allergic pre-medication (dexamethasone, clemastine and ranitidine).[22,20,21,18]. However, cremophor EL in the oral formulation can be expected to have an effect on the absorption of paclitaxel and possible toxic effects on the gastrointestinal tract. In mouse models, it was demonstrated that a 7-fold increase in cremophor EL resulted in an increased recovery in the feces of paclitaxel from 7.6% to 36%, indicating that cremophor EL limits the absorption of paclitaxel.[24] Similar results were obtained in a clinical study in which polysorbate 80 and cremophor EL formulations of paclitaxel were compared. It was found that both Cmax and AUC of paclitaxel were

significantly higher for the polysorbate 80 formulation.[25] Furthermore, three studies have shown that the AUC of paclitaxel did not proportionally increase with increasing drug dose.[26,18,17] However, population pharmacokinetic modeling of phase I and exploratory studies did not reveal a significant dose-dependent bioavailability of paclitaxel. The authors postulated that the small number of patients and the high inter-individual variability possibly masked this cremophor EL effect.[27] Nevertheless, they did find a time-dependent effect of cremophor EL on the absorption of paclitaxel, which could be explained by micellar entrapment of paclitaxel by cremophor EL and subsequent degradation or dilution of these micelles.[27] The bioavailability of cyclosporin is possibly also influenced by cremophor EL. It was shown that the AUC of cyclosporin decreased with increasing paclitaxel dose, which could be explained by micellar entrapment of cyclosporin in the gastrointestinal tract by cremophor EL.[28]

Table 1: Phase II studies with oral paclitaxel

	2nd line NSCLC[22]	**1st line Advanced gastric Cancer[21]**	**2nd line Metastatic breast Cancer[20]**
No. of patients (total / evaluable)	26/23	25/24	29/23
Median age	54	63	50
Schedule	Weekly 90 mg/m2 paclitaxel po bid*	Weekly 90 mg/m2 paclitaxel po bid*	Weekly 90 mg/m2 paclitaxel po bid*
Total no. weekly administrations	228	286	442
Median administrations per patient	8	8	15
Median dose intensity (mg/m2/week)	172	141	97
Anti-tumor activity			
overall response rate (ORR) (complete or partial response)	6 (23%)	8 (32%)	15 (52%)
95% confidence interval ORR	9 – 44%	18 – 52%	34 – 70%
Toxicity			
Neutropenia (grade≥3)	14 (53%)	5 (20%)	15 (52%)
Neurotoxicity (grade≥2)	3 (12%)	0	5 (17%)
Diarrhea (grade≥2)	6 (23%)	6 (24%)	7 (24%)
Vomiting/nausea (grade≥2)	6 (23%)	10 (40%)	10 (34%)
Other grade≥2 toxicity	15	17	31

* 30 minutes prior to each dose, 10 mg/kg cyclosporin was administered.

Oral docetaxel

The results obtained with oral paclitaxel, and the knowledge that docetaxel is also a substrate for P-gp,[29] encouraged us to initiate experiments in wild-type and *mdr1a/1b* $^{-/-}$ mice.[30,31] These preclinical studies showed that the exposure to oral docetaxel was significantly higher in *mdr1a/1b*$^{-/-}$ mice compared to wild type mice. In addition, the exposure to docetaxel in wild type mice could be increased to similar levels as in mdr1a/1b$^{-/-}$ mice by co-administration of cyclosporin.[30] These observations, together with clinical experience of oral paclitaxel in combination with cyclosporin, led to a swift initiation of a clinical proof of concept study. This study investigated the effect of co-administered cyclosporin (15 mg/kg) on the bioavailability of docetaxel (75 mg/m2) in patients with advanced solid malignancies. The docetaxel i.v. formulation was used as drinking solution. It was shown that oral docetaxel administered as single agent exhibited poor bioavailability, whereas in combination with cyclosporin an average apparent bioavailability (AUC ratio: AUC after oral administration divided by AUC after i.v. administration) of 90% was reached.[32]

Subsequently, a phase II study with this combination was performed in patients with metastatic breast cancer (unpublished data: H.H. Helgason et al.). Based on the apparent bioavailability of 90% that was found in the proof of concept study, a fixed weekly dose of 100 mg oral docetaxel in combination with 15 mg/kg cyclosporin was administered to 31 patients that entered the study. In a total of 3 complete and 10 partial responses were seen.

Meanwhile, the aforementioned preclinical study with oral docetaxel in mice continued.[30] It was found that in wild-type mice receiving oral docetaxel as single agent, only 40 % of the dose could be recovered in faeces as parent drug, whereas 30% of the dose was recovered as oxidative metabolites. This suggested that a substantial amount of docetaxel is absorbed in the gastrointestinal tract, indicating that presystemic metabolism plays a role in the apparently low oral bioavailability of docetaxel. A study was initiated in which oral docetaxel was administered in combination with ritonavir to wild-type and mdr1a/1b $^{-/-}$ mice. Ritonavir is a HIV protease inhibitor with strong CYP3A4 inhibiting effects and only minor P-gp inhibiting effects.[33] It was found that inhibiting CYP3A4 with ritonavir dramatically (50-fold) increased the apparent bioavailability of docetaxel in both wild-type and *mdr1a/1b*$^{-/-}$ mice.[30] This indicated that both P-gp and CYP3A4 determine the low oral bioavailability of docetaxel with CYP3A4 being quantitatively the most important.

This concept was evaluated in patients with solid tumors. Patients received 10 or 100 mg oral docetaxel co-administered with 100 mg ritonavir simultaneously. The apparent bioavailability of docetaxel combined with ritonavir was 131% ± 90%. The substantial increase in AUC, with an apparent bioavailability of more than 100%, indicated that ritonavir also inhibited the elimination of systemic docetaxel after oral administration.[34]

The pharmacokinetic results are presented in figure 1. A plasma concentration time

curve of 75 mg/m^2 oral docetaxel without booster[32], 100 mg iv docetaxel and 100 mg oral docetaxel co-administered with 100 mg ritonavir[34], are presented. Because of the advantages of ritonavir compared to cyclosporin boosted docetaxel (favorable side-effect profile, extensive clinical experience with ritonavir boosted drugs and the stronger increased docetaxel exposure), future studies will initially be done in combination with ritonavir.

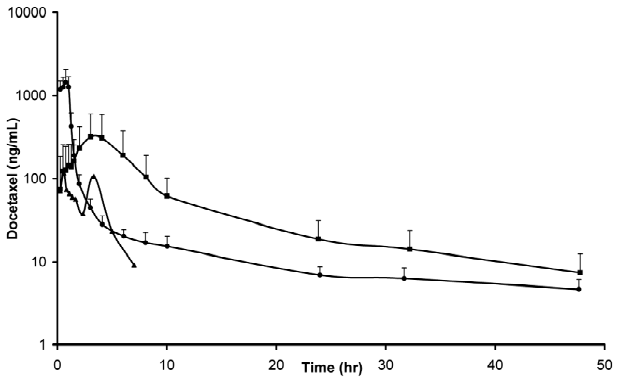

Figure 1: Plasma concentration time curve of docetaxel: 100 mg i.v. (●), 75 mg/m2 oral (▲), and 100 mg oral docetaxel in combination with 100 mg ritonavir (■).

Ongoing studies and future perspectives

The results obtained with oral docetaxel and paclitaxel regimens with coadministered boosters have clearly shown that oral chemotherapy with these drugs is feasible; adequate plasma concentrations were reached, the safety profile observed was comparable to that of i.v. regimens, and the overall response rates (ORR) were high in first line gastric (paclitaxel: ORR 32% (95% confidence interval (CI): 18 – 52%)), second line metastatic breast (paclitaxel: ORR 52% (95% CI: 34 – 70%) and docetaxel: ORR 45% (95% CI: 28 - 62%)) and second line NSCLC (paclitaxel: 23% (95% CI: 9 – 44%)), see table 1. There are however a few hurdles to overcome. The most important limiting factor is the drug formulation used so far. The i.v. formulation is administered as a drinking solution, which has an unfavorable taste, its shelf life is limited and there is higher chance for drug contamination compared to a capsule or tablet. Recently the pharmacy of the Slotervaart Hospital and Netherlands Cancer Institute succeeded in developing a capsule formulation of docetaxel (unpublished data: J.J. Moes et al.). The developed docetaxel capsule formulation was compared with the liquid docetaxel

formulation in patients in a cross-over study. We found a slightly lower bioavailability for the oral capsule formulation, but the inter-patient variability was much lower for the capsule formulation. This is an important finding because a low inter-patient variability enables us to target more precisely within the therapeutic window. The optimal weekly regimen of the oral docetaxel capsules in combination with ritonavir is currently being investigated in a phase I study.

The inter-patient variability is an important factor in limiting the use of oral agents with a narrow therapeutic window. It is therefore of high importance to decrease the inter-patient variability of docetaxel to levels comparable to those of intravenous regimens. Hellriegel et al. found that there is a trend towards a lower inter-patient variability when the bioavailability increases.[35] The inter-patient variability can thus, possibly, be further decreased by increasing the bioavailability of docetaxel. Selection of the optimal booster, the time of intake and the booster dose, are thus of pivotal importance. These parameters are currently being assessed using a minimal number of patients aided with population pharmacokinetic modeling and simulations.

Current research on oral paclitaxel focuses on the selection of the optimal booster-drug combination and on the pharmaceutical development. A new booster is needed because of the immunosuppressive effects of cyclosporin. A possible candidate to replace cyclosporin could be elacridar (GF120918), which has shown to increase the bioavailability of paclitaxel to the same extent as cyclosporin in both preclinical and clinical studies and it lacks the unfavorable immunosuppressive effects.[14,36]

Pre-clinical experiments could also further aid in determining the best strategy for boosting either paclitaxel or docetaxel. Recently, mice were developed that lack the cyp3a4 enzyme. [37] These mice can be cross-bred with mice lacking P-gp (mdr1a/1b$^{-/-}$ mice) or other transporter deficient mice, for instance the apical drug transporter multidrug resistance protein 2 knock-out mice(mrp2$^{-/-}$ mice). These mice models can be used to look more mechanistically into how the different transporters and metabolizing enzymes work together in the absorption, elimination and excretion of docetaxel and paclitaxel. New insights are subsequently swiftly integrated in ongoing clinical trials, optimally leading to selection of a well tolerated oral taxane treatment.

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2

Clinical development of oral docetaxel

Chapter 2.1

From mouse to man: Predictions of human pharmacokinetics of orally administered docetaxel from preclinical studies

S.L.W. Koolen, R.A.B. van Waterschoot, O. van Tellingen, A.H. Schinkel, J.H. Beijnen, J.H.M. Schellens, A.D.R. Huitema

Journal of Clinical Pharmacology (in press)

Abstract

Intravenously administered docetaxel is approved for the treatment of various types of cancer. An oral regimen, in combination with ritonavir, is currently being evaluated in clinical trials. The pharmacokinetics of docetaxel is determined by the activity of the metabolizing enzyme cytochrome P450 3A (CYP3A), and the drug efflux transporter, P-glycoprotein (P-gp). The effect of these proteins on the pharmacokinetics of docetaxel were investigated in different mouse models that lack one or both detoxifying systems. Docetaxel was given to these mice orally or intravenously with or without a strong CYP3A inhibitor, ritonavir. The data of these two preclinical studies were pooled and analyzed using nonlinear mixed effects modeling. The results of the preclinical studies could be integrated successfully, with only a small difference in residual error (33% and 26%, respectively). Subsequently, the model was used to predict human exposure using allometric scaling and this was compared to clinical trial data. It was shown that this model led to adequate predictions of docetaxel exposure in humans.

Introduction

Docetaxel is a semisynthetic taxane derivative originating from the needles of the European yew tree (*Taxus baccata*). It shows activity against various types of cancer.[5] Currently, it is approved for the treatment of breast, non-small-cell-lung, prostate, gastric and head-and-neck cancer. The drug is given in 3-weekly schedules by a 1 hour infusion of 75 – 100 mg/m^2. Our research group is working on the development of an oral docetaxel regimen.[6] An orally available drug could be beneficial in terms of patient convenience, costs and safety. Furthermore, improved anti-cancer activity may be obtained by applying more dose-dense docetaxel schedules that warrant an oral drug formulation.[7,8]

The development of an oral docetaxel regimen was started in different mouse models. It has been demonstrated that the oral administration of docetaxel resulted in a very low systemic exposure. This exposure proved, however, to be significantly higher in *Mdr1a/1b* knockout mice *(Mdr1a/1b*$^{-/-}$*)*.[4] *Mdr1a* and *Mdr1b* encode the two murine isoforms of the drug efflux pump P-glycoprotein (P-gp). These results were swiftly translated into a proof of concept study in humans that investigated orally administered docetaxel in combination with the P-gp and CYP3A inhibitor, cyclosporin, an immunosuppressive agent.[9] It was demonstrated that the exposure to docetaxel in this combination reached therapeutic levels. Anti-tumor activity was subsequently demonstrated in a phase II trial in patients with metastatic breast cancer (manuscript in preparation Helgason et al.) Meanwhile, the aforementioned studies in mice continued and investigated the role of the cytochrome P450 3A (CYP3A) enzyme in the disposition of docetaxel. CYP3A is the primary metabolizing enzyme of docetaxel and is abundantly

present in the gastrointestinal tract and liver. The effect of CYP3A was investigated by co-administration of docetaxel with a strong CYP3A inhibitor, ritonavir. It was found that the inhibition of CYP3A in mice resulted in a very strong increase in exposure to docetaxel.[4] The exposure to docetaxel was higher than that seen in Mdr1a/1b$^{-/-}$ mice. These observations resulted in the initiation of a second proof of concept study to investigate the oral administration of docetaxel in combination with ritonavir in cancer patients.[1] Ritonavir is a HIV-protease inhibitor that is nowadays predominantly used in relatively low doses to enhance the exposure to other protease inhibitors by inhibition of CYP3A.[10]

The study with oral docetaxel in combination with ritonavir in patients demonstrated the feasibility of this concept. The exposure to docetaxel was effectively boosted by a reduced presystemic metabolism in the gut and liver, and a reduced elimination rate of docetaxel.[11,1]

The pharmacology of boosting oral docetaxel by inactivation of either P-gp and/or CYP3A was recently further characterized in novel mouse models; mice that lack CYP3A (*Cyp3a*$^{-/-}$) and mice that lack both P-gp and CYP3A (*Cyp3a/Mdr1a/1b*$^{-/-}$).[2,12] These studies demonstrated that CYP3A and P-gp work together in lowering docetaxel exposure.

This wealth of both preclinical and clinical pharmacologic data allowed us to investigate whether preclinical results can actually be translated to humans.

The aim of this study, therefore, was to predict the exposure to docetaxel in humans after oral administration based on preclinical studies in several (knockout) mouse models using population pharmacokinetic (PK) modeling and simulations. It was hypothesized that by combining the complicated pharmacology of oral and i.v. docetaxel as unraveled in mice, with data of i.v. administered docetaxel to cancer patients[13,14,15,3], accurate predictions of oral docetaxel in humans would be generated, thus enabling rational extrapolation of preclinical data to the human situation.

Methods

Study design

The study design is schematically presented in figure 1. The first step was to develop a population PK model of docetaxel after oral and i.v. administration in mice using data from two mouse model studies (study A and B). The developed mouse PK model was subsequently combined with a well established PK model of i.v. administered docetaxel in cancer patients.[3]

This integrated model was then used to predict oral docetaxel exposure in humans. Finally, these predictions were compared to actual exposure data in humans obtained from a clinical trial.[1]

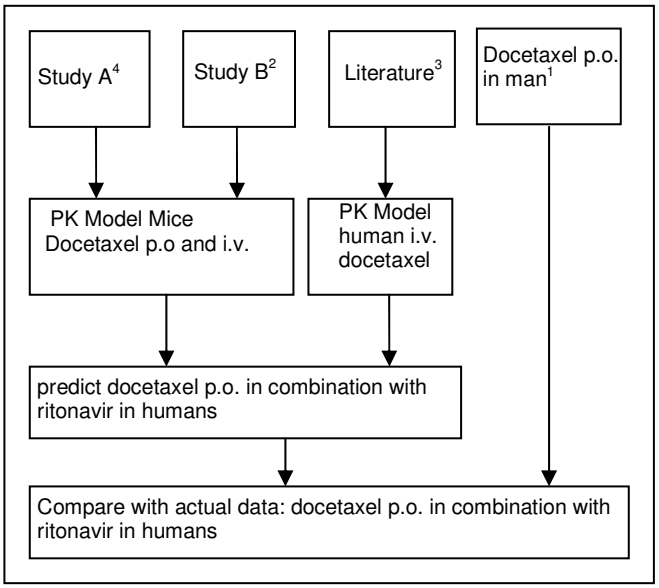

Figure 1: Study design preclinical studies in mice, study A[4] and study B[2] were used to develop a PK model of oral and i.v. docetaxel in mice. The PK model of i.v. docetaxel in humans was obtained from the literature[3]. These two models were combined and used to predict oral docetaxel exposure in humans. The predictions were evaluated by comparing the predicted data with the data obtained in two proof of concept studies in humans[1].

Preclinical model development

The preclinical population PK model was developed using data from two preclinical studies, study A and B. The study designs of these studies are briefly described below.

Study A[4]: The experiments in study A were performed using female FVB wild-type and *Mdr1a/1b*$^{-/-}$ mice. The mice received docetaxel (10 mg/kg) p.o. 30 min after ritonavir (12.5 mg/kg p.o.) or vehicle solution (ethanol/cremophor-EL/propyleneglycol/water, 43:10:25:22 (v/v/v/v) p.o.). Mice were between 10 and 17 weeks old. In total 4 groups of mice were studied: two groups determined by the two genotypes of the mice and these groups received docetaxel with or without ritonavir. Each group consisted of 4 – 7 mice. Multiple blood samples were collected 5, 10, 20 and 45 minutes and 1, 1.5, 2, 3, 4, 6, 8, and 12 hours after administration of docetaxel, by cannulating the jugular vein.[16] Docetaxel was measured with a validated high performance liquid chromatography (HPLC) method.[17]

-Study B[2]: Experiments in study B were done using wild-type, *Mdr1a/1b*$^{-/-}$, *Cyp3a*$^{-/-}$, and *Cyp3a/Mdr1a/1b*$^{-/-}$ double knockout mice. All mice used in this study were male FVB mice and were between 8 and 14 weeks of age. Each mouse received docetaxel on a

single occasion. In total 8 groups of mice were studied: four groups determined by four different genotypes of the mice and these groups received docetaxel either orally or intravenously docetaxel. Each group consisted of 6 – 8 mice. Docetaxel 10 mg/kg was administered by oral gavage or by injection into the tail vein. Multiple blood samples (~40 uL) were collected from the tail vein at 15 and 30 minutes and 1,2,4 and 8 hours after administration of docetaxel. The samples were analyzed using a validated liquid chromatography – tandem mass spectrometry (LC-MS/MS) assay previously described.[18]

Pharmacokinetic data analysis

Population PK modeling was performed using the nonlinear mixed-effects modeling program NONMEM, version VI (Icon Development Solutions, Ellicot City, Maryland, USA).[19] The first-order conditional estimation procedure was used throughout. The adequacy of the tested models was evaluated using both graphical and statistical methods. The log-likelihood ratio test was used to discriminate between hierarchical models differing in only one parameter. A difference in objective function value (OFV) of 6.63, corresponding to a p-value of 0.01, was considered significant.

Standard errors for all parameters were calculated with the COVARIANCE option in NONMEM, and individual Bayesian PK parameters were obtained using the POSTHOC option. The R-based model building aid Xpose (version 4)[20] and Perl speaks NONMEM (PsN) were used for graphical model evaluation. Piraña was used for run deployment and analysis.[21]

Between subject variability was modelled exponentially and residual variability was modelled using a proportional error model. Since the samples in study A and B were measured using different techniques (LC-UV and LC-MS/MS, respectively), separate residual error models were investigated for study A and study B.

Body weight was scaled to a 70 kg in order to provide interpretable PK parameters (i.e. PK parameters for a 70 kg adult). Scaling was performed using allometry with a power coefficient of 0.75 for clearance and 1 for volume of distribution.[22] The weight of each mouse was set at 25 grams. See for example equation 1.

Eq. 1: $$CL = CL_{70} * \left(\frac{weight}{70} \right)^{0.75}$$

(abbreviations: CL: clearance in L/h, CL_{70}: clearance normalized to 70 kg person, weight in kg)

PK model mice: model development

Modeling started with an open 2-compartment model for wild-type mice receiving oral or iv docetaxel. The effects of P-gp and CYP3A (as determined by the different genotypes of the mice) and ritonavir were subsequently added to the model.

Clearance and bioavailability

Clearance and bioavailability of docetaxel in mice were estimated according to similar formulas used for oral docetaxel in humans[11] according to a well-stirred liver model as proposed by Wilkinson et al. [23].

Eq. 2: $$CL = Q * \frac{CL_i}{CL_i + Q}$$

Eq. 3: $$CL_i = \frac{CL_{i0}}{1 + \frac{[RTV]}{K_i}}$$

Where Q is hepatic blood flow, CL_i is intrinsic clearance, CL is the docetaxel clearance, $CL_{i,0}$ is the uninhibited CL_i of docetaxel (the dimension of Q and CL is L/h), [RTV] is the plasma concentration of ritonavir in ng/mL, and K_i is the inhibition constant of ritonavir on docetaxel in ng/mL. Since only total plasma concentrations were available, it was assumed that the blood/plasma ratio was one and the free fraction was independent of the investigated concentration range. Furthermore, plasma concentration data of ritonavir in mice were not available. Therefore, ritonavir PK was allometrically scaled from human data using equation 1. The PK of ritonavir in humans was best described by a single compartment with a lag-time.[24] Distribution volume was estimated to be 96.8 L, and clearance 10.7 L/h. The absorption rate of ritonavir was assumed to be similar to the estimated individual absorption rates of docetaxel found in mice and the lag time, that was observed in humans, was omitted for mice. The hepatic blood flow was set at 140 mL/hr.[25]

Eq. 4:

$$CL_{i0} = \left(CL_{wt} * \theta_{CL}^{Cyp3a-/-} * \theta_{CL}^{Mdr1a/1b-/-} * \theta_{CL}^{Cyp3a/Mdr1a/1b-/-} * \theta_{CL}^{RTV/Mdr1a/1b-/-}\right) * \left(\frac{WT}{70}\right)^{0.75}$$

Eq. 5: $$F = F_{gut} * F_{hep}$$

Eq. 6: $$F_{hep} = \frac{Q}{CL_i + Q}$$

Eq. 7:

$$F_{gut} = F_{wt} * \theta_F^{Cyp3a-/-} * \theta_F^{Mdr1a/1b-/-} * \theta_F^{Cyp3a/Mdr1a/b-/-} * \theta_F^{RTV} * \theta_F^{RTV/Mdr1a/1b-/-}$$

In these formulas CL_{wt} and F_{wt} denote the scaled parameter estimate of a wild-type mouse for clearance and bioavailability, respectively; θ is the estimated fixed effect when the specified gene is knocked out. The specified gene in the formulas is either 1, in case of a knockout mouse or 0 for a wild-type mouse. The same accounts for 'RTV'. When docetaxel is given in combination with ritonavir, this factor is 1, otherwise it is 0. The factors $\theta^{Cyp3a/Mdr1a/1b-/-}$ and $\theta^{RTV/Mdr1a/1b-/-}$ are interaction factors. The log-likelihood ratio test was used to test significance of the different factors.

Model evaluation

The final model was selected based on goodness-of-fit and as given by the objective function value. Furthermore, the model was evaluated by inspecting the visual predictive check plots.[26] The data set was simulated 1000 times. The observed data, the median and the 5th and 95th percentile of the prediction interval were plotted using PsN version 3.0 [27] and Xpose version 4.1.0.[20]

Extrapolation & simulation to humans

The developed preclinical PK model was subsequently extended with a well-established model for i.v. docetaxel in human. The bioavailability and the relative effects of the different detoxifying systems (P-gp and CYP3A4) as identified in mice were incorporated into the model of i.v. docetaxel in cancer patients. The absorption rate constant (Ka) was not scaled since body weight is not a major determinant, THerefore, it was assumed that 50% would be absorbed within the first hour after intake, consequently, Ka of oral docetaxel in humans was set at 0.7 h^{-1}. The hepatic blood flow was set at 80 L/h and the ritonavir plasma concentrations were estimated using a previously described population PK model.[24]

This combined model was used to predict exposure to orally administered docetaxel in humans, for which aim two hypothetical situations were simulated: oral docetaxel 100 mg in humans assuming complete CYP3A inhibition (Sim A), or in combination with 100 mg ritonavir given 1 hour prior to docetaxel (Sim B).

With this model 1000 individuals were simulated for the two aforementioned situations. The simulated results were presented by an adapted visual predictive check. The median and the 50% confidence area of the simulated data were plotted. The model-predicted human exposure was compared to actual human exposure. The median observed concentration time curve in cancer patients of oral docetaxel in combination with 100 mg ritonavir, given one hour prior to docetaxel, was plotted.[1] Patients with histological or cytological proof of cancer, for whom no standard of proven therapeutics exists, were included in this study. Data was available from 15 patients. Plasma samples for PK analysis were drawn at 0.25, 0.5, 0.75, 1, 1.25, 1.5, 2, 3, 4, 6, 8, 10, 24, 36 and 48 hours after docetaxel ingestion. The median concentration time curve of oral docetaxel 100 mg when co-administered with ritonavir 100 mg was calculated.

Furthermore, the mean and coefficient of variation of the area under curve (AUC) of the simulated regimens were calculated. The AUC with extrapolation to infinity was determined using R (version 2.10.0) [28] by employing validated scripts. The simulated AUC values were compared with the observed AUC after administration of 100 mg docetaxel with 100 mg ritonavir given 1 hour prior to docetaxel intake published by Oostendorp et al.[1]

Results

Of in total 70 mice, 474 plasma concentration time points were available for PK analysis. The different mouse strains and treatment strategies are presented in table 1.

Table 1: The number of mice, stratified for mouse strain that received docetaxel 10 mg/kg intravenously (i.v.) or orally (p.o.) with or without co-administration of 12.5 mg/kg ritonavir.

Docetaxel 10 mg/kg	Intravenous administration	Oral administration
Wild-type	5	10
Mdr1a/1b$^{-/-}$	5	13
Cyp3a$^{-/-}$	5	6
Cyp3a/Mdr1a/1b$^{-/-}$	5	8
Wild-type + ritonavir 12.5 mg/kg	-	6
Mdr1a/1b$^{-/-}$ + ritonavir 12.5 mg/kg	-	7

PK model development in mice

It was observed that co-administration of the CYP3A inhibitor, ritonavir, in wild-type mice resulted in a much higher exposure to docetaxel compared to the docetaxel exposure in *Cyp3a*$^{-/-}$ mice, with an estimated gut bioavailability (the fraction of docetaxel that passes the gastrointestinal barrier) of 70% (CV% 30%) and 34% (CV% 23%), respectively. The systemic exposure to docetaxel was also much higher in the *Mdr1a/1b*$^{-/-}$ mice who received ritonavir compared to the exposure in *Cyp3a/Mdr1a/1b*$^{-/-}$ mice (gut bioavailability of 105% versus 52 %). A higher than 100% bioavailability was probably estimated because no data was available of intravenously administered docetaxel in combination with ritonavir. The effect of ritonavir on docetaxel clearance was best described applying equation 2 and 3, with an estimated inhibition constant (Ki) of 0.47 µg/mL (CV%: 60%). No non-linear PK behavior in clearance was observed in the different mouse strains.

In table 2 the final parameter estimates are presented. Separate estimation of volume of distribution (V), inter-compartmental clearance (Q1) or absorption rate constant (Ka) for each mouse strain did not result in improvement of the model. The final model described the observed data well (see figure 2 and 3). The final parameter estimates were estimated with adequate precision; the coefficient of variation (CV%) was low and the results of the visual predictive check demonstrated that the confidence interval and the median of the simulated data were in accordance with the observed data in mice. Goodness of fit plots of the different mice strains and visual predictive check plots are presented in figure 2 and 3.

Table 2: Parameter estimates together with the coefficient of variation (CV) of the population PK model of docetaxel in mice.

Pharmacokinetic parameters in mice (values normalized to 70 kg human)			**Estimate**	**CV (%)**
Ka_{70}	Absorption rate	(h^{-1})	1.3	12%
Q	Hepatic blood flow 25 g mouse (FIXED)	(L/h)	0.14	-
V2	Volume central compartment	(L)	114	4.2%
F_{gut}	Gut bioavailability:			
	F_{WT}	(%)	27%	24%
	$F_{cyp3a\ ko}$	(%)	34%	23%
	$F_{mdr1\ ko}$	(%)	40%	15%
	F_{RTV}	(%)	70%	30%
	$F_{mdr1a\ ko\ +\ RTV}$	(%)	105%	-
	$F_{cyp3a/mdr1a/1b\ ko}$	(%)	52%	-
CL_i	CL_{WT} (intrinsic clearance)	(L/h)	72.9	15%
	$\theta_{CL}^{cyp3a-/-}$	-	0.142	28%
	$\theta_{CL}^{mdr1a/1b-/-}$	-	0.755	13%
	$\theta_{CL}^{mdr1a/1b/cyp3a-/-}$	-	0.207	29%
	$\theta_{CL}^{RTV/mdr1a/1b-/-}$	-	0.191	56%
	CL Mdr1a/1b$^{-/-}$	(L/h)	55.0	
	CL Cyp3a$^{-/-}$	(L/h)	10.4	
	CL Cyp3a/Mdr1a/1b$^{-/-}$	(L/h)	1.6	
	CL Mdr1a/1b$^{-/-}$ mice + RTV	(L/h)	10.5	
Q1	Inter-compartment clearance	(L/h)	5.4	13%
V3	Volume of distribution of peripheral compartment	(L)	146	9.7%
Ki	Inhibition constant RTV – docetaxel	(µg/mL)	0.47	60 %
Interindividual variability in CL		(%)	32%	87%
Interindividual variability in KA		(%)	61%	33%
Interindividual variability in F		(%)	41%	43%
Residual error Study A		(%)	32%	-
Residual error Study B		(%)	26%	-

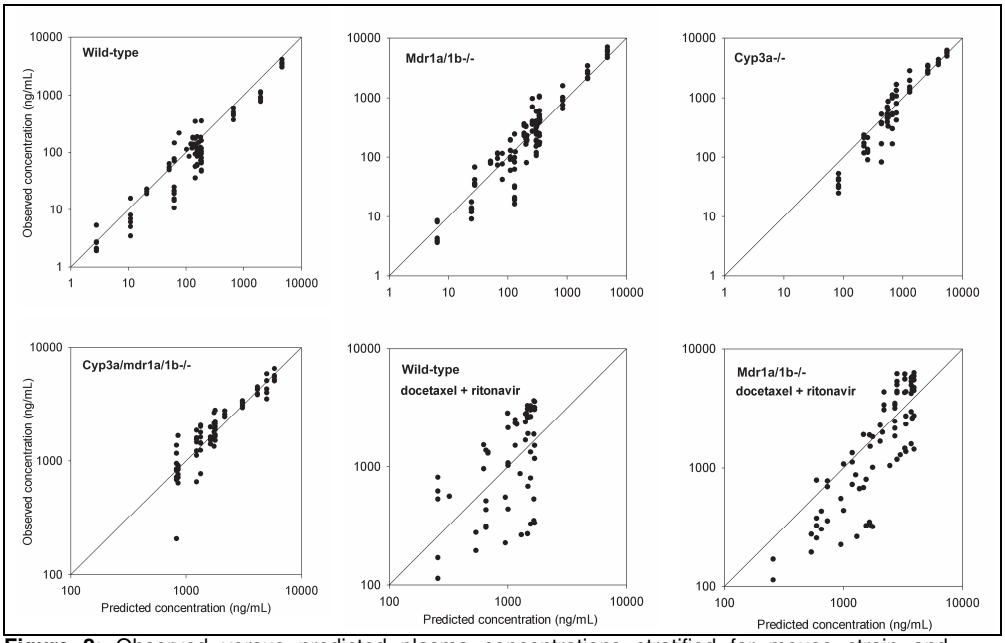

Figure 2: Observed versus predicted plasma concentrations stratified for mouse strain and treatment schedule.

Interaction between CYP3A, P-gp and co-administered ritonavir

The Cyp3a$^{-/-}$ mice demonstrated a 86% reduction in intrinsic clearance (from 72.9 L/h to 10.4 L/h) and the Mdr1a/1b$^{-/-}$ mice demonstrated a 24% reduction in intrinsic clearance (from 72.9 L/h to 55.0 L/h). These data indicate that Cyp3a and P-gp are the most important factors responsible for docetaxel metabolism. The intrinsic clearance in the absence of both CYP3A and P-gp (Cyp3a/Mdr1a/1b$^{-/-}$ mice) was low and accounted for approximately 2% of total docetaxel intrinsic clearance in the presence of these proteins (1.6 L/h versus 72.9 L/h). Based on these clearance estimates, an interaction between CYP3A and P-gp could not be confirmed.

Besides the intrinsic clearance, the interaction between CYP3A and P-gp was also studied at the level of gut bioavailability. Mice lacking Cyp3a and/or P-gp showed a higher gut bioavailability compared to wild-type mice. Mice lacking CYP3A showed a 7% (34% versus 27%) increase in gut bioavailability and mice lacking P-gp showed a 13 % increase (40% versus 27%). The combined loss of both enzymes showed a more than additive increase of 25% in gut-bioavailability (52% versus 27%).

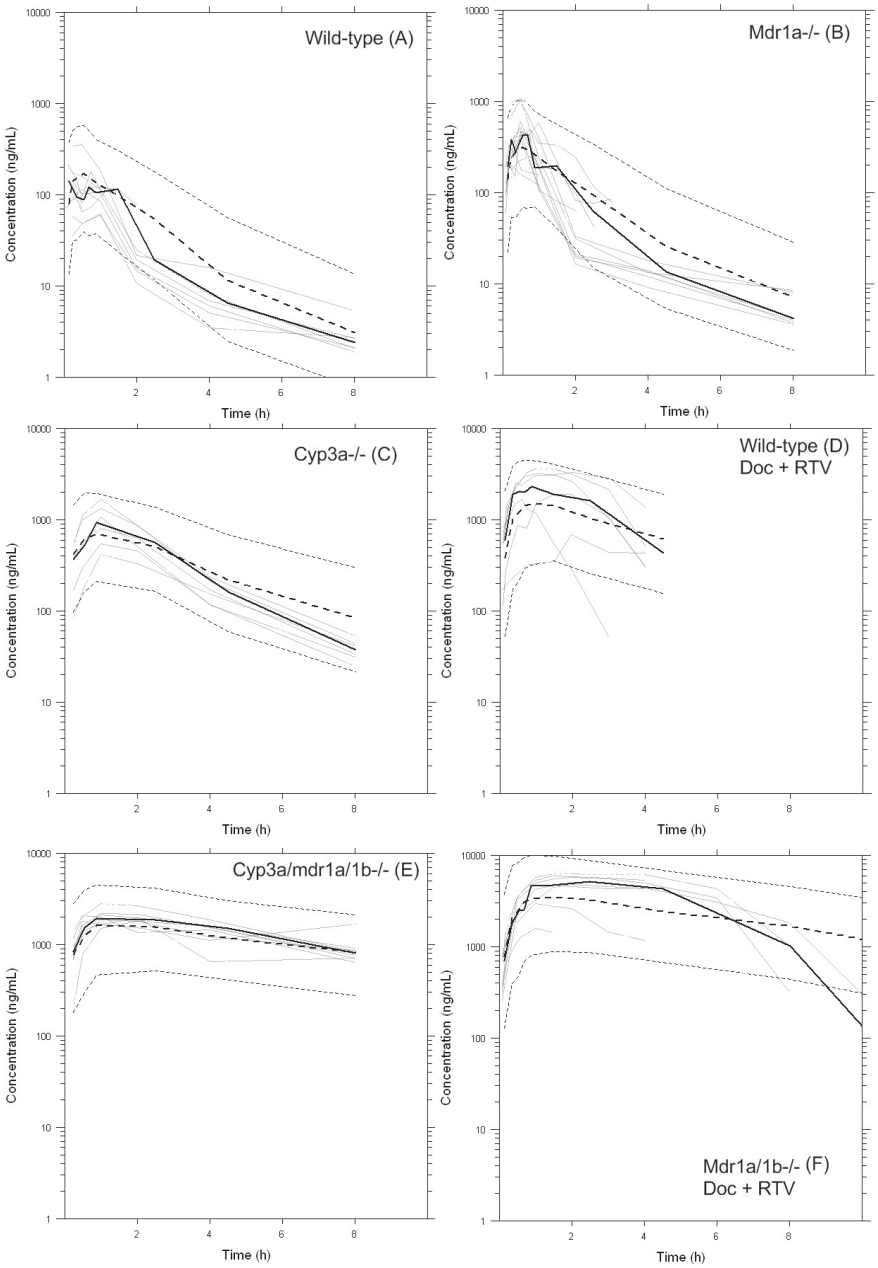

Figure 3: Visual predictive check plots of orally administered docetaxel (10 mg/kg) to wild-type mice [A], Mdr1a/1b$^{-/-}$ knockout mice [B], Cyp3a$^{-/-}$ knockout mice [C], wild-type mice co-treated with 12.5 mg/kg ritonavir [D], Cyp3a/mdr1a/1b$^{-/-}$ knockout mice [E], Mdr1a/1b$^{-/-}$ mice co-treated with 12.5 mg /kg ritonavir [F] . The thin grey lines are the individual observed concentration time profiles and the black solid lines the median observed concentration time profile of docetaxel. The dotted lines represent the median, 5th and 95th percentile of the simulated data.

Predictions of human exposure

The first column in table 3 lists the parameter estimates as determined by Bruno et al[3] of i.v. docetaxel administered to cancer patients. The 2nd – 3rd column were the models that were used for simulating oral docetaxel in two hypothetical situations:

100 mg oral docetaxel in human 1) with complete CYP3A inhibition, and 2) in combination with ritonavir.

The results of the simulations were plotted in figure 4 together with the median concentration time curves found in cancer patients when a dose of 100 mg docetaxel was given orally in combination with ritonavir 100 mg. This figure illustrates that the observed exposure to docetaxel when given orally in combination with 100 mg ritonavir to cancer patients, was reasonably well predicted if complete CYP3A inhibition (figure 4A) was assumed and was under predicted if partial CYP3A inhibition (figure 4B) by ritonavir was assumed using the developed PK model.

The mean observed systemic exposure, measured by AUC_{inf}, after administration of 100 mg docetaxel po with 100 mg ritonavir was 2.8 mg*h/L (CV% 50.8) [1] The mean predicted exposure if complete CYP3A inhibition was assumed was 3.0 mg*h/L (CV%: 56%) and simulation of oral docetaxel in combination with ritonavir resulted in predicted systemic exposure of 1.4 mg*h/L (CV%: 58%)

Table 3: Parameter estimates of i.v. administered docetaxel in humans [3] and the extrapolated parameters for orally administered docetaxel assuming complete CYP3A4 inhibition [Sim A], or in combination with ritonavir [Sim B].

Pharmacokinetic parameters in human			**Ref.** [3,24]	**Sim A**	**Sim B**
CL	clearance	(L/h)	36.7		
Q	*Hepatic blood flow***	*(L/h)*	*80*	80	80
CLi	*Intrinsic clearance***	*(L/h)*	*67.8*	**9.6**	67.8
Ki	RTV inhibition constant	(µg/mL)	-	-	**0.47**
V_2	Volume central compartment	(L)	8.31	8.31	8.31
k_{23}	Transport rate; 2nd to 3rd compartment	(h^{-1})	1.07	1.07	1.07
k_{32}	Transport rate; 3rd to 2nd compartment	(h^{-1})	1.74	1.74	1.74
k_{24}	Transport rate; 2nd to 4th compartment	(h^{-1})	1.28	1.28	1.28
k_{42}	Transport rate; 4th to 2nd compartment	(h^{-1})	0.079	0.079	0.079
ka	Absorption rate	(h^{-1})	-	0.7	0.7
F	Bioavailability	(%)	-	**34%**	**70%**
Ritonavir					
CL	Clearance	(L/h)	10.7	-	10.7
V5	Distribution volume	(L)	96.8	-	96.8
Tlag	Absorption lag time	(h)	0.78	-	0.78
Ka	Absorption rate	(h^{-1})	0.87	-	0.87
Variability docetaxel					
Interindividual variability in CL		(%)	33.5	34%	34%
Interindividual variability in F		(%)	-	**41%**	**41%**
Interindividual variability in V2		(%)	56.1	56%	56%
Interindividual variability in KA		(%)	-	**61%**	**61%**
Residual error		(%)	20.5	20%	20%

**Intrinsic clearance was calculated using the docetaxel clearance determined by Bruno et al. and an estimated hepatic blood flow of 80 L/h.

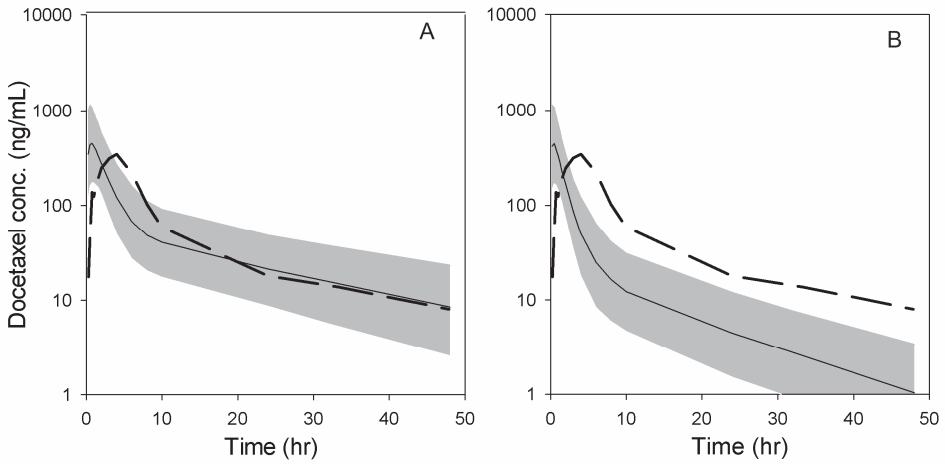

Figure 4: Simulated median (black line), 25th and 75th percentile of the simulated data (grey surface area) of 100 mg docetaxel given to patients in two hypothetically situations; SIM A: complete CYP3A inhibition, SIM B: when given in combination with ritonavir. The dashed line represents the median curves of 100 mg docetaxel given in combination with 100 mg ritonavir in patients obtained in a proof of concept study.[1]

Discussion

We have developed a PK model of orally and intravenously administered docetaxel in mice. The results of two pre-clinical studies could be integrated successfully as demonstrated in the visual predictive checks (figure 3). Corrections for different study design, handling of samples or analytical assays were not required, except for a small difference in residual error, which was slightly higher for study A (32%) compared to study B (26%). The developed model adequately described the PK in different mouse strains. Based on the PK alone, similar conclusions could be drawn compared to the original studies that were analyzed using non-compartmental analysis. E.g. co-administered ritonavir resulted in high exposure to docetaxel and CYP3A is quantitively the most import factor to influence the low systemic exposure to docetaxel when given orally. The original study of Waterschoot et al. was designed to unravel the interplay between CYP3A an P-gp.[2] A functional interplay, as has been proposed by Benet et al.,[29,30] suggests that P-pg works as a gatekeeper to prevent saturation of intracellular CYP3A. In this manner, P-gp would increase the amount of drug metabolized by CYP3A. This has been described as a synergistic relationship. However, the conducted experiments conducted by Van Waterschoot et al.[2], nor the population PK analysis of the data described here, could confirm this hypothesis. The separate effects of CYP3A and P-gp seemed to account for the total intrinsic clearance observed in wild type mice:

Mdr1a/1b$^{-/-}$ mice had a reduction of 17.9 L/h in clearance compared to wild type mice (72.9 L/h versus 55.0 L/h) and Cyp3a$^{-/-}$ mice showed a 62.5 L/h lower clearance compared to wild type mice (72.9 L/h versus 10.4 L/h). Mice with total loss of both proteins showed only a small intrinsic clearance of 1.6 L/h, which is probably a result of minor alternative elimination routes, some of which may have been upregulated due to the combined loss of CYP3A and P-gp.[2]

The gut bioavailability (the percentage of docetaxel that actually passes the intestinal barrier) is 1.3-1.5 fold higher in the single knock out mice (Mdr1a/1b$^{-/-}$: 40%, Cyp3a$^{-/-}$: 34%) and 2 fold higher in the double knock-out mice (52%) compared to wild-type mice (27%).
These percentages may indicate that CYP3A and P-gp work together synergistically, since the bioavailability increases more than additively in mice with combined loss of CYP3A and P-gp. However, as extensively discussed by Van Waterschoot et al.[2], the mechanistic interaction between CYP3A and P-gp should be investigated from the perspective of a double knock-out mouse. From this perspective it is observed that the individual contribution of CYP3A or P-gp in preventing docetaxel from passing the gut barrier is larger in the absence of the other protein. Thus, when one of these proteins is not functioning, than the other protein, exerts its effect more efficiently. Garmire et al observed a similar effect in their in silico model[31] and described this effect as CYP3A – P-gp antagonism. [32]

The exposure to docetaxel is much higher in wild-type mice that received co-administered ritonavir (F_{gut}: 70%) than the exposure in *Cyp3a*$^{-/-}$ mouse (F_{gut}: 34%). This large discrepancy may be explained by inhibition of P-gp by ritonavir, although in vitro experiments failed to demonstrate that ritonavir was unable to inhibit the transport of docetaxel through LLC-MDR1 cells.[4] Another explanation could be upregulation of detoxifying enzymes (transporters, or other metabolizing enzymes) in *Cyp3a*$^{-/-}$ mice.[2] Indeed, some upregulation of Mdr1a has been observed in the liver of Cyp3a$^{-/-}$ mice.[33]
The clearance of docetaxel when co-administered with ritonavir was described by equation 2 and 3, showing a ritonavir concentration-dependent clearance of docetaxel, with an inhibition constant of 0.47 µg/mL in mice. Using this inhibition constant for the prediction of oral docetaxel exposure in man resulted in under-prediction of the docetaxel concentration time curve as determined in a clinical trial (see figure 4B)[1] Population PK analysis of oral docetaxel in man demonstrated a much lower inhibition constant of 0.028 µg/mL[11]. This is probably caused by over-predicted ritonavir plasma concentrations in mice when scaled allometrically. Plasma concentration time data of ritonavir in mice would probably have generated more accurate estimations of docetaxel exposure in man. However, these data were not available.

Extrapolating PK results between species has been reviewed often. In most cases, results of multiple species of different sizes are used to extrapolate to humans.[34,22] Studies that extrapolated PK data to humans using only data of mice, are rare. This is not surprising regarding the large physiological differences between mice and man. In our case, however, we coupled preclinical data on orally administered docetaxel with clinical data on intravenously administered docetaxel. This strategy resulted in predictions that were in good agreement with the observed data on orally administered docetaxel in combination with ritonavir to patients.

Currently, future studies are designed to investigate more thoroughly the formation and pharmacokinetics of docetaxel and ritonavir metabolites after inhibition of, or the absence, of drug-transporters and/or metabolizing enzymes in preclinical mice and *in vitro* studies. The current analysis provides an excellent tool to incorporate the generated data into a single (physiological) model and to extrapolate these data to humans and to preliminary judge the validity of these extrapolated data.

In conclusion, a population pharmacokinetic model was successfully developed of preclinical data. The model provided a good pharmacological insight of boosted orally administered docetaxel and the influence and interaction of P-gp and CYP3A. Furthermore, the developed model provided a tool to extrapolate the results of future preclinical studies to humans and finally, this study demonstrates that the use of population pharmacokinetic analysis in an early, pre-clinical, stage can help estimating the pharmacokinetics accurately in humans and thereby help in optimizing boosted docetaxel regimens for patients.

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Chapter 2.2

Population pharmacokinetics of intravenously and orally administered docetaxel with or without co-administration of ritonavir in patients with advanced cancer

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British journal of clinical pharmacology, 2010, 69(5): 465-74

Abstract

Docetaxel has a low oral bioavailability due to affinity for P-glycoprotein and Cytochrome P450 (CYP) 3A4 enzymes. Inhibition of the CYP3A4 enzymes by ritonavir resulted in increased oral bioavailability.

The aim of this study was to develop a population pharmacokinetic (PK) model and to evaluate and quantify the influence of ritonavir on the PK of docetaxel.

Data from two clinical trials were included in the data analysis, in which docetaxel (75 mg/m^2 or 100 mg) had been administered intravenously or orally (10 mg or 100 mg) with or without co-administration of oral ritonavir (100 mg). Population modeling was performed using non-linear mixed effects modeling. A 3-compartment model was used to describe the i.v. data. PK data after oral administration, with or without co-administration of ritonavir, were incorporated into the model.

Gut bioavailability of docetaxel increased approximately 2-fold from 19 to 39% (CV: 13 %) by ritonavir co-administration. The hepatic extraction ratio and the elimination rate of docetaxel was best described by estimating the intrinsic clearance. Ritonavir was found to inhibit in a concentration dependent manner the intrinsic clearance of docetaxel, which was described by an inhibition constant of 0.028 µg/mL (CV: 36%). A maximum inhibition of docetaxel clearance of more then 90% was reached.

A PK model describing both the PK of orally and intravenously administered docetaxel in combination with ritonavir, was successfully developed. Co-administration of ritonavir lead to increased oral absorption and reduced elimination rate of docetaxel.

Introduction

Docetaxel has significant anti-tumor activity against a range of tumor types and is approved for the treatment of locally advanced or metastatic breast, non-small cell lung (NSCLC), head and neck, gastric and, hormone refractory metastatic prostate cancer at doses ranging from 60 to 100 mg/m^2 administrated as a 1-hour intravenous infusion every 3 weeks. Currently, weekly schedules of docetaxel are increasingly used. The rationale behind weekly administration is that this schedule results in more frequent exposure of docetaxel to tumor cells while lower C_{max} values are reached.[1]

Development of an orally available formulation of docetaxel is of interest because oral administration is preferred over intravenous (i.v.) administration, due to patient convenience, reduced administration costs and the opportunity to investigate more schedule-intensive treatment regimens.[2]

The oral bioavailability of docetaxel is, however, limited due to P-glycoprotein (P-gp) and cytochrome P450 (CYP) metabolizing enzymes, mainly CYP3A4.[3] The development of an oral docetaxel regimen started with co-administration of cyclosporin A, a potent P-gp

inhibitor, resulting in high systemic exposure to docetaxel,[4] however further development of this combination was terminated because preclinical research showed that inhibition of CYP3A4 was even more effective in enhancing the systemic exposure of docetaxel [3]. Inhibition of CYP3A4 by ritonavir, resulted in boosted systemic exposure of oral docetaxel in mice,[3] and in cancer patients.[5] Although several studies have suggested that ritonavir may act as inhibitor of P-gp,[6] *in vitro* studies have shown that ritonavir is not a potent inhibitor of P-gp mediated transport of docetaxel.[3] The enhancement of the systemic exposure of CYP3A4 substrates by ritonavir is already standard practice in the treatment of HIV patients with protease inhibitors.[7,8] The ritonavir dose used for boosting these agents is 100 mg, which is well below its therapeutic dose of twice daily 600 mg. In general these low doses show only limited side effects.[7,8]

A proof-of-concept study[5] of oral docetaxel in combination with ritonavir was performed in patients with advanced solid tumors. The apparent bioavailability (ratio of area under the plasma concentration time curve after oral and intravenous administration) of oral docetaxel (75 mg/m^2) alone was approximately 14%.[4] The apparent bioavailability of 100 mg oral docetaxel in combination with 100 mg ritonavir was above 100%.[5] Considering that a standard weekly docetaxel dose is 35 mg/m2,[9,10,11] systemic exposure to docetaxel needed for an effective weekly docetaxel regimen can be reached with the combination of both drugs. These results were considered promising and formed the basis for further clinical development of this combination.

The pharmacokinetics (PK) of this combination are critical for the further development, either for the evaluation of new formulations as well as for optimization of the design of oral docetaxel / ritonavir regimens (e.g. optimal dose, multiple ritonavir dosing, dosing interval). The PK is, however, not completely understood. The concentration time curves of docetaxel show non-linear pharmacokinetics in the terminal part of the plasma concentration time curve, suggesting a time and/or concentration dependent effect of ritonavir on the metabolism of docetaxel.

The primary objective of this study was to evaluate the influence of ritonavir on the absorption and elimination rate of docetaxel due to inhibition of P-gp and CYP3A4. Secondly, a population PK model, using nonlinear mixed effect modeling (NONMEM) was developed to assess simultaneously the PK of orally and intravenously administered docetaxel with or without co-administration of ritonavir. This model can be used for further development of the combination and to support future trials and schedules.

Materials and methods

Patients

Data were obtained from two clinical trials of whom the inclusion and exclusion criteria were similar.[5,4] Patients with histological or cytological proof of cancer, for whom no standard of proven therapeutics existed, were included in the study. Eligibility criteria

included a performance status ≤ 2 on the World Health Organization (WHO) scale, life expectancy of ≥ 3 months, adequate bone marrow (absolute leukocyte count ≥ $3.0*10^9$/liter, platelets > $100*10^9$/liter), hepatic (serum bilirubin < 20 µmol/litre, aspartate amino transferase and alanine amino transferase ≤ 1.5 times the normal upper limit; in case of liver metastasis amino transferase and alanine amino transferase ≤ 3 times the normal upper limit) and renal function (serum creatinine ≤ 160 µmol/litre and / or clearance ≥ 50 mL/min), no radiotherapy (palliative limited radiation for pain reduction was allowed) or chemotherapy for at least 3 weeks prior to entry and able and willing to swallow oral medication. Exclusion criteria consisted of active bacterial or viral infections, clinical signs of active brain or leptomeningeal metastases, alcoholism, drug addiction, psychiatric disorders leading to inadequate follow-up, pregnancy, or breast feeding and chronic use of H_2-receptor antagonists or proton pump inhibitors. The studies were approved by the Medical Ethics Committee of the Netherlands Cancer Institute and written informed consent was obtained from all patients prior to study entry.

Drug administration

The i.v. formulation of docetaxel (Taxotere; Rhone-Poulenc Rorer/Aventis, Antony, France) was used for both i.v. and oral administration. This formulation contains 0.5 mL polysorbate 80 per 20 mg docetaxel. Furthermore, commercial available ritonavir (Norvir; Abbott, Illinois, USA, 100 mg capsules) was used. Oral drugs were taken with 100 mL tap water after an overnight fast. Patients remained fasted until 1.5 h after docetaxel administration. Standard docetaxel pre-treatment consisting of oral dexamethason 4 mg 1 h before drug administration and 4 mg every 12 h (two times) after drug administration and oral granisetron 1 mg 1 hour before drug administration, was given during all cycles.

Study design

Patients in the first study were randomized into two groups. The first group received on day 1 ritonavir 100 mg and 60 minutes later 10 mg orally administered docetaxel, on day 8 they received 100 mg ritonavir and 10 mg oral docetaxel simultaneously. On day 22, patients received 100 mg i.v. administered docetaxel. Patients continued, if it was considered to be in their best interest, with 3-weekly docetaxel i.v. according to standard practice. The second randomization group followed the same schedule except that day 1 and day 8 were reversed.[5]

The low starting dose of docetaxel was selected for safety reasons because preclinical data in mice revealed that co-administration of ritonavir resulted in a 50-fold increase in systemic exposure to docetaxel.[5,3] After the first PK interim analysis and clinical evaluation, the oral docetaxel dose was increased from 10 mg to 100 mg. In total 22 patients were included in this study.

The second study was a proof of concept study of oral docetaxel plus cyclosporin A.[4] In this study docetaxel was given in combination with cyclosporin A. Data of docetaxel administered simultaneously with cyclosporine A, were not included in the current analysis. However, data of 3 patients receiving oral docetaxel 75 mg/m^2 alone and 14 patients receiving docetaxel i.v. 100 mg/m^2, were included.

PK sample collection, processing and storage procedures

Both studies applied extensive PK sampling during the first 48 hours after administration for both docetaxel and ritonavir; predose, 0.25, 0.5, 0.75, 1, 1.25, 1.5, 2, 3, 4, 6, 8, 10, 24, 36, and 48 hours.[4]

Blood samples were collected in heparinized tubes and centrifuged; plasma was separated and stored at -20°C until analysis. Docetaxel plasma levels were measured in the first study with a validated liquid chromatography coupled with tandem mass spectrometry with a lower limit of quantification (LLQ) of 0.25 ng/mL.[12] In the second study,[4] docetaxel concentrations were determined with a validated high-performance liquid chromatography assay (HPLC) with a LLQ of 10 ng/mL.[13] Plasma concentrations of ritonavir were determined using an isocratic reversed-phase ion-pair, HPLC assay with ultraviolet detection with an LLQ of 50 ng/mL.[14] The accuracies and precisions of both assays felt within $\pm$ 15%. The quality control samples of the assays were prepared separately and, therefore, the results of different assays were considered interchangeable.

Data analyses

Data from the different administration routes were analyzed simultaneously using NONMEM software (version VI; Icon Development Solutions, Ellicot City, Maryland, USA) [15]. Piraña (an interface to NONMEM, and our cluster) was used for run deployment and analysis.[16] The First Order (FO) estimation method with natural logarithmically (Ln) transformed concentration time data was used to develop the model and the First Order Conditional Estimation (FOCE) method was used in order to estimate the final parameter-estimates. Standard errors for all parameters were calculated with the COVARIANCE option in NONMEM. The PK model of docetaxel i.v. has been extensively investigated by Bruno and co-workers.[17,18] The first step was to fit this 3-compartment model to the data of i.v. administrated docetaxel.

Subsequently, a model with an additional depot compartment was fit to data of oral and i.v. administered docetaxel (without co-administration with ritonavir). Different absorption models using a lag-time or a discrete number of transit compartments to mimic a gamma like, asymmetric S-shaped absorption profile, were investigated. This model was extended with the ritonavir pharmacokinetics. Maximum a posterior Bayesian estimates of the PK parameters of ritonavir (clearance (Cl) (L/hr), distribution volume (Vd) (L), and absorption rate constant (Ka) (hr^{-1})) for each individual were calculated using the

POSTHOC option of NONMEM and a previously developed population pharmacokinetic model of ritonavir.[8] From a few patients , ritonavir data was lacking, for these patients, the population parameters established with the ritonavir PK model were used.[8]
The docetaxel CL and bioavailability (F) were subsequently modeled using a model previously applied by Lu et al.[19] according to a well-stirred liver model designed by Wilkinson et al.[20] This model assumes that the drug (in our case docetaxel) is exclusively metabolized by the liver, after possible loss in the gut:

Eq. 1: $$F = F_{gut} * F_{hep} = F_{gut} * \frac{Q}{CL_i + Q}$$

Eq. 2: $$CL = Q * \frac{CL_i}{CL_i + Q}$$

Eq. 3: $$CL_i = \frac{CL_{i0}}{1 + \frac{[RTV]}{K_i}}$$

Where F_{gut} is gut bioavailability (=1 – extraction ratio across gut), F_{hep} is hepatic bioavailability (= 1 – extraction ratio across liver (CL_i/(CL_i +Q)), Q is hepatic blood flow, CL_i is intrinsic clearance, CL is the docetaxel clearance, $CL_{i,0}$ is the uninhibited CL_i of docetaxel, [RTV] is the plasma concentration of ritonavir, and K_i is the inhibition constant of ritonavir on docetaxel. This model was originally designed for total blood clearance.[20] Since we have only studied total plasma concentrations, we have assumed that the blood/plasma ratio is one and the free fraction is one or independent of the investigated concentration range.

The effect of ritonavir on F_{gut} was modeled in an non ritonavir concentration manner.[19]

Eq. 4: $$F_{gut} = F_{doc} * \theta(x)^{RTV}$$

Where F_{doc} is the gut bioavailability of docetaxel without booster (this value is estimated based on 3 patients who received oral docetaxel without ritonavir), θ(x) is the fixed effect to calculate the increase in gut bioavailability when ritonavir is co-administrated, and RTV is an indicator taken the value 1 when ritonavir is given and 0 otherwise.
For the NONMEM analyses, subroutine ADVAN6 TRANS1 was used.

Interindividual and interocassional variability for different PK parameters was estimated using an exponential model. Different models were evaluated for their adequacy to estimate the residual variability. Discrimination between models was based on both

graphical and statistical methods. The significance of an increase in the goodness-of-fit between hierarchical models was tested using the log-likelihood ratio test.[15] A minimal difference of the objective function value (OFV) of more than 10.83, corresponding to a significance level $P < 0.001$ was used for discrimination between two hierarchical models differing in one parameter.
Furthermore, the model was evaluated by visual inspection of the goodness-of-fit plots. Xpose (version 4.0), an R based (version 2.9.0) model building aid, was used for the graphical goodness-of-fit analyses.[21]

For model evaluation, the shrinkage of eta and epsilon were calculated.[22,23] The adequacy of the final model was evaluated by a visual predictive check.[24] The data set was simulated 1000 times. The observed data, the median and the 95% confidence area of the predicted median, and the 5^{th} and 95^{th} percentile of the prediction interval were plotted using PsN (version 3.0) and Xpose (version 4.0).[25,21] Additionally, plots of 2 individuals were depicted showing the observed, predicted and individual predicted concentrations.

The final model was used for simulating different ritonavir dosing strategies in combination with 100 mg docetaxel. The combinations investigated were 50, 100, and 200 mg ritonavir administered simultaneously or 1 hour prior to docetaxel, and two doses of 100 mg ritonavir administered simultaneously or 1 hour prior and 2 hours after docetaxel intake. The $AUC_{(0-\infty)}$ for these dosing strategies were calculated for 1000 simulated patients using NONMEM. The geometric mean and the 90% confidence interval were calculated using the Log-transformed data. The aim of this simulation study was to estimate the expected mean exposure to docetaxel with different ritonavir schedules, and to estimate whether the exposure to docetaxel can be increased by optimizing the ritonavir schedule.

Results

Thirty-six patients were included in the two studies; 20 males and 16 females with median age 54 (range 31-73). PK data were assessed from 72 treatment courses, which are specified in table 1. In total, the data set comprised 1025 docetaxel plasma concentrations and 276 ritonavir plasma concentrations.

Table 1: Number of patients per treatment. (Doc. = docetaxel, RTV = ritonavir, p.o. = oral, i.v. = intravenous).

Dose and administration route	Number of patients with doc. data	Number of patients with RTV data
doc. i.v. 100 mg/m^2	15	
doc. i.v. 100 mg	17	
doc. p.o. 75 mg/m^2	3	
RTV p.o. 100 mg + doc. p.o. 10 mg after 1hr	6	
RTV p.o. 100 mg + doc. p.o. 100 mg after 1hr	15	8
RTV p.o. 100 mg + doc. p.o. 10 mg	5	
RTV p.o. 100 mg + doc. p.o. 100 mg	11	8

Model development

Figure 1 provides an overview of the developed model. The 3-compartment model developed by Bruno and co-workers[17,18] was fit to our i.v. data. The observed concentrations were well predicted and the estimated fixed effects were not relevantly different compared to the estimates as obtained by Bruno et al. The parameter estimates are given in table 2.

A depot compartment was added to the model and fit to the oral data. Different absorption models were investigated for their significance. In an early model development stage, an absorption lag-time or an transit compartments did not relevantly improve the model. In an final stage, however, it was found that a model with a single transit compartment described the data best.

The next step was to add the ritonavir data to the model. Gut bioavailability was modeled according to equation 4. The bioavailability without, and with ritonavir was found to be 19% and 39%, respectively (coefficient of variation (CV): 21 and 13%, respectively). Separate estimates of the bioavailability for sequential and simultaneous administration of docetaxel and ritonavir did not result in a significant model improvement.

The hepatic bioavailability was modeled by calculating the hepatic extraction ratio according to equation 1 and 3. The hepatic blood-flow was fixes at 80 L/hour, since estimating of this parameter resulted in a poor precision and a large estimation confidence interval determined by log-likelihood profiling. This resulted in an estimated

uninhibited intrinsic clearance of docetaxel of 119 L/h (CV: 19%), and an inhibition constant of docetaxel and ritonavir of 0.028 µg/mL (CV: 36%).
The hepatic bioavailability was incorporated in the differential equation of the first compartment by multiplying it with the input amount of docetaxel and by extracting the remaining fraction.

$$\frac{dA(1)}{dt} = -k_{tr} * A(1) * F_{hep} - k_{tr} * A(1) * A(1) * \left(1 - F_{hep}\right)$$; Dose compartment and first pass effect with $A(1)_{t=0} = F_{gut} *$ Dose (F_{hep} and F_{hep}: see eq. 1 and 3)

$$\frac{dA(2)}{dt} = k_{tr} * A(1) * F_{hep} - k_{tr} * A(2)$$; Transit compartment

$$\frac{dA(3)}{dt} = k_{tr} * A(2) - \frac{Cl}{V3} * A(3) - \left(k_{34} + k_{35}\right) * A(3) + k_{43} * A(4) + k_{53} * A(5)$$;
Central docetaxel compartment (Cl: see eq. 2 and 3)

$$\frac{dA(4)}{dt} = k_{34} * A(3) - k_{43} * A(4)$$; 1e peripheral compartment docetaxel

$$\frac{dA(5)}{dt} = k_{35} * A(3) - k_{53} * A(5)$$; 2e peripheral compartment docetaxel

$$\frac{dA(6)}{dt} = -k_{a} * A(6)$$; Dose compartment ritonavir

$$\frac{dA(7)}{dt} = k_{a} * A(6) - k_{70} * A(7)$$; Central compartment ritonavir

Figure 1: Differential equations of the final model. k_{tr}: transit rate constant (2/(mean absorption time; MAT)), CL: docetaxel clearance, V3: volume of the central compartment, k_{xy}: rate constant between compartment x and y.

Elimination
The elimination was modeled using similar equations and parameters as for the hepatic bioavailability. The CL of docetaxel was modeled according to equation 2. The effect of this equation is graphically depicted in figure 2. This figure shows that the docetaxel clearance decreased almost instantaneously after administration of ritonavir. Thus, ritonavir inhibited CYP3A4, thereby reducing the clearance of docetaxel by approximately 90%, after which it gradually returned to baseline level.

Volume of distribution
Separate analyses of the volume of distribution of the central compartment for orally and intravenously administered docetaxel resulted in different estimates of 44L (CV: 17%) and 9.8L (CV: 10%) respectively. It was hypothesized that these differences were

caused by the main excipient of the formulation, polysorbate-80. The assumption that the initial volume after i.v. administration increased after elimination of polysorbate-80 (or its micelles) to a volume similar as for oral administration, was evaluated. The data did not support such a shift in this PK parameter. Therefore, different estimates for the distribution volume were implemented in the final PK model.

Table 2: Estimations of; the basic i.v. model and the final model (IIV = inter individual variability, CV = covariance).

			i.v. Model		**Final model**		
			Typical value (CV%)	IIV (CV%)	Typical value (CV %)	IIV (CV%)	IOV (CV%)
Pharmacokinetic model							
Cl	Total plasma clearance	(L/h)	44.1 (6%)	29.4% (15%)			
$CL_{i,0}$	Intrinsic clearance	(L/h)			113 (19%)	60 % (26%)	22% (127%)
MAT	mean absorption time	(h)			1.3 (21%)	87 % (35%)	52% (29%)
$V2_{iv}$	Volume central compartment (i.v.)	(L)	8.9 (9%)	37.8% (14%)	9.8 (10%)	45 % (27%)	
$V2_{po}$	Volume central compartment (p.o.)	(L)			44.0 (17%)	35 % (27%)	
Q1	Intercompartmental Cl (C2-C3)	(L/hr)	6.1 (7%)		6.9 (12%)		
V3	Volume peripheral compartment	(L)	7.3 (9%)		7.5 (16%)		
Q2	Intercompartmental Cl (C3-C4)	(L/hr)	14.4 (12%)	20.3% (20%)	15.7 (14%)		
V4	Volume peripheral compartment	(L)	388 (11%)		376 (15%)		
F1	Gut bioavailability	(%)			19% (21%)	72 % (45%)	
F_{RTV}	Gut bioavailability + RTV	(%)			39% (13%)	72 % (45%)	44% (35%)
K_i	Inhibition constant: ritonavir - docetaxel	(µg/mL)			0.028 (36%)	122 % (33%)	
Q	Hepatic blood flow (fixed)	(L/h)			80		
CL~V2	Correlation CL~V2					44.6%	
Residual error							
P	Proportional error	(%)	23% (8%)		32% (14%)		
P	Proportional error first four hours after oral administration	(%)			64%		

Error model:

Visual inspection of the concentration-time curves showed higher inter-patient variability for orally, compared to intravenously administered docetaxel. This was largely attributed

to large inter-individual variability (IIV) of the bioavailability and absorption rate of orally administered docetaxel.
Despite the estimation of the IIV in these two parameters, the residual error remained larger for orally administered docetaxel, especially for the ascending part of the curve. Separate estimates of the proportional error for the first four hours after oral administration resulted in an improved fit of the model.

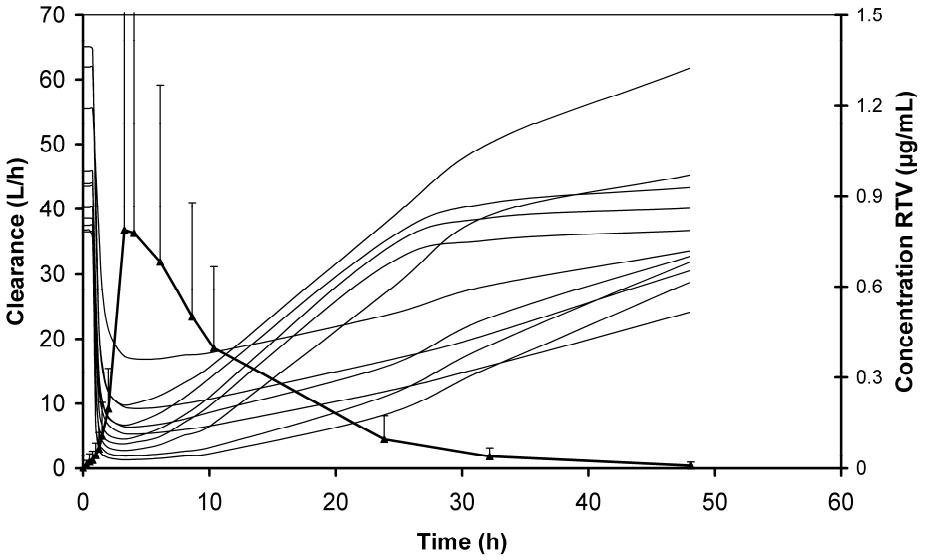

Figure 2: The estimated clearance of docetaxel versus time (hr) in individual patients receiving 100 mg docetaxel and 100 mg ritonavir simultaneously and the average concentration time curve of 100 mg ritonavir (▲).

Model evaluation
Goodness-of-fit plots from the final model showed that both the population and individual predicted concentrations are equally distributed around the line of identity (figures not included).
The eta-shrinkage of the random effects on the inter-individual variability were in the range of 9.2 – 22.5 %, the eta-shrinkage of the random effects on the intra-individual variability were in the range of 22.5 – 70.8 % and the epsilon-shrinkage was 7.5%. The shrinkage of the intra-individual variability were fairly high, but considered adequate for further simulation studies.

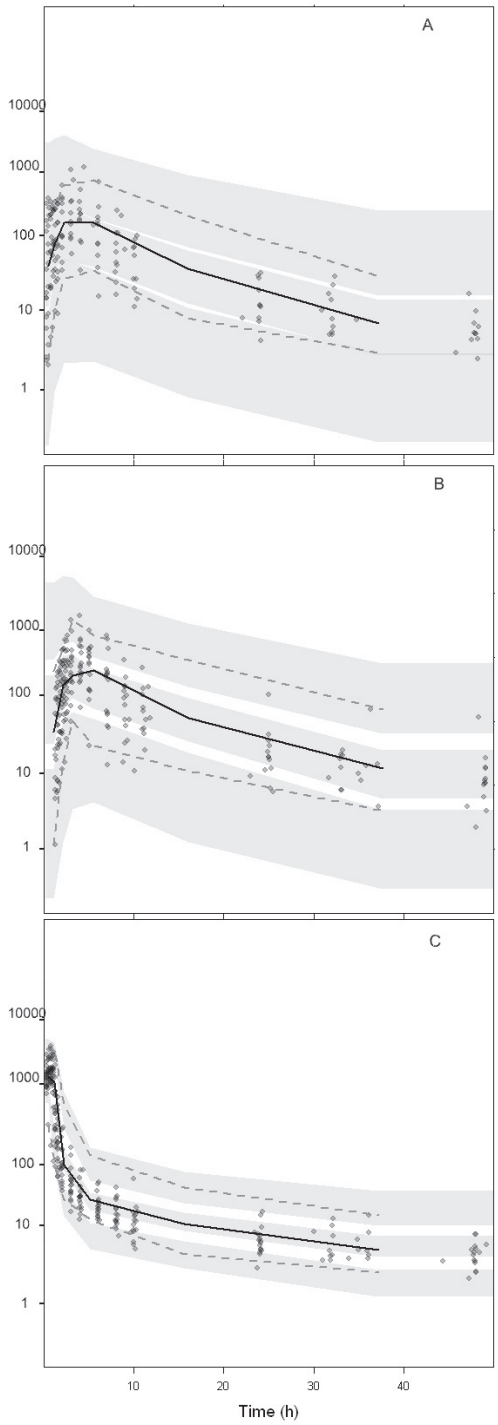

Figure 3: Visual predictive check (VPC) plots: The grey surface is the 90% confidence area of the predicted median, and the 5th and 95th percentile of the prediction interval. The black line is the observed median and the grey dotted lines are the 5th and 95th percentile of the observed data. In figure A oral docetaxel 100 mg and ritonavir 100 mg are given simultaneously, in figure B oral docetaxel 100 mg and ritonavir 100 mg are given 1 hour sequentially and in figure C docetaxel 100 mg is given intravenously. On the y-axis the concentration of docetaxel in ng/mL.

The visual predictive check plots (figures 3A-C) showed that the measured concentrations were well distributed within the 90% confidence area. Furthermore, the 90% confidence interval is relatively large for orally, and small for i.v. administered docetaxel.
Model performance was further investigated by inspecting the observed and predicted concentrations of 6 curves, see figure 4.

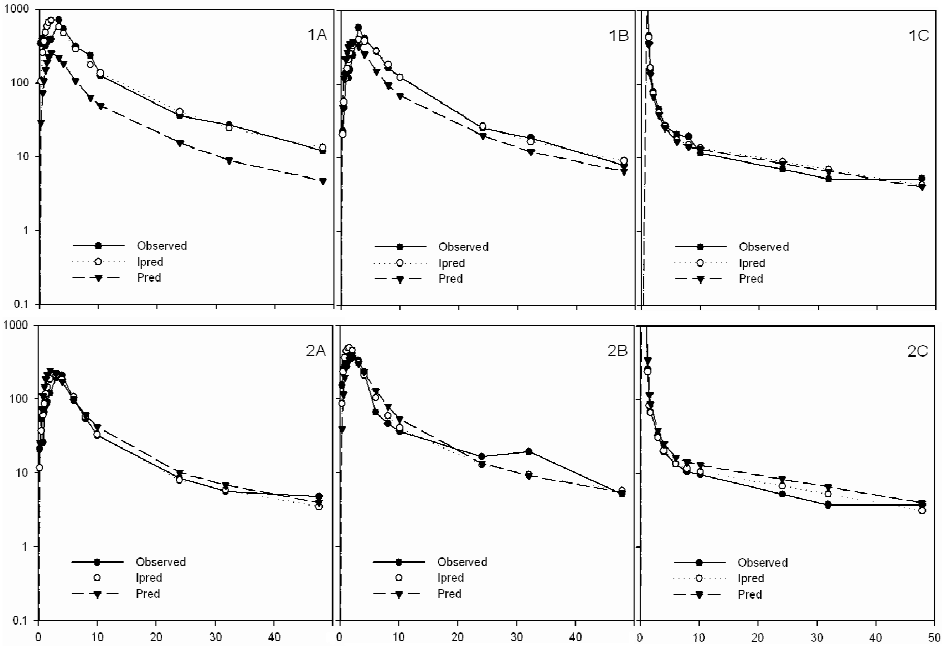

Figure 4: the observed, predicted (Pred) and individual predicted (Ipred) concentrations versus time for two individuals (1 and 2) on 3 occasions: oral docetaxel 100 mg in combination with 100 mg ritonavir (A), oral docetaxel 100 mg given 1 hour after 100 mg ritonavir (B) and 100 mg docetaxel given intravenously (C). On the y-axis the concentration of docetaxel in ng/mL, and to x-axis the time in hour.

Simulation of different ritonavir regimens

The simulations of different ritonavir treatment strategies showed that the apparent oral bioavailability can be marginally increased by increasing the ritonavir dose, or by giving multiple ritonavir doses. The geometric mean of the $AUC_{(0-\infty)}$ and the 90% confidence interval of docetaxel in combination with different RTV treatment regimes are given in table 3. The highest exposure of 100 mg oral docetaxel was seen when 200 mg ritonavir was administered 1 hour prior to docetaxel intake.

Table 3: Simulation of 1000 patients treated with 100 mg docetaxel in combination with 10 different ritonavir (RTV) treatment regimens. The geometric mean of the $AUC_{(0-\infty)}$ (in mg h/L) with the 90% confidence area are given.

	Simultaneous with docetaxel	1 hour prior to docetaxel
Single dose 50 mg RTV	1.07 (1.00 – 1.13)	1.87 (1.77 –1.97)
Single dose 100 mg RTV	1.36 (1.28 - 1.45)	2.56 (2.41 – 2.71)
Single dose 200 mg RTV	1.71 (1.61 - 2.15)	3.32 (3.14 – 3.50)
50 mg RTV -1 or 0 hr + 2 hr post-dose	1.27 (1.19 – 1.35)	2.18 (2.06 – 2.29)
100 mg RTV -1 or 0 hr + 2 hr post-dose	1.63 (1.53 – 1.74)	2.95 (2.79 – 3.12)

Discussion

The amount absorbed of docetaxel increased from 19% without to 39% when co-administrated with ritonavir. *In vitro* results showed that the P-gp mediated transport is hardly influenced by ritonavir.[3] Thus, the reduced pre-systemic clearance by inhibition of CYP3A4 is probably the major determinant in the enhancement of the absorption of docetaxel. The estimated amount of docetaxel absorbed is much lower than the apparent bioavailability ($AUC_{oral}/AUC_{i.v.}$), which was above 100%.[5] This apparent discrepancy can be explained by reduced elimination due to inhibition of CYP3A4 mediated elimination. The exposure to docetaxel can be increased by both improving the passage through the gastrointestinal barrier by inhibiting CYP3A4 and P-glycoprotein in the enterocytes and furthermore by inhibition of the first pass metabolism by the liver. Additionally, the elimination of docetaxel can be decreased as well by reduced metabolism by CYP3A4 enzymes.

The competitive inhibition of hepatic CYP3A4 was assumed to be ritonavir concentration dependent resulting in non-linear elimination of orally administered docetaxel. The total plasma clearance of docetaxel decreased almost instantaneously and gradually returned back to the initial clearance in parallel with declining concentrations of the inhibitor, ritonavir.

An effect of polysorbate-80 on the distribution of docetaxel was previously suggested by Loos et al.[26] Due to the high molecular weight of polysorbate-80 (1310 Da) and the formation of large micelle complexes, absorption of polysorbate-80 after oral administration is not to be expected. Together with a fast elimination,[27] plasma concentrations of polysorbate-80 will be negligible after oral administration, which is in contrast to i.v. administration. I.v. administered docetaxel is present in the circulation in at least three distinguishable forms, polysorbate-bound, protein-bound and free. Non-

linear pharmacokinetics of docetaxel was seen in mice at doses above the therapeutic range.[27] Van Tellingen et al. showed that immediately after infusion of a dose of 100 mg/m^2 of docetaxel, concentrations of polysorbate-80 were found above the critical micelle concentrations (0.009 %v/v).[28] However, soon after administration these concentrations dropped below the detection limit (0.01% v/v) of the assay and most likely under the critical micelle concentration.[27] The authors concluded that polysorbate-80 can result in non-linear pharmacokinetics but not at dose-levels relevant for the clinical situation. The results of the simultaneous analysis of orally and i.v. administered docetaxel showed, however, that volume of the central compartment is small for i.v. administered docetaxel and much higher after oral administration. This indicates that polysorbate-80 micelles might be the cause of a low distribution volume for i.v. administered docetaxel. Micelle breakdown occurs instantaneously and complete. Thus, an instantaneous increase in volume of distribution for i.v. administered docetaxel can be expected after micelle breakdown. This immediate increase was, however, not identifiable in the analysis, probably because a large volume of distribution at steady state was found for docetaxel.

Docetaxel given as a standard 1-hour infusion shows high inter-individual variability, which is associated with variability in efficacy and toxicity.[29] This variability is higher for orally administered docetaxel, which is mainly due to a high variability in absorption. This can clearly be seen in the visual predictive check plots (figure 4). The initial part of the visual predictive check plots show a relatively large 90% confidence area for oral, in contrast to i.v. administered docetaxel. The main risk of a high variability is under-, and over-dosing of patients. Further research is needed to investigate strategies for reducing the inter-individual variability. A possible method could be increasing the ritonavir dose. This is expected to result in a more complete and sustained inhibition of CYP3A4. Within the proof of concept study, patients were given a flat dose of docetaxel, however when the variability is too high, dose individualization based on one or more covariates proven to have a significant influence on the pharmacokinetics, for instance body-surface-area, hepatic function, age, α1-acid glycoprotein,[18] C1236T mutation in the ABCB1 gene[30] and CYP3A4*1B polymorphism[31] could be considered. However, it is questionable whether these covariates are of relevance considering the high inter-patient variability caused by the oral route of administration. Another option, on the condition that the inter-occasion variability is low, is to individualize the dose by therapeutic drug monitoring.

Simulations of different combination regimes have shown that a regimen with 200 mg ritonavir dosed 1 hour prior to docetaxel intake, results in the highest exposure. These results should be evaluated cautiously. The PK model was developed without prior knowledge concerning the dose effect of ritonavir on the oral bioavailability of docetaxel,

and data concerning multiple ritonavir doses were not available. However, these results point out the direction for further clinical development of the combination.
Oral administration of docetaxel in combination with ritonavir has shown to be a promising mode of administration. The PK profile is markedly different compared to intravenously administered docetaxel. The hypothesized differences were quantified in the current analysis. The current model forms a suitable tool for further development of this combination and will support design of further studies and schedules.

In conclusion, co-administration of ritonavir lead to improved oral absorption and a ritonavir-concentration dependent inhibition of CYP3A4, which resulted in a reduced elimination rate for docetaxel. A PK model of docetaxel in combination with ritonavir was successfully developed and will be used to establish optimal combination regimens.

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Chapter 2.3

Weekly oral docetaxel as solid dispersion (ModraDoc001 capsules) in combination with ritonavir is well tolerated and results in high exposure to docetaxel

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Interim analysis
(data were monitored up to the 80/100 mg dose-level)

Abstract

Docetaxel is registered for 1-hour infusions in 3-weekly schedules. Oral administration of docetaxel, however, is feasible when given in combination with ritonavir, which is an inhibitor of the drug metabolizing enzyme CYP3A4. The aim of this study was to investigate the maximum tolerated weekly dose (MTD) of a novel solid dispersion formulation of docetaxel, ModraDoc001, in combination with ritonavir and secondly, to investigate the pharmacokinetics of docetaxel.

Blood sampling for pharmacokinetic analysis was performed. Toxicity was assessed throughout and preliminary anti-tumor activity was determined using RECIST criteria.

Thus far, 35 patients were included in the study. The most frequently observed adverse events were diarrhea (n=23) and fatigue (n=18). Dose limiting toxicities were diarrhea (n=3) at the 30 mg docetaxel /100 mg ritonavir (30/100 mg), 80/100 mg and 60/200 mg dose levels, fatigue (n=1) at 30/100 mg elevated transaminases (n=1) at 60/200 mg and vomiting and nausea (n=1) at 80/100 mg. Docetaxel exposure increased with increasing ModraDoc001 doses, mean docetaxel exposure at the 80/100 mg dose level was 1490 ng/mL*h (CV: 56%). Anti-tumor activity was demonstrated in two patients who had a confirmed partial response.

The study is still ongoing at the time this report was written and therefore, the MTD could not yet be established. Thus far, oral administration of docetaxel by ModraDoc001 capsules in combination with ritonavir was feasible, showed a satisfactory safety profile, high systemic exposure and an inter-patient variability comparable to the i.v. formulation. Furthermore, promising anti-tumor activity was observed at the two highest dose-levels.

Introduction

Docetaxel is a semisynthetic taxane originating from the needles of the European yew tree (*Taxus baccata*) and acts by disrupting the microtubule network. The drug has significant anti-tumor activity against a range of tumor types and is approved for the treatment of breast, non-small cell lung cancer (NSCLC), head and neck, gastric and prostate cancer at doses ranging from 60 to 100 mg/m^2 administered as a 1-hour infusion every 3 weeks. Weekly intravenous (i.v.) schedules of docetaxel result in similar efficacy and a different toxicity profile with a lower incidence of febrile neutropenia compared to 3-weekly schedules.[1,2,3,4] However, weekly schedules are hardly prescribed probably due to patient inconvenience.
An orally available drug formulation of docetaxel would overcome these unpractical aspects of a weekly docetaxel regimen. Additionally, the unfavorable effects of the i.v. formulation vehicle, polysorbate 80 (allergic infusion reactions), are not to be expected after oral administration.[5,6]

Oral administration of docetaxel is, however, hampered by its affinity for the drug efflux-pump P-glycoprotein (P-gp) and the metabolizing enzyme, cytochrome P450 3A4 (CYP3A4), which are both extensively present in the gastrointestinal epithelium and the liver. Furthermore, the solubility of docetaxel is low thereby limiting oral availability further.

In previous proof of principle studies, it was demonstrated that co-administration of oral docetaxel and the CYP3A4 and P-gp inhibitor cyclosporin A (CsA) significantly enhanced the exposure to docetaxel significantly.[7] In a preclinical study was demonstrated that CYP3A4 is largely responsible for the low oral bioavailability of docetaxel.[8] Therefore, a second proof of concept study was initiated with the more selective CYP3A4 inhbitor, ritonavir. This study confirmed that boosting orally administered docetaxel with low-dose ritonavir resulted in high systemic exposure to docetaxel.[9]

Thus far, all clinical trials with orally administered docetaxel were conducted with the i.v. formulation applied as drinking solution (DS), which was unpractical and, due to the poor taste, only moderately tolerated.[7,9,10] Recently, a solid docetaxel capsule formulation (ModraDoc001capsules) has been developed in our institute. To improve the poor aqueous solubility, a solid dispersion formulation of docetaxel was developed.[11]
The primary aim of this study was to assess the safety, dose limiting toxicities and optimal treatment regimen of oral docetaxel in combination with ritonavir. Secondly the pharmacokinetics (PK) of docetaxel were determined.

Patients & Methods

Patients

Patients for whom no standard therapy of proven benefit existed and with a histologically confirmed cancer refractory to current therapies were eligible for the study. Other eligibility criteria included: Age> 18 years; life expectancy > 3 months; no radio- or chemotherapy within the last 4 weeks prior to study entry (palliative limited radiation for pain reduction was allowed). Patients had to have acceptable bone marrow function (neutrophil count $\geq$ 1.5 x 10^9/L; platelet count $\geq$ 100 x 10^9/L), liver function (serum bilirubin level $\leq$20 µmol/L; alanine, and/or aspartate aminotransferase $\leq$ 2.5 times the institutional upper limit of normal (ULN)), renal function (serum creatinine level <160 µmol/L or clearance > 50 mL/min) and a World Health Organization (WHO) performance status $\leq$ 2. Patients were not eligible if they suffered from uncontrolled infectious disease, neurologic disease, bowel obstructions, or symptomatic brain metastases, alcoholism, drug addiction, psychotic disorders leading to inadequate follow-up or pregnancy. Other exclusion criteria were concomitant use of known P-gp or CYP3A4 inhibitors and chronic use of H2-receptor antagonists or proton pump inhibitors. The study protocol was approved by the medical ethics committee of the institute and all patients had to give written informed consent prior to start of the study.

Study design

The study was designed as a two-arm, open-label, non-randomized dose escalation study to define the safety profile, pharmacokinetics and maximum tolerated dose (MTD) of oral docetaxel in a weekly dosing schedule in combination with ritonavir. The MTD was defined as the highest dose resulting in no more than 1/6 probability of causing a dose limiting toxicity (DLT). A DLT was defined as any of the following that occurred during the first four weekly treatment cycles and was determined to be possibly, probably or definitely related to docetaxel: Grade $\geq$ 3 non-haematological toxicity (other than untreated nausea, vomiting or diarrhea), Grade 4 trombocytopenia, Grade 4 neutropenia lasting more than 7 consecutive days, Grade 3 febrile neutropenia and lastly, inability to begin next course of treatment within 3 weeks of scheduled dosing due to toxicity. Dose escalation was performed according to the toxicity and pharmacokinetic profile observed at prior dose levels. The oral docetaxel dose could be increased with minimal steps of 10 mg and maximally with 100% of the previous dose level. The ritonavir dose was allowed to be escalated after the 4^{th} dose level with steps of 100 mg.

All toxicities observed, were graded according to the National Cancer Institute Common Toxicity Criteria for Adverse Events (NCI CTCAE) version 3.0. Tumor evaluation was performed according to RECIST criteria version 1.0.[12]

Arm I: Dose escalation study

The study design is schematically presented in figure 1. Patients treated in the first 4 dose-levels received during the first cycle 20 mg docetaxel i.v. (Taxotere®, Sanofi Aventis, France) administered over a 30 minute infusion in combination with an oral 100 mg ritonavir capsule (Norvir®; Abbott, Illinois, USA) to determine the apparent oral bioavailability of oral docetaxel. For PK assessments blood samples were drawn at baseline, end of infusion and 15, 30 min and 1, 1.5, 2, 4, 7, 10, 24 and 48 hour after infusion. After cycle 1, the first cohort of 3 patients received during the second and subsequent weeks 30 mg docetaxel by oral administration of the docetaxel i.v. formulation (Taxotere®, Sanofi Aventis, France) in combination with 100 mg ritonavir. PK assessments were performed during the second week at baseline, 15, 30, 45 min and 1, 1.5, 2, 4, 7, 10, 24, and 48 hours after dosing. The 30 mg docetaxel dose was considered safe and was selected based on the standard weekly dose of i.v. docetaxel (35 mg/m^2). Patients were considered evaluable for safety if they completed the first 4 treatment weeks or if a DLT occurred in this period, otherwise, a patient was replaced.

After the first cohort, the novel capsule formulation of docetaxel (ModraDoc001 capsules; Slotervaart Hospital, Amsterdam, The Netherlands) became available. A sub-study investigating the PK of these capsules was implemented before further dose escalation. After completion of the sub-study, further dose escalation was performed with ModraDoc001 capsules.

In this sub-study, in total 6 evaluable patients were included. Patients received weekly 30 mg oral docetaxel in combination with 100 mg ritonavir. Patients received during the first and second, or the second and third week (determined by randomization), oral docetaxel by two ModraDoc001 capsules of 15 mg docetaxel. During the other weeks (week 1 or 3 and week 4 and subsequent weeks) patients received 30 mg oral docetaxel as the DS. PK assessments were performed for the first 3 weeks during the first 24 hours after intake. (PK samples were drawn at baseline and after 30 min, 1, 1.5, 2, 3, 4, 6, 8, 10 and 24 hours). This resulted in PK evaluation for one dose of the DS and two doses of ModraDoc001 capsules.

Patients continued treatment with weekly 30 mg docetaxel DS in combination with 100 mg ritonavir until progressive disease or adverse events that required discontinuation of therapy.

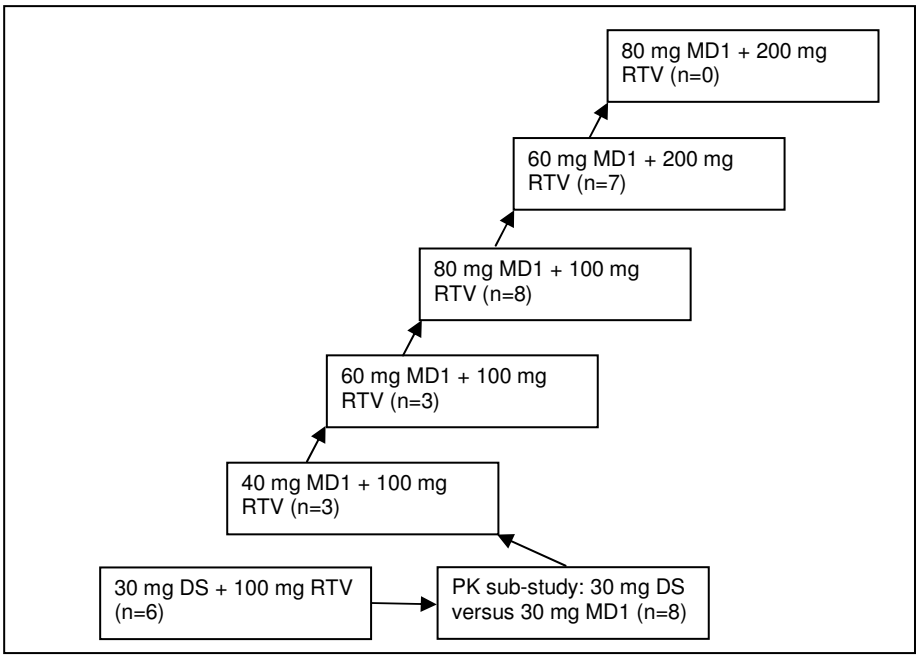

Figure 1: Study schedule. Abbreviations: DS: docetaxel drinking solution, MD1: ModraDoc001 capsules, RTV: ritonavir, n: number of patients.

Drug administration and pre-medication

Oral docetaxel and ritonavir were ingested together with 150 mL tap water. Patients took the study drugs either 1 hour before or 2 hours after a meal. Pre-treatment consisted of 4 mg dexamethason 1 hour prior to, and 12 and 24 hours after docetaxel intake. In addition, patients also received 1 mg granisetron orally 1 hour prior to oral docetaxel / ritonavir to prevent nausea and/or vomiting. Dexamethason pre-medication was omitted in Arm I from dose level 2 and higher. Dexamethason pretreatment aimed at reducing the incidence of allergic infusion reactions, fluid retention, nausea and vomiting. Since these adverse events were not observed after oral intake of docetaxel or well under control with granisetron, dexamethason pre-medication was omitted in dose-level 2 and higher.

Pharmacokinetic analysis

Blood samples for pharmacokinetic analysis of docetaxel were collected in lithium heparinized tubes. Samples were immediately placed on ice and were centrifuged within 1 hour at 1500 x g for 10 minutes at 4°C. Plasma was stored at or below -20°C until analysis. Docetaxel was quantified by use of high-performance liquid chromatography

with tandem mass spectrometric detection (LC-MS/MS) as described by Kuppens et al. [13] with docetaxel-d9 (Toronto Research Chemicals Inc.) as internal standard. The lower limit of quantification of the assay was 0.25 ng/mL docetaxel.

Data analysis

The individual pharmacokinetic parameters were analyzed using descriptive pharmacokinetic methods using R (version 2.10.0) by employing validated scripts. The mean and coefficient of variation (CV) of the following PK parameters were reported: the maximum observed plasma concentration (C_{max}), time to reach C_{max} (T_{max}), clearance (CL/F), distribution volume at steady state (Vss/F), the terminal elimination half-life (t1/2), the area under the plasma concentration-time curve between 0 and the time point of the last quantifiable data point (AUC_{0-t}) or, if possible, with extrapolation to infinity (AUC_{inf}) using the terminal rate constant. Furthermore, the apparent bioavailability (F%) was calculated for patients who received during the first course 20 mg i.v docetaxel in combination with ritonavir. This was done using equation 1:

Eq. 1: $$F\% = \frac{AUC_{\inf}(po)}{AUC_{\inf}(iv)} * \frac{Dose(iv)}{Dose(po)} * 100\%$$

Within-subject variability (WSV) in AUC was calculated if a patient had PK assessments of the same regimen twice. This was calculated using equation 2:

Eq. 2: $$WSV = \frac{1}{n} * \sum \left(\frac{| AUC^{2e} - AUC^{1e} |}{AUC^{1e}} \right)_{1-n} * 100\%$$

Results

Patient characteristics

In total 35 patients were included in this study. Twenty-seven patients were included in the dose escalation study and 8 patients were included in the PK sub-study. Two patients of the first 6 patients treated in sub-study, were not evaluable due to unexpected early clinical deterioration. These patients were replaced. The patient characteristics of all patients are given in table 1.

Table1: Patient characteristics at baseline

	Dose escalation (n=27)	PK sub-study (n=8)
Sex		
Female	11	5
Male	16	3
Age (years)		
Median	59	62
Range	38 - 71	46 – 74
ECOG performance status		
0	14	1
1	10	5
2	3	2
Pathological diagnosis		
NSCLC	11	1
Urothelial cell carcinoma	3	3
Ovarian	2	2
Melanoma	2	-
Anal	2	-
Primary unknown	2	-
Other	5	2
Disease stage		
Locally advanced	2	-
Metastatic	25	8
No. of Prior treatments (surgery, radiotherapy or chemotherapy)		
1	1	-
2	5	-
3	7	2
$\geq$4	14	6

Determination of the maximum tolerated dose

The dose escalation schedule is depicted in figure 1. Six patients were treated at the first dose-level, 30 mg docetaxel DS in combination with 100 mg ritonavir. Two out of first 6 patients went off study after 2 and 3 weekly cycles, respectively, due to rapid clinical deterioration of their disease and were not considered evaluable for safety. A 70 year old male patient treated at the same dose developed study drug related grade 3 diarrhea and grade 3 diarrhea-related fatigue immediately after intake of the first oral docetaxel dose, which both quickly improved after two days. Nevertheless, study treatment was stopped since it was considered not in the best interest for the patient to continue. The 4^{th}, 5^{th} and 6^{th} patient received in total 6 to 20 weekly cycles without the occurrence of severe drug-related toxicity.

Subsequently, eight patients, of whom 6 evaluable, were treated in the PK sub-study. In general, treatment was well tolerated with only mild to moderate adverse events. However, one severe adverse event was seen in a 56 year old female patient with ovarian cancer. She went off study after 5 cycles due to clinical deterioration grade 3 fatigue, which was possibly study treatment related. The pharmacokinetic results in these patients (discussed below) showed a slightly lower systemic exposure to docetaxel after intake of the ModraDoc001 capsules compared to the docetaxel DS.
Overall 10 evaluable patients were treated at the level of 30 mg DS / ModraDoc001 of whom two developed a DLT.

In view of the encountered safety at the level of 30 mg docetaxel the smallest possible dose increase of 10 mg was applied resulting in the new dose level of 40 mg ModraDoc001 in combination with 100 mg ritonavir (40/100 mg dose level).

Three patients were treated at the 40/100 mg dose level without the occurrence of any grade 3-4 toxicity. Subsequently, 3 patients were treated at the 60/100 mg dose level. One patient with grade 1 fatigue prior study start, had a short episode of grade 3 fatigue during this cycle which was possibly related to the study drug. This improved while being on treatment and dose reductions or dose omissions were not needed. The short episode of grade 3 fatigue was therefore not considered a DLT. Since, the other two patients treated with 60/100 mg dose had only mild to moderate toxicity, the dose for the next 3-patient cohort was escalated to 80/100 mg.

In total 8 patients were treated at the 80/100 mg dose-level. Two patients went off study preliminary due to clinical deterioration and withdrawal of consent of a second patient. A third patient developed severe, probably study-treatment related grade 3 diarrhea, vomiting and nausea. None of the other 5 patients developed grade 3-5 toxicity. Based on 6 evaluable patients of whom one patient developed a DLT, the 80/100 mg dose-level was considered safe.

As in a parallel PK study was shown that 200 mg ritonavir resulted in 77% increase in docetaxel exposure compared to 100 mg ritonavir, the next step was to increase the ritonavir dose to 200 mg (Koolen et al. manuscript in preparation; chapter 2.5 of this thesis). For safety reasons the dose of ModraDoc001 was decreased to the previous level of 60 mg.
Seven patients were treated at the 60/200 mg dose-level of whom 1 patient was not evaluable due clinical deterioration of disease after 3 weekly cycles. Overall, 6 evaluable patients were treated at the 60/200 mg dose-level of whom one patient developed a DLT. This 71 year old male patient with NSCLC developed grade 3 diarrhea requiring hospitalization and elevated transaminases: grade 3 AST and ALT elevations. After a

two week dose-delay these laboratory values returned to normal. The patient restarted at the 80/100 mg dose level, but developed after two 2 doses grade 3 fatigue and diarrhea. He continued without the occurrence of severe toxicity at the 60/100 mg dose level.

The sixth dose level was subsequently set at 80/200 mg. This dose level is currently ongoing and the maximum tolerated dose remains therefore to be determined.

Table 2: Adverse events possibly, probably or definitely related to study drug with an overall incidence of 5% or more.

	dose escalation							PK sub-study	
	30/100 mg*	40/100 mg	60/100 mg	80/100 mg	60/200 mg	Total (n = 27)		30/100 mg# (n = 8)	
Adverse event	(n = 6)	(n = 3)	(n = 3)	(n = 8)	(n = 7)	n	%	n	%
Diarrhea	3	-	3	7	5	18	67	5	63
Fatigue	2	1	2	5	2	12	48	6	50
Nausea	2	1	2	6	3	14	52	2	25
Vomiting	1	-	1	4	2	8	30	2	25
Alopecia	2	-	1	3	2	8	30	-	
Stomatitis	1	-	-	2	2	5	19	1	13
Neuropathy	1	-	1	1	2	5	19	-	
Nail changes	-	-	-	3	1	4	15	-	
Constipation	-	-	1	-	1	2	7	1	13
ALT elevation	-	-	-	-	2	2	7	-	
AST elevation	-	-	-	-	2	2	7	-	
Dyspnea	-	-	-	1	1	2	7	-	
Taste alteration	1	-	-	1	-	2	7	-	
Hiccups	1	-	-	-	-	1	4	1	13
Flushes	-	-	-	1	-	1	4	1	13
Abdominal pain	-	-	1	1	-	2	7	-	

*30 mg docetaxel DS in combination with 100 mg ritonavir.

#30 mg docetaxel DS or ModraDoc001 capsules in combination with 100 mg ritonavir.

Adverse events

The most reported adverse events were fatigue (19 patients) and gastrointestinal toxicities including diarrhea (n=23), nausea (n=16), vomiting (n=10) and stomatitis (n=6). Other frequently observed adverse events were alopecia (n=8), neuropathy (n=5) and nail changes (n=4). Apart from one event of grade 1 decreased leukocytes in the 60/200 mg dose level, no hematological toxicities were observed. An overview of all possibly related adverse events is presented in table 2. The grade 3 possibly related adverse events are also separately presented in table 3. Grade 4-5 possibly, probably or definitely related adverse events were not observed.

Short episodes of fatigue typically occurred during the second and third day after study-drug intake. In case of severe fatigue, dose reductions and dose omissions generally resulted in recovery to baseline. Diarrhea commenced mostly within 1 or 2 days after study-drug intake. Symptoms remained mild for most patients after rapid intervention with loperamide, which was given until complaints resolved. Severe diarrhea could be effectively treated with loperamide and i.v. fluids followed by re-start at a lower dose level.

Table 3: grade 3 possibly, probably or definetly related adverse events. (grade 4 – 5 related adverse events were not observed)

	dose escalation							PK sub-study	
	30/100 mg*	40/100 mg	60/100 mg	80/100 mg	60/200 mg	Total (n = 27)		30/100 mg# (n = 8)	
Adverse event	(n = 6)	(n = 3)	(n = 3)	(n = 8)	(n = 7)	n	%	n	%
Fatigue	1	-	1	1	1	4	15	1	13
Diarrhea	1	-	-	1	1	3	11	-	-
Nausea	-	-	-	1	-	1	4	-	-
vomiting	-	-	-	1	-	1	4	-	-
ALT elevation	-	-	-	-	1	1	4	-	-
AST elevation	-	-	-	-	1	1	4	-	-

*30 mg docetaxel DS in combination with 100 mg ritonavir.

#30 mg docetaxel DS or ModraDoc001 capsules in combination with 100 mg ritonavir.

Pharmacokinetics

The pharmacokinetic data are presented in table 4 and the mean concentration time curves are presented in figures 2 and 3.

The results of the PK sub-study, the comparison of the ModraDoc001 capsule formulation with the docetaxel DS, is graphically presented in figure 2. It can be seen that the DS resulted in a slightly higher systemic exposure. The mean $AUC_{0\text{-}24h}$ after the dinking solution was 644 ng/mL*h (CV: 85%) versus 526 ng/mL*h (CV: 36%) and 565 ng/mL*h (CV: 46%) after the first and second ModraDoc001 dose, respectively. However, the inter-individual variability of the docetaxel DS of 85% was much higher than after ModraDoc001 capsules, which was 36% and 46% after the first and second ModraDoc001 dose, respectively.

It seems that Cmax values were higher after administration of the DS and that T_{max} values were longer for ModraDoc001 compared to the DS (table 4).

The mean concentration time curves of each investigated dose level are presented in figure 3A. It was demonstrated that the exposure to docetaxel could be increased with increasing ModraDoc001 doses. (figures 3A and 3B) The highest dose level, 80/100 mg, resulted in a mean docetaxel exposure of 1490 ng/mL*h (CV: 56%).

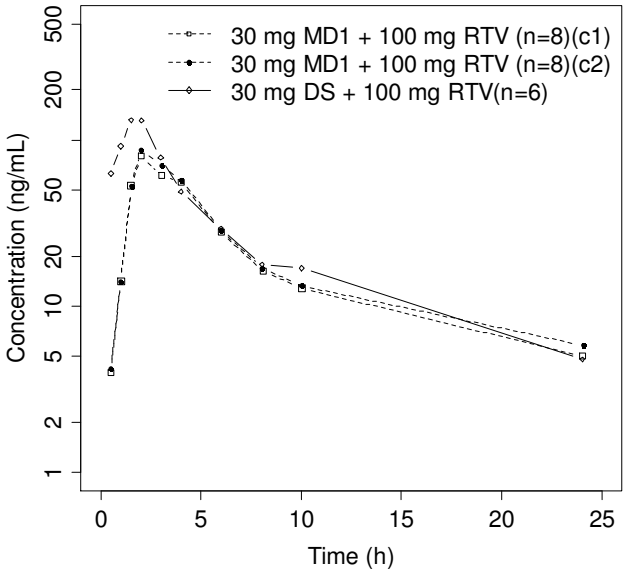

Figure 2: Mean plasma concentration-time curves of patients who participated in Arm II: 30 mg docetaxel drinking solution (DS) boosted by 100 mg ritonavir (RTV), 30 mg ModraDoc001 capsules (MD1) boosted by 100 mg RTV on the 1st and 2nd occasion.

Table 4: Pharmacokinetic parameters. (abbreviations: DS: docetaxel drinking solution; MD1: ModraDoc001 capsules, RTV: ritonavir, N: number of patients, CV: coefficient of variation, EOI: end of infusion, 1st: First cycle, 2nd: second cycle [a]AUC_{0-24h} is presented, [b]n=2 (range is given), [c]n=3, [d]n=6, [e]n=7, [f]n=8, [g]n=10, [h]n=16)

	n	AUC_{0-48h}		AUC_{inf}		C_{max}		T_{max}		t1/2		CL/F		Vss/F		F	
		Mean (ng/mL*h)	CV (%)	Mean (ng/mL*h)	CV (%)	Mean (ng/mL)	CV (%)	Median (h)	range	Mean (h)	CV (%)	Mean (L/h)	CV (%)	Mean (L)	CV (%)	Mean (%)	CV (%)
20 mg IV + 100 mg RTV	19	533	47	592[h]	50	477	44	EOI		17.3[h]	20	41.6[h]	43	1050[h]	51		
30 mg DS + 100 mg RTV	11	644[a,g]	85	765[f]	87	174	92	1.5	0.75 – 2.1	10.7[f]	24	76.4[f]	96	1300[f]	110	61[c]	74
30 mg MD1 + 100 mg RTV (1st)	8	526[a]	36	553[d]	42	101	45	1.8	1.0 – 3.0	9.16[d]	11	62.7[d]	44	819[d]	38		
30 mg MD1 + 100 mg RTV (2nd)	8	565[a]	46	670[e]	46	99.1	40	2.5	1.5 – 3.2	8.73[e]	11	53.8[e]	48	681[e]	50		
40 mg MD1 + 100 mg RTV	3	306	49	334	44	63.4	79	1.6	0.85 – 1.6	15.8	25	143	55	3560	80	50	42
60 mg MD1 + 100 mg RTV	3	1270	58	1360	56	288	60	1.5	1.5 – 2.0	16.4	19	52.3	42	1300	54	87 (76-98)[b]	
80 mg MD1 + 100 mg RTV	8	1490[e]	56	1570	51	264	46	2.0	1.5 – 4.0	12.7	26	60.1	38	1150	57	64[e]	29
60 mg MD1 + 200 mg RTV (1st)	7	943	62	1010	61	113	56	2.0	1.5 – 4.1	13.7	20	80.3	57	1650	74		
60 mg MD1 + 200 mg RTV (2nd)	7	1000	78	1180[d]	78	129	70	2.0	0.77 – 4.0	14.9[d]	20	76.5[d]	59	1690[d]	72		

Docetaxel 20 mg was given intravenously to 19 patients in order to calculate the apparent bioavailability. The mean exposure after 20 mg docetaxel i.v. in combination with 100 mg ritonavir was 533 ng/mL*h with an inter-patient variability of 47%. The mean bioavailability values were 61% for the 30 mg docetaxel DS, 50% for the 40/100 mg dose level, 87% for the 60/100 mg dose level and 64% for the 80/100 mg dose level. These data do not show a dose-dependent effect on the bioavailability of docetaxel.

The within-subject variability could be determined in PK sub-study for 8 patients who received 30 mg ModraDoc001 in combination with 100 mg ritonavir p.o. and for 7 patients who were treated at the 60/200 mg dose level. The intra-patient variability at these dose levels was 31% and 16%, respectively.

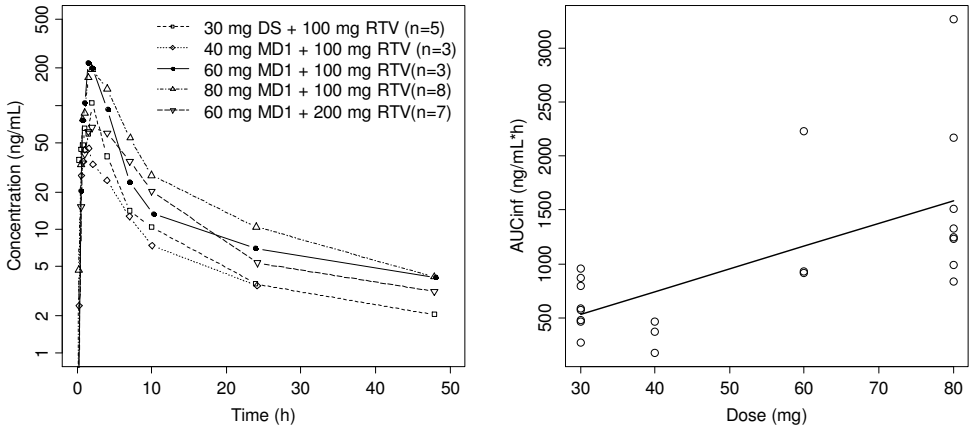

Figure 3: left panel (3A): mean plasma concentration-time curves of the different dose levels, right panel (3B) systemic exposure, measured by AUC_{inf}, versus the administered ModraDoc001 dose in combination with 100 mg ritonavir. (abbreviations: MD1: ModraDoc001 capsules, RTV: ritonavir)

Anti-tumor activity

Patients were treated for a median of 6 one-week cycles (range 2 – 37 one-week cycles). In total 25 patients were evaluable for at least 1 response assessment. Two patients (6% overall) achieved a confirmed partial response. One patient with adenocarcinoma of unknown primary origin treated at the 80/100 mg dose level demonstrated a partial response after 6 one-week cycles which was later confirmed. After 37 one-week cycles this patient is still on treatment. The second patient, with progressive metastatic NSCLC and stable locally advanced prostate carinoma, who was

treated at the 60/200 mg dose level developed severe toxicity after 2 and 6 one-week cycles for which the dose was reduced to 80/100 mg and 60/100 mg respectively. The 60/100 mg dose level was well tolerated. A partial response was observed after 6 cycles, which was later confirmed and after 20 one-week cycles the patient is still ongoing.
Additionally, fourteen patients (40% overall) had stable disease as best response of whom 5 patients had RECIST confirmed stable disease and remained at least 13 weeks on study. At the time of this interim analysis, 5 patients were still ongoing. Response data are summarized in table 5.

Table 5: Treatment duration and response according to RECIST v1.0. Abbreviations: DS: docetaxel drinking solution, MD1: ModraDoc001 capsules, RTV: ritonavir, n: number of patients.

Dose level	No of patients	No of 1-week cycles (median, range)	Best response			
			PR	SD	PD	NE
30 mg DS + 100 mg RTV	6	4.5 (2-20)	-	4 (67%)	-	2 (33%)
30 mg DS / MD1 + 100 mg RTV*	8	5 (2-8)	-	1 (13%)	4 (50%)	3 (37%)
40 mg MD1 + 100 mg RTV	3	6 (5-37)	-	1 (33%)	2 (67%)	-
60 mg MD1 + 100 mg RTV	3	6 (5-6)	-	1 (33%)	2 (67%)	-
80 mg MD1 + 100 mg RTV	8	9.5 (2-37+)	1 (13%)	4 (50%)	-	3 (37%)
60 mg MD1 + 200 mg RTV	7	8+ (3-20+)	1 (14%)	4 (57%)	1 (14%)	1 (14%)
Total	35	6 (2-37+)	2 (6%)	14 (40%)	8 (23%)	11 (31%)

* Pk sub-study, +: patients still on treatment

Discussion

This study demonstrates that the oral administration of ModraDoc001 capsules resulted in high systemic exposure to docetaxel and could safely be given to patients up to 60 mg in combination with 200 mg ritonavir. The MTD has not yet been determined, but regarding the DLTs observed at the 80/100 and 60/200 dose level, we expect it to be close to these dose levels.

Furthermore, it was demonstrated that oral administration of ModraDoc001 capsules at a dose of 30 mg in combination with 100 mg ritonavir resulted in similar exposure to docetaxel compared to the i.v. liquid formulation given as oral DS. The mean exposure was lower for the capsule formulation, however, more importantly the inter-individual variability was also lower. The median inter-individual variability of all ModraDoc001

dose levels was 56% (range 36% - 78%), compared to 85% for the docetaxel DS. Inter-patient variability after i.v. docetaxel was in this study 47% and reported literature values are between 25% and 49%.[14,15] Therefore, it can be concluded that inter-patient variability after oral administration of ModraDoc001 is comparable to i.v. administration.

Major problems observed after standard weekly and 3-weekly docetaxel infusions, such as neutropenia, febrile neutropenia and fluid retention, were not observed in the current study, although the weekly exposure to docetaxel observed in the 3 highest dose-levels of 943 – 1490 ng/mL*h was similar to the standard weekly docetaxel i.v. 35 mg/m^2 regimen (e.g. 1040 $\pm$ 400 ng/mL*h,[16] 1320 $\pm$ 420 ng/mL*h,[14] 1590 $\pm$ 400 ng/mL*h (in combination with irinotecan)[14])

Baker et al demonstrated that the exposure to unbound docetaxel is correlated to the incidence of docetaxel related neutropenia.[17] It is imaginable that docetaxel – protein kinetics is different after oral administration, since docetaxel reaches the circulation without polysorbate and at a much slower rate compared to standard infusion rates.

Fluid retention after i.v administration proved to be related to the cumulative docetaxel dose.[15] In that study 631 patients were treated with 100 mg/m^2 in a 3-weekly schedule of whom 53% developed fluid retention (most patients treated in that study did not receive dexamethason premedication). In the current study, several patients were treated for more than 2 months with weekly ModraDoc001 capsules without dexamethason premedication, however, none of these patients developed fluid retention.

The most observed adverse event in this study was diarrhea with an overall incidence of 66% (23 of 35 patients). This is higher than after weekly or 3-weekly i.v. docetaxel regimens with reported diarrhea incidences of 21% - 40% overall. [3,4] The higher incidence after oral administration can probably be attributed to local effects, due to high docetaxel concentrations in the gastrointestinal tract. A second explanation could be the contribution of ritonavir to the incidence of diarrhea, since gastrointestinal toxicity is one of the main adverse events of ritonavir and ritonavir-boosted regimens.[18] The observed diarrhea in this study was found to be reversible and, in general, well manageable with loperamide. In total 3 patients had severe diarrhea. These patients were adequately treated with i.v. fluids and loperamide and 2 out of these 3 patients restarted treatment at a lower dose.

This study strengthens the concept that docetaxel can be given orally. Administration of docetaxel by an oral solid dispersion (ModraDoc001 capsules) in combination with ritonavir offers great advantages for patients. Major problems observed after intravenous therapy with docetaxel, e.g. fluid retention, hematological toxicity and infusion reactions

were, thus far, not observed. Consequently, high doses of dexamethason to prevent fluid retention and allergic reactions were not required. Proof of anti-tumor activity observed in this study warrants further development of oral docetaxel by means of ModraDoc001 capsules in combination with ritonavir.

Acknowledgements

This work was supported with a grant from ZonMw (project 95100101, project title: Crossing the barrier: from intravenous to effective oral formulations of chemotherapy)

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Chapter 2.4

Concept of boosting orally administered docetaxel by CYP3A4 inhibition

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Abstract

The oral bioavailability of docetaxel is low mainly due to pre-systemic metabolism by cytochrome P450 (CYP) 3A4. In this study we investigated whether oral docetaxel can be boosted by a CYP3A4 inhibitor. The selected CYP3A4 inhibitors were ketoconazole, clarithromycin, ritonavir and grapefruit juice. The results in 12 patients demonstrated a 1.5-10 fold increase in systemic docetaxel exposure for the different inhibitors. Low-dose ritonavir was selected for the future development of an oral docetaxel regimen.

Introduction

Docetaxel is an cytotoxic drug for the treatment of various types of cancer. It is approved for the treatment of metastatic breast, non small cell lung cancer (NSCLC), prostate, gastric cancer and head and neck cancer. Usually it is administered in 3-weekly schedules by 1 hour intravenous (i.v.) infusions. An oral docetaxel regimen is currently in clinical development.[1] This is expected to be beneficial in terms of patient convenience and possibly also in terms of safety and health care costs.[1]

Our previous studies revealed that orally administered docetaxel has low oral bioavailability due to its affinity for the drug efflux pump P-glycoprotein (P-gp) and drug metabolizing cytochrome P450 (CYP) enzymes, especially CYP3A4, both highly expressed in the epithelial layer of the gastrointestinal (GI) tract.[2] We demonstrated in proof of principle studies that systemic exposure to oral docetaxel can be significantly enhanced by co-administration of oral docetaxel with the P-gp and CYP3A inhibitor Cyclosporin A.[3] Furthermore, co-administration of oral docetaxel with the more selective CYP3A4 inhibitor ritonavir proved the hypothesis that inhibition of CYP3A4 largely increases systemic exposure of orally ingested docetaxel.[4]
To further strengthen this concept and to select the most optimal booster drug to enable oral therapy with docetaxel, we performed the current study with the known CYP3A4 inhibitors: ritonavir, ketoconazole clarithromycin and grapefruit juice. This study was conducted with a solid oral dosage form of docetaxel, ModraDoc001 10 mg capsules (Slotervaart Hospital, Amsterdam, The Netherlands).[5] To improve the very poor aqueous solubility of docetaxel, a solid dispersion formulation of docetaxel was developed. It was shown that the reduced particle size, the amorphous nature and the intimate presence of a highly soluble polymer in this formulation results in a significantly improved dissolution of docetaxel. This formulation demonstrated in a proof of concept study to result in similar systemic exposure to docetaxel compared to the drinking solution (i.v. formulation of docetaxel taken orally) but resulted in lower interpatient variability (Koolen et al. manuscript in preparation).

The aims of this study were to investigate the systemic exposure to docetaxel after administration of ModraDoc001 with or without co-administration of one of the CYP3A4 inhibitors ritonavir, ketoconazole, clarithromycin or grapefruit juice and to determine the optimal booster for boosting ModraDoc001.

Patients & Methods

Patients

Patients for whom no standard therapy of proven benefit existed, with a histologically confirmed cancer and for whom docetaxel might be a beneficial treatment option were eligible for the study. Other eligibility criteria included: age $\geq$ 18 years; life expectancy $\geq$ 3 months and no radio- or chemotherapy within the last 4 weeks prior to study entry (palliative limited radiation for pain reduction was allowed). Patients had to have acceptable bone marrow-, liver- and renal function and a World Health Organization (WHO) performance status $\leq$ 2. Patients were not eligible if they suffered from uncontrolled infectious disease, neurologic disease, bowel obstructions, or symptomatic brain metastases, alcoholism, drug addiction, psychotic disorders leading to inadequate follow-up, or if they were pregnant. Other exclusion criteria were concomitant use of known P-gp or CYP3A4 inhibitors and chronic use of H2-receptor antagonists, or proton pump inhibitors. The study protocol was approved by the medical ethics committee of the institute and all patients had to give written informed consent prior to start of the study.

Study Design

This study was designed as a 3-arm open-label, cross-over study with four evaluable patients per arm. Patients were assigned to arm I – III in order of recruitment. Patients assigned to Arm I received during the first week a single dose of 30 mg ModraDoc001 capsules (without booster) and during the second and third week they received 30 mg ModraDoc001 in combination with 400 mg ketoconazole (Nizoral®: Janssen-Cilag, Tilburg, The Netherlands) or 100 mg ritonavir (Norvir®; Abbott, Illinois, USA). Patients assigned to Arm II received the same schedule as in Arm I, but instead of ketoconazole, patients received 2 glasses (400 mL) of grapefruit juice (Coolbest® pink grapefruit juice: Royal Friesland Foods N.V. Meppel, The Netherlands). Patients assigned to arm III received during the first and second week 30 mg ModraDoc001 in combination with 1000 mg clarithromycin (Klacid®: Abbott, Illinois, USA) or 100 mg ritonavir. The sequence of ritonavir or the alternative booster was randomized. Patients continued in week 3 (Arm III) or week 4 (Arm I and II) with weekly ModraDoc001 in combination with 100 mg ritonavir. The 30 mg dose level was selected because this was the highest dose determined to be safe in a parallel ongoing phase I dose escalation study. Patients took the study drugs either 1 hour before or 2 hours after a meal together with 150 mL tap water. Water was not given to patients if the drug was given in combination with

grapefruit juice. Pre-treatment consisted of 4 mg dexamethason 1 hour prior to, and 12 and 24 hours after docetaxel intake. In addition, patients also received 1 mg granisetron orally 1 hour prior to oral docetaxel / ritonavir to prevent nausea and vomiting.

Sampling and analyses

Blood samples for pharmacokinetic analysis were collected during the first, second (all arms) and third (Arm I and II, only) week. Blood samples for docetaxel analyses were drawn at baseline and 30 min, 1, 1.5, 2, 3, 4, 6, 8, 10 and 24 hours after docetaxel intake. Samples were centrifuged within 1 hour at 1.500 x g and plasma was stored at or below -20°C until analysis. Docetaxel plasma concentrations were determined using a validated LC-MS/MS assay. The lower limit of quantification (LLQ) of the assay was 0.25 ng/mL docetaxel. [6]

Inhibitory effect of grapefruit juice in vitro

The grapefruit juice used was investigated for its CYP3A4 inhibitory potential. Ketoconazole (K1003, Sigma Aldrich Co, St Louis, MO, USA) and fresh pink grapefruits (bought at a local supermarket) were used as control. Grapefruit juice was centrifuged at 2.000 g during 5 minutes, diluted 1:1 with potassium phosphate buffer 0.5 M pH 7.5 and the solution was brought at pH 7.5 with sodiumhydroxide 2 M. The CYP3A4 inhibitory effect was investigated using human CYP3A4 microsomes and 7-benzyloxy-4-(trifluoromethyl)-coumarin as CYP3A4 substrate (GenTest, BD Biosciences, Woburn, MA, USA) according to the manual provided by BD Biosciences.[7,8]

Data analysis

The individual pharmacokinetic parameters were analyzed using descriptive pharmacokinetic methods using R (version 2.10.0) by employing validated scripts. The maximum observed plasma concentration (C_{max}), time to reach C_{max} (T_{max}) and the area under the plasma concentration time curve between 0 and the time point of the last quantifiable data point ($AUC_{o\text{-}last}$) were reported. Patient characteristics, toxicities and responses were summarized using descriptive statistics.

Patient evaluation

Before each one-week cycle, a physical examination was performed, hematology and blood chemistry were monitored and toxicities and performance status were monitored. All toxicities were graded according to the National Cancer Institute Common Toxicity Criteria for Adverse Events (version 3.0). Tumor evaluation was performed every 6 weeks using RECIST v1.0 criteria. [9] Patients remained on treatment until they no longer had clinical benefit from treatment or if toxicity led to patient withdrawal. Patients were replaced if they went off study before receiving ModraDoc001 capsules in combination with two different boosters.

Results

Twelve patients were included in this study. One patient, treated in Arm I, had progressive disease after the first two one-week cycles. Therefore, a fifth patient was enrolled in Arm I. Inclusion in Arm II was prematurely stopped after pharmacokinetic data of the first 3 patients demonstrated that grapefruit juice only had a marginal effect on the systemic exposure to docetaxel. Patient characteristics are provided in table 1.

Table 1: patient demographics

	(n=12)
Sex	
Female	5
Male	7
Age (years)	
Median	59
Range	47 – 70
Body surface area (m^2)	
Median	1.9
Range	1.4-2.3
ECOG performance status	
0	3
1	7
2	2
Pathological diagnosis	
NSCLC	5
Endometrial	2
Primary unknown	2
Other	3
Disease stage	
Stage III	2
Stage IV	10

Pharmacokinetics

The mean $AUC_{0\text{-last}}$, C_{max} and the median T_{max}, stratified for each treatment, are given in table 2 and the mean concentration time curves are presented in figure 1. The results demonstrated that the systemic exposure to docetaxel after administration of 30 mg ModraDoc001 without booster, was low (mean $AUC_{0\text{-}8}$ 36.7 ng/mL*h with relative standard error (%CV): 59%). However, after co-administration of 100 mg ritonavir, 400 mg ketoconazole or 1000 mg clarithromycin the exposure to docetaxel increased

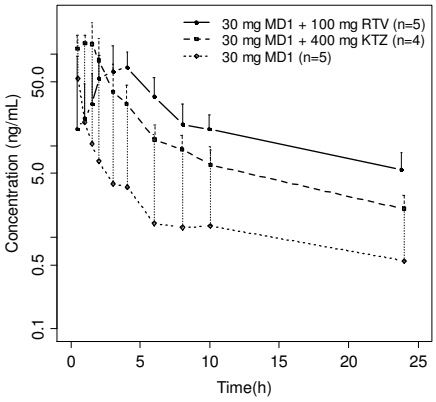
approximately 10 fold (mean $AUC_{0\text{-last}}$ (and %CV): 492 (57%), 450 (42%), 392 (25%) ng/mL*h , respectively). Intra-patient analysis suggested that ritonavir resulted in a slightly higher exposure to docetaxel, compared to ketoconazole or clarithromycin. The mean curves of docetaxel after boosting with ritonavir, ketoconazole and clarithromycin showed apparently different profiles: the C_{max} after boosting with ritonavir was lower and T_{max} was later.

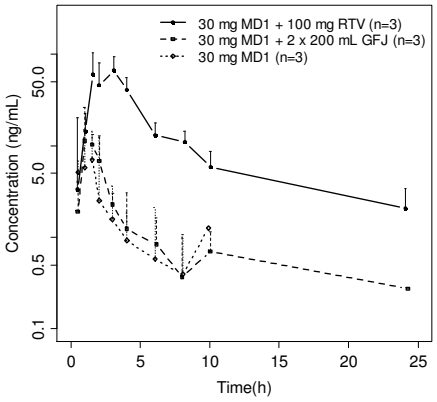
The strong effect observed after boosting ModraDoc001 with ritonavir, ketoconazole or clarithromycin was not observed after co-administration of 2 glasses of grapefruit juice. Intra-patient analysis demonstrated an average 1.5 fold increase in $AUC_{0\text{-last}}$, from 18.6 ng/mL*h (%CV: 62%) to 28.1 ng/mL*h (%CV: 43%).

Table 2: Mean (or median for $T_{max)}$ pharmacokinetic parameters and their relative standard deviation stratified for treatment schedule. Abbreviations: MD1: ModraDoc001, RTV: ritonavir, KTZ: ketoconazole, GFJ: grapefruit juice, CLM: clarithromycin, $AUC_{0\text{-}24h}$ area under the curve from baseline to 24 hours, Cmax: maximum measured plasma concentration, Tmax: time to reach C_{max}, CV: coefficient of variation.

		$AUC_{0\text{-}24h}$		C_{max}		T_{max}	
Treatment	n	Mean (ng/mL*h)	CV(%)	Mean (ng/mL)	CV(%)	Median (h)	range (h)
Arm I							
MD1	5	[a]47.6	40	39.1	81	0.53	0.50-10.0
MD1 + RTV	5	513	58	87.4	52	2.00	0.40-4.02
MD1 + KTZ	4	450	42	178	32	1.00	0.50-1.52
Arm II							
MD1	3	[a]18.6	62	7.66	62	1.00	0.97-2.07
MD1 + RTV	3	290	51	62.7	62	2.00	1.63-2.03
MD1 + GFJ	3	[a]28.1	43	18.1	78	1.50	0.98-2.00
Arm III							
MD1 + RTV	4	[b]659	46	104	68	1.70	1.02-3.02
MD1 + CLM	4	392	25	153	34	1.00	0.98-3.03
Total							
MD1	8	[a]36.7	59	27.3	107	0.98	0.50-10.0
MD1 + RTV	12	[c]492	57	86.7	59	2.00	0.40-4.02

[a]$AUC_{0\text{-}8h}$, [b]n=3 patients, [c]n=11 patients





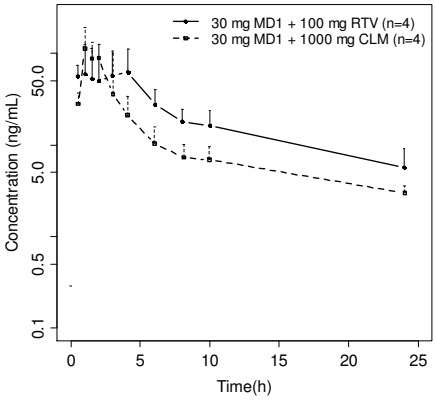


Figure 1: Mean plasma concetration time curves. From left to right: Arm I, Arm II and Arm III

In vitro activity of grapefruit juice

The CYP3A4 inhibitory effect of the grapefruit juice used, was determined *in vitro* (ketoconazole and fresh grapefruits were used as controls). The measured 50% inhibitory concentration (IC50) of the tested grapefruit juice was 4.6 µg/mL versus 10.1 µg/mL of fresh grapefruit juice. Ketoconazole was found approximately 65 times more potent with a measured IC50 of 0.07 µg/mL.

Safety and anti-tumor activity

Overall, the patients were treated for a median of 6.5 one-week cycles (range:2-30) Ten out of 12 patients experienced possibly treatment related adverse events of which fatigue (7 patients), diarrhea (5 patients), nausea (4 patients) and neuropathy (4 patients) were the most predominant. Adverse events were generally mild. Only one grade 3 possibly related adverse event was reported, hallucinations, which was attributed to dexamethason pre-medication. Tumor regression was not observed, although stable disease was achieved in two patients with NSCLC for respectively, 19 and 30 weeks.

Discussion

This study proofs the concept of boosting docetaxel exposure by a CYP3A4 inhibitor. Co-administration of ModraDoc001 capsules with a strong CYP3A4 inhibitor, with the exception of grapefruit juice, resulted in high systemic exposure to docetaxel compared with ModraDoc001 capsules alone.

The exposure to docetaxel after boosting with a ritonavir (100 mg), ketoconazole (400 mg) or clarithromycin dose (1000 mg) resulted in systemic exposure to docetaxel of the same magnitude. These boosters resulted in an approximately 10 fold increase in systemic exposure to docetaxel. However, it should be noted that clarithromycin and ketoconazole were dosed at their maximum therapeutic dose whereas ritonavir was dosed at a low 100 mg dose. This low dose was selected based on the experience gained with boosting orally administered HIV protease inhibitors with ritonavir, to which aim the 100 mg ritonavir dose is usually sufficient.[10] For boosting docetaxel, the optimal ritonavir has not yet been determined. Population pharmacokinetic analysis suggested that the exposure could slightly be further increased with a 200 mg dose.[11]

The mean curves and pharmacokinetic parameters of docetaxel after boosting with ketoconazole or clarithromycin showed some similarities. The mean curve after boosting with ritonavir had however a distinctly different profile with lower C_{max}, longer $T_{max.}$ This may be explained by the reduced gastric emptying rate induced by ritonavir resulting in a longer time for docetaxel to reach Cmax.[12] Secondly, ketoconazole is a strong competitive inhibitor with an inhibition constant (Ki) of 0.03 μM[13] and ritonavir is reported to be a dual inhibitor with both competitive and mechanism-based characteristics with a reported Ki value of 0.15 μM.[14,15,16]. Mechanism-based inhibition of CYP3A is characterized by NADPH, time and concentration dependent irreversible inhibition. Whereas a competitive inhibitor results in rapidly reversible inhibition. This could explain the high C_{max} and short T_{max} time after boosting with ketoconazole versus ritonavir

boosted docetaxel. However, clarithromycin is, like ritonavir, a mechanism based inhibitor[17] but the pharmacokinetic profile shows more similarities with ketoconazole. Lastly, the three CYP3A4 inhibitors are also able to inhibit, although with lower inhibition constants, P-gp. Since, docetaxel is a substrate for P-gp,[18,19,20] the high doses of ketoconazole and clarithromycin could possibly, more effectively than low dosed ritonavir, have resulted in relevant P-gp inhibition thereby promoting the absorption of docetaxel causing high docetaxel Cmax values.

Unlike the other tested CYP3A4 inhibitors, grapefruit juice only modestly increased docetaxel exposure. Grapefruit juice is reported to inhibit CYP3A4 due to several constituents like the furanocoumarines (e.g., bergamottin and 6,7-dihydroxybergamottin).[21,22] Since there is large variation in CYP3A4 inhibiting potential among different brands,[23] the CYP3A4 inhibiting potential was also tested *in vitro*. This assay demonstrated that the selected grapefruit juice was capable of inhibiting CYP3A4. However, the marginal increase in docetaxel exposure after co-administration of grapefruit juice led to the conclusion that grapefruit is not an appropriate booster for orally administered ModraDoc001 capsules.

This study proves the concept of boosting orally administered docetaxel using ModraDoc001 capsules by inhibition of CYP3A4. Low dose ritonavir was selected for the further development of an oral docetaxel regimen, since, ritonavir resulted in high systemic exposure to docetaxel, had a good safety profile and its use as booster is already standard practice in multiple anti –HIV regimens.

Acknowledgements

This work was supported with a grant from ZonMw (project 95100101, project title: Crossing the barrier: from intravenous to effective oral formulations of chemotherapy)

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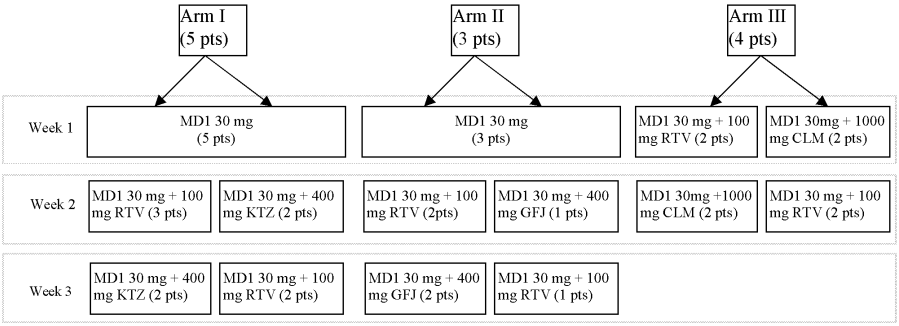
Supplementary figure 1: Study overview and number of patients enrolled. Patients continued in week 3 (arm III) or week 4 (Arm I and II) with weekly ModraDoc001 in combination with 100 mg ritonavir. (abbreviations: MD1: ModraDoc001, pts: patients, RTV: ritonavir, KTZ: ketoconazole, GFJ: grapefruit juice, CLM: clarithromycin)



Chapter 2.5

Boosting oral docetaxel (ModraDoc001) with ritonavir: What is the optimal ritonavir regimen?

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Abstract

Oral administration of docetaxel (ModraDoc001 capsules) is feasible when given in combination with the CYP3A4 inhibitor (booster) ritonavir. The aim of this study was to define the optimal ritonavir regimen to boost docetaxel exposure. The following regimens were investigated: 100 mg or 200 mg ritonavir given simultaneously with oral docetaxel or 100 mg given simultaneously plus 100 mg ritonavir given 4 hours after intake of oral docetaxel. In total, 11 patients were included in this pharmacokinetic study. The 200 mg dose resulted in a 77% increase in systemic exposure to docetaxel compared to boosting with 100 mg ritonavir, and 2 doses of 100 mg ritonavir separated by four hours resulted in a mean 21% increase in docetaxel exposure compared to a single 100 mg ritonavir dose. In conclusion, future development of weekly oral docetaxel will be performed with 200 mg ritonavir, since this is safe, practical for patients and resulted in high systemic exposure to docetaxel.

Introduction

Docetaxel is approved for the treatment of various types of cancer and administered intravenously. It is primarily metabolized by cytochrome P450 (CYP) 3A4 enzymes and its metabolites are subsequently excreted via the bile into the faeces. Oral administration of docetaxel is hampered by extensive pre-systemic metabolism in the gut and liver by CYP3A4 and by active excretion by the drug-efflux pump P-glycoprotein (P-gp). A proof of concept study in cancer patients demonstrated that oral treatment with docetaxel is possible when given in combination with ritonavir.[1] The Human immunodeficiency virus (HIV) protease inhibitor ritonavir is a potent cytochrome P450 (CYP) 3A4 inhibitor. In HIV treatment it is predominantly used at subtherapeutic doses to enhance the systemic exposure to other antiretroviral agents through inhibition of first pass metabolism.[2,3]

It was found that boosting oral docetaxel with ritonavir resulted in an apparent bioavailability (systemic exposure measured by AUC compared to the same dose given intravenously) of more than 100%.[1]

These findings encouraged the development of an oral solid dosage form of docetaxel, ModraDoc001. This capsule formulation (solid dispersion) demonstrated good pharmaceutical characteristics, in terms of solubility and stability, and good clinical pharmacological characteristics, in terms of systemic exposure and interpatient variability.[4]

Pharmacokinetic (PK) studies on the optimal ritonavir booster dose have demonstrated that 100 mg is usually sufficient to boost the systemic exposure of target drugs.[5] This accounts for saquinavir,[6,7] fosamprenavir,[8,9] and elvitegravir.[10] In contrast, exposure to lopinavir[11,5] and indinavir[12] was significantly increased after boosting with 400 mg versus 100 mg ritonavir. These conflicting results warrant clinical assessment of the optimal ritonavir regimen for boosting orally administered docetaxel.

An important difference with oral docetaxel and the boosted protease inhibitors is the schedule. Oral docetaxel has an intended weekly schedule whereas protease inhibitors are given daily or bi-daily. Steady-state pharmacokinetics or pharmacodynamics (prolonged decreased levels of CYP3A4) of ritonavir are then reached, which might result in a lower ritonavir dose required for optimal boosting for a daily or bi-daily regimen. In addition, ritonavir 400 mg dosed 48 hours prior to saquinavir or nelfinavir had a relevant effect on the systemic exposure of these drugs. This indicates a persistent systemic effect of ritonavir on CYP3A4 activity.[13]

In the first proof of concept study, docetaxel was given orally in combination with 100 mg ritonavir given simultaneously or 1 hour prior to docetaxel. There were no significant differences observed between the simultaneous or sequential ritonavir regimens, however, there appeared to be a trend towards higher docetaxel exposure when ritonavir was given 1 hour before docetaxel.[1]

Additionally, population pharmacokinetic analysis of the data of this proof-of-concept study demonstrated that the CYP3A activity, measured by docetaxel clearance, after approximately 4 hours post-dose slowly recovered to normal values.[14]
For safety, the ritonavir dose should be as low as possible. Studies with ascending ritonavir boosting doses showed a relevant increase in the incidence of ritonavir related adverse events with doses higher than 200 mg.[12]
Since, clinical trials conducted thus far, were inconclusive about the optimal ritonavir regimen in combination with oral docetaxel, the aim of this study was to establish the optimal, and safe, ritonavir regimen to boost the exposure to docetaxel after intake of ModraDoc001 capsules.

Patients & Methods

Patients

Patients for whom no standard therapy of proven benefit existed and with a histologically confirmed cancer refractory to current therapies were eligible for the study. Other eligibility criteria included: age≥ 18 years; life expectancy ≥ 3 months; no radio- or chemotherapy within the last 4 weeks prior to study entry (palliative limited radiation for pain reduction was allowed). Patients had to have acceptable bone marrow, liver, and renal function and a World Health Organization (WHO) performance status ≤ 2. Other exclusion criteria were concomitant use of known P-glycoprotein (P-gp) or CYP3A4 inhibitors and chronic use of H2-receptor antagonists or proton pump inhibitors. The study protocol was approved by the medical ethics committee of the institute and all patients had to give written informed consent prior to start of the study.

Study Design

The study was designed as a two-arm open label proof of concept study. Arm I was designed to compare the influence on the systemic exposure to docetaxel after boosting with 100 mg ritonavir given simultaneously or 100 mg given simultaneously plus a second ritonavir dose of 100 mg 4 hours after intake of oral docetaxel. The docetaxel formulation that was initially used was the i.v. formulation (Taxotere; Sanofi-Aventis, France), which was ingested orally. After the first patient this was amended to a novel docetaxel capsule formulation that became available (ModraDoc001: Slotervaart Hospital, Amsterdam, The Netherlands).
The second arm was designed to compare the effect of 100 mg or 200 mg ritonavir given simultaneously with ModraDoc001 capsules on systemic exposure to docetaxel. A schematic overview of the study is depicted by figure 1.

Arm I

Six patients were assigned to Arm I and received weekly oral docetaxel with pharmacokinetic sampling during the first and second week. The 6 patients were randomized into two groups. The first group received during the first cycle 20 mg oral docetaxel in combination with 100 mg ritonavir. During the second week, they received 20 mg oral docetaxel in combination with 2 x 100 mg ritonavir: 100 mg ritonavir given simultaneously plus 100 mg given 4 hours after intake of oral docetaxel. The second randomization group received the two treatment schemes in reversed order. As described above, the first patient received oral docetaxel by the drinking solution (i.v. docetaxel formulation) and subsequent patients received oral docetaxel by ModraDoc001 capsules.

Arm II

After almost completion of Arm I, the study protocol was amended and Arm II was added. Arm II was a proof of concept study written for 4 patients. The patients were randomized into two groups. The first group received 30 mg ModraDoc001 capsules in combination with 100 mg ritonavir during the first week and in combination with 200 mg ritonavir during the second week. Patients randomized into the second group received these schedules in reversed order, see figure 1.

In week 3 and beyond, patients could continue treatment with weekly ModraDoc001 in combination with 100 mg ritonavir for as long they were considered to be receiving benefit. Doses of oral docetaxel (ModraDoc001 capsules) were based on the highest weekly ModraDoc001 dose determined to be safe in a parallel ongoing phase I study. Adverse events were graded according to the National Cancer Institute Common Toxicity Criteria for Adverse Events (NCI CTCAE) version 3.0. Tumor evaluation was performed every 6 weeks using RECIST v1.0 criteria.[15] Patients were considered evaluable when they received the first 2 one-week cycles.

PK sampling

Blood samples for pharmacokinetic analysis were collected during the first and second week at: predose 0.5, 1, 1.5, 2, 3, 4, 5, 6, 8, 10, 24 and 48 hours after intake. Samples were immediately placed on ice and were centrifuged within 1 hour at 1500 x g for 10 minutes at 4^{o}C. Plasma was stored at or below -20 ^{o}C until analysis.

Analytical Assay

Docetaxel was quantified in plasma by use of high-performance liquid chromatography with tandem mass spectrometric detection (LC-MS/MS) as described by Kuppens et al. [16] with docetaxel-d9 (Toronto Research Chemicals Inc.) as internal standard. The lower limit of quantification of the assay was 0.25 ng/mL docetaxel.

Data analysis

The individual pharmacokinetic parameters were analyzed using non-compartmental methods using R version 2.10.0[17] by employing validated scripts. The area under the plasma concentration time curve (AUC) between 0 hrs and infinity (AUC_{inf}) using the trapezoid rule, was determined. Furthermore, the observed maximal plasma concentration (C_{max}), time to maximal plasma concentration (T_{max}) and elimination half life (t1/2) were reported. Patient characteristics, toxicities and anti-tumor activity were summarized using descriptive statistics.

Results

In total 11 patients entered the study; 7 patients in arm I and 4 patients in arm II. One patient in Arm I went off study after the first week due to clinical deterioration and was replaced. Patients' characteristics are briefly summarized in table 1.

Table 1: Patient demographics

	(n=11)
Sex	
Female	3
Male	8
Age (years)	
Median	65
Range	40 – 75
Body surface area (m^2)	
Median	2.0
Range	1.5-2.3
ECOG performance status	
0	6
1	5
Pathological diagnosis	
Urothelial cell carcinoma	3
Esophageal / gastric	3
NSCLC	2
Ovarian	2
Melanoma	1
Disease stage	
Stage III	0
Stage IV	11

Pharmacokinetics

Ten patients were available for pharmacokinetic analysis. Pharmacokinetic parameters are summarized in table 2 and the mean concentration time curves are presented in figure 1.

Table 2: Pharmacokinetic parameters of docetaxel. (abbreviations: N: number of patients, DS: docetaxel drinking solution, MD1: ModraDoc001 capsules, RTV: ritonavir)

Schedule	N	AUC_{inf} (ng/mL*h)		C_{max} (ng/mL)		T_{max} (h)		$T_{1/2}$ (h)	
		Mean	%CV	Mean	%CV	Median	range	Median	%CV
20 mg DS + 100 mg RTV	1	250	-	53.8	-	0.93	-	14.3	-
20 mg DS + 2x 100 mg RTV	1	373	-	48.0	-	0.92	-	14.0	-
20 mg MD1 + 100 mg RTV	5	387	28	53.3	16	1.98	1.00-2.12	12.1	29
20 mg MD1 + 2x 100 mg RTV	5	469	31	62.9	35	1.60	0.98-3.02	16.0	3.5
30 mg MD1 + 100 mg RTV	4	394	25	48.5	41	2.04	1.00-6.07	17.3	20
30 mg MD1 + 200 mg RTV	4	698	12	75.1	27	2.00	1.53-5.98	14.5	14

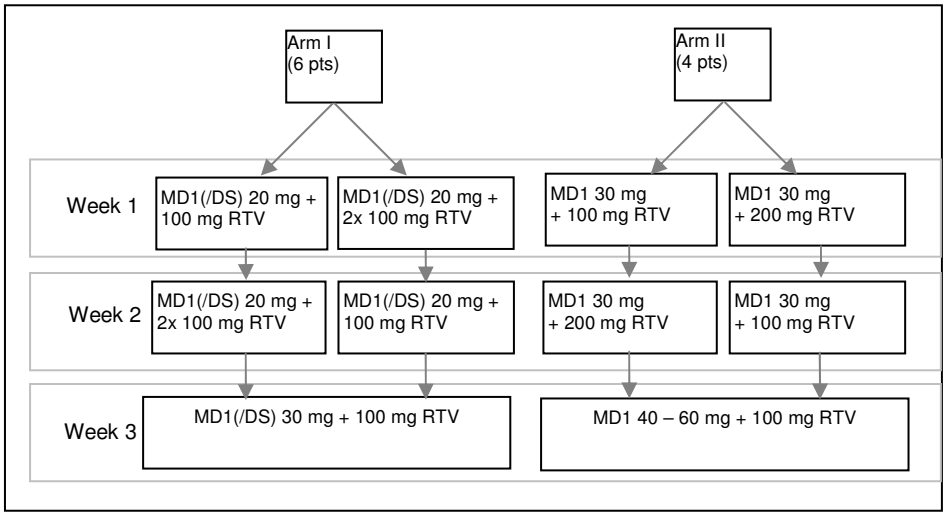

Figure 1: Schematic overview of the study. (abbreviations: N: number of patients, DS: docetaxel drinking solution, MD1: ModraDoc001 capsules, RTV: ritonavir)

Figure 2A shows that the docetaxel curves after boosting with 100 mg or 2x 100 mg ritonavir largely overlap. However, the terminal part (from t = 8 h) of the docetaxel curve decreased at a slower rate for the 2 x 100 mg ritonavir regimen. This resulted in an approximately 21 % increase in systemic exposure to docetaxel: from 387 (%CV: 28%) to 469 ng/mL*h (%CV: 31%). Figure 2A illustrates that the exposure in each individual is higher for the 2x100 mg ritonavir regimen whereas, the C_{max} was not relevantly different between the two groups, which is expected since the C_{max} was observed in all patients before the second ritonavir dose was ingested.

A single 200 mg ritonavir dose resulted in a relevant increase in docetaxel exposure and C_{max} compared to a single dose of 100 mg as demonstrated in 4 patients, see table 2 and figure 2B. The AUC_{inf} increased by 77% from 394 ng/mL*h (%CV: 25%) to 698 ng/mL*h (%CV: 12%) and C_{max} increased by 55% from 48.5 ng/mL (%CV: 41%) to 75.1 ng/mL (%CV: 27%). Figure 3C and 3D show that an increase in exposure and in C_{max} was observed in each patient.

Regarding the elimination rate, provided in table 2, it can be seen that a second ritonavir dose 4 hours after intake of ModraDoc001 capsules resulted in an increased elimination half-life (12.1 versus 16.0 hours). Contrary, a double dose 200 mg versus 100 mg ritonavir did not result in an increased elimination half-life (17.3 versus 14.5 hours). Furthermore, the variability in elimination half-life was lower after boosting with 2 x100 mg (3.5% versus 29%) and 200 mg (14% versus 20 %) ritonavir compared to 100 mg ritonavir.

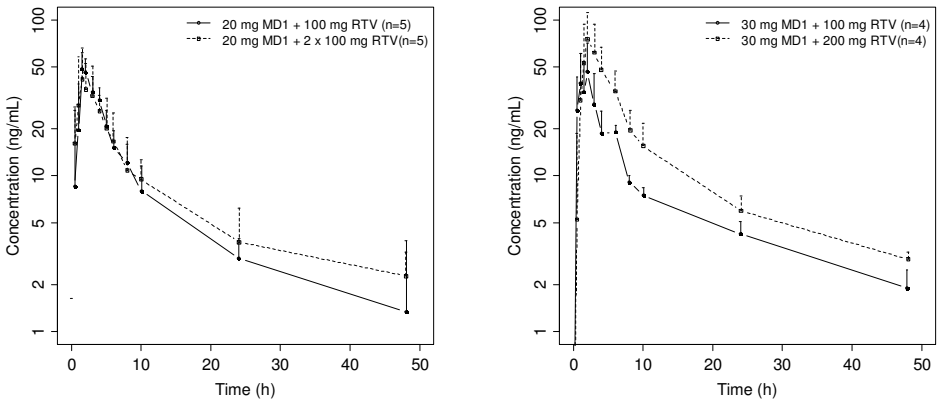

Figure 2: Mean concentration time curves of docetaxel. Left panel (2A): results of Arm I: 20 mg ModraDoc001(MD1) in combination with 100 mg ritonavir or 2x 100 mg ritonavir (ingested simultaneously and 4 hours after intake of ModraDoc001 capsules). Right panel (2B): results of arm II: 30 mg ModraDoc001 in combination with 100 mg or 200 mg ritonavir. (abbreviations: MD1: ModraDoc001 capsules, RTV: ritonavir)

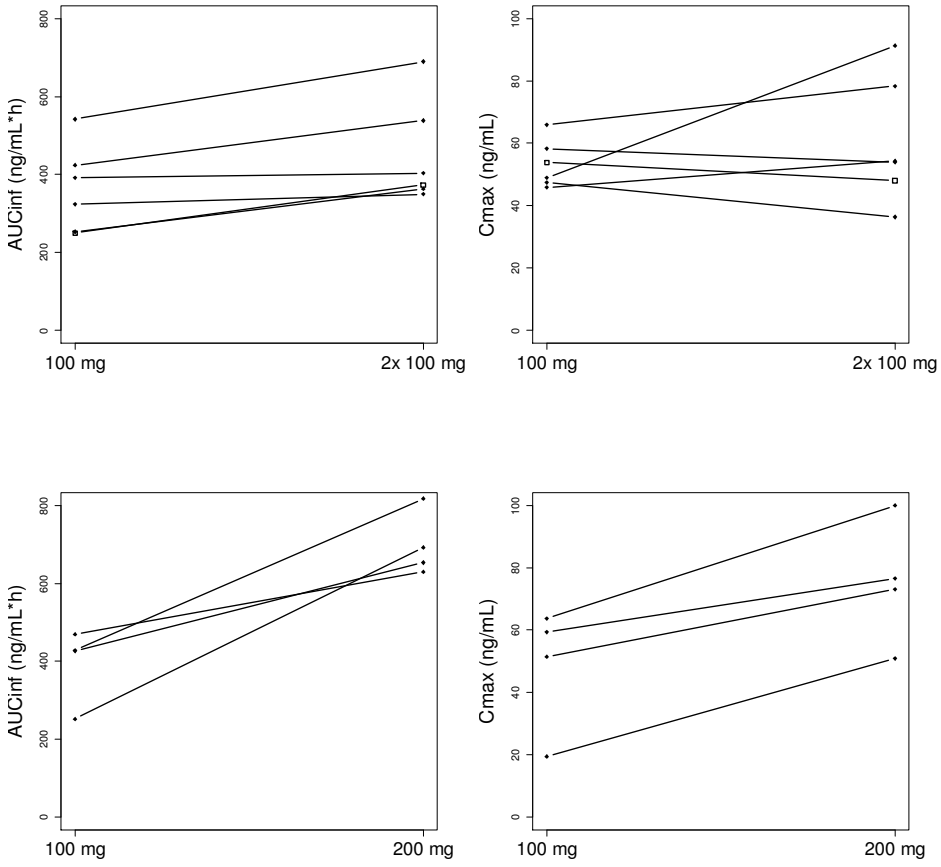

Figure 3: Plots of AUC_{inf} and C_{max} of docetaxel for each individual patient. Upper left (3A): AUCinf after 100 mg or 2 x 100 mg ritonavir (100 mg given simultaneously and 100 mg given 4 hours after intake of oral docetaxel); upper right (3B): C_{max} after 100 mg or 2 x 100 mg ritonavir, lower left (3C): AUC_{inf} after boosting with 100 mg or 200 mg ritonavir, lower right (3D): C_{max} after boosting with 100 or 200 mg ritonavir.

Safety and anti-tumor activity

Overall, patients were treated for a median of 11 weekly cycles (range 1-27 cycles). All patients had possibly treatment-related adverse events. The most frequently reported adverse events were diarrhea (5 patients), nausea (5 patients), fatigue (4 patients) and neuropathy (3 patients). All, except one, adverse events were CTCAE grade 1-2. One patient had a short episode of grade 4 dyspnea, which was possibly study treatment

related. However, after recovery, the patient continued with ModraDoc001 and ritonavir without requiring dose reduction or dose omissions. No objective tumor response was observed albeit that one patient with urothelial cell carcinoma and one patient with esophageal cancer had stable disease for 27 and 21 one-week cycles, respectively.

Discussion

Results of this study show that 200 mg ritonavir concomitantly with docetaxel relevantly increased the systemic exposure to docetaxel (77% in AUC_{inf}) compared to a single 100 mg ritonavir dose. A second 100 mg ritonavir dose 4 hours after administration resulted in a moderate (21 %) increase in docetaxel compared to a single 100 mg ritonavir dose.
The effect of a second ritonavir dose 4 hours after docetaxel intake could be completely attributed to the decreased elimination rate of docetaxel. On the contrary, the 200 mg single ritonavir dose resulted mainly in a decreased first pass effect, regarding the increased C_{max} (55%). Thus, CYP3A4 inhibition in the gastrointestinal epithelium and liver, during first-pass, appeared to be the major determinants of the higher systemic exposure after boosting with 200 mg ritonavir.
The 77% increase in systemic exposure after boosting with 200 mg ritonavir compared to 100 mg ritonavir was higher than expected. Doses of more than 100 mg ritonavir generally resulted in only marginal increases in systemic exposure to target drugs when applied as booster for anti-HIV drugs.[6,7,8,9,10] Indinavir and lopinavir did show relevant increases in systemic exposure with increasing ritonavir booster doses, however these increases were maximally 45 %.[12,11] Although, the sample size of our study was small, an other likely explanation is the differences in schedules between boosted oral docetaxel and boosted protease inhibitors. Daily or bi-daily dosing of ritonavir results in persistent CYP3A4 inhibition, potentially requiring lower doses of ritonavir that are able to effectively boost the target drug.[13,18]
Bierman demonstrated that ritonavir is able to inhibit P-gp regulated transport [19] and Kharasch et al showed that ritonavir given at higher doses (600 - 800 mg daily) to healthy volunteers resulted in enhanced exposure to methadone and fexofenadine due to P-gp inhibition.[20,21] Since, docetaxel is also a substrate for P-gp, as has been demonstrated preclinically[22] and in a clinical trial,[23] inhibition of P-gp could may also explain further increases in docetaxel exposure. However, it is questionable whether higher ritonavir doses will result in relevant further increases in docetaxel exposure, or reduction of inter-patient variability. as, the systemic exposure (measured by AUC) of oral docetaxel after boosting of ModraDoc001 with 100 mg ritonavir was higher than obtained after the same dose of docetaxel given intravenously.[1] Furthermore, the increased ritonavir pill burden and increased risk for ritonavir related adverse events make higher ritonavir doses undesirable.

In conclusion, the clinical development of ModraDoc001 will continue in combination with 200 mg ritonavir ingested simultaneously. This regimen is selected based on [a] the high increase in systemic exposure to docetaxel when given in combination with 200 mg compared to 100 mg ritonavir or 2 x 100 mg [b] it is a practical regimen for patients (all drugs are ingested simultaneously), [c] and higher doses than 200 mg ritonavir are not expected to result in relevant further increases in docetaxel exposure that outweigh the increased pill burden and additional risks for ritonavir related adverse events.

Acknowledgements

This work was supported with a grant from ZonMw (project 95100101, project title: Crossing the barrier: from intravenous to effective oral formulations of chemotherapy)

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3

Pharmaceutical & clinical development of oral paclitaxel

Chapter 3.1

Paclitaxel can be administered orally in combination with CYP3A4 inhibitors as pharmacokinetic boosters

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Interim analysis

Abstract

The oral bioavailability of paclitaxel is low due to extensive pre-systemic metabolism by cytochrome P450 (CYP) 3A4 and CYP2C8 enzymes and due to active excretion in the gut lumen by P-glycoprotein. Previously it has been demonstrated that the exposure to paclitaxel can be significantly increased by co-administration of the P-gp and CYP3A4 inhibitor, Cyclosporin A (CsA). The aim of this study was to investigate whether the systemic exposure to paclitaxel can also be significantly enhanced by co-administration of a CYP3A4 inhibitor. For this aim oral paclitaxel 100 mg was given concomitantly with ketoconazole, clarithromycin or ritonavir. The different CYP3A4 inhibitors resulted in a mean systemic exposure to paclitaxel between 751 – 1280 ng/mL*h. Which is comparable to the exposure after the use of CsA as booster (1030 ng/mL*h).
These results demonstrate that orally administered paclitaxel can be successfully boosted by a CYP3A4 inhibitor. Further clinical development will be conducted with ritonavir as booster due to the extensive clinical experience and the favorable safety profile.

Introduction

Paclitaxel is approved for the treatment of various malignancies and is administered intravenously (i.v.).[1] Oral application of paclitaxel would have several advantages, such as patient convenience and reduced risk of hypersensitivity reactions due to the absence of the solvent Cremophor EL.[2,3] Furthermore, Cremophor EL is responsible for the non-linear pharmacokinetic (PK) behavior of paclitaxel after i.v. administration of paclitaxel (Taxol®).[4]
Oral bioavailability of paclitaxel is, however, limited by its affinity for the drug efflux pump P-glycoprotein (P-gp) and by its extensive presystemic metabolism by cytochrome P450 (CYP) enzymes. It has been demonstrated that co-administration of oral paclitaxel with an inhibitor of P-gp, Cyclosporin A (CsA), resulted in an 8-9 fold increased systemic paclitaxel exposure[5] and more importantly, CsA boosted orally administered paclitaxel demonstrated promising anti-tumor activity.[6,7]

The oral route also has the possibility to further investigate daily, metronomic, paclitaxel regimens. Continuous exposure to low concentrations of paclitaxel has been reported to have anti-tumor activity by inhibiting angiogenesis.[8,9,10] A recent phase II study with 96-hour paclitaxel infusions reported these findings, however, the long infusion times resulted in a high incidence of bacterial infections.[11] Oral administration might greatly improve the safety and feasibility of metronomic therapy. But, daily intake of 15 mg/kg CsA by cancer patients as we have used in previous studies[5,6,7,12] to enable exposure to orally administered paclitaxel, may cause severe adverse events such as

immunosuppression. Therefore, selection of an alternative safe booster to enhance the exposure to paclitaxel is warranted.
Parallel to the development of oral paclitaxel, oral docetaxel is being developed. Oral docetaxel was found to be successfully boosted by ritonavir, a strong CYP3A4 inhibitor. [13,14] However, docetaxel is mainly metabolized by CYP3A4,[15] whereas paclitaxel is metabolized by both CYP3A4 and CYP2C8.[16] The effect of boosting paclitaxel with ritonavir may therefore be less pronounced than of docetaxel. Co-administration of paclitaxel with a P-gp inhibitor primarily aims at increasing the absorption from the gastrointestinal tract. In Mdr1a/1b$^{-/-}$ mice (mice lacking P-gp), CYP enzymes became an important factor limiting the oral bioavailability of paclitaxel. This is illustrated by the fraction of unchanged paclitaxel recovered from the faeces, which was reduced from 86% of the dose in wild-type mice to less than 2% in mice lacking P-gp.[17]
Since CYP2C8 is only expressed in very low amount in the intestines[18] CYP3A4 is mainly responsible for metabolism of paclitaxel in the gastrointestinal tract. Inhibition of (intestinal) CYP3A4 may therefore be an effective strategy to boost orally administered paclitaxel. The most important advantage of ritonavir boosted paclitaxel would be that ritonavir has a demonstrated good safety profile when given daily or bi-daily at doses of 100 – 200 mg.[19,20]

The aim of this study was to investigate whether the exposure to oral paclitaxel can be enhanced by co-administration of a CYP3A4 inhibitor. For this aim paclitaxel was given orally in combination with three different well known CYP3A4 inhibitors: ritonavir, clarithromycin and ketoconazole.

Patients & Methods

Patients

Patients for whom no standard therapy of proven benefit existed, with a histologically confirmed cancer and for whom paclitaxel might be a beneficial treatment option were eligible for the study. Other eligibility criteria included: age $\geq$ 18 years; life expectancy $\geq$ 3 months and no radio- or chemotherapy within the last 4 weeks prior to study entry (palliative limited radiation for pain reduction was allowed). Patients had to have acceptable bone marrow-, liver- and renal function and a World Health Organization (WHO) performance status $\leq$ 2. Patients were not eligible if they suffered from uncontrolled infectious disease, neurologic disease, bowel obstructions, or symptomatic brain metastases, alcoholism, drug addiction, psychotic disorders leading to inadequate follow-up, or if they were pregnant. Other exclusion criteria were concomitant use of known P-gp or CYP3A4 inhibitors and chronic use of H2-receptor antagonists, or proton pump inhibitors. The study protocol was approved by the medical ethics committee of the institute and all patients had to give written informed consent prior to start of the study.

Study Design

This study was a two-step open-label proof of concept study. The first step was to investigate whether orally administered paclitaxel could be boosted by 100 mg ritonavir, a CYP3A4 inhibitor. The effect was compared with CsA 15 mg/kg boosted paclitaxel.
After evaluation of ritonavir,, the study was extended with three additional treatment arms to further proof the concept of boosting paclitaxel with a CYP3A4 inhibitor. In these additional arms two other boosters, clarithromycin and ketoconazole and a higher ritonavir dose (200 mg) were investigated. The study schedule is depicted in Figure 1.

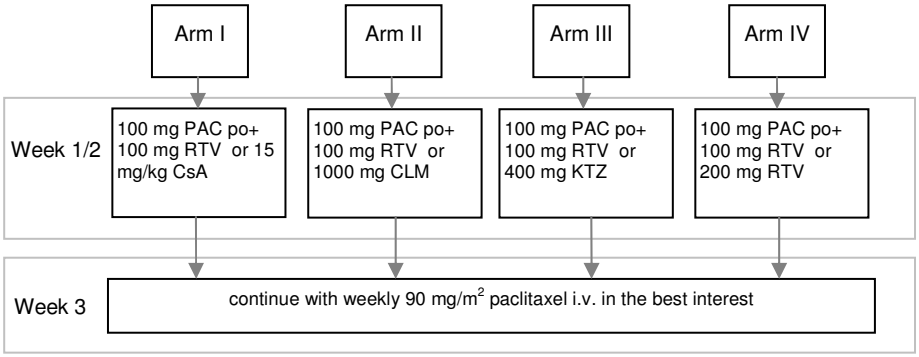

Figure 1: study schedule. (abbreviations: PAC po: orally administered paclitaxel, RTV: ritonavir, CLM: clarithromycin, KTZ: ketoconazole)

Arm I: Four patients received during the first two weeks, on day 1 and 8, 100 mg paclitaxel (Paclitaxel Mayne, i.e. paclitaxel 6 mg/mL, dissolved in ethanol and polyethoxylated castor oil (Cremophor EL) 1:1 w/v, Mayne Pharma, Melbourne Australia) as drinking solution in which paclitaxel is dissolved in ethanol, water and polyethoxylated castor oil (Cremophor EL). Patients were randomized into two groups, the first group received on day 1 paclitaxel in combination with 15 mg/kg CsA p.o. and on day 8 paclitaxel in combination with 100 mg ritonavir p.o. The second randomization group received ritonavir and CsA in reversed order. Both CsA and ritonavir were given 30 minutes prior to paclitaxel intake. Patients took the study drugs either 1 hour before or 2 hours after a light breakfast together with approximately 150 mL water. Pre-treatment consisted of 4 mg dexamethason 1 hour prior to, and 12 and 24 hours after paclitaxel intake. Additionally, patients also received 1 mg granisetron 1 hour prior to oral paclitaxel to prevent nausea and/or vomiting.

Arm II-IV were designed similarly. In arm II 100 mg paclitaxel p.o. was boosted with 100 mg ritonavir or 1000 mg clarithromycin; in arm III 100 mg paclitaxel p.o. was boosted with 100 mg ritonavir or 400 mg ketoconazole and in arm III 100 mg paclitaxel p.o. was boosted with 100 mg or 200 mg ritonavir.

All patients continued in week 3 (day 15) with weekly 90 mg/m^2 paclitaxel i.v. (Paclitaxel Mayne, Mayne Pharma, Melbourne Australia) in their best interest. Patients remained on treatment until they had no longer clinical benefit, or if toxicity led to patient withdrawal.

Sampling and analyses

Blood samples for pharmacokinetic analysis were collected during the first and second week. Blood samples for paclitaxel analysis were drawn at baseline and 30 min, 1, 1.5, 2, 3, 4, 6, 8, 10, 24 and 48 hours after paclitaxel intake. Samples were centrifuged within 1 hour at 1.500 x g and plasma was stored at or below -20°C until analysis. Paclitaxel was quantified in plasma by use of high-performance liquid chromatography with tandem mass spectrometric detection (LC-MS/MS).[21] The lower limit of quantification of the assay was 0.25 ng/mL paclitaxel.

Data analysis

The individual pharmacokinetic parameters were analyzed using descriptive pharmacokinetic methods using R (version 2.10.0) by employing validated scripts. The maximum observed plasma concentration (C_{max}), time to reach C_{max} (T_{max}) and the area under the plasma concentration-time curve between 0 and the time-point of the last quantifiable data point (AUC_{o-t}) were reported, since it was not possible to extrapolate the concentration time curve to infinity for each individual. Patient characteristics and toxicities were summarized using descriptive statistics.

Patient evaluation

Before each one-week cycle, a physical examination was performed, hematology and blood chemistry were monitored and toxicities and performance status were monitored. All toxicities were graded according to the National Cancer Institute Common Toxicity Criteria for Adverse Events (version 3.0). Patients remained on treatment with weekly 90 mg/m^2 paclitaxel i.v. until they no longer had clinical benefit from treatment or if toxicity led to patient withdrawal. Patients were replaced if they went off study before receiving the first two one-week cycles.

Results

In total 17 patients were included in this study. One patient showed clinical deterioration and went off study after the first week. The patient characteristics are briefly summarized in table 1.

Table 1: patient demographics

	overall (n=17)
Sex	
Female	5
Male	12
Age (years)	
Median	59
Range	28 - 80
ECOG performance status	
0	10
1	5
2	2
Pathological diagnosis	
NSCLC	5
Urothelial cell carcinoma	4
Primary unknown	2
Other	6
No. of Prior treatments (surgery, radiotherapy or chemotherapy)	
1	1
2	3
3	4
≥4	9

Pharmacokinetics

The mean pharmacokinetic parameters are given in table 2 and the mean concentration time-curves are given in figure 2. In figure 3A-C the values of $AUC_{0\text{-}24h}$ of each individual patient are plotted enabling intra-patient comparison of the systemic exposure to paclitaxel after the different boosters. The first four patients were included in Arm I. The $AUC_{0\text{-}24h}$ of paclitaxel after boosting with ritonavir was 732 ng/mL*h (coefficient of variation (CV): 59%) versus 1030 ng/mL*h (CV: 12%) after boosting with CsA. Although the mean exposure after boosting with ritonavir was approximately 29 % lower than CsA boosted paclitaxel, this demonstrates that low-dose ritonavir is able to boost paclitaxel exposure after oral administration, regarding the 8-9 fold increase in paclitaxel exposure after boosting with CsA as has been demonstrated previously.[5] Subsequently in Arm II –

IV the effect of clarithromycin and ketoconazole were determined. The results, presented in table 2 and figure 2, demonstrate that both CYP3A4 inhibitors had comparable effects on the systemic paclitaxel exposure and largely resulting in overlapping plasma concentration-time profiles compared to ritonavir boosted paclitaxel.

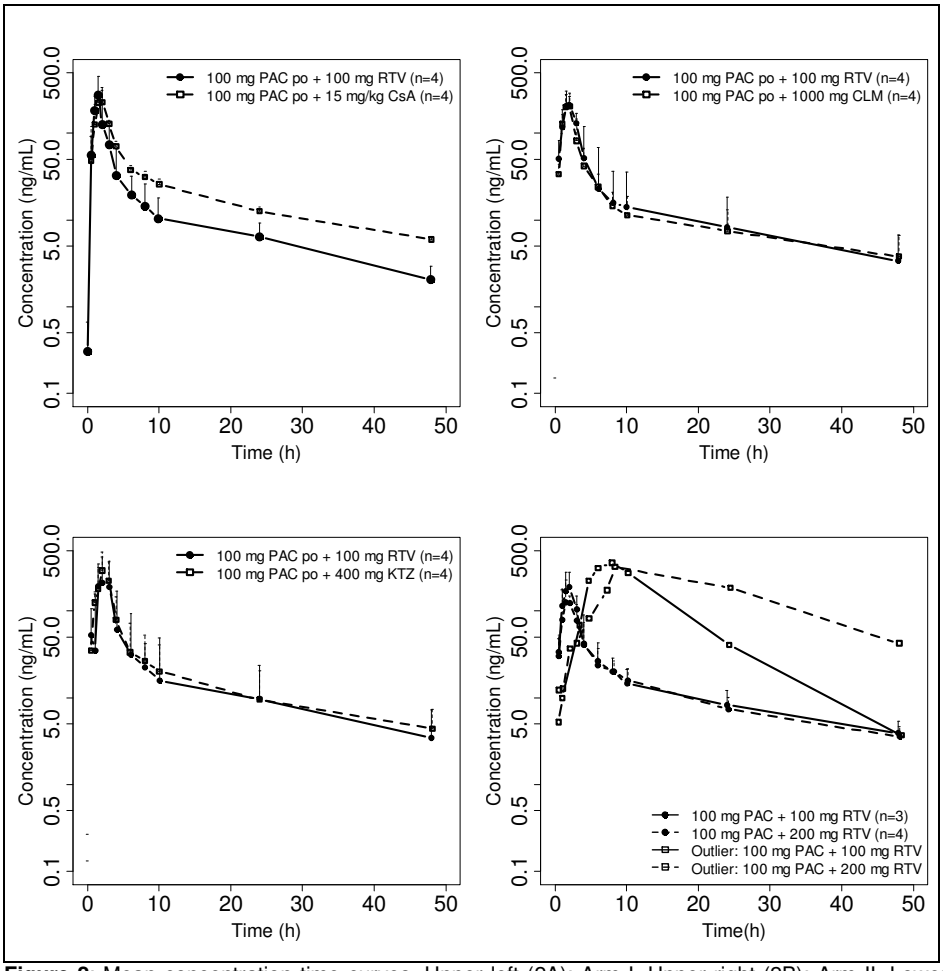

Figure 2: Mean concentration time curves. Upper left (2A): Arm I, Upper right (2B): Arm II, Lower left (2C): Arm III, Lower right (2D): Arm IV (Abbreviations: PAC: paclitaxel drinking solution, RTV: ritonavir, CsA: Cyclosporin A, CLM: clarithromycin, KTZ: ketoconazole)

Table 2: Pharmacokinetic parameters. (abbreviations: PAC: paclitaxel, RTV: ritonavir, CLM: clarithromycin, KTZ: ketoconazole)

			AUC_{0-24h}		C_{max}		T_{max}		$t_{1/2}$	
	Treatment	N	Mean (ng/mL*h)	CV (%)	Mean (ng/mL)	CV (%)	Median (h)	range (h)	Mean (h)	CV (%)
Arm I	PAC + 100 mg RTV	4	732	59	283	67	1.31	0.98-1.98	15.6	37
	PAC + 15 mg/kg CsA	4	1030	12	252	32	1.74	1.08-2.08	18.1	12
Arm II	PAC + 100 mg RTV	4	973	41	224	22	2.02	1.50-4.02	17.1	10
	PAC + 1000 CLM	4	751	39	233	33	1.52	1.50-2.00	20.8	20
Arm III	PAC + 100 mg RTV	4	1280	84	308	51	2.04	1.52-3.08	15.6	3.7
	PAC + 400 mg KTZ	4	1210	66	307	62	2.03	2.00-2.10	16.4	14
Arm IV	PAC + 100 mg RTV	4	1400	113	212	63	2.03	1.52-7.98	16.3	45
	PAC + 200 mg RTV	5	1570	115	226	36	2.00	1.52-8.37	17.4	7.0
Total	PAC + 100 mg RTV	14	1100	85	257	52	1.99	0.98-7.89	16.2	26

In all arms combined mean exposure to paclitaxel (AUC_{0-24h}) with ritonavir 100 mg as booster was 1100 ng/mL*h (n=16, CV: 81%) and the mean AUC with extrapolation to infinity, which could be determined in 15 patients, was 1380 ng/mL*h (CV: 81%).

A double dose of ritonavir, 200 mg versus 100 mg, was investigated in Arm IV in 5 patients of whom 4 were evaluable for PK analysis. One patient had a quickly deteriorating clinical disease after the first week and was replaced. No large effect of doubling the ritonavir dose was observed. One patient, had a more than 3-fold higher systemic exposure to paclitaxcel than the other investigated patients, see figure 2D and 3D. It concerned a 45 year old male black patient with gastric cancer. The concentration time curves of this patient were not taken into account for calculating the mean concentration time curves (figure 2D).

Safety

Safety data were available of 17 patients who received at least one dose of oral paclitaxel. Adverse events that were possibly, probably or definitely related to oral intake of 100 mg paclitaxel in combination with one of the investigated boosters were generally mild. The most reported adverse events were diarrhea (5 patients), nausea (4 patients), fatigue (4 patients) and neuropathy (4 patients) all of which were grade 1. There were no grade 2-5 possibly related adverse events observed.

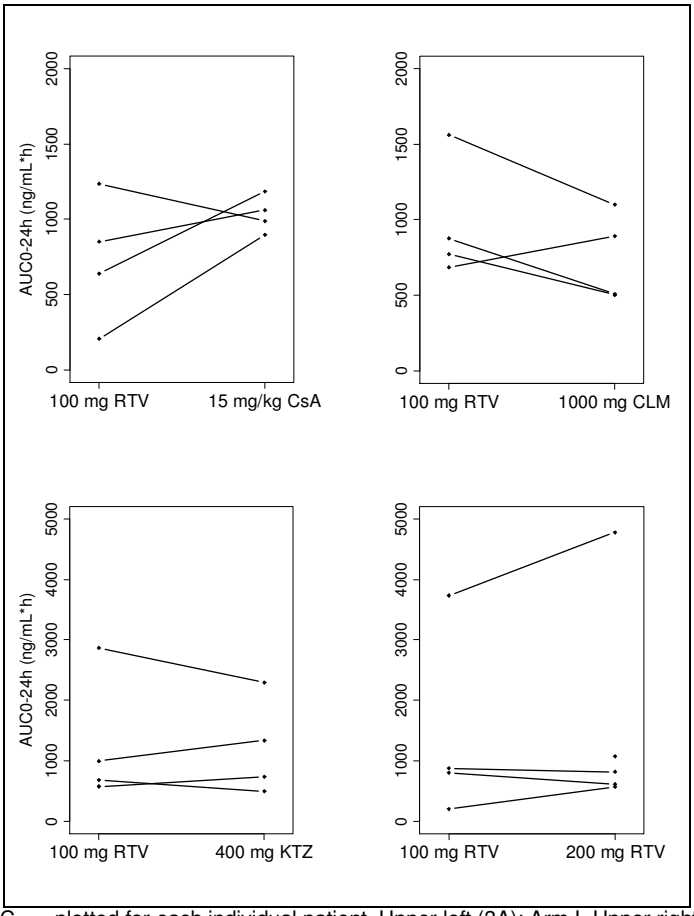

Figure 3: $AUC_{0\text{-}24h}$ plotted for each individual patient. Upper left (3A): Arm I, Upper right (3B): Arm II, Lower left (3C): Arm III, Lower right (3D): Arm IV (Abbreviations: PAC: paclitaxel drinking solution, RTV: ritonavir, CsA: Cyclosporin A, CLM: clarithromycin, KTZ: ketoconazole)

Discussion

This study demonstrates that paclitaxel exposure upon oral administration can be effectively boosted by the CYP3A4 inhibitors ritonavir, clarithromycin and ketoconazole. No relevant differences in the oral PK of paclitaxel were observed comparing CsA ritonavir 100 mg clarithromycin and ketoconazole.

These data form the basis to continue the development of oral paclitaxel in combination with low dose ritonavir. Ritonavir was selected based on the extensive experience gained with ritonavir boosted drugs, the good safety profile of 100 – 200 mg ritonavir and the high increase in systemic exposure to paclitaxel after boosting with ritonavir.

It should be noted that paclitaxel was not given without a booster, in this study. Previous studies demonstrated that 15 mg/kg CsA results in approximately 8-9 fold increase in systemic exposure to paclitaxel.[5,22] This study demonstrated that the effect of 100 -200 mg ritonavir is in the same order.

In this study, and also in previous studies employing orally administered paclitaxel, the booster was given 30 minutes prior to paclitaxel. In studies with oral docetaxel it has been shown that the systemic exposure to docetaxel did not significantly differ when ritonavir was given 1 hour prior to or concomitant with docetaxel.[13] However, the optimal time-interval between ritonavir and paclitaxel remains to be determined.

The optimal dose of ritonavir is probably dependent on the paclitaxel schedule. In anti-HIV treatment, daily or bi-daily doses of more than 100 mg generally result in only marginal further increases in systemic exposure to the target drug compared with a dose of ritonavir of 100 mg.[23] Thus far, the PK data of only two patients were available who received oral paclitaxel in combination with 100 or 200 mg ritonavir. Therefore, no relevant conclusion can be deduced from this data.

In conclusion, ritonavir dosed at 100-200 mg resulted in significant systemic exposure to oral paclitaxel applied as a drinking solution. The concept of improved systemic exposure of oral paclitaxel was further demonstrated by the fact that also the well known CYP3A inhibitors clarithromycin and ketoconazole relevantly increased systemic exposure to oral paclitaxel. For safety reasons ritonavir as booster drug is to be preferred above CsA.

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Chapter 3.2

Pharmaceutical and clinical development of a new oral solid dispersion formulation of paclitaxel

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Abstract

A new oral solid dispersion formulation of paclitaxel (ModraPac001 10 mg capsules) administered in combination with the pharmacokinetic (PK) booster, ritonavir, has been developed and investigated in patients with advanced cancer. Paclitaxel has a very low permeability and a very low aqueous solubility (biopharmaceutical classification system class IV). Therefore a PK booster was used to improve the permeability, and a solid dispersion formulation was developed to improve the solubility of paclitaxel.

Solid dispersions were freeze-dried from tert-butanol (TBA)/water mixtures and characterized by X-ray diffraction and differential scanning calorimetry. In vitro dissolution and long term stability were evaluated. The best performing formulation, a ternary solid dispersion system of paclitaxel, polyvinylpyrrolidone (PVP)-K30 and sodium dodecyl sulphate (SDS), was selected for evaluation against a paclitaxel drinking solution in 4 patients with advanced cancer.

The mean systemic exposure to paclitaxel after oral administration of 30 mg paclitaxel and 100 mg ritonavir was 211 ± 77 ng/mL*hr for ModraPac001 10 mg capsules compared to 267 ± 108 ng/mL*hr for the paclitaxel drinking solution. The measured systemic exposure of the capsules was 21% lower than the paclitaxel drinking solution. However, taking in to account the good pharmaceutical characteristics of ModraPac001, e.g. the preferred drug-handling by personal and patients, the prolonged shelf-life and the ambient storage condition, the lower systemic exposure was considered acceptable. These results encourage further research in to the oral administration of paclitaxel using the ModraPac001 10 mg capsule formulation in combination with the PK booster ritonavir.

Introduction

The intravenous (i.v.) formulation of paclitaxel (Taxol® and several generic products) is approved for the treatment of various malignancies. In principle, oral administration of paclitaxel has several advantages over intravenous administration, such as patient convenience and improved safety due to the absence of the solvents Cremophor EL and ethanol. Furthermore, dexamethason pre-medication is possibly not needed when paclitaxel is given orally. However, paclitaxels oral bioavailability is limited by the poor aqueous solubility, the affinity for the drug efflux pump P-glycoprotein (P-gp) and by the extensive presystemic metabolism by cytochrome P450 (CYP) enzymes.

It was demonstrated that oral administration of paclitaxel and an inhibitor of P-gp, (Cyclosporin A, CsA), resulted in an increased systemic exposure to paclitaxel [1]. In addition to this, phase II studies showed that orally administered paclitaxel, pharmacokinetically boosted by CsA, also had promising anti-tumor activity [2,3,4]. Recently, we demonstrated that orally administered paclitaxel can effectively be boosted

by inhibiting CYP3A4 (Koolen et al. in preparation; chapter 3.1 of this thesis). Ritonavir, one of the tested CYP3A4 inhibitors was selected for further studies. Ritonavir is a HIV protease inhibitor and a potent inhibitor of CYP3A. In HIV therapy it is predominantly used at low doses to enhance the systemic exposure to other protease inhibitors.[5]

To date, all our previous clinical studies with orally administered paclitaxel were conducted with the i.v. formulation applied as a drinking solution. However, this formulation is far from optimal because the drinking solution has a poor taste, is potentially unsafe for healthcare workers due to a high contamination risk, is difficult to dose, and has a poor physical and chemical stability.

Thus far, the poor aqueous solubility of paclitaxel (paclitaxel dihydrate: 1 µg/mL [6]) limited the development of an appropriate formulation for oral administration of paclitaxel. To overcome this limitation we developed a solid dispersion formulation. A solid dispersion is defined as a molecularly dispersed drug in a hydrophilic matrix. The matrix consists of carriers such as PVP, polyethylene glycol (PEG), and sucrose.[7,8,9] They have been successfully applied to improve the solubility and bioavailability of several low-solubility drugs (e.g. griseofulvin, tacrolimus, everolimus, ritonavir and lopinavir.[10] The improved solubility of solid dispersion systems is attributed to the reduced particle size, the dispersion of drug particles in a highly soluble matrix, and to the formation of a more soluble physical state (e.g. an amorphous state).[11]

Liggins et al.[12] previously showed that there are at least three different physical states of paclitaxel, anhydrous paclitaxel, paclitaxel dihydrate and amorphous paclitaxel, each with their own aqueous solubility. The most stable form, paclitaxel dihydrate has an equilibrium solubility of approximately 1 µg/mL, while the less stable anhydrous paclitaxel has a peak solubility of 3.5 µg/mL. Liggins et al. collected no solubility data for amorphous paclitaxel, although other authors reported values of 30 µg/mL.[13]

In this study we show that oral administration of the solid dispersion formulation of paclitaxel (ModraPac001 10 mg capsules) in combination with the PK booster ritonavir results in clinically relevant systemic exposure to paclitaxel.

Materials and Methods

Pharmaceutical development

Paclitaxel was purchased from Indena (Milan, Italy). Various grades (PVP K12, PVP-K17 and PVP-K30) of polyvinylpyrrolidone (PVP) were kindly supplied by BASF (Ludwigshafen, Germany). Tert-butanol (TBA), sodium dodecyl sulphate (SDS) and dimethyl sulfoxide (DMSO) were purchased from VWR (Amsterdam, The Netherlands). Water for Injection (WfI) was obtained from B. Braun (Melsungen, Germany).

Hydroxypropyl-ß-cyclodextrin (HP-ß-CD) was supplied by Roquette (Lestrem, France). Hard gelatin capsules were purchased from Capsugel (Bornem, Belgium).

Preparation of paclitaxel formulations

Paclitaxel formulations were prepared by freeze-drying and/or physical mixing (mortar and pestle). Freeze-drying of paclitaxel formulations was performed in stainless steel boxes (Gastronorm size 1/9) using a freeze dryer (Model Lyovac GT4, GEA Lyophil GmbH, Hürth, Germany) according to a method previous developed by Van der Schoot et al. [14] Capsules were produced by weighing an amount of freeze-dried solid dispersion powder equivalent to 5, 10 or 25 mg drug. The solid dispersion powder was mixed with lactose and SDS using mortar and pestle and encapsulated with a manual capsulation apparatus into size 0 hard gelatin capsules.

Dissolution testing

Dissolution of the capsule formulation was tested according to the European Pharmacopoeia, using a type 2 (paddle) dissolution apparatus (Erweka, Heusenstamm, Germany) filled with 500 to 900 mL WfI or Simulated Intestinal Fluid without pepsin (SIFsp) at 37 °C stirred at 75 or 100 rpm. Samples were collected at various timepoints, filtrated using a 0.45 µm filter and diluted 1:1 v/v with a 1:4 v/v mixture of methanol and acetonitrile. Samples were subsequently analyzed by RP-HPLC-UV. For solubility screening of the paclitaxel formulations, a dissolution screening method was used. Briefly, an amount of powder, equivalent to approximately 4 mg paclitaxel, was added to a 50 mL beaker containing 25 milliliter of WfI at 37 °C and a magnetic stirring bar stirred at 720 rpm. Samples were collected at various time points, filtrated using a 0.45 µm filter and diluted 1:1 v/v with a 1:4 v/v mixture of methanol and acetonitrile. Samples were subsequently analyzed on a reversed phase HPLC system with UV detection (RP-HPLC-UV).

RP-HPLC-UV

Samples were analyzed using an isocratic HPLC-UV system (Thermo Scientific, Breda, The Netherlands) equipped with a reversed phase analytical column (Apex 150 x 4.6 mm 5 µm, C8). The mobile phase consisted of 1 part of methanol, 4 parts of acetonitrile and 5 parts of a 0.02 M ammonium acetate solution. Paclitaxel was detected at 227 nm with a flow of 1.0 mL/min and an injection volume of 10 µL.[15]

X-ray powder diffraction

X-ray powder diffraction measurements were performed on a X'pert pro diffractiometer equipped with an X-celerator (PANanalytical, Almelo, The Netherlands). Samples of approximately 0.5 mm thick were placed in a metal sample holder, placed in the diffractiometer and scanned at a current of 50 mA and a tension of 40 kV. Scan range

was 10-60 degrees 2-theta, with a step size of 0.020 degrees and a scan speed of 0.002 degrees per second.

Modulated differential scanning calorimetry (MDSC)
MDSC measurements were performed on a Q2000 differential scanning calorimeter (TA Instruments, New Castle, DE, USA). Temperature scale and heat flow were calibrated with indium. Samples of approximately 10 mg powder were transferred into Tzero Aluminium pans (TA instruments), hermetically closed and placed in the autosampler. Samples were equilibrated at 20.00 °C, after 5 minutes the samples were heated to 190.00 °C at a speed of 2.00 °C/min. Modulation was performed every 60 seconds at +/- 1.00 °C

Fourier Transform Infrared spectroscopy (FT-IR)
Infared spectra were recorded from 400– 4000 cm^{-1} with a resolution of 2 cm^{-1} on a FT-IR 8400S Spectrophotometer equipped with a golden gate ® (Shimadzu, 's-Hertogenbosch, The Netherlands).
Residual solvents
Residual water was determined with the Karl Fischer method using a Metrohm 758 KFD Titrino (Herisau, Switzerland). Samples of approximately 50 mg were dissolved in 5 mL of preconditioned methanol, the titrant was standardized with 30 mg of WfI.
Residual TBA was determined with gas chromatography (GC) analysis. Samples of approximately 50 mg were dissolved in 5.0 mL of DMSO. The GC system was composed of a Model 5890 gas chromatograph equipped with a flame ionization detector, a split-splitless injector, and a Model 6890 series autosampler (Agilent, Amstelveen, The Netherlands). Separation was achieved with a Crossbond 6% cyanopropylphenyl–94% dimethylpolysiloxane (30 m × 0.53 mm ID × 3.0 µm film thickness) column.[16]

Clinical development
Patients
Patients for whom no standard therapy of proven benefit existed and with a histologically confirmed cancer refractory to current therapies were eligible for the study. Other eligibility criteria included: Age > 18 years; life expectancy > 3 months; no radio- or chemotherapy within the last 4 weeks prior to study entry. Patients had to have a World Health Organization (WHO) performance status $\leq$ 2 and adequate hematological, renal and hepatic function. Patients were not eligible if they suffered from uncontrolled infectious disease, neurologic disease, bowel obstructions, or symptomatic brain metastases. Other exclusion criteria were concomitant use of known P-gp or CYP3A4 inhibitors and chronic use of H2-receptor antagonists or proton pump inhibitors. The

study protocol was approved by the medical ethics committee of the institute and all patients had to give written informed consent prior to start of the study.

Study Design

This study was designed as a randomized, open label proof of concept study. In total 4 evaluable patients with advanced cancer were included. During the first two weeks, patients received 30 mg paclitaxel p.o. and 100 mg ritonavir p.o. Paclitaxel was either formulated as a paclitaxel drinking solution (Paclitaxel Mayne, Mayne Pharma, Melbourne Australia) or the developed solid dispersion formulation (ModraPac001 10 mg capsules, Slotervaart Hospital Amsterdam, The Netherlands). Patients were randomized into two groups. Two patients received ModraPac001 10 mg capsules in the first week followed by the paclitaxel drinking solution in the second week, the other two patients received both formulations in the reversed order. All Patients continued in week 3 with weekly 90 mg/m^2 paclitaxel i.v. (Paclitaxel Mayne, Mayne Pharma, Melbourne Australia) in their best interest. Patients remained on treatment until they no longer had clinical benefit, or if toxicity led to patient withdrawal.

Drug administration

Patients took the study drugs either 1 hour before or 2 hours after a light breakfast. Thirty minutes prior to oral administration or paclitaxel, patients received one capsule of ritonavir 100 mg (Norvir®; Abbott, Illinois, USA). Both ritonavir and paclitaxel were taken in combination with approximately 150 mL tap water. Pre-treatment consisted of 4 mg dexamethason 1 hour prior to, and 12 and 24 hours after paclitaxel intake. Additionally, patients also received 1 mg granisetron orally 1 hour prior to oral administration of paclitaxel and ritonavir to prevent nausea and vomiting.

Premedication for intravenously administered paxclitaxel (in week 3 and beyond) consisted of dexamethason 10 mg p.o. 12 hours prior to paclitaxel infusion and dexemathason 10 mg i.v., clemastine 2 mg i.v. and ranitidine 50 mg i.v 30 minutes prior to paclitaxel infusion.

Patient evaluation

Pretreatment evaluation included a complete physical examination and a review of the medical history. Before each course, a physical examination was performed, and toxicities and performance status were monitored. Hematology and blood chemistry were monitored weekly. All observed toxicities were graded according to the National Cancer Institute Common Toxicity Criteria for Adverse Events (NCI CTCAE) version 3.0. Tumor evaluation was performed every 6 weeks. Tumor responses were evaluated according to RECIST criteria version 1.0.[17]

Pharmacokinetic analysis

The pharmacokinetic profile of both the paclitaxel drinking solution and the ModraPac001 10 mg capsules was determined. Blood samples for pharmacokinetic analysis were collected during the first and second week. Blood samples were drawn in lithium heparinized tubes at baseline and at 0.5, 1, 1.5, 2, 3, 4, 6, 8, 10, 24 and 48 hours after paclitaxel intake. Samples were immediately placed on ice and were centrifuged within 1 hour at 1500 x g for 10 minutes at 4°C. Plasma was stored at or below -20°C until analysis.

Analytical Assay

Paclitaxel was quantified in plasma by use of high-performance liquid chromatography with tandem mass spectrometric detection (LC-MS/MS) described earlier.[18] The lower limit of quantification of the assay was 0.25 ng/mL paclitaxel.

Data analysis

The individual pharmacokinetic parameters were analyzed using descriptive pharmacokinetic methods and validated R scripts (R version 2.10.0).[19] The areas under the plasma concentration-time curves to the last quantifiable sample point (AUC_{0-t}) were estimated by the linear trapezoidal (absorption phase) and logarithmic trapezoidal rule (elimination phase). The areas under the plasma concentration-time curves to infinite time (AUC_{inf}) were calculated by extrapolation. Furthermore, the observed maximum plasma concentration (Cmax) and time to maximal plasma concentration (Tmax) were reported.

Results and discussion

Pharmaceutical development

In our initial experiments we were able to produce amorphous paclitaxel by first dissolving crystalline paclitaxel in tert-butanol/water mixtures and subsequently removing these solvents by freeze-drying. Both X-ray diffraction, MDSC, and FTIR analysis showed that crystalline paclitaxel was converted to amorphous paclitaxel (see figure 1). The next step was to find the best carrier and the optimal drug load. We selected four carriers (PVP-K12, PVP-K17, PVP-K30 and HP-b-CD) and four drug loads (10, 25, 40 and 75% w/w of paclitaxel) to screen for the most optimal solid dispersion composition.

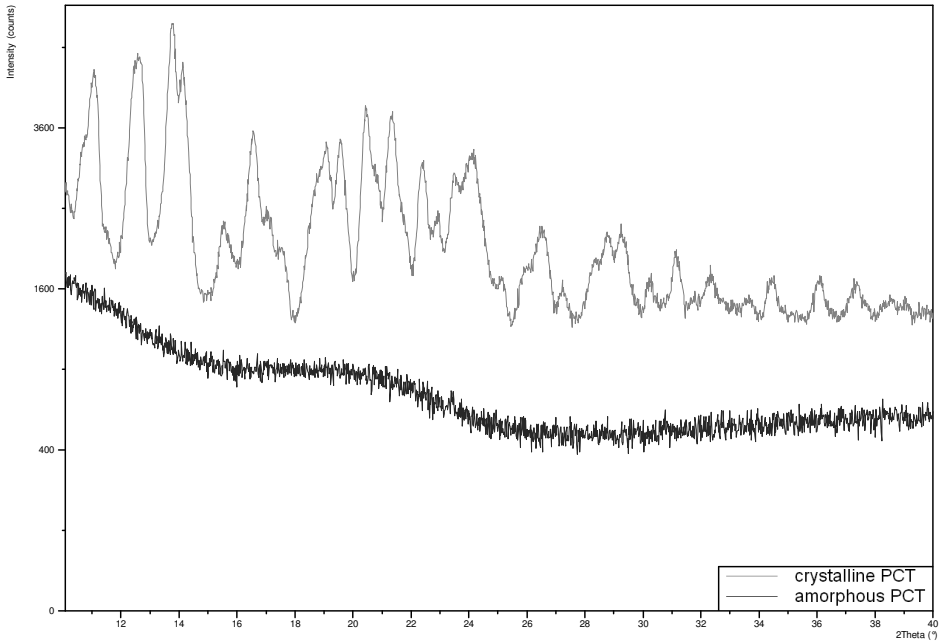

Figure 1a: X-ray diffraction spectra: Crystalline and amorphous paclitaxel

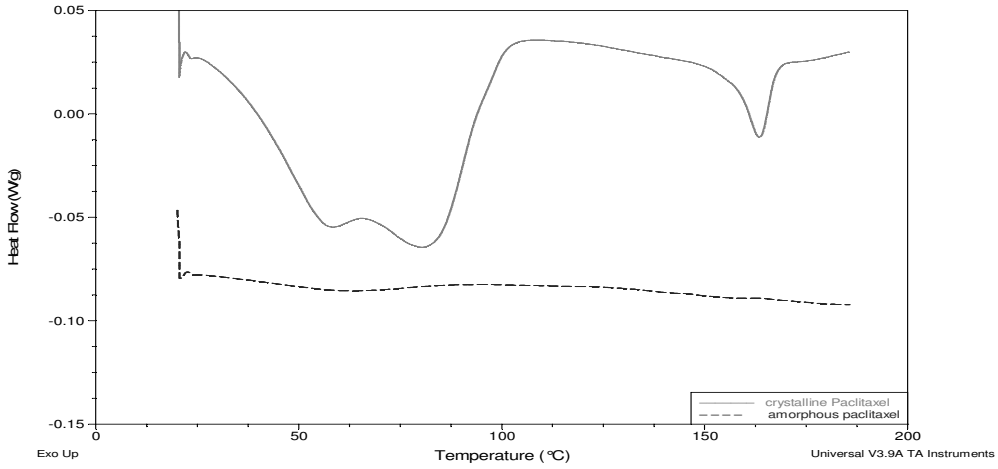

Figure 1b: MDSC thermograms: Crystalline and amorphous paclitaxel

To select the most appropriate carrier, 25 mg paclitaxel capsules were produced from solid dispersions with PVP-K12, PVP-K17, PVP-K30 or HP-b-CD. Subsequently, the solubility of the different capsules were investigated. To test the carriers ability to inhibit the paclitaxel precipitation the target concentration of the dissolution test was set at 50 µg/mL (25 mg in 500 mL), well above the reported maximum solubility of amorphous paclitaxel, 30 µg/mL [20]. The dissolution profiles of the 4 different capsules are shown in figure 2.

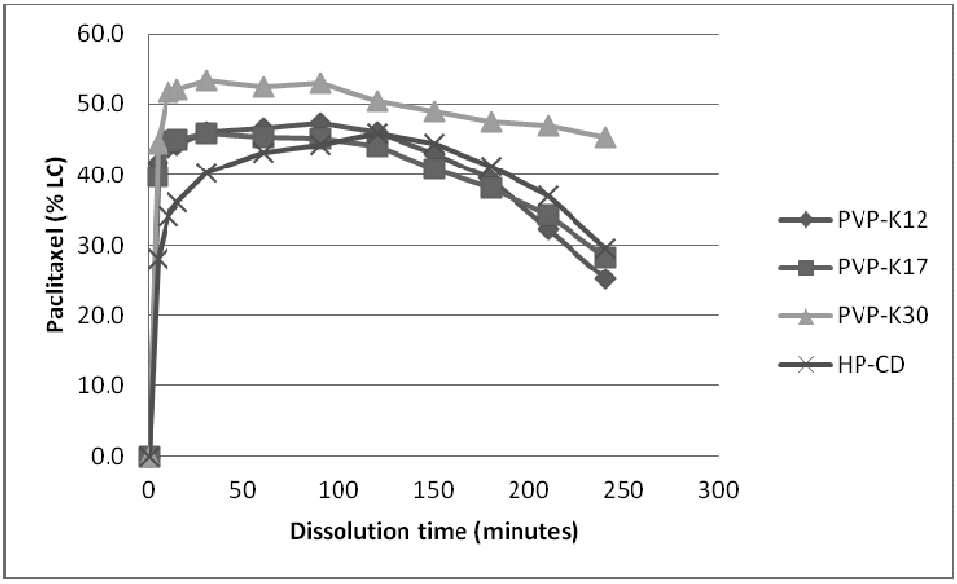

Figure 2: Dissolution profiles of paclitaxel solid dispersion capsules with four different carriers and a drug to carrier ratio of 40% w/w. (25 mg of paclitaxel per capsule, 500 mL WfI, 100 RPM, 37 ℃)

The type of carrier influences the dissolution rate, the maximum solubility and the time and extent of paclitaxel precipitation. The capsule with the paclitaxel/PVP-K30 solid dispersion system releases more than 53% of the total amount within 30 minutes and is able to keep at least 45% of the total amount of paclitaxel in solution for 4 hours. In contrast, the HP-b-CD solid dispersion releases only 40% of the total amount of paclitaxel within 30 minutes and is not able to keep more than 30% of the total amount of paclitaxel in solution for 4 hours. PVP-K12 and PVP-K17 solid dispersions system have a higher initial release than HP-b-CD, but are unable to prevent the precipitation of paclitaxel after 120 minutes. The difference in performance between the three types of PVP is most probably related to the differences in PVP chain length. PVP is believed to inhibit drug precipitation by two mechanisms. One mechanism is the formation of hydrogen bonds between PVP and drug molecules thereby shielding drug particles from each other Increased viscosity of the dissolution medium is another mechanism; the

increased viscosity limits the mobility of the drug molecules which makes it more difficult to form crystals. At higher PVP chain lengths, both mechanisms will be more effective.[21] [22]

To further optimize the paclitaxel solid dispersion the influence of the drug to carrier ratio on the solubility of paclitaxel was investigated. Four solid dispersions with different drug to carrier ratios, and 100% amorphous paclitaxel were tested with the dissolution screening method. For this particular test a paclitaxel target concentration of 150 µg/mL was used, this is approximately 150 times the equilibrium solubility of paclitaxel dihydrate and approximately 5 times the maximum solubility of amorphous paclitaxel.[23] The dissolution screening curves shown in figure 3 are typical for a supersaturated drug, a rapid dissolution to a supersaturated solution is followed by precipitation of the drug after which the equilibrium solubility of the most stable form is reached. The maximum solubility of amorphous paclitaxel is approximately 30 µg/mL, this is in accordance with values reported earlier.[24] However, this solubility can only be reached when the precipitation of paclitaxel is inhibited by a carrier, 100% amorphous paclitaxel precipitates immediately upon contact with water and is therefore not able to reach its maximum solubility. Addition of a carrier to amorphous paclitaxel leads to a higher maximum solubility which is maintained for a longer time period (100% PCT versus 75% PCT). Higher amounts of carrier in the solid dispersion system further improve the dissolution rate, the maximum solubility and the time to precipitation. The solid dispersion with a drug load of 10% w/w (drug to carrier ratio 1/10) showed the best performance.

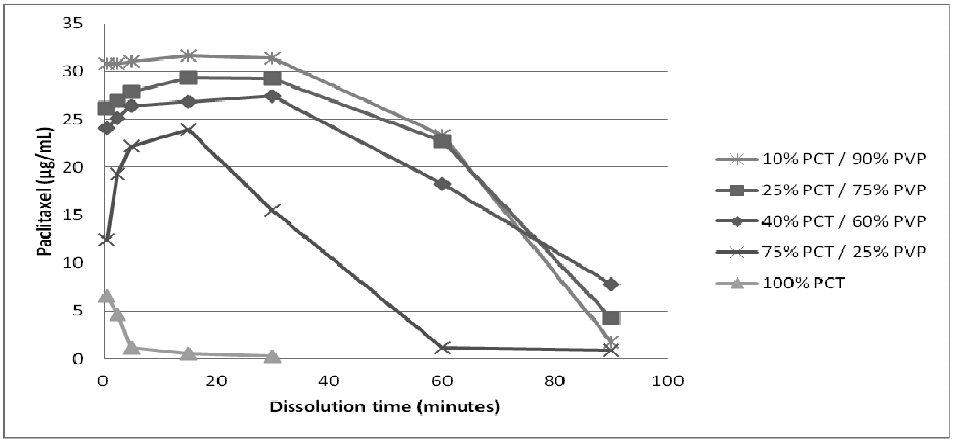

Figure 3: Dissolution screening curves of amorphous paclitaxel and paclitaxel/PVP-K17 solid dispersions with various drug to carrier ratios

To further proof that the paclitaxel solid dispersions resulted in improved solubility, the dissolution profiles of capsules prepared from a solid dispersion powder of paclitaxel and PVP-K17 (SD) were compared to the dissolution profiles of capsules prepared from a physical mixture (PM) of paclitaxel and PVP-K17, using a standard Pharmacopoeial dissolution test (figure 4). The dissolution profiles clearly show the improved dissolution of the paclitaxel solid dispersion capsules. Less than 40% of the total amount of paclitaxel is released from the physical mixture capsules, while almost 100% of the total amount of paclitaxel is released from the solid dispersion capsules. The relative amount of paclitaxel dissolved is lower for the 10 mg physical mixture capsule compared to the 5 mg physical mixture capsule, indicating that the maximum solubility of paclitaxel from the PM is already reached. In contrast, the relative amount dissolved of paclitaxel is the same for both 5 and 10 mg solid dispersion capsules.

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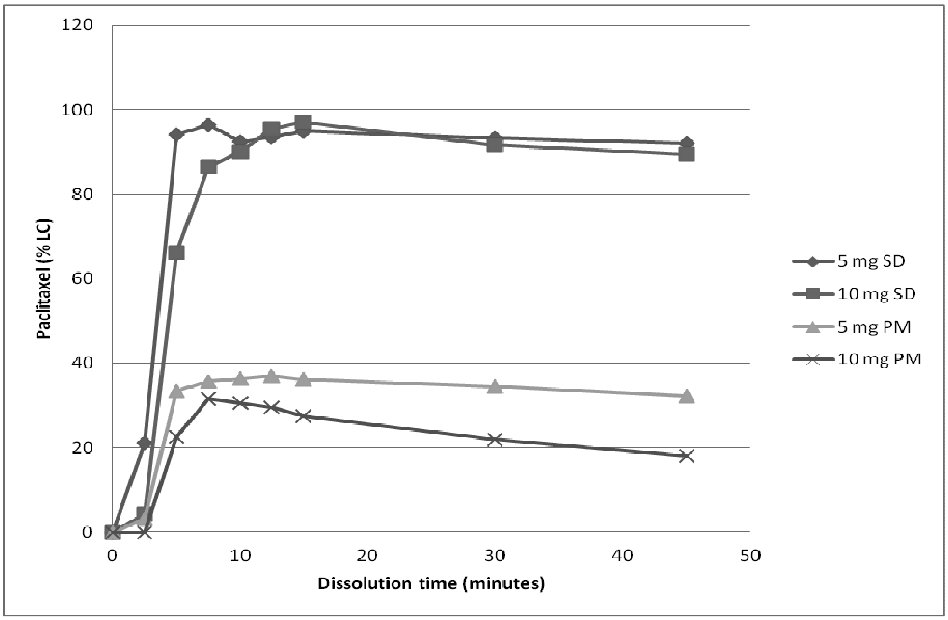

Figure 4: Dissolution profiles of 5 and 10 mg paclitaxel capsules prepared from physical mixtures (PM) or solid dispersions (SD**)** of paclitaxel (20%) and PVP-K17 (80%).

All of the tested solid dispersion systems contained no surfactant to eliminate any effects during the solid dispersion formation. However, several tests showed that a surfactant was necessary for the release of paclitaxel form the capsules. It was therefore decided to add the surfactant, SDS to the capsule by physical mixing it with the solid dispersion

powder. After the most suitable carrier and drug to carrier ratio were selected additional test were performed which proved that incorporation of the surfactant into the solid dispersion was even more effective than addition of the surfactant to the capsule powder (data not shown). Therefore, the final paclitaxel solid dispersion formulation contains 1/11 w/w paclitaxel, 9/11 w/w PVP-K30 and 1/11 SDS (ModraPac001 10 mg capsules).
Quality control testing of the ModraPac001 10 mg capsules showed a very rapid dissolution of paclitaxel, which remaind in solution for at least 4 hours. Retest performed after 12 months of storage at 2 – 8°C and at 25 °C / 60% RH gave no indication of a change in the chemical or physical properties of the ModraPac001 10 mg capsules.

Clinical development

In total 4 patients (3 male and 1 female) were included with a median age of 51 years (range 47 – 63 years). All patients had metastatic disease for which they received at least two lines of prior anti-cancer therapy. Patient 2 was given paclitaxel drinking solution 2 hours after ritonavir intake and patient 4 was given ModraPac001 simultaneously with ritonavir. The selected 30 mg dose was below the standard paclitaxel weekly i.v. (90 mg/m^2) and p.o. (phase II studies: weekly 90 mg/m^2 bi-daily) regimen because of safety considerations and the non-linear pharmacokinetics observed at higher doses of orally administered paclitaxel.[25]
The mean systemic exposure to paclitaxel, measured by AUC_{inf}, after oral administration of 30 mg paclitaxel and 100 mg ritonavir was 211 ± 77 ng/mL*hr for ModraPac001 10 mg capsules compared to 267 ± 108 ng/mL*hr for the paclitaxel drinking solution. The mean maximum plasma concentration was 48 ng/mL after intake of the paclitaxel drinking solution and 42 ng/mL after administration of ModraPac001 (table 1 and figure 5).

Table 1: Pharmacokinetic parameters, AUC_{inf}, $C_{max,}$ and T_{max}, after administration of 30 mg paclitaxel drinking solution (LF) or ModraPac001in combination with 100 mg ritonavir.

	30 mg Paclitaxel LF + 100 mg ritonavir			30 mg ModraPac001 + 100 mg ritonavir		
ID	AUC_{inf} (ng/mL*h)	C_{max} (ng/mL)	T_{max} (h)	AUC_{inf} (ng/mL*h)	C_{max} (ng/mL)	T_{max} (h)
1	371	66	1.97	127	29	2
2	325*	53	2	226	63	2.02
3	247	38	1.07	310	30	2.02
4	125	34	1.52	179**	45	1.6
mean	267	48	1.64	211	42	1.91
CV (%)	108	14	0.44	77	16	0.21

*ritonavir was given 2 hours prior to paclitaxel liquid formulation

**ritonavir was given simultaneously with ModraPac001

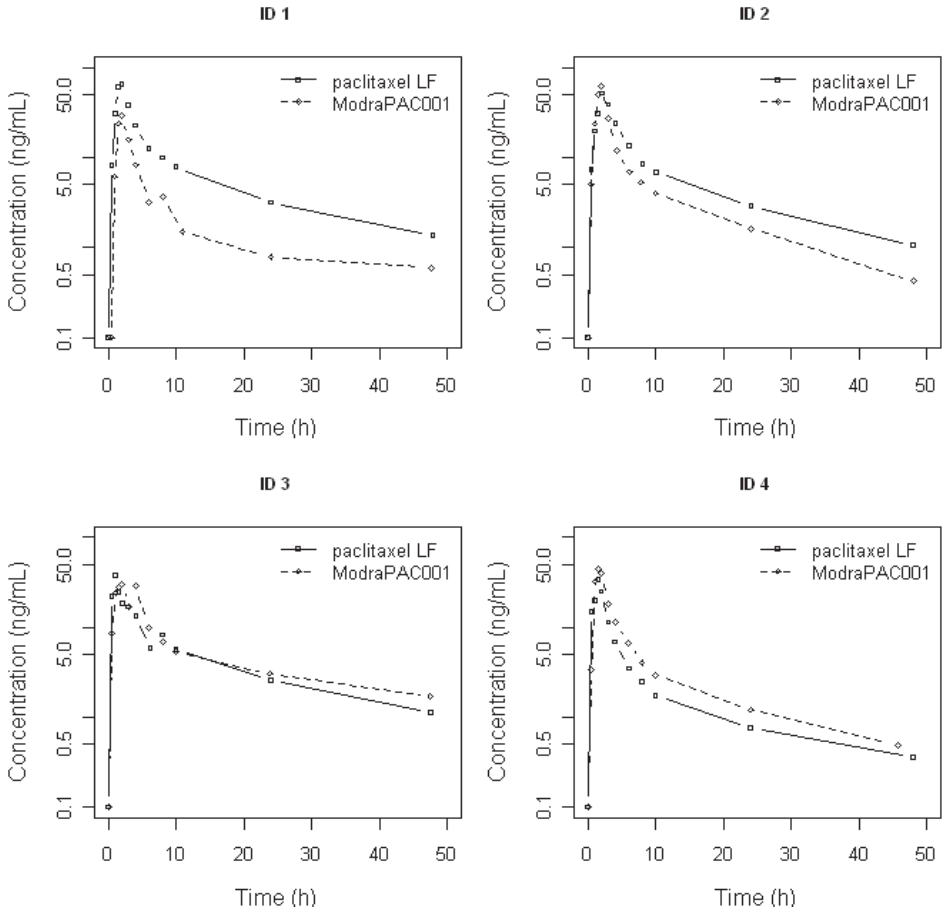

Figure 5: Plasma concentration time curves of the four patients who participated in this study. On the Y-axis the paclitaxel plasma concentration in ng/mL and on the X-axis the time in hour. The solid line represents the concentration time curve of paclitaxel drinking solution (LF) and the dotted line the ModraPac001 capsule.

Adverse events that were possibly related to orally administered paclitaxel, either the paclitaxel drinking solution or ModraPac001 10 mg capsules, were grade 1 nausea (2 patients), voice changes (2 patients), vomiting (1 patient), myalgia (1 patient) and grade 2 pyrexia (1 patient).

This study was designed as a pilot study to demonstrate that the systemic exposure to paclitaxel after intake of ModraPac001 10 mg capsules would be in same the range as after intake of the oral drinking solution of paclitaxel. These results confirm that oral administration of paclitaxel in the form of a solid dispersion capsule formulation is possible. These findings warrant further development of the oral solid dispersion formulation of paclitaxel, ModraPac001 10 mg capsules, in combination with the PK booster ritonavir.

Thus far many oral formulations of paclitaxel were investigated, for example: Paxoral®,[2] SMEOF#3[26] and a polymeric paclitaxel formulation.[27] The development of these formulations was terminated due low systemic exposure, high variability in exposure to paclitaxel and/or unpractical drug administration. Several taxanes have been especially designed for oral administration: BMS275183, ortaxel (IDN-5109),[28] IDN-5390,[29] and milataxel (MAX-321).[30] However, due to high variability in PK, unfavourable safety profile or lack of anti-tumor activity, the clinical development of these compounds was terminated.

In contrast, PK boosted oral administration of paclitaxel has demonstrated anti-tumor activity and acceptable toxicity in 3 phase II studies.[2,3,4] However, further development of the combination of the paclitaxel drinking solution with CsA as PK booster was hampered by the unpractical formulation and the safety profile of CsA. In this study we demonstrated that the oral solid dispersion formulation of paclitaxel, ModraPac001 10 mg capsules, has a pharmacokinetic profile comparable to the drinking solution. Furthermore, ModraPac001 10 mg capsules have favourable pharmaceutical characteristics such as a 12 month stability at ambient conditions, an acceptable taste, and allow safe and practical dosing of a highly toxic anti-cancer agent. These promising results encourage further investigation of oral administration of paclitaxel by administration of ModraPac001 10 mg capsules in combination with ritonavir.

Future plans with oral administration of paclitaxel consist of a phase I study on metronomic paclitaxel by bi-daily dosing of ModraPac001 in combination with ritonavir. Continuous exposure to low concentrations of paclitaxel has been reported to have anti-tumor activity by inhibiting angiogenesis.[31] Metronomic dosing of paclitaxel has proven to inhibit angiogenesis, both in vitro and in vivo, by inhibition of the proliferation and differentiation of endothelial cells.[32,33] A recent phase II study with 96-hour paclitaxel infusions confirmed these findings, however the long infusion times resulted in a high incidence of bacterial infections.[34] Our oral solid dispersion formulation of paclitaxel, ModraPac001 10 mg capsules, might enable successful translation of the metronomic paclitaxel concept into clinical application.

Conclusions

We successfully developed a new solid oral dosage form of paclitaxel using a solid dispersion formulation approach. The solid dispersion proved to be stable for at least 12 months at ambient conditions.

Oral administration of the solid dispersion formulation of paclitaxel, ModraPac001 10 mg capsules, in combination with the PK booster ritonavir resulted in clinically relevant systemic exposure to paclitaxel. The new oral formulation of paclitaxel enables the development of metronomic chemotherapy with paclitaxel.

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4

Pharmacological studies on gemcitabine

Chapter 4.1

Phase I study of oral gemcitabine prodrug (LY2334737) alone and in combination with erlotinib in patients with advanced solid tumors

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Abstract

LY2334737 is an orally available prodrug of gemcitabine. It was designed to overcome the extensive presystemic deamination of orally administered gemcitabine. The objective of this study was to determine the maximum tolerable dose (MTD) and dose limiting toxicities (DLT) of daily LY2334737 with or without erlotinib.

Patients with advanced or metastatic cancer refractory to standard therapy were eligible. Patients were treated with escalating doses of LY2334737 monotherapy 5-50 mg administered once-daily for 14 days of a 21-day cycle. After obtaining the first safety data, patients were also treated with escalating doses of LY2334737 20-40 mg in combination with 100 mg erlotinib. Safety was determined throughout and pharmacokinetics were evaluated. After determination of the MTD, the study was extended with a bioequivalence trial to investigate a novel LY2334737 drug-formulation.

Results: In total 65 patients were treated in this study. The MTD for LY2334737 was 40 mg for both single agent and as combination with 100 mg erlotinib. Fatigue was the most frequent DLT for LY2334737 monotherapy (4 patients) followed by elevated transaminases (2 patients), both observed at the 40-50 mg dose levels. Two patients in the combination arm had DLTs at the 40 mg dose level. These were fatigue and elevated liver enzymes. The most common adverse events were fatigue (n=38), nausea (n=27), vomiting (n=24), diarrhea (n=23), anorexia (n=20), pyrexia (n=18) and elevated transaminases (n=14). The PK showed dose proportional increase in LY2334737 and gemcitabine exposure. The metabolite 2',2'-difluorodeoxyuridine accumulated with an accumulation index of 4.3 (CV%: 20%). In one patient complete response in prostate-specific-antigen was observed for four cycles and stable disease was achieved in 22 patients overall. Additionally, PK analysis demonstrated that the two investigated LY2334737 drug-formulations were bioequivalent.

Conclusion: LY2334737 displays linear pharmacokinetics and the MTD is 40 mg with or without daily 100 mg erlotinib. Signs of anti-tumor activity warrant further development.

Introduction

Gemcitabine (2',2-difluorodeoxycytidine or dFdC) is an anticancer agent approved for the treatment of a variety of solid tumor types, including pancreatic, non-small-cell-lung, ovarian, bladder and breast cancer. The anticancer activity of gemcitabine is mediated through its effects on DNA synthesis in rapidly dividing cells. Gemcitabine must be phosphorylated by deoxycytidine kinase and other intracellular kinases to produce the active forms, gemcitabine diphosphate and triphosphate (dFdC-DP and dFdC-TP). Incorporation of dFdC-TP into DNA during S-phase of cell cycle, results in termination of DNA synthesis, single-strand breaks and eventually cell death.[1,2] Gemcitabine is rapidly metabolized into the active metabolite 2',2-difluorodeoxyuridine (dFdU) by cytidine deaminase that is present at high levels in plasma, red blood cells and liver.[3,4]

Gemcitabine activity and toxicity is highly dependent on the frequency and duration of administration. In previous Phase I and II studies,[5,6,7,8] lower gemcitabine concentrations over longer infusion times were clinically active. As a surrogate marker for tumor uptake and activation of gemcitabine, levels of dFdC-TP were measured in patients' peripheral–blood mononuclear cells (PBMCs). Prolonged gemcitabine infusion resulted in enhanced accumulation of dFdC-TP in PBMCs [9] and in leukemic cells.[10,5] Taken together, these studies indicate that the anti-tumor effect of gemcitabine could be schedule dependent and that lower doses given over longer exposure times might be efficacious.[8] However, pancreatic cancer was not found to be a sensitive tumor type to proof this concept, since prolonged infusion times did not result in improved overall and progression free survival.[11] Nevertheless, preclinical studies in mice with daily administration of gemcitabine demonstrated anti-tumor activity in human colon, lung, and prostate tumor xenograft models (data on file at Lilly).

In view of this, daily and every-other day oral administration of gemcitabine was studied in a previous clinical trial.[12] The pharmacokinetic (PK) data obtained in this trial revealed that this approach was not feasible due to lack of bioavailability. The poor bioavailability was attributed to the extensive first-pass metabolism by deamination of gemcitabine into dFdU. Hence, a new chemical entity, LY2334737, was developed.[13] LY2334737 is a prodrug of gemcitabine, in which the (metabolic) unstable amine group is covalently bound to valproic acid. When LY2334737 is administered orally, its amide bond is slowly hydrolyzed and gemcitabine and valproic acid are released systemically. It is postulated that following LY2334737 administration, gemcitabine will be protected from extensive pre-systemic deamination, resulting in lower conversion into dFdU and thereby in significant exposure to gemcitabine in plasma after oral administration.

Moore et al. demonstrated that the combination of epidermal growth factor receptor (EGFR) inhibitor erlotinib and gemcitabine resulted in a modest albeit statistically

significantly improved survival in patients with advanced pancreatic cancer when compared to gemcitabine alone.[14] Since erlotinib apparently has no overlapping toxicity or PK interactions with gemcitabine[14], the feasibility of the combination of LY2334737 with erlotinib was investigated in this study as well.

The aim of this study was to determine a dose of LY2334737 to be recommended for phase II studies that may be safely administered to patients with cancer as monotherapy and in combination with the EGFR inhibitor erlotinib. During this study, two different formulations of LY2334737 were tested (non-registration formulation and registration formulation designed as NRF and RF, respectively) . The RF formulation had improved pharmaceutical characteristics relative to the NRF in terms of shelf-life conditions and loading capacity of the carrier, resulting in smaller capsules. After the determination of the recommended dose (using the NRF) for testing in phase II studies, this study was extended with a bioequivalence study to investigate the RF formulation of LY2334737.

Patients & Methods

Patient selection

Patients with histologically or cytologically proven cancer (solid tumors only) for whom no treatment of proven benefit existed were eligible. Other eligibility criteria were: age $\geq$ 18, performance status $\leq$ 2 (Eastern Cooperative Oncology Group (ECOG)) and an estimated life expectancy of $\geq$ 12 weeks. Previous therapies for cancer had to be discontinued for at least 30 days before study entry and 6 weeks in case of mitomycin-C or nitrosoureas. Patients had to have adequate bone marrow function, defined as absolute neutrophil count (ANC) $\geq$ 1.5 x 10^9/L, platelets $\geq$ 100 x 10^9/L, and hemoglobin $\geq$ 9 g/dL, and adequate renal and hepatic function defined as serum creatinine $\leq$ 1.5 the upper limit of normal (ULN), bilirubin $\leq$ 1.5 times ULN and alanine transaminase (ALT) and aspartate transaminase (AST) $\leq$ 2.5 times ULN. In case of metastases, AST and ALT $\leq$ 5 times ULN were acceptable. Exclusion criteria were: experimental therapy received within the last 30 days prior to study entry, symptomatic central nervous system malignancy or metastasis, gastrointestinal disease that may interfere with adequate oral absorption and patients who had previous diagnosis of liver cirrhoses, chronic hepatitis, history of, or active alcohol abuse and acute or chronic leukemia. The study was approved by the local medical ethics committee of each hospital and all patients had to give written informed consent. The study was performed at the Netherlands Cancer Institute and the University Medical Center Utrecht.

Study design:

The study is a non-randomized open-label, dose escalation, 3-arm phase I study of LY2334737 as monotherapy (Arm A) and in combination with erlotinib (Tarceva®) (Arm

B). During the study, an additional arm (Arm C monotherapy cross-over replicate) was added by amendment to determine the bioequivalence of a novel LY2334737 drug formulation: RF. Arm B started when at least 15 patients were observed in Arm A (LY2334737 monotherapy) and after at least one treatment-related grade 2 toxicity was observed. These conditions were implemented to reduce the number of patients treated with low, possibly inactive, doses of LY2334737. After completion of Arm A, Arm C was opened. This part of the study was conducted at the recommended dose determined in Arm A.
Safety was determined according to the Common Terminology Criteria for Adverse Events (CTCAE) version 3.0 and preliminary anti-tumor activity was determined using RECIST v1.0.[15] Tumor biomarkers were determined to explore any preliminary anti-tumor activity.

Arms A and B:
The primary objective of Arms A and B of this study was to determine the recommended dose (RD) for phase II studies of LY2334737 alone or in combination with erlotinib. The RD was defined as the maximum tolerated dose (MTD), which is the highest dose of LY2334737 monotherapy or in combination with erlotinib that had no more than 33% probability of causing a dose limiting toxicity (DLT). A DLT was defined as an adverse event observed during the first 21-day cycle of LY2334737 therapy according to any of the following criteria: 1) grade 4 neutropenia lasting $\geq$ 5 days or neutropenic fever, 2) grade 4 thrombocytopenia, 3) any $\geq$ grade 3 nonhematologic toxicity, and 4) interrupted treatment due to toxicity.

After enrollment in the study, patients assigned to Arm A and B received one 5 mg (non-therapeutic) dose of gemcitabine (Gemzar®) via an intravenous (i.v.) push in order to determine the individual gemcitabine clearance and thereafter the bioavailability of gemcitabine after LY2334737 oral administration. Subsequently, a 4- to 7-day washout period was required before initiating LY2334737. The i.v. administration together with the washout period was designated as cycle 1.
In cycle 2, the first cohort of 3 patients received 5 mg LY2334737 every other day (QOD) for 14 days followed by 7 days of rest. This starting dose was selected based on preclinical safety studies and data generated with oral gemcitabine.[12]
On the condition that no drug-related grade 2 (or greater) hematological toxicity and/or non-hematological toxicity (excluding nausea and vomiting without treatment and alopecia) were observed in the first cohort, subsequent cohorts were administered LY2334737 daily (QD) for 14 days followed by 7 days of rest.
The same LY2334737 treatment schedule was applied in Arm B with the addition of daily 100 mg erlotinib. This dose was selected since this is the approved erlotinib dose in combination with gemcitabine for the treatment of pancreatic cancer.

Dose escalation followed the continual reassessment method (CRM), a Bayesian model to efficiently estimate the MTD. The incidence of DLTs across all investigated dose levels is taking into account. Subsequently, a probability distribution of DLT versus the LY2334737 dose is generated. The MTD is determined by the dose at which the probability of DLT is (33%). The CRM potentially escalates the dose too rapidly using single patient cohorts.[16] Modifications based on observed toxicity that limit dose escalations were implemented. This modified CRM has proven to provide greater efficiency than standard dose escalation schemes.[17,16]

Arm C: bioequivalence assessment of a novel LY2334737 drug-formulation

While recruitment in Arm A and B was ongoing, the RF formulation of LY2334737 was developed. In Arm C, the objective was to compare the relative bioavailability of RF with the NRF evaluated in Arm A and B. This was investigated using a 3 period replicate cross-over design. Thirty evaluable patients were planned to enroll into two groups of 15 patients each. Patients received the RF or NRF formulation on day 1, 3 and 5 in the following order: group 1: RF, RF and NRF and group 2: NRF, RF, and NRF. After PK assessments, patients received on 10 consecutive days LY2334737 (starting day 6) as NRF at the recommended phase II dose followed by 7 days of rest. Cycle 1 was defined as the first 5 days of bioequivalence assessment and cycle 2 was defined as the subsequent 10 days daily treatment and 7 days rest period. In cycle 3 (and beyond) , patients received the NRF of LY2334737 as daily dosing for 14 days followed by 7 days of rest.

The two formulations were considered equivalent if the 90 % confidence limits ratio of exposure were within the 0.7 to 1.43 interval (data were analyzed on the log scale). These boundaries are wider than the standard bioequivalence boundaries set forth by the FDA: 0.8 to 1.25.[18] However, since LY2334737 is not a marketed drug and still in phase I clinical testing, a formal bioequivalence trial is not required.

Drug formulation

Intravenous gemcitabine (Gemzar®, Eli Lilly and Company, Indianapolis, USA) is commercially available. The NRF formulation of LY2334737 (Eli Lilly and Company, Indianapolis, USA) was supplied as 5, 15 and 30 mg capsules for oral consumption and the RF of LY2334737 (Eli Lilly and Company, Indianapolis, USA) was supplied as 15 and 20 mg capsules. Two substantial changes have been made in the composition of the RF from the NRF. To facilitate the manufacturing process, the enteric coating is changed from hydroxcyporpyl methylcellulose acetate succinate to methyl methascrylate methacrylic acid copolymer Type A. The second change in order to decrease capsule size, the amount of LY2334737 loaded onto the carrier beads increased from 10% w/w in NRF to 25% in RF. Neither of these 2 changes is expected to cause significant increase

or decrease in exposure between NRF and RF. Erlotinib (Tarceva®, OSI Pharmaceutical, Genetech, Roche) is commercially available.

Safety evaluation

Pre-treatment evaluation included a complete medical history, physical exam, ECG, chest x-ray, vital signs, assessments of adverse events using CTCAE v3.0, the use of concomitant medications, urine or serum pregnancy test and laboratory tests of hematology (hemoglobin, leukocytes, platelets, neutrophils (ANC), lymphocytes, monocytes, eosinophils and basophils) and serum chemistry (total bilirubin, alkaline phosphatase, AST, ALT, creatinine, calcium, glucose, sodium, phosphorus and potassium). Before each cycle a physical exam, assessment of adverse events and notation of concomitant medication were repeated and hematology and serum chemistry were checked. If any grade 2 or grade 3 toxicities were seen with laboratory tests, hematology and serum chemistry were repeated every 2 to 3 days.

Pharmacokinetics

The pharmacokinetics of LY2334737, gemcitabine, dFdU, valproic acid, and, if applicable, erlotinib were monitored during the study. The PK sampling scheme of Arm A and B consisted of sampling cycle 1 after the IV gemcitabine dose on day 1 (predose, end of infusion, 0.5, 1 and 2 hours after infusion) and cycle 2 after LY2334737 dose on day 1 (predose, 0.5, 1, 2, 4, 6, 8 and 24 hours after dosing) and day 14 (predose, 0.5, 1, 2, 4, 6, 8, 24, 48 and 168 hours after dosing). Blood samples for LY2334737 and metabolites were drawn in lithium heparinized tubes containing 0.075 mL of 10 mg/mL tetrahydrouridine (inhibitor of cytidine deaminase). The PK sampling scheme of Arm C consisted of sampling cycle 1 on day 1, 3 and 5 (predose, 0.5, 1, 2, 4, 6 and 8 hours after dosing). The intracellular phosphorylated gemcitabine metabolite levels (dFdC-TP) were monitored on day 1 of cycle 2 (Arm A and B) at 1, 4, 8 and 24 hours after dosing, and day 14 (cycle 2, Arm A and B) at 1, 4, 8, 24, 168 hours after LY2334737 dosing. LY2334737, metabolites and erlotinib concentrations were measured using validated (LC-MS/MS) assays. For the determination of dFdC-TP, approximately 15 mL blood was drawn into sodium heparinized tubes. The tubes were centrifuged at 4°C for 5 minutes at 1500 x g. PBMCs were isolated and dFdC-TP levels were determined as described previously using a validated LC-MS/MS assay.[19] The following pharmacokinetic parameters were determined by non-compartmental analysis using WinNonLin version 5.2 (Pharsight corporation): maximum plasma concentration (Cmax), time to reach Cmax (tmax), area under the plasma concentration time curve from time zero to 24 hours (AUC0-24h), AUC from time zero to infinity (AUCinf) and terminal half life (t1/2). The geometric mean and coefficient of variation and the accumulation index (AUC day14 / AUC day 1) are provided.

The incorporation of gemcitabine into genomic DNA in PBMCs was determined in Arm C. One blood sample (10 mL using EDTA tubes) was collected on day 3 of cycle 1 and day 3, 6 and 10 of cycle 2. After the DNA had been isolated and completely hydrolyzed, the fluorinated nucleotide (dFdC) was detected using LC-MS/MS.

Biomarkers

In arm A and B blood samples were drawn for the determination of antiangiogenesis biomarkers: circulating endothelial cells and vascular endothelial growth factor (VEGF). Blood samples for the determination of circulating endothelial cells were collected on five occasions; twice prior to start (mean baseline value) and subsequently once on day 7, 14 and 21 of cycle 2. Mononuclear cells were isolated and circulating endothelial cells (CECs) were detected using flow cytometry as previously described.[20] Additionally, VEGF was determined in plasma at baseline and day 7 and 14 of cycle 2. Analysis was performed using a commercially available ELISA kit (R&D Systems).

Results

Patient characteristics

A total of 65 patients with histologically or cytologically confirmed advanced solid tumors were treated in this study. Thirty-two patients were entered in Arm A, 10 patient in Arm B and 23 patients in Arm C. The characteristics of these patients are summarized in table 1.

Table1: Patient demographics (n=65)

		Arm A (n=32)	**Arm B (n=10)**	**Arm C (n=23)**
Sex	Female	11	5	10
	Male	21	5	13
Age (years)	Median	61	56.5	55
	Range	43 - 81	24 - 70	36 – 77
ECOG performance status	0	15	4	14
	1	16	6	9
	2	1	-	
Pathological diagnosis	Pancreas	7	1	2
	Mesothelioma	5	-	1
	Colorectal	4	-	7
	Sarcoma	1	3	2
	Melanoma	3	-	2
	Ovary	1	1	2
	Bladder / urinary tract	2	-	2
	Esophagus	1	-	2
	Cholangiocarcinoma	1	1	-
	Stomach	1	1	-
	Other	6	3	3
Disease stage	Stage III	1	-	1
	Stage IV	31	10	22
No. of Prior treatments (surgery, radiotherapy or chemotherapy)	0	5	1	1
	1	5	-	2
	2	4	2	3
	3	3	3	4
	$\geq$4	15	4	13

Dose limiting toxicity and maximum tolerated dose

The LY2334737 dose in the monotherapy arm (Arm A) was rapidly escalated from 5 mg QOD up to 40 mg QD with dose increments of 100%. Since moderate toxicities were observed in the three patients treated at the 40 mg dose level, the magnitude of dose escalation was reduced and the next highest dose level was set at 50 mg QD. Seven patients were treated with 50 mg LY2334737 monotherapy of whom 4 experienced DLTs. The first patient developed after 11 days of LY2334737 treatment grade 3 pyrexia (drug related fever) and grade 4 thrombocytopenia, which both completely recovered after interruption of treatment. A second patient developed after 9 days grade 3 fatigue for which treatment was discontinued. Severe hyponatremia, fatigue and pulmonary embolism were the DLTs of the third patient. These adverse events recovered after discontinuation of LY2334737. The last patient developed grade 3 hyponatremia and fatigue. Regarding the severe toxicity at the 50 mg dose level, it was concluded that this dose was non-tolerable.

Subsequently, the following 3 patients were treated at the next lowest dose level of 40 mg QD at which already three were treated without serious toxicity. However in the expansion cohort two patients developed DLTs. One patient developed grade 3 alanine aminotransferase (ALT) elevations, which partly resolved after a week of rest and a dose reduction. The second patient, a 61 year old male patient with pancreatic cancer, died suddenly which was considered possibly related to LY2334737 intake. This patient was admitted to a hospital because of a partial obstruction of the duodenum due to pancreatic tumor, grade 3 hyponatremia, elevated alkaline phosphatase and transaminases. Four days later the patient complained of dyspnea, low blood pressure and signs of hypoperfusion (hypovolemic shock). The following day the patient experienced abdominal pain, dyspnea, and died suddenly. The events of dyspnea, hypovolemic shock and sudden death were considered possibly related to study treatment.

Subsequently, three patients were treated with 30 mg LY2334737 QD. None of these patients experienced DLTs. Based on the incidence of DLTs, the MTD was estimated, using the CRM, to be 40 mg LY2334737. It was estimated that the median probability of having a DLT at this dose level was 25 %.

The dose escalation arm of LY2334737 in combination with erlotinib (100 mg) started at the 20 mg LY2334737 dose level. DLTs were not observed in the 4 patients treated at this dose level. The following 6 patients were treated at the 40 mg dose level of whom 2 experienced DLTs. These were grade 4 gamma-glutamic transpeptidase (GGT) and grade 3 elevated ALT levels. Both patients discontinued with LY2334737 and erlotinib. The CRM resulted in an estimated probability of 31% having a DLT at the 40 mg LY2334737 and 100 mg erlotinib dose level. Therefore, the MTD for the combination with erlotinib was set at 40 mg LY2334737.

Table 2: Adverse events (all grades) possibly related to study drug with an overall incidence of 5% or more overall.

	Arm A								Arm B				Arm C	
	5 mg*	10 mg	20 mg	30 mg	40 mg	50 mg	Total (n = 32)		20 mg	40 mg	Total (n = 10)		40 mg (n = 23)	
Adverse event	(n = 7)	(n = 4)	(n = 3)	(n = 3)	(n = 8)	(n = 7)	n	%	(n = 4)	(n = 6)	n	%	n	%
Fatigue	1	-	1	3	5	6	16	50	1	5	6	60	16	70
Nausea	-	-	2	2	4	4	12	38	-	4	4	40	11	48
Vomiting	-	1	1	2	3	5	12	38	2	2	4	40	8	35
Diarrhea	-	-	1	2	-	4	7	22	1	5	6	60	10	43
Anorexia	1	1	1	-	4	4	11	34	-	2	2	20	7	30
Pyrexia	-	-	-	1	1	5	7	22	2	4	6	60	5	22
ALT elevation	-	-	-	1	4	2	7	22	-	2	2	20	4	17
AST elevation	-	-	-	1	3	2	6	19	-	2	2	20	5	22
Abdominal pain	2	-	1	1	1	2	7	22	1	1	2	20	2	9
Dysgeusia	-	1	1		3	2	7	22	-	2	2	20	1	4
Weight decreased	-	-	-	1	1	-	2	6	-	2	2	20	6	26
Influenza like illness	-	-	-	-	1	1	2	6	1	3	4	40	2	9
Headache	-	-	-	1	1	1	3	9	-	1	1	10	3	13
Stomatitis	-	-	-	2	-	1	3	9	1		1	10	3	13
Alkaline phosphatase elevation	-	-	-	-	1	2	3	9	-	1	1	10	2	9
Chills	-	-	-	1	-	-	1	3	-	-	-	-	5	22
Dyspnea	-	-	-	-	3	2	5	16	-	1	1	10		
Edema peripheral	-	-	-	-	-	4	4	13	-	-	-	-	2	9
Dry Skin	-	1	1	1	-	-	3	9	1	1	2	20	-	-
Night sweats	-	-	1	-	1	1	3	9	1	-	1	10	1	4
Rash	-	-	-	-	-	-	-	-	3	2	5	50	-	-
Hyponatremia	-	-	-	1	1	2	4	13	-	-	-	-	1	4

Adverse events

A total of 56 out of 65 patients experienced possibly drug-related treatment emergent adverse events. The most frequent were fatigue (with an incidence between 50 – 70% for the different treatment arms), pyrexia and influenza like illness and gastrointestinal disorders including nausea, vomiting, diarrhea, stomatitis, dysgeusia and anorexia. In addition, liver toxicities reported included grade 4 GGT, ALT and AST elevations. An overview of the observed adverse events is provided in table 2. The severity of most toxicities was mild (grade 1-2). A dose-dependent increase in the occurrence of adverse

events was observed, since, patients who were treated with daily 20 mg LY2334737 or less, showed a substantially lower incidence of adverse events compared to patients treated with 30 mg LY2334737 or more. Furthermore, no grade 3 or 4 toxicities were observed in patients treated with 20 mg LY2334737 or less. The observed grade 3 and 4 toxicities at higher dose levels consisted mainly of fatigue, and AST and ALT elevations (table 3).

Table 3: CTC grade 3-4 adverse events possibly related to study drug with an overall incidence of 3% or more.

	Arm A (n=32)		**Arm B (n=10)**		**Arm C (n=23)**	
Adverse event	Grade 3	Grade 4	Grade 3	Grade 4	Grade 3	Grade 4
Fatigue	5 (16%)	-	2 (20%)	-	5 (22%)	-
ALT elevation	4 (13%)	-	1 (10%)	-	3 (13%)	1 (4%)
AST elevation	3 (9%)	-		1 (10%)	1 (4%)	1 (4%)
Hyponatremia	4 (13%)	-		-	1 (4%)	-
Alkaline phosphatase elevation	1 (3%)	-	1 (10%)	-	2 (9%)	-
Dehydration	2 (6%)	-	1 (10%)	-	-	-

The most commonly observed adverse event, fatigue, appeared early after start of LY2334737 and ceased quickly after drug discontinuation. Several patients presented with pyrexia up to grade 3. In general the fever seemed to be well-tolerated by patients and was resistant to treatment with acetaminophen or nonsteroidal anti-inflammatory drugs. Interruption of study medication resulted in recovery of symptoms. Besides fatigue, dose-dependent liver enzyme elevations were the most reported grade 3-4 toxicities. Mostly, these events occurred quickly after the first 14 days of LY2334737 treatment. Treatment discontinuation or a dose reduction to the next lower dose level, mostly resulted in recovery to normal levels.
Hematological toxicity was rarely observed. Grade 3 and 4 thrombocytopenia were observed in two patients treated with 50 mg LY2334737, but no neutropenia of any grade occurred. Severe, grade 3, hyponatremia was observed in 5 patients. This was not expected regarding the working mechanism or the toxicity profile of (i.v.) gemcitabine. A possible explanation could be reduced food intake by patients, since three of the patients with hyponatremia had also LY2334737 related anorexia.

The combination arm of LY2334737 and erlotinib demonstrated a similar toxicity profile compared to LY2334737 monotherapy. However, a few adverse events were more

frequently observed in the combination arm. These were diarrhea (60%), rash (50%), pyrexia (60 %) and influenza-like illness (40%), which is consistent with the known toxicity profile of erlotinib.
In total 12 of the 65 patients discontinued the study due to adverse events. The most common adverse event causing discontinuation was fatigue.

Pharmacokinetics and pharmacodynamics
A total of 49 patients provided blood samples for pharmacokinetic analyses after a single dose of LY2334737 (NRF) and a total of 28 patients provided data after 14 days of consecutive LY2334737 (NRF) administration.
After drug administration, LY2334737 was absorbed rapidly with maximum concentrations reached 2.0 hours (10^{th} and 90^{th} percentile: 1.0 – 6.0 hr) after drug intake. Conversion to gemcitabine occurred rapidly and Cmax of gemcitabine was reached at approximately the same time as LY2334737 at a median of 2.0 hours (10^{th} – 90^{th} percentile: 1.0 -4.9 hrs) after dose. The mean concentration-time curves after 40 mg LY2334737 are provided in figure 1A and the pharmacokinetic parameters are presented in table 4. A dose-proportional increase in LY2334737 and gemcitabine Cmax and AUC was observed, and the median elimination half life of LY2334737 and gemcitabine, determined using all pharmacokinetic data, were 1.77 and 1.83 hours, respectively. The variability in LY2334737 and gemcitabine exposure after administration of 40 mg on day 1, were 37 and 39 %, respectively. The variability in Cmax at this dose level was 62 and 72 %. There was no statistically significant accumulation of LY2334737 and gemcitabine, although figure 2 shows a trend of increasing LY2334737 and gemcitabine exposure after 14 consecutive doses. Valproic acid could not be determined since all samples fell below the lower limit of quantification (10 µg/mL).

Ten patients that participated in Arm B were treated with LY2334737 in combination with erlotinib. The pharmacokinetics of LY2334737 in this combination arm was comparable to LY233437 monotherapy. The geometric mean exposure (AUC0-24h) to erlotinib was 8.2 µg*hr/mL (CV%: 77%) at day 1 and 18 µg*hr/mL (CV%: 120%) at steady state.

In study arm C, a novel LY2334737 drug formulation (RF) was compared with the old drug formulation applied in arm A and B (NRF). Twenty-three patients were included in this part of the study. No differences in systemic exposure to LY2334737 or gemcitabine were observed (AUC ratio LY2334737 RF:NRF of 1.02 with 90% confidence interval of 0.91 – 1.14; AUC ratio gemcitabine RF:NRF of 0.91 with 90% confidence interval of 0.80-1.02). However, the RF showed a significantly lower Cmax compared to NRF, 32.1 ng/mL versus 49.8 ng/mL LY2334737 and 3.12 ng/mL versus 5.24 ng/mL gemcitabine (geometric mean values, 90 % confidence interval of ratio of least squares: 0.55 – 0.79 for LY2334737 and 0.49 – 0.71 for gemcitabine).

Plasma levels of dFdU, the major gemcitabine metabolite, were determined in all patients. dFdU has a long terminal half-life of 88 hours (CV%: 32%) measured in patients treated in Arm A. This means that at day 14, following 14 daily doses, steady state was almost reached (approximately 4 times the terminal half-life). At day 14 the geometric mean Tmax was 4.0 hours (2.0 - 24 hr); Cmax was 1.30 µg/mL (CV%: 19%) and AUC0-24hr was 28.3 µg*hr/mL (CV%: 18%) dFdU, respectively. The PK ratio's (day 14 versus day 1) were 3.6 (CV% 32%) for Cmax and 4.3 (CV% 20%) for AUC0-24hr. The mean concentration time curves of dFdU at day 14 are provided in figure 1B.

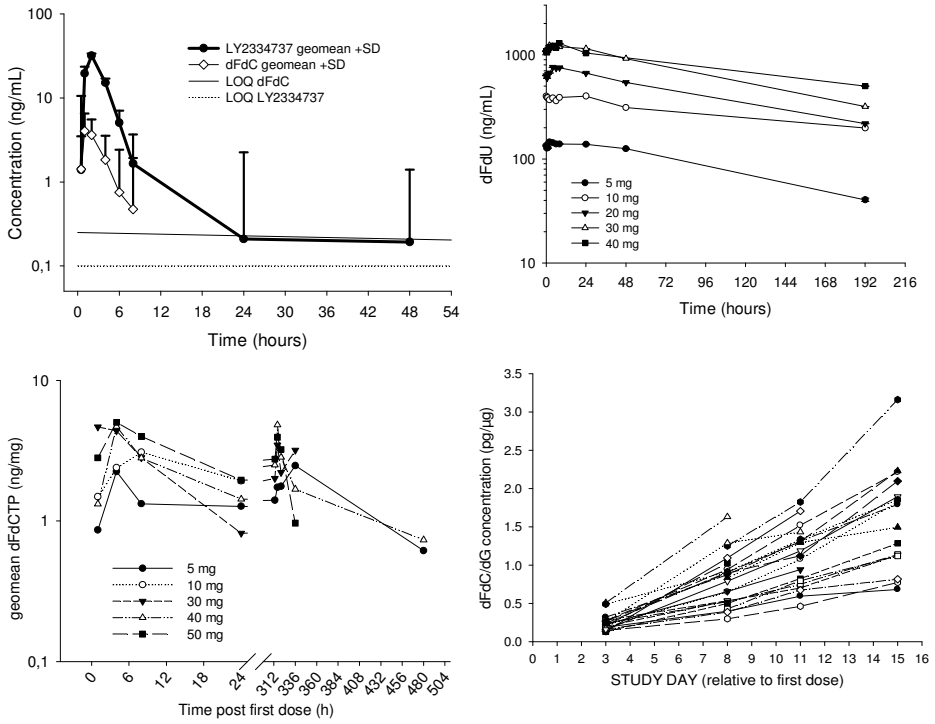

Figure 1: Upper left (1A): geometric mean concentration time curve of LY233477 and gemcitabine (dFdC) following 40 mg. Upper right (1B): geometric mean dFdU concentration time curves after different LY2334737 doses. lower left (1C): concentration time curves of dFdC-TP in PBMCs after different LY2334737 doses. Lower right (1D): dFdC into DNA after daily 40 mg LY2334737.

The active metabolite of gemcitabine, dFdC-TP was measured in isolated PBMC`s. The geometric mean AUC0-24hr at day 1 and day 14 after 40 mg LY2334737 was 80 ng*hr/mg PBMC (CV%: 69%) and 79 ng*hr/mg PBMC (CV%: 47%), respectively. This

corresponds to approximately 27 h*pmol/10^6 cells.[19] The geometric mean concentration-time curves observed after the different dose levels are provided in figure 2C.

In Study Arm C, the incorporation of gemcitabine in genomic DNA was measured in PBMCs. The results of this assay are given in Figure 1D. The level of incorporated gemcitabine increased while patients were on treatment and for most patients a plateau level was not yet observed within the investigated time period of 14 days.

No statistically significant decrease or increase in CEC or VEGF levels during treatment, nor between arm A and B (with or without erlotinib) were observed, although, patients treated in Arm B showed a trend towards decreasing CEC en VEGF upon treatment for 21 days (data not shown).

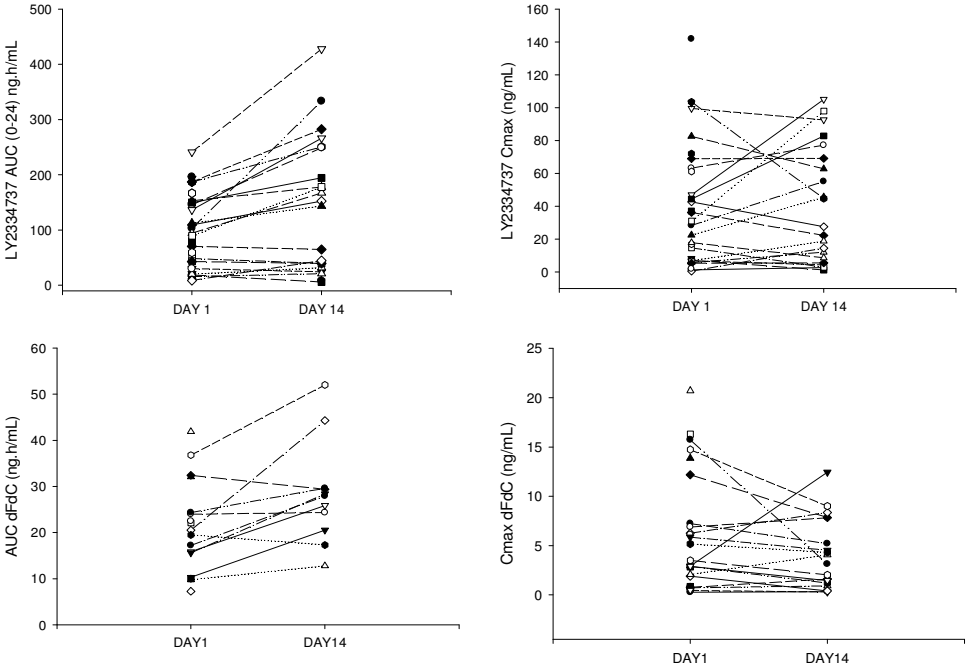

Figure 2: LY2334737 (upper left and right: 2A and 2B) and dFdC (lower left and right: 2C and 2D) PK parameter (AUC and Cmax) on day 1 and day 14.

Table 4: Pharmacokinetics of LY2334737,gemcitabine and dFdU after day 1 and day 14. The geometric mean and coefficient of variation (%) are given.

	LY2334737			gemcitabine (dFdC)			dFdU		
	Cmax (ng/mL)	AUCinf (ng*hr/mL)	T1/2 (hr)	Cmax (ng/mL)	AUCinf (ng*hr/mL)	T1/2 (hr)	Cmax (ng/mL)	AUC0-24hr (ng*hr/mL)	T1/2 (hr)
Day 1									
5 mg (n=7)	5.3 (68)	[f]20.0 (23)	[f]1.6 (32)	0.60 (43)	-	-	61 (24)	1463 (33)	-
10 mg (n=4)	11.6 (110)	52.0 (30)	2.4 (100)	1.37 (125)	[a]6.7	[a]2.8	121 (56)	2511 (58)	-
20 mg (n=2)	39.3 (12)	88.0 (32)	2.6 (152)	3.2 (14)	[a]9.9	[a]1.1	192 (12)	4008 (13)	-
20 mg + erlotinib (n=3)	34.6 (102)	132 (40)	5.2 (20)	2.00 (43)	[b]9.0 (8.8)	[b]2.1 (3)	160 (12)	3169 (22)	-
30 mg (n=3)	44.3 (37)	126 (29)	3.4 (102)	5.25 (55)	16.0 (46)	2.1 (29)	262 (25)	[a]5705	-
40 mg (n=19)*	47.3 (62)	140 (37)	3.0 (123)	5.32 (72)	17.4 (39)	1.9 (72)	296 (29)	[g]5853 (25)	-
40 mg + erlotinib (n=6)	60 (200)	170 (89)	2.0 (91)	6.45 (303)	27.9 (49)	1.6 (40)	320 (48)	[e]7093 (29)	-
50 mg (n=5)	71.3 (61)	173 (31)	3.2 (87)	11.1 (63)	26.6 (42)	1.7 (51)	363 (40)	[c]5753 (130)	-
Day 14									
5 mg (n=5)	5.0 (156)	[d]17.0 (88)	[d]1.5 (31)	[d]0.62 (96)	-	-	152 (43)	3338 (42)	89.3 (20)
10 mg (n=2)	4.0 (50)	80.0 (128)	-	[a]0.4	-	-	[a]401	[a]9355	[a]151
20 mg (n=3)	20.7 (33)	81.0 (73)	5.5 (216)	[b]1.56 (38)	-	-	767 (23.4)	17066 (26)	89.2 (5)
20 mg + erlotinib (n=3)	51.5 (50)	172 (68)	11.3 (86)	3.66 (66)	10.8 (83)	1.7 (17)	873 (3)	18438 (6)	105 (22)
30 mg (n=3)	85.6 (12)	208 (19)	4.0 (91)	7.97 (46)	25.1 (22)	3.7 (105)	1034 (28)	22254 (28)	71.0 (6)
40 mg (n=5)	44.7 (125)	244 (38)	11.4 (374)	4.41 (80)	35.9 (115)	8.7 (298)	1295 (19)	28264 (18)	[d]85.1 (20)
40 mg + erlotinib (n=4)	43.8 (158)	189 (80)	3.4 (99)	4.09 (120)	29.1 (75)	4.8 (146)	977 (18)	21055 (15)	78.2 (31)
50 mg (n=3)	61.2 (39)	340 (32)	5.2 (198)	5.02 (58)	37.0 (33)	3.6 (49)	1292 (30)	[b]32477 (0)	[b]92.0 (34)

* including patients from study arm C who were given on day 1 40 mg of the non-registration formulation. [a]n=1, [b]n=2, [c]n=3, [d]n=4 [e]n=5, [f]n=6, [g]n=15

Anti-tumor activity

A total of 51 patients were evaluable for at least one response assessment. The best response within 22 patients was stable disease. Remarkable were two patients with mesothelioma who had progressive disease prior to start but showed stable disease for 14 and 17 3-weekly cycles, respectively. One patient with prostate carcinoma had proven benefit of the treatment. This patient was assigned to the 40 mg dose-level in combination with daily erlotinib 100 mg. The LY2334737 dose was, due to grade 3 ALT elevations, reduced in cycle 4 and 8 to 30 mg and 20 mg, respectively. Since there were no measurable lesions, this patient was evaluated on prostate specific antigen (PSA), which was 89.4 ug/L at study entry and decreased to 32 µg/L (end of cycle 2). A complete response of PSA was observed after cycle 4 (PSA: 0.6 ug/L) and stabilized for 4 cycles. By the end of cycle 8, progression of PSA was observed (PSA: 20.6 ug/L).

Discussion

This report describes the first in-human study of the oral gemcitabine prodrug LY2334737. The MTD was determined for both monotherapy and in combination with 100 mg erlotinib, to be 40 mg daily administrations of LY2334737 for 14 days followed by one week of rest. Furthermore, the LY2334737 registration formulation was considered bioequivalent to the non-registration formulation.

The most reported adverse events were fatigue, elevated liver enzymes, gastrointestinal toxicity and flu-like illness (including pyrexia), which are adverse events also frequently observed after i.v. administration of gemcitabine. Hematological toxicity was, apart from grade 3/4 thrombocytopenia in two patients treated at the 50 mg dose level, not observed. This is surprising, since hematological toxicity is the predominant dose limiting toxicity of gemcitabine i.v. A possible explanation for this is the lower systemic exposure to gemcitabine over time. At the MTD patients received a cumulative dose of 560 mg LY2334737 (equivalent to 304 mg gemcitabine) in a 21-day cycle, which is significantly lower than standard i.v. gemcitabine regimens (e.g. 1250 mg/m^2 on day 1 and 8 of a 21-day cycle). However, typical gemcitabine related non-hematological toxicities were observed at these relatively low doses of LY2334737. These discrepancies in toxicity profile probably originate in the oral route of administration and the short dosing intervals resulting in continuous exposure to LY2334737 and metabolites. Twice-weekly schedules were previously investigated for i.v. gemcitabine. The weekly schedules were better tolerated, but the most striking difference in toxicity between the weekly and twice-weekly schedule was the incidence of flu-like illness i.e., 63% in the twice weekly group versus 20% for the weekly schedule.[21] Pyrexia and flu-like illness were among the most observed toxicities in this study.

The PK of gemcitabine after intravenous administration is characterized by a high Cmax and rapid decline in gemcitabine plasma levels after the end of the infusion, resulting in a

short lasting exposure to high levels of gemcitabine and metabolites. The continuous exposure to low levels gemcitabine, achieved after daily LY2334737 intake, may contribute to cumulative non-hematological toxicities, whereas the acute high exposure after i.v. gemcitabine results in hematological toxicities.

AST and ALT elevations were, after fatigue, the most reported grade 3/4 toxicities. Studies in mice revealed that after multiple oral doses of gemcitabine accumulation of phosphorylated gemcitabine (dFdC-TP) and phosphorylated dFdU (dFdU-TP) occurred. Especially the accumulation of dFdU-TP in mouse liver was more pronounced following oral administration of gemcitabine compared to intravenous administration.[22] High levels of dFdU-TP were also observed in PBMCs of patients treated with oral gemcitabine. Therefore, the hepatotoxicity after daily intake of LY2334737 may be related to accumulation of gemcitabine metabolites in the liver.

LY2334737 was designed to overcome the extensive presystemic deamination of gemcitabine by cytidine deaminase to dFdU. This was required since systemic exposure to gemcitabine after oral administration was very low.[12]
Despite the reduced presystemic deamination due to the prodrug design, the total exposure to dFdU was still high and accumulation was observed after daily dosing of LY2334737. However, It is important to note that the accumulation ratio (Day14/Day1) of dFdU exposure was 4.45 or 0.75-fold lower than that observed following 2 weeks daily dosing of oral gemcitabine. [12] This indicates that the first pass metabolism of gemcitabine into dFdU is lower following oral LY2334737 compared to oral gemcitabine.

Both deamination of dFdC-monophosphate (MP) to dFdU-MP as well as cellular uptake of dFdU followed by phosphorylation results in the formation of dFdU-TP, which can be incorporated in DNA and RNA. [23] Due to the long terminal half life, dFdU accumulates during the first two weeks of treatment and its plasma concentration is only reduced by about 75 % (approximately twice the terminal half life) during the week of rest. The continuous exposure to dFdU may have contributed to the toxicity profile of daily LY2334737 treatment. Although there was a clear increase in toxicity with dose (see table 2), it was not possible to assess more precisely the relationship between exposure of LY2334737 or its metabolite to toxicity because of both variability in systemic exposure and lack of knowledge of LY2334737 and metabolites concentration prior to systemic exposure at the level of the gut and liver.

The metabolite dFdC-TP and dFdC incorporated into DNA could be detected in peripheral PBMCs, although with high inter- and intra-patient variability. This indicates that the pharmacologically active form of gemcitabine is able to accumulate into these

cells. Previously it has been demonstrated that this is a marker for the anti-tumor activity of gemcitabine. [24,25]
Hints of anti–tumor activity or stable disease was observed in 22 of 51 evaluable patients. Furthermore, a confirmed complete response of PSA was demonstrated in a patient with metastatic prostate carcinoma.

Part C of this study compared two LY2334737 drug formulations: NRF vs RF. The RF showed slightly lower Cmax values. This discrepancy most likely originates in the loading of the carrier beads (an excipient of the capsule formulation), which is much higher for the RF. Dissolution experiments demonstrated that higher loading percentages of LY2334737 onto the carrier beads resulted in lower dissolution rates, possibly resulting in lower Cmax values (data on file at Lilly). However, these discrepancies did not result in differences in exposure to LY2334737 and gemcitabine. Therefore, the NRF and RF were considered bioequivalent and future studies will be conducted with the RF, since this formulation resulted in smaller capsules and was easier to manufacture and store.

In conclusion, this study demonstrates that LY2334737 can be safely administered to patients with solid tumors up to doses of 40 mg/day during 14 days followed by one week of rest with or without daily 100 mg erlotinib. The most frequently observed toxicities were fatigue, gastrointestinal toxicities, elevated liver enzymes and flu-like illness. The pharmacologically active forms of gemcitabine could be detected in isolated mononuclear cells and signs of anti-tumor activity were observed. The recommended dose of LY2334737 for future studies is 40 mg/day.

Acknowledgements

This study was supported financially by Eli Lilly and Company.
The authors thank Susanne K Rhoades and Roberta L Balgobin for managing LY2334737 program, Takeshi Makiuchi for his support for his study support, Joanna Burke and Tess Lam for their statistical support and analysis.

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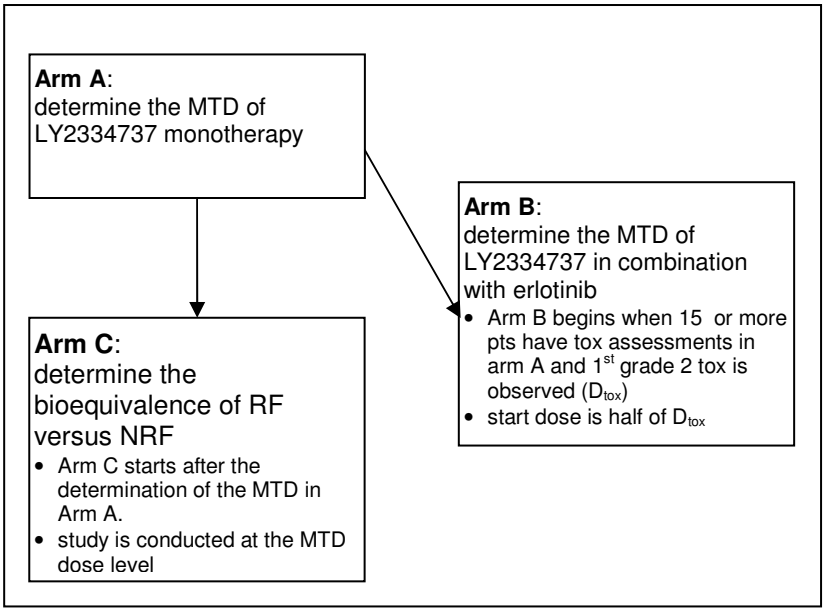
Supplementary tables and figures

supplementary table 1: Treatment duration and response according to RECIST v1.0. (PR: partial response, SD: stable disease, PD: progressive disease, NE: not evaluable)

Regimen	No of patients	Duration in cycles (median, range)	Best response			
			PR	SD	PD	NE
Arm A: 5-20 mg	14	3.0 (2.0 -17)	0	5	7	2
Arm A: 30 – 50 mg	18	2.0 (2.0 -14)	0	4	8	6
Arm B: 20 – 40 mg + erlotinib	10	2.5 (2.0 – 8.0)	0	4	5	1
Arm C: 40 mg	23	3.0 (2.0 – 13)	0	9	9	5
Total	65	3.0 (2.0 – 17)	0	22	29	14

Supplementary figure 1: Study overview (abbreviations: D_{tox}: dose level in arm A at which the first possibly related grade 2 adverse event occurs, tox: toxicity)



Chapter 4.2

Pharmacokinetics of gemcitabine and metabolites in a patient with double sided nephrectomy: A case report and a review of the literature

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Oncologist, 2009; 14(9): 944-8

Abstract

A patient with complete renal failure due to urothelial cell carcinoma related nephrectomy of both kidneys, received palliative chemotherapy with carboplatin and gemcitabine.
The patient received gemcitabine 1000 mg/m2 followed by carboplatin 100 mg. Shortly after, he underwent hemodialysis. Pharmacokinetics of gemcitabine and metabolites in plasma and in peripheral blood mononuclear cells (PBMCs) were monitored.
double-sided nephrectomy and hemodialysis had no influence on gemcitabine pharmacokinetics, however, a high exposure was seen for the main metabolite difluorodeoxyuridine (dFdU) ($AUC_{(0\text{-}51hr)}$: 844 µg/mL*h). During hemodialysis plasma concentrations of dFdU were reduced by 50 %. High concentrations of the intracellular phosphorylated metabolites (gemcitabine-triphosphate and dFdU-triphosphate) were observed: 228 pmol/10^6 cells and 47 pmol/10^6 cells, respectively. The patient tolerated the regimen poorly; adverse events included a grade 4 thrombocytopenia.
Hemodialysis effectively reduced plasma concentrations of dFdU. Furthermore, high concentrations of intracellular phosphorylated metabolites may be related to double sided nephrectomy resulting in a poor tolerability of gemcitabine.

Introduction

Gemcitabine in combination with cisplatin is approved for first line treatment of locally advanced, or metastatic urothelial cell carcinoma. In patients with renal insufficiency cisplatin is often replaced by carboplatin.
Gemcitabine (dFdC), a fluorinated analogue of deoxycytidine, becomes cytotoxic when phosphorylated intracellularly. Gemcitabine is first phosphorylated to gemcitabine monophosphate (dFdC-MP), which is rapidly further phosphorylated to gemcitabine diphosphate (dFdC-DP), and gemcitabine triphosphate (dFdC-TP). dFdC-DP inhibits ribonucleotide reductase, which is necessary for DNA synthesis and dFdC-TP can terminate DNA synthesis by incorporation into the DNA strand.
Gemcitabine is rapidly metabolized by cytidine deaminase into the metabolite 2'2'-difluorodeoxyuridine (dFdU). dFdU is excreted in urine and its elimination depends on renal function.
The metabolite dFdU has been assumed to be non-toxic at concentrations observed in patients, however at high concentrations toxic effects can be expected, as observed in preclinical studies.[1,2] It was hypothesized that the toxicity of dFdU observed in preclinical cell experiments was caused by the intracellular phosphorylation to dFdU-triphosphate (dFdU-TP).[1] The results of a clinical phase I study with orally administered gemcitabine were in accordance with this. Patients tolerated the oral gemcitabine regimen poorly and accumulation of dFdU and high levels of dFdU-TP were observed.[3] Considering these findings, renal impairment is a factor that may complicate gemcitabine treatment. When

renal function is compromised, dFdU will retain in the body, which may result in toxic side-effects. In this case report we present a patient who underwent regular hemodialysis due to complete renal failure and who was treated with carboplatin and gemcitabine for metastatic urothelial cell carcinoma. Plasma gemcitabine and dFdU levels were monitored, including the intracellular levels of the phosphorylated metabolites (dFdC-TP and dFdU-TP) in PBMCs, which are responsible for the efficacy and toxicity of gemcitabine treatment.

Patient and methods

Case

A 72 year old Caucasian male patient was diagnosed with urothelial cell carcinoma, for which he was treated with mitomycin intravesical installations, in 1994. Due to disease progression he underwent right-sided nephrectomy in 2004 and left-sided nephrectomy, ureterectomy and cystoprostatectomy in May 2006. Since the left-sided nephrectomy, the patient was dependent on hemodialysis. In January 2008, he presented with lymph node and lung metastasis for which he received 3 courses of palliative chemotherapy consisting of gemcitabine and carboplatin.

Physical examination demonstrated a moderately ill patient with normal cardiac, pulmonary and hepatic function, WHO performance status of I, body weight 86 kg and height 186 cm. Blood chemistry showed high creatinine levels (12.2 mg/dL).

Patient treatment and hemodialysis

The first cycle of chemotherapy consisted of a single 1-hour intravenous infusion of 100 mg carboplatin followed by hemodialysis 2 hours after infusion. Hemodialysis was performed using a 4-hour bicarbonate dialysis, a blood-flow of 200 mL/min and dialysate flow of 500 mL/min. Therapeutic drug monitoring was applied to determine the platinum exposure. The second cycle, 3 weeks later, consisted of carboplatin and gemcitabine. A 30-minute infusion of gemcitabine 2000 mg (1000 mg/m2) was given on day 1 and day 8. On day 1, treatment started with a 30-minute gemcitabine infusion, immediately followed by a 30-minute infusion of 100 mg carboplatin. Two hours after the end of the carboplatin infusion, hemodialysis was started. The third and last cycle of gemcitabine-carboplatin was started four weeks after start of the second cycle.

Bioanalysis and Pharmacokinetic analysis

The plasma pharmacokinetics of gemcitabine and dFdU were determined on day 1 of the second cycle. Blood samples were drawn before, immediately after infusion and at 0.5, 2.5 (before start hemodialysis), 4.5, 6.5 (end of hemodialysis) and 51 hrs after infusion (after 51 hours, the patient returned to the hospital for regular hemodialysis). Blood

samples were drawn into lithium-heparinized tubes containing 750 µg tetrahydrouridine (THU) (Calbiochem, La Jolla, CA, USA) to prevent ex vivo conversion of gemcitabine into dFdU. Blood samples were immediately placed on ice and centrifuged for 10 minutes at 1500 g. Plasma samples were stored at -80°C until analysis. Gemcitabine and dFdU concentrations were analyzed using a previously described validated liquid chromatography with tandem mass spectrometry (LC-MS/MS) assay. The lower limit of quantification of the assay is 0.5 ng/mL for gemcitabine and 5 ng/mL for dFdU.[4]

The intracellular phosphorylated metabolites, dFdC-TP and dFdU-TP were measured in PBMCs at 4 time points: 0.5, 2.5, 6.5 and 51 hours after gemcitabine infusion. Samples of 15 mL venous blood were drawn in lithium-heparinized tubes and PBMCs were isolated and dFdC-TP concentrations were measured using a previously described validated LC-MS/MS assay.[5] The dFdU-TP concentrations were determined using the same assay, as has been previously described.[3,5]

The data were analyzed using descriptive pharmacokinetic methods by employing PK-Solutions version 2.0 (Summit Research Services, USA). The results were compared with data reported in the literature from patients with normal renal function,[6,7] and complete renal failure.[8,9,10]

Results

The results of the PK analysis are graphically depicted in figure 1 and the calculated PK parameters are summarized in table 1. The results showed, apart from the terminal half-life, no significant difference in dFdC pharmacokinetics compared to literature values from patients with normal renal function. The calculated terminal half-life of dFdC in our patient was much longer (12 hours), probably due to the sensitive analytical assay used, showing the terminal part of the plasma concentration time curve. The pharmacokinetics of dFdU, however, differed compared to patients with normal renal function. Hemodialysis though, effectively reduced dFdU concentrations and resulted in a reduction of 50% in plasma concentrations. After hemodialysis, the plasma levels of dFdU did not decrease further (t½: 242 hr).

The highest concentrations for the intracellular metabolites, dFdC-TP and dFdU-TP, were measured after hemodialysis, 6.5 hrs after the end of infusion. The measured Cmax values for dFdC-TP and dFdU-TP were 228 pmol/10^6 cells and 47 pmol/10^6 cells, respectively. Immediately after the end of infusion the measured concentration for dFdC-TP and dFdU-TP were very low (3 pmol/10^6 cells) and below the limit of quantification, respectively. After 51 hours the concentration of dFdU-TP was higher than the dFdC-TP concentration, indicating that dFdC-TP was eliminated faster than dFdU-TP.

The first cycle, consisting of carboplatin monotherapy, was well tolerated. However, the second cycle consisting of carboplatin in combination with gemcitabine was poorly tolerated. The patient suffered from asthenia grade 2, anorexia with weight loss (3 kg in 14 days), headache, back pain, flu-like symptoms and thrombocytopenia (with a nadir of $16*10^9$ /L). During the third cycle, the gemcitabine dose was reduced by 25% to 750 mg/m2. Unfortunately, his clinical condition quickly deteriorated due to general malaise and further weight loss. Soon after the third cycle, a CT-scan revealed progressive disease and treatment with carboplatin and gemcitabine was stopped.

Table 1: Pharmacokinetic parameters of our patient and reported data of patients with normal renal function and patients with complete renal failure (Case 1-3). The gemcitabine dose was in all patients 1000 mg/m2

	Patients with complete renal failure				Normal renal function Reported data [6,7,9]
	Our patient	Case 1 [9]	Case 2 [8]	Case 3 [8]	
dFdC					
t½ (hr)	12.05[a]	0.32	0.20	0.20	0.15 – 0.37
$AUC_{0-\infty}$ (µg/mL*h)	9.8	5.4	5.4	4.5	7.5 – 11.4
Cmax (µg/mL)	13.1	7.1	14.0	12.0	10 – 18.3
dFdU					
t½ (excluding dialysis) (h)	242	(62[b])	530	241	5 – 16.5
t½ under dialysis (h)	3.4	3.9	2.9	4	
$AUC_{0-\infty}$ (µg/mL*h)	844[d]	1130	369.1[c]	509.7[c]	73 - 251
Cmax (µg/mL)	42.4	39	32	29.6	23.6 – 28.2
dFdC-TP					
Cmax (pmol/10^6 cells)	229	-	-	-	56 ± 14
dFdU-TP					
Cmax (pmol/10^6 cells)	47	-	-	-	-

[a] t½ is relatively long, probably due to sensitive analytical assay showing the terminal part of the plasma concentration time curve [b] t½ calculated over 28 hours, including hemodialysis. [c] $AUC_{(0-28\ hr)}$, [d] $AUC_{(0-51\ hr)}$

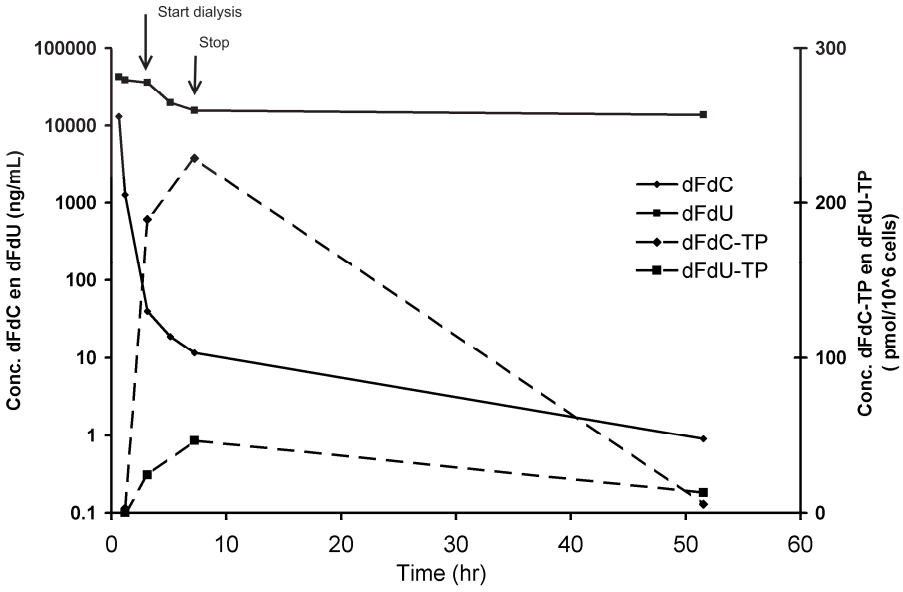

Figure 1: plasma concentration time curve of gemcitabine (dFdC) and dFdU in ng/mL (left y-axis) and concentration time curves of dFdC-TP and dFdU-TP (dotted line) in peripheral blood mononuclear cells (in pmol/10^6 cells; right y axis).

Discussion

This case report demonstrates that dFdU is hardly cleared in patients without renal function. Hemodialysis, however, resulted in a 50% reduction of dFdU plasma concentration levels. Despite this effective clearance of dFdU during hemodialysis, the total dFdU clearance is substantially lower than in patients with normal renal function. Thus, patients on hemodialysis are exposed to high concentrations of dFdU for a considerable length of time. Considering these observations, prolonged exposure to dFdU may result in enhanced toxicity. The question whether it may result in increased anti tumor activity regarding toxicity and efficacy, also remains unanswered.

As pointed out in the introduction, the cytotoxic effect of gemcitabine depends on intracellular phosphorylation to dFdC-DP and dFdC-TP. Veltkamp et al. demonstrated

that dFdU can also be phosphorylated to its triphosphate (dFdU-TP) and subsequently incorporated into DNA and RNA. [1]
Despite the unchanged concentration of dFdU between 6.5 hours and 51 hours after start, the concentration of both intracellular phosphorylated metabolites decreased between 6.5 and 51 hours after start of infusion. This indicates that extracellular dFdU was not the main source for the formation of intracellular dFdU-TP. The clearance of dFdU-TP was, however, less than for dFdC-TP. This could be due to a difference in elimination half-life or due to the formation of dFdU-TP by extracellular dFdU.[1] The intracellular exposure to dFdU-TP is difficult to evaluate due to a limited number of studies in which dFdU-TP levels were measured.

The first cycle, carboplatin monotherapy was well tolerated. The second cycle, carboplatin in combination with gemcitabine, was tolerated poorly. Besides the high dFdU levels, this patient also had high dFdC-TP levels in PBMCs. In a PK study of gemcitabine the maximum dFdC-TP concentrations after administration of 1000 mg/m^2 were approximately 58 pmol/10^6 cells.[6] The concentration of dFdC-TP in this patient was approximately 4 fold higher (228 pmol/10^6 cells). This could be related to the poor tolerability of gemcitabine in this patient.

A few PK studies of gemcitabine have been conducted in patients with a poor to moderate renal function and a few case reports of patients who are completely dependent on hemodialysis have been published. The first case report discussed a 64 year old male patient with pancreatic cancer who received 2 courses of 1000 mg/m2 gemcitabine followed by hemodialysis after 24 hours. No unexpected toxicity was observed in this patient. The pharmacokinetics of this patient are listed in table 1 (case 1).[9] A second case report discussed an 81 year old male patient with pancreatic cancer who was treated with 650 mg/m2 gemcitabine (on day 1, 8 and 15 of a 4 week schedule) followed by hemodialysis 5.5 hours after the end of infusion. No severe ($\geq$ grade 2) toxicity was observed. In this patient, hemodialysis resulted in a 46% reduction in dFdU plasma concentrations.[10] Matsuda et al. presented 5 Japanese patients with pancreatic carcinoma and chronic renal failure who received 800 -1000 mg gemcitabine. One patient who was not on hemodialysis developed grade 3 neutropenia and thrombocytopenia, which recovered immediately after hemodialysis. The other four patients were on standard hemodialysis, which was initiated within 24 hours after gemcitabine. Two of them had to stop with gemcitabine due to hematological toxicity. Unfortunately, pharmacokinetics were not monitored in these patients.[11] The last case report discussed two Japanese patients with urothelial cell carcinoma and complete renal failure, who received 1000 mg/m^2 gemcitabine. Grade 1-2 fever was observed in both patients. Soon after the start of the gemcitabine treatment, the patients stopped due

to progressive disease. The pharmacokinetics of these two patients are listed in table 1 (case 2 and 3).[8]
A phase I study was conducted in patients with renal dysfunction (creatinine levels 1.6 – 3.2 mg/dL, median 1.8 mg/dL). The investigators observed severe toxicity in 4 of the 15 patients even at reduced doses of 650 mg/m^2 and 850 mg/m^2. However, they were unable to correlate the observed toxicity with any pharmacokinetic parameter of gemcitabine or dFdU.[12] A second phase I study was conducted in 18 patients divided in 4 groups based on EDTA-Cr^{51} plasma clearance ($\geq$ 80 mL/min; $\geq$60 and <80; $\geq$30 and < 60; and $\geq$30 and <80 plus renal insufficiency induced by previous chemotherapy). Gemcitabine 500 to 1000 mg/m2 was administered intravenously. The authors did not observe a significant impact of mild to moderate renal insufficiency on gemcitabine pharmacokinetics (dFdC and dFdU).[13] The toxicity observed in this phase I trial was mild; overall 17% of the patients had grade IV thrombocytopenia.

The toxicity observed in patients who were dependent on hemodialysis or had impaired renal function was generally mild or comparable with toxicity seen in patients with normal renal function, which indicates that gemcitabine can be given safely to patients with renal failure. However, the total number of patients is too small to draw definitive conclusions. Unfortunately, in none of the studies or case reports the pharmacologically active phosphorylated metabolites were measured. These data are essential to evaluate whether the reduced dFdU elimination in patients with impaired renal function, also results in increased concentrations of phosphorylated metabolites and thus, whether these patients are at higher risk for gemcitabine related toxicity.

In our patient high intracellular concentrations of the phosphorylated metabolites were measured, which may be related to the double sided nephrectomy. Hemodialysis effectively reduced dFdU and should therefore preferably be initiated after gemcitabine infusion on the same day.

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5

Conclusions and perspectives

Conclusions and Perspectives

The aim of this thesis was to investigate the oral application of the frequently used anticancer agents docetaxel, paclitaxel and gemcitabine. Oral application of these agents may be beneficial since oral therapy has many advantages for both patient and healthcare. The drug can be taken at home without hospital admission, which is generally preferred by patients. Furthermore, an orally available drug enables the use of more chronic treatment regimens.
However, when given orally, these drugs hardly reach the systemic circulation. The taxanes have a low bioavailability due to affinity for drug transporters, especially ABCB1 (P-glycoprotein, P-gp), extensive first pass metabolism by cytochrome P450 3A4 (CYP3A4) and poor drug solubility. Gemcitabine has a very low oral bioavailability due to extensive first-pass deamination by cytidine deaminase.

In previous studies it has been demonstrated that the low systemic exposure to docetaxel is primarily determined by CYP3A4 in gut and liver. Inhibition of CYP3A4 in mice using low-dose ritonavir was found to increase the systemic exposure in mice by 50-fold. In patients the apparent bioavailability of docetaxel increased to more than 100% after co-administration of ritonavir.
The use of a drug-drug interaction to enable treatment with an orally administered drug raises many additional pharmacological questions; what booster should be used? When should the booster be administered and at what dose and what is the safety of the booster drug itself in such approach? The studies described in this thesis investigated the pharmacology of boosting orally administered docetaxel more thoroughly in order to determine the optimal boosted oral docetaxel regimen. We demonstrated that the concept of boosting docetaxel is possible with any strong CYP3A4 inhibitor (ritonavir, ketoconazole, clarithromycin and grapefruit juice). This further proofs the principle of the concept. We selected low-dose ritonavir for the further development of boosted orally administered docetaxel, since a single dose of 100-200 mg of ritonavir resulted in high systemic exposure to docetaxel, had a good safety profile and since ritonavir is used as booster as standard practice in multiple anti-HIV regimens.
To further understand the pharmacokinetic and pharmacological interaction between docetaxel and ritonavir, data from previously conducted preclinical and clinical trials were analyzed more thoroughly using nonlinear mixed effects modeling (NONMEM). It was found that the interaction between docetaxel and ritonavir followed a well stirred liver model as proposed by Wilkinson et al.[1] With this model it was demonstrated that co-administration of ritonavir led to improved oral absorption and a ritonavir-concentration dependent inhibition of CYP3A4, which resulted in a reduced elimination rate for docetaxel. Besides a better insight into the pharmacology of the drug-drug interaction

between docetaxel and ritonavir, the developed model also provided a tool to further optimize the timing and dose of ritonavir.

In chapter 2.2, the developed PK model was used to evaluate in silico the influence of different ritonavir regimens on docetaxel exposure. It was demonstrated that the exposure to docetaxel could be increased with increasing ritonavir doses, however, these effects were not very prominent. To confirm these simulation results, a proof-of-concept study was conducted in cancer patients (Chapter 2.5). In this study orally administered docetaxel was boosted with 100 or 200 mg ritonavir (docetaxel and ritonavir were ingested simultaneously). The higher ritonavir doses resulted in remarkably higher exposures to docetaxel compared to boosting with 100 mg ritonavir. The plasma concentration time curves, presented in chapter 2.5, clearly demonstrated that this effect could almost completely be attributed to increased bioavailability. This is illustrated by increased maximal plasma concentration and similar terminal elimination half life. In the same study, also a second 100 mg ritonavir dose was given to patients 4 hours after docetaxel - ritonavir intake. This second ritonavir dose resulted in a decreased elimination rate and only a modest increase in systemic exposure to docetaxel.
Based on the aforementioned simulation study and the proof-of-concept study, the 200 mg ritonavir dose given concomitantly with oral docetaxel was selected for future studies. The data and simulation studies indicated that most likely the exposure to docetaxel can be increased further with doses higher than 200 mg or multiple ritonavir doses. However, these additional effects are expected to be small and do not outweigh the increased pill burden and additional risks for ritonavir related adverse events.

Most studies described in this thesis were conducted with a novel solid docetaxel formulation, ModraDoc001 capsules. To improve the poor aqueous solubility, a solid dispersion formulation of docetaxel has been designed in house. A similar formulation was developed for paclitaxel, ModraPac001 capsules. Both formulations gave high systemic exposure to docetaxel or paclitaxel (chapter 2.3 and chapter 3.2, respectively), when given in combination with a booster drug. At this moment, more data are available of the ModraDoc001 compared to the ModraPac001 capsules. These data show that the inter-patient variability for the capsule formulation is significantly lower compared to the orally administered intravenous formulation, (see chapter 2.3) and that this variability is in the same order as after intravenous administration. This is an important finding since high variability may lead to severe toxicity or under-dosing.

The safety of weekly oral docetaxel in combination with ritonavir was determined in a dose escalation study of which an interim analysis is presented in chapter 2.3. The maximum tolerated dose remains to be established, but the safety profile and systemic

exposure to docetaxel observed thus far, are promising and warrant further development of this regimen. Major treatment limiting adverse effects observed after intravenous therapy with docetaxel, e.g. fluid retention, hematological toxicity and infusion reactions were not observed. Consequently, high doses of dexamethason to prevent fluid retention and allergic reactions were not required. Furthermore, the anti-tumor activity observed at the two highest dose-levels strengthens the concept that oral administration of docetaxel is feasible and potentially active.

Two important findings on orally administered paclitaxel are discussed in this thesis; the development of the solid paclitaxel formulation (chapter 3.2) and the fact that orally administered paclitaxel can be boosted with a CYP3A4 inhibitor (chapter 3.1). Paclitaxel is predominantly metabolized by two enzymes: CYP3A4 and CYP2C8. However, in the intestines, paclitaxel is mainly metabolized by CYP3A4, since CYP2C8 is only expressed in very low amounts in the intestines. Consequently, inhibition of (intestinal) CYP3A4 by ketoconazole, clarithromycin or ritonavir resulted in high systemic exposure to orally administered paclitaxel. Based on the extensive experience gained with ritonavir boosted drugs, the good safety profile and the high increase in systemic exposure to paclitaxel, ritonavir was selected for the future development of orally administered paclitaxel.
These findings form the basis for a recently initiated study on metronomic dosing of ritonavir boosted paclitaxel. Continuous exposure to low concentrations of paclitaxel is reported to have anti-tumor activity by inhibiting angiogenesis. Metronomic dosing of paclitaxel has proven to inhibit angiogenesis by inhibiting the proliferation and differentiation of endothelial cells *in vitro* and *in vivo*. The paclitaxel capsule formulation, ModraPac001, may be an excellent formulation to test this concept in the clinic for the first time.

In chapter 4 of this thesis two pharmacological studies on gemcitabine are presented. Gemcitabine has a poor oral bioavailability due to extensive first pass metabolism by cytidine deaminase. To circumvent this rapid deamination into 2',2- difluorodeoxyuridine (dFdU), a gemcitabine prodrug was developed, LY2334737. The unstable amine group in this molecule was covalently bound to valproic acid. In chapter 4.1 the results are discussed of the first-in-human study of this novel compound. It was demonstrated that the prodrug increased the bioavailability to gemcitabine and reduced the accumulation of the gemcitabine metabolite, dFdU compared to orally administered gemcitabine. Nevertheless, the exposure to this metabolite was high and the metabolite accumulated due to its long terminal half life. The contribution of dFdU to the toxicity profile of gemcitabine is not completely understood. *In vitro* studies demonstrated that dFdU is more than 1000 times less active than gemcitabine. However, the measured concentrations of dFdU are more than 1000 times higher than the exposures to gemcitabine after oral administration of the prodrug. Since, *in vivo* and *in vitro* studies

demonstrated that dFdU is able to enter the cell and that phosphorylated forms of dFdU can be found, a contribution of dFdU to the anti-tumor activity and toxicity of gemcitabine treatment is likely.

At the maximum tolerated dose of LY2334737 patients received a cumulative dose of 560 mg (equivalent to 380 mg gemcitabine) in a 21-day cycle, which is significantly lower than standard i.v. gemcitabine regimens (e.g. 1250 mg/m^2 on day 1 and 8 of a 21-day cycle). Nevertheless, typical gemcitabine related non-hematological toxicities (fatigue, gastrointestinal toxicities, elevated liver enzymes and pyrexia) were observed at these relatively low doses. These discrepancies in toxicity profile probably originate in the oral route of administration and the short dosing intervals resulting in continuous exposure to LY2334737 and metabolites. Oral administration of gemcitabine is still limited by the extensive first-pass metabolism of this drug and therefore, for further clinical development of this concept this issue needs to be addressed.

Future perspectives

The studies described in this thesis warrant the further development of orally administered taxanes. Currently, plans for phase II clinical testing of ritonavir boosted ModraDoc001 are made for maintenance or second line treatment of patients with non-small-cell-lung-cancer (NSCLC). However, many pharmacological questions remain to be answered. For example what are the metabolic routes of boosted orally administered taxanes, since these are most likely altered when the main metabolizing enzyme, CYP3A4, is inhibited? In mice it was shown that when CYP3A4 was inhibited, or missing (cyp3a4$^{-/-}$ knock-out mice), the role of ATP-binding cassette transporters (ABC) like P-gp and multi-drug-resistance associated protein 2 (MRP2) in excreting docetaxel became more profound (discussed in chapter 2.1). To determine these metabolic routes, new analytical assays using liquid chromatography coupled to tandem mass spectrometry (LC-MS/MS) are currently being developed. With these assays the pharmacokinetics of the different metabolites can be determined.
Furthermore, when the taxanes are boosted with ritonavir, resulting in inhibition of the major metabolizing enzyme of docetaxel, pharmacogenetic variations in the genes encoding the ABC transporters could be of increasing importance, whereas these polymorphisms hardly effect taxane pharmacokinetics when given intravenously. Blood samples for pharmacogenetic analyses were drawn from all patients discussed in this thesis and are planned to be analyzed shortly.

The maximum tolerated dose of daily LY2334737 treatment followed by 7 days of rest has been determined in a study presented in chapter 4.1 of this thesis. This gemcitabine

prodrug was designed to overcome the extensive presystemic metabolism by cytidine deaminase to dFdU. Indeed, the levels of dFdU after administration of the prodrug were lower than the levels of this metabolite after oral administration of gemcitabine. However, the levels of dFdU were still very high and possibly negatively affected the safety profile of LY2334737 treatment. Another strategy to improve the systemic exposure to gemcitabine and decrease the exposure to dFdU could be achieved by inhibiting cytidine deaminase. This boosting strategy might be possible with tetrahydrouridine. This strong inhibitor of cytidine deaminase already demonstrated to significantly reduce presystemic metabolism of gemcitabine in preclinical studies.

Concluding, the oral application of the taxanes was found to be feasible. The presented pharmacokinetic data demonstrated that the systemic exposure to the taxanes was high and signs of antitumor activity were observed after treatment with ritonavir boosted ModraDoc001 capsules. The extensive first-pass metabolism of gemcitabine and LY2334737 precludes further development of these compounds.

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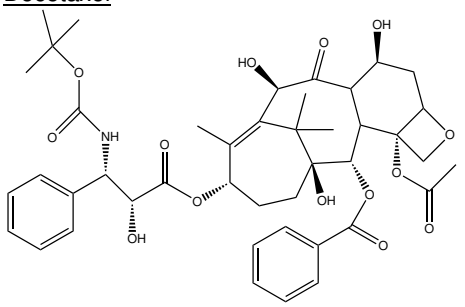
Chemical structures

Chemical structures of investigated molecules

Chapter 1-3:

Docetaxel



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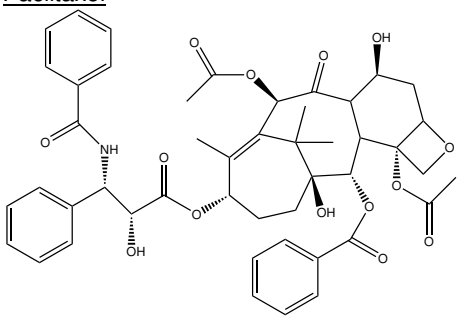

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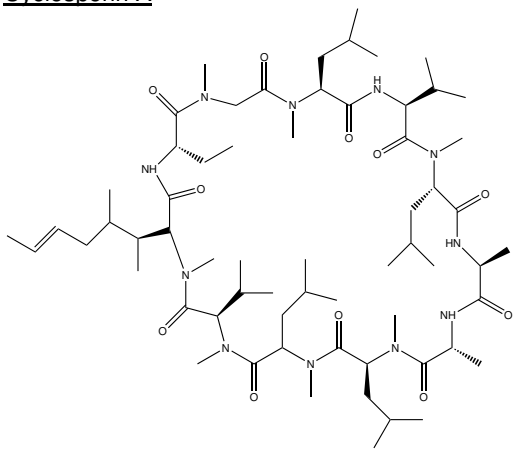
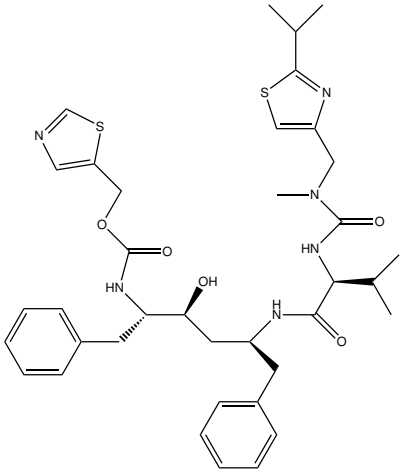
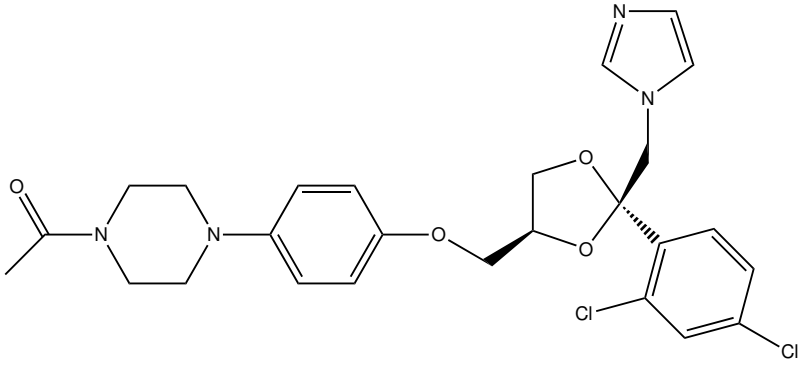


Ritonavir



Ketoconazole



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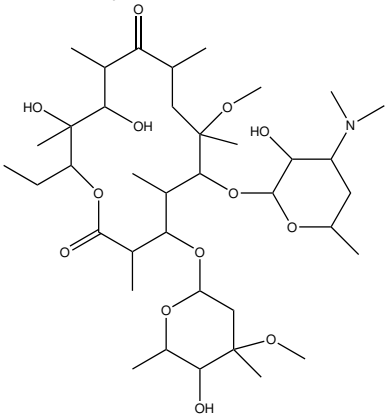
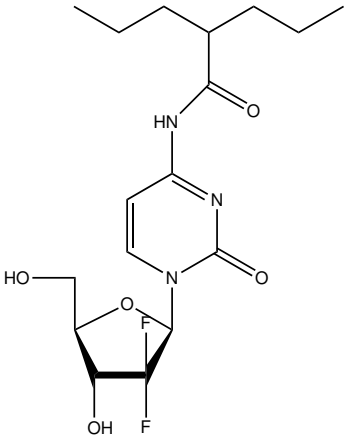
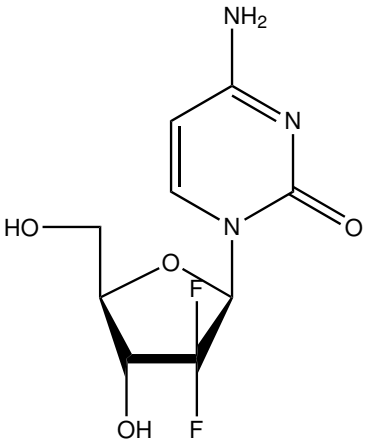


Chapter 4:

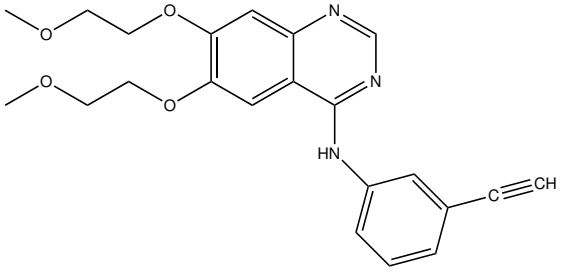
LY2334737



Gemcitabine



Erlotinib



Samenvatting

Samenvatting: conclusies en toekomstperspectief

Het doel van het onderzoek beschreven in dit proefschrift was om de orale toepassing van docetaxel, paclitaxel en gemcitabine te onderzoeken. Orale toediening heeft veel voordelen; ten eerste geeft de patiënt in het algemeen de voorkeur aan orale toediening, dit is immers minder invasief en de medicijnen kunnen thuis worden ingenomen. Verder zijn de kosten van orale toediening waarschijnlijk lager en is het mogelijk om de medicijnen chronisch, dat wil zeggen dagelijks of twee maal daags, in te nemen.
Echter, indien deze medicijnen oraal worden ingenomen bereiken zij slechts in zeer geringe mate de bloedbaan. De taxanen (docetaxel en paclitaxel) hebben een zeer lage biologische beschikbaarheid doordat zij een substraat zijn voor drug-transporters, met name P-glycoproteïne (P-gp), snel worden gemetaboliseerd in de darmwand en de lever door cytochroom P450 (CYP) enzymen en door de slechte oplosbaarheid. Gemcitabine heeft een lage orale biologische beschikbaarheid vanwege het first-pass metabolisme door cytidine deaminase.

Uit voorgaande studies blijkt dat de lage biologische beschikbaarheid van docetaxel met name wordt bepaald door CYP3A4 in lever en darmen. Remming van CYP3A4 in muizen met een lage dosering ritonavir (de booster-drug) resulteerde in een sterke, 50-maal, toename in systemische blootstelling aan docetaxel. De schijnbare biologische beschikbaarheid (de AUC na orale toediening gedeeld door de AUC na intraveneuze toediening van dezelfde dosering) in mensen na remming van CYP3A4 was hoger dan 100%.
Het gebruik van een drug-drug interactie ten behoeve van de behandeling met oraal docetaxel brengt veel additionele farmacologische vragen met zich mee: welke booster moeten we gebruiken? Wanneer en in welke dosering moet de booster worden toegediend en wat is de veiligheid van deze aanpak? De studies beschreven in hoofdstuk 2 van dit proefschrift hadden als doel de farmacologie van ‘boosted’ oraal docetaxel te onderzoeken en daarnaast het bepalen van het optimale oraal docetaxel regime. We hebben aangetoond dat een hoge blootstelling aan docetaxel mogelijk is na toediening van een sterke CYP3A4 remmer (ketoconazol, claritromycine en ritonavir). Een lage dosering ritonavir, 100-200 mg, werd op basis van deze studie geselecteerd voor de verdere ontwikkeling. Deze keuze werd gemaakt op basis van de hoge biologische beschikbaarheid aan docetaxel bij gelijktijdig toedienen van ritonavir, het goede veiligheidspatroon van laag gedoseerd ritonavir en de reeds uitgebreide ervaring met het gebruik van ritonavir als booster in de behandeling van HIV.

Om de farmacokinetische interactie tussen docetaxel en ritonavir beter te begrijpen, zijn data van oraal en intraveneus toegediend docetaxel van voorgaande preklinische en

klinische studies nader onderzocht met behulp van Nonlinear Mixed Effects Modeling (NONMEM). Aangetoond werd dat de interactie tussen docetaxel en ritonavir goed beschreven kan worden door een 'well-stirred' lever model zoals ontwikkeld door Wilkinson et al. Met dit model werd aangetoond dat gelijktijdige toediening van ritonavir zorgt voor een toename in docetaxel absorptie en een ritonavir-concentratie afhankelijke remming van CYP3A4 activiteit. Dit resulteerde in een afname van de docetaxel eliminatie.

Het ontwikkelde model werd ook toegepast om de optimale timing en dosering van ritonavir te schatten (hoofdstuk 2.2). Middels computer simulaties werd voorspeld dat de blootstelling aan docetaxel slechts in geringe mate kon worden verhoogd middels een hogere dosering ritonavir. Om deze simulatie resultaten te bevestigen werd een proof-of-concept studie uitgevoerd waarin oraal docetaxel werd gegeven met 100 of 200 mg ritonavir. Anders dan voorspeld zorgde de 200 mg dosering voor een sterke toename (77%) van de systemische blootstelling aan docetaxel, zie hoofdstuk 2.5. De plasma concentratie tijd curven lieten duidelijk zien dat dit effect met name kon worden toegeschreven aan een hogere biologische beschikbaarheid, de Cmax was sterk gestegen en de terminale delen van de docetaxel curven liepen parallel. In dezelfde studie werd oraal docetaxel ook gegeven in combinatie met 100 mg ritonavir tegelijkertijd en een tweede ritonavir capsule van 100 mg 4 uur na docetaxel inname. Deze tweede ritonavir capsule resulteerde in een verdere afname van de docetaxel eliminatie snelheid. Echter, dit had slechts een gering effect op de totale systemische blootstelling aan docetaxel.
Op basis van de hierboven genoemde simulatie studie en de proof-of-concept studie, werd een dosering van 200 mg ritonavir met gelijktijdige inname van docetaxel, geselecteerd voor de verdere ontwikkeling van oraal docetaxel. De data geven aan dat hogere ritonavir doseringen de blootstelling aan docetaxel nog verder zouden kunnen verhogen. Echter deze toename is waarschijnlijk klein en zal niet opwegen tegen de hogere kans op ritonavir gerelateerde bijwerkingen.

Veel studies beschreven in dit proefschrift zijn uitgevoerd met een nieuwe docetaxel formulering: ModraDoc001 capsules. Om de slechte oplosbaarheid van docetaxel te verbeteren, is er een 'solid-dispersion' ontwikkeld. Op dezelfde manier is een vergelijkbare formulering voor oraal paclitaxel ontwikkeld, ModraPac001. Beide formuleringen resulteerden in een hoge systemische blootstelling aan docetaxel of paclitaxel (respectievelijk hoofdstuk 2.3 en hoofdstuk 3.2). De data van ModraDoc001 lieten een significant lagere variatie in blootstelling zien in vergelijking met de drinkoplossing van docetaxel (orale toediening van de intraveneuze docetaxel formulering) en vergelijkbaar met de interpatiënt variatie na intraveneuze therapie,

hoofdstuk 2.3. Dit is een belangrijke vinding aangezien een hoge interpatiënt variatie mogelijk leidt tot ernstige toxiciteit of onderdosering.

De veiligheid van oraal docetaxel in combinatie met ritonavir werd onderzocht in een dosis escalatie studie, waarvan een interim analyse staat beschreven in hoofdstuk 2.3. De studie is momenteel nog bezig en de maximum tolereerbare dosering kon dus nog niet worden vastgesteld. Ernstige bijwerkingen die bij intraveneuze toediening voorkomen, zoals overgevoeligheidsreacties, hematologische toxiciteit en vochtretentie, zijn tot nu toe niet gezien. Wel werd er bij een aantal patiënten ernstige diarree geconstateerd. Tumor regressie werd aangetoond bij 2 patiënten behandeld op de 2 hoogste dosis niveaus. Dit versterkt het concept dat orale therapie met docetaxel mogelijk is en moedigt verder onderzoek naar de orale toepassing aan.

Twee belangrijke vindingen voor de orale toepassing van paclitaxel staan beschreven in dit proefschrift; de ontwikkeling van een capsule formulering van paclitaxel (hoofdstuk 3.2) en de ontdekking dat oraal toegediend paclitaxel ook mogelijk is door remming van CYP3A4 (hoofdstuk 3.1). Paclitaxel wordt met name gemetaboliseerd door 2 enzymen, CYP2C8 en CYP3A4. Echter, in de darmen wordt paclitaxel vooral door CYP3A4 gemetaboliseerd omdat CYP2C8 hier slechts in zeer geringe mate voorkomt. Waarschijnlijk als gevolg hiervan resulteert remming van (intestinaal) CYP3A4 met ketoconazol, claritromycine of ritonavir in een hoge blootstelling aan oraal toegediend paclitaxel.
Deze twee vindingen vormen de basis voor een recent geïnitieerde studie naar metronoom ritonavir-boosted paclitaxel. In in vitro en in vivo experimenten is reeds aangetoond dat continue blootstelling aan lage concentraties paclitaxel resulteert in anti-tumor activiteit door remming van de proliferatie van endotheelcellen. Dit resulteert in remming van de bloedvatengroei (angiogenese). De paclitaxel capsuleformulering in combinatie met ritonavir vormt een goede kandidaat om dit concept verder te onderzoeken.

In hoofdstuk 4 worden twee farmacologische studies van gemcitabine gepresenteerd. Gemcitabine heeft een lage orale biologische beschikbaarheid vanwege uitgebreid ‘first-pass’ metabolisme door cytidine deaminase in 2’,2-difluorodeoxyuridine (dFdU). Om dit te voorkomen is een pro-drug van gemcitabine ontwikkeld, LY2334737. De onstabiele amine groep is in dit molecuul covalent gebonden aan valproinezuur. In hoofdstuk 4.1 worden de resultaten beschreven van de eerste studie van LY2334737 in patiënten. De farmacokinetiek liet zien dat de biologische beschikbaarheid van gemcitabine toenam en dat de accumulatie van dFdU lager was in vergelijking met orale toediening van gemcitabine. Desondanks was de blootstelling aan dFdU hoog en accumuleerde dFdU door de lange halfwaardetijd. De bijdrage van dFdU aan het toxiciteitsprofiel van

gemcitabine is nog niet volledig bekend. In vitro studies toonden aan dat dFdU meer dan 1000 maal minder actief is in vergelijking met gemcitabine. Echter de gemeten concentraties van dFdU zijn ook meer dan 1000 keer hoger dan de blootstelling aan gemcitabine. Daarom is een bijdrage van dFdU aan het toxiciteitsprofiel en de anti-tumor activiteit aannemelijk, ook omdat in vivo en in vitro studies aangetoond hebben dat dFdU in staat is om de cel binnen te dringen en net als gemcitabine in staat is om in de cel te fosforyleren.

Op het niveau van de maximaal verdraagbare dosering van LY2334737 ontvingen patiënten een cumulatieve dosering van 560 mg (equivalent aan 380 mg gemcitabine) in een 21-daagse kuur. Dit is veel minder dan standaard i.v. gemcitabine regimes (bijvoorbeeld 1250 mg/m^2 op dag 1 en 8 van een 21-daagse kuur). Desondanks, werd typische gemcitabine non-hematologische toxiciteit (vermoeidheid, gastro-intestinale bijwerkingen, verhoogde leverenzymen en koorts) gezien op deze relatief lage doseringen. Dat deze veelal ernstige bijwerkingen toch voorkomen, komt waarschijnlijk door de orale toedieningsvorm en de korte doseerintervallen, hetgeen resulteert in continue blootstelling aan LY2334737 en metabolieten. Orale toepassing van gemcitabine wordt ook bij toediening van de pro-drug (LY2334737) gelimiteerd door het uitgebreide 'first-pass' metabolisme. Voor de toekomstige ontwikkeling van oraal gemcitabine zal dit eerst moeten worden opgelost.

Toekomstperspectief

De studies beschreven in dit proefschrift tonen aan dat orale toepassing van de taxanen mogelijk is en rechtvaardigen de verdere ontwikkeling daarvan. Op dit moment zijn er reeds plannen gemaakt om ritonavir-boosted ModraDoc001 te onderzoeken bij de tweedelijns behandeling van niet-kleincellig longcarcinoom (NSCLC). Echter, veel farmacologische vraagstukken dienen nog beantwoord te worden. Bijvoorbeeld, wat zijn de metabolisme-paden van 'boosted' oraal toegediende taxanen? In muizen is aangetoond dat wanneer CYP3A4 is geremd of ontbreekt, de rol van P-gp en multi-drug-resistance associated protein 2 (MRP2) in de eliminatie van docetaxel in toenemende mate van belang wordt (besproken in hoofdstuk 2.1). Om de metabole routes van docetaxel en paclitaxel te bestuderen, worden momenteel nieuwe analytische assays ontwikkeld. Met behulp van deze methodes kan de farmacokinetiek van de diverse metabolieten bepaald worden.

De gemcitabine pro-drug was ontworpen om het uitgebreide 'first-pass' metabolisme door cytidine deaminase in dFdU te omzeilen. Dit was deels gelukt aangezien de dFdU spiegels lager waren dan na orale toediening van gemcitabine. Echter, de dFdU spiegels waren nog altijd erg hoog en hadden mogelijk een negatief effect op de veiligheid van de

behandeling. Een andere strategie om de blootstelling aan gemcitabine na orale toediening te verhogen en de blootstelling aan dFdU te verlagen, is remming van cytidine deaminase. Remming van cytidine deaminase is mogelijk met tetrahydrouridine. Deze sterke remmer heeft reeds in preklinische studies aangetoond dat het de blootstelling aan gemcitabine sterk kan verhogen.

Concluderend kan worden gesteld dat orale toediening van taxanen mogelijk is. De gepresenteerde farmacokinetiek data lieten zien dat de blootstelling aan de taxanen hoog was en tekenen van anti-tumor activiteit werden gezien na behandeling met ModraDoc001 in combinatie met ritonavir. Het uitgebreide ‘first-pass’ metabolisme van gemcitabine en LY2334737 beperkt momenteel de orale toepassing van deze stoffen.

Dankwoord

Dankwoord

Dit proefschrift is het resultaat van de samenwerking met velen. Ik heb er erg veel van geleerd en graag zou ik dan ook een aantal mensen in het bijzonder willen noemen.
Allereerst wil ik de patiënten bedanken die hebben meegedaan aan de studies beschreven in dit proefschrift.
Mijn promotoren Jan Schellens en Jos Beijnen wil ik bedanken voor de ruim 4 jaar dat ik in hun onderzoeksgroep heb mogen werken. Beste Jan, jij was mijn eerste aanspreekpunt. Ik heb onze samenwerking erg gewaardeerd. Je interesse en enthousiasme voor het onderzoek en alle resultaten werkten aanstekelijk. Maar bovenal heb ik erg veel van je geleerd en daar ben ik je dankbaar voor.
Mijn co-promotor, Alwin Huitema, wil ik graag bedanken voor de intensieve begeleiding van de diverse PK studies. Jouw analytisch vermogen en kunst om nimmer de rode draad uit het oog te verliezen, waren van groot belang voor dit proefschrift.
Een groot aantal studies beschreven in dit proefschrift waren niet mogelijk geweest zonder de ontwikkeling van de ModraDoc en ModraPac capsules. Graag zou ik dan ook bij deze Johannes Moes en zijn begeleider Bastiaan Nuijen willen bedanken.
De uitvoering van de N07DOW studie vergde veel tijd en inspanning van bijna iedereen binnen de farmacologie groep. Allereerst wil ik de nurse practitioners bedanken voor de wekelijkse begeleiding en controle van alle patiënten. In het bijzonder zou ik Marianne en Annemarie willen noemen voor het up-to-date houden van het verpleegkundig protocol, hetgeen niet eenvoudig was met 10 amendementen en 13 verschillende behandelarmen. Daarnaast zou ik alle artsen, Jan, Serena, Neeltje en Marja, willen bedanken voor de behandeling van de patiënten en het oplossen van de diverse queries. Alle kinetiekverpleegkundigen van afdeling 4C onder aanvoering van Thea zou ik graag willen bedanken voor de samenwerking en de vele bloedafnames.
Voor de kwaliteitswaarborging en dataverwerking van de studie ben ik veel dank verschuldigd aan de mensen van de wetenschappelijke administratie en statistiek, met name Song, Brigitte, Harriët en Bojana. Beste Harriët, de database begint dankzij jouw kennis van SAS nu echt vorm te krijgen. Beste Bojana, het afgelopen jaar hadden we bijna wekelijks overleg wat snel en goed verliep. Jouw kennis en ervaring hebben de studie in korte tijd op een hoger niveau gebracht, heel erg bedankt daarvoor.
Alle medewerkers van het trialbureau zou ik graag willen bedanken voor het registreren van alle patiënten, maar ook voor jullie hulp met al mijn CRF vragen. Jolanda, heel erg bedankt voor de diverse administratieve zaken en jouw hulp met de promotiemappen.

Voor de analytische ondersteuning zou ik graag alle mensen van het laboratorium van de apotheek willen bedanken. In het bijzonder zou ik Hilde Rosing en Bas Thijssen willen

bedanken. Verder zou ik ook graag mijn collega OIO's Robert, Bas en Jeroen willen bedanken voor jullie hulp met de tri-fosfaat, pergolide en ritonavir assays!
Mijn collega OIO's in de keet en op afdeling 2C van het NKI wil ik bedanken voor de gezelligheid, hulp en interesse. Met veel plezier kijk ik terug op de diverse OIO weekenden en borrels!
Ron, bedankt voor al je hulp met NONMEM en uiteraard voor de ontwikkeling van Pirana! Verder zal ik ons uitstapje naar de Franse Côte d'Azur niet snel vergeten. Coen, fijn dat je me op de valreep de mogelijkheden van R hebt laten zien. Rik, bedankt voor je hulp met de DOW studie.
Mijn paranimfen en oud-collegae Maarten en David wil ik bedanken voor de gezelligheid. Leuk dat jullie mijn paranimfen willen zijn!

Familie en vrienden wil ik bedanken voor jullie interesse in mijn onderzoek en voor de nodige afleiding. En als allerlaatste wil ik Corine bedanken. Wat ben ik blij dat ik jou ben tegengekomen!

Rest mij nog het MODRA team erg veel succes te wensen bij de verdere ontwikkeling van oraal docetaxel en paclitaxel!

Stijn, Utrecht 2010

Curriculum Vitae

Curriculum Vitae

Stijn Leonard Willem werd op 14 september 1980 geboren te Weert. In 1999 behaalde hij het atheneum diploma aan het Bisschoppelijk college te Weert. In datzelfde jaar werd begonnen met de studie farmaceutische wetenschappen aan de Universiteit Utrecht. Ter afsluiting van zijn doctoraalprogramma werd een wetenschappelijke stage verricht aan de R&D laboratoria van de L.V.M.H. cosmetica groep in Saint-Jean de Braye, Frankrijk. Tijdens deze stage heeft hij meegeholpen met de ontwikkeling en validatie van immunoassays. In 2004 werd het doctoraalexamen afgelegd en in augustus 2006 werd het apothekersdiploma behaald. Aansluitend begon hij met een promotieonderzoek onder begeleiding van prof.dr. Jan Schellens, prof.dr. Jos Beijnen en co-promotor dr. Alwin Huitema. Naast het promotieonderzoek werd tevens de opleiding tot klinisch farmacoloog gevolgd.

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