

Clinical pharmacology of novel anticancer agents

Focus on oral formulations

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Clinical pharmacology of novel anticancer agents

Focus on oral formulations

Klinische farmacologie van nieuwe antikanker medicijnen

Focus op formuleringen voor orale toediening

(met een samenvatting in het Nederlands)

Proefschrift

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“You treat a disease, you win, you lose.
You treat a person, I guarantee you, you’ll win, no matter what the outcome.”

Robin Williams in Patch Adams

Voor Mirte

Preface

In this thesis the clinical pharmacology of novel oral anti-cancer agents is discussed. Apart from the clinical development of the anti-cancer agents, a modelling approach to correlate exposure of oral docetaxel combined with ritonavir to toxicity, the development of an assay to detect circulating endothelial cells in peripheral blood, a proof of principle study to prevent ototoxicity after cisplatin therapy and a cost-analysis study of treatment costs on non-small cell lung cancer are presented in this thesis.

Oral taxane formulations:

The taxanes docetaxel and paclitaxel are registered for the treatment of solid tumors [1, 2]. Currently both docetaxel and paclitaxel are administered intravenously, because of poor oral bio-availability. In *Chapter 1* their cellular and clinical pharmacological properties are described in a review [3].

In *Chapter 2* the clinical development of oral formulations of both docetaxel and paclitaxel is described. The oral administration of docetaxel and paclitaxel could improve patient convenience, as administration can occur more flexibly and at home without the need for an indwelling intravenous catheter. Furthermore it could potentially increase the patients safety as the excipients associated with hypersensitivity reactions and peripheral neuropathy (Cremophor EL for paclitaxel and Polysorbate-80 and ethanol for docetaxel) [4, 5] are not needed in the oral formulations. Finally it might result in a reduction in costs as administrations can occur at home rather than on a day care unit. Oral administration is, however difficult, as both taxanes have a poor oral bioavailability, as a result of limited water solubility and high first-pass effect. In order to improve solubility novel solid dispersion formulations of both docetaxel and paclitaxel were developed by the pharmacy of the Netherlands Cancer Institute. The docetaxel formulations are denoted ModraDoc001 capsule and ModraDoc006 tablet and the paclitaxel formulations are denoted ModraPac001 capsule and ModraPac005 tablet [6–8]. The increase in water solubility alone is insufficient to obtain clinically relevant exposure to docetaxel or paclitaxel. To decrease the first-pass effect, which is the result of metabolism by the cytochrome P450 (CYP)3A4 enzyme and involvement of drug-transporters, such as P-glycoprotein (P-gp), Multidrug Resistance-associated Protein 2 (MRP 2) and Organic Anion-Transporting Polypeptides (OATP)1B1/1B3 [9–11], a so called booster drug is needed. These booster drugs generally inhibit the enzyme and/or the transporters that are the major determinants of the first pass effect. In *Chapter 2.1* several potential booster drugs known to inhibit P-gp and/or CYP3A4 are investigated with the aim to find the most optimal booster for orally administered docetaxel. The study was performed as a cross-over study, wherein docetaxel was administered as the ModraDoc001 capsule.

On the basis of the results obtained in *Chapter 2.1* the protease inhibitor ritonavir was selected for the further clinical development in phase I clinical studies. Ritonavir is a strong CYP3A4 inhibitor,

however it also inhibits several drug transporters e.g. P-gp and OATP1B1/1B3 [11, 12]. In a phase I dose-escalation study the once weekly oral administration of the ModraDoc001 capsule combined with ritonavir (ModraDoc001/r) was investigated. The result of this trial are presented in *Chapter 2.2*. After completion of the dose-escalation with the ModraDoc001 capsule/ritonavir, the ModraDoc006 tablet became available and was implemented in a new dose-escalation arm of the study. The ModraDoc006 tablet was also administered in a once weekly schedule and combined with ritonavir (ModraDoc006/r). In the dose-escalation in a once weekly administration with ModraDoc001 capsule/ritonavir the variability in exposure to docetaxel increased with dose. To potentially lower the variability in exposure a second dose-escalation trial was started employing a bi-daily once weekly administration schedule (e.g. administration of ModraDoc/ritonavir in the morning and afternoon of day 1). As in the first study the dose-escalation with ModraDoc001/r was completed first and thereafter the ModraDoc006 tablet/r was implemented and investigated in a new arm of the study. The results of this study are presented in *Chapter 2.3*. The results of the dose-escalation with ModraDoc006/r were promising with clinically relevant exposure to docetaxel, an acceptable safety profile and promising anti-tumor activity. Further development in phase II and III clinical development is therefore pursued. *Chapter 2.4* describes the study design process of the phase II study planned with ModraDoc006/r in the orphan indication angiosarcoma. In this non-inferiority study ModraDoc006/r will be compared to intravenous paclitaxel the current standard of care. *Chapter 2.5* describes the proof-of-concept study of boosting uptake of orally administered paclitaxel by CYP3A4 or P-gp inhibition. The study was performed as a cross-over study and aimed to determine the most optimal booster of orally administered paclitaxel (as a drinking solution), the optimal booster could very well have been different from docetaxel, as paclitaxel is also metabolized by CYP2C8 [3]. Ritonavir was, however also determined to be the optimal booster for orally administered paclitaxel. After completion of the proof-of-concept study described in *Chapter 2.5* the ModraPac001 capsule, became available for clinical use and a phase I dose-escalation study investigating low dose metronomic (LDM) administration of paclitaxel combined with ritonavir was started. During the trial the development of the ModraPac005 tablet was completed and this formulation was then implemented and used in combination with ritonavir in the remainder of the study. The dose-escalation study of the ModraPac001 capsule and ModraPac005 tablet co-administered with ritonavir in a LDM schedule is described in *Chapter 2.6*. In support of the clinical development of ModraDoc001 capsule/r and ModraDoc006 tablet/r pharmacokinetic modeling was performed and a pharmacokinetic model was developed [13]. After completion of the pharmacokinetic model, the toxicity data obtained in the phase I trials described in *Chapter 2.2 and Chapter 2.3* was used for the pharmacokinetic-toxicodynamic model. The modeling development and the results are presented in *Chapter 2.7*.

Circulating endothelial cells (CEC):

Circulating endothelial cells (CEC) are a suggested biomarker of LDM treatment [14, 15]. Their numbers were found to be elevated in cancer patients [16, 17], where they are thought to play an important role in the growth of and the repair of tumor vasculature [14]. It is expected that as a result of anti-angiogenic therapy, as given in the dose-escalation study with oral paclitaxel *Chapter 2.6*, their numbers will decline as an early indicator of anti-tumor response. In *Chapter 3* the development of and the preliminary testing of the method to enumerate CEC in whole blood of cancer patients and healthy volunteers by CD34 bead enrichment followed by flow-cytometric measurement is described.

P53-HDM2 interaction inhibitors:

The p53 protein is a key mediator of apoptosis [18]. The gene encoding for p53 (TP53) is mutated in about 50% of human tumors and the occurrence of the mutation is considered an early event in tumorigenesis [19]. In about 50% of tumors, where the gene is not mutated (wild-type) the function of p53 is often down-regulated otherwise, for example by upregulation or overexpression of the Human Double Minute protein 2 (HDM2, or MDM2 in mice). HDM2 down-regulates the function of p53 by direct binding, thereby promoting its degradation [20]. A novel class of drugs prevents the binding of HDM2 to p53, by binding to the p53 binding pocket of HDM2. By preventing degradation of p53, it is expected that p53 can perform its role in apoptosis. In preclinical mouse models this results in anti-tumor activity, in tumors that were TP53 wild-type [21]. In *Chapter 4.1* the results of the dose-escalation study of the HDM2-p53 interaction inhibitor, SAR405838 in monotherapy is presented. The study was performed in patients, who were TP53 wild-type (exons 2-10) [22]. In *Chapter 4.2* the phase I dose-escalation study of the combined treatment with SAR405838 and the MEK inhibitor pimasertib is presented. This combination of a MEK inhibitor and a HDM2-p53 interaction inhibitor showed promising results in preclinical *in vitro* and *in vivo* studies. In the preclinical studies the combination showed to have a synergistic anti-cancer effect in TP53 wild-type tumors, harboring a mutation in B-RAF, K-RAS or N-RAS [23]. Based on the promising preclinical studies a phase I clinical study was initiated. Patients with a tumor that was confirmed TP53 wild-type (exons 2-10) and harboring a mutation in either B-RAF, K-RAS or N-RAS were included.

Dovitinib:

The multi-tyrosine kinase inhibitor dovitinib was evaluated in both phase II and phase III clinical development [24–26]. In support of the clinical development a drug-drug interaction study with the strong CYP1A2 inhibitor fluvoxamine was needed, as CYP1A2 is the second most important enzyme involved in the metabolism of dovitinib, with the most important enzyme being CYP3A4 [27]. In this drug-drug interaction study the steady-state pharmacokinetics of dovitinib alone and

after combination treatment with fluvoxamine was compared in order to establish the effect of CYP1A2 inhibition on the exposure to and the metabolism of dovitinib. The results of this study are presented in *Chapter 5*.

Cisplatin induced ototoxicity:

The platinum agent cisplatin is used in the treatment of both childhood and adult solid tumors. The total dose, that can be administered is limited, due to cumulative toxicity. One of these cumulative toxicities is ototoxicity. Previously it was shown that the anti-oxidant sodium thiosulfate (STS) can reduce the severity of ototoxicity when administered intravenously [28]. The major down side of intravenous STS administration is, that it also diminishes the anti-tumor efficacy of cisplatin. Local application to the middle ear, by trans tympanic administration is not expected to result in systemic exposure, while it might prevent ototoxicity, as STS can diffuse over the round window into the middle ear. The feasibility and safety of STS administrations to the middle ear in patients treated with cisplatin for advanced solid tumors were investigated in a proof-of-principle study, which is described in *Chapter 6*.

Cost analysis of non-small cell lung cancer treatment:

Treatment of cancer has become more expensive over time. In the past costs associated with hospitalization were the major cost drivers in non-small cell lung cancer (NSCLC) [29]. However, with the introduction of novel anti-cancer agents, that are generally more expensive a change in the cost-drivers might have occurred in recent years. In a retrospective cost-analysis, described in *Chapter 7*, the costs and cost-drivers of NSCLC for the period 2006-2012 in the Netherlands Cancer Institute are presented and placed in perspective in comparison to a previous cost analysis study in the Netherlands that looked at the treatment of NSCLC in 2005 [29].

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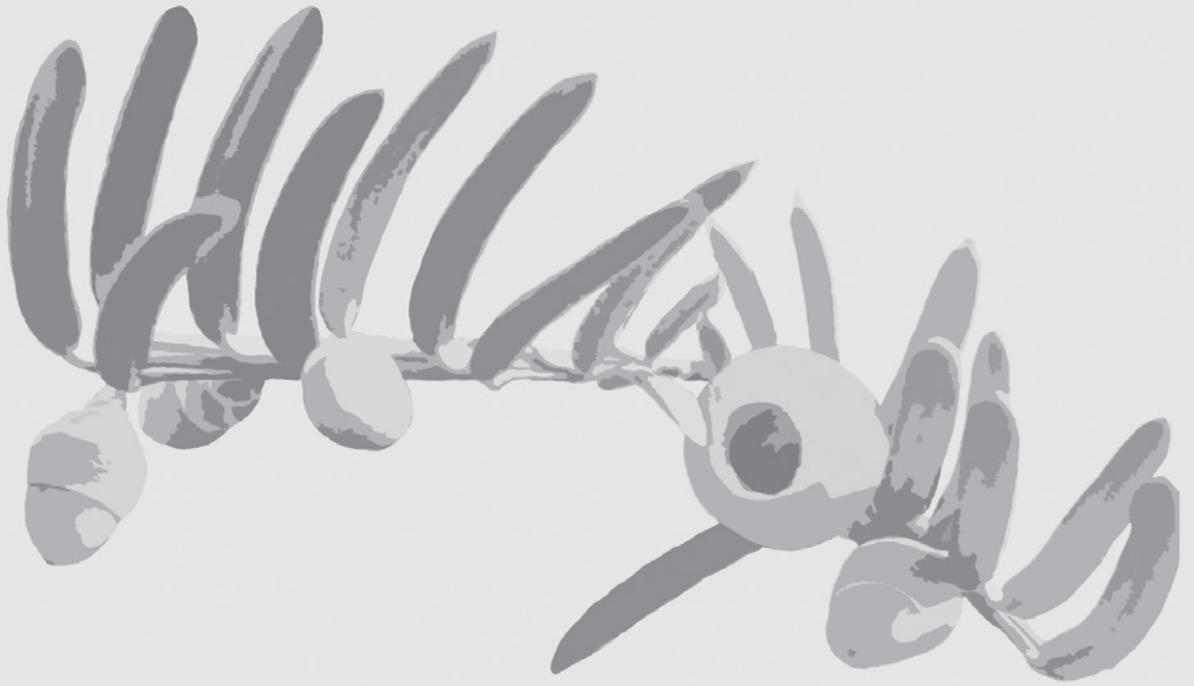
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Chapter 1

Pharmacology of taxanes



Chapter 1

Cellular and clinical pharmacology of the taxanes docetaxel and paclitaxel - a review.

Vincent A. de Weger

Jos H. Beijnen

Jan H.M. Schellens

Anticancer Drugs. 2015 Feb; 26 (2): 240

Summary:

Paclitaxel and Docetaxel are active against a range of human cancers. The antitumor activity is based on stabilization of the microtubule dynamics and thereby disruption of the cell cycle. The taxanes are administered as intravenous solutions in a short administration schedule. Distribution of both taxanes is rapid with large volumes of distribution and significant binding to plasma proteins. The metabolism of paclitaxel is mediated primarily by the P450 cytochrome enzymes CYP2C8 and CYP3A4 whereas docetaxel is only metabolized by CYP3A4. The most common toxicities after intravenous administration are neutropenia, hypersensitivity reactions, neurotoxicity and alopecia. Several new administration forms are in development, albumin bound paclitaxel (Abraxane®) has recently been registered. Oral formulations have been developed for the taxanes and several are now undergoing phase I trials. New formulations might improve efficacy, safety and could be easier to use.

Introduction

Paclitaxel was first discovered in the 1970's. The extract of the bark of the western yew tree *Taxus brevifolia* was found to have activity against murine tumors. The effective component, paclitaxel, was identified in 1971 by Wani et al. as summarized in the preceding article [1]. Limited availability of the western yew and problems with the formulation of paclitaxel (Taxol®) and especially because of hypersensitivity reactions, development of paclitaxel was suspended for almost a decade. The delay in the development of paclitaxel prompted the search for other taxanes. In 1981 a French group discovered a new taxane drug docetaxel. Docetaxel (Taxotere®) is a semisynthetic product produced by esterification of 10-deacetyl Baccatin III, which by itself has no cytotoxic activity. The 10-deacetyl Baccatin III is isolated from the needles of the European yew tree. *Taxus baccata* a more renewable source [2,3]. The availability problems of paclitaxel were eventually resolved when synthetic paclitaxel was developed and made available [4]. Clinical activity against a wide range of solid tumors has been shown for the taxanes, especially in the treatment of breast, ovarian, prostate, gastric, non-small cell lung cancer and head and neck cancers that are covered in subsequent articles within this supplement [5–10].

In this review the preclinical and clinical pharmacokinetics of the taxanes docetaxel and paclitaxel will be discussed and an insight into possible future directions for treatment with taxanes as oral formulation or as low dose metronomic therapy will be presented.

Chemotherapeutic mechanism of action (MTD/LDM)

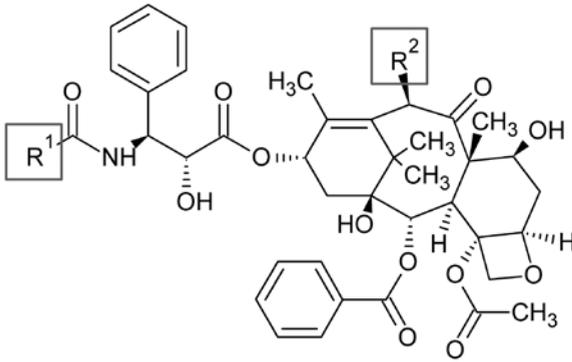
The taxanes are chemically very similar and therefore have a comparable mode of action. They share a taxane ring with a four-membered oxetan side ring at positions C4 and C5 and an ester side chain at C13. Docetaxel and paclitaxel differ at two places R1) a tert-butoxy moiety instead of a benzamide phenyl group on the position on the lateral C13 side chain and R2) a hydroxyl group instead of an acetyl group on the C10 position (figures 1) The side chain at C13 is important since this part binds to the microtubules leading to the formation of stable tubulin bundles, disrupting the physiological assembly and disassembly of microtubules in a guanosine triphosphate independent way. As a result cell proliferation is inhibited by halting the cell cycle at the metaphase/anaphase boundary and by formation of an incomplete metaphase plate of chromosomes induced by the stabilization of the microtubule dynamics [11,12]. A second mode of action of taxanes has been identified, which is an effect on the tumor vasculature and micro-environment when administered continuously at low doses. This form of therapy is better known as low dose metronomic therapy (LDM). These low doses are cytotoxic to proliferating endothelial cells thereby inhibiting tumor vasculogenesis,

whereas tumor cells are insensitive to the low drug level exposure [13,14]. In preclinical studies LDM therapy with taxanes has shown potential in several tumor types [15,16].

On a molar basis docetaxel is more potent in inducing cytotoxicity than paclitaxel both in vitro and in tumor xenografts. Docetaxel has also been found to be slightly more active as a tubulin assembly promoter and stabilizer [11]. The affinity for the binding site to tubulin is about 2-fold higher for docetaxel, than that for paclitaxel. Intracellular levels are higher and cellular efflux is slower for docetaxel compared to paclitaxel in in-vitro models at similar concentrations [2,11,17]. Clinical studies comparing docetaxel to paclitaxel are limited. Phase II/III studies in breast cancer, ovarian cancer and gastric cancer comparing docetaxel and paclitaxel in combination with another chemotherapeutic agent all found no differences in overall survival. Toxicity profiles were different, though mild for all schedules [18–21].

“Classic” High dose chemotherapy:

In phase I trials docetaxel was first administered as an intravenous (IV) formulation in a 2 weekly schedule with infusion durations of 1 or 2 hours. Because of toxicity a 3 weekly schedule was further explored. Subsequently, longer infusion durations ranging from 1-24 hours and weekly versus 3 weekly schedules were investigated. Infusion durations longer than 6 hours were associated with increased toxicity, especially mucositis. The most relevant side effect of docetaxel is neutropenia which is correlated with area under the plasma concentration-time curve (AUC) and duration of exposure $>0.20 \mu\text{mol/L}$ of docetaxel [22,23]. A large meta-analysis of weekly versus 3 weekly administration showed a comparable overall survival, however febrile neutropenia was more severe in the 3 weekly schedule. No differences were observed for other docetaxel-related toxicities [24]. In the early stages of the development of paclitaxel infusion durations ranged from 3-24 hours. It was expected that with longer infusion durations the hypersensitivity reactions were less common and less severe. In a randomized study that used a bifactorial design, patients were randomized to receive 175 or 135 mg/m^2 of paclitaxel over either 3 or 24 hours [25]. Prophylactic medication to prevent hypersensitivity reactions was given in all schedules. No differences were shown in the frequency or severity of hypersensitivity reactions observed in the 4 groups investigated in this study, nor was there a difference in progression free survival or disease free survival in the groups investigated. On the other hand, significant differences were observed in the incidence of neutropenia; 71% vs 18% of patients developed neutropenia in the 24 hour vs 3 hour infusion schedule respectively. Results of this study performed by Eisenhauer et al. favored the shorter administration scheme of paclitaxel, because of the lower frequency of neutropenia in this schedule. The higher dose level investigated of 175 mg/m^2 , showed a better efficacy in ovarian cancer, compared to the 135 mg/m^2 dose-level [25].



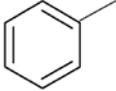
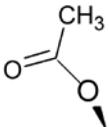
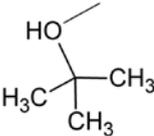
	R ¹	R ²
paclitaxel		
docetaxel		

Figure 1: The basic structure of paclitaxel and docetaxel, the boxes indicate the sites of differences between paclitaxel and docetaxel. The table shows the moiety attached at these sites for paclitaxel and docetaxel.

The most common toxicities seen after IV administration of the taxanes are hypersensitivity reactions and neutropenia. The hypersensitivity reactions –at times severe and life threatening—are seldom seen beyond the first or second course and take place shortly after starting the infusion. With premedication the frequency and severity of hypersensitivity reactions are controllable, and these often resolve within 15 minutes of interrupting the infusion, and do not recur upon restarting the infusion at slower rates. Factors predisposing to severe hypersensitivity reactions remain unclear and both the taxanes and the pharmaceutical excipients are thought contribute [3,26]. Neutropenia is the most important toxicity and found to be dose-limiting in phase I trials; it is associated with the time-period the drug levels are above plasma concentrations of 0.1 $\mu\text{mol/l}$ for paclitaxel [27,28]. In adjuvant treatments containing taxanes, the extent and duration of neutropenia are improved

by the sequential administration of granulocyte colony-stimulating factor (g-csf). Anemia and thrombocytopenia are rare side-effects for both taxanes. Other common side effects include sensory neurotoxicity, diarrhea, stomatitis, nausea/vomiting, skin toxicity and alopecia [12,29]. Specific to docetaxel is a unique fluid retention syndrome, characterized by edema, pleural effusion and ascites. Fluid retention is related to the cumulative dose of the drug and usually resolves after docetaxel is stopped and it is less severe when prophylactic corticosteroids and histamine receptor antagonist are administered [12].

Low dose metronomic therapy

The taxanes when administered as low dose metronomic therapy (LDM) have shown promising results *in vitro* and *in vivo*. Muta et al. found that docetaxel and paclitaxel are toxic to endothelial cells in rats at very low concentrations. Others have shown effectiveness with paclitaxel or docetaxel when administered at low concentrations in mice bearing implanted prostate and gastric tumors [16,30]. The antiangiogenic effect is lost or greatly diminished in maximum tolerated dose (MTD) based treatment schedules, presumably because effects on the tumor vessel micro-environment are lost during the long drug free breaks. Furthermore, cremophor EL used in the pharmaceutical intravenous formulation of paclitaxel may diminish the antiangiogenic activity of paclitaxel [31]. This potentially higher anti-angiogenic of docetaxel *vis-a-vis* paclitaxel has only been shown pre-clinically [32]. Because of the different modes of action of classical MTD-based therapy versus LDM-based therapy, LDM therapy may still show activity even after tumors have become taxane resistant. A single clinical study employing the LDM concept has been published, in which patients were treated with continuous infusion of paclitaxel. However, because of complications related to the infusion (thrombosis, infections) the study has not progressed into phase III trials [33]. The main reason that no other clinical studies have been performed is the lack of a commercially available oral dosage form of either paclitaxel or docetaxel. Several oral formulations though are now under investigation [34,35]. Possibly potentiation of anti-angiogenic agents in combination with taxanes is covered in a subsequent article.

Metabolism (cellular/clinical pharmacology)

The pharmacokinetic profiles of paclitaxel and docetaxel reveal fast tissue distribution and large volumes of distribution 182 liters/m² and 74 liters/m², respectively. Peak plasma levels are reached at the end of IV administration. The pharmacokinetic behavior of paclitaxel and docetaxel is best described by a 3 compartment non-linear model. With peak plasma concentrations after iv administration reaching 5 µM for paclitaxel at a dose of 175 mg/m² as a 3h infusion and 3 µM for

docetaxel at a dose of 100 mg/m² given as a 1h infusion. The systemic clearance of paclitaxel is on average 350 ml/min/m² and of docetaxel 300 ml/min/m² [12,29].

Intravenous administration:

Paclitaxel and docetaxel are administered as IV solutions with pharmaceutical vehicles cremophor EL/ethanol and polysorbate 80/ethanol respectively, since both compounds have poor water solubility. In plasma paclitaxel and docetaxel are about 90% bound to serum albumin and α 1-acid glycoprotein, with a minor lipoprotein-bound contribution [36]. In animal studies a similar plasma protein binding is observed ranging from 70-76% in rats and 89-95% in mice [17,37].

Tissue distribution is extensive, rapid and similar for paclitaxel and docetaxel. Preclinical results in animals have shown high levels in most tissues. The highest concentrations are found in the liver, bile ducts, the intestine, as well as the intestinal contents. The absorption into the central nervous system and the testis is limited. The brain is protected by effective efflux of the taxanes by P-glycoprotein (P-gP). As was shown by studies in Mdr1a knockout mice (Mdr1a encodes for P-gP) the accumulation of paclitaxel was 3-fold higher in the brain whereas no major differences are observed in other tissues [38]. Exposure to docetaxel and paclitaxel is relatively high in tumor tissue compared to other tissues, whereas peak tumor levels are relatively low. However, because of slow elimination from tumor tissue the AUC in tumor tissue is about 5-fold higher compared to plasma [17,36,39].

Oral administration:

Oral absorption of the taxanes is limited, because of poor aqueous solubility, extensive first pass metabolism by intestinal CYP3A enzymes and active excretion from intestinal cells by P-gP. P-gP and CYP3A have proven to be the most important limiting factors in uptake of paclitaxel, with minor contributions of other enzymes or transporters as was shown in animal knockout studies and humanized CYP3A mice models [40–44]. In the past preclinical studies demonstrated that the uptake of the taxanes can be boosted by inhibiting P-gP and CYP3A by co-administration of cyclosporin A or ritonavir which resulted in promising activity and good safety in clinical studies [41,45]. Oral administration of docetaxel in mice together with the CYP3A inhibitor ritonavir increased the plasma levels of docetaxel about 50-fold in preclinical studies [41]. Tissue distribution and plasma protein binding were comparable after IV and oral administration.

Metabolism and Excretion:

Metabolism after initial uptake/IV administration of the taxanes proceeds mainly by liver cytochrome P450 enzymes, most importantly by CYP3A and for paclitaxel also by CYP2C8 [46,47]. The metabolism of paclitaxel is comparable between mice and humans, whereas in rat the 2m-hydroxypaclitaxel is the major metabolite which is not detected in humans. The major metabolite in humans

6 α -hydroxypaclitaxel is formed by metabolism by CYP2C8 at the C6 position of the taxane ring. The second metabolite 3'-p-hydroxypaclitaxel is the hydroxylation product of the phenyl moiety at the C3' position of the C13 side chain. The formation of this metabolite is mediated by CYP3A. The 6 α ,3'-p-dihydroxypaclitaxel is the result of metabolism by both enzymes [48,49]. The metabolites and the metabolic pathways are depicted in figure 2.

The principal site of metabolism of docetaxel is the tert-butylpropionate side chain which undergoes oxidation of one of the methyl groups forming the M2 metabolite. M1, M3 and M4 are either formed by CYP3A enzymes or formed by spontaneous cyclisation of the unstable aldehyde and acid intermediate metabolites of the alcohol into 2 diastereoisomers (M1 and M3) and a ketone metabolite (M4) [50]. The CYP2C8 enzyme is incapable of binding to docetaxel, because of the side-chains that differ between paclitaxel and docetaxel. In figure 3 docetaxel is shown with its major metabolites and the metabolic pathway involved [47]. The metabolites of both taxanes have little to no antitumor activity. For instance, the 6 α -hydroxypaclitaxel metabolite of paclitaxel is 30 times less cytotoxic as paclitaxel [48]. The metabolites of docetaxel showed very limited activity in a study conducted by Sparreboom et al [51].

Excretion of the taxanes by hepatic clearance is significant with 70-80% being excreted into bile by P-gP and MRP-2 transporters either as metabolites or as parent drug. Renal clearance contributes only marginally and is about 5-10% [12,29].

In animals the taxanes and their metabolites have also been found in the intestine after cannulation of the bile duct in *mdr1a* wild-type mice whereas in *mdr1a* knockout mice this excretion was limited [40]. Therefore, it can be concluded that the taxanes are excreted directly into the intestinal lumen by intestinal P-gP. The excretion via the bile was similar in both groups, in the *mdr1a*^{-/-} group, however, paclitaxel was almost completely excreted as metabolites whereas in wild-type mice around 40% was excreted as unchanged drug [40]. In P-gP and *cyp3a* knockout mouse metabolism of paclitaxel is largely dependent on MRP-2 (ABCC2), both after IV and oral administration. Combinations of inhibitors of CYP3A and P-gP could, therefore, further improve the oral bioavailability of the taxanes [42,52].

Drug drug interactions (DDI)

The taxanes are metabolized by CYP2C8 and CYP3A enzymes of the cytochrome P450 system. Inhibitors or inducers of these enzymes could therefore mediate a drug-drug interaction increasing or decreasing the plasma levels of docetaxel or paclitaxel, respectively. Importantly, CYP3A inducers - for, instance ketoconazole, macrolide antibiotics and ritonavir - when co-administered with paclitaxel or docetaxel have shown to increase plasma levels of these drugs by inhibition of metabolism of

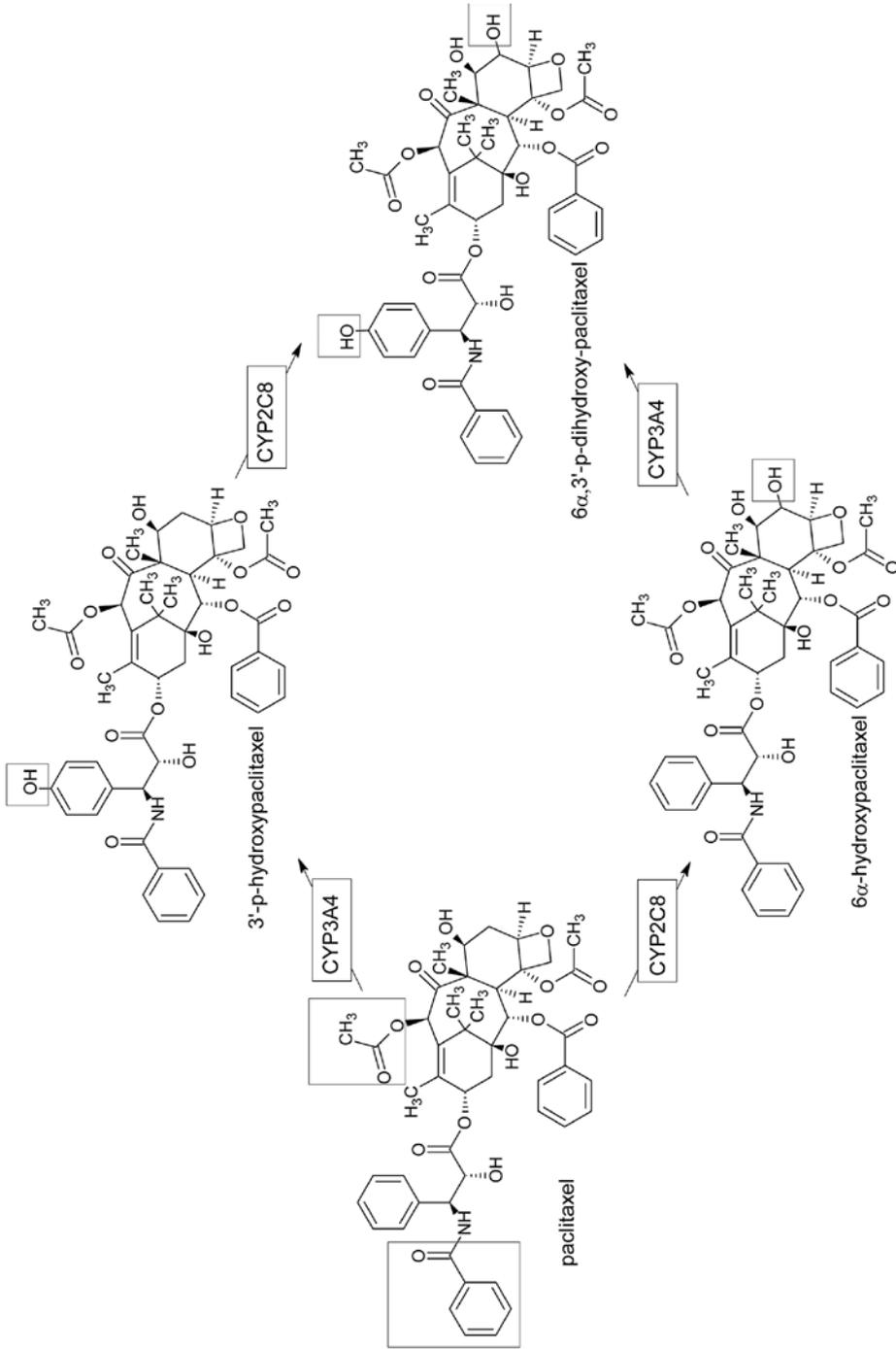


Figure 2: Paclitaxel with the major metabolites and the enzymatic which moiety of the molecule is metabolized. The boxes indicate which moiety of the molecule is metabolized.

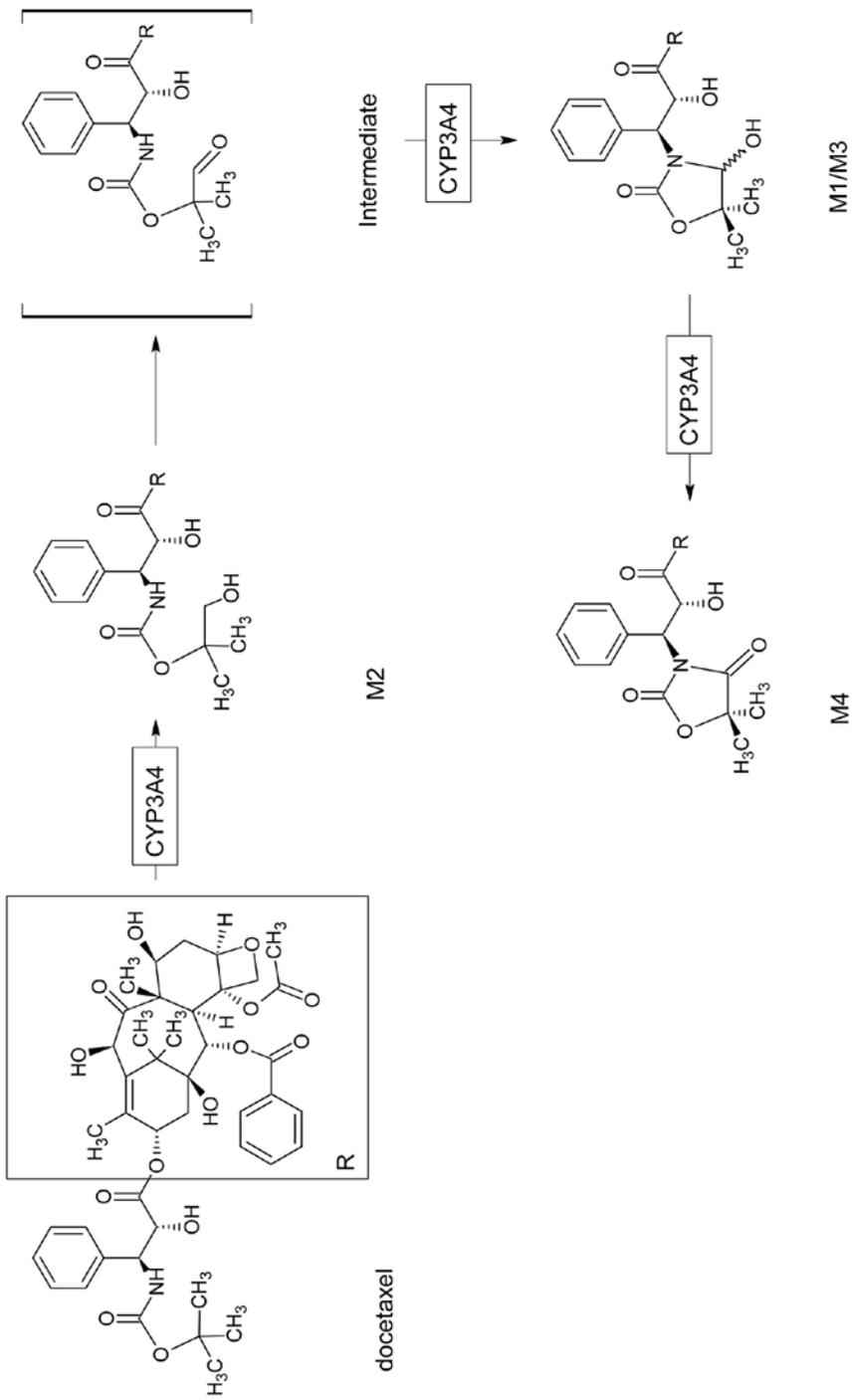


Figure 3: Docetaxel and the major metabolites and the enzymatic enzymes which moiety of the molecule is metabolized.

the taxanes after IV and oral administration [41,53]. Inhibitors of P-gP have also shown significant drug-drug interaction with the taxanes. Elacridar and cyclosporin A are potent inhibitors of P-gP and have shown to increase plasma levels of the taxanes after both oral and IV administration [45]. Concomitant medication that significantly induces or inhibits any of these enzymes or transporters should be avoided. Special care should be taken when an oral formulation of the taxanes becomes available, since these interactions have proven to be more significant in mice studies [41].

Current/Novel regimens

As covered subsequently, new IV formulations of the taxanes are under investigation as well as efforts to improve the availability after oral administration or reduce the side effects. For IV administration albumin-bound paclitaxel (Abraxane®) has been developed and initially registered for the treatment of breast cancer. In a phase II trial in metastatic breast cancer with albumin-bound paclitaxel combined with gemcitabine a confirmed response rate of 50% was reached, a median duration of response of 6.9 months and an overall survival at 6-months of 60% of patients. Most commonly reported grade 3/4 toxicities were neutropenia (42%), fatigue (28%), anemia and dyspnea (both 14%) [54]. Another phase II trial in breast cancer showed a good safety profile of albumin-bound paclitaxel in a group of women heavily pretreated with a taxane. Severe neutropenia was seen in less than 5% of patients [55]. Eventually, it has found a role in pancreatic cancer [56] and in NSCLC [57], and is further covered in these respective articles.

Today several strategies for development of an oral formulation of both taxanes are under investigation. Lian et al. have used nanomicelles to improve uptake of orally ingested paclitaxel. The uptake was significantly improved, in animal studies, compared to an oral administered paclitaxel IV solution [35]. At our Institute boosting of the oral absorption by improving the aqueous solubility and by limiting first-pass metabolism by co-administration of inhibitors of P-gP and CYP3A by cyclosporin A or ritonavir has shown promising results in vitro as well as in vivo. The booster drug cyclosporin A, initially employed in phase I and II trials has been replaced by ritonavir because of its better safety profile and because ritonavir is well established and registered for this purpose in combinations with several HIV-protease inhibitors [45]. Phase I trials employing ritonavir as an oral booster for both docetaxel and paclitaxel are currently ongoing at our Institute, with both weekly and continuous LDM regimens being under investigation [34]. The MTD chemotherapy is given either as a single dose or as a twice daily once a week dose in combination with ritonavir in a phase I dose escalation study. The preliminary results of the comparison of capsule and tablet formulations have been published [58]. Dose escalation thus far has shown that this strategy is safe and well tolerated. The maximum tolerated dose has been defined as 60 mg docetaxel weekly with

100 mg ritonavir or as a twice-daily dose of 20 mg docetaxel with twice daily 100 mg ritonavir. The most common dose limiting toxicities in these studies are diarrhea, nausea and fatigue. Exposure in these dose levels in terms of AUC_{inf} is comparable to the AUC_{inf} after weekly IV administration of 35 mg/m² [59,60].

In a phase I trial paclitaxel is administered in an LDM schedule. This study is conducted as a dose-escalation study with a continuous twice daily administration of paclitaxel given as an oral solid dispersion formulation, as described by Moes et al [34], and ritonavir. Dose escalation has thus far proven that this combination can be administered safely at a twice daily dose of 15 mg paclitaxel with twice daily 100 mg ritonavir. The most common drug related adverse events observed in the first dose levels were fatigue, diarrhea and nausea, mostly not exceeding grade 1. The maximum tolerated dose in this study has not been reached yet. Thus far no hypersensitivity reactions were seen in any of these studies even though premedication with corticosteroids and antihistamine was omitted. Further preclinical and clinical studies have to be performed to show the future potential of this combination as an oral anti-cancer therapy.

Conclusions/future perspectives

The taxanes are an important group of anti-cancer agent used in daily practice in the treatment of a range of tumors types, with an extensive first pass metabolism after oral administration. Important in the metabolism and excretion are CYP3A, CYP2C8, MRP-2 and P-gP. Intravenous administration has the limitations that patients have to visit the hospital on a regular basis and that the excipients polysorbate 80 and Cremophor EL may induce hypersensitivity reactions. Oral administration, would be easier to administer and could possibly reduce the incidence of hypersensitivity reactions. The low bioavailability after oral administration of these drugs has proven to be difficult to overcome [58]. By co-administration of ritonavir with newly developed oral formulations bio-availability has greatly improved and this could potentially open up new opportunities for future treatment with the taxanes.

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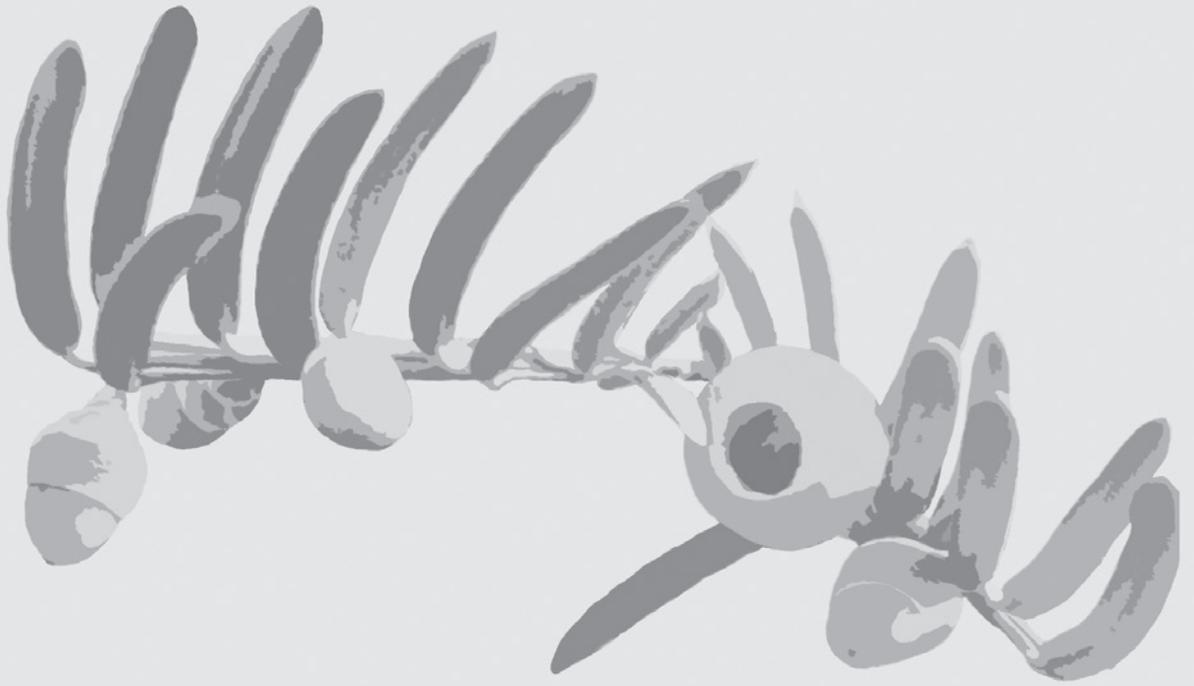
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Chapter 2

Oral taxanes



Chapter 2.1

Boosting of orally administered docetaxel by CYP3A4 inhibition.

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Abstract:

Background: The oral bioavailability of docetaxel is low due to a high first-pass effect. In this study the use of CYP3A4 inhibitors to boost the uptake of orally administered docetaxel was investigated.

Methods: This was a five arm cross over study performed in patients with solid tumors. The boosting effect of ritonavir (either as single 100 mg or 200 mg dose or split 2x 100 mg dose), clarithromycin (1000 mg), ketoconazole (400 mg), or grapefruit juice (2x 200 ml) on orally administered docetaxel as ModraDoc001 was investigated.

Results: Overall twenty-one patients were enrolled. Docetaxel exposure ($AUC_{0-24} \pm$ standard deviation (SD)) was statistically significant increased after co-administration of ModraDoc001 with either ritonavir as 200 mg single dose (528 ± 96.3 ng/ml*h) or as 100 mg single dose (280 ± 69.2 ng/ml*h), clarithromycin (392 ± 98.9 ng/ml*h) or ketoconazole (451 ± 196 ng/ml*h), as compared to oral ModraDoc001 administration without a booster (36.7 ± 21.7 ng/ml*h). In contrast, docetaxel exposure was not significantly affected after co-administration of ModraDoc001 with grapefruit juice.

Conclusion: Oral uptake of docetaxel as ModraDoc001 can be boosted by concomitant treatment with several CYP3A4 inhibitors. Co-administration of a single 200 mg dose of ritonavir led to the highest docetaxel exposure.

Introduction:

The anti-cancer drug docetaxel is currently administered intravenously (iv), because of poor water-solubility and high first pass metabolism. The iv administration has two major down-sides, which could be overcome by oral administration: 1) it is invasive, since an indwelling iv catheter is needed; 2) solvents added to the formulation, like alcohol and polysorbate 80 in the case of docetaxel, increase the risk of hypersensitivity reactions [1].

The oral bioavailability of docetaxel is however low and several challenges need to be taken into account when developing an oral docetaxel formulation. First, docetaxel has a high-first pass effect, which is caused by its affinity for drug-transporters e.g. P-glycoprotein (P-gp, MDR1, ABCB1), multidrug resistance-associated protein 2 (MRP2, ABCC2), and organic anion-transporting polypeptide (OATP)1B1/1B3, that are involved in the excretion of docetaxel back into the gut lumen either directly or via the bile [2–5] and by metabolism into inactive metabolites by the cytochrome P450 (CYP) enzyme CYP3A4 present in both the gastro-intestinal tract and the liver [4,6–8]. Prior studies have shown that the bio-availability of orally administered docetaxel can be increased by inhibition of CYP3A4 and / or P-gp [9–12].

A second challenge is related to the poor water solubility of docetaxel. For this reason a solid dispersion powder of docetaxel has been produced, formulated into capsules (denoted ModraDoc001) and previously tested in a proof-of-concept clinical study showing acceptable systemic exposure to docetaxel, when co-administered with 100 mg ritonavir [10].

In the past the uptake of orally administered docetaxel and paclitaxel from the gastro-intestinal (GI) tract has been boosted using the P-gp and CYP3A4 inhibitor cyclosporin A (CsA) [9,11,13,14]. CsA is however not an ideal drug to be used as a booster, since its toxicity profile includes nephrotoxicity and myelosuppression. The protease inhibitor ritonavir might be a more appropriate alternative booster drug. In effect, ritonavir has been used at sub-therapeutic dosages as a booster drug of other protease inhibitors in HIV treatment, due to its ability to inhibit CYP3A4 at low dosages, with only very limited side-effects [15].

We performed a proof of concept study in which the novel oral docetaxel formulation ModraDoc001 capsule was co-administered with ritonavir (i.e., as single 100 mg or 200 mg dose or split 2x 100 mg dose, respectively) or clarithromycin, ketoconazole, or grapefruit juice, all well-known CYP3A4 inhibitors, and the effect on docetaxel exposure was compared to the exposure observed after administration of ModraDoc001 without a booster drug.

Patients and Methods:

Adult patients with advanced solid tumors and a World Health Organization (WHO) performance status of ≤ 2 were eligible for the trial. Other inclusion criteria were no radio- or chemotherapy within 4 weeks prior to start study treatment (limited palliative radiation for pain reduction was allowed), and adequate bone marrow, liver and renal function. Relevant exclusion criteria consisted of concomitant medication interacting with CYP3A4 and / or P-gp and chronic use of proton pump inhibitors or H2-receptor antagonists. The study protocol was approved by the medical ethics committee of the Netherlands Cancer Institute. All patients had to give written informed consent prior to start study activities. The study was registered under identifier ISRCTN32770468 (ISRCTN register).

Study design:

An open-label cross-over design was employed. All patients received oral docetaxel as ModraDoc001 (10 mg docetaxel per capsule). The study consisted of five arms. In arm I and II different schedules of ritonavir (i.e. 100 mg, 200 mg and 2x 100 mg 4 hours apart) were co-administered with ModraDoc001, whereas in the arm III, IV and V clarithromycin, ketoconazole and grapefruit juice were co-administered, respectively. A minimum of 4 up to a maximum of 5 evaluable patients were enrolled in each arm. In all arms patients were randomized in two groups (i.e., A and B); patients enrolled in group B received the same treatment as the corresponding group A but in reversed order during weeks 1 and 2. A study overview is presented in table 1.

In week 1 and week 2 patients in arm I received 20 mg ModraDoc001 in combination with 100 mg ritonavir (Norvir, Abbott, Illinois, USA) or 20 mg ModraDoc001 in combination with 2x 100 mg ritonavir (100 mg ritonavir simultaneously with ModraDoc001 and 100 mg 4 hours after the intake of ModraDoc001). In week 1 and week 2 patients in arm II received 30 mg ModraDoc001 combined with 100 mg ritonavir or 30 mg ModraDoc001 combined with 200 mg ritonavir as single dose. In week 1 and week 2 patients in arm III received 30 mg ModraDoc001 with 100 mg ritonavir or 1000 mg clarithromycin. In arm IV and V patients received 30 mg ModraDoc001 without a booster in week 1. In week 2 and week 3 patients in arm IV received 30 mg ModraDoc001 in combination with 100 mg ritonavir or 400 mg ketoconazole, whereas in arm V patients received 30 mg ModraDoc001 in combination with 100 mg ritonavir or with 400 ml of grapefruit juice (Coolbest pink grapefruit juice: Royal Friesland Foods N.V., Meppel, the Netherlands).

Study drug was administered with 150 ml of tap water in fasted condition. Patients enrolled in arm V took ModraDoc001 with grapefruit juice instead of water. All patients were pretreated with granisetron (1 mg, 1 hour prior to ModraDoc001) and dexamethasone (4 mg 1 hour prior and 12 and 24 hours after docetaxel intake).

After completion of the first two or three weeks of treatment (where co-administration of ModraDoc001 with different booster drugs was explored) patients were allowed to continue treatment with weekly ModraDoc001 co-administered with 100 mg ritonavir. Adverse events were graded according to the National Cancer Institute Common Toxicity Criteria for Adverse Events (NCI-CTCAE) version 3.0 [16].

Pharmacokinetic sampling and analyses:

Blood samples for PK analysis were collected during the first, second (all arms) and third week (arms IV and V) of treatment pre-dose, 0.5, 1, 1.5, 2, 3, 4, 6, 8, 10 and 24 hours after intake of study drug(s). In arms I and II additional sampling occurred 5 hours and 48 hours after intake. Samples were centrifuged within 1 hour at 1500 g for 10 minutes. Plasma was stored at -20 °C or below until analysis. Docetaxel plasma concentrations were determined using a validated liquid chromatography-tandem mass spectrometry (LC-MS/MS) assay, with 0.25 ng/mL docetaxel as lower limit of quantification [17].

The individual non-compartmental PK parameters were determined using validated scripts in the software package R (version 3.0.1). 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}), the area under the plasma-concentration time curve from $t=0$ up to $t=24$ (AUC_{0-24}), the area under the plasma-concentration time curve from $t=0$ up to infinity (extrapolated (AUC_{0-inf}) using the terminal rate constant), and the terminal half-life ($t_{1/2}$). Paired t-tests were performed to compare the exposure after the different boosters employed, using PK of evaluable patients only).

Results:

Twenty-two patients (seven male and fifteen female) entered the study and were treated in the arms I – V, as shown in table 1. Two patients in arm IV were replaced: one patient discontinued treatment after two weeks due to rapid tumor progression, and the other one did not start study treatment due to clinical deterioration. All other patients were evaluable for PK. The median age was 62 years (range 47-74 years). Most patients had a WHO performance status of 0 or 1 (86%). The most common tumor types were non-small cell lung cancer (NSCLC; 6 patients), urothelial cell carcinoma (3 patients), unknown primary (3 patients) and ovarian (3 patients). Arm V was prematurely discontinued after assessment of PK data of the first three patients demonstrating that grapefruit juice only marginally increased exposure to docetaxel. Patient characteristics are shown in more detail in table 2.

Table 1: Study overview: Patients included in arms I to V and the treatment given during weeks 1-3 and beyond.

	Week 1	Week 2	Week 3 or 4 and Beyond
Arm I (n=5)	Cross-over (group A and B) 30 mg MD1 + 100 mg RTV or 30 mg MD1 + 2x 100 mg RTV*		30 – 60 mg MD1 + 100 mg RTV
Arm II (n=4)	Cross-over (group A and B) 30 mg MD1 + 100 mg RTV or 30 mg MD1 + 200 mg RTV		
Arm III (n=4)	Cross-over (group A and B) 30 mg MD1 + 100 mg RTV or 30 mg MD1 + 1000 mg CLM		
Arm IV (n=6)	MD1 30 mg	Cross-over (group A and B) 30 mg MD1 + 100 mg RTV or 30 mg MD1 + 400 mg KTZ	
Arm V (n=3)	MD1 30 mg	Cross-over (group A and B) 30 mg MD1 + 100 mg RTV or 30 mg MD1 + 2x 200 ml GFJ	

* 100 mg ritonavir simultaneous with MD1 and 100 mg ritonavir 4 hours after the intake of ModraDoc001. Abbreviations: MD1 = ModraDoc001, RTV = ritonavir, CLM = clarithromycin, KTZ = ketoconazole, GFJ = grapefruit juice.

Table 2: Patient demographics

	(n=22)
Sex	
Female	7
Male	15
Age (years)	
Median	62
Range	(47-74)
ECOG performance status	
0	9
1	10
2	3
Pathologic diagnosis	
NSCLC	6
Urothelial cell carcinoma	3
Unknown primary	3
Ovarian	3
Endometrial	2
Other	5

Abbreviations: NSCLC: non-small cell lung cancer

Pharmacokinetics

The AUC_{0-24h} , AUC_{0-inf} , C_{max} , T_{max} and $t_{1/2}$, the standard deviation (SD) and coefficient of variation (CV) of docetaxel are presented in table 3. The mean plasma-concentration time curves \pm SD are shown in figures 1 A to E for all treatment arms.

Table 3: Docetaxel pharmacokinetics after administration of oral docetaxel (as ModraDoc01) without / with boosters.

	Number of patients	AUC_{0-24h} (ng/ml*h) Mean \pm SD (CV)	AUC_{0-inf} (ng/ml*h) Mean \pm SD (CV)	C_{max} (ng/ml) Mean \pm SD (CV)	T_{max} (hours) Mean \pm SD (CV)	$T_{1/2}$ (hours) Mean \pm SD (CV)
ARM I						
20 mg MD1 + 2x 100 mg RTV	N=5	324 \pm 104 (32%)	460 \pm 160 (35%)	62.9 \pm 21.8 (35%)	1.74 \pm 0.845 (49%)	17.1 \pm 1.79 (10%)
20 mg MD1 + 100 mg RTV	N=5	294 \pm 59.1 (20%)	395 \pm 123 (31%)	53.3 \pm 8.55 (16%)	1.73 \pm 0.464 (27%)	14.6 \pm 2.36 (16%)
ARM II						
30 mg MD1 + 200 mg RTV	N=4	528 \pm 96.3 (18%)	698 \pm 83.7 (12%)	75.2 \pm 20.1 (27%)	2.88 \pm 2.08 (72%)	14.5 \pm 1.99 (14%)
30 mg MD1 + 100 mg RTV	N=4	280 \pm 69.2 (25%)	395 \pm 97.3 (25%)	48.5 \pm 20.0 (41%)	2.79 \pm 2.24 (81%)	17.8 \pm 3.17 (18%)
ARM III						
30 mg MD1 + 1000 mg CLM	N=4	392 \pm 98.9 (25%)	442 \pm 99.0 (22%)	153 \pm 52.4 (34%)	1.52 \pm 1.01 (66%)	11.8 \pm 3.75 (32%)
30 mg MD1 + 100 mg RTV	N=4	558 \pm 354 (63%)	795 \pm 329* (41%)	105 \pm 72.3 (69%)	1.88 \pm 0.86 (46%)	11.2 \pm 3.64* (32%)
ARM IV						
30 mg MD1 + 400 mg KTZ	N=4	451 \pm 196 (41%)	474 \pm 196 (41%)	178 \pm 56.2 (32%)	1.01 \pm 0.566 (56%)	7.73 \pm 0.951 (12%)
30 mg MD1 + 100 mg RTV	N=5	513 \pm 298 (58%)	659 \pm 343 [‡] (52%)	87.4 \pm 45.5 (52%)	2.30 \pm 1.35 (59%)	10.4 \pm 3.66 [‡] (35%)
30 mg MD1	N=5	47.6 \pm 18.9 [‡] (40%)	NR	39.1 \pm 31.8 (81%)	2.51 \pm 4.21 (168%)	NR
Arm V						
30 mg MD1 + 2x 200 ml GFJ	N=3	28.1 \pm 12.0 [‡] (43%)	NR	18.1 \pm 14.1 (78%)	1.50 \pm 0.511 (34%)	NR
30 mg MD1 + 100 mg RTV	N=3	290 \pm 147 (51%)	317 \pm 163 (51%)	62.7 \pm 38.6 (61%)	1.89 \pm 0.228 (12%)	7.78 \pm 0.729 (9.4%)
30 mg MD1	N=3	18.6 \pm 11.5 [‡] (62%)	NR	7.66 \pm 4.71 (62%)	1.35 \pm 0.627 (47%)	NR

Pharmacokinetic parameters presented for the arms I – V. Abbreviations: AUC_{0-inf} = area under the plasma-concentration time curve from time 0 with extrapolation to infinity, C_{max} = maximum plasma concentration, T_{max} = time to reach C_{max} , SD = standard deviation, CV = coefficient of variation, MD1 = ModraDoc001, RTV = ritonavir, KTZ = ketoconazole, CLM = clarithromycin, GFJ = grapefruit juice, N = number of patients, NR = not reported.

*n = 3, [‡]n = 4, [§]n=10, [‡] AUC_{0-8h} is reported (last measurable time point).

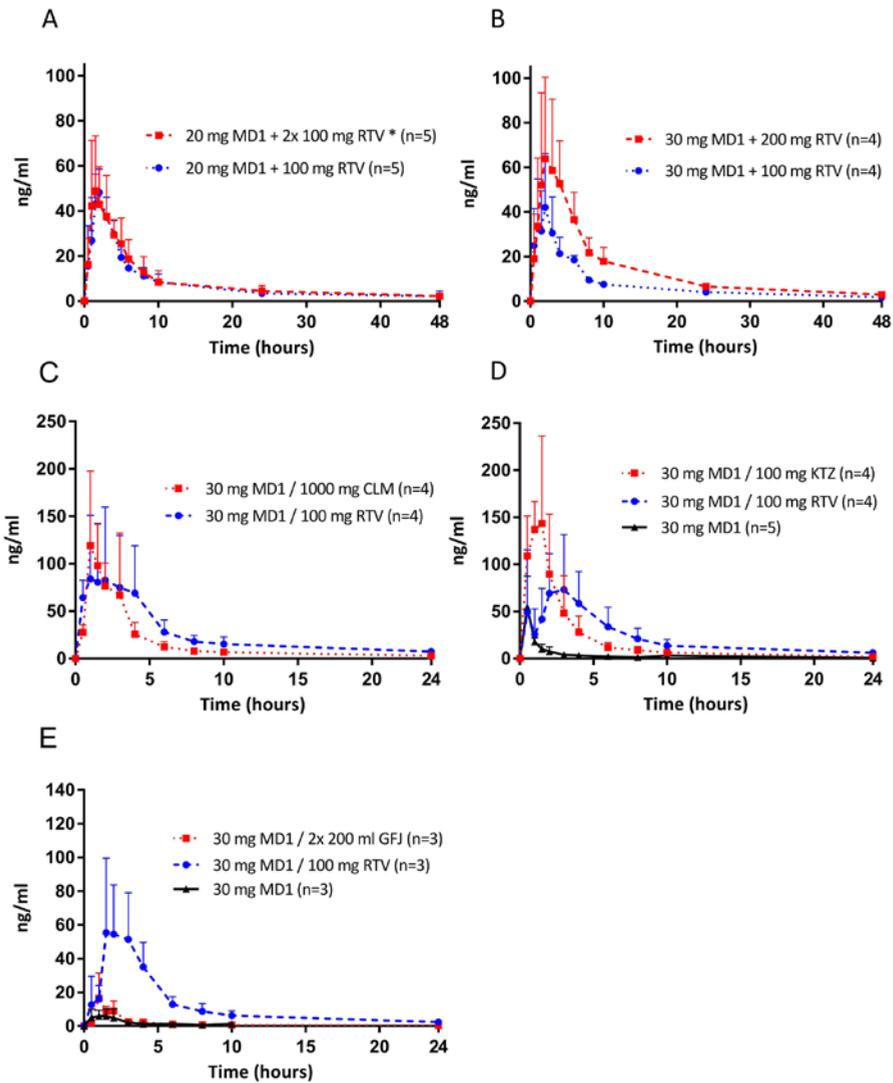


Figure 1: Plasma concentration-time curves of docetaxel

Mean plasma concentration-time curves of docetaxel \pm standard deviation in arms I to V in figures 1A to 1E, respectively. * 100 mg ritonavir simultaneous with MD1 and 100 mg ritonavir 4 hours after the intake of ModraDoc001. Abbreviations: MD1 = ModraDoc001, RTV = ritonavir, CLM = clarithromycin, KTZ = ketoconazole, GFJ = grapefruit juice.

In arm I no significant increase in the docetaxel systemic exposure (AUC_{0-24}) was observed after co-administration of ModraDoc001 with 2x 100 mg ritonavir 4 hours apart (10%; $p = 0.14$) as compared to co-administration of a single dose of 100 mg ritonavir. $AUC_{0-24} \pm SD$ was 324 ± 104 and 294 ± 59.1 ng/ml*h after 2x 100 mg ritonavir and 1x 100 mg ritonavir, respectively.

In arm II a statistically significant increase by 89% in docetaxel exposure was observed after co-administration of ModraDoc001 with a single 200 mg ritonavir dose as compared to a single 100 mg ritonavir dose ($AUC_{0-24} \pm SD$: 528 ± 96.3 and 280 ± 69.2 ng/ml*h, respectively; $p = 0.011$). $C_{max} \pm SD$ (maximum plasma concentration) increased significantly by 55% (75.2 ± 20.1 and 48.5 ± 20.0 ng/ml, respectively; $p < 0.01$). Of note, co-administration of ModraDoc001 with one single dose of 100 mg ritonavir led to a statistically significant increase in docetaxel exposure ($AUC_{0-24} \pm SD$) of about 12-fold, as compared to administration of ModraDoc001 without a booster (472 ± 288 and 36.7 ± 21.7 ng/ml*h, respectively).

In arms III - V the systemic exposure to docetaxel after administration of ModraDoc001 alone (i.e., without a booster) was low (mean $AUC_{0-24} \pm SD$: 36.7 ± 21.7 ng/ml*h; mean $C_{max} \pm SD$: 27.3 ± 29.1 ng/ml). After co-administration of 100 mg ritonavir, 1000 mg clarithromycin or 400 mg ketoconazole systemic exposure to docetaxel increased by 10-fold. The mean $AUC_{0-24} \pm SD$ was not significantly different between the 3 boosters (i.e., 472 ± 288 , 392 ± 98.9 and 451 ± 196 ng/ml*h, respectively, $p > 0.05$). $C_{max} \pm SD$ of docetaxel was 87.1 ± 51.4 , 153 ± 52.4 and 178 ± 56.2 ng/ml after co-administration of ModraDoc001 with ritonavir, clarithromycin and ketoconazole, respectively. T_{max} ($\pm SD$) of docetaxel differed per booster evaluated: it was shortest after co-administration of ketoconazole (1.01 ± 0.566 hours), intermediate after clarithromycin (1.52 ± 1.02 hours), and longest after co-administration of ritonavir (2.06 ± 0.957 hours).

Co-administration of ModraDoc001 with grapefruit juice did not result in a significant increase in docetaxel exposure ($AUC_{0-8} \pm SD$: 28.1 ± 12.0 ng/ml*h; $C_{max} \pm SD$: 18.1 ± 14.1 ng/ml). Of note, in vitro studies performed in order to evaluate the CYP3A4 inhibitory effect (in terms of 50% inhibitory concentration (IC50)) of the grapefruit juice tested in this study indicated that ketoconazole was approximately 65 times more potent than the grapefruit juice used in the study (IC50 0.07 μ g/ml vs 4.6 μ g/ml, respectively).

Safety

The combination of ModraDoc001 with the co-administered drugs was generally well tolerated. Adverse events did not exceed grade 2 severity during the pharmacokinetic phase (first 2 or 3 weeks of treatment). Most common adverse events were fatigue (29%), nausea (29%) and diarrhea (24%).

Discussion:

This study provides further proof of concept that oral docetaxel uptake can be increased by CYP3A4 inhibition. Co-administration of ModraDoc001 with one single dose of 100 mg ritonavir resulted in a significant 12-fold increase in docetaxel AUC_{0-24} (\pm SD) as compared with ModraDoc001 without a booster. Doubling of the ritonavir dose co-administered with ModraDoc001 to 200 mg resulted in a significant further increase in exposure to docetaxel (i.e., AUC_{0-24}) by 89%. In contrast, a second administration of 100 mg ritonavir 4 hours after the first administration did not result in a significant increase in docetaxel exposure as compared to a single 100 mg ritonavir dose. ModraDoc001 combined with the strong CYP3A4 inhibitors ketoconazole (400 mg), clarithromycin (1000mg) and ritonavir (100mg) resulted in a more than 10-fold increase in systemic exposure, as compared to administration without a booster. It should be noted that both ketoconazole and clarithromycin were administered at their maximum therapeutic dose. The dose of ritonavir on the other hand was relatively low and in line with other studies where a 100 mg ritonavir dose is used to boost the uptake of other HIV protease inhibitors [18,19].

The plasma concentration-time curves and PK parameters of docetaxel after co-administration of ModraDoc001 with clarithromycin and ketoconazole showed similarities. The concentration-time curve of docetaxel after co-administration of ritonavir showed a lower C_{max} and a later T_{max} as compared with ModraDoc001 alone. This is likely due to delayed gastric emptying caused by ritonavir. Increasing the ritonavir dose from 1x 100 mg to 1x 200 mg did not result in a later T_{max} whereas C_{max} significantly increased after a 200 mg ritonavir dose.

The increase in AUC_{0-24} and C_{max} observed in arm II after co-administration of ModraDoc001 with a single dose of 200 mg ritonavir could be explained by additional inhibition of either CYP3A4 or of drug-transporters (e.g. P-gp, organic anion-transporting polypeptide (OATP)1B1/1B3) able to affect docetaxel pharmacokinetics, as ritonavir is also an inhibitor of several drug transporters, albeit at different plasma concentrations [20,21].

In contrast, docetaxel plasma concentrations were not significantly affected by co-administration of ModraDoc001 and grapefruit juice. This could be explained by the very weak inhibition of CYP3A4 observed in our *in vitro* models by the grapefruit juice employed in this study, which was clearly inferior to ketoconazole.

The results of this study clearly indicate statistically significantly higher docetaxel exposure by increasing the dose of ritonavir (from 1x 100 mg to 1x 200mg) and by co-administration of ketoconazole and clarithromycin. When translating these results to clinical practice temporary interruption of ModraDoc001/ritonavir treatment might be recommended in the case concomitant treatment with ketoconazole and / or clarithromycin is indicated, as continued treatment with ModraDoc001/ritonavir might result in increased docetaxel related toxicity by higher docetaxel plasma exposure.

Conclusion:

Oral bio-availability of docetaxel as ModraDoc001 can be increased by co-administration of strong CYP3A4 inhibitors, i.e., ritonavir, ketoconazole and clarithromycin. In contrast, co-administration of ModraDoc001 with grapefruit juice did not exert significant effect on docetaxel exposure. Ritonavir is considered the most optimal booster for further investigation in clinical testing, based on the limited toxicity at the dose administered.

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J. Schellens and J. Beijnen are (part-time) employees and shareholder of Modra Pharmaceuticals BV, a spin-out company of the Netherlands Cancer Institute, developing oral taxane formulations.

M. Keessen is a current employee of Modra Pharmaceuticals BV.

The other authors declare that they have no conflict of interest

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Chapter 2.2

A phase I dose escalation study of once weekly oral administration of docetaxel as ModraDoc001 capsule or ModraDoc006 tablet in combination with ritonavir.

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Submitted

Abstract:

Purpose: Oral bioavailability of docetaxel is poor. Absorption could be improved by development of pharmaceutical formulations based on docetaxel solid dispersions, denoted ModraDoc001 capsule and ModraDoc006 tablet (both 10 mg) and co-administration of ritonavir, an inhibitor of CYP3A4 and P-glycoprotein. In this study the safety, maximum tolerated dose (MTD), recommended phase II dose (RP2D), pharmacokinetics and preliminary antitumor activity of oral docetaxel combined with ritonavir in a once weekly (QW) continuous schedule was investigated.

Experimental design: Patients with metastatic solid tumors were included. Dose-escalation was performed using a classical 3+3 design. Pharmacokinetic sampling was performed for up to 48 hours after drug administration. Safety was evaluated using CTCAE v3.0. Anti-tumor activity was assessed according to RECIST v1.0.

Results: Sixty-seven patients were treated at weekly docetaxel dosages ranging from 30-80 mg in combination with 100 or 200 mg ritonavir. Most common toxicities were nausea, vomiting, diarrhea and fatigue, mostly of grade 1-2 severity. No hypersensitivity reactions were observed. The area under the plasma concentration-time curve (AUC_{0-48}) of docetaxel at the RP2D of QW 60 mg ModraDoc001 capsule with 100 mg ritonavir was 1000 ± 687 ng/ml*h and for QW 60 mg ModraDoc006 tablet with 100 mg ritonavir the AUC_{0-48} was 1790 ± 819 ng/ml*h. Nine partial responses were reported as best response to treatment.

Conclusion: Oral administration of once weekly docetaxel as ModraDoc001 capsule or ModraDoc006 tablet in combination with ritonavir is feasible. The RP2D for both formulations is 60 mg ModraDoc with 100 mg ritonavir. Anti-tumor activity is considered promising.

Introduction:

The anti-cancer agent docetaxel is registered for the treatment of breast, gastric, prostate, head and neck cancer and non-small cell lung cancer (NSCLC) as an intravenous (iv) solution [1]. The iv route of administration has limitations which might be overcome by oral administration. First an indwelling iv catheter is needed for administrations and patients have to visit the hospital to receive docetaxel, whereas in case of an oral formulation administration would be more flexible and could potentially reduce costs of treatment as patient do not need to be admitted to a day-care unit [2]. A second advantage is that the solvents ethanol and polysorbate-80 are not needed in an oral formulation. Polysorbate-80 is considered to be at least partially the causative agent of the hypersensitivity reactions that occur during or shortly after administration of iv docetaxel [3,4]. Oral bioavailability of docetaxel is however low, due to poor water solubility and high-first pass effect. The poor water solubility could be improved by the development of a solid dispersion formulation by freeze- or spray drying of crystalline docetaxel in combination with a hydrophilic carrier and surfactant [5,6]. These solid dispersions consist of (more) amorphous docetaxel, and have a very small particle size and increased surface area, as compared to crystalline docetaxel. In combination with the hydrophilic carrier this results in an increased apparent water solubility. Two solid dispersion formulations have been developed and tested in the clinic: the ModraDoc001 capsule (10 mg docetaxel, freeze dried) [5] and the ModraDoc006 tablet (10 mg docetaxel, spray dried) [6]. The high first-pass effect of docetaxel is the result of metabolism in the intestinal lumen and the liver by the cytochrome p450 (CYP) enzyme CYP3A4 and active excretion from intestinal cells and via the bile into the gut lumen by drug transporters, such as P-glycoprotein (P-gp) (MDR1/ABC1), multidrug resistance-associated protein 2 (MRP2,ABCC2) and organic anion-transporting polypeptides (OATP)1B1/1B3 [7–9]. In both pre-clinical and clinical studies the bioavailability of docetaxel was effectively increased by co-administration of inhibitors of either CYP3A4 or P-gp [10–12]. Several candidates of so called booster drugs have been investigated ultimately resulting in the selection of the CYP3A4 and P-gp inhibitor ritonavir [11,13]. Ritonavir has shown to be safe even at higher dosages than used in this study in the treatment of HIV and it has been reported to be a safe and good booster of other protease inhibitors metabolized by CYP3A4, such as lopinavir [14,15]. In this phase I dose-escalation study two dose-escalation arms of once weekly (QW) dosing of oral docetaxel were investigated, exploring an oral drinking solution and subsequently ModraDoc001 capsule in combination with ritonavir (arm 1) and ModraDoc006 tablet in combination with 100 mg ritonavir (arm 2), respectively. The drinking solution was replaced by the capsule formulation after one dose-level, because of its poor taste, poor stability and limited dosing accuracy. The study was designed to establish safety, the maximum tolerated dose (MTD) and the recommended phase II dose (RP2D) of the ModraDoc001 capsule and ModraDoc006 tablet, respectively, when

co-administered with ritonavir. Secondary aims included pharmacokinetics (PK) of docetaxel and preliminary anti-tumor activity.

Patients and methods:

Study design and treatment schedule

In this phase I, open-label, dose-escalation study three oral docetaxel formulations were investigated: an oral docetaxel drinking solution (Taxotere®, Sanofi Aventis, Paris, France), ModraDoc001 10 mg capsule and ModraDoc006 10 mg tablet. The study consisted of two dose-escalation arms as shown figure 1. In the first arm patients were treated in week 1 with QW 20 mg intravenous docetaxel, as a 30 minute infusion (Taxotere®, Sanofi Aventis, Paris, France) with an oral dose of 100 mg ritonavir. In week 2 and thereafter patients received QW 30 mg docetaxel as drinking solution (Taxotere®) combined with 100 mg ritonavir at dose-level 1. At the subsequent dose-levels patients received QW oral ModraDoc001 capsules [5] combined with 100 mg ritonavir (ModraDoc001 capsule / 100 mg ritonavir) in week 2 and beyond. After escalation to 3 additional dose-levels the ritonavir dose was increased from 100 mg to 200 mg and patients did not receive iv docetaxel in week 1 anymore. The increase of the ritonavir dose was based on the findings of another study suggesting that a 200 mg ritonavir dose could significantly increase docetaxel exposure (unpublished data).

After completion of the dose-escalation with ModraDoc001 capsule / 100 mg or 200 mg ritonavir, investigation of the ModraDoc006 tablet formulation [6] was performed in arm 2 of the study at two dose-levels in combination with a fixed dose of 100 mg ritonavir, starting at the QW 60 mg ModraDoc006 tablet / 100 mg ritonavir dose, corresponding to the RP2D for the ModraDoc001 capsule / 100 mg ritonavir.

Pre-medication consisted of granisetron 1 mg 1 hour prior to ModraDoc administration during cycle 1 and 2. Granisetron was thereafter administered as needed. Patients were allowed to continue treatment with the oral drinking solution, ModraDoc001 capsule or ModraDoc006 tablet in combination with ritonavir until disease progression or intolerable toxicity.

A classical 3+3 dose-escalation design was used: 3 patients were enrolled at each dose level and the dose was escalated if no dose-limiting toxicity (DLT) occurred. If one DLT was observed in 1 out of 3 patients the dose was expanded to 6 patients. If either 2 out of 3 or 2 out of 6 patients experienced a DLT at a dose, this dose was deemed non-tolerable. The previously tested lower dose was then expanded to 6 patients to assess the safety of this dose-level. The maximum tolerated dose (MTD) was defined as the dose at which DLTs occurred in <2 out of 6 patients.

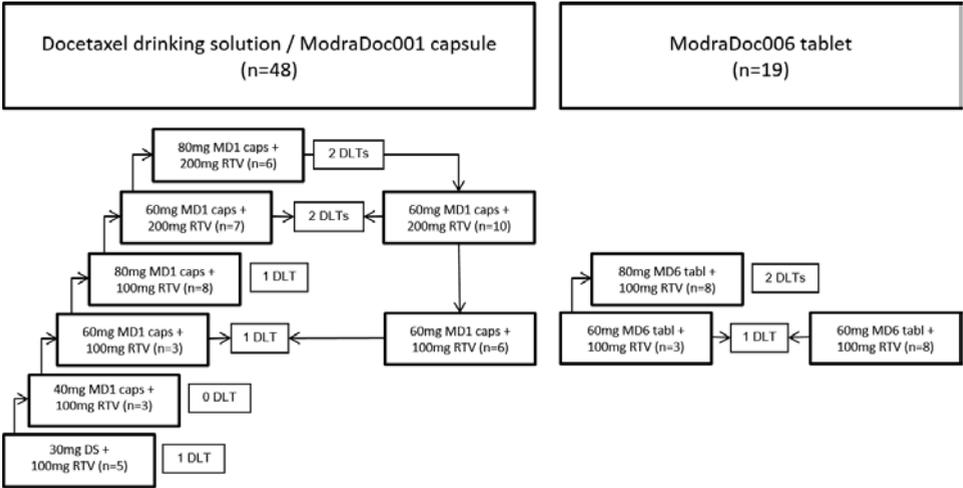


Figure 1: study schedule dose escalation performed with the capsule and tablet formulation. N = number of patients treated at a dose-level. In the box the number of dose-limiting toxicities (DLT(s)) observed at each dose-level. Abbreviations: MD1 = ModraDoc001, MD6 = ModraDoc006, caps = capsule, tabl = tablet, RTV = ritonavir, n = number of patients. DLT = dose-limiting toxicity.

Dose-escalation of docetaxel as ModraDoc001 capsule or ModraDoc006 tablet was based on the safety evaluation and the pharmacokinetic data. All patients who received at least one dose of docetaxel in combination with ritonavir were considered evaluable for safety. All patients who completed the first 4 weeks of treatment were considered evaluable for DLT as well as patients with treatment interruption before week 4 due to adverse events (AEs) matching the DLT criteria. Patients not completing the first 4 weeks of treatment due to reasons not related to docetaxel (as drinking solution, ModraDoc001 capsule or ModraDoc006 tablet) or ritonavir were replaced. The MTD was expanded to a maximum of 12 DLT evaluable patients.

DLT was defined as any of the following events occurring in the first 4 weeks of treatment considered to be at least possibly, probably or definitely related to docetaxel (as drinking solution, ModraDoc001 capsule or ModraDoc006 tablet) or ritonavir: grade 3 or 4 non-hematologic toxicity (other than untreated nausea, vomiting or diarrhea), grade 3 and 4 nausea, vomiting or diarrhea despite maximal support, grade 4 thrombocytopenia or grade 4 neutropenia for more than 7 consecutive days, grade 3 or 4 febrile neutropenia, and/or inability to begin the next course within 3 weeks of scheduled dosing due to toxicity.

Patient eligibility:

Patients ≥18 years old with metastatic solid tumors were eligible. Other inclusion criteria were WHO performance status of ≤2, a life expectancy of at least 3 months, and adequate bone marrow, renal

and hepatic function. Patients taking concomitant treatment being strong P-gp and/or CYP3A4 inhibitors were excluded. Patients who had symptomatic cerebral or leptomeningeal metastases or pre-treated with any anticancer treatment within four weeks prior to the first dose of oral docetaxel (as drinking solution, ModraDoc001 capsule or ModraDoc006 tablet) were also excluded from the study (radiotherapy on a limited field for pain palliation was allowed). The study protocol was approved by the Medical Ethics Committee of the Netherlands Cancer Institute. All patients had to provide written informed consent prior to start of treatment. The study was registered under identifier ISCRTN322770468 (ISCRTN register).

Study procedures:

During the first 6 weeks of treatment patients were seen weekly at the outpatient clinic for safety evaluation consisting of a physical examination, registration of adverse events according to the National Cancer Institute's Terminology Criteria for AE's version 3.0 (NCI-CTCAE v3.0) [16] and their relation to study treatment, registration of concomitant medication and laboratory assessments consisting of hematology and serum chemistry. After the first 6 weeks of treatment patients were seen every 2 weeks. Tumor response evaluation was performed after 6 weeks and every 8 weeks thereafter in accordance with RECIST version 1.0 [17].

Pharmacokinetics:

PK blood sampling was performed at pre-defined time-points at day 1 and 8 or 15 of treatment. Samples for docetaxel PK were drawn from a peripheral intravenous catheter pre-dose, post infusion and 0.25, 0.5, 1, 1.5, 2, 4, 7, 10, 24 and 48 hours after infusion of iv docetaxel. PK sampling for docetaxel after oral administration of ModraDoc001 capsule / 100 mg or 200 mg ritonavir was performed pre-dose, 0.25, 0.75, 1, 1.5, 2, 4, 7, 10, 24 and 48 hours after dosing. Sampling for evaluation of ritonavir PK was performed pre-dose, 0.5, 1, 2, 7, 10 and 24 hours after administration. PK sampling for docetaxel and ritonavir after administration of ModraDoc006 tablet / 100 mg ritonavir was performed pre-dose, 0.5, 1, 1.5, 2, 3, 4, 7, 10, 24 and 48 hours after administration. All samples were collected in lithium heparin tubes of 4 ml. Samples were centrifuged within 1 hour at 1500 g for 10 minutes at 4 °C and stored at -20 °C until docetaxel and ritonavir quantification. Docetaxel was quantified in plasma by a high-performance liquid chromatography assay with tandem mass spectrometric detection (LC-MS/MS) as developed by Kuppens et al [18] and later on by the LC-MS/MS method as described by Hendriks et al [19]. All ritonavir samples were analyzed according to the method of Hendriks et al [19]. Stable isotopically labelled docetaxel and ritonavir were used as internal standards. The lower limit of quantification of the assay was 0.5 ng/mL for docetaxel and 2.0 ng/mL for ritonavir. The assay was validated according to FDA guidelines [20] and the principles of Good Laboratory Practice (GLP).

Data analysis:

Individual non-compartmental PK parameters were determined using validated scripts in the R software package (version 3.01) [21]. The mean, standard deviation (SD) and coefficient of variation (CV) for the following PK parameters were calculated: maximum concentration (C_{max}), time to reach C_{max} (T_{max}), the area under the plasma concentration-time curve between $t=0$ and the last PK time point at 48 hours (AUC_{0-48}) and with extrapolation to infinity (AUC_{0-inf}), and terminal half-life ($t_{1/2}$).

Results:

Patients

Overall 67 patients were included in the study, of which 5 patients received docetaxel as the drinking solution, 43 patients as ModraDoc001 capsule and 19 patients as ModraDoc006 tablet. Individual dose-levels investigated and number of patients included per arm are presented in figure 1. One patient enrolled at the 80 mg ModraDoc006 tablet / 100 mg ritonavir dose-level did not start treatment due to clinical deterioration. Patients had a median age of 58 (range 36 - 78) and 59 (range 47 - 75) years in the ModraDoc001 capsule and ModraDoc006 tablet arm, respectively. Overall, the majority of patients were males (55%) and had a WHO PS ≤ 1 (93%). Patient demographics are presented in more detail in table 1.

Safety and tolerability:

Adverse events:

The docetaxel drinking solution, ModraDoc001 capsule and ModraDoc006 tablet (all combined with ritonavir) were well tolerated. Toxicity observed was mostly of grade 1 or 2 severity. Treatment-related toxicity (i.e., considered possibly, probably or definitely related to study drug by the investigator) occurring in $>5\%$ of patients or grade ≥ 3 is presented in table 2. The most commonly reported toxicities were diarrhea (70%), nausea (67%), fatigue (67%) and vomiting (42%).

Dose-limiting toxicity:

Seven patients experienced one or more DLTs during dose-escalation of ModraDoc001 capsule / 100 or 200 mg ritonavir. Reported DLTs were grade 4 neutropenia and dehydration (both observed in one patient), grade 3 diarrhea (6x), nausea (2x), vomiting (2x), fatigue (2x), elevated AST (2x), elevated ALT (2x), mucositis (1x) and anorexia (1x).

A total of 3 patients experienced DLTs during dose-escalation of ModraDoc006 tablet / 100 mg ritonavir. They were grade 3 diarrhea (1x), neutropenic fever (1x), and mucositis (1x). One patient had a DLT based on a delay of treatment for more than 3 weeks as a result of grade 2 treatment-related

toxicities. DLT events are shown per patient and dose-level in table 3.

Based on the observed DLTs the MTD for the ModraDoc001 capsule was QW 60 mg ModraDoc001 capsule / 200 mg ritonavir. The MTD was however not considered to be the RP2D, as at this dose-level several patients required dose modifications due to treatment related toxicity, that was not dose-limiting. The RP2D was determined as QW 60 mg ModraDoc001 capsule / 100 mg ritonavir. Based on the DLTs and the overall toxicity observed, the MTD and RP2D for the ModraDoc006 tablet were determined as QW 60 mg ModraDoc006 tablet / 100 mg ritonavir.

Table 1: Baseline patient characteristics

Formulation	Oral drinking solution or ModraDoc001 capsule	ModraDoc006 tablet
Number of patients	n=48	n=19
Gender		
Male	27 (56%)	10 (53%)
Female	21 (44%)	9 (47%)
Age,		
median (range), years	58 (36-78)	59 (47-75)
WHO performance status		
0	21 (44%)	12 (63%)
1	22 (46%)	7 (37%)
2	5 (10%)	0
Primary tumor type		
NSCLC	22 (46%)	8 (42%)
UCC	5 (10%)	0
Ovarian	3 (6%)	1 (5%)
Unknown primary	3 (6%)	1 (5%)
Anal	3 (6%)	1 (5%)
Breast	2 (4%)	1 (5%)
Cholangiocarcinoma	1 (2%)	2 (11%)
Head and neck	0 (0%)	2 (11%)
Melanoma	2 (4%)	0
Other	8 (17%)	3 (16%)
Prior therapy		
- Chemotherapy	48 (100%)	17 (89%)
- Radiotherapy	33 (69%)	9 (47%)
- Surgery	26 (54%)	9 (47%)

Abbreviations: NSCLC = non-small cell lung cancer, UCC = Urothelial cell carcinoma

Table 2: Adverse event reported related (possibly, probable or definitely related) to oral docetaxel occurring in >7% of patients or grade ≥3, per dose-level.

n = 66 patients	DS 30 mg		MD1 40 mg		MD1 60 mg		MD1 80 mg		MD1 60 mg		MD1 80 mg		MD6 60 mg		MD6 80 mg		% event				
	RTV 100 mg	Gr3	RTV 100 mg	Gr1-2	RTV 100 mg	Gr1-2	RTV 100 mg	Gr3	RTV 200 mg	Gr1-2	RTV 200 mg	Gr3	RTV 100 mg	Gr1-2	RTV 100 mg	Gr3		n event			
Diarrhea	1	1	0	0	3	0	9	2	9	2	0	3	2	0	8	1	0	5	0	46	70%
Nausea	2	0	1	0	2	0	10	2	11	0	0	5	0	0	7	0	0	4	0	44	67%
Fatigue/malaise	3	0	1	0	1	1	9	2	9	2	0	2	2	0	7	0	0	5	0	44	67%
Vomiting	1	0	0	0	1	0	6	2	4	0	0	3	0	0	8	0	0	3	0	28	42%
Alopecia	1	0	0	0	1	0	5	0	7	0	0	2	0	0	4	0	0	2	0	22	33%
Mucositis	1	0	0	0	0	0	3	0	5	0	0	2	1	0	2	0	0	1	1	16	24%
Constipation	0	0	0	0	1	0	1	0	3	0	0	3	0	0	0	0	0	1	0	9	13%
Weight loss	0	0	0	0	0	0	2	0	3	0	0	0	0	0	3	0	0	1	0	9	13%
Abdominal pain	0	0	0	0	0	0	2	0	1	0	0	1	0	0	1	0	0	3	0	8	12%
Dysgeusia	1	0	0	0	0	0	1	0	0	0	0	0	0	0	4	0	0	2	0	8	12%
Nail changes	0	0	0	0	0	0	3	0	4	0	0	0	0	0	0	0	0	0	0	7	10%
Anorexia	0	0	0	0	0	0	3	0	1	0	0	0	1	0	2	0	0	0	0	7	10%
Sensory neuropathy	0	0	0	0	1	0	2	0	4	0	0	0	0	0	0	0	0	0	0	7	10%
Neutropenia	0	0	0	0	0	0	0	0	1	1	1	1	0	1	0	0	0	0	1	6	9%
Abdominal cramps	0	0	0	0	0	0	0	0	0	0	0	1	0	0	4	0	0	1	0	6	9%
AST increased	0	0	0	0	0	0	0	0	0	2	0	0	0	0	0	0	0	1	0	3	4%
Dyspnea	0	0	0	0	0	0	0	0	2	0	0	0	0	0	0	0	0	0	0	3	4%
Leukocytopenia	0	0	0	0	0	0	0	0	1	0	0	0	1	0	0	0	0	0	1	3	4%
ALT increased	0	0	0	0	0	0	0	0	0	2	0	0	0	0	0	0	0	0	0	2	3%
Thrombocytopenia	0	0	0	0	0	0	0	0	0	1	0	1	0	0	0	0	0	0	0	2	3%
Respiratory insufficiency	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	1	1%
Dehydration	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	1	1%
Gastritis	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	1	1%
Duodenal ulcer	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	1	1%
Neutropenic fever	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	1	1%

Abbreviations: DS = docetaxel drinking solution, MD1 = ModraDoc001, MD6 = ModraDoc006, caps = capsule, tabl = tablet, RTV = ritonavir, n = number of patients, gr = grade

Table 3: Dose-limiting toxicities observed in the dose-escalation with the ModraDoc001 capsule and ModraDoc006 tablet.

Dose-level	Dose-limiting toxicity (CTCAE v3.0)
ModraDoc001 capsule dose-escalation	
30 mg DS / 100 mg RTV	Grade 3 diarrhea
60 mg MD1 / 100 mg RTV	Grade 3 vomiting and nausea
80 mg MD1 / 100 mg RTV	Grade 3 diarrhea, vomiting and nausea
60 mg MD1 / 200 mg RTV	Grade 3 diarrhea, elevated AST and ALT
	Grade 3 diarrhea, fatigue and elevated AST and ALT
80 mg MD1 / 200 mg RTV	Grade 4 dehydration, grade 3 diarrhea and mucositis
	Grade 4 neutropenia, grade 3 diarrhea, fatigue and anorexia
ModraDoc006 tablet dose-escalation	
60 mg MD6 / 100 mg RTV	Grade 3 diarrhea
80 mg MD6 / 100 mg RTV	Grade 3 neutropenic fever, mucositis
	Delay of >3 weeks due to grade 2 toxicities

Abbreviations: DS = docetaxel drinking solution MD1 = ModraDoc001, MD6 = ModraDoc006, RTV = ritonavir.

Serious adverse events:

A total of 61 SAEs were reported in patients treated with the oral drinking solution / 100 mg ritonavir or ModraDoc001 capsule / 100 or 200 mg ritonavir, of which 23 (38%) were considered related to study treatment. The most commonly observed treatment-related SAEs were diarrhea (occurring in 6 patients, 29%), nausea and vomiting (both occurring in 3 patients, 14%).

A total of 28 SAEs were reported in patients treated with ModraDoc006 tablet / 100 mg ritonavir, of which 8 (29%) were considered related to study treatment. Treatment-related SAEs were mucositis (2x, 25%), diarrhea (1x, 13%), fatigue (1x, 13%), nausea (1x, 13%), neutropenic fever (1x, 13%), and respiratory insufficiency (1x, 13%). The last SAE resulted in a treatment related death (reported as a separate SAE) occurring in a 64 year old female patient with an esophageal carcinoma. After 11 weeks of treatment with ModraDoc006 tablet / ritonavir the patient was admitted with respiratory insufficiency, which could not clearly be attributed to underlying disease or to an infectious origin. The event was therefore considered to be possibly related to ModraDoc006 / ritonavir.

Pharmacokinetics:

PK parameters of docetaxel are presented in table 4 for iv docetaxel, the oral docetaxel drinking solution, the ModraDoc001 capsule and the ModraDoc006 tablet formulation, respectively. In figure 2 the mean plasma concentration-time curves of docetaxel per dose-level \pm standard deviation are presented for in A the ModraDoc001 capsule / 100 mg ritonavir, in B ModraDoc001 capsule / 200 mg ritonavir (B) and in C the ModraDoc006 tablet / 100 mg ritonavir.

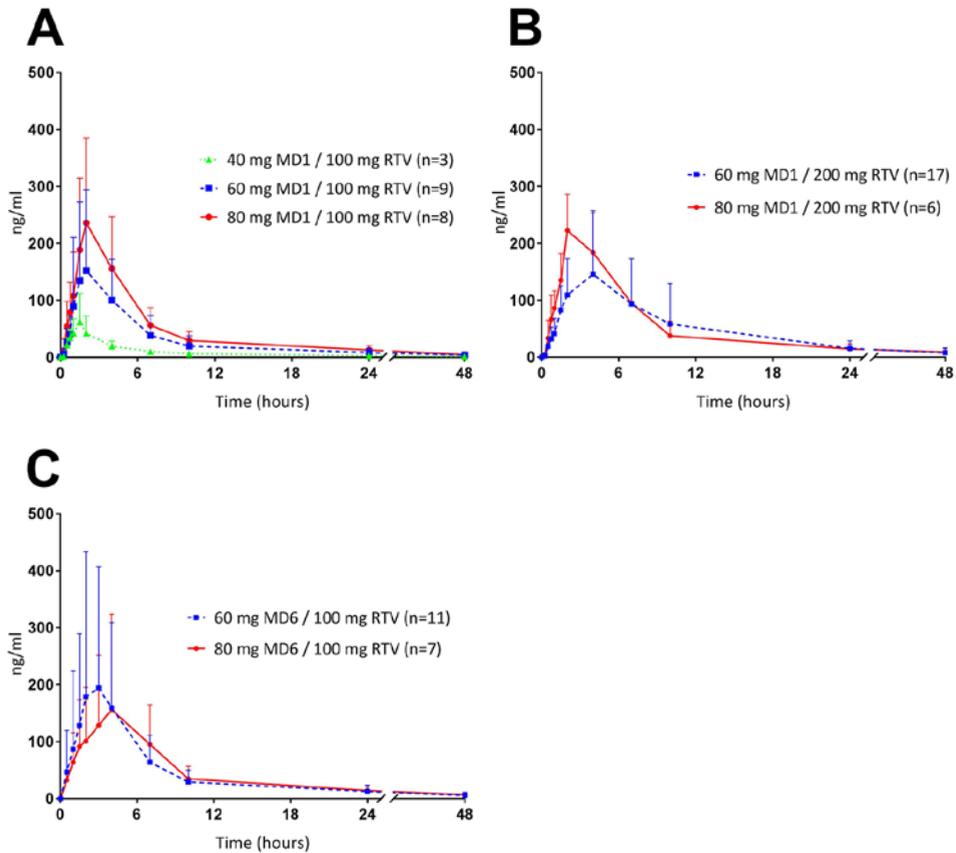


Figure 2: Plasma concentration time curves of docetaxel
 The mean plasma concentration-time curves of docetaxel \pm standard deviation in A) the ModraDoc capsule (MD1) / 100 mg ritonavir (cycle 2) in combination with either 100 mg (RTV), in B) the ModraDoc capsule / 100 mg ritonavir(MD1)(cycle 1) and in C) the ModraDoc tablet (MD6) / 100 mg ritonavir (cycle 1).
 Abbreviations: MD1 = ModraDoc001, MD6 = ModraDoc006, RTV = ritonavir, n = number of patients.

The mean $AUC_{0-48} \pm SD$ and $C_{max} \pm SD$ after iv administration of 20 mg docetaxel / 100 mg oral ritonavir were 537 ± 255 ng/ml*h and 478 ± 208 ng/ml, respectively. The mean $AUC_{0-48} \pm SD$ and $C_{max} \pm SD$ after administration of 30 mg docetaxel drinking solution / 100 mg ritonavir were 488 ± 250 ng/ml*h and 161 ± 183 ng/ml, respectively. The estimated bio-availability of the drinking solution was 85% (SD \pm 59%). The mean AUC_{0-48} and C_{max} of docetaxel increased with dose for the ModraDoc001 capsule. C_{max} of docetaxel did not increase with the increase of ritonavir from a 100 mg to a 200 mg dose, while an increase in the docetaxel AUC_{0-48} was observed with the increase in ritonavir dose. The mean $AUC_{0-48} \pm SD$ at the RP2D of 60 mg ModraDoc001 / 100 mg ritonavir and at the MTD of 60 mg ModraDoc001 / 200 mg ritonavir were 1000 ± 687 ng/ml*h and 1631 ± 1268

Table 4: Pharmacokinetic parameters of once daily dosing of docetaxel as an oral drinking solution (DS), ModraDoc capsule or tablet combined with either 100 mg or 200 mg ritonavir*.

	Docetaxel intravenous 20 mg oral ritonavir 100 mg (n=19)	Docetaxel DS 30 mg ritonavir 100 mg (n=5)	ModraDoc001 caps 40 mg ritonavir 100 mg (n=3)	ModraDoc001 caps 60 mg ritonavir 100 mg (n=9)
C_{max} (ng/mL)	478 ± 208 (43%)	161 ± 183 (113%)	63.4 ± 50.0 (79%)	177 ± 136 (77%)
AUC_{0-48} (ng/mL*h)	537 ± 255 (48%)	488 ± 250 ^a (51%)	306 ± 150 (49%)	1000 ± 687 (69%)
AUC_{0-inf} (ng/mL*h)	598 ± 281 (47%)	476 ± 308 ^b (65%)	337 ± 146 (43%)	1097 ± 764 (70%)
T_{max} (h)	EOI	1.35 ± 0.469 (35%)	1.32 ± 0.40 (31%)	2.45 ± 0.985 (40%)
$T_{1/2}$ (h)	17.6 ± 3.91 (22%)	16.6 ± 3.89 ^b (23%)	18.1 ± 5.93 (33%)	16.4 ± 1.53 (9.3%)

*Results are shown for the first dose of oral docetaxel. Abbreviations: DS = drinking solution, caps = capsule, tabl = tablet. C_{max} = maximum concentration measured; AUC_{0-48} = Area under the plasma concentration-time curve from 0 to the last time point at 48 hours;

ng/ml*h, respectively. The mean $C_{max} \pm SD$ at the RP2D and at the MTD for ModraDoc001 was 177 ± 136 ng/ml and 170 ± 106 ng/ml, respectively. The bio-availability of ModraDoc001 capsule / 100 mg ritonavir was 65% (SD ± 22%).

Mean AUC_{0-48} and C_{max} reached at the MTD / RP2D of 60 mg ModraDoc006 tablet / 100 mg ritonavir were 1492 ± 1449 ng/ml*h and 229 ± 241 ng/ml, respectively. The relatively high AUC_{0-48} and C_{max} observed at the MTD were driven by the results obtained in one patient, reporting an AUC_{0-48} that was 5.2 fold higher (5590 ng/ml*h) and C_{max} that was 5.7 fold higher (914 ng/ml) as compared to the other patients treated with the same dose. This was the same patient who experienced a death event possibly related to docetaxel treatment.

T_{max} was 1 hour later for the ModraDoc006 tablet, as compared to the T_{max} of the ModraDoc001 capsule, independently of the docetaxel dose (3.47 ± 1.6 hours and 2.29 ± 0.93 hours, respectively). Mean $T_{1/2}$ was comparable for the ModraDoc001 capsule and the ModraDoc006 tablet: $T_{1/2}$ was 16.4 ± 3.7 hours and 17.5 ± 3.3 hours, respectively, independently of the docetaxel dose administered.

Anti-tumor activity:

A total of 41 out of the 48 patients treated with the oral drinking solution or ModraDoc001 capsule (combined with 100 mg or 200 mg ritonavir) were evaluable for efficacy. A total of 6 patients (15%)

ModraDoc001 caps 80 mg ritonavir 100 mg (n=8)	ModraDoc001 caps 60 mg ritonavir 200 mg (n=17)	ModraDoc001 caps 80 mg ritonavir 200 mg (n=6)	ModraDoc006 tabl 60 mg ritonavir 100 mg (n=11)	ModraDoc006 tabl 80 mg ritonavir 100 mg (n=7)
264 ± 122 (46%)	170 ± 106 (62%)	226 ± 56.1 (25%)	229 ± 241 (105%)	178 ± 163 (91%)
1483 ± 687 (52%)	1631 ± 1268 (78%)	1790 ± 819 (46%)	1493 ± 1449 (97%)	1471 ± 935 (64%)
1581 ± 795 (50%)	1308 ± 710 ^c (52%)	2014 ± 1630 (80%)	1709 ± 1678 ^d (97%)	1598 ± 1279 ^e (80%)
2.19 ± 0.752 (34%)	4.03 ± 2.45 (61%)	2.37 ± 0.792 (33%)	3.37 ± 1.50 (44%)	4.59 ± 1.71 (37%)
13.9 ± 3.96 (28%)	16.5 ± 4.17 ^c (25%)	17.6 ± 3.64 (21%)	17.4 ± 3.56 ^d (20%)	17.5 ± 3.08 ^e (18%)

AUC_{0-inf} = Area under the plasma concentration-time curve from 0 to infinity; T_{max} = time at which C_{max} was measured; t_{1/2} = terminal half-life, EOI = end of infusion. ^a: n= 4 (incomplete PK of 1 patient), ^b: n=3, ^c: n=12, ^d: n= 10, ^e: n=5 (unreliable regression).

reported a partial response, of which 3 were confirmed after a minimum of 4 weeks. No complete responses were observed. A total of 23 patients had stable disease as best response to treatment. Median time on study in patients experiencing clinical benefit was 19 (range 3 - 72) weeks. A total of 14 out of the 19 patients treated with ModraDoc006 tablet / 100 mg ritonavir were evaluable for efficacy. Three patients (21%) experienced a partial response, of which 1 was confirmed after a minimum of 4 weeks. No complete responses were observed. Seven patients had stable disease as best response to treatment. Median time on study in patients experiencing clinical benefit was 13 (range 6 - 28) weeks.

Discussion:

In this dose-finding study the continuous QW administration of oral docetaxel as ModraDoc001 capsule or ModraDoc006 tablet co-administered with ritonavir was explored. Based on the observed dose-limiting toxicities, the MTD was established as QW 60 mg ModraDoc001 capsule / 200 mg ritonavir and QW 60 mg ModraDoc006 tablet / 100 mg ritonavir.

Treatment related-toxicity was mostly of grade 1 and 2 severity and was manageable with dose modifications and interruptions. The most commonly reported treatment-related adverse events

consisted of non-hematological toxicities.

Of note, hypersensitivity reactions and fluid retention (well-known adverse events reported for the docetaxel iv formulation) were not observed despite the lack of pre-medication with corticosteroids. This is probably due to the fact that oral ModraDoc001 capsule and ModraDoc006 tablet formulations lack the excipient polysorbate-80 [3,4]. Furthermore no grade 3 peripheral neuropathy or other neurotoxicity was observed, while this is reported in about 3% of patients after weekly iv docetaxel [22]. Partial (13 patients, 19%) or complete hair loss (9 patient, 13%) (alopecia) was reported in 22 patients, whereas for weekly iv docetaxel partial and complete hair loss are observed in 12.5% and 58.3% of patients, respectively [23]. The incidence of severe neutropenia and neutropenic fever was limited (6 patients (9%) and 1 patient (1.5%), respectively) and was dose-limiting in only 1 patient. These findings are in line with a meta-analysis published by di Miao and colleagues showing a reduction in bone marrow suppression (in particular neutropenia) and a slight increase in non-hematological toxicity when a weekly iv docetaxel schedule was compared with a 3-weekly administration [24]. The occurrence of grade 3 diarrhea was relatively high with the oral formulations (11%) and dose limiting. The exposure to docetaxel in terms of AUC_{0-inf} and the variability in AUC_{0-inf} at the RP2D for both the ModraDoc001 capsule and the ModraDoc006 tablet formulations were in a concentration range comparable to continuous weekly iv docetaxel dosed at 30–36 mg/m² [25–27] given according to either a continuous or an intermittent weekly schema (i.e. 3 weeks of in a 4 weekly cycle).

Anti-tumor activity of ModraDoc001 capsule / 100 mg or 200 mg ritonavir and ModraDoc006 tablet / with 100 mg ritonavir was reported in known docetaxel sensitive tumors: partial responses were observed in 3 patients with NSCLC, in 2 patients with an unknown primary tumor and in 1 patient with ovarian, head and neck, anal and esophageal carcinoma, respectively. This preliminary activity is considered promising.

The safety, PK and efficacy results of this study further support the proof-of-concept that co-administration of a P-gp / CYP3A4 inhibitor with a known drug substrate is feasible, safe and able to improve the oral bioavailability of the substrate drug. Although both docetaxel formulations (i.e., ModraDoc001 capsule and ModraDoc006 tablet) explored in the present study appear to show similar results. The ModraDoc006 is preferred from a pharmaceutical point of view, as described by Sawicki et al [6] and will be likely selected for further clinical development.

Conclusion:

Administration of the novel oral formulations of docetaxel as ModraDoc001 capsule and ModraDoc006 tablet in combination with ritonavir according to a QW schedule is feasible and safe. The RP2D was determined as 60 mg ModraDoc001 capsule / 100 mg ritonavir or 60 ModraDoc006 tablet / 100 mg ritonavir. Toxicity appears manageable. Anti-tumor activity is considered promising. Further clinical investigation is warranted.

Ethical standards and conflict of interest:

The study protocol was approved by the local Medical Ethics Committee and all patients had to give written informed consent. The ISRCTN register identifier is ISRCTN32770468.

J.H. Beijnen and J.H.M. Schellens have received a grant for translational research (ZonMw code 40-41200-98-004).

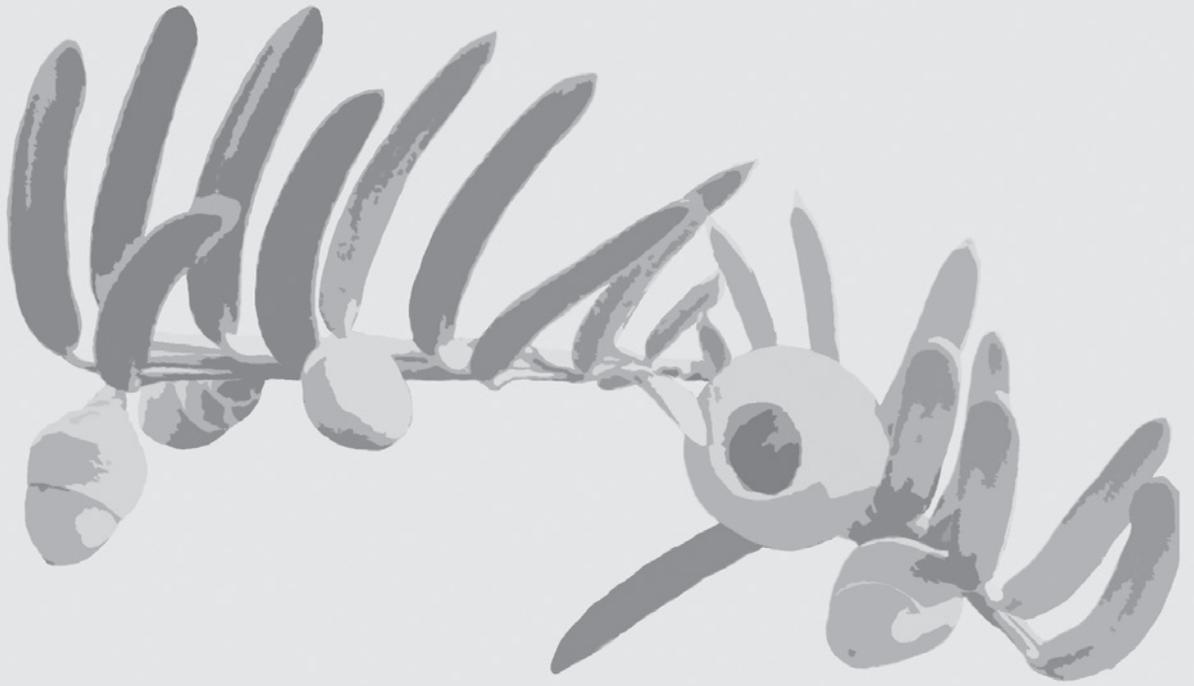
The study was performed as investigator initiated study funded by the Netherlands Cancer Institute. J.J. Moes, B. Nuijen, J.H. Beijnen and J.H.M. Schellens are patent holders on oral taxane formulations.

The other authors declare that they have no conflict of interest.

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Chapter 2.3

A dose-escalation study of bi-daily once weekly oral docetaxel as ModraDoc001 or ModraDoc006 combined with ritonavir.

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Abstract:

Introduction: Two solid dispersions of docetaxel (denoted ModraDoc001 capsule and ModraDoc006 tablet (both 10 mg)) were co-administered with 100 mg ritonavir (/r) and investigated in a bi-daily once weekly (BIDW) schedule. Safety, maximum tolerated dose (MTD), pharmacokinetics (PK) and preliminary activity were explored.

Methods: Adult patients with metastatic solid tumors were included in two dose-escalation arms. PK sampling was performed during the first week and the second or third week. Safety was evaluated using CTCAE v3.0. Anti-tumor activity was assessed every 6 weeks according to RECISTv1.0.

Results: ModraDoc001 capsule/r and ModraDoc006 tablet/r were administered to 17 and 28 patients, respectively. The most common adverse events were nausea, vomiting, diarrhea and fatigue, mostly of grade 1-2 severity. Grade 3/4 neutropenia/neutropenic fever was observed in two patients (4%). The MTD was determined as 20/20 mg ModraDoc001/r and 30/20 mg ModraDoc006/r (morning/afternoon dose) once weekly. The mean area under the plasma concentration-time curve (AUC_{0-48}) \pm standard deviation at the MTD for ModraDoc001/r and ModraDoc006/r were 686 ± 388 ng/ml*h and 1126 ± 382 ng/ml*h, respectively. Five partial responses were reported as best response to treatment.

Conclusion: Oral administration of BIDW ModraDoc001/r or ModraDoc006/r is feasible. The once weekly 30/20 mg ModraDoc006 tablet/r dose-level was selected for future clinical development. Anti-tumor activity is promising.

Introduction:

Docetaxel is a semi-synthetic analog of paclitaxel and which is derived from the European yew, *Taxus baccata*. Docetaxel has proven anti-tumor activity against several solid tumors and has been approved for the treatment of squamous cell head and neck cancer (SCCHN), non-small cell lung cancer (NSCLC), prostate, breast and gastric cancer at a dose of 75 mg/m² or 100 mg/m² intravenously (iv) every three weeks [1].

Oral administration of taxanes is hampered by two factors: 1) poor water solubility and 2) high first-pass effect due to high affinity for drug transporters (e.g., P-glycoprotein (P-gp), multidrug resistance-associated protein 2 (MRP2)) and metabolism by the cytochrome P450 enzyme (CYP) CYP3A4, all abundantly present in the intestine and the liver [2]. The poor water solubility has been improved by the development of a solid dispersion formulation by freeze- or spray- drying of crystalline docetaxel in combination with a hydrophilic carrier and surfactant [3,4]. This solid dispersion consists of fully amorphous docetaxel and has a decreased particle size, as compared to the crystalline docetaxel powder and thereby an increased surface area. This combination results in an increased apparent water solubility. Two solid dispersion formulations have been developed and tested in the clinic: the ModraDoc001 capsule (10 mg docetaxel, freeze dried) [3] and the ModraDoc006 tablet (10 mg docetaxel, spray dried) [4].

The first pass-effect of docetaxel can be decreased by co-administration of an inhibitor of P-gp and/or CYP3A4. In the past the concept of boosting with the P-gp and CYP3A4 inhibitor cyclosporin A (CsA) to inhibit first-pass effect has successfully been applied in phase I and II clinical trials, after intake of a docetaxel drinking solution [5,6]. CsA is, however, not an ideal booster because of its side-effects which include bone marrow suppression and renal failure after prolonged use. The protease inhibitor ritonavir on the other hand could be an ideal booster drug as it is a strong inhibitor of CYP3A4 and a moderate inhibitor of P-gp. Furthermore, it has been used for many years as a booster drug to increase plasma levels of other protease inhibitors, such as lopinavir, in the treatment of human immunodeficiency virus (HIV). At the booster dose of 100 mg it has shown good tolerability, with the most common side-effects being mild gastro-intestinal toxicity [7].

The aim of the current study was to investigate safety and feasibility of the co-administration of oral docetaxel as ModraDoc001 capsule or as ModraDoc006 tablet, both in combination with ritonavir in a bi-daily once weekly (BIDW) schedule. Secondary objectives included pharmacokinetics (PK) and preliminary anti-tumor activity.

Patients and methods:

Study design and treatment schedule

In this phase I, open-label, dose-escalation study two oral docetaxel formulations, ModraDoc001 capsule [3] and ModraDoc006 tablet [4] both containing 10 mg docetaxel, were investigated. Dose-escalation was performed with both formulations. Dosing of patients occurred in a BIDW schedule. A dose of 100 mg ritonavir (Norvir®, Abbott, Illinois, U.S.) was co-administered with both ModraDoc formulations. Administration occurred after a 2 hour fast prior and 1 hour after ModraDoc and ritonavir. The dose-levels investigated are presented in figure 1.

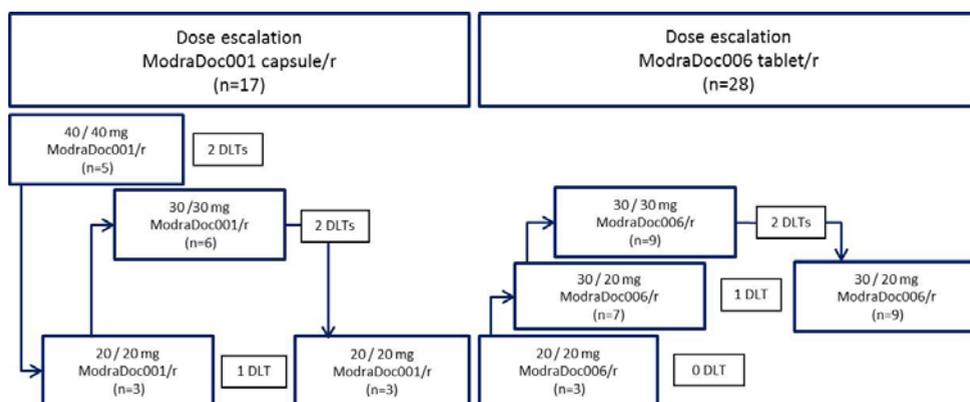


Figure 1: Study overview

Dose-escalation as performed for ModraDoc001 and ModraDoc006; n = number of patients included, r = ritonavir, DLT = dose-limiting toxicity, BIDW = bi-daily weekly, QW = once weekly.

The study started with dose-escalation with the ModraDoc001 capsule formulation, starting from a BIDW 40 / 40 mg (morning / afternoon dose) ModraDoc001 capsule with BIDW 100 mg ritonavir (r) simultaneously with oral docetaxel (ModraDoc001/r). This starting dose was based on the results of another phase I trial exploring a once daily-once weekly (QW) administration of ModraDoc001 capsule/r [8]. The starting dose proved to be intolerable, due to dose-limiting toxicities (DLT), after which a dose de-escalation was applied.

Subsequently, the ModraDoc006 tablet formulation became available for clinical testing and was implemented in a new dose-escalation cohort. The starting dose for this cohort corresponded to the maximum tolerated dose (MTD) observed for the capsule formulation: BIDW 20 / 20 mg ModraDoc006 tablet with BIDW 100 mg ritonavir simultaneous with oral docetaxel (ModraDoc006 tablet/r).

The BIDW administrations of study drugs were performed with a 7 - 12 hour interval. Premedication consisted of 1 mg granisetron 1 hour prior to both administrations. If patients did not experience

nausea or vomiting during the first weeks of treatment, administration of granisetron was omitted after week 3. Patients were allowed to continue study treatment until disease progression or intolerable toxicity.

Dose-escalation in both cohorts was performed according to a classic 3+3 design [9]. In brief, at all dose-levels 3 patients were enrolled. If no DLT occurred, the dose was escalated. If a DLT was observed in 1 out of 3 patients the dose was expanded to 6 patients. If 2 or more out of a maximum of 6 patients experienced a DLT at a certain dose, this dose was considered non-tolerable. The previously explored lower dose level was then expanded to 6 patients. MTD was defined as the dose at which DLT occurred in < 2 out of 6 patients. Dose escalation was based on safety and PK data observed.

Patients who received at least one dose of study drug were considered evaluable for safety. The DLT period was defined as the first four weeks of treatment. Patients who did not complete the first four weeks of treatment due to reasons not related to ModraDoc/r were replaced. The MTD was expanded to a maximum of 12 patients.

DLT was defined as any of the following events occurring within the first four weeks of treatment and considered to be possibly, probably or definitely related to ModraDoc/r: grade 3 or 4 non-hematologic toxicity, grade 3 and 4 nausea, vomiting and/or diarrhea despite maximum support, grade 4 thrombocytopenia or grade 4 neutropenia for more than 7 consecutive days, febrile neutropenia and/or inability to begin the next course of treatment within 7 days of scheduled dosing due to toxicity other than stated above.

Patient eligibility:

Patients 18 years or older with either histological or cytological proof of cancer for whom no standard treatment options were available were eligible. Other inclusion criteria included World Health Organization (WHO) performance status (PS) of ≤ 2 , life expectancy ≥ 3 months and adequate bone marrow function (neutrophil count $\geq 1.5 \times 10^9/L$ and platelets of $\geq 100 \times 10^9/L$), renal function (serum creatinine ≤ 1.5 times the institutional upper limit of normal (ULN) or creatinine clearance ≥ 50 ml/min as calculated with the Cockcroft-Gault formula) and hepatic function (alanine aminotransferase (ALT) and aspartate aminotransaminase (AST) ≤ 2.5 times the ULN, bilirubin ≤ 1.5 times the ULN). The use of concomitant medication being a strong P-gp or CYP3A4 inhibitor was not allowed during the study. Patients with symptomatic cerebral or leptomeningeal metastases were also excluded. The study protocol was approved by the Medical Ethical Committee of the Netherlands Cancer Institute and all patients had to provide written informed consent prior to start of study procedures. The study was registered in clinicaltrial.gov under identifier: NCT01173913.

Study procedures:

A complete medical history including concomitant medication, physical examination, laboratory evaluation and a radiologic tumor assessment were performed at baseline. Patients were seen weekly on the outpatient clinic during the first 6 weeks of treatment and subsequently every 2 weeks. Adverse events (AE) were collected according to the National Cancer Institute's Terminology Criteria for AE's version 3.0 (NCI-CTCAE v3.0) [10]. Tumor response evaluation was performed every 6 weeks according to Response Evaluation Criteria in Solid Tumor (RECIST) version 1.0 [11].

Pharmacokinetics:

Pharmacokinetic (PK) blood sampling was performed on day 1 and 15 of treatment for the capsule formulations and on day 1 and 8 for the tablet formulation. Samples were drawn pre-dose, 0.5, 1, 1.5, 2, 3, 4, 6, 7, 7.5, 8, 8.5, 9, 10, 11, 24 and 48 hours after the first administration. The second administration of the study drug was performed 7 hours after the first administration. Samples were collected in lithium heparin tubes of 4 ml. Samples were centrifuged within 1 hour at 1500 g, for 10 minutes at 4 °C. Plasma was stored in a pre-labeled 2 ml eppendorf tubes at -20 °C until docetaxel and ritonavir quantification. Docetaxel and ritonavir concentrations were determined in plasma by a LC-MS/MS method as described by Hendrikx et al [12]. Stable isotopically labeled docetaxel and ritonavir were used as internal standards. The lower limit of quantification of the assay was 0.5 ng/mL for docetaxel and 2.0 ng/mL for ritonavir. The assay fulfills current FDA guidelines for bioanalytical validation [13].

Data analysis:

The individual non-compartmental pharmacokinetic parameters were determined using validated scripts in the R software package (version 3.01) [14]. The mean, standard deviation (SD) and coefficient of variation (CV) for the following PK parameters were calculated: maximum concentration (C_{max}) after the first and second dose of oral docetaxel (C_{max} 1 and C_{max} 2), time to reach C_{max} 1 and 2 (T_{max} 1 and 2, respectively), the area under the plasma concentration-time curve between $t=0$ and the last PK time point at 48 hours (AUC_{0-48h}), and if possible with extrapolation to infinity (AUC_{0-inf}) and the terminal half-life ($t_{1/2}$).

Results:

Patients characteristics:

Table 1 shows the characteristics of the included patients. In the dose-escalation cohort of the ModraDoc001 capsule a total of 17 patients were enrolled, 9 (53%) male and 8 (47%) female patients. The median age was 60 years (range 41-77). The majority of patients had an WHO PS \leq 1 (94%). All patients had received prior chemotherapy, and 53% and 41% had received prior radiotherapy and/or surgery, respectively (table 1). Patients were treated at three dose-levels ranging from BIDW 20 / 20 mg ModraDoc001 /r to BIDW 40 / 40 mg ModraDoc001/r (fig.1).

In the dose-escalation cohort of the ModraDoc006 tablet formulation a total of 28 patients were enrolled, 17 (61%) male and 11 (39%) female patients. The median age was 58 years (range 47-76). The majority of patients had an ECOG PS \leq 1 (97%). Previous treatments included chemotherapy (93%), radiotherapy (64%) and surgery (36%) (table 1). Three dose-levels were investigated ranging from BIDW 20 /20 mg ModraDoc006/r to BIDW 30 / 30 mg ModraDoc006/r (fig.1).

Table 1: Patient demographic and baseline characteristics

	ModraDoc001 capsule/r	ModraDoc006 tablet/r
Number of patients	n=17	n=28
Gender		
Male	9 (53%)	17 (61%)
Female	8 (47%)	11 (39%)
Age,		
median (range), years	60 (41-77)	58 (47-76)
WHO performance status		
0	8 (47%)	13 (47%)
1	8 (47%)	14 (50%)
2	1 (6%)	1 (3%)
Primary tumor type		
NSCLC	11	10
Neuro endocrine carcinoma	0	4
Urogenital	3	3
Head and Neck	0	3
Anal	1	1
Ovarian	1	1
Other	1	6
Prior therapy		
- Chemotherapy	17 (100%)	26 (93%)
- Radiotherapy	9 (53%)	18 (64%)
- Surgery	7 (41%)	10 (36%)

Abbreviations: n = number of patients, NSCLC = non-small cell lung cancer

Table 2: Treatment-related adverse events observed in $\geq 10\%$ of patients treated with ModraDoc001 capsule or ModraDoc006 tablet formulations in combination with ritonavir or \geq grade 3.

Name of AE	ModraDoc001/r 40 / 40 mg (n=5)			ModraDoc001/r 20 / 20 mg (n=6)		ModraDoc001/r 30 / 30 mg (n=6)	
	Gr 1-2	Gr 3	Gr 4	Gr 1-2	Gr 3	Gr 1-2	Gr 3
Diarrhea	3	0	0	3	0	4	1
Fatigue	4	1	0	4	0	3	2
Nausea	2	0	0	1	1	3	1
Vomiting	1	0	0	2	0	3	0
Anorexia	3	0	0	0	0	4	1
Mucositis	1	1	0	0	0	1	0
Alopecia	1	0	0	1	0	1	0
Weight loss	1	0	0	2	0	2	0
AST increased	0	0	0	1	0	2	0
Dysgeusia	0	0	0	0	0	1	0
ALT increased	0	0	0	0	1	1	0
Constipation	0	0	0	2	0	0	0
Anemia	1	1	0	0	0	2	0
Pain, abdomen	1	0	0	2	0	1	0
Nail toxicity	0	0	0	0	0	0	0
Abdominal cramps	0	0	0	0	0	1	0
Dehydration	0	1	0	0	0	0	1
Leukocytopenia	1	0	1	0	0	0	0
Neutropenia	1	0	1	0	0	0	0
Epistaxis	0	0	0	0	0	0	1
Febrile neutropenia	0	0	1	0	0	0	0
Hyponatremia	0	1	0	0	0	0	0
Hypoalbuminemia	0	0	0	0	0	0	0
Hypokalemia	0	0	0	0	0	0	0
Hemorrhage, upper GI	0	0	0	0	0	0	1
INR increased	0	0	0	0	0	0	1
Lymphocytopenia	0	1	0	0	0	0	0
Edema peripheral	0	0	0	0	0	0	0
Gastritis	0	0	0	0	0	0	0
Pneumonia	0	0	0	0	0	0	0
Rash	0	0	0	0	0	0	0

Abbreviations: n = number of patients, BIDW = bi-daily once weekly, AST = aspartate aminotransferase, ALT = alanine transaminase, GI = gastro-intestinal tract, INR = international normalized ratio.

2.3

ModraDoc006/r 20 / 20 mg (n=3)		ModraDoc006/r 30 / 20 mg (n=16)		ModraDoc006/r 30 / 30 mg (n=9)			n = 45	
Gr 1-2	Gr 3	Gr 1-2	Gr 3	Gr 1-2	Gr 3	Gr 4	Total	%
2	0	12	0	3	1	0	29	64%
1	1	4	2	3	0	0	25	56%
2	0	9	1	4	1	0	25	56%
1	0	7	0	3	1	0	18	40%
0	0	1	1	2	2	0	14	31%
0	0	4	3	0	2	0	12	27%
0	0	3	0	3	0	0	9	20%
0	0	2	0	2	0	0	9	20%
0	0	1	0	3	0	0	7	16%
0	0	4	0	2	0	0	7	16%
0	0	1	0	3	0	0	6	13%
0	0	3	0	1	0	0	6	13%
0	0	2	0	0	0	0	6	13%
0	0	2	0	0	0	0	6	13%
0	0	2	1	3	0	0	6	13%
0	0	2	0	2	0	0	5	11%
0	0	0	0	0	2	0	4	9%
0	0	0	0	0	0	1	3	7%
0	0	0	0	0	0	1	3	7%
0	0	0	0	1	0	0	2	4%
0	0	0	0	0	1	0	2	4%
0	0	0	0	0	1	0	2	4%
0	0	0	0	1	1	0	2	4%
0	0	0	1	1	0	0	2	4%
0	0	0	1	0	0	0	1	2%
0	0	0	0	0	0	0	1	2%
0	0	0	0	0	0	0	1	2%
0	0	0	0	1	1	0	1	2%
0	0	1	0	0	0	0	1	2%
0	1	0	0	0	0	0	1	2%
0	0	0	1	0	0	0	1	2%

Safety and tolerability:

An overview of adverse events considered to be (possibly, probably or definitely) related to the study medication is presented in table 2 for ModraDoc001 capsule/r and ModraDoc006 tablet/r, respectively.

The most common adverse events reported with ModraDoc001 capsule/r were fatigue (82%), diarrhea (65%), anorexia (47%) and nausea (47%), mostly being of grade 1 or 2 severity. Fatigue grade 3 occurred in 3 patients while grade 3 diarrhea, anorexia and nausea were all seen in 1 patient. The most common adverse events observed with ModraDoc006 tablet/r were diarrhea (64%), nausea (61%), vomiting (43%) and fatigue (39%), mostly of grade 1 and 2 severity. Mucositis grade 3 was observed in 3 of 16 (19%) patients treated at the 30/20 mg docetaxel dose.

Table 3: Dose-limiting toxicities (every line represents one patient).

Dose-level	Dose-limiting toxicity (CTCAE v3.0)	SAE
Capsule dose-escalation		
20 / 20 mg ModraDoc001/r	Grade 3 nausea, elevated ALT	No
30 / 30 mg ModraDoc001/r	Grade 3 anorexia, epistaxis and hemorrhage upper gastro-intestinal tract	Yes
30 / 30 mg ModraDoc001/r	Grade 3 diarrhea, nausea, dehydration	Yes
40 / 40 mg ModraDoc001/r	Grade 4 febrile neutropenia, grade 3 mucositis (oral cavity), dehydration, hyponatremia	Yes
40 / 40 mg ModraDoc001/r	Grade 3 fatigue	No
Tablet dose-escalation		
30 / 20 mg ModraDoc006/r	Grade 3 nausea and mucositis	Yes
30 / 30 mg ModraDoc006/r	Grade 3 diarrhea, vomiting, dehydration and anorexia	Yes
30 / 30 mg ModraDoc006/r	Grade 3 neutropenic fever, dehydration, mucositis	Yes

Abbreviations: SAE = serious adverse event, BIDW = bi-daily once weekly, QW = once weekly, r = ritonavir, ALT = alanine transaminase,

Dose limiting toxicity:

Overall 8 patients experienced dose-limiting toxicities (table 3). A total of 5 patients treated with the ModraDoc001 capsule/r reported 13 AEs that were considered as dose-limiting: grade 4 neutropenic fever, grade 3 nausea (2x), dehydration (2x), diarrhea, mucositis, elevated ALT, epistaxis and upper gastro-intestinal tract bleeding, fatigue and hyponatremia. All AEs occurred in 1 patient except for nausea and dehydration. Three patients treated with the ModraDoc006 tablet/r reported 9 AEs that were considered as dose-limiting: grade 3 mucositis (2x), dehydration (2x), diarrhea, nausea, vomiting, anorexia and neutropenic fever. All AEs occurred in 1 patient except for mucositis and dehydration.

Based on the observed DLTs, the MTD was BIDW 20 / 20 mg ModraDoc001/r for the capsule formulation, and 30 mg (morning dose) and 20 mg (afternoon dose) once weekly ModraDoc006/r for the tablet formulation.

Serious Adverse Events (SAE):

A total of 21 SAEs were reported in the ModraDoc001 capsule/r cohort, of which 18 (86%) were considered related to study treatment. The most common treatment-related SAEs were dehydration, nausea and mucositis, all occurring in two patients.

A total of 65 SAEs were reported in the ModraDoc006 tablet/r cohort, of which 23 (42%) were considered related to study treatment. The most common treatment-related SAEs were mucositis and vomiting, both occurring in 4 patients. Other common treatment-related SAEs were diarrhea, nausea and dehydration, all occurring in 2 patients.

Treatment discontinuations – Dose modifications:

In the ModraDoc001 capsule cohort, the most common reason for permanent discontinuation of study treatment was progression of disease (PD) (11 patients, 65%), followed by adverse events (5 patients, 29%), and withdrawn by the principle investigator (1 patient, 6%). Dose-reductions were reported in 2 patients (12%).

In the ModraDoc006 tablet cohort, the most common reason for permanent discontinuation of study treatment was PD (21 patients, 75%), followed by adverse events (6 patients, 22%). Dose reductions were reported in 3 patients (11%), 1 patient required two dose reductions because of prolonged grade 1 thrombocytopenia.

Pharmacokinetics:

PK parameters are presented in table 4 (both formulations) and plasma concentration-time curves are presented in figures 2A and 2B for the capsule and tablet dose-levels, respectively. The mean C_{max} for the ModraDoc001 capsule was reached after 2.33 and 9.42 hours after the first and second dose in cycle 1, respectively (T_{max}) independently of dose. C_{max} and AUC_{0-inf} increased with dose. C_{max} at the MTD of 20/20 mg ModraDoc001/r was 35.7 (coefficient of variation (CV) 62%) and 78.2 (CV 79%) ng/ml after the first and second dose in cycle 1, respectively and AUC_{0-48h} at the MTD in cycle 1 was 686 ng/ml*h (CV 57%). The terminal half-life ($t_{1/2}$) was 14.5 (CV 28%) hours in cycle 1 independent of dose.

Table 4: Pharmacokinetic parameters on docetaxel exposure (cycle 1).

	Modra- Doc001 capsule/r 20 / 20 mg	Modra- Doc001 capsule/r 30 /30 mg	Modra- Doc001 capsule/r 40 / 40 mg	Modra- Doc006 tablet/r 20 / 20mg	Modra- Doc006 tablet/r 30 / 20 mg	Modra- Doc006 tablet/r 30 / 30 mg
	(n=6)	(n=6)	(n=5)	(n=3)	(n=16)	(n=9)
C_{max} 1 ng/ml	35.7 ± 22.1 (62%)	75.8 ± 43.8 (58%)	102 ± 97.0 (95%)	33.0 ± 7.79 (24%)	69.4 ± 46.0 (66%)	98.9 ± 50.4 (51%)
C_{max} 2 ng/ml	78.2 ± 61.9 (79%)	150 ± 78.7 (53%)	151 ± 169 (112%)	83.8 ± 13.5 (16%)	102 ± 46.4 (45%)	197 ± 104 (53%)
AUC₀₋₄₈ ng/ml*h	686 ± 388 (57%)	1508 ± 874 (58%)	1818 ± 1799 (99%)	702 ± 187 (27%)	1126 ± 382 (34%)	1598 ± 834 (52%)
AUC_{0-inf} * ng/ml*h	791 ± 500 * (63%)	1367 ± 155 * (11%)	2799 ± 3107 * (111%)	781 ± 188 * (24%)	1418 ± 429 * (30%)	1602 ± 814 * (51%)
T_{max} 1 hours	1.99 ± 1.04 (52%)	2.59 ± 1.68 (65%)	2.46 ± 1.05 (43%)	3.03 ± 1.04 (34%)	2.84 ± 1.49 (52%)	2.52 ± 1.02 (40%)
T_{max} 2 hours	9.02 ± 0.545 (6.0%)	9.08 ± 0.990 (11%)	10.3 ± 0.417 (4.0%)	9.20 ± 0.778 (8.5%)	9.48 ± 1.27 (13%)	9.11 ± 0.856 (9.4%)
t_{1/2} * hours	14.6 ± 1.30 * (8.9%)	18.4 ± 4.93 * (27%)	12.9 ± 1.37 * (11%)	14.8 ± 5.23 * (35%)	17.8 ± 4.79 * (27%)	15.6 ± 2.22 * (14%)

Abbreviations: C_{max} 1 = maximum concentration measured after the first dose; C_{max} 2 = maximum concentration measured after the second dose; AUC₀₋₄₈ = Area under the plasma concentration-time curve from 0 to the last time point at 48 hours; AUC_{0-inf} = Area under the plasma concentration-time curve from 0 to infinity; T_{max} 1 = time at which C_{max} 1 was measured; T_{max} 2 = time at which C_{max} 2 was measured t_{1/2} = terminal half-life; BIDW = bi-daily once weekly, QW = once weekly.

* Patients with unreliable regression were removed from the analyses for AUC_{0-inf} and T_{1/2}: n = 5, 5, 2, 3, 10, 8 for the columns 1 to 6 respectively.

The mean C_{max} for the ModraDoc006 tablet was reached after 2.76 and 9.33 hours after the first and second dose in cycle 1, respectively (T_{max}) independently of dose. C_{max} and AUC_{0-inf} increased with dose. C_{max} at the MTD of 30/20 mg ModraDoc006/r was 69.4 (CV 66%) and 102 (CV 45%) ng/ml after the first and second dose in cycle 1, respectively and AUC_{0-48h} at the MTD in cycle 1 was 1126 ng/ml*h (CV 34%). The AUC_{0-48h} at the MTD for the ModraDoc006 tablet/r in cycle 2 was 1537 ng/ml*h (CV 40%), a significant increase of 36% in comparison to cycle 1 (paired T test (for patients for whom PK data of cycle 1 and 2 was available; p = 0.0049). The terminal half-life (t_{1/2}) was 12.5 (CV 24%) hours in cycle 1 independent of dose.

Anti-tumor activity:

A total of 14 out of the 17 patients treated with ModraDoc001 capsule/r were evaluable for efficacy. One patient with NSCLC had a partial response (PR), five patients had stable disease (SD) and eight patients had PD as best response to treatment. The objective response rate was 1/14 (7%). Median time on study in patients experiencing clinical benefit (SD + PR at 6 weeks) was 10 (range 3 - 21) weeks. A total of 17 out of the 28 patients treated with ModraDoc006 tablet/r were evaluable for efficacy. Four patients had a PR (2 patients with a SCCHN and 2 with a NSCLC), five patients had SD and seven patients had PD as best response to treatment. The objective response rate was 4/17 patients (24%). Median time on study in patients experiencing clinical benefit (SD + PR at 6 weeks) was 18 (range 11 - 54) weeks. In figure 3 the time on study is shown for all patients who started treatment. Figure 4 presents the best changes in tumor volume per patient.

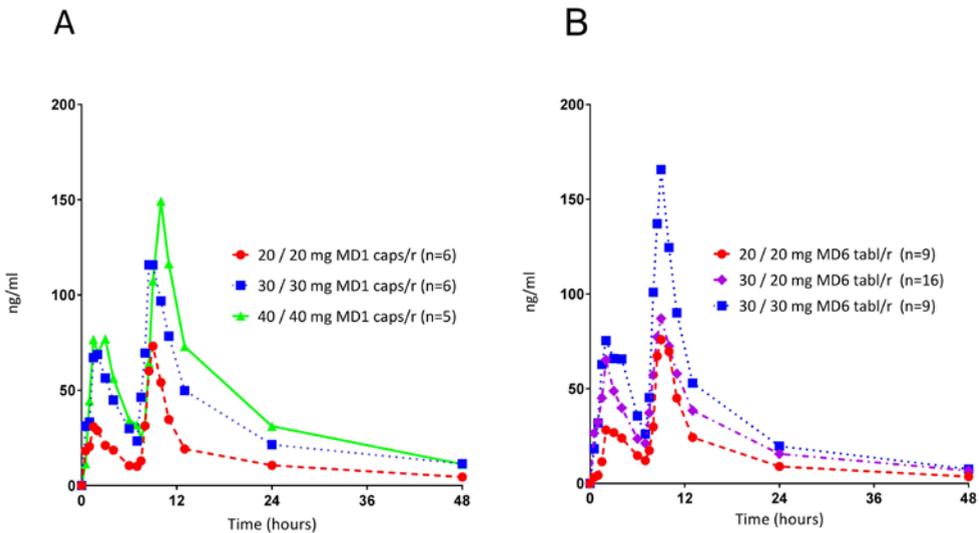


Figure 2: Plasma concentration-time curves of docetaxel

Mean plasma concentration-time curves of ModraDoc001 capsule with ritonavir (fig. A) and ModraDoc006 tablet with ritonavir (fig. B) at all dose-levels evaluated. BIDW = bi-daily once weekly, QW = once weekly, MD1 caps = ModraDoc001 capsule, MD6 tabl = ModraDoc006 tablet, r = ritonavir.

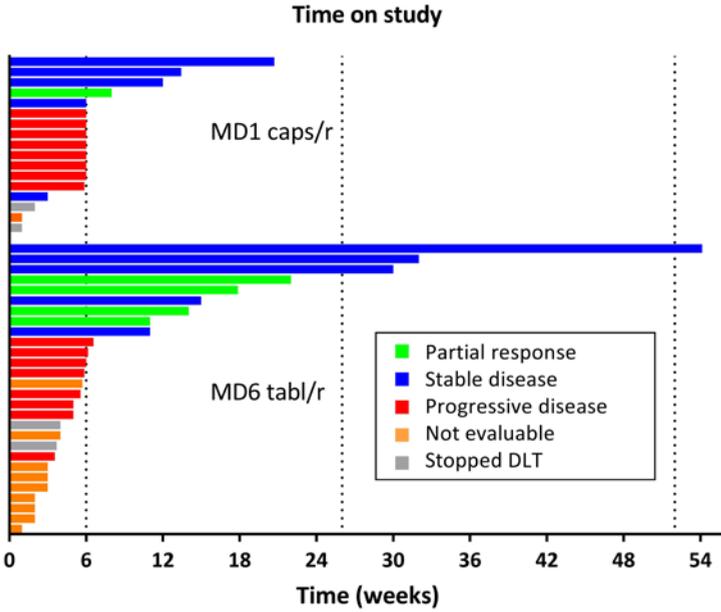


Figure 3: Time on study
 One line represents one patient. Shown separately for the ModraDoc001 capsule and ModraDoc006 tablet formulation. Time on study is the time from first study drug intake to last intake of study medication. Dotted-lines: first line, first tumor evaluation (6 weeks), second and third line 6 and 12 months of treatment, respectively. Abbreviations: MD1 caps = ModraDoc 001 capsule; MD6 tabl = ModraDoc006 tablet, r = ritonavir; DLT = dose-limiting toxicity.

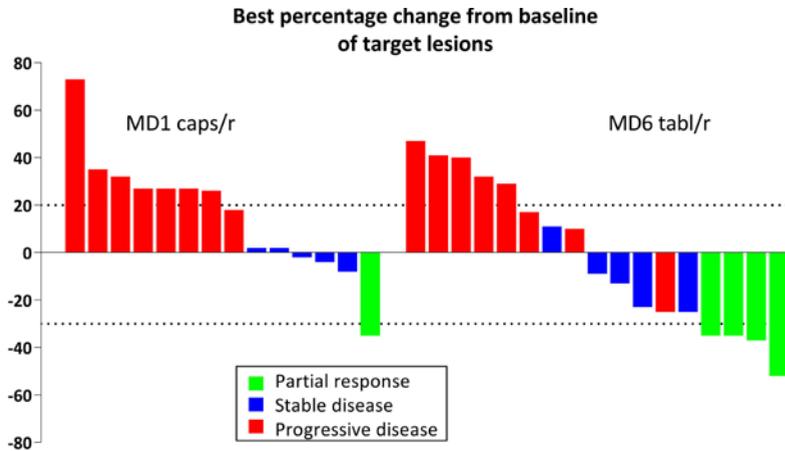


Figure 4: Best percentage change in tumor volume from baseline as per RECIST 1.0.
 Abbreviations: MD1 caps = ModraDoc001 capsule, MD6 tabl = ModraDoc006 tablet, r = ritonavir; DLT = dose-limiting toxicity.

Discussion:

In this dose-finding study the BIDW administration of oral docetaxel as the ModraDoc001 capsule or the ModraDoc006 tablet formulation co-administered with ritonavir was explored according to a classical 3+3 dose escalation design. Based on the observed dose-limiting toxicities, the MTD and RP2D was BIDW 20 / 20 mg ModraDoc001/r, and BIDW 30 / 20 mg ModraDoc006/r.

Treatment related-toxicity was mostly of grade 1 and 2 severity and was manageable with dose modifications and interruptions. No unexpected safety signals were observed considering the known safety profile of the already registered iv docetaxel formulation. Of note, no hypersensitivity reactions and in addition only 1 event (2%) of grade 3 fluid retention (well-known adverse events reported for the docetaxel iv formulation) were observed despite the lack of pre-medication with corticosteroids. This is probably due to the fact that ModraDoc001 and ModraDoc006 do not contain Polysorbate-80: the excipient which is used in the iv formulation and which causes the hypersensitivity reactions [15,16]. No grade 3 peripheral sensory neuropathy was observed. Alopecia was reported in about 20% of patients in both cohorts. The most commonly reported treatment-related adverse events consisted of non-hematological toxicities, with mucositis, diarrhea and nausea being dose limiting. The incidence of severe neutropenia and neutropenic fever was limited to one event in both cohorts (4% of all patients). These findings are in line with a meta-analysis published by di Miao and colleagues showing a reduction in bone marrow suppression (in particular neutropenia) and a slight increase in non-hematological toxicity when a weekly iv docetaxel schedule was compared with a 3-weekly iv administration [17].

Moreover, the exposure to docetaxel in terms of AUC_{0-inf} at the MTD for the ModraDoc006 tablet formulation was in the same range as once weekly iv docetaxel of 30-36 mg/m² [18–20]. Furthermore the observed inter-patient variability for ModraDoc006 tablet/r is in line with those previously reported for iv docetaxel [20,21]. Anti-tumor activity of ModraDoc001/r and ModraDoc006/r was reported in known docetaxel sensitive tumors: partial responses were observed in 3 out of 21 patients with NSCLC (14%) and in 2 out of 3 patients with SCCHN (66%). This preliminary activity is considered promising [22,23].

The safety, PK and activity results of this study further support the proof-of-concept that co-administration of a P-gp/CYP3A4 inhibitor in combination with a known drug substrate is feasible, safe and able to improve the oral bioavailability of the substrate drug. The use of the booster drug ritonavir is not expected to result in safety issues, as its use in daily HIV treatment in combination with other protease inhibitors is found to be safe and well tolerable [24].

Finally, from a pharmaceutical point of view the ModraDoc006 tablet formulation is preferable over the ModraDoc001 capsule formulation for three reasons, as described by Sawicki et al [4]: 1) The solid dispersion used in the tablet is fully amorphous due to the spray drying method applied. This

fully amorphous state results in a super saturation and an increased solubility, as compared to the freeze dried solid dispersion used in the capsule. 2) The tablet formulation tablet is stable for up to 24 months, whereas the dissolution of the capsule capsules worsens in this time period. 3) The spray-drying method in combination with tablet manufacturing is more efficient, more robust, faster and industrially applicable, which makes it more suitable for large scale productions [4]. Based on these properties the tablet formulation was selected for the further clinical development of oral docetaxel.

Conclusion:

Administration of the novel oral formulation of docetaxel as ModraDoc001 capsule and ModraDoc006 tablet in combination with ritonavir according to a continuous BIDW schedule appears to be feasible and safe. The MTD/RP2D was determined as BIDW 30 / 20 mg ModraDoc006 tablet/r (morning/afternoon dose). The tablet formulation was selected for the further development, because of its improved pharmaceutical properties. Toxicity thus far was manageable. Anti-tumor activity is considered promising. The combination of ModraDoc006 tablet/r therefore warrants further investigation in phase II and III clinical trials.

Acknowledgements - conflicts of interest:

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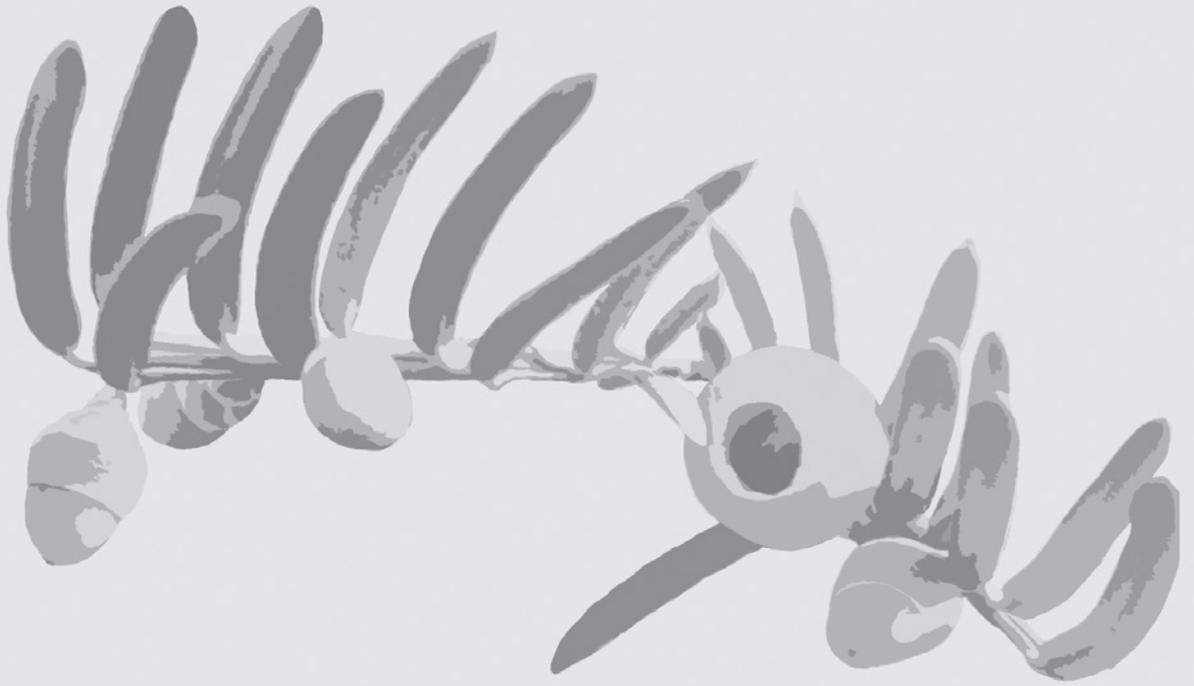
J.H. Beijnen and J.H.M. Schellens have received a grant for translational research (ZonMw code 40-41200-98-004). B. Nuijen, J.H. Beijnen and J.H.M. Schellens are patent holders on oral taxane formulations.

The other authors declare that they have no conflict of interest.

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Chapter 2.4

Rationale and study design process of a randomized phase II study in angiosarcoma investigating oral docetaxel with ritonavir (ModraDoc006/r) versus intravenous paclitaxel: The ANGIODOC study, an EORTC study.

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In preparation

Abstract:

Background: Angiosarcoma is a rare and aggressive form of soft tissue sarcoma. Treatment in advanced/metastatic disease consists of intravenous (iv) chemotherapy with either paclitaxel or doxorubicin. This study aims to show non-inferiority in terms of progression free survival (PFS) of oral docetaxel (as ModraDoc006 tablet) in combination with ritonavir (r), (ModraDoc006/r), versus iv paclitaxel in angiosarcoma.

Methods/design: A randomized hybrid non-inferiority phase II study will be performed in which up to 80 patients with advanced or metastatic angiosarcoma will be included. Patients will be randomized 1:1 over the iv paclitaxel arm and the investigational arm ModraDoc006/r. Paclitaxel will be administered iv on days 1, 8 and 15 of a 28 day cycle at a dose of 80 mg/m² and ModraDoc006/r will be given on days 1, 8, 15 and 22 of a 28 day cycle at a dose of 30 / 20 mg ModraDoc006/r (morning/afternoon docetaxel dose) with 100 mg ritonavir. Secondary endpoints include overall survival, overall response rate, safety, and quality of life (QoL). The sample-size was calculated on the basis of the mean PFS of iv paclitaxel treatment based on a literature review, which was predicted to be 6 months. The non-inferiority margin under H₀ was set at a Hazard Ratio (HR) of 2, meaning a reduction in median PFS from 6 to 3 months. The HR under H₁ was set at 0.75 meaning an increase in PFS of 6 to 8 months. In total 58 events in the 80 patients enrolled need to be observed to assess non-inferiority with these margins with 90% power (5%, two sided-significance level). Safety will be assessed according to CTCAE criteria v4.03 and anti-tumor activity will be measured according to RECIST 1.1. QoL will be evaluated using the QLQ-C30 questionnaire. In case of slow accrual (defined as less than 60 patients included within the first 24 months) the study can be prematurely discontinued after inclusion of at least 60 patients.

Conclusion: Results of this study might indicate a place of ModraDoc006 /r as first or second line treatment of angiosarcoma.

Background:

Angiosarcoma

Angiosarcoma is a rare (~1-2%) and aggressive form of soft tissue sarcoma [1]. Radical surgery is the treatment of choice for local disease, often followed by radiotherapy, as radical excision is difficult to achieve, because of the invasive and often multifocal nature of angiosarcoma [2,3]. Cytotoxic chemotherapy represents the standard treatment in patients with unresectable or metastatic disease. Despite systemic treatment the prognosis remains poor with a reported median progression free survival (PFS) ranging from 3.7 to 9.5 months [4–9] and median overall survival (OS) of about 7.6 months [10]. Anthracyclines and taxanes are the cytotoxic agents generally employed in angiosarcoma with comparable activity [4,6,10–13]. Both paclitaxel and docetaxel have shown activity in angiosarcoma with response rates ranging from 18-62%, but paclitaxel is mostly used in clinical practice [6,7,10]. The reported 6-month progression free survival rate (PFR) with intravenous (iv) paclitaxel is 24-57% [4,9,10]. Paclitaxel is administered as a 3-weeks on / 1 week off schedule at a dose of 80 mg/m² (day 1, 8, 15 every 28 days). In a case series, 9 patients with cutaneous angiosarcoma were treated with weekly iv docetaxel (25 mg/m²). In 6 out of 9 patients an objective response was observed (e.g. partial or complete response) [6], suggesting that docetaxel has activity in this population.

The anti-tumor activity of paclitaxel versus docetaxel has only been compared in three randomized studies performed in ovarian, gastric and non-small cell lung cancer, always in combination with a second cytotoxic agent. In all these studies activity of the two taxanes was comparable [14–16]. Suggesting the taxanes are likely to be interchangeable in the treatment of solid tumors.

Oral docetaxel

The iv administration of taxanes has several disadvantages. It is invasive and patients need to visit the hospital for administration. Furthermore the commonly observed hypersensitivity reactions are likely caused by the excipients, (i.e. Cremophor EL and polysorbate-80), that are present in the iv formulations of paclitaxel and docetaxel, respectively. Oral administration would allow for a more convenient administration and might reduce the incidence of hypersensitivity reactions as polysorbate-80 is not needed in the oral formulation of docetaxel [17]. However, oral bioavailability of docetaxel is poor because of poor aqueous solubility and extensive first pass effect caused by metabolism by liver and intestinal CYP3A4 and excretion by drug transporters e.g. P-glycoprotein (P-gp) [18]. In pre-clinical and in clinical studies the bioavailability of orally administered docetaxel improved significantly by co-administration of the CYP3A4 and P-gp inhibitor ritonavir [19,20]. The aqueous solubility was improved by the development of a solid dispersion formulation, denoted ModraDoc006 tablet (10 mg docetaxel) [17]. The concept of improving oral bio-availability by CYP3A4

and P-gp inhibition was further investigated in two phase I dose-escalating studies in patients with solid tumors in which ModraDoc006 was combined with 100 mg ritonavir (ModraDoc006/r) resulting in a significant increase in oral bio-availability. The exposure to docetaxel reached at the maximum tolerated dose of weekly 30/20 mg (morning/afternoon dose; both with 100 mg ritonavir) ModraDoc006/r is comparable to a weekly iv docetaxel dose of 35 mg/m², in terms of the area under the plasma concentration-time curve (AUC) [20–23]. Administration of oral docetaxel in combination with ritonavir, as ModraDoc006/r was proven tolerable and safe. Common toxicities observed in the phase I trials employing weekly administration regimens were nausea, diarrhea, fatigue, vomiting and alopecia. Generally toxicity was manageable and of grade 1-2 severity. Furthermore hypersensitivity reactions did not occur in either of the two phase I trials, allowing treatment without premedication corticosteroids [22,23]. The oral administration of docetaxel could therefore potentially improve patient safety, and quality of life. Furthermore it might reduce treatment costs, as administrations can occur at home instead of at the day-care unit [24]. The primary aim of the current phase II study is to show that the PFS of patients with advanced stage angiosarcoma treated with ModraDoc006/r is non-inferior to “standard” iv paclitaxel. Secondary aims include overall response rate (ORR), PFR at 6, 9 and 12 months, overall survival (OS), quality of life (QoL) and safety of ModraDoc006/r and iv paclitaxel, respectively.

Study design and scientific advice:

The study was designed in two phases as scientific advice from the European Medicines Authority (EMA) was sought after completion of a first study protocol draft (Study design I). Suggestions provided by the EMA were implemented in the final study design (Study design II).

Study design I

In the first draft protocol a non-comparative single stage A'Hern design was employed [25]. Herein the primary objective was to demonstrate a 6 month PFR of >20% with ModraDoc006/r. The 20% PFR at 6 months was selected on the basis of the results of the study published by Penel et al, where administration of iv paclitaxel led to a PFR at 6 months of 24% [10]. The alpha was set at 0.1 (one-sided) and a power of 90% was selected to detect a PFR 6 months of 20%. Based on these assumptions, 11 patients out of 36 needed to be progression-free at 6 months to meet pre-specified criteria. In that case the activity of ModraDoc006/r would have been considered sufficient to warrant further investigation. A dropout of around 10% was expected and therefore a total of 40 patients were planned to be enrolled in the ModraDoc006/r arm. In order to gain insight on the activity of iv paclitaxel in the selected study population a second arm was planned where around 20 patients

would have been included according to a 2:1 randomization schema. Patients could continue treatment until progression of disease or unacceptable toxicity. The major disadvantage of this design was that it was not a comparative design, therefore no formal comparison of the activity of iv paclitaxel versus ModraDoc006/r could be performed. A second disadvantage of the design was the selection of PFR 6 months as a primary endpoint rather than a time based endpoint (e.g. PFS or OS).

EMA (European Medicine Agency) advice

Scientific advice was sought by EMA. The primary question was whether the proposed study design and sample-size would be acceptable to support marketing authorization. During the procedure several alternative comparative (hybrid) non-inferiority designs were discussed, together with their feasibility. In the final advice letter EMA suggested three main points for consideration: 1) A comparative study design (e.g. a non-inferiority design) was to be preferred over a non-comparative study; 2) A more solid time to event endpoint was preferred over the binary PFR 6 months, as primary endpoint (e.g. PFS or OS); and 3) an increase in the sample size was encouraged.

Study design II (final)

Based on the above suggestions the study was re-designed to a 1:1 randomized comparative hybrid non-inferiority study with PFS as primary endpoint [26]. The null hypothesis of inferiority is used, but the sample size is chosen to have adequate power to detect a modest treatment benefit under the alternative hypothesis. Since the difference between the non-inferiority margin Δ and the treatment effect used for the sample size calculation is larger than between Δ and 1 (classical non-inferiority design alternative hypothesis), the hybrid design requires a smaller sample size than the pure non-inferiority design. One could hypothesize that due to the limited toxicities seen so far with weekly ModraDoc006/r, patients would be able to take the drug for a longer period of time, which could translate into a modest treatment benefit, justifying the use of such a design. The reported median PFS in studies in angiosarcoma patients after iv paclitaxel varies between studies. The estimated median PFS for iv paclitaxel based on a literature search is 6 months. The studies included in this calculation with their respective PFS results and the number of patients included are presented in table 1.

No formal sample-size calculation was performed for study design II. Instead power calculations were performed under different scenarios of H0 and H1 (given a one-sided 5% alpha and assuming a median PFS of 6 months in the paclitaxel arm) to assess the value of the design for different sample sizes (see table 2 for a sample-size of 80 patients and table 3 for a sample-size of 60 patients). The non-inferiority margin hazard ratio (HR) under H0 was set at 2, i.e. a reduction from 6 to 3 months in median PFS. This non-inferiority margin was considered acceptable by the members of the EORTC-

Soft-Tissue and Bone Sarcoma Group, when the safety and QoL in the ModraDoc006/r treated patients would be better as compared to the standard iv paclitaxel.

Table 1: Calculation of median progression free survival, based on a literature review of angiosarcoma patients.

Study	Number (n) of patients included	Median PFS (months)	n * Median PFS
Schlemmer et al [7]	32	7.6	243
Penel et al [10]	30	4	120
Italiano et al [12]	75	5.8	435
Letsa et al [28]	12	7	84
Ray-Coquard al [9]	49	6.6	323
Apice et al [29]	17	4.6	78
Byeon et al [30]	21	5.7	120
Total	236		1403

Average median PFS n = 236 1403 / 236 = 5.9 months

Calculation of median PFS for iv paclitaxel on available literature. As in these studies also patients with a WHO PS of 2 were included median PFS was considered to be about 6 months for iv paclitaxel. Abbreviations: PFS = progression free survival, iv = intravenous, n = number of patients, WHO = World Health Organization, PS = performance score.

Table 2: Power calculations for a sample-size of 80 patients

Number of events	Number of pts	Accrual duration	Total duration	HR under H0	HR under H1	PFS under H1	Power under H1
58	80	30 M	35 M	2	1	6 M	83%
58	80	30 M	35 M	2	0.9	6.7 M	91%
58	80	30 M	35 M	2	0.8	7.5 M	96%
58	80	30 M	35 M	2	0.78	7.7 M	97%
58	80	30 M	35 M	2	0.75	8 M	98%

Table 3: Power calculation for a sample-size of 60 patients

Number of events	Number of pts	Accrual duration	Total duration	HR under H0	HR under H1	PFS under H1	Power under H1
41	60	24 M	32 M	2	1	6 M	71%
41	60	24 M	32 M	2	0.9	6.7 M	81%
41	60	24 M	32 M	2	0.8	7.5 M	90%
41	60	24 M	32 M	2	0.78	7.7 M	91%
41	60	24 M	32 M	2	0.75	8 M	93%

As a result the sample size was increased to 80 patients, in order to increase the power of the study. An early feasibility stopping rule was implemented based on accrual in the first 24 months. If accrual is less than 60 patients in this period, the study can be stopped after inclusion of the 60th patient. The phase II study in angiosarcoma will be performed by Modra Pharmaceuticals BV, a spin-out company of the Netherlands Cancer Institute, in collaboration with the European Organization for Research and Treatment of Cancer (EORTC). The study will be performed as an open-label phase II trial comparing the PFS of patients with advanced unresectable angiosarcoma treated in the first or second line with iv paclitaxel or with oral ModraDoc006/r. Stratification factors include line of treatment (e.g. first or second line) and etiology (i.e., radiotherapy induced versus de novo angiosarcoma). A schematic overview of the study is shown in figure 1.

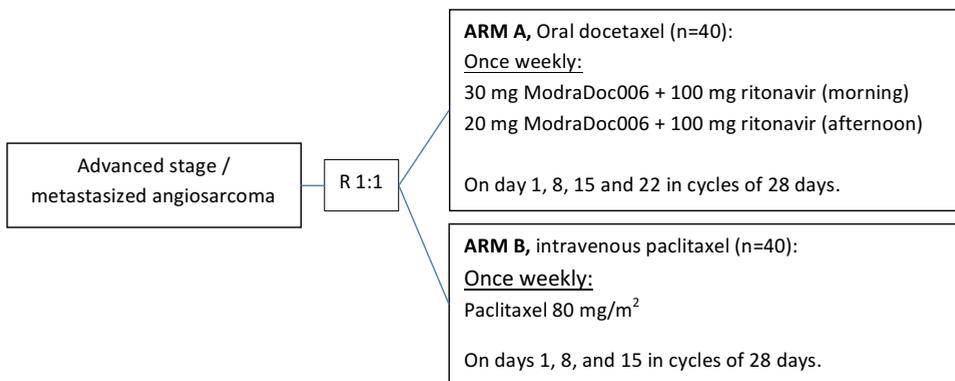


Figure 1: Study design

Study design: A total of 80 patients will be enrolled in this phase II trial randomized over arms A and B. If accrual is less than 60 patients within 24 months, the trial may be stopped prematurely after inclusion of at least 60 patients. (R = randomization; n = number of patients)

Objectives

The primary objective of this study is to show that the PFS in the intention-to-treat (ITT) population of patients with angiosarcoma treated with ModraDoc006/r is non-inferior to iv paclitaxel. Secondary objectives are to compare the ORR, OS, PFR at 6, 9 and 12 months, PFS in the per protocol population, QoL and the safety of ModraDoc006/r and iv paclitaxel.

Inclusions criteria

Patients of 18 years or older, who have a histological or cytological proof of advanced stage angiosarcoma, for whom no curative surgery or radiation therapy exist will be enrolled. Patients must have a life expectancy of at least 12 weeks and a World Health Organization (WHO) performance score of 0 or 1. Minimal acceptable laboratory values include: ANC of $\geq 1.5 \times 10^9$ /L, platelet count

of $\geq 100 \times 10^9$ /L, adequate hepatic function (as defined by serum bilirubin $\geq 1.5 \times$ upper limit of normal (ULN), ALT and AST $\geq 2.5 \times$ ULN ($5 \times$ ULN if liver metastases are present)) and adequate renal function (as defined by serum creatinine $\geq 1.5 \times$ ULN or creatinine clearance ≥ 50 ml/min (by Cockcroft-Gault or MDRD formula)). Patients are required to have measurable disease according to Response Criteria in Solid Tumors version 1.1 (RECIST v1.1) [27] and to be able to swallow oral medication. Finally patients should provide written informed consent.

Exclusion criteria

Relevant exclusion criteria are previous treatment with a taxane or more than 1 prior line of chemotherapy for angiosarcoma. Patients should not use medication that modulates MDR and/or CYP3A are excluded, as well as patients who received radiotherapy within 3 weeks prior to study enrollment, unless this concerns single dose radiotherapy for palliation on non-target lesions. Patients who have a bowel obstruction or motility disorders that may influence the absorption of study drugs will not be allowed to participate, as well as patients who have symptomatic brain metastases or leptomeningeal metastases. Finally patients who have another active malignancy or had a malignancy in the past three years with the exception of adequately treated cervical carcinoma in situ and non-melanoma skin cancer are also excluded from the study.

Interventions

Patients will be screened at baseline on in- and exclusion criteria, after having signed informed consent. Baseline measurements include a tumor assessment and a QoL assessment (QLQ-C30 questionnaire). Subsequently patients will be randomized between to treatment arms (arm A and B). In arm A patients will receive bi-daily once weekly 30 / 20 mg (morning/afternoon dose) ModraDoc006 tablet in combination with bi-daily once weekly 100 mg ritonavir (taken simultaneously with ModraDoc006) on days 1, 8, 15 and 22 of a 28 day cycle (continuous weekly schedule). In arm B patients will be treated with iv paclitaxel at a dose of 80 mg/m² on days 1, 8 and 15 of a 28 days cycle. Patients will be followed at the outpatient clinic for safety every week during the first four weeks and every cycle (4 weeks) thereafter. Tumor assessments will be performed according to RECIST v1.1 every 8 weeks. The QoL assessment will be repeated after cycle 1 and 2 (4 weeks) and every two cycles (8 weeks) thereafter. A final QoL assessment will be performed at end of treatment.

Concomitant treatment

Patients in arm A will receive ModraDoc006/r without premedication in the first cycle. If considered in the best interest of the patient, supportive medication may be given at the discretion of the

investigator and according to good clinical practice. In arm B patients will be given iv paclitaxel according to local protocol including standard pre-medication.

Dose-reductions

Dose-reductions of oral docetaxel will be performed in 10 mg steps. The first reduction will be to 20 / 20 mg ModraDoc006/r and the second dose-reduction will be to 20 / 10 mg ModraDoc006/r. The ritonavir dose will not be changed. A maximum of two dose reductions is allowed. In patients requiring a dose reduction no dose re-escalation is allowed. Guidelines for dose modifications are specified in the study protocol.

Dose-reductions for paclitaxel will be allowed according to local treatment guidelines as considered necessary by the treating physician.

Logistics and administrative arrangements

The study protocol will be revised and approved by the accredited medical ethical committees of all participating sites and will be performed in compliance with the declaration of Helsinki and ICH-GCP. The study will be coordinated by the EORTC and will be performed as an European multi-center study. Prior to study start patients will be randomized over the two study arms using a minimization algorithm.

Discussion:

The current study aims to assess whether treatment with oral docetaxel (as ModraDoc006) combined with ritonavir is as effective as iv paclitaxel in terms of median PFS in patients with angiosarcoma. Based on the results from the phase I trials with ModraDoc006/r it is expected that oral administration will improve safety and QoL as compared to iv paclitaxel treatment. Although a pharmacoeconomic analysis is not included in the current study, a reduction in costs is to be expected.

The hybrid design offers the possibility to assess non-inferiority with a relatively small sample size, compared to standard non-inferiority designs. At the same time the hybrid design might not have adequate power to establish non-inferiority when the new treatment is equivalent to the standard treatment. The loss of power is even more dramatic when the new treatment performs worse than the standard treatment. Indeed, when testing non-inferiority employing a hybrid design the intent-to-treat analyses tend to dilute treatment differences. The per-protocol analyses should be added to support the robustness of the primary non-inferiority analysis [26].

In this study both patients treated in the first- and second line are included because angiosarcoma

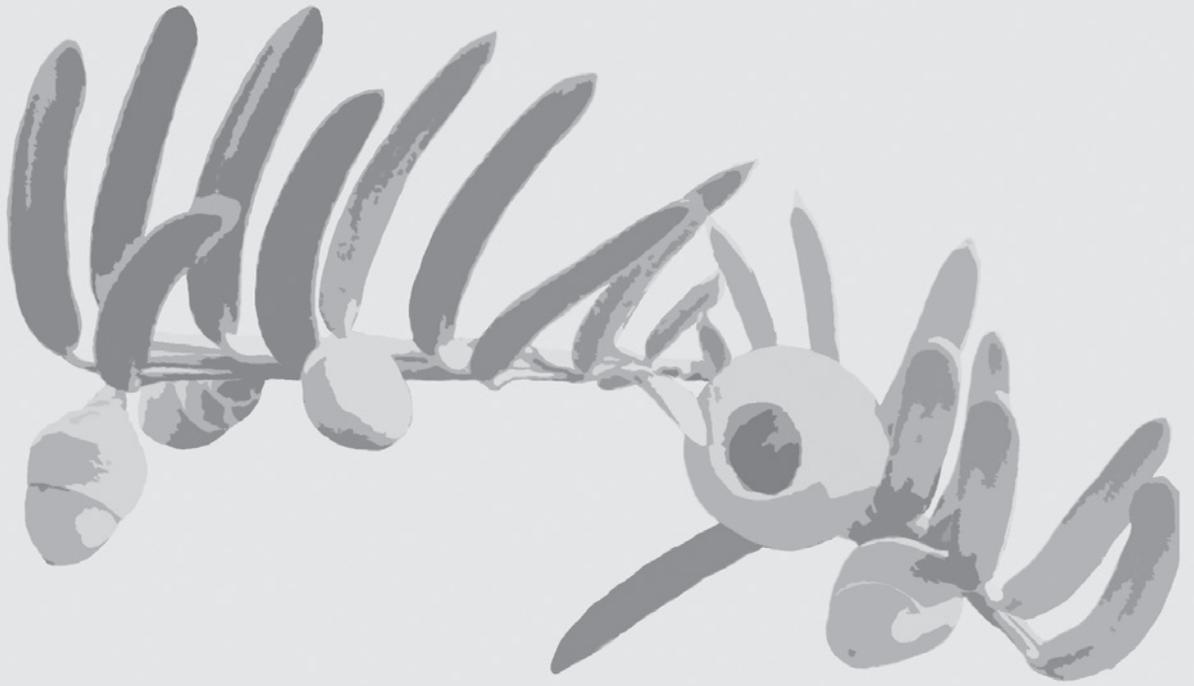
is a rare disease and the standard of care differs between countries, being iv paclitaxel the preferred first-line agent in some countries and doxorubicin in others. Therefore, limiting inclusion to first line treatment only would challenge the feasibility of the trial. A discussion regarding the proposed comparator to be employed in the study was held with EMA during the scientific advice procedure. According to the EMA physicians choice would be preferred as comparator. However, considering that it is currently unclear whether PFS under treatment with taxanes or doxorubicin is comparable, it was felt that implementation of iv paclitaxel as comparator would allow a better comparison in PFS (primary study endpoint). A comparison with iv docetaxel treatment was not considered appropriate as it does not represent the standard of care in Europe.

In conclusion, the current study aims to evaluate activity of the novel oral docetaxel formulation ModraDoc006/r in patients with angiosarcoma. If similar antitumor activity in comparison with the standard iv paclitaxel will be observed, associated with a more favorable toxicity profile leading to an improvement in patient's QoL, the results might be supportive for a place of ModraDoc006/r as first or second line treatment of angiosarcoma.

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Chapter 2.5

Boosting of orally administered paclitaxel by CYP3A4 inhibition.

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Abstract:

Purpose: The oral bioavailability of paclitaxel is low due to high first pass metabolism by cytochrome P450 (CYP)3A4 and affinity for the drug transporter P-glycoprotein (P-gp) present in the gastrointestinal tract and the liver. In this study the boosting effect of the co-administrated CYP3A4 and/or P-gp inhibitors, ritonavir, ketoconazole, clarithromycin and cyclosporin A on the systemic exposure to orally administered paclitaxel was investigated.

Methods: The study was a four arm open-label cross over study: in three arms the boosting of orally administered paclitaxel by 100 mg ritonavir was compared to either 400 mg ketoconazole, 1000 mg clarithromycin or 15 mg/kg cyclosporin A. In the fourth arm the boosting of a 100 mg dose of ritonavir was compared with a 200 mg ritonavir dose. Patients included had advanced solid tumors.

Results: Seventeen patients were enrolled in four arms. The magnitude of boosting by cyclosporin A, ketoconazole and clarithromycin was in line with that observed for ritonavir. The paclitaxel area under the plasma concentration time curve AUC_{0-24h} (\pm standard deviation) were 1033 (\pm 122), 1207 (\pm 803), 749 (\pm 297) and 918 (\pm 631) ng/ml*h, respectively. Doubling of the ritonavir dose resulted in a minor further increase of 22% in AUC_{0-24h} in the exposure to paclitaxel ($p = 0.60$).

Conclusions: Oral uptake of paclitaxel can be boosted via both CYP3A4 and/or P-gp inhibition. Ritonavir, ketoconazole, clarithromycin and cyclosporin A all boosted the oral uptake of paclitaxel. A dose of 100 mg ritonavir is considered adequate for boosting of orally administered paclitaxel.

Introduction:

The taxane paclitaxel is administered intravenously (iv) and is widely applied in the treatment of various malignancies [1]. Oral application of paclitaxel has several advantages. It improves patient convenience as the patient would not have to visit the hospital for drug administration and an indwelling iv catheter would not be needed. However, the oral bioavailability of paclitaxel is limited because of high-affinity for the drug-transporter P-glycoprotein (P-gp, MDR1, ABCB1), which is highly expressed in the epithelial layer of the gastro-intestinal tract and actively excretes paclitaxel into the gut lumen either directly or via the bile [2,3], and extensive pre-systemic metabolism by the cytochrome P450 (CYP) enzyme CYP3A4 [3,4]. In the past the uptake of orally administered paclitaxel from the gastro-intestinal (GI) tract was boosted using the P-gp and CYP3A4 inhibitor cyclosporin A (CsA) [5–8]. Boosting with CsA resulted in an 8-9 fold increase in systemic exposure to paclitaxel and encouraging anti-tumor activity was observed in three clinical phase II studies [8–11].

Oral administration would enable the investigation of daily doses or allow for more frequent administration schedules of paclitaxel, for instance in a “metronomic” schedule. The aim of metronomic therapy is to reach a continuous low concentration of the administered drug (e.g. paclitaxel) over a prolonged period of time. These low concentrations are generally not sufficient to obtain a direct anti-tumor effect. The endothelial cells of the vasculature are however, sensitive to these concentrations and will die as a result [12]. As the endothelial cells are needed for the formation of novel blood vessels, tumor-angiogenesis would be prevented or hampered, thereby resulting in an anti-tumor effect. The administration of such a metronomic schedule has shown potential in preclinical studies [13–15] and in a phase II study in which paclitaxel was administered as a continuous 96-hour infusion [16]. The phase II study was however complicated by a high incidence of bacterial infections, which were attributed to the long infusion times and the indwelling catheter. The bacterial infections ultimately resulted in early closure of the study. Oral administration of paclitaxel in such a schedule would be ideal, however CsA as booster is not practical as its use might result in adverse events, such as bone marrow suppression and renal failure [17]. Therefore, a booster drug with a better safety profile is needed.

The development of oral paclitaxel was paralleled by the development of an oral formulation of docetaxel, of which the oral bioavailability could significantly be boosted by the strong CYP3A4 inhibitor ritonavir (RTV) [18,19]. This is ideal for docetaxel as it is a substrate of P-gp and it is only metabolized by CYP3A4 [20]. Paclitaxel, however is metabolized by both CYP3A4 and CYP2C8 [21]. CYP2C8 is primarily expressed in the liver and its gastro-intestinal expression is low [22]. As it is expected that the first-pass effect is mainly the result of intestinal metabolism, the role of CYP2C8 in limiting the oral bioavailability of paclitaxel might be limited. Therefore, inhibition of CYP3A4 alone could be sufficient to boost uptake of orally administered paclitaxel.

The aim of the current study was to explore whether the exposure to orally administered paclitaxel can be enhanced by co-administration of CYP3A4 inhibitors and/or P-gp inhibitors. Paclitaxel was orally administered as a drinking solution with two selected strong CYP3A4 inhibitors (clarithromycin and ketoconazole), the P-gp and strong CYP3A4 inhibitor RTV or the previously studied strong P-gp and CYP3A4 inhibitor CsA.

Patients and Methods:

Patients:

Patients with advanced solid tumors of ≥ 18 years were eligible for the trial. Other inclusion criteria were no radio- or chemotherapy within the last 4 weeks (limited palliative radiation for pain reduction was allowed). Patients were required to have acceptable bone marrow, liver and renal function and a World Health Organization (WHO) performance status of ≤ 2 . Exclusion criteria included concomitant medication interacting with CYP3A4 and/or P-gp, chronic use of proton pump inhibitors or H₂-receptor antagonists and a life expectancy of less than 3 months. The study protocol was approved by the medical ethics committee of the Netherlands Cancer Institute. All patients had to give written informed consent prior to start of study activities. The study was registered in the ISRCTN database under identifier ISRCTN 32770468.

Study design:

This study was designed as a two-step open-label proof of concept study. In step one the boosting of orally administered paclitaxel by a 100 mg RTV dose was compared with boosting by a CsA dose of 15 mg/kg. In step two three additional arms were opened to further proof the concept of boosting of orally administered paclitaxel via CYP3A4 inhibition. In these arms, two other boosters (i.e. clarithromycin and ketoconazole) and a higher RTV dose of 200 mg were investigated. A study overview is presented in table 1.

Arm I – IV

All arms were designed similarly. Four evaluable patients were enrolled into each of the four arms. Patients in arm I received, 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, Mayne Pharma, Melbourne, Australia) as a drinking solution in which paclitaxel was dissolved in ethanol, water and Cremophor EL. Patients were randomized into two groups, group I received on day 1 oral paclitaxel in combination with 15 mg/kg oral CsA and on day 8 oral paclitaxel in combination with 100 mg oral RTV. Group II received the same treatment in reversed order. Both CsA and RTV

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 150 ml of tap water. Pre-treatment consisted of 4 mg dexamethasone 1 hour prior to, 24 and 48 hours after paclitaxel intake. Additionally, patients received 1 mg of granisetron 1 hour prior to oral paclitaxel to prevent nausea and/or vomiting. Patients in arms II-IV were randomized and (pre)treated as described for arm I, with the difference that in arm II 100 mg oral paclitaxel was boosted with 100 mg RTV or 1000 mg clarithromycin; in arm III 100 mg oral paclitaxel was boosted with 100 mg RTV or 400 mg ketoconazole, and in arm IV oral paclitaxel was boosted with a 100 mg or a 200 mg dose of RTV.

Table 1: Study overview. Number of patients included in arms I to IV and the treatment administered in weeks 1-2. Thereafter, all patients received weekly paclitaxel iv 90 mg/m² in their best interest.

	Week 1 or Week 2 (cross-over)	Week 3 and beyond
Arm I (n=4)	100 mg PAC + 15 mg/kg CsA or 100 mg PAC + 100 mg RTV	Weekly paclitaxel 90 mg/m ² iv
Arm II (n=4)	100 mg PAC + 1000 mg CLM or 100 mg PAC + 100 mg RTV	
Arm III (n=4)	100 mg PAC + 400 mg KTZ or 100 mg PAC + 100 mg RTV	
Arm IV (n=5)	100 mg PAC + 100 mg RTV or 100 mg PAC + 200 mg RTV	

Abbreviations: CsA = cyclosporin A, PAC = paclitaxel, RTV = ritonavir, CLM = clarithromycin, KTZ = ketoconazole, iv = intravenously.

After completion of the pharmacokinetics in the first two weeks patients were allowed to continue on iv paclitaxel at a weekly dose of 90 mg/m² (Mayne, Mayne Pharma, Melbourne, Australia). Patients remained on treatment until they no longer had clinical benefit, or toxicity led to patient withdrawal.

Safety:

Adverse events were graded according to the National Cancer Institute Common Toxicity Criteria for Adverse Events (NCI-CTCAE) version 3.0 [23]. Patients were considered evaluable when they completed the pharmacokinetics (PK) in week 1 and 2. Patients who were not evaluable for the PK were replaced.

Pharmacokinetic sampling and analyses:

Blood samples for PK analysis were collected during the first and second week: pre-dose, 0.5, 1, 1.5, 2, 3, 4, 6, 8, 10, 24 and 48 hours after intake of study drug. Samples were centrifuged within 1 hour at 1500 g for 10 minutes. Plasma was stored at -20 °C in the pharmacy until analysis. Paclitaxel plasma concentrations were quantified using a validated high-performance liquid chromatography with tandem mass spectrometric detection (LC-MS/MS) assay, with a lower limit of quantification of 0.25 ng/mL paclitaxel [24].

The individual non-compartmental PK parameters were determined using validated scripts in the software package R (version 3.0.1). The mean, standard deviation (SD) 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}), the area under the plasma-concentration time curve from t=0 to 24 hours (AUC_{0-24h}) and AUC to infinity (AUC_{0-inf}) was extrapolated using the terminal rate constant. The terminal half-life ($T_{1/2}$) was also calculated.

To evaluate whether observed differences in paclitaxel exposure for the boosters evaluated in the arms were statistically significantly different paired t-tests were performed. Only PK evaluable patients were evaluated.

Results:

Patients

In the study 17 patients were enrolled into the arms I-IV. One patient in arm IV did not complete the two weeks of PK due to clinical deterioration and was replaced. All other patients were evaluable for the PK. Of the 17 patients included 5 were male (29%) and 12 female (71%), the median age of patients was 59 (range 29-80) years. The most common tumor types were non-small cell lung cancer (NSCLC)(5 patients) and urothelial cell carcinoma (4 patients). The patient characteristics are summarized in table 2.

Pharmacokinetics

The mean PK parameters with standard deviations (SD) and coefficient of variation (%CV) are presented per arm in table 3. The mean plasma concentration-time curves for the four arms are shown in figure 1. The AUC_{0-24h} (SD) in arm I was 1033 (\pm 122) ng/ml*h and 732 (\pm 429) ng/ml*h for boosting with CsA and RTV, respectively ($p = 0.24$). This provided proof that uptake of paclitaxel can be boosted via CYP3A4 inhibition using low dose RTV. Although the boosting was less efficient and AUC_{0-24h} was approximately 29% lower as compared to CsA, this provided sufficient rationale

to continue with arms II to IV. In arms II and III the effect of boosting with clarithromycin and ketoconazole respectively was determined. Both drugs increased the bioavailability of oral paclitaxel and plasma levels were comparable to those reached with RTV as a booster. In arm II the AUC_{0-24h} (SD) for paclitaxel boosted with clarithromycin and RTV were 749 (\pm 297) and 970 (\pm 400) ng/ml*h, respectively ($p = 0.23$) and in arm III the AUC_{0-24h} (SD) for paclitaxel boosted with ketoconazole and RTV were 1207 (\pm 803) and 1267 (\pm 1062) ng/ml*h, respectively ($p = 0.78$). In arm IV the doubling of the RTV dose from 100 mg to 200 mg resulted in a minor further increase in paclitaxel exposure in terms of AUC_{0-24h} from 629 (\pm 370) to 770 (\pm 230) ng/ml*h, respectively ($p = 0.60$). In arm IV one patient had a 6-fold higher exposure to paclitaxel in terms of AUC_{0-24h} as compared to the other patients. This concerned a 45 year old male patient of African descent with gastric cancer. This patient was pretreated with surgery, for his gastric tumor and was expected to have a normal bowel function. On review, his co-medication did not contain any drugs that were not allowed per protocol. Analysis of known single nucleotide polymorphisms resulting in reduced CYP enzyme activity did not show the CYP2C8*3, CYP3A4*22 or the CYP3A5*3 polymorphism in this patient. The analysis of ritonavir PK did not show any abnormalities. The PK results and the concentration-time curves of this patient are presented separately in table 3 and figure 1, respectively as this patients was considered an outlier.

Table 2: Patient demographics

	n = 17
Sex	
Female	5
Male	12
Age (years)	
Median	59
Range	(29-80)
WHO performance status	
0	10
1	4
2	3
Pathologic diagnosis	
NSCLC	5
Urothelial cell carcinoma	4
Primary unknown	2
Other	6

Abbreviations: NSCLC = non-small cell lung cancer, n = number of patients included, WHO = World Health Organization.

Table 3: Paclitaxel pharmacokinetics after administration of oral paclitaxel with the evaluated booster drugs.

	Number of patients	AUC _{0-24h} (ng/ml* ^h) mean ± SD (%CV)	AUC _{0-4h} (ng/ml* ^h) mean ± SD (%CV)	C _{max} (ng/ml) mean ± SD (%CV)	T _{max} (hours) mean ± SD (%CV)	T _{1/2} (hours) mean ± SD (%CV)
ARM I						
100 mg PAC + 15 mg/kg CsA	n=4	1033 ± 122 (12%)	1413 ± 120 [#] (8.5%)	252 ± 80 (32%)	1.66 ± 0.47 (28%)	18.1 ± 2.19 [#] (12%)
100 mg PAC + 100 mg RTV	n=4	732 ± 429 (59%)	706 ± 370 [#] (52%)	283 ± 189 (67%)	1.40 ± 0.45 (33%)	18.9 ± 3.69 [#] (20%)
ARM II						
100 mg PAC + 1000 mg CLM	n=4	749 ± 297 (40%)	1041 ± 497 (48%)	232 ± 76.8 (33%)	1.64 ± 0.24 (15%)	20.8 ± 4.18 (20%)
100 mg PAC + 100 mg RTV	n=4	970 ± 400 (41%)	1269 ± 617 (49%)	223 ± 49.4 (22%)	2.39 ± 1.12 (47%)	17.1 ± 1.76 (10%)
ARM III						
100 mg PAC + 400 mg KTZ	n=4	1207 ± 803 (67%)	1542 ± 1003 (65%)	305 ± 189 (62%)	2.04 ± 0.04 (2.1%)	17.2 ± 3.55 (21%)
100 mg PAC + 100 mg RTV	n=4	1267 ± 1062 (84%)	1608 ± 1331 (83%)	306 ± 156 (51%)	2.17 ± 0.66 (30%)	16.3 ± 1.66 (10%)
ARM IV						
100 mg PAC + 200 mg RTV*	n=4	770 ± 230 (30%)	1002 ± 285 (28%)	202 ± 71 (35%)	1.77 ± 0.29 (17%)	17.5 ± 1.37 (7.8%)
100 mg PAC + 100 mg RTV*	n=3	629 ± 370 (59%)	829 ± 458 (55%)	162 ± 110 (68%)	1.86 ± 0.29 (16%)	19.6 ± 3.14 (16%)
<i>Outlier:</i>						
100 mg PAC + 200 mg RTV	n=1	4893	8382	324	8.37	NR
100 mg PAC + 100 mg RTV	n=1	3733	4130	360	7.98	NR
All patients						
100 mg PAC + 100 mg RTV*	n=15	918 ± 631 (69%)	1150 ± 829 [§] (72%)	249 ± 135 (54%)	1.96 ± 0.76 (39%)	17.8 ± 2.62 [§] (15%)

Pharmacokinetic parameters presented for the arms I – IV. *PK data of the outlier in arm IV were removed from the analyses and are shown separately. AUC_{0-4h}: area under the plasma-concentration time curve from time 0 to infinity, AUC_{0-24h}: area under the plasma-concentration time curve from time 0 to 24 hours, C_{max}: maximum plasma concentration, T_{max}: time to reach C_{max}. Abbreviations: SD = standard deviation, %CV = coefficient of variation, PAC = paclitaxel, CsA = cyclosporin A, RTV = ritonavir, CLM = clarithromycin, KTZ = ketoconazole, N = number of patients, NR = not reported unreliable regression, [#] n= 3: one patient was not reported, due to unreliable regression, [§] n= 14, one patient was not reported, due to unreliable regression.

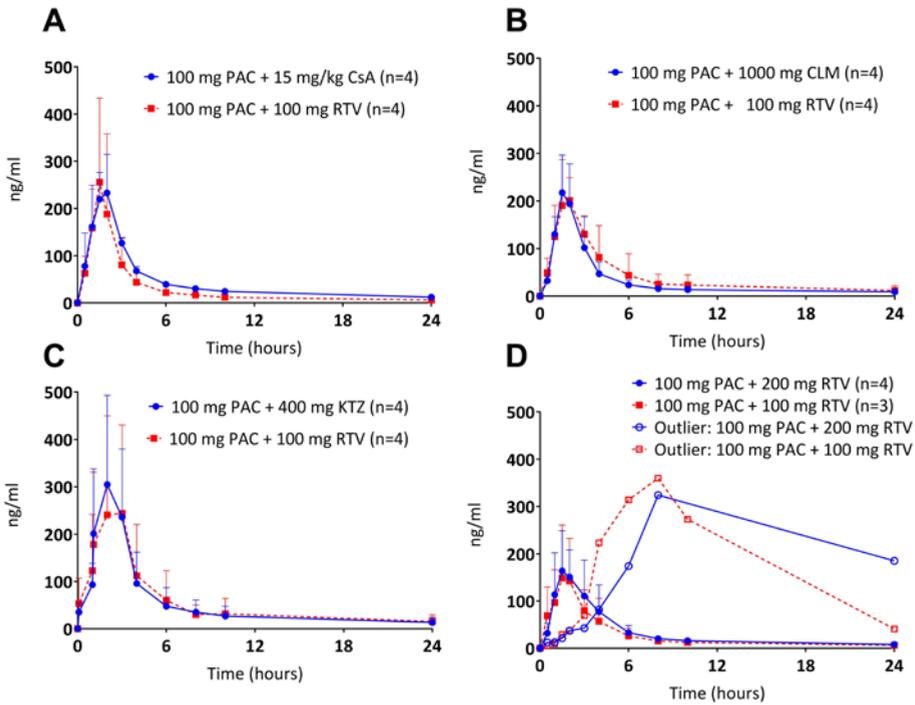


Figure 1: plasma concentration-time curves
 The mean plasma concentration-time curves for arms I to IV, in figures 2A - 2D respectively. Error bars represent the standard deviation of the mean. The patient with outlying PK is shown separately in figure 2D. Abbreviations: CsA = cyclosporin A, PAC = paclitaxel, RTV = ritonavir, CLM = clarithromycin, KTZ = ketoconazole, n = number of patients.

Safety of oral paclitaxel (first 2 weeks)

Safety data were available for all patients. Adverse events that were possibly, probably or definitely related to oral administration of paclitaxel and/or the administered boosters were generally mild. The most common adverse events were diarrhea (5 patients), fatigue (4 patients), nausea (3 patients) and sensory neuropathy (3 patients). No grade 3 or higher treatment related adverse events were reported.

Discussion:

Uptake of orally administered paclitaxel can considerably be boosted by P-gp and/or CYP3A4 inhibition. No significant differences in exposure to paclitaxel were observed for the four boosters applied in this study.

The results of this study formed the basis for the development of oral paclitaxel in combination

with a low dose of RTV in a metronomic schedule. RTV is ideal as a booster, because of its well-known and good safety at low dosages. Furthermore, it is widely used as booster of other protease inhibitors in HIV treatment [25]. In this study oral paclitaxel administration without a booster has not been evaluated. In a previous study exposure to oral paclitaxel with and without the booster CsA has been investigated. In that study the increase in systemic exposure of paclitaxel was about 8 to 9-fold higher after administration of oral paclitaxel combined with 15 mg/kg CsA as compared to administration of oral paclitaxel alone [11]. Given these results an indirect comparison can be made for RTV. As boosting of oral paclitaxel with a 15 mg/kg CsA resulted in a 29% higher AUC_{0-inf} as compared to boosting with a 100 mg RTV dose in this study. Combined, this indicates that the increase in exposure to paclitaxel after oral administration with the booster RTV is about 5-fold higher as compared to paclitaxel administration without a booster.

Administration of the booster drug in this study and in previous studies with oral paclitaxel generally occurred 30 minutes prior to oral administration of paclitaxel. It has been shown in studies with oral docetaxel that the systemic exposure to docetaxel did not differ when the booster (RTV) is administered 1 hour prior or administered simultaneously with docetaxel [19]. RTV used as booster for protease inhibitor therapy in HIV treatment is generally also administered simultaneously [26]. However, to our knowledge no other studies on the best moment of administration have been performed, neither have been published. The optimal time-interval for the paclitaxel and RTV administration remains therefore to be determined. Based on our studies with paclitaxel or docetaxel, administration of RTV should be shortly before or together with the taxane as this seems to be the optimal time point and the most practical for patients.

The increase of the RTV dose from 100 mg to 200 mg did not result in a further significant increase in the systemic exposure to paclitaxel. For protease inhibitors, it has been shown that dosages higher than 100 mg RTV generally did not result in a further increase in the exposure of the co-administered protease inhibitor [26]. The result obtained here is therefore in line with these studies.

It should be noted that RTV is administered at a relatively low dose of 100 mg, since the maximum tolerated dose is bi-daily 600 mg [27]. The low RTV dose is known to be very safe with at most grade 1 gastro-intestinal toxicity [28]. The other boosters investigated have all been administered close to or at their maximum tolerable daily dose. These high dosages of the booster could potentially result in toxicity caused by the booster. This provides an additional rationale for the use of RTV as a booster especially as frequent daily dosages will be applied in metronomic dosing.

Conclusion:

Systemic exposure to paclitaxel upon oral administration can be increased by co-administration of 100 mg RTV. The boosting of RTV was however not as efficient as with 15 mg/kg CsA. The concept of boosting of oral paclitaxel via CYP3A4 inhibition was further confirmed by co-administration of two other CYP3A4 inhibitors, clarithromycin and ketoconazole. The use of RTV as a booster is preferred above the others as it is administered at a relatively low and safe dose. Metronomic dosing of oral paclitaxel with the booster RTV (100 mg) is currently being explored in an ongoing phase I trial (registered as NTR3632 in the Netherlands trial register (NTR)).

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Compliance with ethical standards:

The study was performed in compliance with the Declaration of Helsinki and its amendments. All patients provided informed consent prior to participation. The study was registered in the ISRCTN database under identifier: ISRCTN 32770468.

Conflicts of interest:

This work was supported by a ZonMw grant awarded to Jos Beijnen and Jan Schellens (project 95100101, project title: Crossing the barrier: from intravenous to effective oral formulations of chemotherapy).

J. Schellens, J. Beijnen and B. Nuijen are patent holder on oral taxane formulations. J. Schellens and J. Beijnen are shareholders and employees (part time) of Modra Pharmaceuticals BV, a spin off company developing oral taxanes.

M. Keessen is an employee of Modra Pharmaceuticals BV.

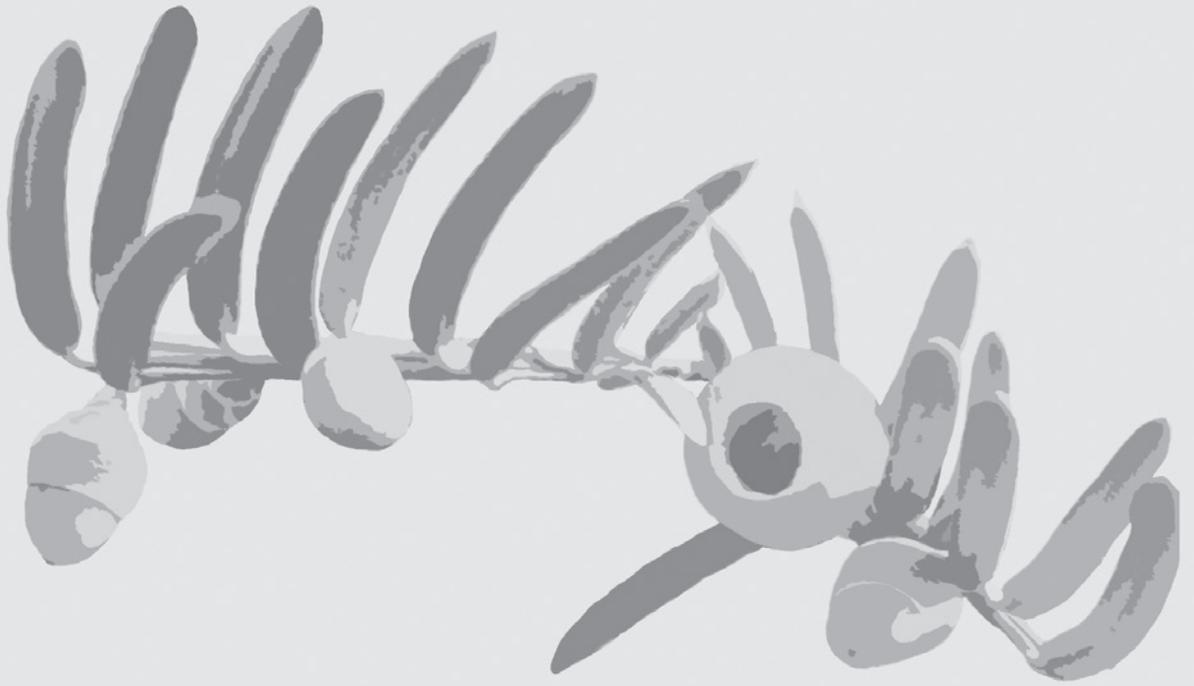
The other authors declare to have no conflict of interest

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Chapter 2.6

A phase I dose-escalation study of low dose-metronomic chemotherapy with oral paclitaxel in combination with ritonavir (ModraPac001/r and ModraPac005/r).

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Abstract:

Background: ModraPac001 capsule and ModraPac005 tablet are novel pharmaceutical formulations containing paclitaxel as a solid dispersion. Oral administration of these formulations of paclitaxel is feasible in combination with a low dose of the CYP3A4 inhibitor ritonavir enabling low-dose metronomic (LDM) treatment. The aims of this study were to determine the safety, feasibility, maximum tolerated dose (MTD) and pharmacokinetics (PK) of LDM paclitaxel as ModraPac001 or ModraPac005 in combination with a 100 mg ritonavir dose (ModraPac001/r or ModraPac005/r).

Methods/patients: Adult patients with advanced solid tumors were enrolled according to a classical 3 + 3 design. Paclitaxel was administered bi-daily (BID) as ModraPac001/r up to the BID 20 mg dose-level. Thereafter, the ModraPac005 tablet replaced the ModraPac001 capsule. Safety was assessed according to CTCAE 4.02. PK sampling was performed on days 1, 2, 8 and 22.

Results: In total 37 patients were enrolled in 9 dose-levels (BID 2.5 mg up to BID 30 mg paclitaxel in combination with 100 mg ritonavir). Common treatment related toxicities were fatigue, diarrhea and nausea. Adverse events considered dose-limiting were: nausea (3x), febrile neutropenia, neutropenia, dehydration and vomiting. The MTD was determined as BID 20 mg ModraPac005/r. The maximum plasma concentration of paclitaxel and the area under the plasma concentration-time curve of 0 to 24 hours (AUC_{0-24h}) at the MTD were 34.6 ng/ml (Coefficient of Variation (CV) 79%) and 255 ng/ml*h (CV 62%), respectively. Stable disease was the best response to treatment observed in 15 out of 30 evaluable patients.

Conclusion: LDM therapy with paclitaxel is feasible and safe. The MTD was determined as BID 20 mg ModraPac005 in combination with BID 100 mg ritonavir.

Introduction:

The taxane paclitaxel is administered intravenously in the treatment of solid tumors [1] as a three weekly or weekly regimen aimed at obtaining a direct cytotoxic effect on tumor cells. In recent years continuous low dose metronomic therapy (LDM) has shown potential as anti-cancer treatment [2–4]. In this form of therapy classical cytotoxic drugs such as paclitaxel are administered at daily low dosages. LDM treatment has multiple potential mechanisms of actions, these include anti-angiogenesis, stimulation of the immune-system and possibly direct tumor cell targeting [5]. The potential of LDM paclitaxel was observed in both preclinical models and in a clinical study [6–8]. In the clinical study paclitaxel was given weekly as a continuous 96-hour infusion at a total daily dose of 10 mg/m² in combination with bi-daily 400 mg celecoxib. Four out of the 20 patients with metastatic melanoma included experienced clinical benefit (i.e., stable disease or partial response). However, administration was complicated by catheter related infections likely due to the 4 day continuous infusion. Ultimately the study was prematurely discontinued because of these infections [6]. LDM without an oral formulation of paclitaxel is, therefore considered to be impractical and not feasible due to the risk of catheter related infections. Specifically for oral metronomic therapy two paclitaxel solid dispersions ModraPac001 capsule [9] and ModraPac005 tablet [10] were developed by the pharmacy of the Netherlands Cancer Institute. Oral administration of paclitaxel is hampered by its poor bioavailability after oral administration, because of poor water solubility and high first pass effect. The water solubility was efficiently improved by the development of a solid dispersion formulation containing, besides paclitaxel, a hydrophilic carrier and surfactant. The first pass effect could be decreased by the co-administration of a P-glycoprotein (P-gp) and/or a cytochrome P450 (CYP) 3A4 inhibitor [11,12]. P-gp and CYP3A4 are the most important drug transporter and the major enzyme, respectively, involved in the first pass effect of oral paclitaxel [13,14]. Paclitaxel is also metabolized by CYP2C8, but this enzyme is not considered to be involved in its first pass effect, as it is barely expressed in the gastro-intestinal tract [15]. Previously cyclosporin A (CsA), a strong P-gp and CYP3A4 inhibitor was used to boost paclitaxel uptake, in both phase I and II clinical studies [12,16–19]. CsA is, however, not ideal for use in LDM treatment, as frequent administrations may cause bone marrow suppression and nephrotoxicity. Therefore, boosting with other CYP3A4 and/or P-gp inhibitors was investigated and the strong CYP3A4 and moderate P-gp inhibitor ritonavir was selected for the development in LDM treatment. Ritonavir might be the ideal booster drug, as it has proven to improve efficiently the uptake of a range of different protease inhibitors in HIV treatment with negligible toxicity at the dosages given for this purpose (i.e., 100 to 200 mg daily) [20].

Several pharmacodynamic markers have been suggested in LDM treatment. In this study two pharmacodynamics markers were measured: thrombospondin-1 (TSP-1) and circulating endothelial cells (CEC). TSP-1 is a potent endogenous inhibitor of angiogenesis that has been shown to be upregulated in animal studies with LDM treatment with paclitaxel, whereas normal levels of TSP-1 are found after high dose paclitaxel treatment [4,21,22]. CEC have a role in the neo-vascularization and have been shown to be upregulated in cancer patients. In clinical studies with LDM treatment numbers of CEC declined during treatment [23,24].

The aim of the current study was to determine the maximum tolerated dose (MTD) and the recommended phase II dose (RP2D) of oral paclitaxel as ModraPac001 capsule and/or ModraPac005 tablet in combination with the booster ritonavir in a metronomic continuous bi-daily (BID) schedule. Secondary aims included assessment of pharmacokinetics, pharmacodynamics and preliminary anticancer activity.

Patients and Methods:

Study design:

This study was performed as an open-label dose-escalation trial investigating the metronomic BID continuous administration of oral paclitaxel as ModraPac001 2.5, 5 and 10 mg capsules and as ModraPac005 10 mg tablets, both developed by the Pharmacy of The Netherlands Cancer Institute, Amsterdam, The Netherlands. ModraPac001 and ModraPac005 were always co-administered with a 100 mg ritonavir dose (Norvir®, Abbott, Illinois, USA) (denoted ModraPac001/r or ModraPac005/r). Starting from the BID 20 mg ModraPac001/r dose-level the ModraPac005 tablet was implemented in the protocol. The tablet was implemented after inclusion of the first two patients at this dose-level who were treated with the ModraPac001 capsule. ModraPac005 tablet/r has thereafter fully replaced ModraPac001 capsule/r.

The BID administrations occurred with a 7 to 12 hour window. ModraPac001 capsule/r and ModraPac005 tablet/r were taken after a fasting period of 1.5 hours prior and 1 hour after administration. The study was approved by medical ethical board of the Netherlands Cancer Institute and registered in the Dutch trial registry (NTR3632).

Patients:

Patients with metastatic malignant disease of 18 years or older with a World Health Organization (WHO) performance score (PS) ≤ 2 were included. Other inclusion criteria were acceptable bone marrow, renal and hepatic function and no radio- or chemotherapy within the last 4 weeks prior to study start. Patients who were pregnant, breast feeding, unwilling to use reliable contraception, or

patients on CYP3A modulating drugs were excluded from the study. Other exclusion criteria were symptomatic brain or leptomeningeal metastases. Patient who had preexisting treatment related toxicity of grade >1, patients who had an active infectious disease or HIV were also excluded from the trial.

Pre-study procedures consisted of a complete medical history including concomitant medication, physical examination, baseline symptoms, a pregnancy test in female patients of child bearing potential, laboratory assessments including hematology serum chemistry and urinalysis and a radiologic tumor assessment with CT and / or MRI. Patients were seen weekly at the outpatient clinic for a safety evaluation consisting of registration of adverse events (AE) according to the National Cancer Institute's Common Terminology Criteria for AE's version 4.02 (NCI-CTCAE v4.02) [25] and their relationship to study treatment, registration of concomitant medication and for a laboratory assessment, consisting of hematology and serum chemistry. Tumor response evaluation was performed every 6 weeks according to Response Evaluation Criteria in Solid Tumor (RECIST) version 1.1 [26]. A treatment cycle consisted of 3 weeks. Study treatment was continued until disease progression, intolerable toxicity despite dose-reductions or failure to comply with the study protocol. Dose-escalation was performed according to a classical 3+3 design: at all dose-levels 3 patients were enrolled, if no dose-limiting toxicity (DLT) occurred, the dose was escalated. If 1 DLT was observed in 1 out of 3 patients the dose was expanded up to maximally 6 patients. If 2 or more out of a maximum of 6 patients experienced a DLT at a certain dose, this dose was considered non-tolerable. The previous lower dose level was then expanded to 6 patients. The MTD was defined as the dose at which DLT occurred in less than 2 out of 6 patients. Paclitaxel dose in the different escalation cohorts was based on safety and PK profiles observed during the study. The RP2D was defined as the highest dose at which no DLT were observed in the 6 patients included.

All patients who had received at least 1 dose of study drug were evaluable for safety. Patients were considered evaluable for DLT after completion of the first cycle (3 weeks). Patients who did not complete the first cycle due to reasons not related to ModraPac001/r or ModraPac005/r were replaced. At the MTD and RP2D at least 6 patients evaluable for safety were included.

DLT was defined as any of the following events occurring during the first cycle of treatment considered to be possibly, probably or definitely related to ModraPac001/r or ModraPac005/r: grade 3 or 4 non-hematologic toxicity (other than untreated nausea, vomiting or diarrhea), grade 3 and 4 nausea, vomiting and diarrhea despite maximum support, grade 4 thrombocytopenia or grade 4 neutropenia for more than 7 consecutive days, grade 3 or 4 febrile neutropenia and inability to begin the next course within 3 weeks of scheduled dosing due to drug-related toxicity other than stated above.

Pharmacokinetics:

Pharmacokinetic (PK) sampling was performed during cycle 1 day 1 pre-dose, 1, 1.5, 2, 2.5, 3, 4, 6, 7, 8, 8.5, 9, 9.5, 10, 11, 13 and 24 hours after the first drug administration. Steady-state trough levels were collected on cycle 1 day 8 and cycle 2 day 1 (i.e. day 22). Samples were collected in lithium heparin tubes of 4 ml, tubes were then centrifuged within 1 hour at 1500 g for 10 minutes at 4 °C. Plasma was stored in a pre-labeled 2 ml eppendorf tube and stored at -20 °C until paclitaxel quantification. Paclitaxel concentrations were determined in plasma by a validated LC-MS/MS method as described by Hendriks et al [27]. Carbon-13-labeled analogues were used as internal standards. The lower limit of quantification was 0.5 ng/ml paclitaxel. The assay fulfills current FDA guidelines for bioanalytical validation.

The individual non-compartmental PK parameters were determined using validated scripts in the software package R (version 3.0.1). The mean, standard deviation (sd) and coefficient of variation (CV) of the following PK parameters were reported: maximum observed plasma concentration (C_{max}), time to reach C_{max} (T_{max}), both C_{max} and T_{max} were reported after the first and the second dose of ModraPac001/r and ModraPac005/r, the area under the plasma-concentration time curve from t=0 up to the last data point at 24 hours ($AUC_{0-24hrs}$), and the terminal elimination half-life ($t_{1/2}$).

Pharmacodynamics:

Samples for pharmacodynamics (PD) were drawn on cycle 1 day 1, 2 and 8, cycle 2 day 1 (i.e. day 22), cycle 3 day 1 (i.e. day 43), every 6 weeks thereafter and at progression of disease. PD samples were drawn prior to administration of ModraPac001/r or ModraPac005/r.

From the first 19 patients a citrate, theophylline, adenosine and dipyridamole (CTAD; Becton Dickinson) containing tubes was drawn for investigation of plasma thrombospondin-1 (TSP-1) levels. Thereafter 3 CTAD tubes were drawn for enumeration of circulating endothelial cells (CEC) and to measure TSP-1. CTAD tubes were used to prevent platelet activation during sample handling.

Total levels of plasma thrombospondin-1 (TSP-1) were measured in duplicate with an enzyme-linked immunoassay (ELISA) according to the manufacturer's instructions (thrombospondin-1 Immunoassay Kit, Quantikine, cat nr DTSP10, R&D systems) in platelet rich plasma. CTAD tubes were centrifuged at 120 g for 15 minutes at 4 °C. The middle fraction of plasma (650 µl) was collected and then transferred to a 2 ml eppendorf tube. Thrombocyte levels were then quantified on a Coulter Counter. Samples were thereafter stored at -20 °C until further processing. Samples were thawed at room temperature on the day of analysis. Subsequently, samples were refrozen in liquid nitrogen for 1 minute release TSP-1 from platelets, as TSP-1 is scavenged by platelets from the circulation [28]. Before analysis, samples were diluted 100 times in Calibrator Diluent RD5-33.

Blood of the two CTAD tubes collected for CEC enumeration was prepared according to the method developed by Burylo et al [Chapter 3].

Results:

Patients:

Overall 37 patients were enrolled over 9 dose-levels, as shown in figure 1. In total 21 patients received the ModraPac001 capsule/r and 16 patients the ModraPac005 tablet/r. The majority of patients were male (57%) and had a WHO PS of 0 or 1 (95%). Most common tumor types included were NSCLC (9 patients), ovarian, mesothelioma and urothelial cell carcinoma (3 patients each). A more detailed overview of patient characteristics is shown in table 1.

Table 1: Patient characteristics

Age		
mean (range)	61	(37-81)
Gender		
Male	21	(57%)
Female	16	(43%)
Performance status		
0	13	(35%)
1	22	(60%)
2	2	(5%)
Primary tumor type		
NSCLC	9	(24%)
Ovarian cancer	3	(8%)
Mesothelioma	3	(8%)
Urothelial cell carcinoma	3	(8%)
Renal cell carcinoma	2	(5%)
Adenoid cystic carcinoma	2	(5%)
Anal carcinoma	2	(5%)
Neuro endocrine carcinoma	2	(5%)
Head and neck carcinoma	2	(5%)
Colorectal cancer	1	(3%)
Esophageal cancer	1	(3%)
Unknown primary tumor	1	(3%)
Mixed baso-squamous skin carcinoma	1	(3%)
Carcinoma of the major duodenal papilla	1	(3%)
Cholangiocarcinoma	1	(3%)
Leiomyosarcoma	1	(3%)
Osteosarcoma	1	(3%)
Thymic carcinoma	1	(3%)
Prior treatment		
Chemotherapy	34	(92%)
Radiotherapy	17	(46%)
Surgery	17	(46%)

Abbreviation: NSCLC = non-small cell lung cancer

Table 2: Adverse events related to ModraPac001 capsule/r or ModraPac005 tablet/r occurring in > 10% of patients or being of grade ≥ 3 severity.

	BID 2.5 mg MP1/r	BID 5 mg MP1/r	BID 7.5 mg MP1/r	BID 10 mg MP1/r	BID 15 mg MP1/r	
Name of related AE	Gr 1-2	Gr 1-2	Gr 3	Gr 1-2	Gr1-2	Gr1-2
Fatigue / malaise	2	1	1	1	3	1
Nausea	0	0	0	2	1	1
Diarrhea	1	2	0	1	1	0
Vomiting	0	0	0	0	0	1
Weight loss	0	1	0	2	0	0
Sensory neuropathy	0	3	0	0	1	0
Alopecia	0	0	0	0	0	0
Anorexia	0	0	0	0	0	1
Stomatitis / mucositis	0	0	0	0	0	0
Anemia	0	0	0	1	0	0
Dehydration	0	0	0	0	0	0
Dry skin	0	1	0	0	1	0
Leucocytopenia	0	0	0	0	0	0
Neutropenia	0	0	0	0	0	0
Febrile neutropenia	0	0	0	0	0	0

Abbreviations: AE = adverse events, Gr = grade, MP1 = ModraPac001 capsule, MP5 = ModraPac005 tablet, r = ritonavir

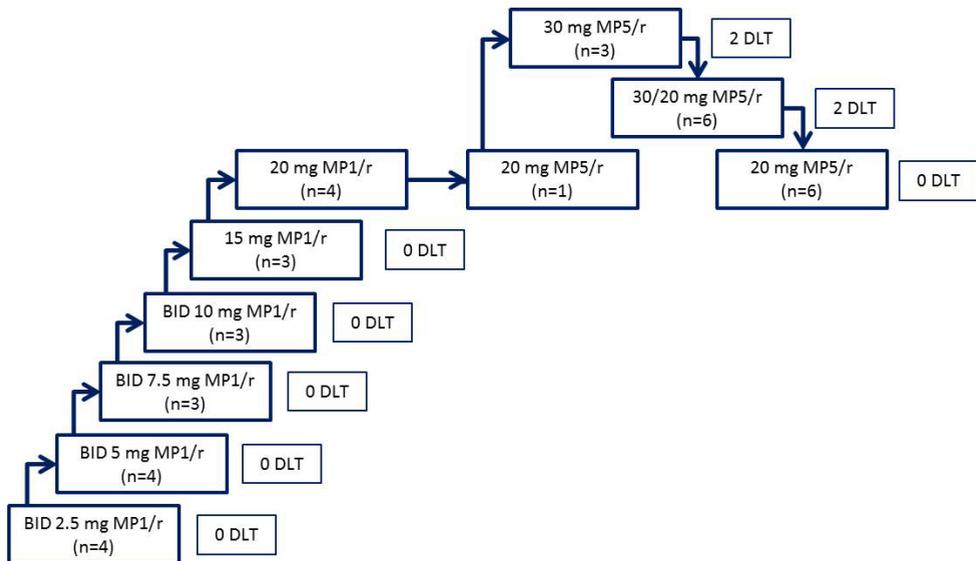


Figure 1: Dose-escalation schedule of ModraPac001 capsule/r and ModraPac005 tablet/r

Abbreviations: BID = bi-daily, MP1 = ModraPac001 capsule, MP5 = ModraPac005 tablet, r = ritonavir, n = number of patients, DLT(s) = dose-limiting toxicity

BID 20 mg MP1/r	BID 20 mg MP5/r	30/20 mg MP5/r		BID 30 mg MP5/r		Total events	% of patients
Gr 1-2	Gr 1-2	Gr 1-2	Gr 3-4	Gr 1-2	Gr 3-4		
0	7	3	2	4	0	25	68%
1	3	2	1	4	2	17	46%
1	1	1	1	1	2	12	32%
1	2	1	0	3	1	9	24%
0	0	0	0	4	0	7	19%
0	1	1	0	0	0	6	16%
0	0	1	0	4	0	5	14%
0	1	0	0	2	0	4	11%
0	1	1	0	1	1	4	11%
0	1	1	0	0	0	3	8%
0	0	0	0	1	2	3	8%
0	0	0	0	1	0	3	8%
0	0	0	1	0	1	2	5%
0	0	0	1	0	1	2	5%
0	0	0	1	0	0	1	3%

Safety:

The most common AEs observed considered related to study drug were fatigue (68%), nausea (46%), diarrhea (32%) and vomiting (24%). A summary of all related AEs that occurred in >10% of patients or that were grade ≥ 3 is presented in table 2.

A total of 7 AEs, which occurred in 4 patients were considered DLTs. DLT at the BID 30 mg ModraPac005 tablet/r dose were grade 3 nausea (1x), grade 4 neutropenia (1x) and grade 3 febrile neutropenia (1x). The neutropenia grade 4 lasted for 7 consecutive days until recovery to grade 3, the fever resolved after 1 day of antibiotic treatment. The patient was retreated after a dose-reduction at the BID 20 mg ModraPac005 tablet/r dose-level, however, after 2 weeks of treatment a second dose-reduction to BID 10 mg ModraPac005 tablet/r was needed, because of development of grade 2 neutropenia.

The DLTs observed at the 30/20 mg ModraPac005 tablet/r dose-level were grade 3 dehydration (1x), vomiting (1x) and nausea (2x). A summary of DLTs per patient is provided in table 3. The MTD was determined as BID 20 mg ModraPac005 in combination with BID 100 mg ritonavir.

Table 3: Adverse events that qualified as dose-limiting toxicity, presented per dose-level, each line represents one patient.

Dose level	Adverse event
BID 30 mg ModraPac005/r	Grade 3 Nausea
BID 30 mg ModraPac005/r	Grade 4 Neutropenia, grade 3 febrile neutropenia
30/20 mg ModraPac005/r	Grade 3 Dehydration, nausea, vomiting
30/20 mg ModraPac005/r	Grade 3 Nausea

Abbreviations: r = ritonavir

Serious Adverse Events (SAE), dose-modifications and reason for discontinuation:

A total of 25 AEs were reported, of these 9 (36%) were considered to be at least possibly treatment related. Treatment-related SAEs were dehydration (4x), nausea (3x), febrile neutropenia (1x), neutropenia (1x), and increased creatinine (1x). None of the treatment related SAEs were fatal.

Dose-modifications were needed in 4 (11%) patients. At the BID 30 mg ModraPac005 tablet/r dose-level 2 (5%) patients required a dose reduction to BID 20 mg ModraPac005/r, due to treatment related AEs of whom one patient needed a second dose reduction to BID 10 mg ModraPac005/r. At the 30/20 mg ModraPac005/r dose level, also 2 (5%) patients required a dose reduction to BID 20 mg ModraPac005 tablet/r, due to treatment related AEs. The main reason for study treatment discontinuation was disease progression (24 patients; 65%), followed by adverse events (8 patients; 22%) and patients request (5 patients; 13%).

Pharmacokinetics:

The plasma concentration-time curves of paclitaxel (as ModraPac001 capsule/r) are shown in figure 2A. The plasma concentration-time curves for paclitaxel at the two highest dose-levels of ModraPac001 capsule/r and all ModraPac005 tablet/r dose-levels are shown in figure 2B. Pharmacokinetic parameters of all dose-levels are presented in table 4. $AUC_{0-24hrs}$ and C_{max} increased with dose up to the BID 15 mg ModraPac001 capsule/r cohort. At the BID 20 mg ModraPac001 capsule/r cohort the $AUC_{0-24hrs}$ and C_{max} did not further increase. At the BID 20 mg dose the ModraPac005 tablet was introduced. At the next dose level (i.e., BID 30 mg ModraPac005 tablet)/r a disproportionally high AUC_{0-24} was observed following the switch from the ModraPac001 capsule to the ModraPac005 tablet formulation. The AUC_{0-24} and C_{max} at the MTD of BID 20 mg ModraPac005 tablet/r were 293 (sd \pm 182) ng/ml*h and 34.6 (sd \pm 27.2) ng/ml, respectively.

Table 4: Pharmacokinetic parameters of paclitaxel after administration of ModraPac001 capsule/r or ModraPac005 tablet/r.

	BID 2.5 mg MP1/r (n=4)	BID 5 mg MP1/r (n=4)	BID 7.5 mg MP1/r (n=3)	BID 10 mg MP1/r (n=3)	BID 15 mg MP1/r (n=3)	BID 20 mg MP1/r (n=4)	BID 20 mg MP5/r (n=7)	30/20 mg MP5/r (n=6)	BID 30 mg MP5/r (n=3)
C_{max} ng/ml	2.52 ± 1.25 (50%)	4.45 ± 0.73 (16%)	8.09 ± 2.65 (33%)	9.10 ± 2.43 (27%)	18.4 ± 4.52 (25%)	13.7 ± 10.1 (73%)	26.4 ± 13.3 (13%)	57.9 ± 12.5 (22%)	85.2 ± 7.19 (8%)
C_{max} ng/ml	1.61 ± 1.12 (70%)	3.95 ± 2.42 (61%)	8.17 ± 3.32 (41%)	7.79 ± 2.24 (28%)	19.8 ± 2.10 (11%)	13.6 ± 9.10 (67%)	34.6 ± 27.2 (79%)	39.7 ± 11.7 (30%)	124 ± 9.02 (7%)
AUC_{0-24hrs} ng/ml²h	23.1 ± 20.6 (89%)	39.3 ± 24.3 (62%)	76.2 ± 27.3 (36%)	81.3 ± 34.4 (42%)	166 ± 17.4 (11%)	121 ± 53 (44%)	255 ± 159 (62%)	373 ± 61.2 (16%)	720 ± 125 (17%)
T_{max} hours	1.38 ± 0.47 (34%)	1.74 ± 0.64 (37%)	1.67 ± 0.74 (45%)	1.67 ± 0.72 (43%)	1.97 ± 0.85 (43%)	2.29 ± 1.15 (50%)	1.87 ± 0.56 (30%)	1.95 ± 0.61 (31%)	1.51 ± 0.05 (3.1%)
T_{max} hours	8.88 ± 0.75 (8%)	9.38 ± 0.24 (2.5%)	8.53 ± 0.45 (5%)	9.18 ± 0.31 (3.4%)	8.98 ± 0.50 (6%)	12.7 ± 7.33 (58%)	8.73 ± 0.67 (8%)	8.55 ± 0.51 (6%)	8.68 ± 0.59 (7%)
t_{1/2} hours	4.74 ± 2.73 (58%)	6.43 ± 3.73 (58%)	6.76 ± 2.14 (32%)	6.56 ± 0.94 (14%)	7.69 ± 0.92 (12%)	15.1 ± 14.8 (97%)	6.92 ± 1.54 (22%)	7.71 ± 1.66 (22%)	6.18 ± 1.57 (25%)

Values are presented as mean ± standard deviation and coefficient of variation (%). Abbreviations: BID = bi-daily, MP1 = ModraPac001, MP5 = ModraPac005, r = ritonavir, C_{max} 1 = maximum concentration measured after first dose, C_{max} 2 = maximum concentration measured after second dose, AUC_{0-24hrs} = area under the plasma concentration-time curve from 0 up to time = 24hrs, T_{max} 1 = time at which the maximum concentration was measured after the first dose, T_{max} 2 = time at which the maximum concentration was measured after the second dose, t_{1/2} = terminal half-life.

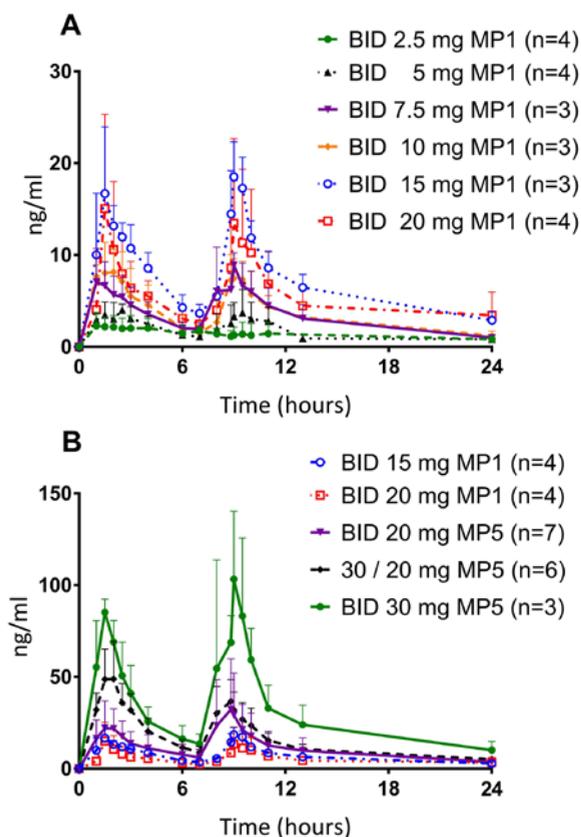


Figure 2: Plasma concentration time curves of paclitaxel per dose-level
 Abbreviations: BID = bi-daily, MP1 = ModraPac001 capsule, MP5 = ModraPac005 tablet, r = ritonavir.

No accumulation of paclitaxel was observed in C_{trough} levels up to the BID 20 mg ModraPac005 tablet/r dose-level. In the 30/20 mg ModraPac005/r cohort accumulation of paclitaxel was observed with a 1.8 fold higher C_{trough} on day 22, as compared to day 8. None of the patients treated at the BID 30 mg ModraPac005 tablet/r dose-level had a sample collected on day 22, as all patients had discontinued treatment at this dose prior to sampling at this time-point due to AEs.

Pharmacodynamics:

No significant correlation between response to treatment and TSP-1 and CEC levels was observed in the overall study population for TSP-1 and CEC levels. The mean fold change from baseline \pm sd in TSP-1 and CEC levels are presented in table 5, by treatment response (i.e. stable disease and progressive disease). Mean CEC levels appeared to rise in patients with progressive disease. This was however driven by one patient in whom CEC levels increased up to 12-fold and 7-fold at the end of cycle 1 and cycle 2, respectively. TSP-1 levels showed a clear increase after initiation of treatment

in one patient, who was treated at BID 30 mg ModraPac005/r. After an initial increase, TSP-1 level decreased after discontinuation of treatment, due to neutropenia. After restart of ModraPac005 tablet/r at the BID 20 mg dose-level a gradual increase was again observed. In the other patients no significant correlation of TSP-1 levels with treatment was observed.

Table 5: CEC and TSP-1 levels during treatment by tumor response.

Day of treatment	CEC levels	CEC levels	TSP levels	TSP levels
	Stable disease	Progressive disease	Stable disease	Progressive disease
	Mean ± sd(n=8)	Mean ± sd (n=6)	Mean ± sd (n=15)	Mean ± sd (n=14)
0	0.00 ± 0.00 (n=8)	0.00 ± 0.00 (n=6)	0.00 ± 0.00 (n=15)	0.00 ± 0.00 (n=14)
1	0.37 ± 0.43 (n=8)	0.10 ± 0.44 (n=6)	-0.029 ± 0.15 (n=15)	0.055 ± 0.12 (n=13)
8	0.44 ± 0.60 (n=8)	0.53 ± 0.99 (n=6)	0.13 ± 0.26 (n=15)	0.094 ± 0.28 (n=13)
22	0.52 ± 0.72 (n=8)	2.48 ± 4.97 (n=6)	0.029 ± 0.19 (n=15)	0.090 ± 0.094 (n=14)
43	0.87 ± 0.68 (n=8)	1.88 ± 4.21 (n=3)	-0.007 ± 0.25 (n=12)	-0.027 ± 0.15 (n= 8)
85	0.91 ± 0.87 (n=2)	-	0.42 ± 0.36 (n=4)	-

CEC and TSP levels are expressed as fold change from baseline, where baseline value was put at 0. Abbreviations: sd = standard deviation; n = number of patients.

Efficacy:

No objective responses were observed in this trial. The best response to treatment was stable disease reported in 15 patients. Average time on study for patients with stable disease was 11 (range 4 – 23) weeks. Progressive disease was seen in 15 patients and 7 patients were not evaluable for tumor response. In 4 patients a prolonged stable disease was observed (> 3 months).

Discussion:

The LDM administration of oral paclitaxel as ModraPac001 capsule and ModraPac005 tablet with the booster ritonavir (denoted as ModraPac001 capsule/r and ModraPac005 tablet/r is feasible and safe. The MTD and RP2D is defined as BID 20 mg ModraPac005 tablet/r. The study is currently ongoing within an expansion phase at the RP2D/MTD in which 16 additional patients will be included. Toxicity was generally mild, being grade 1-2 severity and manageable with supportive care and/or dose-reductions. Treatment related fatigue was the most common adverse event observed, rarely exceeding grade 2. One patient discontinued treatment due to fatigue considered to be related to ModraPac005 tablet/r. In this study two patients developed grade 4 neutropenia, one in each of the two highest dose-levels with the ModraPac005 tablet/r. Thus far, no neutropenia occurred

at the RP2D of BID 20 mg ModraPac005 tablet/r, but only 6 evaluable patients were included. As ModraPac001 capsule and ModraPac005 tablet do not contain Cremophor EL, commonly observed side-effects caused by this excipient (e.g. hypersensitivity reactions) did not occur in this study [29]. ModraPac001 capsule/r and ModraPac005 tablet/r could be administered without any premedication (i.e. without steroids).

After the switch to the ModraPac005 tablet a disproportionately high AUC_{0-24} was observed in the BID 30 mg ModraPac005 tablet/r dose-level. As the exposure to paclitaxel of the BID 20 mg ModraPac005 tablet/r dose-level was also higher than of the BID 20 mg ModraPac001 capsule/r dose-level, this increase is likely to be attributable to the switch in formulation. As no intra-patient comparison of the formulations was performed it remains to be determined whether the ModraPac001 capsule and ModraPac005 tablet are bio-equivalent. Since the bio-equivalence was not determined the BID 20 mg ModraPac005 tablet/r dose-level was expanded to 6 evaluable patients to determine the MTD/RP2D.

No objective anti-tumor responses were observed. This might be attributed to patient selection as the current study was performed in a patient population with a variety of solid tumors and mostly with extensive pretreatment. A more selective population should be sought to further explore the anti-tumor activity of LDM paclitaxel treatment as ModraPac005 tablet/r.

Two potential PD biomarkers of treatment response were evaluated (i.e. TSP-1 and CEC levels). These markers did not predict treatment response. It should be noted that this can be due to the highly heterogeneous patients population, the large variety of tumor types and the lack of partial responses in the study. The usefulness of TSP-1 and CEC as biomarkers in LDM treatment with paclitaxel can, therefore, not fully be evaluated. Further exploration of the usefulness of CEC and TSP-1 is needed and valuable data could be obtained in the expansion phase of the current study. A fasting period of 1.5 hours prior and 1 hour after each study drug intake was implemented, as the effect of co-administration of food on the uptake of paclitaxel from the gastro-intestinal tract is currently unknown. While fasting was feasible in this phase I study, the long fasting periods during the day of treatment are impractical for patients and have relevant impact on patient quality of life. When a food-effect is ruled out more flexible administrations would be possible, which would improve patient convenience. This could potentially improve future compliance to treatment. A food-interaction study is, therefore, implemented in the expansion phase of the study. Herein the effect of food on the exposure to paclitaxel will be investigated in accordance with the guidelines of the food and drug administration (FDA) [30].

Conclusion:

Metronomic treatment with bi-daily ModraPac001 capsule/r or ModraPac005 tablet/r is feasible and reasonably well-tolerated. The identified MTD/RP2D is BID 20 mg ModraPac005 tablet/r. Further clinical development of the concept is warranted.

Conflict of interest and financial support:

J.H. Beijnen and J.H.M. Schellens have received a grant for translational research (ZonMw code 40-41200-98-004).

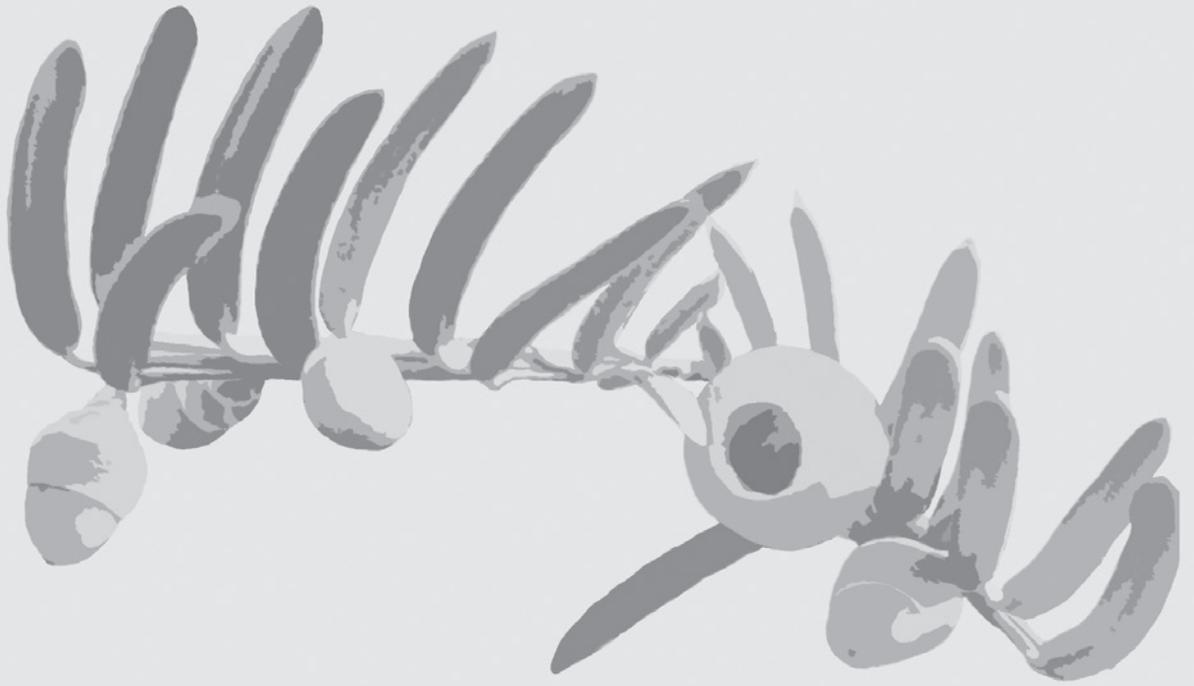
J.J. Moes, B. Nuijen, J.H. Beijnen and J.H.M. Schellens are patent holders on oral taxane formulations. J.H. Beijnen and J.H.M. Schellens are employees and shareholders of Modra Pharmaceuticals BV, the company developing the ModraDoc formulations.

E. Sawicki is an employee of Modra Pharmaceuticals BV the company developing the ModraDoc formulations

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Chapter 2.7

Quantification of the pharmacokinetic- toxicodynamic relationship of oral docetaxel co-administered with ritonavir

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Interim analysis

Abstract

Aims: Oral formulations of docetaxel have been successfully developed as an alternative for intravenous administration. By co-administration of oral docetaxel with the strong enzyme inhibitor ritonavir the plasma exposure of docetaxel is boosted. In dose-escalation trials, the maximum tolerated doses for two different dosing regimens were established and dose-limiting toxicities (DLTs) were recorded. The aim of this study was to establish a pharmacokinetic (PK)-toxicodynamic (TOX) model to quantify the relationship between docetaxel exposure and DLT and to optimise the dose regimen of this combination treatment.

Methods: In total 68 patients were included in the current analysis, among which 12 patients showed a DLT in a four-week observation period. A PK-TOX model was developed. Simulation based on the PK-TOX model was performed.

Results: The final PK-TOX model consisted of an effect compartment representing hypothetical harmful effect caused by docetaxel, which was linked to the probability of a DLT. The recovery rate from this effect compartment was estimated as 0.28 day^{-1} (relative standard error, 47%). In contrast to cumulative area under plasma concentration-time curve (AUC), the amount of docetaxel in the effect compartment was a significant predictor for DLT. Simulations of once-daily 60 mg and twice-daily 30 mg followed by 20 mg of oral docetaxel suggested that 11% and 31% of patients, respectively, would have a probability of > 25% to develop a DLT in a four-week period.

Conclusion: In conclusion, this PK-TOX model can be used to evaluate the dose regimens of the combination of docetaxel and ritonavir and to explore alternative dose regimens.

Introduction:

Oral administration of docetaxel is currently in clinical development as a convenient alternative for intravenous administration [1]. A solid dispersion capsule formulation with improved dissolution characteristics compared to crystalline docetaxel, ModraDoc001 capsule, was initially developed [2] followed by a further improved solid dispersion tablet formulation, ModraDoc006 [3]. A major limitation for oral administration of docetaxel is its low bioavailability due to transport by P-glycoprotein (Pgp) and metabolism by Cytochrome P450 3A4 (CYP3A4) [4–6]. In order to boost docetaxel exposure after oral administration, ritonavir is co-administered, which strongly boosts oral bioavailability of docetaxel by inhibiting CYP3A4 [7].

The ModraDoc capsules and tablets were studied in two dose-escalation trials with co-administration of ritonavir [2, 8–10]. The maximum tolerated doses (MTDs) of ModraDoc/r (r refers to 100 mg ritonavir tablet) were explored. Similar to intravenous docetaxel [6], neutropenia and fatigue were seen as dose-limiting toxicities (DLTs), but the most frequently observed DLTs were gastrointestinal toxicities, such as diarrhoea, vomiting, nausea, and anorexia [10].

Pharmacokinetic (PK)-pharmacodynamic (PD) modelling and simulation has proved to be a useful tool to study the PK-toxicodynamic (TOX) relationships [11]. PK-TOX models quantify the relationship between drug exposure and toxicity and can be used to further evaluate the dose regimen and explore alternative dose regimes by simulation studies [12, 13].

The aims of the current study were: 1) to establish a PK-TOX model for oral docetaxel co-administered with ritonavir based on the accumulated data from the clinical trials; 2) to optimise the dosing schedules of ModraDoc/r formulations and support the clinical drug development.

Methods:

Clinical studies

Docetaxel as ModraDoc capsules or tablets was given weekly once-daily [2, 8, 9] or twice-daily [10] in two phase I studies where a classical 3+3 dose escalating schema was employed. DLTs were evaluated over a four-week treatment period. In total, 68 patients were evaluable for DLT assessment, among which 44 patients received a weekly once-daily dose and 24 patients received a weekly twice-daily dose. In total, 12 patients developed a DLT within the first four weeks (DLT period), of which 9 patients showed a DLT by the second week. Detailed information on the number of patients and DLTs included in the current analysis is shown in Table 1.

Table 1: Overview of the information on the dose levels and dose-limiting toxicity included in the model development

	Once-daily dosing [2, 8, 9]		Twice-daily dosing [10]	
	ModraDoc001 capsule	ModraDoc006 tablet	ModraDoc001 capsule	ModraDoc006 tablet
Tested dose levels (mg/day)	40, 60, 80	60, 80	40, 60, 80	40, 50, 60
Number of patients				
total	37	7	17	7
Dose-limiting toxicity	8		4	
Dose-limiting toxicity at week 1	1		1	
Dose-limiting toxicity at week 2	5		2	
Dose-limiting toxicity at week 3	2		0	
Dose-limiting toxicity at week 4	0		1	

Model development

Sequential modelling of PK and TOX was used to establish the full PK-TOX model [14]. Firstly, an integrated PK model of oral docetaxel and ritonavir was used in this analysis as described in Chapter 4.1. The individual parameter estimates of docetaxel and ritonavir were generated from the PK model and used as input in the subsequent PK-TOX modelling.

The probability of the occurrence of DLTs was estimated by logistic regression. Several measures for drug exposure, such as cumulative area under plasma concentration-time curve (AUC), were considered in the logistic regression model. An effect compartment model relating drug exposure to the probability of DLT was also considered.

Model evaluation

The models were required to reach successful minimisation with plausible and precise parameter estimates. For hierarchical models, the significant level in the drop of objective function values (dOFV) was defined as $p < 0.01$ (degree of freedom = 1, dOFV = 6.6).

Simulations

In the simulation study the following outcome measures were studied: 1) the simulated probability of DLT was compared to the observed incidence; 2) the time course of the probability of DLT was investigated for both once-daily and twice-daily regimens; 3) the differences in the probability of DLT were shown between once-daily and twice-daily regimens.

Simulations of the probability of DLT were performed based on the PK-TOX model in 1,000 patients at each dose regimen for four weeks. For all simulations the ModraDoc tablet was used as the formulation. The simulated doses included: weekly once-daily 60 mg and 80 mg; weekly twice-daily

20 mg (20/20 mg), 30 mg followed by 20 mg (30/20 mg), and 30 mg (30/30 mg). A 100 mg ritonavir tablet was co-administered at each drug intake. The commonly defined MTD in rule-based designs of dose-escalation studies is the dose at which the probability of DLT is < 33%. Therefore, this value was used as a cut-off value in the simulations as comparison with the observed incidences of DLT. In addition, in model-based designs of dose-escalation studies, a pre-defined target toxicity rate was mostly set between 10–33% [15]. Accordingly, a target probability for a DLT was chosen as the probability of DLT incidence of 25% when further evaluating the recommended phase II dose.

Software

Model estimations and simulations were performed using NONMEM (version 7.3.0, ICON Development Solutions, Ellicott City, MD, USA) [16] together with a gfortran compiler with Piraña used as graphical interface [17]. The model estimation used second-order conditional (Laplacian) estimation method. R (version 3.0.3) was used for pre-processing of the data, plotting and calculating the significance of DLT predictor [18].

Results:

Model development

The final PK-TOX model that described the occurrence of DLTs in patients taking ModraDoc/r is shown in Figure 1. The parameter estimates are listed in Table 2.

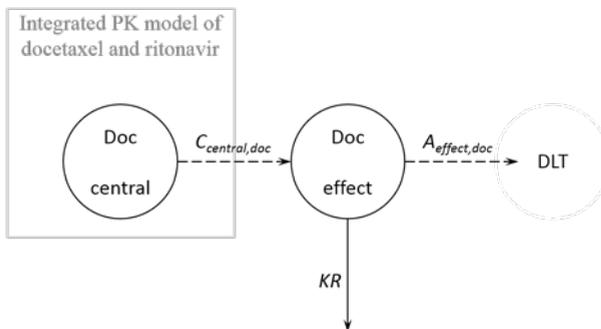


Figure 1. Schematic representation of the pharmacokinetic-toxicodynamic model for oral docetaxel. $A_{effect,doc}$ amount in the effect compartment of docetaxel; $C_{central,doc}$ concentration levels of docetaxel in central compartment; DLT, dose-limiting toxicity; Doc, docetaxel; KR , recovery rate constant.

Table 2: Parameter estimates of pharmacokinetic-toxicodynamic model of oral docetaxel co-administered with ritonavir

Parameters	Units	Estimate	RSE (%)
Recovery rate constant (KR)	day ⁻¹	0.28	47
Intercept (B_0)	-	-13.4	31
Slope (B_1)	-	2.2	28

RSE, relative standard error

The cumulative AUC of docetaxel was not a significant predictor of the probability of DLT. It overpredicted the probability of DLT in weeks 3 and 4 of the DLT period. Therefore, an effect compartment representing hypothetical harmful effect was introduced. The input of the effect compartment was the concentration of docetaxel in the central compartment, the output was modelled as a first order recovery rate. Subsequently, the log-transformed amount in the effect compartment ($A_{effect,doc}$) was related to the probability of DLT. $A_{effect,doc}$ was a significant predictor of the probability of DLT ($p < 0.01$). Compared to the model using the cumulative AUC as a predictor for DLT, the final model showed a strongly improved fit (dOFV of 10.6 points).

The differential equation of the effect compartment and the logistic regression function are shown in Eq. 1–3.

$$\frac{dA_{effect,doc}}{dt} = C_{central,doc} - KR \cdot A_{effect,doc} \quad (\text{Eq.1})$$

$$t = B_0 + B_1 \cdot \log(A_{effect,doc}) \quad (\text{Eq.2})$$

$$\text{Pr} = \frac{\exp(t)}{1 + \exp(t)} \quad (\text{Eq.3})$$

Where $A_{effect,doc}$ represents the amount of the docetaxel in the effect compartment, $C_{central,doc}$ represents the concentration of docetaxel in the central compartment, KR represents the recovery rate of the effect compartment, t is a linear function of log-transformed $A_{effect,doc}$, with B_0 and B_1 as intercept and slope, respectively, Pr is the logistic function representing the probability of DLT.

The KR was estimated as 0.28 day⁻¹ (relative standard error, 47%), translating into a recovery half-life of 2.5 days. Based on the estimated B_0 and B_1 (Table 2), the $A_{effect,doc}$ when Pr is 50%, 33% and 25%, respectively, was calculated as 490 µg-h/L, 356 µg-h/L and 295 µg-h/L. Figure 2 shows the predicted Pr curve with corresponding $A_{effect,doc}$. It can be seen that 100% of patients without DLT, and 83% patients with DLT in four weeks have a Pr < 33%; 98% of patients without DLT, and 67% patients with DLT have a Pr < 25%.

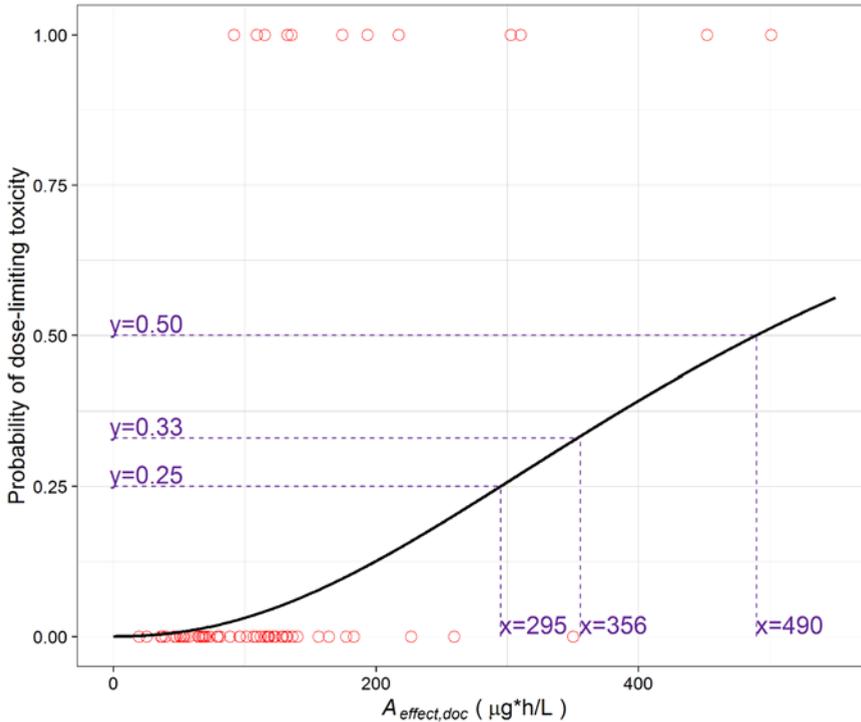


Figure 2: Probability of dose-limiting toxicity predicted by the amount in the effect compartment of docetaxel. $A_{effect,doc}$, amount in the effect compartment of docetaxel. The solid curve represents the probability of dose-limiting toxicity (DLT) predicted by $A_{effect,doc}$; the empty circles present the observed DLT events corresponding to $A_{effect,doc}$; the dashed lines show the $A_{effect,doc}$ when the probability is predicted at 0.25, 0.33, and 0.5, respectively.

The final model was successfully minimised. The parameter estimates of the final PK-TOX model showed acceptable precision (RSE < 50%, Table 2).

Simulations

Figure 3 shows the model simulated Pr with a cut-off of 33% versus the observed DLT incidence. At weekly once-daily 60 mg of docetaxel, 7% of patients in the simulation showed Pr > 33%, and no DLT was observed in 9 patients; at once-daily 80 mg, 14% in the simulation had a Pr > 33% while two DLTs out of four patients (2/4) were observed. For 20/20 mg, 15% of patients had a Pr > 33% while zero out of three (0/3) was observed; for 30/20 mg, the Pr increased to 22%, with one out of seven patients (1/7) developed DLT; for 30/30 mg, Pr further increased to 32%, and two out of six patients (2/6) showed a DLT.

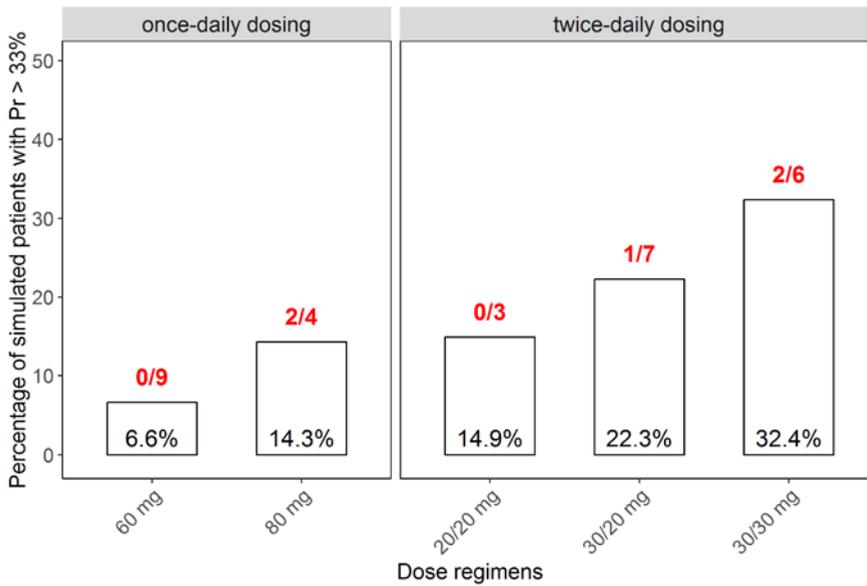


Figure 3: Percentage of simulated patients with > 33% of probability of dose-limiting toxicity at different dose regimens and the observed incidence of dose-limiting toxicity. Pr, probability of dose-limiting toxicity. The bars and the numbers inside the bars show the percentage of simulated patients with Pr > 33% at different dose regimens; the numbers above the bars indicate the observed number of patients with dose-limiting toxicity out of the total evaluable patients treated at that dose.

In order to visualise the change of the $A_{effect,doc}$ over time, Figure 4 shows the median and 10%–90% percentile of simulated $A_{effect,doc}$ for once-daily 60 mg and twice-daily 30/20 mg doses in four weeks. In both dose regimens, the $A_{effect,doc}$ increased within the first two weeks, afterwards steady-state was almost reached (further accumulation in $A_{effect,doc}$ was 3% and 5% for once-daily dose and twice-daily dose, respectively). By week 4, the median of $A_{effect,doc}$ for twice-daily dose was 74% higher than for once-daily dose, which translated into a higher Pr for this regimen.

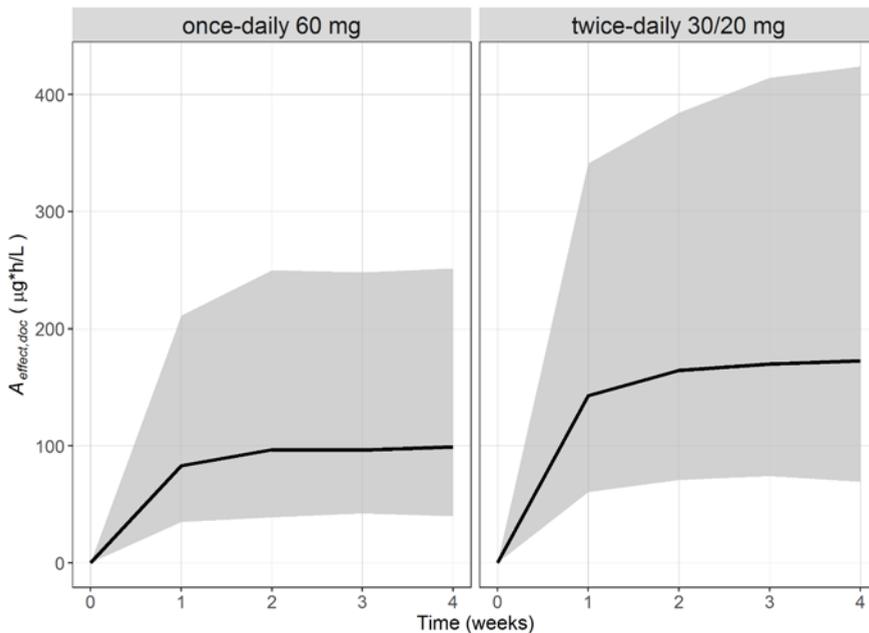


Figure 4: Simulated amount in the effect compartment of docetaxel over four weeks at once-daily and twice-daily dose regimens ($n = 1,000$). $A_{effect,doc}$, amount in the effect compartment of docetaxel. The solid curve represents the simulated median of $A_{effect,doc}$ over time; the grey areas show the simulated $A_{effect,doc}$ between 10% and 90% percentiles.

Quantitatively, Figure 5 shows the cumulative percentage of patients with the target of Pr of 25% at each week for once-daily 60 mg and twice-daily 30/20 mg doses. The newly occurred incidence of DLTs lowered gradually over treatment time. The majority of DLTs, 67% for 60 mg and 73% for 30/20 mg, developed within the first two weeks. This was supported by the dynamic change in $A_{effect,doc}$ over treatment period.

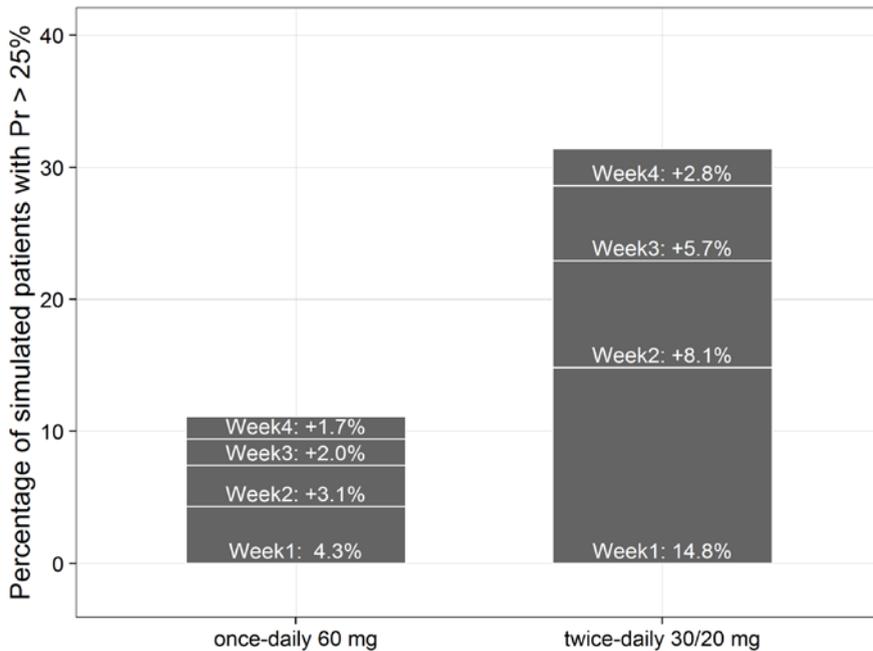


Figure 5: Cumulative percentage of simulated patients with > 25% of probability of dose-limiting toxicity at each treatment week with once-daily and twice-daily dose regimens (n = 1,000). Pr, probability of dose-limiting toxicity. This figure shows the cumulative percentage of patients with Pr > 25%. The increment of patients at each week was indicated. In total, 11.1% of patients receiving once-daily 60 mg, and 31.4% of patients receiving twice-daily 30/20 mg of docetaxel with Pr > 25%.

Discussion:

A PK-TOX model relating the docetaxel exposure to the probability of DLT was developed. This model was developed on data from two dose-escalation trials in which on each dose level only a few individuals were evaluated. Furthermore, docetaxel PK shows wide inter-patient variability. These two issues challenge dose finding of oral docetaxel. The currently developed model enables the *in silico* evaluation of the different dose levels tested. Furthermore, alternative dosing regimens can be evaluated, albeit that extrapolations outside the conditions on which the model was developed should be performed with caution.

The $A_{effect,doc}$ proved to predict the probability of DLT better than cumulative AUC in the plasma. In Figure 4, it can be seen that the amount in the effect compartment, representing hypothetical harmful effect, reached steady-state at approximately week 2 and accordingly the probability on DLT reached steady-state at the same time. This is in agreement with the clinical observation that

75% of DLTs occurred within the first two weeks. The recovery half-life of the effect compartment was estimated at 2.5 days, with relative good precision given the limited dataset.

The PK-TOX simulation supported the MTDs found in dose-escalation trials. Among the evaluated dose regimens, the MTDs of interest of ModraDoc006 for the weekly once-daily dose is 60 mg, and for the twice-daily dose is 30/20 mg (both regimens in combination with 100 mg ritonavir). For once-daily dose regimens, the model simulation agreed with the percentage of DLTs that have occurred at 60 mg (7% vs. 0/9) but suggested much lower incidences of DLTs at 80 mg (14% vs. 2/4), which is lower than the model predicted Pr at the 30/20 mg twice daily regimen. For twice-daily dose regimens, the simulation supported the findings in 20/20 mg (15% vs. 0/3) and 30/30 mg (32% vs. 2/6), while indicated a slightly higher incidences of DLTs at 30/20 mg (22% vs. 1/7). However, this comparison should be interpreted with caution due to the low number of patients on the different dose levels.

Conclusion

A PK-TOX model was built for the prediction of the probability of DLT in oral docetaxel co-administered with ritonavir. The simulation of the PK-TOX model suggested that both once-daily 60 mg and twice-daily 30/20 mg doses of ModraDoc tablet could be safe to use in the clinic.

2.7

Conflict of interests:

Bastiaan Nuijen, Jos Beijnen and Jan Schellens are inventors and hold a patent on oral ModraDoc formulations. Jos Beijnen is CEO and Jos Beijnen and Jan Schellens are shareholders in Modra Pharmaceuticals, a spinout company developing oral taxane formulations.

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Chapter 3

Circulating endothelial cells



Chapter 3

Enrichment of circulating endothelial cells by CD34 microbeads followed by enumeration using flow cytometry.

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Abstract:

Background: Circulating endothelial cells (CECs) are a potential biomarker of angiogenesis. CECs increase in numbers after vessel injury. Higher CECs numbers are reported in cancer patients. Most methods for CECs detection and enumeration rely on flow cytometry (FCM), however, there is no agreement on CECs phenotype and the detection method to be used. This leads to uncertainty about the clinical applicability and variation between studies on CEC numbers reported.

Objective: To develop a selective and accurate method for CECs enumeration in peripheral blood by enrichment followed by FCM in healthy volunteers (HV) and cancer patients.

Methods: Samples were enriched using CD34 microbeads, stained with nuclear dye and anti-CD14, CD15, CD45, CD34 and CD146 antibodies. Putative CECs were examined for Weibel-Palade bodies (WPBs) using anti von Willebrand factor (vWF) antibody and fluorescence microscopy. Linear range of detection (R^2), recovery and precision (CV%) were defined in three experiments by spiking a known number (range 12-12800 CECs/4ml) of surrogate endothelial cells in peripheral blood. Sample storage was determined at -80°C for up to 2 months.

Results: Sorted CECs showed vWF in the WPBs. The relationship between spiked and detected surrogate cells was $R^2 = 1.0$, recovery 94.0-101.4%, and CV% 1.0-18.4%. Recovery \pm standard deviation (within-run day 1-3) were respectively $102.5\% \pm 8.2$, $97.8\% \pm 4.6$, $99.1\% \pm 7.7$, and after 2 months $94.3\% \pm 15.3$. The median CECs/ml in patients was 24.1 versus 14.4 in HVs.

Conclusion: This method for selective, sensitive, and reliable CECs analysis by FCM allows for investigation of CECs as a biomarker in clinical research.

Introduction:

Angiogenesis contributes to the process of tumor development, proliferation and metastasis by formation of new vessels. Inhibition of angiogenesis could therefore result in growth stabilization and ultimately regression of tumors. This has led to the development of anti-angiogenic agents that inhibit the vascular endothelial growth factor (VEGF) for instance bevacizumab or its receptor (VEGFR) such as sorafenib, and sunitinib. The availability of these new anti-angiogenic agents has driven the search for biomarkers that reflect the extent of disease activity and the effect of therapy. However no adequate, easily applicable, predictive and prognostic biomarker has been identified at present. The absence of a biomarker for angiogenesis also impedes selection of patients who might benefit most from these agents.

A candidate biomarker for angiogenesis are circulating endothelial cells (CECs) [1–6]. CECs are mature endothelial cells that have shed from the vessel wall, for instance as a response to injury. Due to poor vessel formation, CECs are more commonly seen in cancer patients [1,7–9]. Their role in angiogenesis is unclear, however, it is hypothesized that they may contribute to tumor vessel formation by stimulating pro-angiogenic factors [10]. The estimated median CEC numbers reported vary from 8–18 CECs/ml in healthy subjects to 11,900 CECs/ml in cancer patients [7,11]. However, reported results are conflicting about the relationship between CECs and their prognostic and predictive value as a biomarker. Important factors contributing to discrepancies between studies evaluating CECs as a biomarker include the variety of applied methods, different CEC phenotype definitions, and analysis of activated or resting CEC subsets [1,12,13]. Numerous methods for the CECs enumeration rely on immunomagnetic bead isolation followed by microscopy like the fully automated CellSearch® method. Others have used flow cytometric methods (FCM) with a sample enrichment step using immunomagnetic beads [7,14–18].

The advantages of the use of FCM for CECs analysis are the opportunity of simultaneous analysis of a wide spectrum of antigens increasing the selectivity, while decreasing the analysis time. The commonly used antigens defining CECs include CD31, CD34, CD105, CD144, CD146, von Willebrand-factor (vWF), Ulex Europaeus Lectin-1. However, an incorrectly designed FCM-based CEC analysis method may lead to overestimation of the CEC count as result of the phenotypic overlap with hematopoietic cells, thrombocytes and apoptotic or dead cells, and their resulting micro-particles [16,19]. The selectivity of a method can be improved by addition of the pan-hematopoietic antigen CD45, and a nuclear and/or viability dye that can exclude thrombocytes, dead cells, and micro-particles [8,20]. Moreover, false-positive counts caused by the non-specific binding of antibodies to the Fc-receptors on hematopoietic cells can be prevented by using a blocking buffer.

The advantages of using immunomagnetic beads for enrichment for analysis of rare cells in the blood circulation are: depletion of redundant blood cells, improved sample purity and sample

volume reduction. However, important disadvantages are: a possible low yield of recovered cells, time consuming, and high cost. The success of using immunomagnetic beads for enrichment depends on the size of the rare cell population to be isolated, the negative or positive selection approach chosen, and the level of expression of the target antigen.

In this report we describe a validated method for the enumeration of CECs from peripheral blood by FCM. The aim of this study was to develop a selective and accurate method for enumeration of CECs in both fresh and cryopreserved (at -80°C) peripheral blood of healthy volunteers and cancer patients. This method uses sample enrichment by CD34 magnetic microbeads, subsequent staining for endothelial antigens (CD34, CD146) and hematopoietic (CD14, CD15, CD45) antigens, followed by a nuclear staining using Hoechst33258. To ensure that exclusively CECs were enumerated, cells that were expected to be CECs based on the predefined phenotype were isolated using a cell sorting system followed by intracellular staining of vWF, which is stored in the Weibel-Palade bodies (WPBs) a cell organelle specific for CECs. Sorted cells were then morphologically examined under a microscope to confirm their CEC phenotype. The recovery and coefficient of variation of the method were assessed in peripheral blood samples spiked with the surrogate Human Umbilical Vein Endothelial Cells (HUVECs). The stability of the endogenous CECs was assessed in peripheral blood samples cryopreserved at -80°C for up to 2 months.

Methods:

Patients and volunteers

Circulating endothelial cell numbers were studied in two clinical protocols. In the first protocol, registered in the Netherlands Trial Register as NTR3632, daily oral administration of paclitaxel co-administered with ritonavir was investigated. In this trial CECs were collected prior to start and at day 2, 8, 22, 43 and every 6 weeks thereafter. The second study was designed to evaluate CEC numbers in both cancer patients and healthy volunteers (HV). From patients included in a clinical phase I trial samples were drawn prior to start and weekly thereafter for up to five times. The treatments administered are presented in Table 1. HVs were asked to provide five samples at weekly intervals. Both studies were approved by the ethics committee of the Netherlands Cancer Institute and both patients and HVs were fully informed and had to provide written informed consent prior to sample collection. Patients with a WHO performance score of 0 to 2 were included in the clinical studies.

Table 1: Characteristics of the patients and healthy volunteers.

Characteristics	Patients	Healthy Volunteers
Total number	39	11
Male	21 (54%)	5 (45%)
Female	18 (46%)	6 (55%)
Mean age (range) years	59 (38-74)	34 (26-49)
Performance score		11
0	19 (49%)	
1	19 (49%)	
2	1 (3%)	
Tumor type		
Colorectal	12 (31%)	
Lung	8 (21%)	
Miscellaneous	19 (49%)	
Prior treatment		
Systemic therapy	31 (79%)	
Number of prior lines (range)	2 (0-6)	
Radiotherapy	15 (38%)	
Surgery	17 (44%)	
Treatment in study		
Docetaxel	11 (28%)	
Cisplatin/pemetrexed/ FGF	2 (5%)	
MEKi + antiHER2/3	9 (23%)	
Metronomic paclitaxel	17 (44%)	

Blood sample collection

Peripheral blood was drawn in 4 ml CTAD (citrate, theophylline, adenosine and dipyridamole) tubes (Becton Dickinson B.V., The Netherlands), in triplicate from HVs and in duplicate or triplicate from cancer patients. The blood was transferred into a 50 ml tube and 46 ml of erythrocyte lysis buffer (ELB) (0.15M ammonium chloride) was added for 15 minutes at room temperature (RT), centrifuged at 500G for 7 minutes at 4°C. Thereafter lyses buffer was removed and the cell pellet was resuspended in 50 ml of phosphate buffered saline (PBS) (Gibco Life Technologies) supplemented with 0.5% BSA and 2 mM EDTA (EDTA; ethylenediaminetetraacetic acid) (Gibco Life Technologies) (hereafter washing buffer), washed by centrifugation at 500G for 7 minutes at 4°C. Pelleted cells were resuspended in 1 ml of 4% formaldehyde and incubated for 15 minutes at RT. The cell suspension was then washed in 49 ml of washing buffer and centrifuged at 500G for 7 minutes at 4°C. Pelleted peripheral blood mononuclear cells (PBMC) was enriched using the CD34 MicroBead kit (Miltenyi Biotec B.V., The Netherlands) and the magnetic-activated cell sorting (MACS) method, or the PBMC pellet was resuspended in 1.5 ml of freezing medium, which consisted of RPMI 1640 (Gibco Life Technologies, Fisher Scientific, The Netherlands), 20% fetal bovin serum (FBS) (Sigma-Aldrich Chemie N.V., The Netherlands) and 10% dimethylsulfoxide (DMSO) (Sigma-Aldrich), and transferred into

a 2 ml Eppendorf tube (Eppendorf Nederland B.V. The Netherlands) and cryopreserved using a freezing container Nalgene Mr.Frosty™ (Thermo Fisher Scientific, The Netherlands) at -80°C until further processing.

HUVEC isolation and purification

HUVECs were isolated by Trypsin digestion. Briefly, the umbilical cord vein was rinsed with PBS, Trypsin-EDTA (0.5%) was injected into the vein and incubated for 5 minutes at 37°C to digest. Digested content was flushed into a 50 ml tube, washed in RPMI 1640 with 10% FBS and centrifuged at 1200 rpm for 10 minutes. Isolated HUVECs were resuspended in 40 µl human FcR blocking reagent (Miltenyi Biotec B.V.) and 50 µl BSA 4% (Sigma-Aldrich Chemie N.V.) in PBS and incubated for 1 hour on ice. Then mouse anti-human anti-bodies were added to a HUVEC sample: CD14-PE-Cy7 (clone TÜK4), CD15-PE-Cy7 (VIMC6), CD34-APC (AC136), CD45-FITC (5B1), CD146-PE (541-10B2). As a control served: 1) unstained cells, 2) cells stained with CD14-PE-Cy7, CD15-PE-Cy7, CD34-APC, CD45-FITC, mouse isotype IgG1-PE (clone IS5-21F5) (Miltenyi Biotec B.V.), incubated for 1 hour on ice. Samples were washed in washing buffer and centrifuged at 1000G for 4 minutes at 4°C. HUVECs were purified from the digested content, by fluorescence-activated cell sorting (FACS) sorting on a FACS Aria IIu equipped with three lasers (excitation wavelength: 405 nm, 488 nm, and 633 nm), a 85 µm nozzle and Diva software (Becton Dickinson B.V.). HUVEC phenotype was: CD14⁻, CD15⁻, CD45⁻, CD34⁺, CD146⁺ and Hoechst33258⁺. HUVECs were sorted into a 2 ml Eppendorf tube containing HUVEC optimized growth medium EGM™-2 BulletKit™ (Lonza Benelux B.V., The Netherlands) containing 2% FBS and VEGF. Collected HUVECs were directly used for further culturing or stored at -80°C as follows: HUVECs were centrifuged at 1000G for 4 minutes at 4°C, the pellet was resuspended in freezing medium consisting of HUVEC optimized growth medium EGM™-2 supplemented with 20% FBS and 10% DMSO.

HUVEC culture and immunostaining for morphology examination

Purified HUVECs were cultured in the HUVEC optimized growth medium EGM™-2 BulletKit™. The cells were cultured according to manufacturer's instructions in a 25cm² flask till confluent. Confluent cells were harvested using trypsin-EDTA and seeded on glass coverslips in a 12 well plate and cultured till confluent. The medium was removed, the cells were washed in PBS, and then fixed in cold (-20°C) methanol (Sigma-Aldrich Chemie N.V.) for 1 minute on ice. After methanol fixation, the cells were washed in PBS and blocked in PBS supplemented with 10% FBS for 45 minutes at RT, followed by incubation for 15 minutes at RT in the dark with polyclonal sheep anti-human vWF-FITC (1:1000) (Abcam, United Kingdom), an IgG isotype-FITC antibody was used to define the background (Abcam, United Kingdom) and Hoechst33258 (100µM) (Sigma-Aldrich Chemie N.V.). The specimen was washed in PBS, dehydrated and inversely layered on a microscopic glass with

mounting medium VectaShield H-1000 in between. The background of the anti-vWF-FITC and its specificity was defined by staining PBMCs, freshly isolated and FACS sorted HUVECs, and cultured HUVECs (supplementary data). The specimen was analyzed on a Zeiss Axio Observer Z1 inverted microscope (Carl Zeiss B.V., The Netherlands) equipped with Hamamatsu ORCA AG Black and White CCD-camera (Hamamatsu Photonics, The Netherlands) and analyzed with ZEN lite software (Carl Zeiss B.V.).

Peripheral blood sample enrichment and immunostaining

The cryostored PBMC samples were quickly defrosted in a water bath at 37°C, centrifuged at 1000G for 4 minutes at 4°C, pelleted sample was washed in washing buffer and centrifuged at 1000G for 4 minutes at 4°C. Pelleted PBMCs were resuspended in 40 µl FcR blocking reagent and 50 µl BSA 4% in PBS and incubated for 1 hour on ice. Washing buffer was added to achieve a final volume of 500 µl, then 20 µl of CD34 MicroBeads was added, samples were incubated with overhead rotation for 1 hour at 4°C. Unbound CD34 MicroBeads were washed away twice by adding washing buffer, centrifuged at 300G for 10 minutes at 4°C, and the washing buffer was discarded. The magnetically labeled cells were isolated using a MACS column and OctoMACS Separator magnetic fields. The magnetically labeled cells were eluted twice from the column with 1.8 ml washing buffer and centrifuged at 1000G for 4 minutes at 4°C. Enriched samples were then immunostained with a mix of mouse anti-human anti-bodies: CD14-Pe-Cy7, CD15-Pe-Cy7, CD34-APC, CD45-FITC, CD146-Pe and Hoechst33258(100µM). As a control served: 1) unstained cells, 2) cells stained with CD14-Pe-Cy7, CD15-Pe-Cy7, CD34-APC, CD45-FITC, and isotype IgG1-PE. The FCM analysis of CECs was done on a FACS Fortessa (Becton Dickinson B.V.) equipped with four lasers (excitation wavelength: 405 nm, 488 nm, 561 nm and 640 nm), a compensation matrix was set up using a single stained sample. The CECs number was defined by acquiring the samples to completion. The CECs phenotype was defined as: CD14-, CD15-, CD45-,CD34+, CD146+, Hoechst33258+. Hematopoietic cells were defined as: CD14+, CD15+, CD45+, CD34-, CD146-, Hoechst33258+. The gating strategy is shown in the Figure 1.

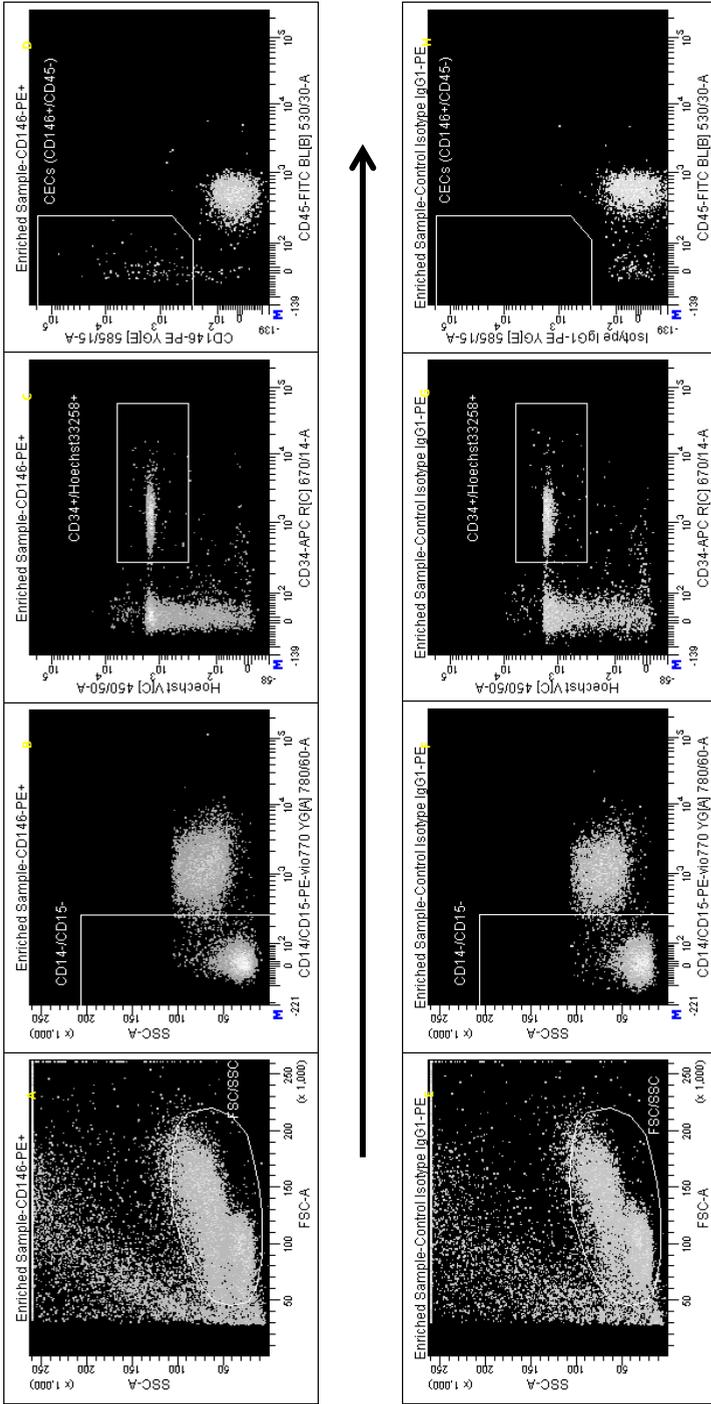


Figure 1: Five-color flow cytometry analysis of CECs. CECs were analyzed using FACS Diva software and sequential gating strategy. The black arrow depicts the gating direction. Doublets were excluded (FSC-A versus FSC-H, plot not shown) prior to gate A. A) Gate A selects for mononuclear cells, and excludes debris and platelets, B) Gate B (dump channel) is derived from gate A and excludes possible contamination by monocytes, macrophages and neutrophils, C) Gate C is derived from gate B and shows the gate with CD34⁺-nucleated Hoechst33258⁺ population, D) Gate D is derived from gate C and selects nucleated, CD45⁺CD34⁺CD146⁺ circulating endothelial cells. Panels E-H represent an enriched control sample stained with an isotype IgG1-PE. Abbreviations: FACS-fluorescence-activated cell sorting, FSC-A – forward scatter area, FSC-H – forward scatter height, SSC-A – side scatter area.

CEC sorting, vWF staining and morphology analysis

PBMCs samples were prepared, enriched and immunostained as described above, however, CD45-FITC was substituted by CD45-PerCP (Becton Dickinson B.V.) to enable the use of polyclonal sheep anti-human vWF-FITC. The putative CECs were sorted into a 0.8 ml tube, washed with PBS supplemented with 4% FBS, centrifuged at 1000G for 4 minutes at 4°C. The cell pellet was resuspended in Perm/Wash (1X) (Becton Dickinson B.V.) in washing buffer supplemented with 4% FBS, incubated 1 hour at RT to block for non-specific anti-body binding, incubated 15 minutes at RT in the dark with vWF-FITC (1:1000). The putative CECs were then washed and centrifuged twice at 1000G for 4 minutes at 4°C in Perm/Wash (1X) in washing buffer with 4% FBS, transferred onto a glass slide, dehydrated and protected by mounting medium VectaShield H-1000 (Vector Laboratories, Inc., USA) and a coverslip. The fluorescent labeled cells were viewed as described.

Linearity, recovery and precision using HUVECs

Purified HUVECs were resuspended in 40 µl FcR blocking reagent and 50 µl BSA 4% in PBS and incubated for 1 hour on ice, then immunostained with CD146-PerCP (Becton Dickinson B.V.) for 1 hour on ice, washed in washing buffer and centrifuged at 1000G for 4 minutes at 4°C. According to parameters used previously for CECs sorting and morphology examination, a homogenous population of CD146-PerCP pre-labeled HUVECs were FACS sorted in order to select those HUVECs that resemble the endogenous CECs in terms of size and marker expression. Then pre-labeled HUVECs were spiked by the FACS sorter at the theoretical range of 12-12800 HUVECs into three 4 ml CTAD tubes with peripheral blood and processed as described above, and into three 0.8 ml tubes containing washing buffer to prepare reference samples. The theoretical input is the number of cells planned to be spiked into a sample with the FACS machine. However, as some less viable HUVECs might crush under the pressure of the FACS sorter or more than one cell at a time might have been sorted, the actual number of spiked HUVECs might differ from the theoretical input. The linear relationship (R^2) was defined at 12, 50, 200, 800, 1600 and 12800 HUVECs spiked per 4 ml, the recovery and coefficient of variation (precision) were evaluated at 12, 50 and 200 HUVECs spiked per 4 ml.

Sample stability in storage

The stability of the processed peripheral blood in freezing medium in storage at -80°C was defined in samples of HVs and cancer patients. Briefly, peripheral blood was collected from four HVs in nine 4 ml CTAD tubes and from four cancer patients in six 4 ml CTAD tubes. Three samples of each donor were directly processed as described above, after fixation the samples were stored in freezing medium at -80°C for 1 and 2 months, and were enriched, immunostained and analyzed by FCM after the planned storage time.

Effect of ELB on CECs recovery and comparative analysis with CD34 and CD146 beads, and without sample enrichment

The effect of ELB on CEC recovery, the recovery using CD146 beads and CEC analysis without enrichment was tested according to the methods described in the supplementary data.

Statistical analysis

The FCM data were analyzed using FACS Diva software by Becton Dickinson B.V.. The regression analysis and the Mann Whitney U-test were performed using GraphPad Prism 6 (GraphPad Software, Inc., USA).

Results:

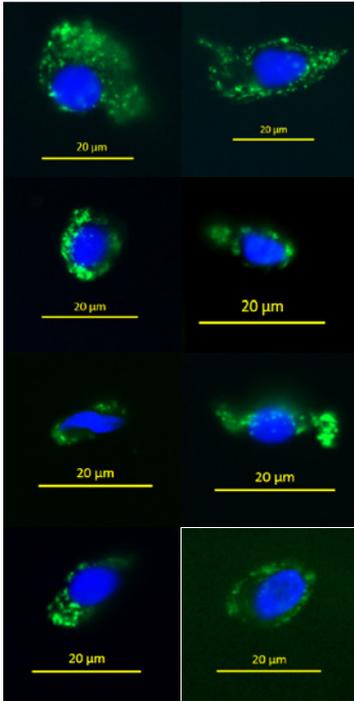
Morphology analysis of HUVECs and putative CECs

The putative CECs were evaluated under a fluorescence microscope for the presence of the WPBs and vWF. As positive control served HUVECs cultured on glass coverslips and as an additional negative control, the CD45 positive hematopoietic and lymphoid cells were sorted and stained with vWF antibody. The microscopic evaluation of putative CECs showed presence of WPBs, which confirmed the endothelial origin of sorted CECs as the WPBs are elongated organelles distributed throughout the cytoplasm of CECs as shown in Figure 2. The varying degree of the vWF staining and nuclear disintegration implicates that these CECs were in different viability stages including apoptotic and necrotic cells. In addition, the sorted control cells stained weakly/non-specifically positive for vWF. These cells had a segmented or rounded nucleus and WPBs could not be identified implicating that these cells were not of endothelial origin, but more likely to be hematopoietic cells.

Assay linearity

The regression analysis of the relationship between the actual number of spiked HUVECs (x) and detected HUVECs (y) was $R^2=1.0$ (Figure 3). The overall within-run (including all ranges) recovery \pm standard deviation was $96.9\% \pm 5.6$ (range 94.0-101.4) and CV% of 5.8% (1.0-18.4) (Table 2, Figure 4).

2A. Endogenous CECs



2B. HUVEC

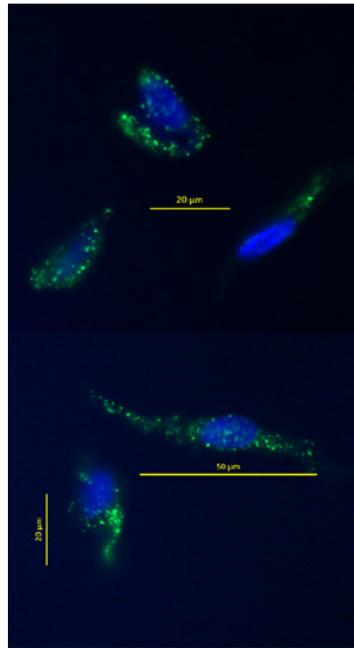


Figure 2: Morphology analysis of putative CECs and HUVEC. 2A) FACS sorted endogenous CECs from a healthy volunteers peripheral blood, 2B) HUVECs (control sample) isolated from an umbilical cord and FACS sorted. CECs and HUVEC were stained with an FITC-labeled anti-vWF antibody (green) and nuclear DNA stain Hoechst33258 (blue). Abbreviations: HUVEC-human umbilical vein endothelial cells, vWF-von Willebrand factor, FACS-fluorescence-activated cell sorting.

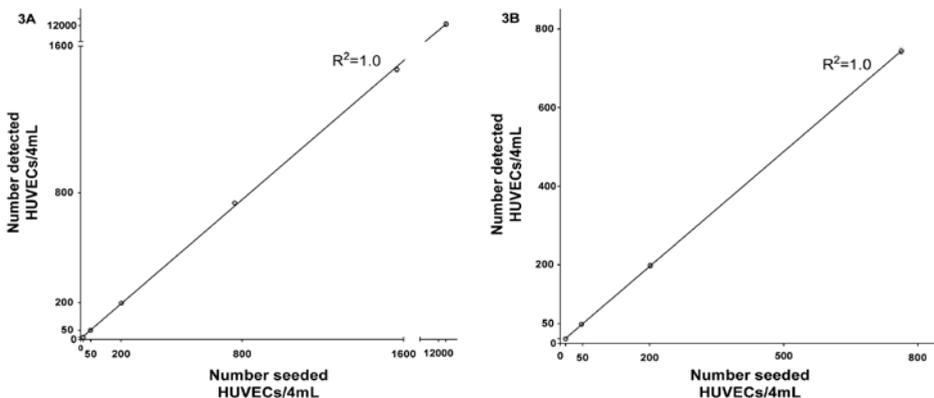


Figure 3: Linear regression analysis. The relationship (R^2) between the reference samples seeded with HUVECs (x) versus detected numbers in peripheral blood of seeded HUVECs (y) at the range of 12, 50, 200, 800, 1600 and 12800 in 4 ml of peripheral blood. Figure 3A represents the relationship R^2 at the full range of seeded HUVECs and figure 3B shows the lower four ranges of seeded HUVECs. Abbreviations: HUVEC-human umbilical vein endothelial cells.

Table 2: Results of the linearity assessment, recovery and reproducibility between the reference samples (actual) and spiked samples (detected).

Spiked HUVECs/4ml		Detected			Recovery %			Mean	S.D.	WR CV%
Theoretical	Actual	1	2	3	1	2	3			
12	12	13	10	*	108.3	83.3	*	95.8	17.7	18.4
50	48	48	48	50	100.0	100.0	104.2	101.4	2.4	2.4
200	201	201	202	190	99.3	99.8	93.9	97.7	3.3	3.4
800	763	738	750	*	96.7	98.3	*	97.5	1.1	1.1
1600	1567	1488	1473	1459	95.0	94.0	93.1	94.0	0.9	1.0
12800	12827	12445	12238	11795	97.0	95.4	92.0	94.8	2.6	2.7
Overall within-run								96.9	5.6	5.8

HUVECs were spiked at the theoretical range of 12, 50, 200, 800, 1600 and 12800 in 4 ml of peripheral blood of healthy volunteers. Theoretical number is the number of HUVEC wanted to seed. Actual number is the mean number of obtained HUVECs in the reference samples. Detected number is the number of measured HUVEC in spiked blood samples. The overall within-run recovery and CV% was calculated as the mean recovery of the assay at all levels of spiked HUVECs together. Abbreviations: HUVEC-Human Umbilical Vein Endothelial Cells, S.D.-standard deviation, WR-within-run, CV-coefficient of variation. *Missing due to technical problems during measurement acquisition.

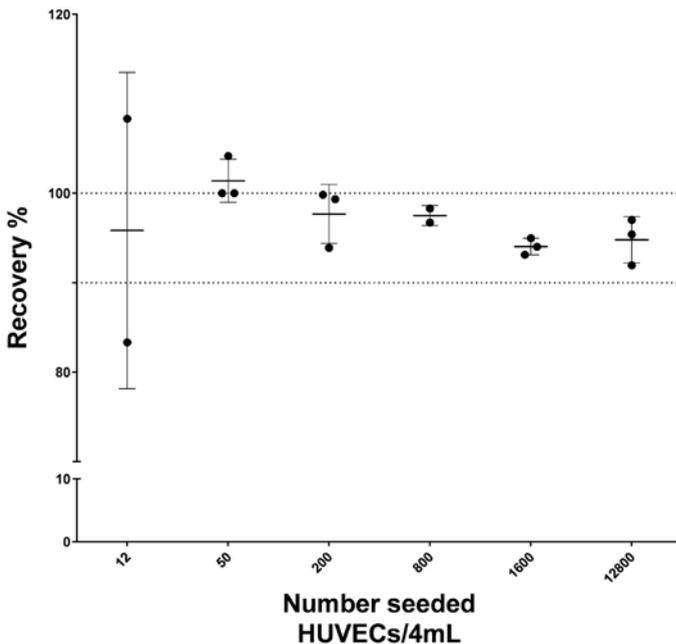


Figure 4: Recovery and reproducibility of seeded HUVECs/4mL in peripheral blood compared to the reference samples. The bars represent the mean and the standard deviation of the mean. Abbreviations: HUVEC-human umbilical vein endothelial cells.

Table 3: Within-run and between-run recovery and coefficient of variation.

WR	Spiked HUVECs/4ml		Detected			Recovery %			S.D.	WRCV%	
	Theoretical	Actual	1	2	3	1	2	3			Mean
Day 1	12	10	11	11	9	113.8	113.8	93.1	106.9	11.9	11.2
	50	42	39	45	45	92.9	107.1	107.1	102.4	8.2	8.1
	200	173	170	166	173	98.5	96.1	100.2	98.3	2.0	2.1
Overall within-run									102.5	8.2	8.0
Day 2	12	10	10	10	10	100.0	100.0	100.0	100.0	0.0	0.0
	50	41	43	42	39	104.0	101.6	94.4	100.0	5.0	5.0
	200	177	174	161	162	98.1	90.8	91.4	93.4	4.1	4.4
Overall within-run									97.8	4.6	4.7
Day 3	12	12	10	13	*	83.3	108.3	*	95.8	17.7	18.4
	50	48	47	49	50	98.6	102.8	104.9	102.1	3.2	3.1
	200	201	201	202	190	99.8	100.3	94.4	98.2	3.3	3.4
Overall within-run									99.1	7.7	7.6
Between-run									99.8	7.0	7.0

The actual number of spiked and recovered HUVECs/4mL performed in triplicate in peripheral blood of healthy volunteers in three independent runs of the assay. The overall within-run recovery of each day is the mean recovery percentage calculated at all levels of spiked HUVECs together, and accompanying standard deviation and CV%. The between-run recovery is the mean of all within-run means recovery percentages, and accompanying standard deviations and CV%. Abbreviations: HUVEC-Human Umbilical Vein Endothelial Cells, S.D.-standard deviation, WR-within-run, CV-coefficient of variation. * Missing due to technical problems during measurement acquisition.

Within-run and between-run recovery and reproducibility

The assay recovery and reproducibility was defined in three independent experiments, at three days by seeding CD146-PerCP pre-labeled HUVECs using a FACS sorter into fresh peripheral blood and washing buffer at the theoretical input of 12, 50, and 200 HUVECs per 4 ml in triplicate. The actual number of spiked HUVECs in the reference samples was defined by FCM analysis. At day-1 the overall within-run recovery was 102.5% \pm 8.2 and CV% 8.0%, on day-2 97.8% \pm 4.6 and 4.7%, and on day-3 99.1% \pm 7.7 and 7.6% (Table 3, Figure 5). The between-run recovery was 99.8% \pm 7.0 and CV% of 7.0%.

Sample stability in storage at -80°C

The stability of endogenous CECs was investigated in samples stored at -80°C for up to 2 months. The number of CECs detected in freshly processed and analyzed samples (time 0) was compared to the number of CECs detected after 1 month (time 1) and 2 months (time 2) of storage. This was done for samples of cancer patients (n=4) and HVs (n=4). The mean recovery of CECs after 1 month of storage varied from 83.8% \pm 15.1 to 89.3% \pm 2.5 and a CV% of 2.8 to 18% (Table 4, Figure 6). The within-run mean recovery was 86.8% \pm 7.8 and a CV% of 9.0%. After 2 months of storage the mean

Table 4: Stability test. Measurements of the CECs in the samples of cancer patients and healthy volunteers stored at -80°C for 1 month and 2 months.

Donor	Detected time 0						Detected 1 month						Recovery % 1 month									
	1		2		3		Mean	S.D.	CV%	1	2	3	Mean	S.D.	CV%	1	2	3	Mean	S.D.	CV%	
HV1	56	72	64	64.0	8.0	12.5	52	56	62	56.7	5.0	81.3	87.5	96.9	88.5	7.9	8.9					
HV2	55	59	56	56.7	2.1	3.7	49	49	46	48.0	1.7	86.5	86.5	81.2	84.7	3.1	3.6					
HV3	61	56	50	55.7	5.5	9.9	52	51	37	46.7	8.4	93.4	91.6	66.5	83.8	15.1	18.0					
HV4	48	39	34	40.3	7.1	17.6	37	35	36	36.0	1.0	91.7	86.8	89.3	89.3	2.5	2.8					
	Overall within-run																					
P1	113	135		124.0	15.6	12.6	102	146		124.0	31.1	82.3	117.7		100.0	25.1	25.1					
P2	716	726		721.0	7.1	1.0	748	679		714.0	48.8	103.7	94.2		99.0	6.8	6.8					
P3	195	166		181.0	20.5	11.4	169	155		162.0	9.9	93.6	85.9		89.8	5.5	6.1					
P4	47	62		55.0	10.6	19.5	73	59		66.0	9.9	133.9	108.3		121.1	18.2	15.0					
	Overall within-run																					
															102.5	17.3	16.7					
Donor	Detected time 0						Detected 2 months						Recovery % 2 months									
	1		2		3		Mean	S.D.	CV%	1	2	3	Mean	S.D.	CV%	1	2	3	Mean	S.D.	CV%	
HV1	74	69	69	70.7	2.9	4.1	69	78	*	73.5	6.4	97.6	110.4	*	104.0	9.0	8.7					
HV2	113	126	78	105.7	24.8	23.5	93	124	96	104.3	17.1	88.0	117.4	90.9	98.7	16.2	16.4					
HV3	61	56	60	59.0	2.6	4.5	49	55	50	51.3	3.2	83.1	93.2	84.7	87.0	5.4	6.3					
HV4	52	40	36	42.7	8.3	19.5	50	37	29	38.7	10.6	117.2	86.7	68.0	90.6	24.8	27.4					
	Overall within-run																					
															94.3	15.3	16.2					

Recovery percentage and CV% were calculated relative to the samples processed and analyzed the same day (time 0). The overall within-run recovery is the mean recovery percentage calculated at all samples together, and accompanying standard deviation and CV%. Abbreviations: P-patients, HV-healthy volunteers, WR-within-run, CV-co-efficient of variation. * Missing due to complication during sample drawing.

recovery varied from $87.0\% \pm 5.4$ and $104\% \pm 9.0$, and a CV% of 6.3% to 27.4%, the within-run recovery was $94.3\% \pm 15.3$ and CV% of 16.2%.

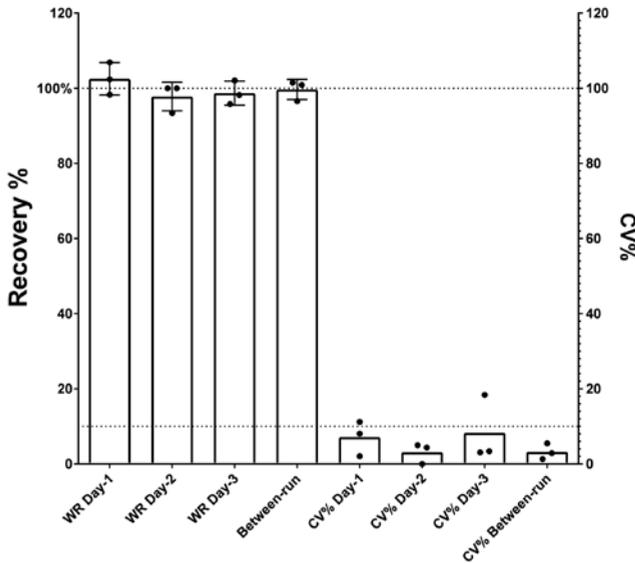


Figure 5: Recovery and reproducibility of seeded HUVECs/4mL in peripheral blood performed in three independent days. Overall within-run (WR) and between-run recovery and coefficient of variation (CV) percentages of HUVECs seeded in 4 mL peripheral blood performed in three independent days, plot accompanying table 3. The bars represent the within-run recovery percentage and accompanying standard deviation and CV% of each day. The between-run recovery is calculated as the mean recovery for all within-run means recovery percentages, and accompanying standard deviations and CV%. Abbreviations: WR-within-run, CV-coefficient of variation.

CEC levels in cancer patients and HVs

The number of CECs was investigated in 39 patients with metastatic cancer and in 11 HVs who served as a reference group to assess CEC numbers in healthy individuals (supplementary Table 1). The number of CECs in patients varied from 2.1 to 361 CECs/ml whole blood with a mean of 27.2 ± 17.8 CECs/ml (median 24.1), whereas in HVs it varied from 5.8 to 26.7 CECs/ml with a mean 14.3 ± 2.3 CECs/ml (median 14.4) (Figure 7 and 8). The median number of CECs at baseline in cancer patients was significantly different from the numbers in HVs (24.1 versus 14.4 CECs/ml respectively, Mann Whitney test, $p=0.0386$) (Table 5, Figure 8 and Supplementary Table 2 and 3). The median CECs number of 9.2 CECs/ml at baseline in patients with lung cancer ($n=8$) was not significantly different from HV ($p>0.05$). The median CECs number was significantly different from HV in patients with colorectal cancer (CRC)(median 22.2, $p=0.0348$, $n=11$) and in patients with miscellaneous tumors (median 29.5, $p=0.0047$, $n=20$) (Table 5, Figure 8).

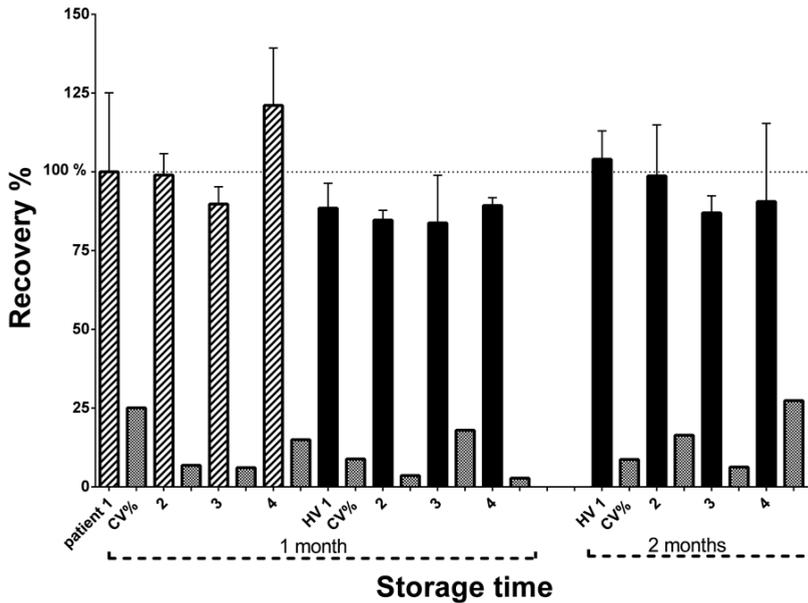


Figure 6: Stability of samples in cryostorage. Samples of cancer patients (n=4) and healthy volunteers (n=4) were stored at -80°C for 1 month and 2 months. Mean recovery percentages and CV% were calculated relative to samples processed and analyzed the same day (time 0). The bars represent the mean recovery percentage of a triplicate (for HV, slashed bars) and duplicate (patients, black bars) measurement and accompanying standard deviation of the mean recovery, and CV% (checkered bars). Abbreviations: HV-healthy volunteers, CV-coefficient of variation.

Effect of ELB on CECs recovery

The effect of ELB on endogenous CECs recovery was tested in Ficoll Paque and CPT Hypaque isolated CECs samples from HV whole blood. The CEC number was compared with the CEC number of samples drawn in CTAD tubes followed by ELB (as described in our method). The number of CECs isolated using CPT and CTAD tubes (supplementary Table 1) in the sample of HV1 were 1.0 ± 1.4 (\pm s.d.) CECs/4 ml and 40.0 ± 5.7 CECs/4 ml, respectively, and HV2 contained 2.5 ± 2.1 CECs/4 ml and 32.0 ± 7.1 CECs/4 ml, respectively.

Fifty six ml of whole blood was separated using Ficoll Paque into a PBMC fraction and a pass through fraction (supplementary Figure 1). The PBMC fraction of HV1 contained 2.7 CECs/4 ml, after ELB treatment 3.0 CECs/4 ml, and the pass through fraction contained 35.1 CECs/4 ml, and HV2 contained 4.0 CECs/4 ml in the PBMC fraction, after ELB treatment 4.0 CECs/4 ml, and 23.6 CECs/4 ml in the pass through fraction. In total, 56 ml of whole blood sample of HV1 contained 40.8 CECs/4 ml and HV2 contained 31.6 CECs/4ml.

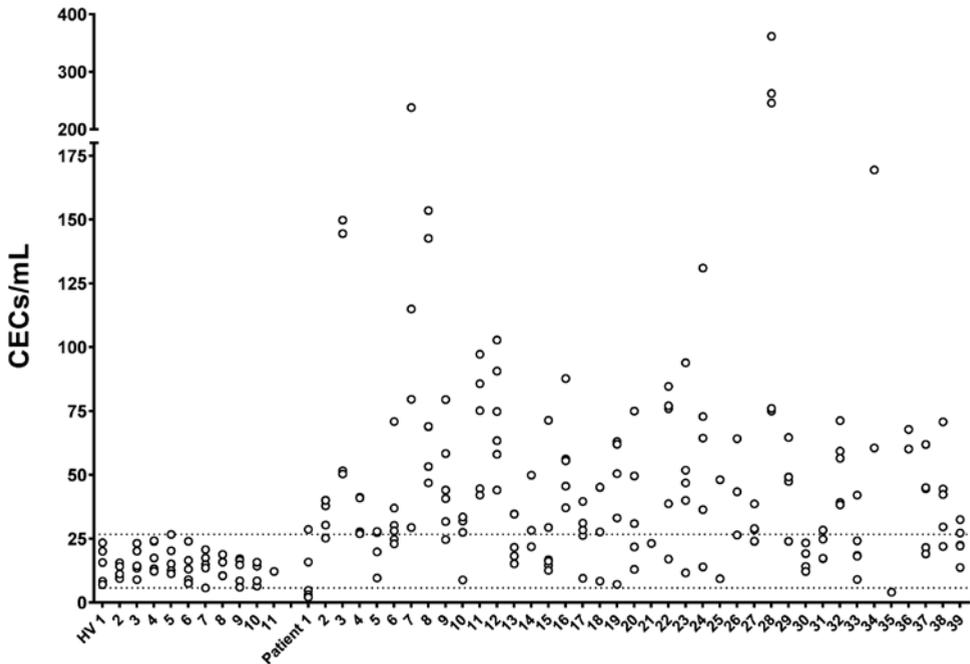


Figure 7: The range of the enumerated CECs/ml in individual healthy volunteers and cancer patients. The dashed lines represent the lower range of 5.8 CECs/ml and the upper range of 26.7 CECs/ml in HV (HV1 to 11). The CECs in cancer patients (1 to 39) varied from 2.1-361.2 CECs/ml. Abbreviations: HV-healthy volunteers.

Comparative analysis with CD34 and CD146 beads, and without enrichment

The samples for the comparative CEC analysis were drawn in CTAD tubes and prepared according to our method. The CD34 beads enriched samples (supplementary Table 6) of HV1 contained 27.3 CECs/ml (109.3 ± 4.2 CECs/4 ml), using CD146 beads was 12.4 CECs/ml (49.7 ± 9.7 CECs/4 ml), whereas without enrichment the number of CECs measured in ml of blood was 30.0 CECs/ml. Respectively, HV2 contained 19.0 CECs/ml (76.0 ± 2.6 CECs/4ml), 6.75 CECs/ml (27.0 ± 3.6 CECs/ml), and 15.0 CECs/ml.

Discussion:

Here, we presented the results of a polychromatic FCM method for enumeration of the absolute number of CECs in peripheral blood of patients with metastatic cancer and HVs. The method includes a CD34 bead sample enrichment. CECs were phenotypical defined as: nucleated cells (Hoechst positive), positive for CD34 and CD146, and negative for CD14, CD15 and CD45. The specificity and reproducibility of the assay were defined by spiking surrogates - the HUVECs - of

the circulating endothelial cells into peripheral blood and by morphologic examination using fluorescence microscopy.

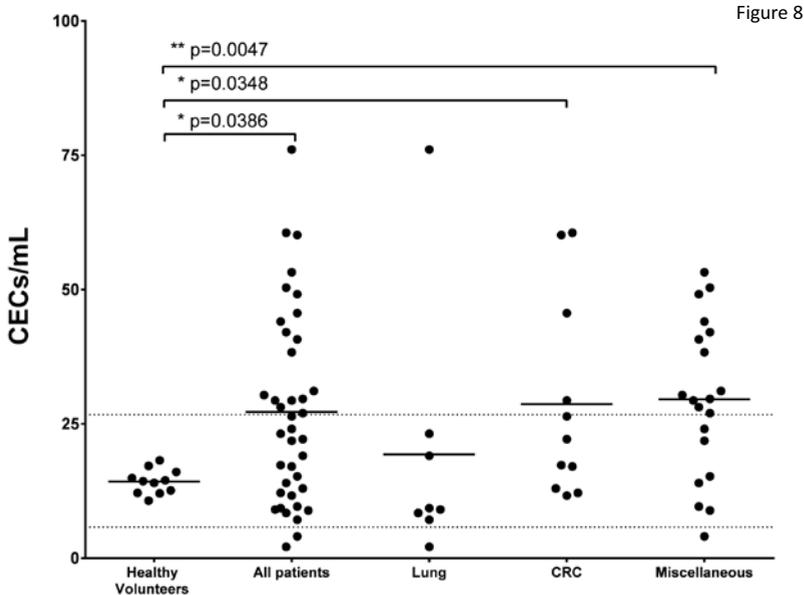


Figure 8: CEC numbers enumerated in healthy volunteers (n=11) and in patients (n=39) with metastatic cancer. CECs number in a sample drawn at baseline prior to start of treatment. The median numbers of CECs were significantly different in CRC patients (p=0.0348) and patients with miscellaneous tumor types (p=0.0047)(* Mann Whitney U-test, P<0.05). The solid line represents the median CECs number, and the dashed line represents the lower and the upper range of the enumerated CECs in HV. Abbreviations: CRC-colorectal cancer, CECs-circulating endothelial cells, HV-healthy volunteers.

CECs are rare cells in the blood circulation, enrichment allows for analysis of a large sample volume by reducing its original volume, it overcomes long sample acquisition and large data files. However, enrichment may introduce bias into the analysis by selecting cells that are more abundant and/or highly express the target marker, as compare to the cells of interest. CECs were previously successfully isolated by positive selection using CD146 magnetic beads, and negative selection using CD15 magnetic microbeads [7,14,17]. In a single experiment we have tested if CD146 magnetic microbeads qualify as an alternative for CD34 bead enrichment of endothelial cells with low CD34 expression. We were able to detect CECs in HVs after CD146 bead enrichment, however, their number was significantly lower compared to samples enriched using CD34 beads and samples prepared without enrichment. A lower number of detected CECs in CD146 enriched samples may be explained by the predominant presence of CD45+/CD146+ cells in the analyzed samples, which

are likely activated T cells. Furthermore, the widely used CD146 surface marker is expressed on the apical side and at the cell-cell junctions, its loss is related to detachment of endothelial cells [21,22]. Hence, CD146 expression level could have led to an incomplete absolute enrichment, especially in the presence of competitor cells, and underestimation of CEC number [20]. In the FCM analysis we have seen that this marker is more heterogeneous and weaker than CD34. The results of the number of CECs in CD34 bead enriched versus non-enriched samples were similar, showing that CD34 beads have not introduced a bias. Therefore, CD34 beads are a better alternative for versatile enrichment of endothelial cells of different origins. CD34 is a surface marker expressed on normal and tumor vascular cells [23]. It is present on micro- and macro-endothelial vessels, e.g. coronary artery endothelial cells, lung microvascular endothelial cells, and liver endothelial cells [24–28]. In addition, the CD34 cell population in peripheral blood is small, CD34 beads are therefore more suitable to select the CECs from between redundant blood cells, and the technical selection process using these microbeads is very practical. Moreover, previously, CD34 beads were used reliably for the isolation of HUVECs, endothelial colony-forming cells, and for the detection of CECs in blood of patients after myocardial infarction, yet, they have not previously been applied for CECs enumeration [15,16,29,30]. The utility of magnetic microbeads and MACS sorting for enrichment was in our lab also applied in the detection and enumeration of circulating tumor cells [31]. Furthermore, enrichment reduced the sample volume, which results in more manageable samples for FCM analysis, as larger data files result in machine's software limitations [7]. We confirmed the usefulness of the enrichment method for the selection of CECs by morphological analysis after FACS sorting CECs, nuclear DNA staining and intracellular staining of the vWF in the WPBs. Isolated CECs had a large nucleus and the vWF was clearly present in the WPBs, a characteristic specific for endothelial cells. Therefore, the presence of other cells expressing these markers, like mesenchymal or hematopoietic cells can be excluded [32,33]. Furthermore fluorescence microscopy images by others are comparable with the CECs images we have made, which further emphasizes the specificity of our method [9,34,35]. The performance of the method in terms of recovery, linearity, reproducibility and sensitivity, was further explored in fresh peripheral blood samples spiked with HUVECs, and long term stability in cryostored samples at -80°C. The regression analysis demonstrated that the method is capable to detect 12-12800 CECs/4ml with high and reproducible recovery (overall recovery of 96.9% and CV% of 20%). The high assay recovery and reproducibility were further underscored in three subsequent experiments with spiked HUVECs (overall recovery of 99.8% and CV% of 7.0%). In a number of experiments we have observed recoveries higher than 100%. A probable cause of recoveries exceeding 100% was the variation within the number of spiked HUVECs in the reference samples. The reference samples used for the regression analysis were subject to variation ranging from 1.8-18.4%. In addition, one cell difference accounts for 8.3% change in recovery at the level of 12 HUVECs/ml, hence, the samples spiked with HUVECs at the lower range are subject to the greatest

variation. In comparison with studies employing enrichment followed by FCM this method showed to be more stable over a wide range of spiked HUVECs without indications for significant cell loss or overestimation of CEC numbers [3,9,17]. A previous study showed that CECs could be cryostored and enumerated after 14 days [8]. With our method we were able to store samples at -80°C for 1 and 2 months with a high yield of CECs (102.5% after 1 month and 94.3% after 2 months). The coefficient of variation remained below the predefined 20%, which further emphasizes the robustness of this method. Storage allows for simultaneous analyzes of samples drawn at different time points from the same patient within two months and increases comparability and reproducibility between measurements. The variation in reproducibility of the FCM measurements was higher in fresh than in cryostored samples possibly due to sample handling. Nevertheless, delayed sample analysis for up to 2 months did not lead to significant cell loss allowing more flexible use of this method. A remark could be made on the sensitivity of our method that was defined using selected HUVECs the cell type that most closely resemble CECs. These surrogate cells do express the same surface markers as CECs, though, the expression can be different from endogenous CECs. Furthermore HUVEC are generally larger than CECs. Another remark is on the use of ELB that showed in one study to influence CEC number in samples treated with ELB. CECs in the circulation generally have a low viability, ELB might therefore disrupt these cells resulting in underestimation of CEC levels. In general, non-viable cells change morphologically: membrane integrity and cytosolic volume decreases and the cell density increases due to cell shrinkage [36,37]. To investigate CECs (resting, active and apoptotic CECs) in relation to cancer several studies applied Ficoll Paque, CPT or ELB to isolate them from whole blood [1,3,38–40]. CPTs also contain Ficoll Hypaque to separate PBMCs from granulocytes, erythrocytes, platelets and dead cells. They are easy to handle and deliver reproducible results, whereas Ficoll Paque and ELB methods are more laborious, deliver different cell purity, cell quality and recovery [41,42]. In addition, to isolate rare cells using Ficoll Paque requires experience, unlike CPT and ELB methods. ELB was widely used in other methods and seems to be harmless for freshly spiked HUVECs but might influence the viability of CECs [14]. In order to define the effect of the ELB on CECs we have isolated CECs from whole blood using Ficoll Paque. We observed no difference in CEC counts between ELB treated and untreated samples. The number of detected CECs was low and comparable with the samples prepared using CPT. In contrast, higher numbers of CECs were detected in the parallel drawn CTAD samples of the same volunteers, prepared according to our method. Additionally, we collected and analyzed the pass through of the Ficoll Paque separation and found relatively high numbers of CEC below Ficoll Paque. The sum of CECs numbers from below and above Ficoll Paque was comparable with the number of CECs in CTAD the samples prepared according to our method. This provides further prove that ELB does not affect CEC numbers. The presence of CECs below Ficoll Paque indicates that if the non-viable CECs are the subject of investigation the Ficoll Paque or CPT methods may be useless.

The different phenotype definitions, poor validation, sample preparation approaches, and the lack of sensitivity of FCM are the factors limiting comparability of CEC numbers between studies [43]. However, enrichment using CD34 beads may overcome the questionable correctness of methods quantifying the absolute number of CECs as their percentage of $\geq 100,000$ events within only 100-200 μl of the sample $[\text{CECs \%} \times \text{white blood cell count}]/100$ [1,12,44,45]. We observed that acquisition of an equivalent of 1 ml of whole blood sample of a HV to completion (prepared by ELB of 4 ml of whole blood without enrichment) produced ten files of $\geq 500,000$ total events and resulted in only 0-6 CEC events per file. However, a CD34 bead enrichment reduced sample volume and produced a data file of $< 100,000$ total events. Still, the significantly higher CECs level in our patients is in agreement with previous reports employing FCM and CellSearch® methods [1,7–9,35,46]. The CEC levels were more variable in patients than in HVs, and generally CEC numbers increased during treatment, suggesting treatment induced vascular damage or disease progression. Furthermore, the investigated patient group was heterogeneous, patients had multiple tumor types and have participated in multiple trials with differing experimental cancer therapies. The systemic therapy that they received before this study may have impaired the normal and the tumor vasculature. Consequently, these patients might have been more prone to vascular damage caused by the current therapy and, subsequently, were more likely to have increased CEC numbers. In this regard, to answer the question whether CECs are a potential predictive and/or prognostic biomarker the CECs level should be assessed in the context of a randomized study, in a pre-specified tumor type. Additionally, implementation of the cancer-associated endothelial markers CD133 and CD276 in our method might provide specific information on the tumor vasculature for example in relation to anti-angiogenic therapy [47–49].

Conclusion:

In conclusion, we have developed a highly selective, sensitive, robust and reliable method for enumeration of CECs in whole blood samples of cancer patients that can be stored for up to 2 months. To our knowledge this is the first method that shows to be a highly reproducible and reliable method for CEC enumeration by a sample enrichment using CD34 magnetic microbeads, followed by polychromatic FCM. This method can be implemented to study the role of CECs as a biomarker in clinical trials, efforts to further study the usefulness of CEC enumeration employing this method are currently ongoing.

Conflict of interest statement

The authors declare that they have no conflicts of interest in the research.

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Supplementary data

Materials and method:

Ficoll Paque

Peripheral blood of two healthy volunteers was drawn in 14 CTAD buffered (Citrate, Theophylline, Adenosine and Dipyridamole) tubes of 4 ml. The blood samples of each volunteer were merged, diluted 1:1 with phosphate buffered saline (PBS) at room temperature (RT), and 16 ml of the blood/PBS mix was layered on 12.5 ml Ficoll Paque (GE Healthcare Life Sciences, The Netherlands) in a 50 ml tube. The samples were centrifuged at 1800 rpm for 20 minutes with no break at RT. The layer containing peripheral blood mononuclear cells (PBMC) was carefully collected, merged, resuspended in PBS, centrifuged at 500 G for 7 minutes at 4°C, and the supernatant was removed. The pellet of cells was resuspended in 10 ml of PBS, well mixed and divided in two equal volumes in 50 ml tubes. One sample portion (PBMCs of 28 ml of whole blood) was directly fixed in 4% formaldehyde and incubated for 15 minutes at RT. The other portion of PBMCs from 28 ml of whole blood was treated with erythrocyte lysis buffer (ELB)(0.15 M ammonium chloride solution) for 15 minutes at RT and thereafter centrifuged at 500 G for 7 minutes at 4 °C. Then the ELB treated sample was fixed in 4% formaldehyde. After fixation the samples were washed in FACS buffer and centrifuged at 500 G for 7 minutes at 4 °C. After centrifugation the samples were ready for CEC isolation, as described in the materials and methods section of the manuscript.

Ficoll Paque pass through

The cells that had passed the Ficoll Paque, that normally would have been discarded, were collected of each volunteer and treated with ELB for 15 minutes at RT, centrifuged 500 G for 7 minutes at 4 °C, washed in FACS buffer and centrifuged at 500 G for 7 minutes at 4 °C. The pellet containing cells of 56 ml of whole blood was fixed in 4% formaldehyde for 15 minutes at RT and then washed in FACS buffer at 500 G for 7 minutes at 4 °C. After centrifugation the samples were ready for CEC isolation as previously described.

CPT Ficoll™ Hypaque™ Solution tube

Peripheral blood of two healthy volunteers was drawn in duplicate BD Vacutainer® cell preparation tube (CPT) (Becton Dickinson, The Netherlands) tubes of 4 ml, containing sodium citrate as an anticoagulant. The blood samples were centrifuged at 1500 G for 25 minutes at RT. The upper layer containing PBMCs was transferred into a 50 ml tube and washed in FACS buffer (up to 50 ml), centrifuged at 500 G for 7 minutes at 4°C. The sample was then fixed in 4% formaldehyde, washed

and CECs were isolated as described in the materials and method section of the manuscript.

CTAD tube followed by red-blood-cell lysis buffer

Peripheral blood of two healthy volunteers was drawn in duplicate in CTAD tubes of 4 ml and processed as described in the materials and method section of the manuscript.

CEC isolation from peripheral blood using magnetic microbeads

Peripheral blood of two healthy volunteers was drawn in eight CTAD tubes of 4 ml. A triplicate of whole blood samples of each volunteer were processed, enriched using CD34-conjugated magnetic microbeads and stained as described in the materials and method section of this manuscript. Also for a comparative analysis, a triplicate of whole blood samples of the same volunteers were enriched using 20 μ l of CD146-conjugated magnetic microbeads (Miltenyi Biotec B.V.) in the same manner as using CD34 microbeads. The absolute number of CECs was also defined in 4 ml of whole blood samples without enrichment. These samples were prepared as the enriched samples, however, addition of magnetic microbeads was omitted. After enrichment and staining the total sample volume was fully analyzed (to completion of the sample). And of the samples without enrichment an equivalent of 1 ml of the whole blood sample was fully analyzed on the FACS Fortessa and equipped with the FACSDiva software. Due to the memory saturation of the FACSDiva software multiple event files were collected, each file contained at least 500,000 single cells (FSC-A versus FSC-H). The total number of CECs in the non-enriched samples was calculated as the sum of CECs of all files.

Tabulated results:

Supplementary table 1: Results of the number of detected CECs in a total of 4 ml of whole blood drawn from two volunteers isolated using CTAD and CPT tubes.

Volunteer	Detected CECs/4ml					
	CPT			CTAD		
	1	2	Mean \pm s.d.	1	2	Mean \pm s.d.
1	0	2	1.0 \pm 1.4	44	36	40.0 \pm 5.7
2	1	4	2.5 \pm 2.1	27	37	32.0 \pm 7.1

CD34 beads enriched samples. CTAD = cells isolated using CTAD tubes according to our method, CPT = cells isolated using CPT tube.

Supplementary table 2: Results of the number of detected CECs in a total of 56 ml of whole blood drawn from two volunteers in CTAD tubes and the number of CECs in samples treated with ELB.

Volunteer	Detected CECs/4ml				
	Ficoll PT	Ficoll + ELB	Ficoll	Total CECs	
1	35.1	3.0	2.7	40.8	
2	23.6	4.0	4.0	31.6	

CD34 beads enriched samples. Ficoll PT = pass through fraction containing cells that passed Ficoll and were treated with ELB; Ficoll + ELB = PBMCs fraction treated with ELB; Ficoll = PBMCs fraction without ELB treatment.

Supplementary table 3: Results of the number of detected CECs in 4 ml of whole blood drawn from two volunteers in CTAD tubes.

Volunteer	CD146 beads					CD34 beads					No enrichment
	Detected CECs/4ml				Mean CECs/ml	Detected CECs/4ml				Mean CECs/ml	Detected CECs/ml
	1	2	3	Mean \pm s.d.		1	2	3	Mean \pm s.d.		
1	52	58	39	49.7 \pm 9.7	12.4	114	106	108	109.3 \pm 4.2	27.3	30.0
2	30	23	28	27.0 \pm 3.6	6.75	79	75	74	76.0 \pm 2.6	19.0	15.0

The CECs were analyzed in samples after enrichment using CD34- and CD146-conjugated magnetic microbeads and samples without enrichment.

Supplementary table 4: Mann-Whitney statistical analysis of the difference between medians of the CECs level in healthy volunteers and the baseline CECs levels in cancer patients.

Mann Whitney test				
Column D	All patients	CRC	Lung	Miscellaneous
Column A	vs. Healthy Volunteers			
P value	0.0386	0.0348	0.3950	0.0047
Two-tailed P value	Two-tailed	Two-tailed	Two-tailed	Two-tailed
Sum of ranks in column A,D	192.5, 1083	94.50, 158.5	121, 69	109, 387
Mann-Whitney U-test	126.5	28.50	33	43
Difference between medians				
Median CECs/ml of column A	14.35, n=11	14.35, n=11	14.35, n=11	14.35, n=11
Median CECs/ml of column D	24.08, n=39	22.17, n=11	9.21, n=8	29.53, n=20

Abbreviations: vs.-versus, CRC-colorectal cancer, ns-non-significant.

Supplementary table 5: CEC measurement results of the individual healthy volunteer and the patients with colorectal cancer, lung cancer and other tumor types.

Healthy Volunteer	Male/ Female	D1	D2	D3	D4	D5				Mean CECs/ml	S.D.		
1	m	20.08	7.08	8.33	23.42	15.67				14.92	7.15		
2	m	11.25	9.42	14.17	15.58	10.00				12.08	2.68		
3	m	20.17	14.42	23.25	13.50	9.00				16.07	5.65		
4	m	17.50	12.25	24.25	23.92	13.25				18.23	5.69		
5	m	26.67	11.33	12.58	20.25	15.17				17.20	6.30		
6	f	13.08	16.50	7.50	9.00	24.00				14.02	6.60		
7	f	13.58	20.75	5.75	14.92	17.50				14.50	5.60		
8	f	10.50	15.83	10.67	15.92	18.83				14.35	3.64		
9	f	6.00	8.50	14.83	16.58	17.17				12.62	5.05		
10	f	8.50	8.50	6.50	15.83	14.25				10.72	4.07		
11	f	12.17								12.17	0.00		
Patient	Cancer type	Male/ Female	D1 Baseline	D2	D3	D4	D5	D6	D7			Mean CECs/ml	S.D.
1	lung	f	2.13	3.13	4.75	28.63	15.88				10.90	11.34	
2	other	m	30.38	40.00	25.25	40.13	37.88				34.73	6.63	
3	other	f	50.38	51.63	149.75	144.50				99.07	55.54		
4	other	m	27.00	41.00	41.25	27.75				34.25	7.95		
5	other	f	9.63	19.88	27.88	27.38				21.19	8.53		
6	other	f	28.13	28.00	37.00	23.00	70.88	24.75	30.25	34.57	16.62		
7	other	m	29.38	79.63	115.00	182.75	237.88				128.93	82.62	
8	other	f	53.25	46.88	69.00	142.63	153.50	68.88			89.02	46.68	
9	other	f	40.75	44.08	31.75	24.63	79.50	58.38			46.52	19.82	
10	other	m	8.88	33.50	31.92	27.50				25.45	11.33		
11	other	m	42.08	85.75	97.25	75.17	44.58				68.97	24.69	
12	other	f	44.08	63.42	102.83	58.08	74.83	90.67			72.32	21.70	

Supplementary table 5: Continued.

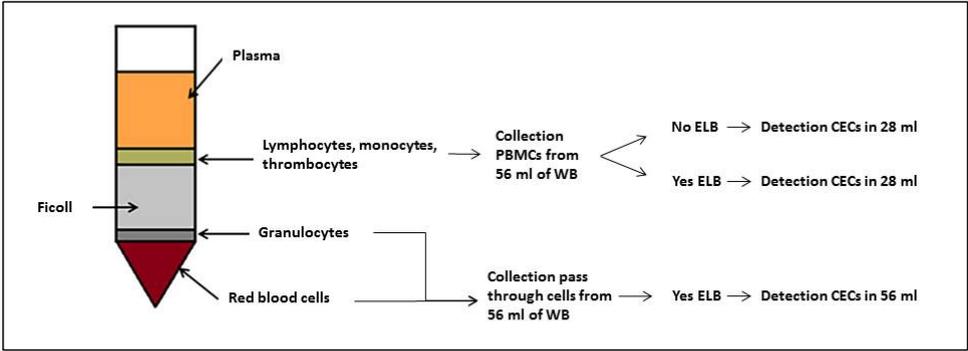
Patient	Cancer type	Male/ Female	D1 Baseline	D2	D3	D4	D5	D6	D7	Mean CECs/ml	S.D.
13	other	f	15.25	18.25	21.63	34.63	34.75			24.90	9.22
14	other	f	21.88	28.25	49.88					33.34	14.68
15	CRC	m	29.38	16.25	12.63	14.63	16.75	71.38		26.84	22.61
16	CRC	f	45.63	87.75	55.50	56.25	37.13			56.45	19.18
17	other	m	31.13	28.38	26.25	39.63	9.50			26.98	11.01
18	lung	m	8.42	27.67	27.67	45.17	45.08			30.80	15.26
19	lung	f	7.17	62.00	63.08	33.17	50.50			43.18	23.46
20	CRC	m	13.00	21.83	30.92	75.00	49.58			38.07	24.70
21	lung	f	23.17							23.17	0.00
22	CRC	m	17.08	84.67	77.08	75.92	38.75			58.70	29.32
23	CRC	m	11.67	93.92	51.83	46.83	40.00			48.85	29.60
24	other	m	14.00	36.42	64.42	72.83	131.00			63.73	44.24
25	lung	f	9.33	48.08						28.71	27.40
26	CRC	m	26.42	43.42	64.17					44.67	18.91
27	other	f	24.08	29.00	28.50	23.92	38.67			28.83	5.99
28	lung	m	76.08	75.00	262.25	361.75	245.83			204.18	125.53
29	other	f	49.17	24.00	64.67	47.42				46.32	16.78
30	CRC	m	12.17	19.17	14.17	23.33	12.33			16.23	4.87
31	CRC	f	17.33	24.83	28.42	17.25				21.96	5.59
32	other	m	38.33	39.17	71.25	59.33	56.50			52.92	14.07
33	lung	m	9.08	18.17	18.50	24.17	42.08			22.40	12.26
34	CRC	m	60.58	169.42						115.00	76.96
35	other	m	4.08							4.08	0.00
36	CRC	f	60.17	67.83						64.00	5.42
37	lung	m	19.08	45.00	61.92	44.58	21.58			38.43	17.97
38	other	f	29.67	42.33	70.75	44.50	22.00			41.85	18.61
39	CRC	m	22.17	27.25	22.50	13.75	32.50			23.63	6.94

Study protocol 1: patient 1 to 17; protocol 2: 18 to 39. Abbreviations: S.D.-standard deviation, CRC-colorectal cancer, D-blood draw 1 to 7.

Supplementary table 6: Analysis of statistical parameters in healthy volunteers and cancer patients.

	Healthy Volunteers	All patients	Lung	CRC	Miscellaneous
Number of values	11	39	8	11	20
Median CECs/ml	14.35	24.08	9.21	22.17	29.53
Minimum	10.72	2.13	2.13	11.67	4.08
Maximum	18.23	76.08	76.08	60.58	53.25
Lower 95% CI of mean	12.72	21.45	-0.6851	16.27	22.81
Upper 95% CI of mean	15.80	32.99	39.30	41.11	36.34

The mean CECs/ml of healthy volunteers is a mean value of all individual measurements. Abbreviations: CI-confidence interval, CRC-colorectal cancer.

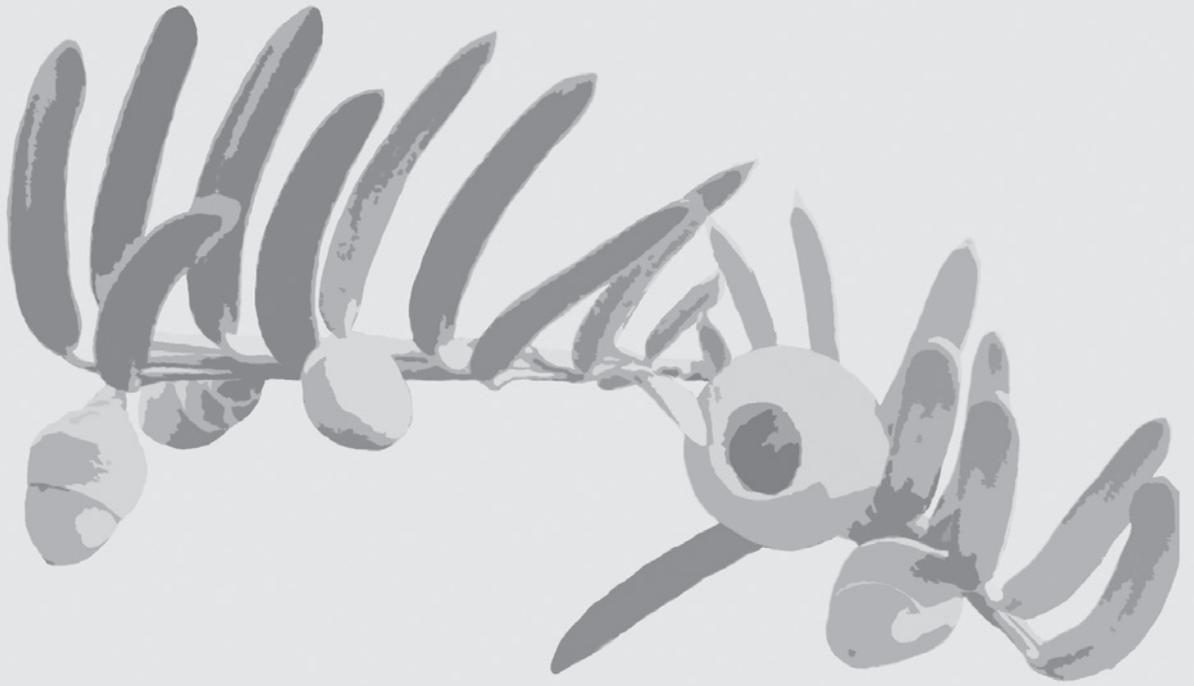


Supplementary figure 1. Schematic representation of the sample preparation for the ELB effect testing.



Chapter 4

HDM-2 inhibitors



Chapter 4.1

**A phase I study of SAR405838,
a novel HDM2 antagonist,
in patients with solid tumours.**

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Eur J Cancer. 2017 May; 76: 144-151.

Abstract:

Purpose: In tumours with wild-type *TP53*, the tumour-suppressive function of p53 is frequently inhibited by HDM2. This Phase I, dose-escalating study investigated the maximum tolerated dose (MTD), safety, pharmacokinetics (PK) and pharmacodynamics (PD) of SAR405838, an HDM2 inhibitor, in patients with advanced solid tumours (NCT01636479).

Methods: In dose escalation, patients with any locally advanced/metastatic solid tumour with *TP53* mutation prevalence below 40%, or documented as *TP53* wild-type, were eligible. In the MTD expansion cohort, only patients with de-differentiated liposarcoma (DDLPS) were included. Primary endpoints were MTD and efficacy in the MTD expansion cohort. Secondary endpoints included safety, PK and PD biomarkers.

Results: Seventy-four patients were treated with SAR405838 (50–800 mg once daily [QD], 800–1800 mg weekly, and 1800 mg twice weekly). Two patients treated with SAR405838 400 mg QD had thrombocytopenia as a dose-limiting toxicity (DLT). The MTD for the QD schedule of SAR405838 was 300 mg QD. No DLTs were observed with the weekly schedule; one patient had a DLT of nausea with the 1800 mg twice-weekly dose. Treatment with SAR405838 was associated with increased plasma MIC-1, reflecting p53 pathway activation. In the DDLPS MTD cohort, 89% of patients had *HDM2* amplification at baseline and no *TP53* mutations were observed; best response was stable disease in 56% and progression-free rate at 3 months was 32%.

Conclusion: SAR405838 had an acceptable safety profile with limited activity in patients with advanced solid tumours. The MTD of SAR405838 was 300 mg QD; MTD was not reached with the weekly schedule.

Introduction:

Loss of the tumour-suppressive function of p53 is an important step in tumourigenesis. While loss of p53 is often due to somatic *TP53* mutations, some tumours still harbour wild-type *TP53* [1]. In these cases, biological function of p53 is frequently inhibited by the mouse double minute 2 protein (MDM2; HDM2 in humans) [2–4]. HDM2 inhibits activation of p53 target genes by binding to the transactivation domain of p53 and promoting its degradation. The HDM2 gene is amplified and/or its gene product is overexpressed in several tumour types, including de-differentiated liposarcoma (DDLPS) [5,6]. Disrupting the interaction between HDM2 and p53 using small-molecule antagonists, leading to reactivation of p53, has shown encouraging antitumour activity *in vitro* and *in vivo* [7,8]. Therefore, there is rationale for investigating treatment with HDM2 inhibitors in patients with p53 wild-type tumours.

SAR405838 is an oral spirooxindole derivative antagonist of HDM2, which binds selectively to HDM2 with an inhibitory constant (K_i) value of 0.88 nM [8]. Preclinical data have shown that SAR405838 treatment results in robust p53 pathway activation, leading to p53-dependent cell-cycle arrest and apoptosis *in vitro* and *in vivo* [8]. SAR405838 treatment resulted in tumour regression or complete tumour growth inhibition in multiple mouse xenograft tumour models. In addition, SAR405838 treatment in HDM2-amplified osteosarcoma xenograft models resulted in complete tumour regression.

This Phase I, first-in-human study was conducted to determine the maximum tolerated dose (MTD), safety, pharmacokinetics (PK) and pharmacodynamics (PD) of SAR405838 in patients with solid tumours, including an MTD expansion cohort of patients with DDLPS (NCT01636479).

Methods:

Study design

This was a Phase I, open-label, dose-ranging, dose-escalating, safety, PK and PD study of SAR405838 administered orally in adult patients with advanced solid tumours. Once-daily (QD), once-weekly (QW) and twice-weekly (BIW) oral administration schedules of SAR405838 were evaluated. The primary endpoints were MTD and efficacy in the MTD cohort (including progression-free rate [PFR] at 3 months). Secondary endpoints included safety, PK, PD (change in macrophage inhibitory cytokine-1 [MIC-1] levels in plasma), and tumour genetics status (including baseline tumour *TP53* mutation and *HDM2* gene copy number status) in tumour samples and plasma.

The protocol was approved by all involved Independent Ethics Committees and Institutional Review Boards. The clinical trial was conducted in compliance with all applicable international and national

laws and regulations, and adhered to the principles outlined in the Helsinki declaration. Written, informed consent was obtained from each patient prior to study participation.

Patient population

Eligible patients were aged ≥ 18 years with a histologically or cytologically confirmed solid tumour for whom no further effective standard treatment was available. Eligible patients had disease that was locally advanced or metastatic, and measurable as defined by Response Evaluation Criteria in Solid Tumors (RECIST) version 1.1 [9]. For dose escalation, patients with any solid tumour having a reported *TP53* mutation prevalence below 40% [10], or documented as *TP53* wild-type, were eligible. For the MTD expansion cohort, only patients with DDLPS were included. Patients were required to have an Eastern Cooperative Oncology Group performance status (ECOG PS) of 0 or 1 and life expectancy ≥ 12 weeks.

Dose escalation and dose-limiting toxicities

An adaptive design was employed in dose escalation, with at least two patients evaluable for dose-limiting toxicity (DLT) required in each cohort and a maximum dose escalation of 100% permitted. The starting dose was 50 mg QD. The primary DLT evaluation period was the first cycle (3 weeks), but late-onset DLTs were also considered in dose-escalation decisions (up to 6 weeks).

DLTs were defined as any of the following treatment-related events: haematological – grade 4 thrombocytopenia, grade 4 neutropenia, or grade 3 febrile neutropenia; non-haematological – grade 3 fatigue persistent for more than 7 days, or persistent (>48 hours) grade 2 nausea, vomiting or anorexia despite the use of medical intervention; or any toxicities resulting in an interruption of the scheduled study treatment by >7 days.

Statistical methods

It was anticipated that approximately 42 DLT-evaluable patients would be enrolled in the dose-escalation phase, with an expected assessment of approximately 7 dose levels. A total of 16 efficacy-evaluable patients were to be included in the MTD expansion cohort (estimated enrolment of approximately 20 patients). Given an assumed true 3-month PFR of 45% in this population, a 3-month PFR $\leq 21\%$ was to be rejected at a 1-sided 10% level with $>80\%$ power if the observed 3-month PFR was at least 37.5% (6 patients progression free at 3 months). Given an assumed true overall response rate (ORR) of 40% in this population, an ORR $\leq 12.5\%$ was to be rejected at a 1-sided 10% level with $>80\%$ power if the observed ORR was at least 31.3% (≥ 5 responders).

Safety assessments

Safety was assessed by evaluation of adverse events (AEs), DLTs, changes in vital signs, 12-lead

electrocardiograms, physical examinations, ECOG PS and clinical laboratory tests (including haematology, coagulation, blood chemistry and urinalysis). AEs were graded according to the National Cancer Institute Common Terminology Criteria for Adverse Events version 4.0 [11].

Pharmacokinetic assessments

Blood samples (predose, 1, 2, 4, 6, 8 and 24 h) for assessment of SAR405838 concentrations were collected on Days 1 (all cohorts) and 8 (QD dosing cohorts only) along with trough on Day 15 of Cycle 1 and Day 1 of each subsequent cycle. PK parameters, calculated from the blood concentration data using standard non-compartmental methods, included maximum concentration (C_{\max}), time to reach C_{\max} (t_{\max}), area under the plasma concentration–time curve (AUC) from time 0 to 24 hours (AUC_{0-24}), terminal half-life ($t_{1/2z}$) and apparent total body clearance (CL/F).

Food effect was evaluated at specific dose levels. On Day 1 of Cycle 2 (for cohorts with formal food effect assessment versus fasted) or from Day 1 of Cycle 1 for cohorts only treated fed, patients received a moderate-fat breakfast within 30 minutes prior to dosing. A 24 h blood sample collection was performed on Day 1 of Cycle 2 when applicable. Food effect was assessed from fed/fasted comparison on C_{\max} and AUC_{0-24} or from direct comparison of exposure parameters.

Pharmacodynamic assessments

Blood samples for peripheral PD biomarkers analyses, including MIC-1, were collected during screening, on Days 1, 2, 8, 9 and 15 of Cycle 1, and on Day 1 of each subsequent cycle (optional). MIC-1 protein concentrations were measured in plasma samples using an analytically validated enzyme-linked immunosorbent assay (ELISA) (Quantikine® Human GDF-15 immunoassay).

Tumour genetic status

Tumour tissue was collected for assessment of baseline tumour *TP53* mutation and *HDM2* gene copy number status (optional in dose-escalation phase, mandatory in MTD expansion cohort) with an optional follow-up tumour biopsy in patients with disease progression after having initially benefited from study treatment. Tumour genetic status was assessed as previously described [12] (Supplementary Methods). Optional collection of blood samples for plasma isolation and cell-free DNA extraction was done for this study and assessed as previously described [12] (Supplementary Methods).

Efficacy assessments

Tumour response was investigator assessed by RECIST version 1.1 [9]. Assessments were made at least every 2 cycles, or less frequently if indicated.

Results:

Patient population

Forty-five patients were treated on a continuous dosing schedule of SAR405838 50 mg QD (n = 3), 100 mg QD (n = 3), 200 mg QD (n = 3), 300 mg QD (n = 28), 400 mg QD (n = 7) and 800 mg QD (n = 1). Median age was 61 years (range 22–82; Table 1). Median number of prior treatments was 2 (range 1–7). The most common primary tumour type was liposarcoma (56%).

Twenty-nine patients received an intermittent dosing schedule of SAR405838 400 mg x 2 (interval of 4 hours) QW (n = 4), 800 mg QW (n = 3), 1200 mg QW (n = 10), 1800 mg QW (n = 6) and 1800 mg BIW (n = 6). Median age was 60 years (range 38–78; Table 1). Median number of prior treatments was 2 (range 1–7). The most common primary tumour type was liposarcoma (34%)

Mean duration of treatment was 14 weeks for patients on a continuous dosing schedule and 11 weeks for patients on an intermittent dosing schedule. In the continuous dosing population, 32 patients (71%) discontinued due to disease progression, six (13%) due to AEs and five (11%) due to other reasons. In the intermittent dosing population, 20 patients (69%) discontinued the study due to disease progression, five (17%) due to AEs, one (3%) due to poor compliance to the protocol and two (7%) due to other reasons.

Table 1: Patient baseline characteristics

	Daily dosing (n = 45)	Weekly dosing (n = 29)
Median age, years (range)	61 (22–82)	60 (38–78)
Male, n (%)	22 (49)	14 (48)
ECOG PS, n (%)		
0	24 (53)	11 (39)
1	21 (47)	17 (61)
Missing	0	1 (3)
Tumour type, n (%)		
Liposarcoma	25 (56)	10 (34)
Gastrointestinal	5 (11)	5 (17)
Skin (melanoma)	3 (7)	0
NSCLC	0	2 (7)
Other	12 (27)	12 (41)
Median number of prior anticancer therapies (range)	2 (1–7)	2 (1–7)

ECOG PS, Eastern Cooperative Group performance status; NSCLC, non-small cell lung cancer.

Dose escalation and dose-limiting toxicities

During the dose-escalation phase, two DLTs of grade 4 thrombocytopenia occurred at the 400 mg QD dose level (one patient in Cycle 1 and one patient in Cycle 2). After the first case of grade 4

thrombocytopenia was seen, the DLT period for the QD schedule was increased from 1 to 2 cycles based on the late onset of the thrombocytopenia. An additional seven patients were enrolled at the 300 mg QD dose level and did not experience a DLT; therefore, the 300 mg QD dose level was chosen as the MTD for the continuous dosing schedule. Twenty-one patients with DDLPS were treated in the MTD expansion cohort. Of these, one patient had treatment-related grade 4 thrombocytopenia in Cycle 2 and one patient had treatment-related grade 4 thrombocytopenia in Cycle 5. No DLTs were observed with the QW schedule; at the 1800 mg BIW dose level, one patient had a DLT (prolonged grade 2 nausea). However, due to no further increase in exposure at rising weekly doses, further dose-escalation in the weekly cohort was stopped.

Table 2: Treatment-related AEs of any grade occurring in >10% of patients and all treatment-related grade ≥ 3 AEs

Preferred term, n (%)	Daily dosing (n = 45)	Weekly dosing (n = 29)	All (N = 74)
Any treatment-related AE	33 (73)	27 (93)	60 (81)
Nausea	18 (40)	19 (66)	37 (50)
Fatigue	14 (31)	15 (52)	29 (39)
Decreased appetite	6 (13)	12 (41)	18 (24)
Vomiting	4 (9)	13 (45)	17 (23)
Diarrhoea	9 (20)	8 (28)	17 (23)
Thrombocytopenia	9 (20)	2 (7)	11 (15)
Any grade ≥ 3 treatment-related AE	6 (13)	1 (3)	7 (9)
Thrombocytopenia	6 (13)	0	6 (8)
Neutropenia	2 (4)	1 (3)	3 (4)

AE, adverse event.

Safety

The most frequently occurring AEs regardless of causality were nausea (59%), fatigue (58%) and vomiting (42%); the most frequently occurring grade ≥ 3 AE was thrombocytopenia (8%; supplementary Table 1). The most frequently occurring treatment-related AEs were nausea (50%), fatigue (39%) and decreased appetite (24%); the most frequently occurring treatment-related grade ≥ 3 AEs were thrombocytopenia (8%) and neutropenia (4%; Table 2). The most frequently occurring grade 3/4 haematological laboratory abnormality was lymphopenia (16% in the continuous dosing cohort and 10% in the intermittent dosing cohort).

Table 3: SAR405838 PK parameters

Cohort	Cycle/Day	PK parameter, median [range](n) or mean ± SD (n; CV%)			
		t _{max} , h	C _{max} , ng/mL	AUC _{0–24} , ng.h/mL	t _{1/2} , h
QD 50 mg	C1/D1	2 [1.97–2](3)	407 ± 191 (3; 47)	4700 ± 2510 (3; 53)	26.8 ± 12.5 (3; 47)
	C1/D8	2.03 [1.98–3.95](3)	499 ± 251 (3; 50)	6420 (1)	11.9 (1)
QD 100 mg	C1/D1	6 [1.97–6.17](3)	520 ± 127 (3; 24)	8830 (1)	26.8 (1)
	C1/D8	3.97 [2–8.22](3)	954 ± 743 (3; 78)	7900 [3270–12500](2)	15.3 [13.6–16.9](2)
QD 200 mg	C1/D1	4.05 [2–5.93](3)	956 ± 393 (3; 41)	15100 [8550–21700](2)	17.4 [13.2–21.6](2)
	C1/D8	4.13 [4.05–8.08](3)	910 ± 379 (3; 42)	15800 [11700–19800](2)	14.3 (1)
QD 300 mg	C1/D1	4.02 [2–4.05](7)	1380 ± 438 (7; 32)	19600 ± 7490 (6; 38)	16 ± 2.87 (5; 18)
	C1/D8	2.01 [1.97–6](6)	1490 ± 505 (6; 34)	17400 ± 4000 (4; 23)	16.6 ± 5.17 (3; 31)
QD MTD 300 mg	C2/D1_FED	6.04 [2.57–8](4)	998 ± 626 (4; 63)	15300 [6240–24400](2)	12.6 (1)
	C1/D1	4 [1–9.9](21)	1510 ± 758 (21; 50)	13000 ± 6640 (4; 51)	17 ± 4.66 (4; 28)
	C1/D8	4 [1–8.25](12)	1780 ± 963 (12; 54)	40900 (1)	16.9 (1)
QD 400 mg	C2/D1_FED	4 [2–6](9)	1250 ± 507 (9; 41)	24900 [19800–29900](2)	16.8 (1)
	C1/D1	4 [2–8](7)	2170 ± 1200 (7; 55)	32300 ± 20100 (5; 62)	18.5 ± 9.91 (4; 54)
	C1/D8	2.02 [2–4.12](6)	2160 ± 1040 (6; 48)	36100 ± 22200 (4; 61)	19.5 ± 5.94 (4; 30)
QD 800 mg	C2/D1_FED	4.13 [4–4.25](2)	824 [817–831](2)	15400 (1)	20.7 (1)
	C1/D1	4 (1)	1800 (1)	24600 (1)	19.4 (1)
	C1/D8	2.02 (1)	2240 (1)	30300 (1)	11.8 (1)
QW BID 400 mg	C1/D1	6.17 [6.02–8.02](3)	1960 ± 484 (3; 25)	32400 ± 9850 (3; 30)	12 ± 3.72 (3; 31%)
	C2/D1 FED	7 [6–8](2)	3630 [2740–4510](2)	57000 [42300–71700](2)	11.3 [10.9–11.8](2)
QW 800 mg	C1/D1	1.98 [1.07–4](3)	1620 ± 300 (3; 19)	21000 ± 3370 (3; 16)	16.4 ± 2.61 (3; 16)
	C2/D1 FED	8.17 (1)	3410 (1)	57600 (1)	10.4 (1)
QW 1200 mg	C1/D1	3 [1.97–8.03](6)	3310 ± 1460 (6; 44)	42900 ± 21700 (5; 51)	15.6 ± 4.37 (5; 28)
	C2/D1 FED	4.01 [2–5.98](4)	5270 ± 2550 (4; 49)	65500 ± 32000 (4; 49)	12.1 ± 4.92 (3; 41)
FED QW 1200 mg	C1/D1	5.11 [3.98–9.83](4)	3300 ± 1520 (4; 46)	54300 ± 19200 (3; 35)	20.1 ± 10.7 (3; 53)
FED QW 1800 mg	C1/D1	5 [1.05–10.1](6)	4590 ± 839 (6; 18)	78000 ± 22200 (5; 28)	NR
FED QW D1D2 1800 mg	C1/D1	7.4 [2.02–28.1](6)	4010 ± 1670 (6; 42)	57300 ± 23600 (6; 41)	17 ± 13.4 (6; 79)

For n ≥ 3 results reported as mean ± SD (n; CV%) for all parameters except for t_{max} reported as median [range] (n). For n = 1: value (1), for n = 2 mean [range] (2).

Twenty-two patients (30%) had a serious AE (SAE); five of which (7%) were treatment-related. Eleven patients (15%) had AEs leading to treatment discontinuation, including thrombocytopenia (5%) and nausea (5%). Twelve patients (16%) had dose modifications due to AEs. Twenty-eight patients (38%) died during the study; most commonly due to disease progression (26 patients, 35%). Two patients died due to other causes (pneumonia and urosepsis, and clinical deterioration). No treatment-

related deaths were reported.

Pharmacokinetics

Table 3 summarises SAR405838 PK parameters. Figure 1 shows plasma SAR405838 concentration–time profiles. In fasted state, SAR405838 mean C_{\max} up to ~3000 ng/mL was achieved. SAR405838 exposure (AUC_{0-24} and C_{\max}) was dose-proportional with doses of up to 400 mg, but not above this dose. The apparent $t_{1/2}$ of SAR405838 was in the 10–21-hour range. After repeated dose (QD), a limited systemic accumulation (<2-fold increase) was observed and generally decreased with dose.

Food consumption tended to decrease drug absorption for 300 mg or 400 mg doses. Overall, a dose-related increase of both C_{\max} and AUC_{0-24} could be achieved in the 50–1800 mg dose range, with food consumption contributing to exposure increase (mean C_{\max} up to ~4500 ng/mL) for the higher doses given QW.

Pharmacodynamics

SAR405838 administration was associated with dose-dependent increase in plasma MIC-1, peaking at 8 hours post-treatment on Cycle 1 Day 1, with mean induction of 5-fold compared with same-day baseline at doses ≥ 1200 mg. MIC-1 levels were reduced by 24 hours in a manner that was reflective of SAR405838 exposure. Patients who had a DLT had the highest MIC-1 levels. Plasma MIC-1 protein AUC_{0-24} (marker of p53 pathway activation) correlated positively with SAR405838 AUC_{0-24} at Cycle 1 Day 1 (QD and QW schedules combined) and at Cycle 1 Day 8 (QD schedules; Figure 2).

Thrombocytopenia seemed to correlate with higher SAR405838 exposure over 1 week (AUC_{0-168}) achieved with QD schedules. However, the higher C_{\max} achieved in the QW schedule did not seem to correlate with thrombocytopenia (Supplementary Figure 1).

Tumour genetic status

Tumour baseline biopsies were available for 20 patients in the DDLPS MTD expansion cohort, with 17 containing sufficient DNA for genetic analysis; 89% of patients exhibited *HDM2* amplification and no *TP53* mutations were observed [12]. However, mutations in *TP53* were observed in plasma cell-free DNA in multiple patients during the study, indicating emergence of *TP53* mutations in response to treatment. In addition, *HDM2* copy number increased during time on treatment. Full mutational analysis of emerging *TP53* mutations in patients with DDLPS has been published previously [12].

Efficacy

No objective responses were observed; 38 of 66 evaluable patients (58%) had stable disease as best response. In evaluable patients with DDLPS, 22 of 31 patients (71%) had stable disease. In the MTD

DDLPS expansion cohort, 10 of 18 patients (56%) had stable disease; PFR at 3 months was 32%. Changes in target lesion diameters were not dose-dependent; percentage change from baseline in sum of target lesions is shown in Figure 3.

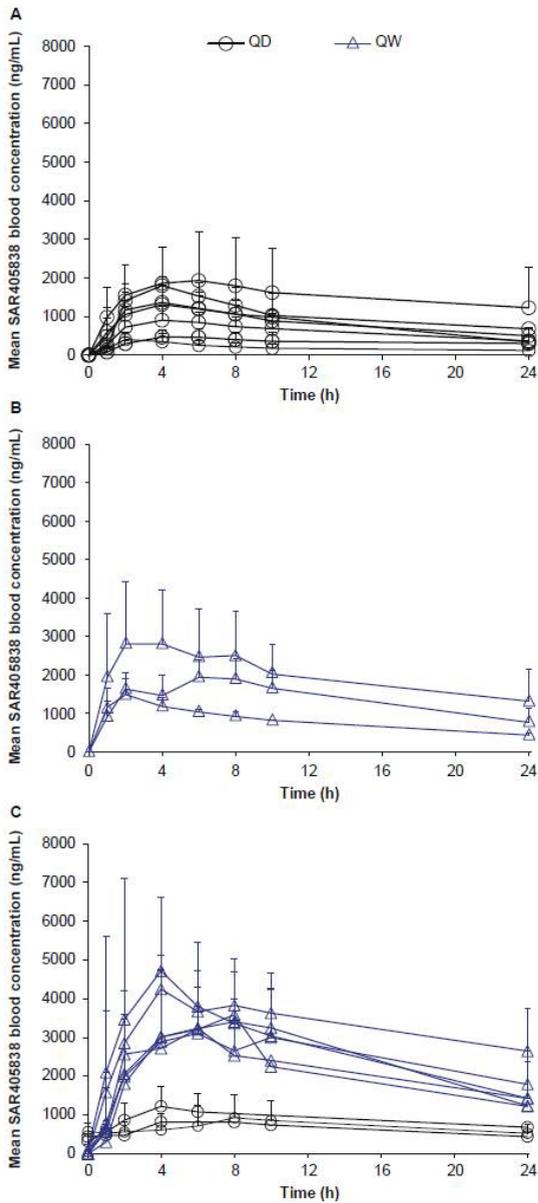


Figure 1: Mean plasma SAR405838 concentration over time. A) Single dose (QD or QW) fasted; B) repeat dose (QD) fasted; C) single dose (QW) or repeated dose (QD) fed QD, once daily; QW, once weekly.

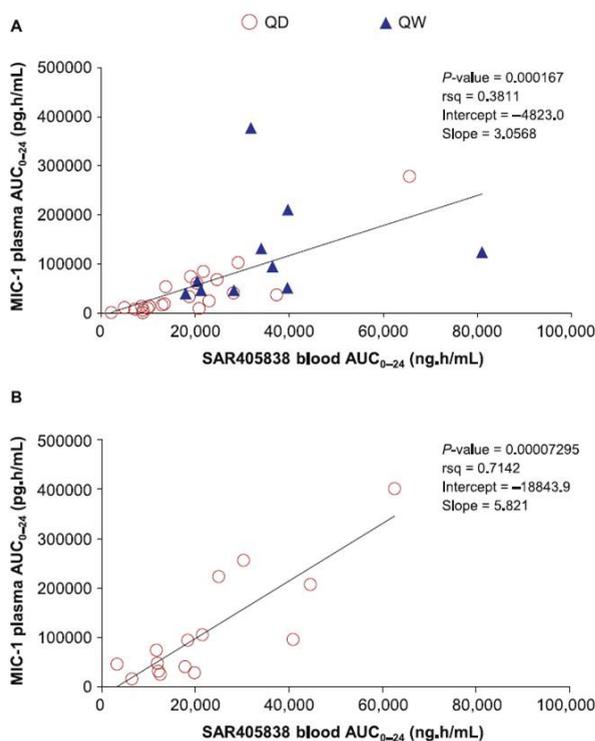


Figure 2: Correlation between SAR405838 blood AUC_{0-24} and MIC-1 plasma AUC_{0-24} : A) QD and QW cohorts at Cycle 1 Day 1/Day 2; B) QD cohort at Cycle 1 Day 8/Day 9 AUC , area under the concentration–time curve; MIC-1, macrophage inhibitory cytokine-1; QD, once daily; QW, once weekly.

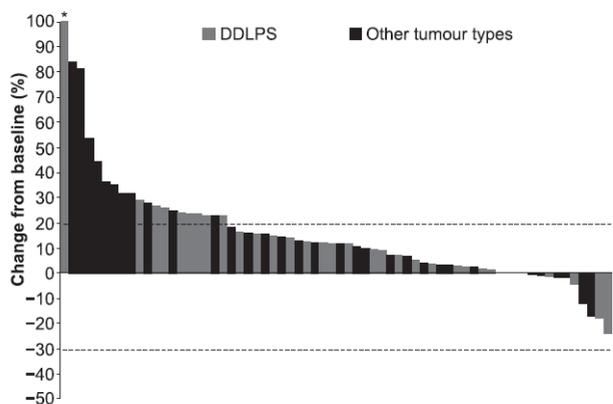


Figure 3: Maximum percentage change from baseline in the sum of target lesion diameters. Dotted lines represent partial response (-30%) and progressive disease (+20%).

*Patient had 265% increase.

DDLPS, de-differentiated liposarcoma.

Discussion:

This Phase I, first-in-human study was conducted to determine the MTD, safety, PK and PD of SAR405838 in patients with solid tumours. The MTD for SAR405838 in a continuous schedule was established as 300 mg QD. Two patients treated with SAR405838 400 mg QD had DLTs of grade 4 thrombocytopenia. The MTD was not reached with the weekly schedule. No DLTs were observed in the QW cohorts, although the 1800 mg BIW dose level schedule was only moderately tolerated and could be considered as a maximum administered dose (1 DLT observed out of 6 evaluable patients; in addition another patient had an AE meeting definition of DLT in Cycle 3).

The most frequently occurring treatment-related grade ≥ 3 AE was thrombocytopenia, consistent with other drugs in the *HDM2* antagonist class [13–15]. The observed thrombocytopenia was notable for its long duration. Thrombocytopenia correlated with SAR405838 exposure in the QD cohort. Treatment with SAR405838 was associated with plasma MIC-1 secretion, reflecting p53 pathway activation.

Although no objective responses were observed, disease stabilization occurred in the majority (58%) of patients. In patients with DDLPS, 71% had stable disease. Efficacy was consistent with previous Phase I studies of HDM2 inhibitors [14–16]. Disease control rate was comparable with results from clinical trials of approved agents in soft tissue sarcoma, such as trabectedin [17], eribulin [18] and pazopanib [19]. The lack of objective responses observed may be due in part to emergence of *TP53* resistance mutations, which were observed in circulating tumour DNA [12].

In summary, SAR405838 had an acceptable safety profile in patients with advanced solid tumours. The MTD of the QD schedule was SAR405838 300 mg QD; MTD was not reached with the weekly schedule. Although no responses were observed with single-agent SAR405838, MIC-1 modulation and the safety profiles support further evaluation of SAR405838 within combination regimens.

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Conflict of interest statement:

ALC has received honoraria from Pharmamar, Pfizer, Novartis, GSK, Lilly and Amgen. AW consults for Eli Lilly, receives research support from Sanofi and his institution receives research support from Amgen, Daiichi-Sankyo, Eli Lilly, Karyopharm, Kolltan, Merck, Plexikon and Sanofi. KH and SM are employees of Sanofi. GT is an employee of Sanofi and holds stock in Sanofi. WZ is a former employee of Sanofi and holds stock in Sanofi. KT is a contractor with Sanofi. JS, MDJ, MD, ML and VDW have no conflicts to declare.

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Supplementary Material

Supplementary Methods

Patients and samples

Pre-treatment surgical or core needle tumour biopsies were obtained from 20 patients participating in the expansion cohort. Tumour biopsies were fixed in formalin and embedded in paraffin (FFPE) blocks. Sample collection for this study was approved by the ethics committees of participating institutions. 5 µm sections were stained with haematoxylin and eosin according to standard procedures and examined by an expert pathologist to confirm the diagnosis of DDLPS and to ensure the presence of at least 30% tumour cells. At least five 10 µm scrolls were cut from each FFPE block and placed into sterile 1.5 mL tubes for DNA extraction. DNA was extracted from tumour biopsies using the QIAamp® DNA FFPE Tissue kit (Qiagen). Blood (3–4 mL) was collected into EDTA tubes from patients at baseline and before treatment on Day 1 of every other cycle for as long as patients participated in the study. Collection of blood samples for plasma isolation and DNA extraction was optional for this study.

Tumour genetic status

MDM2 amplification in baseline tumour biopsies was assessed by TaqMan® quantitative PCR (Applied Biosystems), using *RNaseP* as the reference gene (*MDM2* probe Hs01463512_cn, TaqMan® Copy Number Reference Assay RNase P). Relative quantitation was performed using the $\Delta\Delta C_t$ method and a normal healthy human donor DNA sample was used as the calibrator. Amplification was defined as having greater than five copies of *MDM2* using the mean of triplicate measurements. Tumour mutation profile was assessed using the Ion AmpliSeq™ Cancer Hotspot Panel v2 (Life Technologies). Tumours were sequenced to a median coverage of at least 19,000X. Mutations were called using MuTect, Strelka and SomaticIndelDetector (<http://www.broadinstitute.org/cancer/cga/>). Oncotator was used to annotate mutation calls. Tumours were declared *TP53* wild-type if no non-synonymous mutations were called.

Plasma preparation and cfDNA isolation from plasma

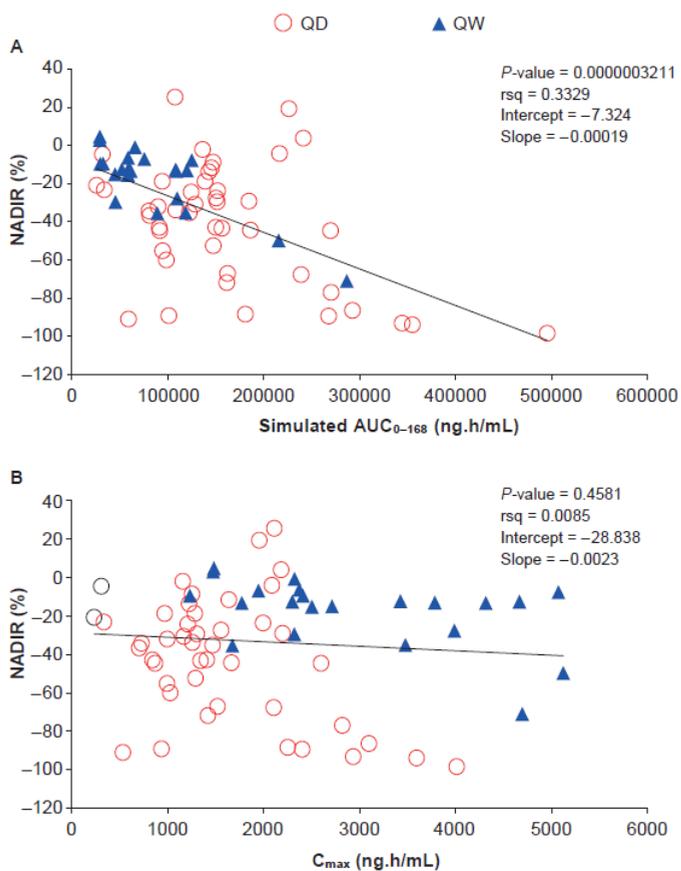
Plasma was prepared at clinical sites within 15–30 minutes from blood draw using double centrifugation as previously described. Blood samples were processed first by centrifugation at 1600 (+ 150) g for 10 minutes. The supernatant was transferred to a fresh 2 mL tube and was centrifuged again at 3000 (+ 150) g for 10 minutes. The resulting supernatant was transferred into a 3.5 mL polypropylene tube and stored at -80°C until cfDNA isolation. This process typically yielded

approximately 1.2 mL of plasma for DNA isolation. For cfDNA isolation, we used either a manual or an automated process. For manual extraction, we used the QIAamp® Circulating Nucleic Acid Kit (QIAGEN, Catalogue # 55114) using the QIAvac 24 Plus (QIAGEN, Catalogue # 19413) according to the manufacturer’s recommended procedures. For automatic cfDNA preparation, we used the QIASymphony DSP Virus/Pathogen Kit (QIAGEN, Catalogue # 937055) using the QIASymphony SP system (QIAGEN, Catalogue # 9001751).

Supplementary Table 1: AEs of any grade, regardless of causality, occurring in >10% of patients and grade ≥ 3 AEs occurring in >2% of patients

Preferred term, n (%)	Daily dosing (n = 45)	Weekly dosing (n = 29)	All (n = 74)
Any AE	43 (96)	29 (100)	72 (97)
Nausea	23 (51)	21 (72)	44 (59)
Fatigue	24 (53)	19 (66)	43 (58)
Vomiting	15 (33)	16 (55)	31 (42)
Diarrhoea	14 (31)	13 (45)	27 (36)
Decreased appetite	13 (29)	12 (41)	25 (34)
Constipation	11 (24)	9 (31)	20 (27)
Dyspnoea	9 (20)	7 (24)	16 (22)
Cough	6 (13)	7 (24)	13 (18)
Dry mouth	6 (13)	7 (24)	13 (18)
Thrombocytopenia	9 (20)	2 (7)	11 (15)
Abdominal pain	8 (18)	2 (7)	10 (14)
Headache	6 (13)	4 (14)	10 (14)
Dyspepsia	7 (16)	3 (10)	10 (14)
Anaemia	5 (11)	4 (14)	9 (12)
Dry skin	6 (13)	3 (10)	9 (12)
Pyrexia	4 (9)	5 (17)	9 (12)
Dysgeusia	4 (9)	4 (14)	8 (11)
Any grade ≥ 3 AE	15 (33)	8 (28)	23 (31)
Thrombocytopenia	6 (13)	0	6 (8)
Neutropenia	2 (4)	1 (3)	3 (4)
Fatigue	2 (4)	0	2 (3)
General physical health deterioration	0	2 (7)	2 (3)

AE, adverse event.



Supplementary Figure 1: Correlation between SAR405838 PK parameters and platelet count. A) AUC_{0-168} ; B) C_{max} . AUC, area under the concentration–time curve; C_{max} , maximum concentration; PK, pharmacokinetics; QD, once daily; QW, once weekly.



Chapter 4.2

**A phase I study of the HDM2 antagonist SAR405838
combined with the MEK inhibitor pimasertib in patients
with advanced solid tumors.**

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Abstract

Background: The combination of a MEK1/2 inhibitor and a HDM2 inhibitor has shown a synergistic effect in pre-clinical studies. This phase I, open-label, dose-escalation study evaluated the safety, of combination therapy with the HDM2 inhibitor SAR405838 and the MEK1/2 inhibitor pimasertib administered orally once daily (QD) or twice daily (BID) in advanced solid tumors.

Methods: Patients with locally advanced or metastatic solid tumors with documented wild type *TP53* and *RAS* or *RAF* mutations were enrolled. Primary endpoints were maximum tolerated dose (MTD) and recommended phase II dose. Secondary endpoints included safety, pharmacokinetics (PK) and pharmacodynamics (PD) and tumor response.

Results: Twenty-six patients were treated with SAR405838 200 or 300 mg QD plus pimasertib 60 mg QD or 45 mg BID. The MTD was SAR405838 200 mg QD plus pimasertib 45 mg BID (highest total daily dose of pimasertib). The most common dose-limiting toxicity (DLT) was thrombocytopenia. The most frequently occurring treatment-related adverse events (AEs) were diarrhea (81%), increased blood creatine phosphokinase (77%), nausea (62%) and vomiting (62%). No significant drug–drug interactions were observed. The PD biomarkers MIC-1 and pERK were respectively upregulated and down regulated in response to study treatment. In 24 efficacy-evaluable patients, 1 patient (4%) had a partial response and 63% had stable disease (SD).

Conclusion: Although SAR405838 and pimasertib could not be administered at the single-agent MTDs when combined, preliminary antitumor activity suggests potential added benefit of restoring p53 activity while inhibiting the MAPK pathway in *TP53* wild-type and *MAPK*-mutated malignancies (67% of patients achieved \geq SD).

Introduction:

The tumor suppressor p53 has a pivotal role in preventing tumorigenesis through the induction of cell cycle arrest and apoptosis [1]. *TP53* is the most frequently mutated gene in human cancer; however, some tumors still harbor wild-type *TP53* [2]. In these cases, wild-type *TP53* function is usually inhibited by the negative regulator mouse double minute 2 (MDM2, HDM2 in humans). HDM2 binds to the transactivation domain of p53 and acts as a p53-specific E3 ubiquitin ligase, leading to degradation in the proteasome. Overexpression of HDM2 has been reported in various tumor types, and small-molecule inhibitors of HDM2 have demonstrated antitumor activity in preclinical studies [3,4].

SAR405838 is an oral, selective, spirooxindole derivative antagonist of HDM2 [4]. SAR405838 treatment results in p53 pathway activation, leading to p53-dependent cell-cycle arrest and apoptosis in preclinical models [4]. SAR405838 monotherapy was investigated in a phase I dose-escalation study in patients with solid tumors, including a maximum tolerated dose (MTD) expansion cohort of patients with de-differentiated liposarcoma (DDLPS) [5]. The main dose-limiting toxicity (DLT) observed was thrombocytopenia; the MTD and recommended phase II dose (RP2D) of SAR405838 was 300 mg once daily (QD) [5].

MEK is a key component of the MAPK signaling pathway, which is integral to the proliferation and survival of cancer cells [6]. Activation of the GTPase RAS leads to a phosphorylation cascade via the kinases RAF, MEK and ERK, that results in the activation of oncogenic gene expression. Activating mutations in the upstream components B-RAF or RAS (K-RAS, N-RAS, and H-RAS) are the most frequent cause of upregulation of the MAPK signalling pathway [7]. However, targeting RAS directly has been unsuccessful to date. Therefore, strategies for inhibiting the MAPK pathway have focused on inhibiting B-RAF and MEK (MEK1 and MEK2 isoforms). MEK inhibitors have demonstrated encouraging activity in preclinical studies and preliminary clinical activity in solid tumors exhibiting RAS pathway activation [8].

Pimasertib is an oral, selective, small-molecule inhibitor of MEK1/2. In a phase I dose-escalation trial DLTs included skin rash, acneiform dermatitis, ocular events and stomatitis [9]. The MTD for pimasertib monotherapy using either a QD or a twice-daily (BID) regimen is 90 mg and 60 mg, respectively; the recommended phase II dose (RP2D) of pimasertib monotherapy was determined at 60 mg BID. Pimasertib is currently being investigated in phase I/II clinical trials in a number of tumor types, and has demonstrated preliminary clinical activity as monotherapy and in combination with other agents [10,11].

Together, the p53 and MAPK pathways are the most frequently mutated tumor suppressor and oncogene pathways. Preclinical studies have provided rationale to test the combination of SAR405838 and pimasertib in tumors with wild-type *TP53* and MAPK pathway activation. In preclinical

RAS pathway-activated, *TP53* wild-type xenograft melanoma models (UACC62), a therapeutic benefit was observed for the SAR405838 and pimasertib combination over the activity of either single agent; durable tumor regression was observed with the combination [12].

This phase I, dose-escalation study evaluated the safety, pharmacokinetics (PK) and pharmacodynamics (PD) of SAR405838 combined with pimasertib administered QD or BID in advanced solid tumors.

Methods:

Study design

This was a Phase I, open-label, dose-escalation, safety, PK, and PD study of SAR405838 administered QD and combined with pimasertib administered either QD or BID in 21-day cycles in adult patients with advanced solid tumors (NCT01985191). Both study medications were administered orally using a gelatin capsule formulation. Patients fasted for 2 hours prior to and 1 hour after each dose. Each dose of SAR405838, except at Cycle 1 Day 1, was to be taken immediately after pimasertib administration, preferably in the morning of each day. Treatment could have continued until precluded by toxicity, noncompliance, progression, or death.

The primary endpoints were MTD and RP2D of SAR405838 and pimasertib combination therapy in patients with locally advanced or metastatic solid tumors. Secondary endpoints included safety, PK, PD, tumor response as well as determination of the impact of study combination regimen on the genetic status of *TP53/RAS* when compared with baseline.

Patient population

Patients eligible for inclusion were ≥ 18 years of age with a histologically or cytologically confirmed solid tumor with documented wild type *TP53* and *RAS/RAF* mutations, for which no further effective standard treatment was available. Eligible patients had locally advanced or metastatic disease with at least one measurable lesion defined by Response Evaluation Criteria in Solid Tumors (RECIST) Version 1.1 [13], an Eastern Cooperative Oncology Group performance status (ECOG PS) of 0 to 1, life expectancy ≥ 12 weeks, and sufficient bone marrow function.

This clinical trial adhered to the principles outlined in the Helsinki declaration and was conducted in compliance with all applicable international and national laws and regulations. The protocol was approved by all relevant Institutional Review Boards/Independent Ethics Committees. All patients provided written, informed consent.

Dose escalation and dose-limiting toxicities

A 3+3 design was used for dose escalation. The starting dose was SAR405838 200 mg QD/pimasertib 45 mg BID. Cohorts of 3 to 6 patients were enrolled sequentially in ascending dose levels (DLs) per the protocol and decisions of the Study Committee (Investigators and Sponsor) based on the occurrence of DLTs within the first two cycles. Patient should have taken at least 80% of each study medication in order to be evaluable for DLT, unless precluded by the occurrence of DLT. After confirmation of safety at the first DL1 of SAR405838 200 mg QD/pimasertib 45 mg BID, dose escalation was pursued independently and concomitantly according to the schedule of pimasertib. Using the pimasertib QD schedule, dose escalation was to sequentially proceed to DL2a (SAR405838 200 mg QD/pimasertib 60 mg QD) then DL3a (SAR405838 300 mg QD/pimasertib 60 mg QD). Using the pimasertib BID schedule, dose escalation was to sequentially proceed to DL2b (SAR405838 300 mg QD/pimasertib 45 mg BID) then DL3b (SAR405838 300 mg QD/pimasertib 60 mg BID). If 1 of 3 patients experienced a DLT in the first 2 cycles, the cohort was expanded to 6 patients for confirmation. If a DLT was observed in at least 2 out of a maximum of 6 patients at a DL, this was considered the maximum administered dose. The MTD was the highest DL where at most 1 patient of the cohort experienced a DLT.

A DLT was defined as any of the following drug-related adverse events (AEs) occurring during the first 2 cycles of treatment (Days 1 to 42): an AE that in the opinion of the safety committee was of potential clinical significance such that further dose escalation would expose patients to unacceptable risk; any grade ≥ 3 non-hematological toxicity (excluding grade 3 fatigue persistent for less than 7 days, grade 3 vomiting or diarrhea if controlled within 2 days with adequate therapy, asymptomatic grade 3 creatinine phosphokinase [CPK] elevation, grade 3 aspartate aminotransferase/alanine aminotransferase [AST/ALT] elevations < 7 days in duration, grade 3/4 alkaline phosphatase [ALP] elevations in the context of bone metastasis, or grade 3 hypertension that can be controlled within a week with oral antihypertensives); any grade ≥ 3 thrombocytopenia; any grade 4 neutropenia or febrile neutropenia; any grade 4 anaemia; retinal vein occlusion; left-ventricular ejection fraction decrease $> 20\%$ from baseline or a decrease $> 10\%$ if baseline ejection fraction is 50%; Hy's law; any treatment delay > 2 weeks due to drug-related adverse effects; any severe or life-threatening complication or abnormality not defined in National Cancer Institute Common Terminology Criteria for Adverse Events (NCI-CTCAE) that is attributable to the therapy; and any toxicities resulting in an inability to complete at least 80% of planned trial medication doses during the first 2 cycles.

Safety assessments

Safety was assessed by the evaluation of AEs, DLTs, changes in vital signs, ECOG PS, physical examinations, 12-lead electrocardiograms (ECGs), determination of left-ventricular ejection fraction (LVEF), ophthalmologic examinations, and clinical laboratory tests (including hematology, coagulation, blood chemistry, and urinalysis). AEs were graded according to NCI-CTCAE version 4.03 [14].

Pharmacokinetic assessments

Blood samples were collected on Days 1 (for pimasertib only), 2, 3, 8, and 15 of Cycle 1, on Days 1 and 2 of Cycle 2, and on Day 1 of Cycles 3 and 4, to determine the whole blood and plasma concentrations of SAR405838 and pimasertib, respectively. Calculation of PK parameters included maximum concentration (C_{max}), time to reach maximum concentration (t_{max}), time corresponding to the last concentration above the lowest limit of quantification (t_{last}), area under the concentration versus time curve (AUC) from time 0 to time t (AUC_{0-t} ; 12 hours for BID or 24 hours for QD), AUC from time 0 to t_{last} (AUC_{last}), AUC extrapolated to infinity, AUC over the dosing interval (AUC_{tau}), and terminal half-life ($t_{1/2z}$).

Pharmacodynamic assessments

Blood samples for peripheral PD biomarkers analyses for both SAR405838 and pimasertib, including macrophage inhibitory cytokine-1 (MIC-1) levels in plasma and phosphorylated extracellular signal-regulated kinases (pERK) levels in peripheral blood mononuclear cell (PBMC), were collected on Days 1, 2, 3, 8, and 15 of Cycle 1, on Days 1 and 2 of Cycle 2, and Day 1 of Cycles 3 and 4. MIC-1 protein concentrations were measured in plasma samples using an analytically validated Enzyme-Linked ImmunoSorbent Assay (ELISA) assay (Quantikine® Human GDF-15 immunoassay). pERK levels were determined by evaluating median value changes in response to ex-vivo stimulation with or without Phorbol 12-Myristate 13-Acetate (PMA) over time in whole blood samples (CD 45+ and Lymphocyte populations) by flow cytometry (BD FACSCanto™ II instrument). Stimulated pERK levels were calculated as % (PMA-DMSO)/DMSO.

Molecular profiling assessments

Plasma preparation and cell free DNA (cfDNA) isolation from plasma

Plasma was prepared at clinical sites within 15 to 30 minutes from blood draw using double centrifugation as described in [15]. Blood samples were processed first by centrifugation at 1600 (+ 150) g for 10 minutes. The supernatant was transferred to a fresh 2 ml tube and was centrifuged again at 3000 (+ 150) g for 10 minutes. The resulting supernatant was transferred into a 3.5 ml polypropylene tube and stored at -80°C until cfDNA isolation. This process typically yielded

approximately 1.2 ml of plasma for DNA isolation. For cfDNA isolation, we used the QIAamp® Circulating Nucleic Acid Kit (QIAGEN, Catalog # 55114) using the QIAvac 24 Plus (QIAGEN, Catalog # 19413) according to the manufacturer's recommended procedures.

Targeted sequencing library preparation and mutation analysis

In order to monitor tumor genetic status using liquid biopsies, we developed a targeted deep sequencing assay for mutation detection based on a hybrid-capture target enrichment strategy [15]. Mutation analysis was done as previously described [15].

Efficacy assessments

Radiological tumor assessments were made at least every 2 cycles or less frequently, if indicated. Tumor response was investigator assessed using RECIST 1.1. A partial response (PR) or complete response (CR) must have been confirmed on a second examination performed at least 4 weeks apart, in order to be documented as a confirmed response.

Results:

Patient population

Twenty-six patients with advanced cancer were treated: DL1 SAR405838 200 mg QD/pimasertib 45 mg BID (n = 7); DL2a SAR405838 200 mg QD/pimasertib 60 mg QD (n = 4); DL2b at SAR405838 300 mg QD/pimasertib 45 mg BID (n = 7); and DL3a SAR405838 300 mg QD/pimasertib 60 mg QD (n = 8). Patient baseline characteristics are shown in Table 1. Median age was 59.5 years (range 45–79 years). Patients had advanced cancer diagnoses, most common tumor types being colorectal (42%) or non-small cell lung cancer (31%). Median number of prior anticancer therapies was 3 (range 1–7). Twenty-one of the 26 (81%) patients discontinued the study due to disease progression and 5 (19%) discontinued due to AEs.

Table 1: Patient baseline characteristics

	All patients (n = 26)
Median age, years (range)	59.5 (45–79)
Male, n (%)	16 (62)
ECOG PS, n (%)	
0	10 (38)
1	16 (62)
Tumor type, n (%)	
Colorectal	11 (42)
NSCLC	8 (31)
Melanoma	1 (4)
Other	6 (23)
Median number of prior anticancer therapies (range)	3 (1–7)
Tumor molecular status, n (%)	
KRAS mutations	24 (92)
BRAF mutations	1 (4)
NRAS mutations	1 (4)

ECOG PS, Eastern Cooperative Group performance status, NSCLC, non-small cell lung cancer.

Dose escalation and dose-limiting toxicities

Three patients experienced a DLT out of 12 DLT-evaluable patients treated with the BID pimasertib-based regimen: one of 6 evaluable patients experienced 2 DLTs at DL1 (grade 2 pustular rash and grade 2 thrombocytopenia that led to dose interruptions and <80% of dose completion), and 2 of 6 evaluable patients experienced 1 DLT at DL2b (1 patient with grade 2 thrombocytopenia, for which the SAR405838 dose was interrupted and not resumed, and 1 patient with grade 4 increased lipase). DL3b was not tested.

One patient experienced a DLT out of 8 DLT-evaluable patients treated with the QD pimasertib regimen: no DLT occurred in 3 DLT-evaluable patients treated at DL2a, and 1 of 5 DLT-evaluable patients experienced a DLT at DL3a (grade 3 thrombocytopenia). At that same dose level, an AE meeting DLT definition (grade 2 thrombocytopenia) occurred in cycle 4, post-DLT evaluation period that led to discontinuation of study medication in an additional patient. A sixth patient was not recruited to complete the cohort as DL3a was considered not tolerated.

The MTD was SAR405838 200 mg QD plus pimasertib 45 mg BID (highest total daily dose of pimasertib).

Table 2: Treatment-related AEs (related to SAR405838 and/or pimasertib) of any grade occurring in >20% of patients and all treatment-related grade ≥ 3 AEs

Preferred term, n (%)	SAR405838 200 mg QD + pimasertib 45 mg BID (n = 7)	SAR405838 200 mg QD + pimasertib 60 mg QD (n = 4)	SAR405838 300 mg QD + pimasertib 45 mg BID (n = 7)	SAR405838 300 mg QD + pimasertib 60 mg QD (n = 8)	All patients (n = 26)
Any treatment-related AE	7 (100)	4 (100)	7 (100)	8 (100)	26 (100)
Diarrhea	6 (86)	2 (50)	7 (100)	6 (75)	21 (81)
Blood CPK increased	6 (86)	3 (75)	6 (86)	5 (63)	20 (77)
Nausea	2 (29)	4 (100)	4 (57)	6 (75)	16 (62)
Vomiting	5 (71)	3 (75)	4 (57)	4 (57)	16 (62)
Edema peripheral	5 (71)	2 (50)	3 (43)	1 (13)	11 (42)
Fatigue	2 (29)	1 (25)	4 (57)	2 (25)	9 (35)
Dermatitis acneiform	4 (57)	0	4 (57)	1 (13)	9 (35)
Decreased appetite	3 (43)	2 (50)	3 (43)	1 (13)	9 (35)
Ejection fraction decreased	4 (57)	1 (25)	3 (43)	1 (13)	9 (35)
Rash	2 (29)	2 (50)	2 (29)	2 (25)	8 (31)
Retinal detachment	4 (57)	0	4 (57)	0	8 (31)
Rash pustular	2 (29)	1 (25)	2 (29)	2 (25)	7 (27)
Macular detachment	3 (43)	0	2 (29)	2 (25)	7 (27)
Stomatitis	4 (57)	1 (25)	0	2 (25)	7 (27)
Folliculitis	3 (43)	0	2 (29)	1 (13)	6 (23)
Any grade ≥ 3 treatment-related AE	5 (71)	1 (25)	4 (57)	4 (50)	14 (54)
Blood CPK increased	2 (29)	0	1 (14)	0	3 (12)
Fatigue	1 (14)	0	1 (14)	1 (13)	3 (12)
Thrombocytopenia	0	0	1 (14)	2 (25)	3 (12)
Amylase increased	0	1 (14)	1 (25)	0	2 (8)
Lipase increased	1 (14)	0	1 (14)	0	2 (8)
Diarrhea	0	0	0	1 (13)	1 (4)
Dermatitis acneiform	1 (14)	0	0	0	1 (4)
Ejection fraction decreased	1 (14)	0	0	0	1 (4)
Oral fungal infection	0	0	1 (14)	0	1 (4)
Pneumonitis	0	0	1 (14)	0	1 (4)
Stomatitis	1 (14)	0	0	0	1 (4)
Cheilitis	1 (14)	0	0	0	1 (4)
Aphthous ulcer	1 (14)	0	0	0	1 (4)
Palmar-plantar erythrody- saesthesia syndrome	1 (14)	0	0	0	1 (4)
Asthenia	0	0	1 (14)	0	1 (4)

AE, adverse event; CPK, creatine phosphokinase.

Safety

Mean duration of treatment was 21 weeks (7 cycles per patient) for SAR405838 and pimasertib. The most frequently occurring AEs regardless of causality were diarrhea (81%), increased blood CPK (77%), vomiting (73%), nausea (69%) and fatigue (58%). The most frequently occurring grade ≥ 3 AEs regardless of causality were pulmonary embolism, fatigue and thrombocytopenia (15% each). The most frequently occurring treatment-related AEs were diarrhea (81%), increased blood CPK (77%), nausea (62%) and vomiting (62%; Table 2). The most frequently occurring grade ≥ 3 treatment-related AEs were increased blood CPK (12%), fatigue (12%) and thrombocytopenia (12%; Table 2). AEs of interest included increased blood CPK (77%; grade ≥ 3 12%), decreased ejection fraction

Table 3: Summary of PK parameters for SAR405838 and pimasertib when administered in combination

Cohort	Cycle/ Day	SAR405838 PK parameter, median [range](n) or mean \pm SD (n; CV%)			
		t_{max} , h	C_{max} , ng/mL	C_{max} , Rac	
DL1	C1D2	2 [1.5–6] (7)	1570 \pm 876 (7; 56)	0.71 \pm 0.31 (6; 43)	
	C2D1	1.8 [1.5–4] (6)	1250 \pm 668 (6; 54)	–	
DL2a	C1D2	2 [1.5–6] (3)	1500 \pm 634 (3; 42)	0.97 \pm 0.4 (3; 42)	
	C2D1	2.8 [1.5–4] (4)	1050 \pm 581 (4; 55)	–	
DL2b	C1D2	3 [2–4] (6)	1100 \pm 547 (6; 50)	0.92 \pm 0.37 (5; 41)	
	C2D1	1.5 [0–2] (6)	1100 \pm 361 (6; 33)	–	
DL3a	C1D2	4 [4–24] (7)	1490 \pm 939 (7; 63)	1.3 \pm 0.54 (5; 41)	
	C2D1	2 [1.5–4] (6)	1440 \pm 583 (6; 40)	–	
Cohort	Cycle/ Day	Pimasertib PK parameter, median [range](n) or mean \pm SD (n; CV%)			
		t_{max} , h	C_{max} , ng/mL	C_{max} , Rac ^a	C_{max} , combination effect ^a
DL1	C1D1	0.5 [0.5–2] (7)	218 \pm 108 (7; 49)	1.1 \pm 0.28 (6; 25)	1.1 \pm 0.78 (7; 73)
	C1D2	1.5 [0.5–2] (7)	200 \pm 102 (7; 51)	---	---
	C2D1	1.5 [0.5–1.5] (6)	226 \pm 70.6 (6; 31)	---	---
DL2a	C1D1	1.5 [0.5–1.5] (4)	301 \pm 69.9 (4; 23)	0.97 \pm 0.39 (4; 41)	0.97 \pm 0.24 (4; 24)
	C1D2	1 [0.5–2] (4)	303 \pm 134 (4; 44)	---	---
	C2D1	1.5 [1.5–2] (4)	269 \pm 134 (4; 50)	---	---
DL2b	C1D1	1.5 [0.5–1.5] (7)	184 \pm 109 (7; 59)	1.2 \pm 1.1 (6; 87)	0.99 \pm 0.25 (7; 26)
	C1D2	1.5 [1.5–2] (7)	182 \pm 110 (7; 61)	---	---
	C2D1	1.5 [0.5–2] (6)	147 \pm 93.8 (6; 64)	---	---
DL3a	C1D1	1 [0.5–4] (8)	221 \pm 98.3 (8; 44)	1.8 \pm 1.1 (4; 61) ^b	1.1 \pm 0.41 (7; 39)
	C1D2	1.5 [0.5–2] (7)	200 \pm 84.4 (7; 42)	---	---
	C2D1	1.5 [0.5–1.5] (5)	243 \pm 65.4 (5; 27)	---	---

^a Rac = C2D1 vs C1D2 ratio; combination effect = C1D2 vs C1D1 ratio. ^b Mean Rac high due to 2 patient exhibiting low C_{max} on C1D1

(38%; grade ≥ 3 4%), retinal detachment (31%; no grade ≥ 3), macular detachment (27%; no grade ≥ 3), thrombocytopenia (19%; grades ≥ 3 15%) and increased troponin T (19%; no grade ≥ 3). The most common grade ≥ 3 hematologic laboratory abnormality was lymphocyte count decreased (19%); the most common grade ≥ 3 biochemistry laboratory abnormality was increased ALP (15%). Fourteen patients (54%) had a serious AE (SAE), most commonly disease progression (12%), constipation (8%) and accidental overdose (8%). Four patients (15%) had a treatment-related SAE, including macular detachment, pneumonitis, diarrhea, nausea, vomiting and accidental overdose (4% each). Five patients (19%) had an AE leading to permanent treatment discontinuation, including rash pustular, dyspnea, nausea, vomiting, increased blood CPK, and ECG T-wave inversion (4% for

AUC_{0-24'} ng.h/mL	AUC_{0-24'} Rac	t_{1/2'} h
19300 ± 8570 (7; 44)	0.68 ± 0.27 (6,40)	16.1 ± 7.75 (3; 48)
14600 ± 7080 (6; 49)	–	10.8 (2)
20800 ± 7540 (3; 36)	1.1 ± 0.53 (3,50)	8.86 (1)
15600 ± 8720 (4; 56)	–	10.0 (1)
16300 ± 10300 (6; 63)	1.1 ± 0.83 (5,77)	15.5 ± 4.33 (3; 28)
15000 ± 4650 (6; 31)	–	13.4 ± 2.38 (3; 18)
21400 ± 13500 (7; 63)	1.3 ± 0.68 (5,53)	13.3 ± 4.94 (3; 37)
21200 ± 13700 (6; 64)	–	14.9 ± 11.1 (3; 74)

AUC_{0-12'} ng.h/mL	AUC_{0-12'} Rac^a	AUC_{0-12'} combination effect^a	t_{1/2'} h
755 ± 382 (7; 51)	1.2 ± 0.3 (6; 26)	0.94 ± 0.21 (7; 22)	4.76 ± 1.17 (7; 24)
689 ± 324 (7; 47)	---	---	NR
862 ± 350 (6; 41)	---	---	NR
1120 ± 410 (4; 37)	0.95 ± 0.34 (4; 36)	1 ± 0.13 (4;12)	5.38 ± 0.276 (4; 5)
1130 ± 422 (4; 37)	---	---	NR
1040 ± 504 (4; 48)	---	---	NR
779 ± 594 (7; 76)	0.94 ± 0.36 (6; 38)	0.95 ± 0.17 (7; 18)	4.71 ± 1.39 (7; 30)
684 ± 367 (7; 54)	---	---	NR
532 ± 166 (6; 31)	---	---	NR
935 ± 469 (8; 50)	1.1 ± 0.25 (4; 24)	1.1 ± 0.16 (7; 14)	5.25 ± 1.83 (7; 35)
917 ± 390 (7; 43)	---	---	NR
765 ± 314 (5; 41)	---	---	NR

AUC, area under the curve; C_{max}, maximum concentration; CV, coefficient of variance; DL, dose level; NR, not reported; PK, pharmacokinetic; SD, standard deviation; t_{1/2'}, terminal half-life; t_{max'}, time to maximum concentration.

4.2

each), and fatigue (8%). Additionally, 17 patients had SAR405838 dose modification, reduction, or omission and 18 patients had pimasertib dose modification, reduction, or omission. Four patients died during the study, with three deaths occurring in the post-treatment period. All deaths were due to disease progression with no treatment-related deaths reported.

Pharmacokinetics

Table 3 summarizes SAR405838 and pimasertib PK parameters. Figure 1 shows SAR405838 and pimasertib concentration-time profiles. PK of SAR405838 and pimasertib when administered in combination was similar to the PK of SAR405838 or pimasertib when administered as monotherapy. Due to respective drug variability, only a substantial drug-drug interaction may have been evidenced. The dose increase (50%) between dose levels DL1/DL2a (SAR405838 200 mg) and dose levels DL2b/DL3a (SAR405838 300 mg) did not result in SAR405838 exposure increase. The dose increase (33%) between dose levels DL1/DL2b (pimasertib 45 mg BID) and dose levels DL2a/DL3a (pimasertib 60 mg BID) resulted in pimasertib exposure increase.

Pharmacodynamics

Inhibition of pERK was evaluated in PMA-stimulated PBMCs; $\geq 80\%$ pERK inhibition was observed at C1D1, C1D2 and C2D1, at or around the t_{max} of pimasertib, at most dose levels and was maintained during 4 hours (Figure 2). Pimasertib dosing at 45 mg and 60 mg induced similar inhibition, which was not affected by SAR405838 administration.

Induction of MIC-1, a non-tumor specific soluble protein regulated by p53, was evaluated.

MIC-1 elevation (mean 3.5 +/- 0.8 fold vs baseline) was observed in all patients at C1D2 and C2D1, peaking at 6 hours on both days. SAR405838 dosing at 200 or 300 mg induced a similar increase, which was not affected by pimasertib administration (Figure 3). A patient with confirmed PR (endometrial tumor) had the greatest increase in MIC-1 (8.8-fold) in the DL1 cohort.

Molecular profiling

The mutation status of *TP53*, *RAS* family genes and *BRAF* in ctDNA derived from plasma of 25 patients (one sample missing) was used to correlate with that from tumor tissue at screening. There was a partial concordance between tumor tissue (data provided by the clinical sites) and plasma ctDNA data collected at screening. 16/25 plasma samples had detectable level of *KRAS* (n = 14), *BRAF* (n = 1) or *NRAS* (n = 1) mutations which were 100% concordant to reported tumor DNA genotype. 3/25 plasma samples had detectable mutations in *TP53* gene with a frequency ranging from 0.35% to 18%).

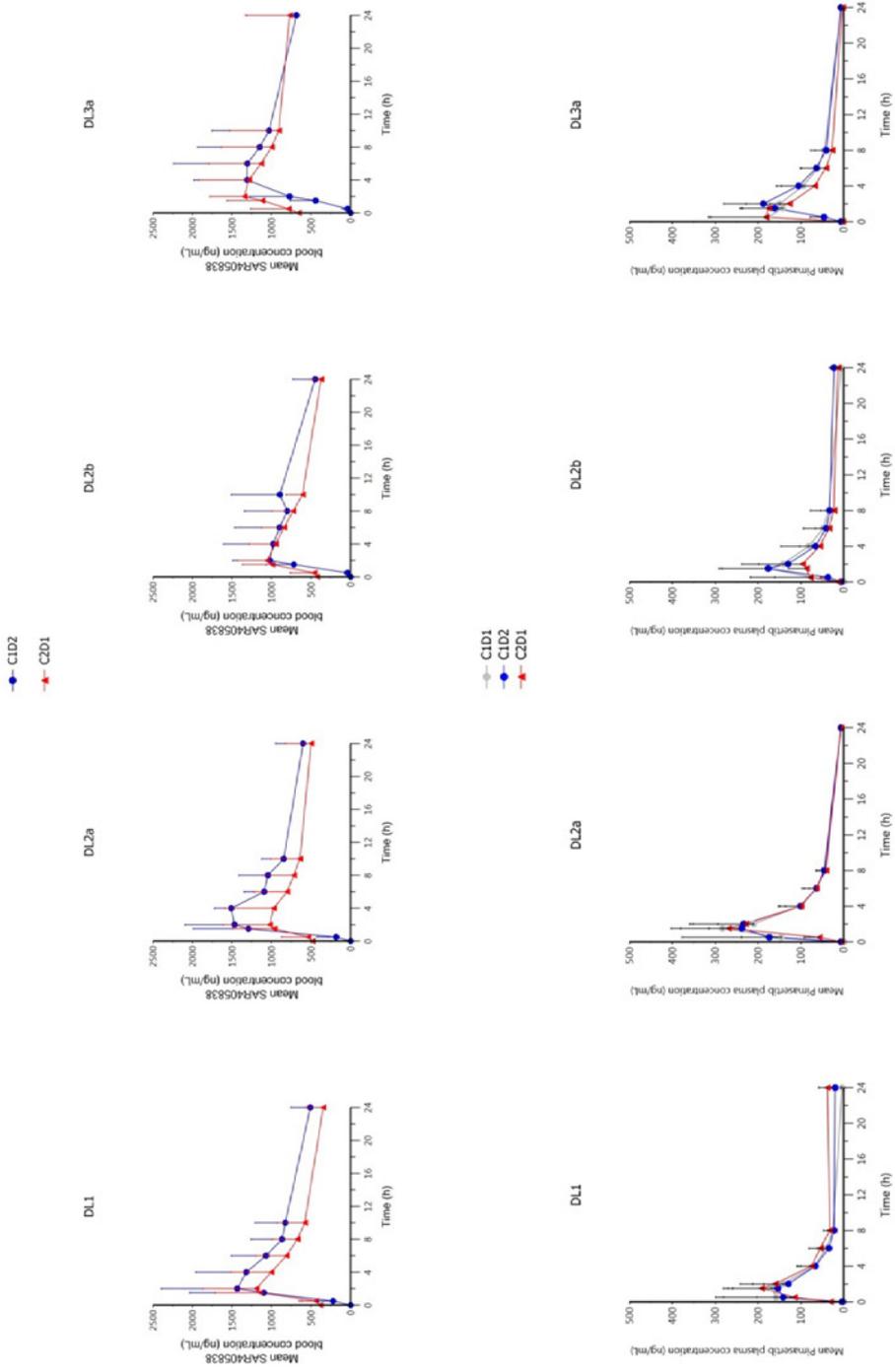


Figure 1: Mean plasma concentration–time profiles for A) SAR405838 and B) pimasertib

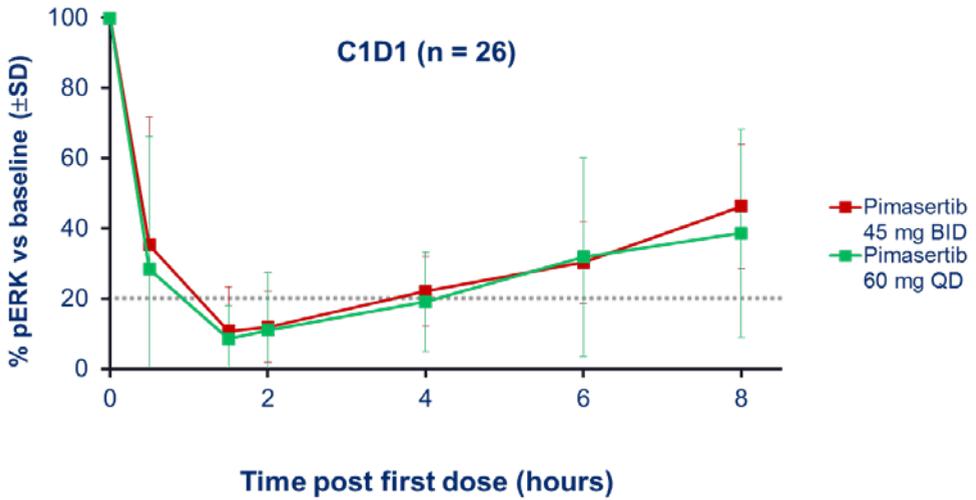


Figure 2: Inhibition of pERK in PMA-stimulated PBMCs
 BID, twice daily; ERK, extracellular signal-regulated kinase; PBMC, peripheral blood mononuclear cell; PMA, phorbol myristate acetate; QD, once daily; SD, standard deviation.

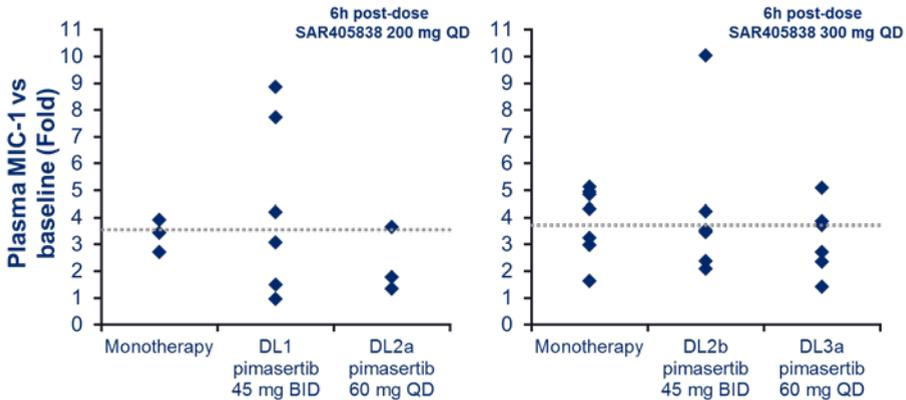


Figure 3: Induction of MIC-1 at steady state (between 1 and 3 weeks of administration).
 Each point represents an individual patient.
 BID, twice daily; DL, dose level; MIC-1, macrophage inhibitory cytokine 1; QD, once daily.

Mutation status was also analyzed to look at the emergence of mutations after study treatment. Samples from 13 patients were collected. *de novo* TP53 mutations were only seen in two patients after study treatment and this occurred after cycle 3.

Efficacy

In 24 efficacy-evaluable patients, the best overall response was PR for 1 patient (4%) with endometrial adenocarcinoma (stage III) at SAR405838 200 mg QD/pimasertib 45 mg BID. Stable disease (SD) was the best overall response observed for 15 patients (63%), including patients with colon (7 patients, 4 at stage IV and 1 each at stages I, II, and III), lung (5 patients, all stage IV), pancreatic (1 patient, stage IV), and intrahepatic bile duct (1 patient, stage IV) cancer, and skin melanoma (1 patient, stage I). Six patients had prolonged SD of > 6 months. Eight patients (33%) had progressive disease as best response. Best percentage change in target lesion diameters is shown in Figure 4; changes in target lesion diameters were variable and were generally not dose-dependent; however, tumor shrinkage was mostly observed in the pimasertib BID-based regimens. Duration of treatment is shown in Figure 5.

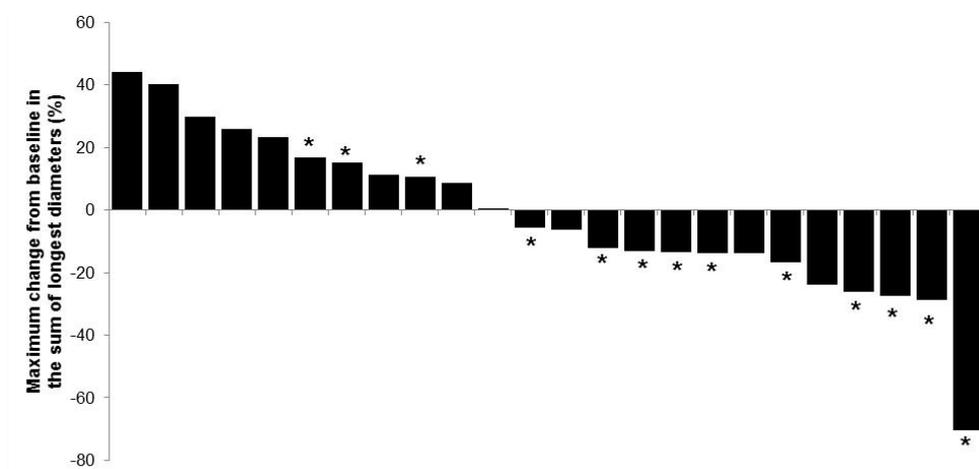


Figure 4: Maximum percentage change from baseline in the sum of target lesion diameters.

Dotted lines represent threshold required for partial response (-30%) and progressive disease (+20%).

* Pimasertib BID.

Discussion:

This phase I, dose-escalation study evaluated the safety, PK and PD of combination therapy with SAR405838 and pimasertib (QD and BID) in advanced solid tumors. The MTD was SAR405838 200 mg QD plus pimasertib 45 mg BID. The main DLT was thrombocytopenia. The safety observations of thrombocytopenia are consistent with other drugs in the HDM2 antagonist class [16–18], and the mechanism of action [19].

At the MTD, significant dose interruptions and reductions occurred after cycle 2 due to late toxicities and poor tolerance. Single-agent MTDs of SAR405838 and pimasertib could not be administered in

combination, due to overlapping toxicity. The most common treatment-related AEs were diarrhea and blood CPK increase. Compared with pimasertib alone, there seems to be an increase in rate of occurrence of CPK increase and drop in LVEF, mainly observed with the BID schedule [9]. This increased toxicity is likely based on a pharmacodynamic interaction, with SAR405838 and is to be attributed to activation of the p53 pathway leading to an increase in apoptosis in healthy tissue. Compared with pimasertib BID regimen, the QD regimen induced less diarrhea, vomiting, electrolytes imbalance, skin reaction, ocular events, CPK increase and drop in LVEF. In addition, AEs meeting DLT definition occurring late and responsible for dose delay and reduction occurred in the BID-pimasertib schedule. Therefore the QD schedule appeared to be better tolerated; however, patients had a shorter duration of treatment compared with the BID schedule

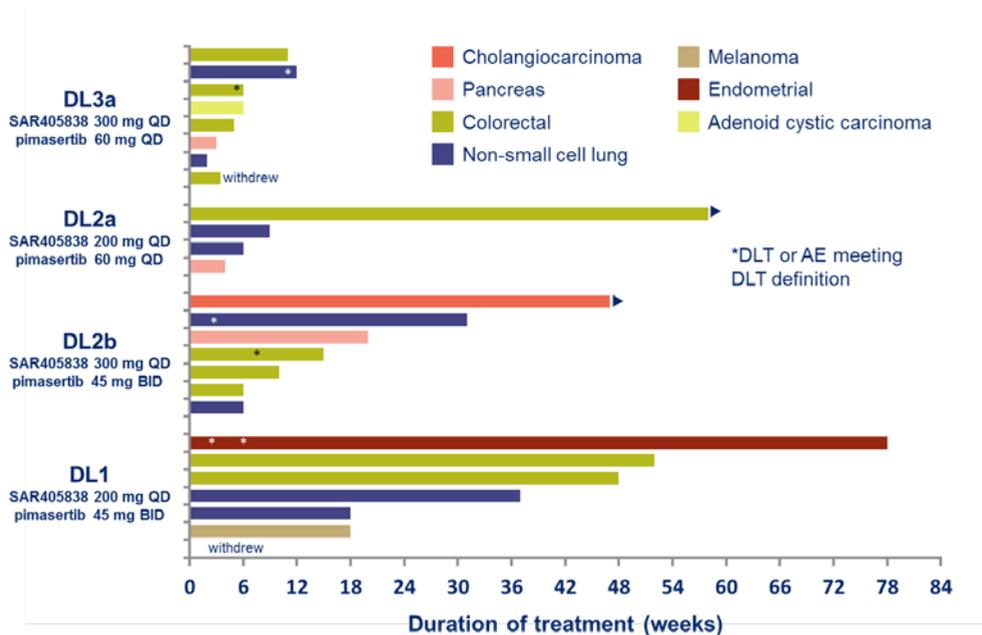


Figure 5: Duration of treatment
 AE, adverse event; BID, twice daily; DL, dose level; DLT, dose-limiting toxicity; QD, once daily.

No significant drug–drug interactions were observed. Considering respective variability of SAR405838 and pimasertib, the PK profiles were generally consistent with previous data for each drug alone. Only a substantial drug–drug interaction may have been evidenced. PD effect of both agents was demonstrated at all doses tested. The PD biomarkers MIC-1 and pERK were respectively upregulated and down regulated in response to study treatment.

There was only 64% concordance for *KRAS* mutation presence between tumor tissue and plasma samples where *KRAS* mutations were reported in the tumor but not in the plasma samples. There were also some discrepancies for *TP53* mutations where 3 mutations were detected in plasma and not in the 3 patient tumor samples. This could be explained by the difference in date of collection for both samples or difference in assay sensitivity. For *KRAS* mutations discrepancies, tissue heterogeneity where a rare mutated clone would have been detected in a specific region of the tumor but not found in the plasma sample could also be a relevant explanation.

The best overall response was PR for 1 patient with an endometrial tumor. For the majority of patients (63%) the best overall response was SD. Prolonged SD (> 6 months) was observed in 4 patients. Preliminary antitumor activity suggests potential for restoring p53 activity while inhibiting the MAPK pathway in TP53 wild-type malignancies with MAPK pathway activation. The data suggest that the pimasertib BID may have increased MAPK pathway inhibition and tumor shrinkage compared with the QD regimen.

In the phase I monotherapy study in solid tumors, emergence of *TP53* mutations in patients being treated with SAR405838 was shown [15]. Molecular profiling data in this combination study could suggest that combination with pimasertib could circumvent the emergence of *TP53* mutations and thus resistance to HDM2 antagonist.

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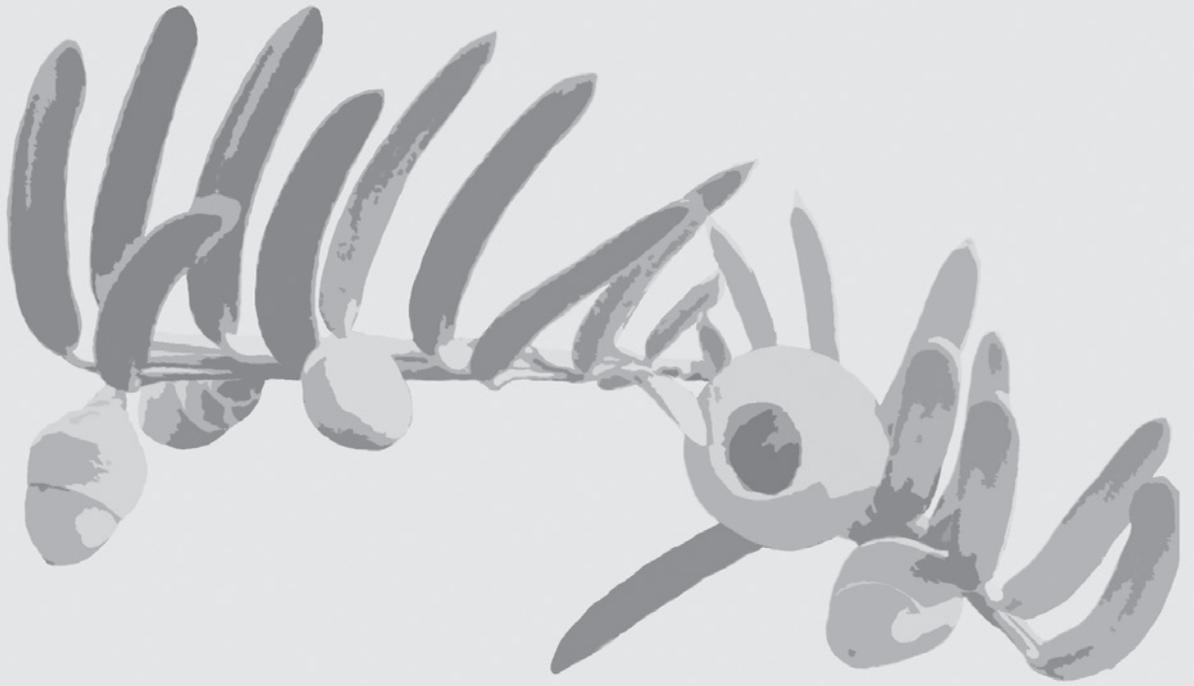
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Chapter 5

Dovitinib



Chapter 5

A drug-drug interaction study to assess the effect of the CYP1A2 inhibitor fluvoxamine on the pharmacokinetics of dovitinib (TKI258) in patients with advanced solid tumors.

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Abstract:

Purpose: Dovitinib is an orally available multi tyrosine kinase inhibitor which inhibits VEGFR 1-3, FGFR 1-3, and PDGFR. This study was performed to investigate the potential drug-drug interaction of dovitinib with the CYP1A2 inhibitor fluvoxamine in patients with advanced solid tumors.

Methods: Non-smoking patients of ≥ 18 years with advanced solid tumors, excluding breast cancer, were included. Patients were treated at a dose of 300 mg in a 5 days on / 2 days off schedule. Steady-state pharmacokinetic assessments of dovitinib were performed with or without fluvoxamine.

Results: Forty-five patients were enrolled; 24 were evaluable for drug-drug interaction assessment. Median age was 60 years (range 30-85). At steady state the geometric mean for dovitinib (Coefficient of Variation% (CV%)) of the area under the plasma concentration-time curve (AUC_{0-72hr}) and maximum concentration (C_{max}) were 2880 ng/mL*h (47%) and 144 ng/mL (41%), respectively. Following administration of dovitinib in combination with fluvoxamine the geometric mean of dovitinib AUC_{0-72h} and C_{max} was 8290 ng/mL*h (60%) and 259 ng/mL (45%), respectively. The estimated geometric mean ratios for dovitinib AUC_{0-72h} and C_{max} (dovitinib + fluvoxamine vs. dovitinib alone) were 2.88 (90% Confidence interval (CI): 2.58, 3.20) and 1.80 (90% CI: 1.66, 1.95). This effect is considered a moderate drug-drug interaction.

Conclusions: Fluvoxamine co-administration resulted in a 80% increase in C_{max} and a 188% increase in AUC_{0-72h} of dovitinib. Given the increase in exposure to dovitinib observed, patients are at risk of dovitinib related toxicity. Dovitinib should therefore not be co-administered with moderate and strong CYP1A2 inhibitors, without dose reduction.

Introduction:

The tyrosine kinases (TK) are important mediators of signal transduction in human cells. Activation of TK leads to diverse biological processes such as growth, differentiation, metabolism, and apoptosis. Activating mutations in TK are important drivers in the development of malignant disease. The multi-tyrosine kinase inhibitor (TKI), dovitinib (TKI258) binds to several TK including and primarily: the vascular endothelial growth factor receptors (VEGFR 1-3), fibroblast growth factor receptors (FGFR 1-3) and platelet-derived growth factor receptor (PDGFR), thereby disrupting the signaling by their respective growth factors, vascular endothelial growth factor (VEGF), fibroblast growth factor (FGF) and platelet-derived growth factor (PDGF) [1].

Dovitinib has been investigated in three phase I dose-escalation trials and the dose of 500 mg on a 5 days on / 2 days off schedule has been selected for the further clinical development [2–4]. Clinical development continued in both phase II and III trials in a variety of malignancies (e.g. renal cell carcinoma and gastro-intestinal stromal cell tumor (GIST)) [5–14].

Dovitinib is extensively metabolized by the cytochrome P450 (CYP) enzymes CYP3A4 and CYP1A1/1A2 (CYP1A) [15]. CYP1A is estimated to account for approximately 1/3 of the *in vivo* metabolism of dovitinib. In pre-clinical models, dovitinib was found to be a moderate CYP3A4 inhibitor and a strong CYP1A2 inducer, a moderate CYP2C19 inducer and a weak CYP2C9 inducer. The induction of CYP1A2 by dovitinib was further investigated in a drug-drug interaction (DDI) study with caffeine, which is a known CYP1A2 substrate. The area under the plasma concentration-time curve (AUC) for caffeine was reduced by 96% after co-administration of dovitinib [16]. This induction of CYP1A2 was further highlighted in a clinical phase I study in which the co-administration of dovitinib with erlotinib was investigated. The study was terminated early, because of a significant drug-drug interaction (DDI). Induction of CYP1A2 by dovitinib was the likely cause of this DDI, that resulted in a decrease of 97% in erlotinib concentrations [17], an effect that is comparable to the interaction observed in the study with caffeine.

To evaluate the effect of CYP1A2 inhibition on the metabolism of dovitinib, the strong inhibitor of CYP1A2, fluvoxamine [18,19] was co-administered in this study. In preclinical human liver microsomal studies, fluvoxamine was shown to be a potent inhibitor of CYP1A2, with K_i values of 0.08-0.28 μM and 0.04 μM for caffeine and ethoxyresorufin, [20,21] respectively. The 100 mg dose of fluvoxamine is expected to achieve peak concentrations close to 0.1 μM [22] suggesting a sufficient inhibitory effect on CYP1A2. Based on the approximately 1/3 of the metabolism of dovitinib that can be accounted for by CYP1A2, an increase of about 33% in dovitinib plasma concentrations was expected. The actual increase in exposure to dovitinib might be higher due to the inherent PK variability of dovitinib. Therefore, as a safety precaution, a reduced dose of 300 mg on a 5 days on /

2 days off schedule was selected for the pharmacokinetic (PK) phase of this study, rather than the maximum tolerated dose (MTD) of 500 mg on a 5 days on / 2 days off schedule.

Materials and methods:

Patients:

Patients with histological or cytological proof of advanced solid tumors, excluding breast cancer, were allowed to enroll in this study. Other inclusion criteria were provision of written informed consent prior to study start, age 18 years or older, an ECOG performance status ≤ 1 , a life expectancy of ≥ 3 months, and laboratory values of the hematology, renal function and hepatic values within a safety threshold. Exclusion criteria were brain metastases, another active malignancy, administration of other targeted therapy within the last two weeks prior to study start. Patients expected to receive or to use moderate to strong inhibitors/inducers of CYP3A4 or CYP1A2 (including tobacco) from five days prior to start for inhibitors and thirty days prior to study start for inducers until after completion of the PK-phase were excluded. Alcohol use exceeding one drink a day was not allowed from three days prior to and until after completion of the PK phase, woman planning to become pregnant or woman pregnant or breast feeding were also not allowed to enroll.

Study Design:

The study was performed in two parts: in part I the effect of fluvoxamine co-administration on dovitinib pharmacokinetics was investigated. In this PK phase patients received dovitinib in a 5 days on 2 days off schedule at a daily dose of 300 mg. Blood samples for steady state PK for dovitinib were collected on days 19-22 (starting on week 3 day 5). On day 22 (week 4 day 1) patients started with a 100 mg QD fluvoxamine dose for one week (days 22-28). On day 26 (week 4 day 5), fluvoxamine was administered 5 minutes prior to dovitinib, and blood samples for PK for dovitinib were collected on days 26-29 (starting week 4 day 5).

After completion of the PK phase patients were allowed to continue dovitinib treatment after a dosing interruption of 5-11 days, to give sufficient time for fluvoxamine washout. Patients continued dovitinib at 500 mg QD in a 5 days on / 2 days off schedule in the clinical treatment phase. Patients were allowed to continue dovitinib until disease progression or intolerable toxicity.

Evaluability criteria:

A minimum of 16 evaluable patients were planned to be enrolled to determine the influence of fluvoxamine on the steady state PK of dovitinib. Patients were evaluable for the DDI part if two full PK profiles of dovitinib (dovitinib alone and dovitinib with fluvoxamine) were available. Full PK was

defined as: a patient did not vomit within 4 hours after receiving dovitinib on the days of blood collection for PK, did receive ≥ 7 of the first 10 scheduled doses of dovitinib, and did receive the next 4 consecutive days of dosing prior to the days of blood collection for PK. Additionally, the patient had to have taken fluvoxamine for 7 consecutive days following the completion of PK collection for dovitinib alone, with no vomiting within 4 hours of fluvoxamine administration on the day of PK profile collection for dovitinib and fluvoxamine.

Safety evaluations:

Safety assessments consisted of monitoring and recording of all AEs, including serious adverse events (SAE), the regular monitoring of electrocardiograms, cardiac imaging (using echocardiogram or multi gated acquisition (MUGA) scan), chest X-ray, hematology, serum chemistry, urinalysis, coagulation, thyroid function test, routine monitoring of vital signs (respiratory rate, weight, body temperature, sitting pulse and sitting blood pressure), and ECOG PS. Assessments were usually performed at Baseline/Screening, PK phase (Day 1, Day 12, Day 19, Day 26) and in the clinical phase (Week 1 Day 1, Week 4 Day 1 onwards every 4 weeks) and at the end of treatment.

Pharmacokinetic measurements:

Pharmacokinetic sampling for dovitinib was performed on days 19 and 26 at the following time points: pre-dose, 2, 4, 6, 9, 24, 48 and 72 hours after administration. Non-compartmental PK analyses were conducted on the full PK profiles of dovitinib. The primary PK parameters determined were area under the plasma concentration time curve time = 0-72h (AUC_{0-72h}) and maximum plasma concentration (C_{max}) for dovitinib with and without fluvoxamine. Secondary PK parameters were AUC_{0-24h} , time to reach C_{max} (T_{max}), terminal half-life ($T_{1/2}$) and apparent oral steady state clearance (CL_{ss}/F).

Efficacy:

The response to treatment was determined by the investigator using the Response Evaluation Criteria in Solid Tumors (RECIST) 1.1. The local Investigator's measurements were used for analysis and for treatment decision making. Evaluation was performed using either CT or MRI scans every 8 weeks.

Statistical analyses:

A formal statistical analysis was conducted to explore the relative bioavailability of dovitinib co-administered with fluvoxamine as compared to dovitinib alone. A linear mixed effects model was fitted to the log-transformed steady state PK parameters (AUC_{0-72hr} and C_{max}). Treatment, as the fixed effect, and patients as random effect were included in the model. For the mixed model analysis, dovitinib + fluvoxamine was the test treatment and dovitinib alone the reference. The

model based, between-treatment mean differences (dovitinib + fluvoxamine – dovitinib alone) and the corresponding two-sided 90% confidence intervals (CIs) were calculated on the log-scale. The between-treatment differences and 90% CIs were then back transformed to the original scale to obtain the geometric mean ratios (dovitinib + fluvoxamine/dovitinib alone) and the corresponding 90% CIs. Steady state PK parameters for dovitinib were summarized by treatment. Safety of dovitinib was evaluated for the PK and clinical treatment phases together by summarizing the frequency and severity of AEs. Efficacy data are presented as assessed by the investigators.

Results:

Overall, 45 patients were enrolled. Patients had a median age of 60 years (range 30-85), with more male (60%) than female (40%). All patients had a good performance status according to the Eastern Cooperative Oncology Group (ECOG) performance score of 0 or 1. The most common tumor types were colorectal cancer (14 patients), adenoid cystic carcinoma (5 patients), ovarian cancer (4 patients) and pancreatic cancer (4 patients). A more detailed overview of the patient characteristics is shown in table 1.

Twenty-five patients completed the PK phase as per protocol, of which 24 were considered evaluable for the pharmacokinetic analysis set (PAS). Twenty patients discontinued prior to completion of the PK phase: 13 for adverse events, 3 due to non-compliance with study treatment and physician decision, one each due to progressive disease, protocol deviation and subject/guardian decision. Patients who did not complete the PK phase were allowed to continue treatment, if considered in their best interest in the treatment phase.

Thirty-three patients continued into the treatment phase. Reasons for treatment discontinuation in these patients were: progression of disease in 24 patients, adverse events in five patients, death, not treatment related, in two patients, and study termination by the sponsor (patients were allowed to continue on dovitinib in another protocol), in two patients.

Pharmacokinetics:

The PK parameters of dovitinib when administered alone and with fluvoxamine are displayed in table 2. At steady state after three weeks of treatment with dovitinib (Day 19) the geometric mean (Coefficient of Variation% (CV%)) of AUC_{0-72h} and C_{max} was 2880 ng/mL*h (47%) and 144 ng/mL (41%), respectively. Following administration of dovitinib in combination with fluvoxamine the geometric mean of dovitinib (Day 26) AUC_{0-72h} and C_{max} increased to 8290 ng/mL*h (60%) and 259 ng/mL (45%), respectively. The estimated geometric mean ratios for dovitinib AUC_{0-72h} and C_{max}

(dovitinib + fluvoxamine vs. dovitinib alone) were 2.88 (90% CI: 2.58, 3.20) and 1.80 (90% CI: 1.66, 1.95), respectively as presented in table 3. This indicates that fluvoxamine increased the dovitinib AUC_{0-72h} by 188% and C_{max} by 80%, a 2.9- and 1.8-fold increase respectively.

Table 1: Patient demographics

	All patients (n=45)
Age (years)	
Median (range)	60 (30-85)
Sex n (%)	
Female	18 (40%)
Male	27 (60%)
Race n (%)	
Caucasian	41 (91.1%)
Black	2 (4.4%)
Asian	2 (4.4%)
ECOG performance status: n (%)	
0	21 (47%)
1	24 (53%)
Tumor type	
Colorectal	14
Adenoid cystic carcinoma	5
Ovarian	4
Pancreatic	4
NSCLC	3
Urothelial Cell carcinoma	3
Other	12

Abbreviations: n = number of patients, NSCLC = non-small cell lung cancer

Based on the fold change, the interaction on C_{max} is considered a weak interaction as the increase is <2-fold, however the interaction on AUC_{0-72h} is considered moderate as the increase was ≥ 2 and ≤ 5 -fold [23]. Consistent with increased AUC_{0-72h} and C_{max} , geometric mean of dovitinib CLs/F decreased from 157 to 77 L/h and $T_{1/2}$ increased from 14 to 20 hours for dovitinib + fluvoxamine vs dovitinib alone respectively.

Safety:

All patients included reported at least one adverse event suspected to be study drug related. The most commonly reported toxicities were diarrhea (60%), nausea (47%), and fatigue (45%). Fourteen patients (31%) discontinued the study drug due to adverse events regardless of study drug relationship. Forty percent of patients experienced grade ≥ 3 toxicity. The most common grade 3 toxicity was fatigue and occurred in 18% of patients; other grade 3 events included diarrhea and pulmonary embolism in two patients each. Additionally, one patient had a grade 4

hypertriglyceridemia. In table 4 all adverse events suspected to be related to the study drug which occurred in >5% of patients or were grade ≥ 3 are presented. Six (13.3%) on-treatment deaths (within 30 days of the last dose of dovitinib), all due to disease progression, occurred on study. Two additional deaths were reported which occurred more than 30 days after the last dose of dovitinib, one due to disease progression and one due to unknown causes.

Table 2: Summary of dovitinib pharmacokinetic parameters by treatment (Pharmacokinetic analysis set of Dovitinib) AUC_{0-72h}: area under the plasma concentration time curve t=0-72h; AUC_{0-24h}: area under the plasma concentration time curve t=0-24h; C_{max}: maximum plasma concentration observed; T_{max}: time to reach C_{max}; T_{1/2}: terminal half-life; CLss/F: apparent oral steady state clearance; N= number of patients included in the analyses.

	AUC _{0-72h} (ng/mL*h)	AUC _{0-24h} (ng/mL*h)	C _{max} (ng/mL)	T _{max} (h)	T _{1/2} (h)	CLss/F (L/h)
Dovitinib n=24						
Mean (CV%)	3180 (46%)	2240 (42%)	155 (39%)	3.97 (1.90-9.00)	14.4 (14%)	157 (41%)
Geo-mean (CV%)	2880 (47%)	2060 (43%)	144 (41%)	Median (range)	14.2 (14%)	145 (43%)
Dovitinib + Fluvoxamine n=24						
Mean (CV%)	9450 (48%)	5080 (50%)	281 (40%)	4.27 (1.75-9.00)	20.5 (25%)*	77.3 (48%)*
Geo-mean (CV%)	8290 (60%)	4600 (50%)	259 (45%)	Median (range)	19.9 (26%)*	69.8 (49%)*

CV%=Coefficient of variation (%) ; CV% = (SD/mean)*100; CV% geo-mean=sqrt(exp(variance log transformed data)-1)x100. * n = 19

Table 3: Summary of statistical analysis of primary pharmacokinetics parameters for dovitinib (Pharmacokinetic analysis set of Dovitinib)

PK parameter (unit)	Treatment	n	Adjusted Geo-mean	Comparison(s)	Treatment comparison 90% CI		
					Geo-mean ratio	Lower	Upper
AUC _{0-72h} (ng/ml*h)	Dovitinib	24	2880	Dovitinib+fluvoxamine: Dovitinib	2.88	2.58	3.20
	Dovitinib + fluvoxamine		8290				
C _{max} (ng/ml)	Dovitinib	24	144	Dovitinib+fluvoxamine: Dovitinib	1.80	1.66	1.95
	Dovitinib + fluvoxamine		259				

AUC_{0-72h}: area under the plasma concentration time curve t = 0-72 h; C_{max}: maximum plasma concentration observed; N = Number of patients; Geo-mean=geometric mean. Adjusted geo-mean, geo-mean ratio and 90% confidence interval (90% CI) are all determined from a mixed effect model and back transformed from log scale. The model for log transformed PK parameter AUC_{0-72h} and C_{max} includes treatment as a fixed factor and patient as a random factor.

Efficacy:

The best tumor response while on study, as assessed by the investigators was partial response in three patients (adenoid cystic carcinoma, urothelial cell carcinoma and endometrial carcinoma, all in 1 patient). Nine-teen patients had stable disease and eleven had progressive disease at the

first evaluation. In figure 1 the time on the PK phase and the treatment phase is presented for all patients. Seven patients had prolonged benefit of dovitinib treatment and continued treatment with dovitinib > 6 months.

Table 2: Adverse events (all grades, in >5% of patients or grade ≥ 3), suspected to be related to the study drug, by preferred term and maximum grade.

All patients (n=45)					
Preferred term	Grade 1 n (%)	Grade 2 n (%)	Grade 3 n (%)	Grade 4 n (%)	All grades n (%)
Total	7 (15.6)	20 (44.4)	17 (37.8)	1 (2.2)	45 (100.0)
Diarrhoea	16 (35.6)	9 (20.0)	2 (4.4)	0	27 (60.0)
Nausea	11 (24.4)	9 (20.0)	1 (2.2)	0	21 (46.7)
Fatigue	10 (22.2)	4 (8.9)	6 (13.3)	0	20 (44.4)
Vomiting	13 (28.9)	4 (8.9)	1 (2.2)	0	18 (40.0)
Decreased appetite	5 (11.1)	4 (8.9)	0	0	9 (20.0)
Weight decreased	3 (6.7)	6 (13.3)	0	0	9 (20.0)
Rash	5 (11.1)	2 (4.4)	0	0	7 (15.6)
Dysgeusia	6 (13.3)	0	0	0	6 (13.3)
Headache	1 (2.2)	4 (8.9)	0	0	5 (11.1)
Abdominal pain upper	4 (8.9)	0	0	0	4 (8.9)
Constipation	2 (4.4)	2 (4.4)	0	0	4 (8.9)
Dyspnea	3 (6.7)	1 (2.2)	0	0	4 (8.9)
Palmar-plantar erythrodysesthesia syndrome	2 (4.4)	1 (2.2)	1 (2.2)	0	4 (8.9)
Abdominal pain	1 (2.2)	1 (2.2)	1 (2.2)	0	3 (6.7)
Dry mouth	2 (4.4)	1 (2.2)	0	0	3 (6.7)
Hypertriglyceridemia	0	0	1 (2.2)	1 (2.2)	2 (4.4)
Pulmonary embolism	0	0	2 (4.4)	0	2 (4.4)
Stomatitis	1 (2.2)	0	1 (2.2)	0	2 (4.4)
Cholesterol increased (blood)	0	0	1 (2.2)	0	1 (2.2)
Epilepsy	0	0	1 (2.2)	0	1 (2.2)
Febrile neutropenia	0	0	1 (2.2)	0	1 (2.2)
Hypertension	0	0	1 (2.2)	0	1 (2.2)
Left ventricular dysfunction	0	0	1 (2.2)	0	1 (2.2)
Leukopenia	0	0	1 (2.2)	0	1 (2.2)

n=number of patients

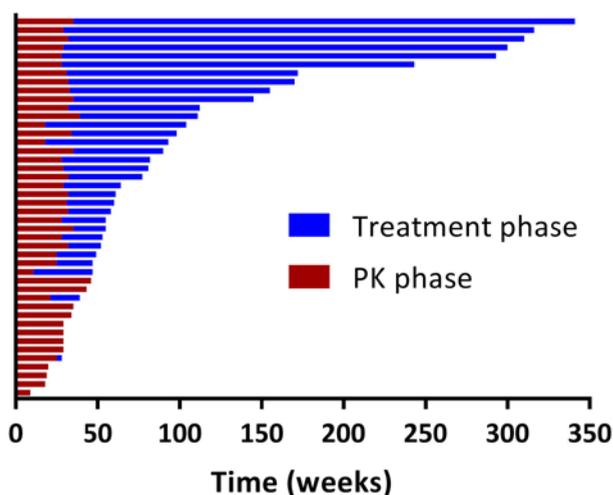


Figure 1: Time on study

Time on study for all 45 patients enrolled. In red the duration patients were in the pharmacokinetic phase (PK) and in blue the time patients continued in the treatment phase.

The dashed vertical line represents the first scheduled tumor assessment (8 weeks).

Discussion:

The co-administration of fluvoxamine with dovitinib resulted in an increase in dovitinib AUC_{0-72h} of 188% and C_{max} of 80%. Fluvoxamine is therefore considered to be a moderate inhibitor of dovitinib metabolism.

The increase in AUC_{0-72h} observed in this study was far greater than expected. Based on the role of CYP1A2 in the metabolism of dovitinib, the increase was predicted to be about 33%. The increase of AUC_{0-72h} was however 188%, about 6-fold higher than expected. It is unlikely that the increase can entirely be attributed to CYP1A2 inhibition alone, as CYP3A4 is normally the major enzyme involved in dovitinib metabolism. The precise importance of CYP1A2 and CYP3A4 in the metabolism of dovitinib after prolonged use is unclear. Over time CYP1A2 will be induced, whereas CYP3A4 will be inhibited by dovitinib. This likely results in a higher dependence on CYP1A2 for metabolism of dovitinib after prolonged use. The 100 mg fluvoxamine dose has likely inhibited CYP1A2 entirely, even after the induction of CYP1A2 by dovitinib. Given the potential increase of metabolism via CYP1A2, the inhibition by fluvoxamine might explain at least in part the higher than expected change in both AUC_{0-72h} and C_{max} . Another potential contributing factor is the additional inhibition on CYP3A4 caused by fluvoxamine, as it is not only an inhibitor of CYP1A2 but also a weak CYP3A4 inhibitor. The combination of CYP1A2 and CYP3A4 inhibition might together provide the explanation for the 188% increase in the dovitinib AUC_{0-72h} . It is unlikely that metabolism of fluvoxamine was

altered as this is primarily dependent on CYP2D6 [24], and dovitinib is not known to inhibit or induce this CYP enzyme.

A 188% increase in AUC_{0-72h} could result in dovitinib related adverse events. In order to mitigate this risk, commonly used drugs such as ciprofloxacin and oral contraceptives, that are strong and moderate CYP1A2 inhibitors, respectively, should not be given with dovitinib, or a dose reduction of dovitinib should be applied. The dovitinib dose should be reduced to 1/3 of the standard dose, given the 2.9 fold increase in AUC_{0-72h} observed in this study after the combined administration of fluvoxamine and dovitinib. Further studies are needed to investigate the role of CYP3A4 inhibition in order to further identify the mechanism behind the large increase in AUC_{0-72hr} observed in this study.

Conclusion:

The DDI observed in this study is of clinical relevance given the potential increase in plasma exposure to dovitinib, as this might result in increased dovitinib related toxicity. Prior to start of a moderate to strong CYP1A2 inhibitor dovitinib treatment should be stopped or the dose should be reduced, in order to mitigate the risk of toxicity.

Acknowledgements:

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Compliance with ethical standards:

The study was performed in compliance with the Declaration of Helsinki and its amendments. All patients provided informed consent prior to participation. The study was registered in clinicaltrials.gov (identifier: NCT01700270).

Funding/conflict of interest:

This study (NCT01700270) was funded by Novartis Pharmaceuticals. The authors declare to have no conflict of interest.

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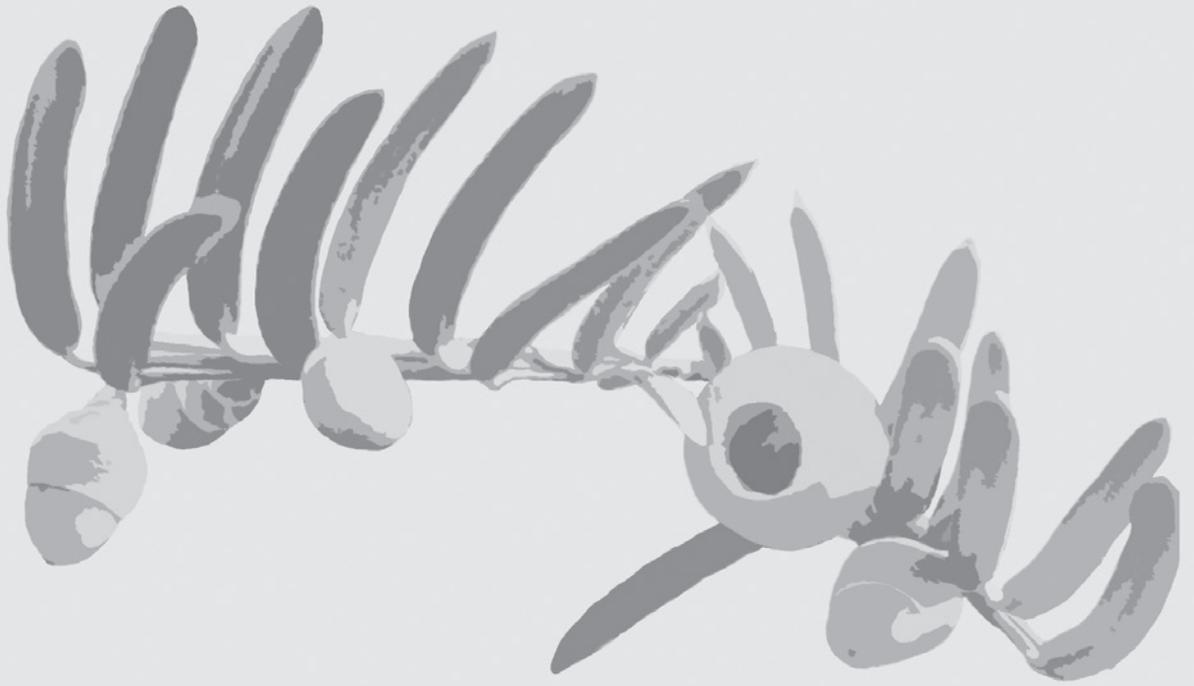
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Chapter 6

Thiosulfate otoprotection



Chapter 6

Sodium thiosulfate middle ear injections to prevent cisplatin induced ototoxicity in patients treated for solid tumors: A proof of concept study.

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Interim analysis

Abstract:

Background: The use of the anticancer agent cisplatin is limited due to cumulative toxicity, for example ototoxicity. Currently there is no effective method to prevent the development of treatment induced hearing loss. This study aimed to reduce ototoxicity of cisplatin by transtympanic injection of the antioxidant sodium thiosulfate (STS) into the middle ear. Secondary aims were to determine the safety of STS gel application and the pharmacokinetics of cisplatin.

Method: Patients of 18 years or older with advanced solid tumors, treated at a cisplatin dose of $\geq 75 \text{ mg/m}^2$ were eligible. In arm A, either placebo gel or 0.1 M STS gel was applied to the middle ear via a grommet. In arm B, one ear was treated with STS gel administered transtympanically while the other ear was left untreated. In both arms, randomization for the treatment ear was performed prior to start of cisplatin treatment. Sensorineural hearing loss (SNHL) was monitored by audiometry before cisplatin treatment and after every cisplatin course. Outcome measures used were the Pure-Tone Averages (PTAs) at 1-2-4 kHz and 8-10-12.5 kHz.

Results: In arm A, 8 patients were included, of which 6 received the STS and placebo gel. None of the patients showed ototoxicity in terms of CTCAE v4.03 or $> 10 \text{ dB}$ hearing loss at the specific PTAs. In arm B, 3 patients were included, of which 2 received STS gel. In 1 patient treatment related hearing loss at 8 and 10 kHz was observed. This patient endured 15 dB more loss in the placebo treated ear at 8 kHz as compared to the STS treated ear. None of the 8 patients treated had toxicity related to gel administration.

Conclusion: Transtympanic application of the STS gel was feasible and safe in our patients treated with high-dose cisplatin chemotherapy for advanced malignancies. Preliminary efficacy was seen in the one patient that suffered hearing loss at the ultra-high frequencies, which was to a lesser degree in the STS treated ear.

Introduction:

Cisplatin is a widely used, anti-cancer agent in the treatment of both childhood and adult solid tumors. The number of cycles and the total dose of cisplatin that can be administered is limited, due to cumulative toxicity that consists of neuropathy, nephropathy and ototoxicity. The nephropathy can largely be prevented by additional intravenous hydration prior to and after infusion of cisplatin. However, for ototoxicity and neuropathy no preventive strategy is available today [1,2].

Cisplatin induces sensorineural hearing loss (SNHL), shown in animal studies to be the result of damage to the outer hearing cells within the organ of Corti and the marginal cells within the stria vascularis. Hearing loss progresses from (ultra-) high-frequencies to low frequencies [3]. The toxicity worsens with each administration of cisplatin and always starts shortly after treatment (i.e. days). It is characterized by symmetric bilateral and irreversible perceptive hearing loss. Tinnitus is considered an early sign of the development of ototoxicity and occurs in 25 - 50% of patients treated with cisplatin [4,5].

Ototoxicity is thought to be the result of both direct and indirect cytotoxic effects of cisplatin. The direct effect is the result of cross-linking of (mitochondrial) DNA within the outer hearing cells. The cross-linking of DNA results in cell damage and cell death, as a result reactive oxygen species (ROS) are formed. ROS will cause further (indirect) damage to the outer hair cells, thereby increasing the ototoxic effects of cisplatin [6]. Therefore, preventive strategies that aim to reduce the production of ROS within the inner ear can be of value to reduce cisplatin related SNHL.

In preclinical models it has been shown that anti-oxidants (e.g. N-acetylcysteine and sodium thiosulfate (STS)) can prevent cisplatin induced hearing loss after both intravenous and transtympanic administration [7]. In humans, the antioxidant N-acetylcysteine has been shown to prevent the development of cisplatin induced ototoxicity in proof-of-principle studies [8,9]. In our institute intravenous (iv) STS rescue, has previously successfully been applied in a randomized phase III trial in patients with advanced Head and Neck Squamous Cell Carcinoma (HNSCC). In this trial 3 courses of iv cisplatin at a 100 mg/m² dose were compared with 4 courses of intra-arterial cisplatin at a dose of 150 mg/m² followed by STS rescue started 30 minutes after cisplatin infusion given as an intravenous bolus of 9 g/m² followed by intravenous 12 g/m² as a 6 hour continuous infusion. Although overall toxicity between treatment arms was found to be comparable [10], there was a difference in the incidence of ototoxicity, which occurred approximately 10% less often in the patients treated with intra-arterial cisplatin followed by STS rescue [11].

There is, however a major down side to the use of systemic STS, as it does not only counteract the toxic effects, but also diminishes the anti-tumor activity of cisplatin by direct binding to the active form of the drug [12]. This was also a possible explanation for the lack of survival benefit of the more dose intensified intra-arterial cisplatin treatment regimen in the phase III trial [10]. Therefore, a topical administration method for STS, not affecting the systemic cisplatin exposure is needed. Transtympanic administrations may be an ideal strategy in this respect, as this technique has been applied and found to be safe in Meniere's disease, sudden sensorineural hearing loss and in ototoxicity studies with N-acetylcysteine. Furthermore, these administrations showed reduction of patients symptoms [8,9,13–15], providing proof of principle that administration to the middle ear can result in clinically relevant drug exposure to the inner ear.

This study aims to proof the concept that transtympanic STS gel application to the middle ear can prevent cisplatin induced ototoxicity in patients with advanced solid tumors treated with cisplatin at a dose of ≥ 75 mg/m². Secondary aims included safety, feasibility and pharmacokinetics of cisplatin in plasma measured as total platinum and unbound platinum.

Patients and methods:

Study design:

Patients:

Patients with advanced solid tumors of 18 years or older, with a World Health Organization (WHO) performance score (PS) of 0 or 1, who were to be treated with cisplatin at a dose of ≥ 75 mg/m² were eligible. If patients were to receive concomitant radiotherapy to the head and neck, the total radiotherapy dose on the cochlea and/or the middle ear was not allowed to exceed 10 Gray (Gy), as higher dosages to the cochlea or middle ear might result in radiotherapy induced hearing loss [16]. Other inclusion criteria were acceptable bone-marrow, renal and hepatic function. Exclusion criteria were prior systemic high-dose cisplatin or oxaliplatin treatment, relevant otological history (e.g. conductive hearing loss, vertigo) and known symptomatic brain or leptomeningeal metastases. The study was performed in two arms. Arm A was a single-blind, placebo controlled, proof-of-concept study. In arm A, the ear to be treated with STS gel was assigned by randomization. The other ear was treated with placebo gel.

Hereafter, a different application method was investigated in arm B, which was a non-blinded, non-placebo controlled proof-of-concept arm. In arm B, the ear to be treated with STS was allocated by randomization and the contra-lateral ear was left untreated.

Patients enrolled in both arms A and B were screened prior to study for eligibility. A safety follow-up was performed within 3 weeks prior to start of each cycle and earlier than 4 weeks after the last

cycle, which consisted of a physical examination, registration of adverse events according to the National Cancer Institute's Common Terminology Criteria for Adverse Events version 4.0.3 (CTCAE) [17] and their relation to study treatment (STS or placebo gel injection), registration of concomitant medication and safety laboratory assessments consisting of hematology and serum chemistry and audiometry.

STS and placebo gel application:

In arm A two syringes with 3 ml hyaluronic acid based gels, one without (placebo) and one with 0.1 M STS, were prepared by the pharmacy of the Netherlands Cancer Institute, on the day of cisplatin administration. In arm B only the STS containing gel was prepared by the pharmacy.

Syringes in both arm A and arm B were warmed to body temperature (37 °C) for 30 minutes in an incubator (CULTURA® M, Almedica AG, Giffers, Swiss) to prevent hypo- or hypercaloric clinical symptoms such as vertigo and nausea. In arm A, the gels were administered through a preplaced grommet using a (Braun, Pencan 25G) needle connected to a 10 cm infusion line (BD Becton Dickenson connecta 10 cm). In arm B, a grommet was placed for venting and the STS gel was applied directly through the lower posterior quadrant of the eardrum using the 25G needle connected to a 10 cm infusion line (BD connecta 10 cm). Prior to the puncture of the eardrum topical anesthesia using a gauze sprayed with xylocaine 10% (lidocaine 100 mg/ml, AstraZeneca, Cambridge, United Kingdom) was applied.

During the injections the patient was lying in a hospital bed with the head end positioned 30 degrees upwards. After the administration of the gel the patients head was tilted 45 degrees along the longitudinal-axis with the infused ear upwards to allow the gel to have contact with the round window. Patients remained in this position for 30 minutes. Thereafter, the procedure was repeated in the other ear (arm A). In arm A, the gel was first administered to the right ear followed by the left ear, independent of treatment.

In both arm A and arm B, three hours after the administration of the gel (the second administration in arm A) the cisplatin infusion was started. Cisplatin was infused as a 4 hour infusion and premedication and pre- and post-hydration were given according to local protocol. If concomitant chemotherapy was to be administered this was given as per local protocol.

Audiometry:

Audiometry was performed in a sound proof booth by a trained speech therapist using the Decos Audiology Workstation. Air conduction (AC) thresholds were measured at 0.25, 0.5, 1, 2, 4, 8, 10, and 12.5 kHz. After obtaining the AC thresholds, the bone conduction (BC) thresholds were measured at 0.5, 1, 2 and 4 kHz. Frequencies from 0.25 to 8 kHz are presented in dB Hearing Level (HL) and the ultra-high frequencies from 8 to 12.5 kHz are presented in dB Sound Pressure Level (SPL). Three

Pure Tone Averages (PTAs) were calculated from the AC thresholds at three different frequencies: for the frequency range relevant for speech perception in quiet this was the average AC threshold at 0.5-1-2 kHz, for speech perception in noise at 1-2-4 kHz and for the perception of high sounds (e.g. music or nature) at 8-10-12.5 kHz. Audiometry was performed pre-treatment (referred to as the baseline hearing level), after each cisplatin cycle and three months posttreatment. Hearing loss of ≤ 10 dB is widely accepted to be non-significant (i.e. CTCAE v4.03, the ASHA guidelines or the TUNE grading scale [18–20]). Therefore only hearing thresholds shifts higher than 10 dB were considered to be clinically relevant differences.

Pharmacokinetics:

Pharmacokinetic (PK) sampling was performed in both arm A and arm B. Samples were drawn: pre-dose, at end of cisplatin infusion and 1, 2, 3, 4 and 18 hours thereafter. Blood was collected in a 10 ml heparin tube. After collection the tube was centrifuged at 1500 g for 10 minutes at 4 °C. Of the plasma 2 ml was transferred to a 2 ml Eppendorf tube and stored at -20 °C until analyses (total platinum). Two plasma ultra-filtrate tubes (Centrifree® ultra-filtrate tubes, Merck Millipore Ltd, Tullagreen, Ireland) were filled with plasma. Centrifree tubes were then centrifuged at 1800 g for 10 minutes at room temperature. Ultra-filtrate was then transferred to a 2 ml Eppendorf and stored until analyses at -20 °C (unbound platinum).

The white blood cell (WBC) pellet was, removed from the heparin tube using a pipette. Remaining red blood cells in the sample were lysed in a 0.15 M ammonium chloride lyses buffer. Samples were then centrifuged at 2000 g for 5 minutes at 4 °C and lyses buffer was discarded after centrifugation. The cells were then washed twice in phosphate buffered saline (PBS), by centrifugation at 2000 g for 5 minutes at 4 °C. After washing the samples were stored in nuclear buffer at -80 °C until processing (DNA-adduct levels). At the time of sample measurement WBC samples were thawed and prepared for measurement in accordance with the method as described by Brouwers et al. for inductively coupled plasma mass spectrometric (ICP-MS) [21]. Prior to analyses the DNA concentration in the samples was measured using a Nanodrop, ND1000 (Isogen, Life sciences). Platinum (Pt) levels were then measured by the pharmacy of the Netherlands Cancer Institute using validated ICP-MS methods. The lower level of quantification (LLOQ) was 7.5 fg Pt / μg DNA for DNA-adducts levels and the LLOQ was 7.50 ng/L for unbound and total platinum [21,22]. Adduct levels were then calculated using the DNA concentration and the concentration of platinum measured in the samples and expressed as the amount of platinum per μg DNA.

PK parameters were calculated using validated scripts in the software package R version 3.0.1 [23]. The maximum observed plasma concentration (C_{max}), the area under the plasma concentration-time curve from start of the cisplatin infusion (time = 0) to 22 hours ($\text{AUC}_{0-22\text{h}}$) were reported for total platinum levels, unbound platinum levels and DNA adduct levels in WBC. The area under the

plasma concentration-time curve from start of the cisplatin infusion (time = 0) with extrapolation to infinity using the terminal rate constant ($AUC_{0-\infty}$) was calculated and reported for unbound platinum levels only.

Results:

In arm A, 8 patients were registered of whom 6 patients were treated with STS and placebo gel. Two patients did not start treatment: one patient experienced pain during placement of the grommet and withdrew consent, the other patient withdrew consent without a formal reason. In arm B, a total of 3 patients was registered of whom 2 were treated with the STS gel. The third patient withdrew consent prior to start, as the logistics were considered to troublesome. Baseline characteristics of the 8 patients, who received treatment are shown in table 1 per treatment arm. Seven patients were male (88%). Patients had a median age of 59 (range 46 – 67) years. All patients received cisplatin at a dose of 75 mg/m² as cisplatin combination chemotherapy. Patients received a median of 3.5 (range 1 - 4) cycles. One patient received only 1 cycle, as this patient developed nephropathy and was therefore switched to carboplatin. Application of the gels was discontinued after the switch to carboplatin.

Table 1: Baseline Patient characteristics

	Arm A (n = 6)	Arm B (n = 2)
Age		
median (range)	59 (46-67) years	61 (59-62) years
Gender		
Male	5 (83%)	2 (100%)
Female	1 (17%)	0
WHO Performance Score		
0	5 (83%)	2 (100%)
1	1 (17%)	0
Tumor type		
NSCLC	3 (50%)	1 (50%)
Mesothelioma	3 (50%)	0
Thymus carcinoma	0	1 (50%)
Number of cycles cisplatin		
median (range)	3 (1-4) cycles	4 (NA)

Abbreviations: NSCLC = non-small cell lung cancer; WHO = World Health Organization.

Safety and feasibility of gel application:

After placement of the grommet, all patients reported upon request that they experienced a change

in the perception of sound, which could not be objectified by audiometry, as none of the patients showed a change in hearing thresholds after cycle 1, as compared to baseline audiometry (without the grommet). Therefore, this change in hearing could not be scored according to CTCAE v4.03.

Application of the gels in arm A was limited by back flow of the gel alongside the needle (through the grommet) during administration. On average a volume of 0.2 ml of gel was administered per ear. An air-fluid level remained behind the eardrum after administration, an indication that the middle ear was not completely filled with gel.

The application technique did improve by application by direct transtympanic puncture of the eardrum in arm B. A larger volume of on average 0.4 ml of STS gel could be administered to the middle ear. No air-fluid level remained behind the eardrum after application.

The gel application was safe and feasible. Patients did not report any pain related to the injections in arm A. In arm B the transtympanic administration was well tolerated after application of the local anaesthesia. Patients in both arm A and arm B did report fullness of the middle ear upon injection, which disappeared within 1 hour after application of the gel. More importantly, no otitis media or perceptive hearing loss as a result of gel application occurred in the study during the treatment or in the follow-up period (up to 3 months after the last administration).

All other reported AEs were to be attributed to cisplatin doublet treatment, or the malignant disease under study. Three patients required a dose-delay, because of AE, which were nausea and fatigue in one patient and neutropenia in the other two patients. No serious adverse events were reported in the study.

Hearing loss during cisplatin treatment:

Mean hearing levels (in dB HL) for AC and BC at the baseline and follow-up audiometry for all patients treated in arm A are depicted in figure 1. In none of the patients included in arm A clinically significant hearing loss (defined as a deterioration of more than 10 dB on any frequency) was observed. Consequently, pure tone averages PTA 0.5-1-2 kHz, PTA 1-2-4 kHz and PTA 8-10-12.5 kHz also did not show any clinically relevant changes as compared to baseline (figure 2).

Cisplatin related tinnitus was reported in two patients (33%), which was bilateral in one patient and did involve only the placebo ear in the other patient.

Mean hearing levels (in dB HL) for AC and BC at the baseline and follow-up audiometry for both patients treated in arm B are depicted in figure 3. In one of the two patients included in arm B clinically significant hearing loss was observed. In this patient a loss of 10 dB and 5 dB was observed at 8 kHz and 10 kHz, respectively in the STS treated ear, whereas a loss of 25 dB and 20 dB was observed at the respective frequencies in the placebo ear. The pure tone averages PTA 8-10-12.5 kHz did however not show a clinically relevant change in this patient. The other patient included did not show any clinically relevant changes in hearing. No tinnitus was reported in arm B thus far.

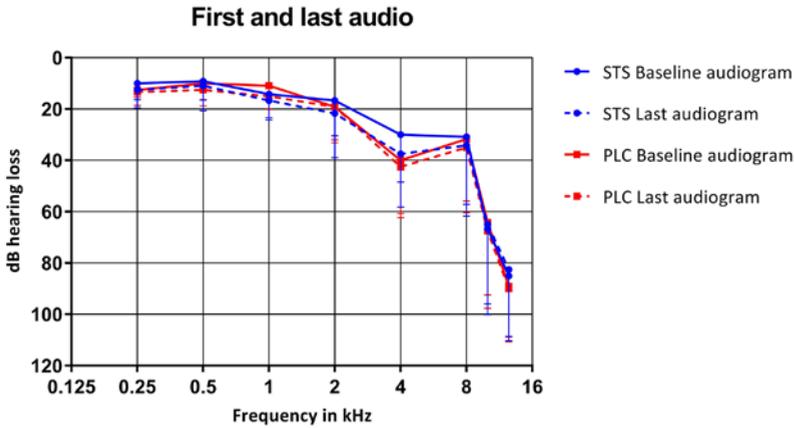


Figure 1: Hearing loss for the air conduction thresholds (arm A)
Hearing loss for the air conduction thresholds (in dB) per treatment (arm A). The mean and standard deviation are shown of all frequencies of the first audiogram (baseline) and the last audiogram of all patients included in arm A. STS = sodium thiosulfate, PLC = placebo, kHz = kilo Hertz, dB = decibel.

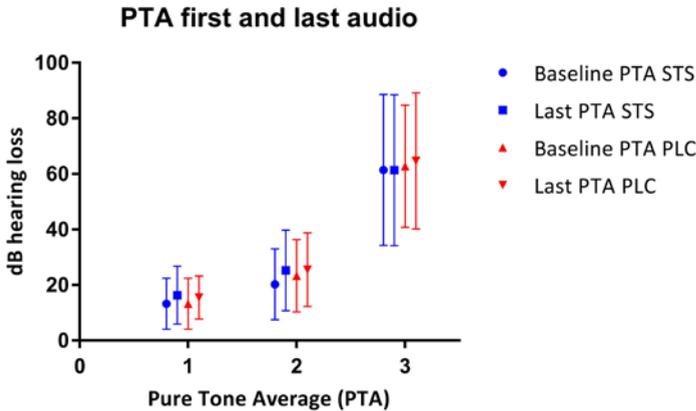


Figure 2: Pure Tone Averages (arm A)
Pure Tone Averages stratified for treatment of patient included in arm A, the mean and standard deviation for the baseline and last audiogram are shown. PTA 1 = 0.5, 1, 2 kHz, PTA 2 = 1,2 and 4 kHz, PTA 3 = 8, 10, 12.5 kHz. Abbreviations: STS = sodium thiosulfate, PLC = placebo, kHz = kilo Hertz, dB = decibel, PTA = pure tone average.

Pharmacokinetics:

Concentration-time curves for total and unbound platinum and DNA adduct levels in WBC are presented in figure 4, corresponding PK parameters C_{max} , AUC_{0-22h} and AUC_{0-inf} are shown in table 2. The mean $AUC_{0-inf} \pm$ standard deviation (SD) for total platinum and unbound platinum were $169 \pm 105 \mu\text{g/ml}\cdot\text{h}$ and $4.44 \pm 1.06 \mu\text{g/ml}\cdot\text{h}$, respectively. Mean C_{max} for total platinum and unbound platinum \pm SD were $2.30 \pm 0.47 \mu\text{g/ml}$ and $0.766 \pm 0.21 \mu\text{g/ml}$, respectively. $AUC_{0-inf} \pm$ SD and $C_{max} \pm$ SD for platinum adduct levels in WBC were $7.96 \pm 2.08 \text{ pg Pt} / \mu\text{g DNA} \cdot \text{h}$ and $0.713 \pm 0.43 \text{ pg}$

Pt / μg DNA, respectively. One patient was removed from the adduct level analyses as a result of a technical error made in the WBC isolation. Pharmacokinetic results of arm B were not yet available at the time of this interim analysis.

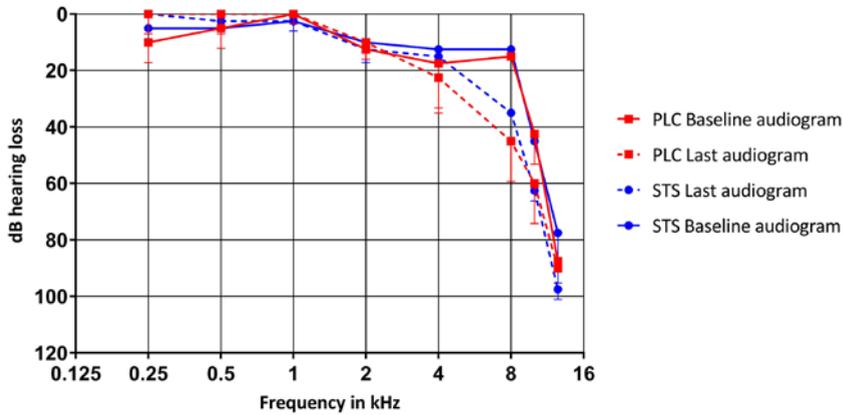


Figure 3: Hearing loss for the air conduction thresholds (arm B)
Hearing loss for the air conduction thresholds (in dB) per treatment (arm B). The mean and standard deviation are shown of all frequencies of the first audiogram (baseline) and the last audiogram of all patients included in arm B. STS = sodium thiosulfate, PLC = placebo, kHz = kilo Hertz, dB = decibel.

Table 2: Pharmacokinetic parameters of total platinum, unbound platinum and adduct levels in white blood cells (arm A).

		Total platinum (n=6)	Unbound platinum (n=6)	Adduct levels in WBC (n=5)
C_{\max} $\mu\text{g/ml}$ or $\text{pg Pt} / \mu\text{g DNA}$	Mean \pm SD (CV)	2.30 ± 0.47 (21%)	0.766 ± 0.205 (27%)	0.713 ± 0.431 (60%)
	range	1.81 - 2.85	0.560 - 1.050	0.449 - 1.480
$AUC_{0-22\text{hrs}}$ $\mu\text{g/ml} \cdot \text{h}$ or $\text{pg Pt} / \mu\text{g DNA} \cdot \text{h}$	Mean \pm SD (CV)	32.3 ± 3.85 (12%)	2.94 ± 639 (21%)	7.96 ± 2.08 (26%)
	range	29.9 - 37.0	2.13 - 3.71	(5.97 - 11.3)
$AUC_{0-\text{inf}}$ $\mu\text{g/ml} \cdot \text{h}$	Mean \pm SD (CV)	NR	4.44 ± 1.06 (24%)	NR
	range		3.46 - 6.21	

Abbreviations: C_{\max} = maximum observed concentration, $AUC_{0-22\text{hrs}}$ = area under the plasma concentration-time curve from $t = 0$ to 22 hours, $AUC_{0-\text{inf}}$ = area under the plasma concentration-time curve with extrapolation to infinity, $T_{1/2}$ = terminal half-life, WBC = white Blood Cells, n = number of patients, NR = not reported, due to unreliable regression, SD = standard deviation, CV = coefficient of variation.

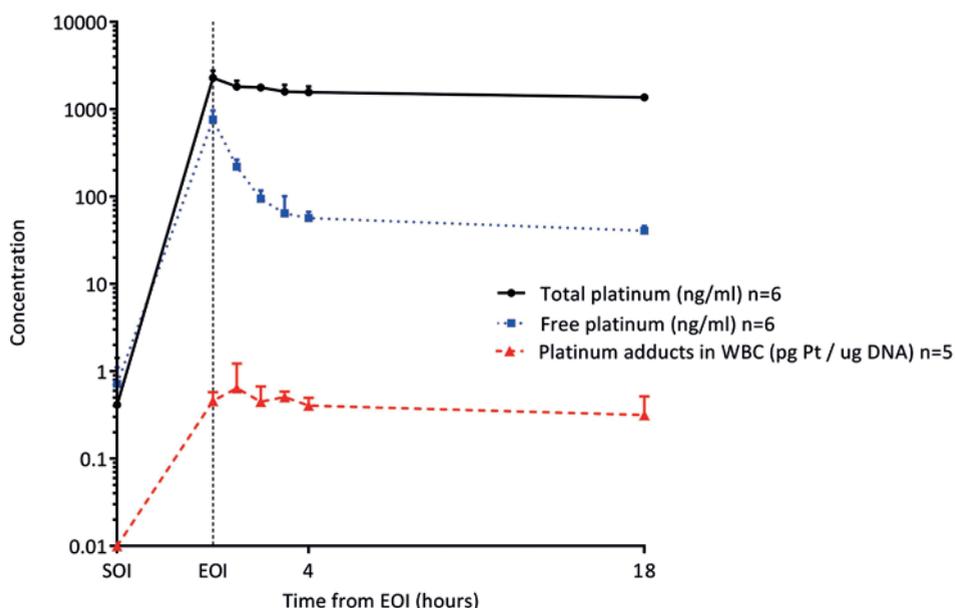


Figure 4: Concentration-time curves of total platinum, unbound platinum and DNA adduct levels in white blood cells in patients treated with 75 mg/m² of cisplatin. SOI = start of infusion, EOI = end of infusion.

Discussion:

Transtympanic middle ear application of STS and placebo gel turned out to be feasible and safe. No toxicities related to gel administration were observed. Application of the gel in arm A was not optimal, as backward flow alongside the needle resulted in limited middle ear infusion of the gel through the grommet. At best a volume of about 0.3 ml could be applied. By adjusting the administration method to a direct puncture of the eardrum posterior to the grommet, the intended volume of 0.4 – 0.5 ml of STS gel could be administered.

It is known that STS diminishes the efficacy of cisplatin when administered intravenously [12]. In our study, a cisplatin dose of at least 75 mg/m² was administered to patients. When considering an average body surface area (BSA) of 1.8 m², patients received about 135 mg (≈ 0.43 mmol) of cisplatin. The amount of STS administered ranged from 0.1 - 0.5 ml of a 0.1 M STS solution (0.01 - 0.05 mmol). As the inhibitory effect of STS is the result of direct binding in a 1:1 ration no more than 10% of the molar weight of the administered cisplatin dose, could be neutralized. This is only when all STS would be available in the systemic circulation. This is, however not the case as STS has a poor oral bio-availability of about 8% [24].

A comparison of the cisplatin PK results with literature is difficult, as it was previously shown, that STS bound platinum is detected, as unbound platinum by the ICP-MS method applied in this study [25]. A comparison of the unbound fraction is therefore not useful. The levels observed were however in line with results of a previous study [26]. A comparison on the DNA adduct levels was also not possible as data in literature are based on different methodologies of adduct measurement.

We were able to show preliminary efficacy of STS gel application on the development of ototoxicity in one patient, in whom the treatment induced high-frequency loss in the STS treated ear was smaller than in the non-treated ear. In a second patient tinnitus was only observed in the placebo ear after 3 cycles of cisplatin at a dose of 75 mg/m², which could have been an early indication of otoprotection by the STS gel.

However, the incidence of ototoxicity was lower than expected beforehand. This might have been caused by the small sample-size and inclusion of some patients who already had some loss in the high frequencies (e.g. ≥ 8 kHz), as patients who already have hearing loss at start of treatment are generally less susceptible to develop ototoxicity [27]. Higher daily dosages of cisplatin (e.g. 100 mg/m²) would likely result in more severe hearing loss [11]. Finally, increasing the number of cycles administered will also result in increased ototoxicity [5]. The current study should, therefore focus on patients, who are to receive cisplatin at a dose of 100 mg/m² as for example given to patients with SCCHN.

Conclusions:

Transtympanic administration of a STS or placebo gel via a grommet or by direct puncture of the eardrum is safe and feasible. Direct transtympanic injection is favorable when compared to administration through a grommet, as it is accompanied by a larger gel volume administered to the middle ear. Otoprotection by topical STS was observed in the only that had clinically relevant cisplatin induced hearing loss. Therefore, to further assess the whether STS application can prevent cisplatin induced hearing loss, the remainder of this study and future studies should focus on inclusion of patients enduring more intense cisplatin treatment regimens and/or more treatment cycles.

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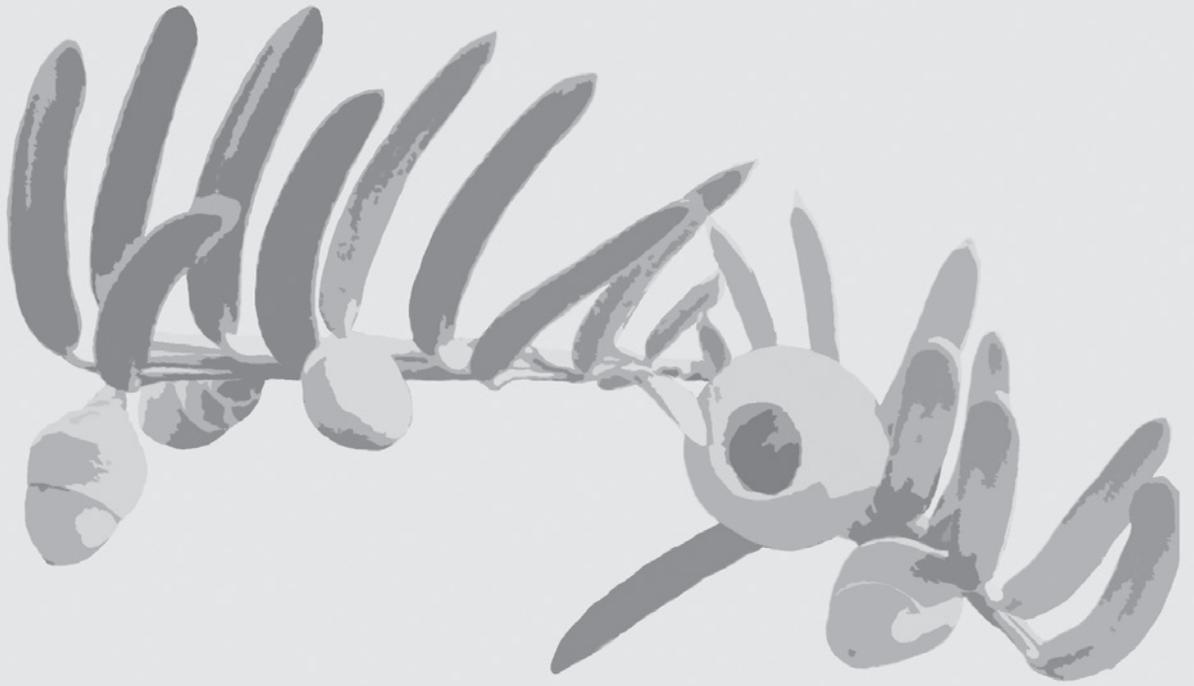
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Chapter 7

Cost analyses of NSCLC



Chapter 7

Changing costs of metastatic non-small cell lung cancer in the Netherlands.

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Published online first: Lung Cancer

Abstract:

Objectives: The primary objective of this study was to identify the total intramural cost of illness of metastatic non-small cell lung cancer (NSCLC) in the Netherlands between 2006-2012. Secondary objective was to identify whether changes in cost patterns of metastatic NSCLC have occurred over the last years.

Methods: Patients diagnosed with metastatic NSCLC between 1-1-2006 and 31-12-2012, who had follow-up to death or the date of data cut-off and no trial participation were included. A structured chart review was performed using a case report form. Data collection started after diagnosis of metastatic NSCLC and ended at death or April first, 2015. Data regarding outpatient visits, clinical attendance, oncolytic drug use, imaging, lab tests, radiotherapy and surgery were collected.

Results: Sixty-seven patients were included with a median age of 67 years. The median follow-up was 234 days. On average patients had 28 outpatient visits and 11 inpatient days. Oncolytic drugs were administered to 76% of the patients. Mean per patient expenditures amounted up to €17,463, with oncolytic drugs (€6,390) as the main cost driver. In comparison with the time-period of 2003-2005 total per patient per year expenses decreased by 44%. The contribution to total yearly costs of oncolytic drugs increased from 18% to 35%, while costs for inpatient stay decreased from 52% to 28% of total expenditures.

Conclusion: Outcomes in this study demonstrate that average treatment costs for metastatic NSCLC in The Netherlands Cancer Institute amount to €17,463. Compared to a prior study the average cost for metastatic NSCLC over time in the Netherlands has decreased. A shift of main cost drivers seems to have occurred from inpatient stay, to oncolytic drugs as main contributor. The shift towards treatment cost might become more visible with the introduction of immunotherapy. These results mark the importance of up-to-date cost of illness studies.

Background:

Lung cancer is the fourth most common cancer in the Netherlands [1]. With over 12,000 diagnoses in 2015, it accounts for over 10,000 annual deaths [1].

The treatment and prognosis of NSCLC depends on the stage of disease at diagnosis. Metastatic NSCLC (stage IV) is incurable and has a poor prognosis with a five-year overall survival (OS) rate of 4% [2]. Treatment guidelines for metastatic NSCLC consist of palliative treatment with oncolytic drugs, radiotherapy, surgery, a combination of these treatments or best supportive care [3,4].

Cancer treatment is currently highly discussed, because of expensive treatments involved; insight in the baseline costs is therefore essential to allow for highly valid cost-effectiveness calculations and decision-making. The treatment of metastatic NSCLC constitutes a large burden on healthcare in terms of costs [5–7], especially now expensive immunotherapy strategies become widely available. In order to gain insight in the NSCLC healthcare burden, Pompen et al. (2009) conducted a retrospective by-chart-review cost of illness (COI) study in 102 patients diagnosed with advanced NSCLC during 2003–2005 in the Netherlands [6]. The estimated intramural healthcare burden of metastatic NSCLC amounted up to €32,386 per patient/year in this time period. Inpatient stay was the main contributor and oncolytic drugs were the second largest contributor [6].

Pompen et al. provided a useful insight in NSCLC costs for the period 2003–2005. Expenses on cancer treatment have however increased two-fold in the period of 2003–2011 as reported by Dutch authorities from 2.4 to 4.8 billion euros a year [8]. In the same time span, expenditures on oncolytic drugs in The Netherlands increased more than three-fold [9]. Relatively new oncolytic drugs for NSCLC i.e. pemetrexed and new orally dosed tyrosine kinase inhibitors e.g. erlotinib, gefitinib and crizotinib all obtained marketing approval and were implemented in the standard of care [10].

These new oncolytic drugs have a substantially higher price per dose than older oncolytic drugs [11]. Data on the healthcare burden of metastatic NSCLC treatment in the Netherlands, have however not been reported since the study by Pompen et al.

Therefore, we aimed to determine total intramural COI in patients diagnosed with metastatic NSCLC in the period of 2006–2012. The secondary objective of this study was to identify changes in cost patterns over time.

Methods:

Patient selection

Healthcare utilization data was obtained by a retrospective medical chart review. Charts were provided by the Netherlands Cancer Institute (NCI; a cancer referral center in Amsterdam). Patients were selected using a search combining data from the Electronic Patient Database (EPD) and the cancer registry database. The search included the following criteria: diagnosis of stage IV NSCLC between 1-1-2006 and 31-12-2012, according to the AJCC lung cancer staging version 7; follow-up in the NCI from diagnosis until death or until the data cut-off date of April first, 2015; no registered clinical trial participation in the NCI at any time during treatment for NSCLC.

Exclusion

All patient records retrieved from the search were individually assessed on in- and exclusion criteria. When during data collection the patient record was found to be incomplete or a patient was diagnosed with a secondary primary tumor in the follow-up period the patient record was excluded from the analyses.

Data collection

Data collection was standardized using an electronic Case Report Form (eCRF). Data was retrieved from the first visit after diagnosis of metastatic NSCLC, until death or end of data collection on April first, 2015. Data collected consisted of patients characteristics and healthcare utilization data.

Demographic characteristics: age, gender, date of birth, ECOG (Eastern Cooperative Oncology Group) performance score (PS), site of metastases, date of diagnose, date of disease progression and date of death were collected. When applicable, the reason for exclusion was recorded.

Healthcare utilization data consisted of: date and type of inpatient visit; date, type and length of outpatient visit; date, type and dose of oncolytic drugs; date and frequency of radiotherapy; number and type of laboratory tests; date and type of surgical procedures. Data concerning imaging was directly extracted from the Hospital Information System (HIS). Dates of all events were registered and all data were collected by one investigator (WK).

Unit costs

Unit costs were based on Dutch guidelines [11–13] or on available literature, if costs were not available in the guidelines [14]. If both sources did not provide sufficient information, unit costs were based on prices as determined by another academic hospital [15]. Because cost on surgical procedures were largely unknown, these costs were not considered in this analyses and were therefore accounted as being zero.

Cost comparison

Outcomes as published by Pompen et al. were extracted from the published article [8]. In this study Pompen and colleagues report on two patient populations: group A received best supportive care (BSC) and group B received second-line treatment in addition to BSC. The weighted mean cost/patient/year for both groups was considered the mean cost per patient for that population. In order to correct for differences in follow-up time, all cost outcomes, excluding cost for oncolytic therapy, from this study were converted from cost/patient into cost/patient/year, using the reported mean follow-up.

Results:

In total 67 patients were found eligible for data collection. In 115 patients follow-up was incomplete and eighteen were not eligible for the study, an overview of reasons for exclusion of patients is shown in figure 1.

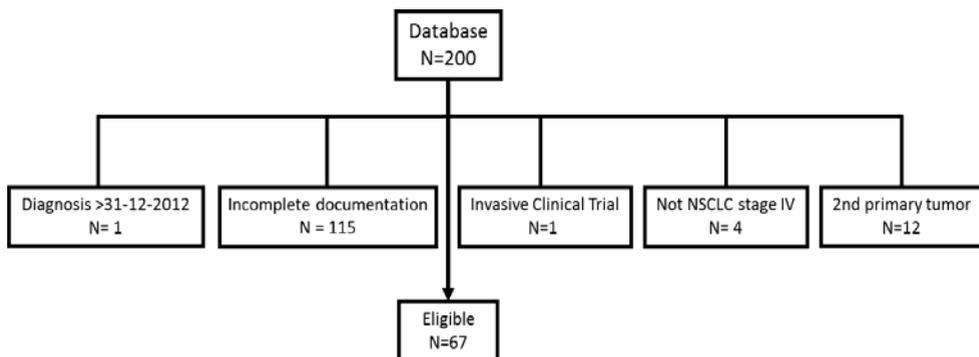


Figure 1: exclusion after individual assessment.

Patient Demographics

Of 67 eligible patients 30 were male (45%) and 37 female (55%), mean age at diagnosis was 67 for male patients and 55 years for female patients. Overall patients had a good PS of 0 to 2, only three patients with a PS greater than 2 were included in the study. The baseline characteristics are presented in table 1.

Table 1: Patient characteristics of all included patients. Abbreviations: ECOG: Eastern Cooperative Oncology Group.

Patient Characteristics		%	
Total			
	Women	37	55
	Men	30	45
Age (years)			
	Men, median	67	
	Range	33 - 89	
	Women, median	55	
	Range	24 - 76	
Performance status (ECOG)			
	0-2	51	76
	>2	3	4
	Unknown	13	19
Year of Diagnosis			
	<1-1-2010	25	37
	>31-12-2009	42	63
Site of Metastases			
	Adrenal	9	13
	Bone	25	37
	Brain	16	24
	Kidney	3	4
	Liver	8	12
	Lung	9	13
	Lymfe	4	6
	Mediastinal	2	3
	Neck	5	7
	Other	14	21
Follow-up (days)			
	Mean	348	
	Median	234	
	Range	24 - 2829	
Histology			
	Adenocarcinoma	34	51
	Large Cell Carcinoma	15	22
	Squamous Cell Carcinoma	13	19
	Unknown Carcinoma	5	7
Mutations			
	EGFR Mutation	4	6
	KRAS Mutation	9	13
	Unknown Mutation	54	81

Mean follow-up time was 348 days (median 234 days) and three patients were alive on the data cut-off date of April first 2015. Bone (37%) and brain metastases (24%) were the most frequent sites of distant metastases. Adenocarcinoma (51%) was the most common histological subtype, followed by large cell carcinoma (22%) and squamous cell carcinoma (19%). KRAS and EGFR mutations were found in 13% and 6% of patients, respectively.

Total costs

A detailed representation of mean cost per patient is provided in table 2. Total mean cost per patient amounted to €17,463 (median: €14,824, range €624 to €68,099). Oncolytic drugs (€6,390; 37%) were the most important cost driver followed by costs for inpatient stays (€4,766; 27%). Total cost of outpatient visits accounted for €3,156 and imaging costs amounted to €1,835. Costs of laboratory tests (€328) was the smallest contributor to healthcare resources used. Total cost per patient for radiotherapy was €987.

Table 2: healthcare resource utilizations and costs. IOD: Intravenous Oncologic Drugs, OOD: Oral Oncologic Drugs.

Cost per healthcare resource	Unit Cost	Mean	Median	Range	Costs	% Treated
Oncolytic Drugs (OD)						
IOD	€ 727 ¹¹	6.8	6	0-28	€ 4,924	65.7
OOD	€ 69 ¹¹	21.3	0	0-425	€ 1,466	29.9
Total OD					€ 6,390	76.1
Outpatient Visit						
Accident/Emergency	€ 151 ¹²	0.1	0	0-2	€ 9	4.5
Day Care	€ 159 ¹²	3.9	2	0-24	€ 615	59.7
Dietician	€ 27 ¹²	0.04	0	0-1	€ 1	4.5
Medical Specialist (MS)	€ 129 ¹²	15.3	11	0-51	€ 1,970	97.0
Nurse	€ 28 ¹²	0.3	0	0-2	€ 8	0.3
Phone Consult MS	€ 65 ¹²	8.2	5	0-33	€ 529	88.1
Other Outpatient	€ 80 ¹²	0.3	0	0-7	€ 25	0.1
Total Outpatient		28.0	24	0-76	€ 3,156	98.5
Inpatient Stay						
Inpatient Day	€ 218 ¹²	0.3	0	0-4	€ 71	20.9
inpatient Night	€ 437 ¹²	10.7	5	0-47	€ 4,694	64.2
Total Inpatient Days		11.1	5	0-49	€ 4,766	65.7
Laboratory						
Blood Test	€ 16 ^{13,14}	15.1	11	0-48	€ 236	89.6
Culture	€ 16 ^{13,14}	4.0	0	0-68	€ 64	43.3
Mutation Analysis	€ 209 ¹³	0.01	0	0-1	€ 3	1.5

Table 2: Continued.

Cost per healthcare resource	Unit Cost	Mean	Median	Range	Costs	% Treated
Pathology	€ 36 ¹³	0.7	0	0-5	€ 25	43.3
Total Laboratory tests		19.8	14	0-95	€ 328	91.0
Imaging						
CTRT	€ 160 ^{13,15}	1.3	1	0-5	€ 210	58.2
CT - scan	€ 202 ^{13,15}	3.3	3	0-15	€ 675	76.1
ECG	€ 18 ¹³	0.9	0	0-6	€ 16	40.3
PET-scan	€ 1,300 ¹³	0.2	0	0-3	€ 252	13.4
X-Ray	€ 54 ^{13,15}	5.7	4	0-24	€ 308	89.6
Ultrasound	€ 61 ^{13,15}	0.8	0	0-4	€ 50	47.8
MRI	€ 198 ¹³	1.1	0	0-17	€ 210	46.3
MRRT	€ 196 ¹³	0.1	0	0-1	€ 12	6.0
Revision	€ 18 ¹³	0.1	0	0-1	€ 1	6.0
Scintigraphy	€ 133 ¹³	0.03	0	0-2	€ 4	1.5
Other imaging	€ 115 ¹³	0.9	0	0-8	€ 98	38.8
Total Imaging		14.3	14	0-44	€ 1,835	98.5
Radiotherapy						
No. Fractions	€ 111 ¹⁴	8.9	8	0-33	€ 987	80.6
Total Radiotherapy		8.9	8	0-33	€ 987	80.6
Surgery						
Lobectomy	€ 0	0.01	0	0-1	€ 0	1.5
Pericardium Puncture	€ 0	0.01	0	0-1	€ 0	1.5
Peripheral Infusies	€ 0	0.1	0	0-3	€ 0	7.5
Pleurodesis	€ 0	0.2	0	0-4	€ 0	6.0
Puncture	€ 0	0.03	0	0-1	€ 0	3.0
Peripheral Catether	€ 0	0.03	0	0-1	€ 0	3.0
Resection Metastases	€ 0	0.01	0	0-1	€ 0	1.5
Diagnostic Pleural Puncture	€ 0	0.01	0	0-1	€ 0	1.5
Stent placement	€ 0	0.04	0	0-3	€ 0	1.5
Other surgery	€ 0	0.1	0	0-1	€ 0	7.5
Total Surgery		0.5	0	0-6	€ 0	23.9
Total					€ 17,463	100.0

Utilization

The mean oncolytic drug use amounted up to 6.8 doses of Intravenously administered Oncolytic Drugs (IOD) and 21.3 doses (days) of Orally administered Oncolytic Drugs (OOD). The most frequently used healthcare utilization were outpatient visits. On average patients were seen 28 times. Medical specialist visits, medical specialist phone consults and day care visits were the most frequently used types of outpatient visits, respectively 15.3, 8.2 and 3.9 visits.

Patients had a mean of 11.1 inpatient days which was associated with 2.9 hospitalizations or ward transfers per patient. Most commonly used laboratory test was blood analyses which was used 15.1 times per patient. Chest X-ray and CT-scan were the most frequently used imaging methods, respectively 5.7 and 3.3 times per patient. In total a mean of 8.9 fractions of radiotherapy and 0.5 surgical procedures per patient were registered.

Incidence of treatment

Ninety-eight point five percent of patients visited the outpatient clinic (one patient was only treated during an inpatient stay). Oncolytic drugs were prescribed to 76.1% of patients. Imaging was performed in 98.5% of patients. Hospitalization occurred in 65.7% of patients. Laboratory tests were performed in 91.0% of patients. Radiotherapy was given to 80.6% of patients. Surgical procedures occurred in 23.9% of patients during the follow-up period.

Oncolytic drugs

A detailed overview of oncolytic drugs used is shown in table 3. Cost for IOD was higher than cost for OOD, €4,924 and €1,466 respectively. Gemcitabine (2.1 doses) was the most frequently administered IOD. Pemetrexed, however was the largest contributor to the costs (€3,961) and was also the drug most frequently used (47.8% of patients). Of all OOD, erlotinib was the most prescribed. On average patients received 17.1 days of treatment with erlotinib. It was also the most frequently used (26.9%) and the biggest contributor to costs (€1,265) for OOD.

Cost comparison

Inclusion criteria differed slightly between this study and the study of Pompen et al. Here we only included patients diagnosed with stage IV NSCLC regardless of the PS. Pompen et al. included patients with stage IIIB or stage IV NSCLC and with a PS of ≤ 2 . In result Pompen et al. reported a mean of 12.5 months follow-up in group A (BSC) (N=74) and 14.4 months follow-up in group B (second-line treatment and BSC) (N=28). Whereas average follow-up reported in this study was 11.4 months (348 days). The cost comparison is shown in table 4. Compared to Pompen *et al.* (2003-2005) mean cost per year of NSCLC treatment decreased by 43% (from € 32,386 to € 18,010 per patient per year). Mean per patient per year expenses on all contributors decreased, except for expenses

on oncolytic drugs and outpatient visits. Mean cost of inpatient stay decreased from €16,777 to € 5,001 per patient per year. Figure 2 shows the comparison of the two studies on cost distribution data per patient per year. Mean per patient expenses on oncolytic drugs increased from 18% to 35%, while mean per patient per year expenses on inpatient stay decreased from 52% to 28%. Mean per patient per year expenses on outpatient visits increased from 8% to 18%. Mean per patient per year expenses on outpatient visits increased from 8% to 18%.

Table 3: detailed representation of oncolytic drugs utilization.

Cost of oncolytic drug use	Cost per mg	Mean Doses	Median Doses	Range	Mean Cost	% Treated
Intravenous Oncolytic Drugs (mean dose)						
Carboplatin (574 mg)	€ 0.33 ¹¹	1.4	0	0-10	€ 267	41.8
Cisplatin (95 mg)	€ 0.47 ¹¹	1.2	0	0-24	€ 53	29.9
Docetaxel (132 mg)	€ 5.49 ¹¹	0.3	0	0-6	€ 217	7.5
Gemcitabine (2092 mg)	€ 0.09 ¹¹	2.1	0	0-17	€ 398	32.8
Paclitaxel (150 mg)	€ 2.62 ¹¹	0.01	0	0-1	€ 6	1.5
Pemetrexed (876 mg)	€ 2.66 ¹¹	1.7	0	0-11	€ 3,961	47.8
Other IOD*	€ 2.88 ¹¹	0.03	0	0-2	€ 22	1.5
Total IOD		6.8	6	0-28	€ 4,924	65.7
Oral Oncolytic Drugs (mean dose)						
Afatinib (280 mg)	€ 1.65 ¹¹	0.2	0	0-14	€ 96	1.5
Erlotinib (144 mg)	€ 0.51 ¹¹	17.1	0	0-425	€ 1,265	26.9
Gefitinib (77 mg)	€ 0.35 ¹¹	3.9	0	0-217	€ 105	4.5
Total OOD		21.3	0	0-425	€ 1,466	29.9
Total					€ 6,390	76.1

*Other IOD include multiple IOD with different prices per mg, a weighted mean is presented



Figure 2: cost distribution extracted from Pompen et al. and cost distribution of this study. All cost are presented per patient per year, except for oncolytic drugs which was presented as cost per patient.

Table 4: cost of illness per patient per year extracted from publication of Pompen *et al.* compared to outcomes (cost/patient/year) from this study.

Healthcare resource (cost/patient/year)	Pompen <i>et al.</i>	This study
Oncolytic Drugs (cost/patient)*	€ 5,711	€ 6,390
Outpatient Visit	€ 2,502	€ 3,312
Inpatient Stay	€ 16,777	€ 5,001
Laboratory + Imaging	€ 4,350	€ 2,271
Radiotherapy	€ 3,045	€ 1,036
Surgery	€ 0	€ 0
Total Costs	€ 32,386	€ 18,010

*Cost of oncolytic drugs was expressed as costs per patient.

Discussion:

Our results show that the average mean intramural cost of metastatic NSCLC amounted up to €17,463 per patient in the period 2006-2012 in the NCI. The costs consisted mainly of expenses on oncolytic drugs and inpatient stays, which contributed to 37% and 27% respectively of the total treatment costs.

Compared to the period 2003-2005 total cost per patient per year of metastatic NSCLC in The Netherlands decreased, while expenses on oncolytic drugs and outpatient visits rose. The decrease in cost could mainly be attributed to a decrease in cost of inpatient stays. The reduction in hospitalizations can partially be attributed to less cisplatin administrations (1.2 doses versus 3.3 doses), since patients were not admitted to receive treatment. A second factor contributing to the reduced healthcare costs is the special referral role of the NCI, which biases our population to a more gradual disease course. This referral role could in addition result in unregistered care in local hospitals. In order to counteract this, patients treated in other hospitals were excluded from the study. However it is likely that some of the local care was not registered at the NCI and therefore unregistered in this study. Finally our population differed from the population by Pompen *et al.* as they included patients with stage IIIb and stage IV NSCLC (according to the AJCC lung cancer staging version 6, not reported), where this study looked at stage IV disease according to the AJCC lung cancer staging version 7. As a result patients with pleural effusion were now scored as having stage IV disease rather than stage IIIb. A bias remains towards lower costs in our study, since no stage IIIb patients without pleural effusion were included.

Nevertheless the dramatic decrease in inpatient stays seems to confirm the hypothesis that new oncolytic drugs and treatments are successfully diminishing the number and the length of hospitalizations in this population.

Expenses on oncolytic drugs have increased significantly. This increase is even more notable since only 76.1% versus 100% of patients were treated with oncolytic drugs in this study and Pompen *et al.* respectively. The increase in cost of oncolytic drugs can be attributed to costs of the oncolytic drugs pemetrexed and erlotinib, these account for 82% of costs on oncolytic drugs. Drug prices of 2017 were used to correct for significant changes in drug pricing, for instance the patent of pemetrexed expired in 2016 [16]. However new immunotherapeutic drugs (e.g. nivolumab and pembrolizumab) have recently obtained marketing approval for the treatment of NSCLC. Therefore costs on oncolytic drugs are likely to increase further as these immunotherapeutic drugs are even more costly.

In order to put our results into perspective, cross validation was performed with other international COI studies. This is difficult as treatment standards for metastatic NSCLC differ between countries and hospitals. The most recent study [17] estimated mean hospital costs for 71 patients diagnosed in 2008 in Spain to be €15,044 [17], while other studies estimated mean hospital cost at €22,066 in Switzerland (1998) [18], and at 11,996 AUD in Australia (2005-2006) [19], which equals to approximately €8500. Based on literature data our findings of mean total cost of € 17,463 for metastatic NSCLC seem to be comparable to international series, but because of great differences in costs between countries no hard conclusions can be drawn. Differences in distribution of cost are more straightforward to compare. All studies reported by Kang *et al.* [19] reported inpatient stay to be the main cost driver in the United States, Canada, Ireland, Australia and the Netherlands [6]. The most recent study however, reported oncolytic therapy as the main cost driver in patients treated in Spain (2008) [17]. This is in line with our presumption that there might be a shift in cost distribution towards oncolytic drugs, which is mainly influenced by new expensive oncolytic drugs. The observed differences in cost distribution and total costs per patient per year between 2003-2005 and 2006-2012 are striking. This indicates that COI studies are sensitive to the time period in which the study is conducted. NSCLC treatment with nivolumab today costs about €1500-€3000,- per dose [11] and patients received a median number of cycles ranging between 6 and 8 doses, depending on tumor histology [20]. This will most likely result in increased expenses on oncolytic drugs. This highlights further that COI studies reflect the current situation and that they are unreliable for long-term use. COI studies provide insights in cost patterns of a certain illness and are the basis for cost-effectiveness studies. Therefore transparent and up-to-date research on COI, is of critical importance to allow for high quality cost-effectiveness research.

Even though our study was carefully designed, the study has limitations. The NCI is actively participating in clinical trials in all stages of drug-development. A selection bias has therefore likely occurred, as patients included in trials are generally the ones with a good performance status and better survival. Secondly, the NCI is a tertiary hospital to which patients are referred to from general and academic hospitals in the Netherlands. This special role biases the population towards patients with more severe disease grades as compared to the population that general and academic

hospitals probably would have. The fact that our research was solely performed in de NCI could have influence the population and therefore treatment costs. Furthermore, the study period is quit long (2006 till 2012), which might have created a heterogeneous group. Though treatment guidelines did not change dramatically, though over the study period.

In our study more women were included at relatively young age, compared to men. This difference is not consistent with cancer incidence and mortality in the Netherlands, which reports a higher incidence of lung cancer in men [1,21]. The referral function of the NCI and an increase in smoking among females partially explain the higher number of female patients with NSCLC included in our trial.

Costs per patient were not normally distributed but skewed to the right. In addition the wide range of follow-up used in this study could potentially result in higher costs, as analysis showed that patients with longer follow-up tend to have a higher cost of treatment ($R^2 = 0.1709$). Differences in study population and follow-up time between our study and Pompen et al. could explain some of the loss of costs. However, costs of treatment per patient do not divide equally over time and extrapolation could be a dangerous step in presenting real world outcomes. In extend, total costs of treatment are more relevant for interpretation of cost patterns, as survival is an important factor contributing to total costs, which should not be ignored when calculating treatment costs. Therefore correction for follow-up was only done in order to make comparison possible between our study and Pompen et al. and not to present real world costs.

Our research was solely focused on cost utilization. Real world COI studies also include extramural healthcare. In order to put intramural costs in perspective Pacolet et al. calculated that intramural costs accounted for 81% of the whole treatment cost of lung cancer in The Netherlands in 2005 [22]. If this is still the case in 2015, than our results could be used to perform a realistic estimation of total real world cost of metastatic NSCLC.

Conclusion:

Mean intramural cost of treatment of metastatic NSCLC patients treated in our cancer referral center amounted to €17,463. Compared to 2003-2005 the average cost for metastatic NSCLC over time in the Netherlands has decreased. A shift of main cost drivers seems to have occurred from inpatient stay in 2003-2005, to oncolytic drugs as the main contributor. This shift is largely attributable to new expensive oncolytic drugs, as they raise oncolytic drug expenses. Meanwhile a decrease in hospital admissions costs occurred. However as a result of the referral function of the NCI, hospital admissions might be underestimated. Our results mark the importance of up-to-date COI studies as we showed that they are sensitive to new developments, this might become of higher importance

as the expensive immunotherapeutic drugs are currently entering the market. We expect that the trend of decreasing costs over time will not be representative as immunomodulatory drugs will drive the trend into the opposite direction.

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Chapter 8

Conclusions and perspectives

Oral taxanes:

Docetaxel:

The results of the phase I clinical trials with “classic high dose chemotherapy” with oral docetaxel as ModraDoc001 or ModraDoc006, co-administered with 100 mg ritonavir (ModraDoc001/r and ModraDoc006/r, respectively) support the further clinical development of ModraDoc006/r (*Chapter 2.2 and 2.3*). The toxicity observed in the phase I trials is in line with that of intravenous docetaxel in a weekly schedule [1]. However, the hypersensitivity reactions and severe (grade ≥ 3) peripheral neuropathy were not observed in the phase I studies despite the fact that dexamethasone pretreatment was omitted. The incidence of low grade diarrhea (e.g. grade 1-2) appears to be increased as compared to a weekly intravenous schedule. The incidence of grade ≥ 3 diarrhea was comparable to studies with intravenous docetaxel. The occurrence of severe grade ≥ 3 diarrhea could be correlated with docetaxel systemic exposure rather than exposure in the gastro-intestinal tract, in both mice and humans [2]. The reduction in overall grade ≥ 3 toxicity potentially results in an improvement of the safety profile of orally administered docetaxel as compared to intravenous docetaxel.

The anti-tumor activity observed with ModraDoc/r is promising with partial responses observed in known docetaxel sensitive tumors (e.g. non-small cell lung cancer (NSCLC) and squamous cell carcinoma of the head and neck (SCCHN)) [3]. The observed activity warrants further development especially given the potential improvement of the safety profile and the potential to reduce treatment costs. Costs might be reduced, as a result of treatment at an outpatient clinic, rather than at the hospital day care unit [4].

The clinical development of ModraDoc006/r is therefore continued in phase I - III clinical trials. A phase I dose-finding study in castration resistant prostate cancer (CRPC) is currently ongoing. As is a combination treatment study in locally advanced prostate cancer, in which oral docetaxel is combined with hormonal- and radiotherapy [5]. A food interaction study with ModraDoc006/r in patients with solid tumors has been initiated recently. A phase II trial in the orphan indication of angiosarcoma has been designed in collaboration with the European Organization for Research and Treatment of Cancer (EORTC), as described in *Chapter 2.4*. The phase III development currently focusses on CRPC, since this appears to be the population in which a non-inferiority study versus intravenous docetaxel has the best feasibility, as docetaxel remains the first-line chemotherapeutic agent of choice in CRPC.

The development of ModraDoc006/r in prostate cancer necessitates careful evaluation of the dose of ModraDoc006 and ritonavir to be employed, as lower exposure to docetaxel (about 50%) in terms of the area under the plasma concentration-time curve up to infinity ($AUC_{0-\infty}$) is reported in studies investigating CRPC [6,7], as compared to studies in other solid tumors [8,9]. The precise mechanism underlying the reduced exposure is unknown, but it is likely caused by upregulation of CYP3A4

and/or drug-transporters (e.g. P-glycoprotein (P-gp), multidrug resistance associated protein 2 (MRP 2) and organic anion-transporting polypeptide (OATP) 1B1/1B3) involved in the excretion of docetaxel. Preliminary results of the ongoing phase I trial with bi-daily once weekly ModraDoc006/r in patients with CRPC already show a lower maximum plasma concentration (C_{\max}) and $AUC_{0-\infty}$ of both docetaxel and ritonavir. Observed C_{\max} and $AUC_{0-\infty}$ for both drugs are about 70% lower as compared to the phase I trial with the same schedule (*Chapter 2.3*). The potential upregulation of CYP3A4 and the drug-transporters might be of greater importance for the oral route given the presence of a first-pass effect after oral administration. It is hypothesized that by administration of a higher dose of ritonavir (e.g. bi-daily 200 mg) the docetaxel exposure can be increased to an exposure to docetaxel comparable to previously reported levels in CRPC, as the higher ritonavir dose potentially results in additional inhibition of CYP3A4, P-gp, MRP 2 and OATP1B1/1B3.

Paclitaxel:

The “continuous low dose metronomic (LDM) treatment” with oral paclitaxel combined with ritonavir was feasible in the phase I trial, described in *Chapter 2.6*. The preliminary anti-tumor activity was, however, disappointing. Therefore, combination treatment with other agents given in a LDM treatment schedule might be needed to obtain satisfactory anti-tumor activity. Potential chemotherapeutic agents to consider are capecitabine, cyclophosphamide or methotrexate, which all can be administered in a LDM treatment schedule (i.e. bi-daily for 2 weeks, with 1 stop week for capecitabine). Another option would be to combine ModraPac005/r with inhibitors of Vascular Endothelial Growth Factor (VEGF) or its receptor (VEGFR) (e.g. bevacizumab). None of these potential combinations have been evaluated in the clinic. Pre-clinical work investigating these combinations would therefore be of great importance, as it could direct choices for the further clinical development based on the results on the activity and toxicity of these combinations.

Another option to improve on the activity could be to better select patients based on tumor characteristics and/or on tumor types, although no specific tumor type sensitive to metronomic treatment has been described. Some have suggested to select highly vascularized tumors given the anti-angiogenic mechanism of action of LDM treatment, again evidence to support the hypothesis is lacking.

The currently used continuous bi-daily administration schedule would be more feasible if ModraPac005/r could be administered with food, as the long fasting periods of about 5 hours per day are impractical for patients. A food interaction study is needed and will be implemented in an additional cohort of the ongoing phase I trial. A food-effect interaction with ritonavir has already been reported in the literature: after co-administration with a moderate- or a high-fat meal, the reported C_{\max} and $AUC_{0-\infty}$ of ritonavir were about 20-23% lower after administration with food [10]. The composition of the meal did not appear to modulate the effect on the exposure. It is therefore

to be expected, that as a result of lower ritonavir levels paclitaxel exposure will also be reduced. Whether this reduction is of clinical relevance is to be awaited.

The evaluated plasma biomarkers in the LDM study with ModraPac/r, i.e. thrombospondin-1 and circulating endothelial cells (CEC), failed thus far to show a correlation with treatment response. Such a relationship could however, not be studied satisfactorily due to lack of tumor response so far in the trial. A need for a validated biomarker of LDM treatment remains. Potential biomarkers could be proteins known to be involved in angiogenesis, such as circulating plasma VEGF or plasma angiopoietin-2 levels [11,12]. Although, as for CEC only correlations with baseline ANG-2 levels and survival have been described. Studies investigating levels in response to treatment are unavailable today, it remains therefore to be determined whether levels decline in patients having a treatment response [12,13]. These biomarkers are of even greater importance as they may guide the further development of a combination treatment.

Future studies with LDM paclitaxel should therefore focus on (1) the identification of patient populations that may benefit from LDM paclitaxel treatment and (2) potential combinations with other anticancer drugs, that may increase the anti-angiogenic effect.

Circulating Endothelial Cells:

A highly sensitive method to measure CEC in both healthy volunteers and cancer patients was developed, employing CD34 microbead enrichment followed by enumeration by flow-cytometry (*Chapter 3*). The proposed method for CEC enumeration allows for storage of samples for up to 2 months, which enables for more flexible sample preparation and measurement. Despite the fact that samples can be stored, the method remains laborious and relatively expensive.

We were able to confirm a previous finding, that CEC numbers are higher in cancer patients, as compared to healthy volunteers [14]. We were however unable to show a correlation with tumor response after LDM paclitaxel treatment, as no responses were identified. Furthermore CEC appear to be an unreliable biomarker to assess anti-cancer treatment efficacy, as their numbers do not only relate to changes in tumor volume, but may also change, as a result of the anti-cancer treatment by itself [14]. The clinical use of CEC as a biomarker in cancer might therefore be limited, especially given the rapid development of other biomarkers that also assess treatment efficacy and that might prove to be more specific for tumor response (e.g. circulating tumor DNA and circulating tumor cells). Additionally CEC numbers are affected by comorbidity of patients, as for example in several cardiovascular diseases in which increases in CEC numbers have been described [15,16]. In these diseases CEC numbers might prove to be a useful biomarker. This could potentially be studied while employing the current method.

HDM2-p53 interaction inhibitors:

The HDM2 (human double minute 2 homolog)-p53 interaction inhibitors are a novel class of anti-cancer agents and their potential usefulness in the clinic requires further investigation. In the monotherapy phase I trial, there were no objective anti-tumor responses (e.g. partial or complete responses) and activity was limited to prolonged stable disease (*Chapter 4.1*). The results in terms of anti-tumor efficacy of our study are in line with studies performed with other HDM2-p53 interaction inhibitors administered as monotherapy. Although partial responses have been observed with other agents of the same class, these have been limited to patients with dedifferentiated or well-differentiated liposarcoma [17–19].

Thrombocytopenia was the only dose-limiting toxicity observed in the monotherapy phase I trial. Thrombocytopenia is not an unexpected adverse event, as the p53 pathway is of importance in hematopoiesis and especially in the megakaryocytopoiesis [20]. In the study with SAR405838 no effect on the maturation of other lines involved in the hematopoiesis was reported, whereas in other studies neutropenia did occur and was found to be dose-limiting in some patients [17–19]. Induction of pharmacodynamic markers (e.g. macrophage inhibitory cytokine-1 (MIC-1)), known to be upregulated upon activation of the p53 pathway [21] was shown in several studies with these agents [17–19,22]. This provides proof of concept on the mechanisms of action of these agents, showing that they do reactivate the p53 pathway. However, the reactivation of p53 alone appears insufficient to obtain anti-tumor activity in most solid tumors. Development of SAR405838 as monotherapy is therefore currently only warranted in liposarcoma. In liposarcoma the function of p53 is down-regulated by specific amplification of the 12q 14-15 locus, on which the HDM2 gene is located. This provides a rationale for the efficacy specifically observed in liposarcoma [23], as HDM2 amplification is rare in other solid tumors.

Future strategies in the development of this class of compounds should therefore aim to explore combinations with for example existing anti-neoplastic agents, that induce DNA damage. As the p53-HDM2 interaction inhibitors have shown to be synergistic with for example doxorubicin and cisplatin in preclinical studies [24].

Another potential combination is described in *Chapter 4.2*. The anti-tumor activity of the combination of the MEK inhibitor pimasertib with SAR405838 appeared promising with 6 patients out of 26 (23%) treated having a prolonged stable disease (> 6 months) and 1 patient (4%) with a partial response (duration of response 78 weeks). In the study the target inhibition of both MEK (measured as reduction in levels of phosphorylated ERK) and HDM2 (measured as increase in MIC-1 levels) was shown, levels also correlated with clinical response. The treatment clearly showed a synergistic effect on anti-tumor activity. The combination proved however to result in more toxicity and already at lower dose-levels as in single agent treatment. This effect could not be

attributed to a pharmacokinetic drug-drug interaction and is therefore more likely to be the result of pharmacodynamic synergy, that does not only affect tumor tissue, but also the healthy tissues. The most common dose-limiting toxicity was again thrombocytopenia, other dose-limiting toxicities were primarily related to pimasertib (e.g. lipase increase, acneiform skin rash). As a result of the toxicity observed the study was terminated prematurely by the sponsor.

The combinations of MEK-, PI3K- and mTOR inhibitors with HDM2-p53 interaction inhibitors have all shown to be promising in preclinical studies [25,26] and further investigation in the clinic is warranted. The mechanism underlying the synergy observed in the pre-clinical models is likely the result of inhibition of MEK-, PI3K and mTOR, as these normally would drive cells towards proliferation and an anti-apoptotic state. By inhibition of these kinases in combination with HDM2 inhibition, p53 is allowed to perform its normal roll more efficiently. The most optimal combination of agents and the most optimal dosages at which they need to be combined should be determined in more detail in preclinical trials in order to prevent severe toxicity in future clinical trials.

Dovitinib:

Dovitinib did not improve the progression-free survival in the phase III superiority trial versus sorafenib as third-line treatment of patients with metastatic renal cell carcinoma [28]. Moreover, the anti-tumor activity observed in other phase II trials in selected tumor types was insufficient to warrant further development [29–32]. The clinical development of dovitinib was therefore halted. The drug-drug interaction study described in *Chapter 5* does however provide valuable information, when dovitinib development is reinitiated in for example a combination treatment. The observed drug-drug interaction is clinically relevant, since commonly used drugs such as amiodarone and ciprofloxacin are also inducers of CYP1A2 and might also increase dovitinib exposure to toxic levels. The precise mechanism underlying the observed drug-drug interaction is not understood, as it cannot completely be attributed to CYP1A2 inhibition alone. A possible explanation is the inhibition of CYP3A4 by dovitinib, that could result in (1) a more important role of CYP1A2 in the metabolism of dovitinib over time and (2) higher fluvoxamine levels which in turn might have led to stronger inhibition of CYP1A2 and thereby higher dovitinib levels. An interaction on the major enzyme involved in fluvoxamine metabolism, CYP2D6, is not to be expected, since dovitinib is not a known inhibitor of this enzyme. The results of the clinical study in which the effect of CYP3A4 inhibition on dovitinib metabolism are, however not publically available. Whether results of this study provide support for either of the hypothesis can therefore not be assessed.

The current study, also underscores the importance of carefully designing a drug-drug interaction study, especially when the drugs investigated are effecting multiple CYP enzymes and are

metabolized themselves via multiple routes. As the interpretation of the results can be rather difficult, as is the case in this study. The current study would have greatly benefited from the measurement of fluvoxamine levels, as it would have allowed us to assess the effect of dovitinib on fluvoxamine plasma levels. An increase in fluvoxamine levels for example would direct towards an interaction in which CYP3A4 is involved.

Sodium thiosulphate otoprotection:

The results of the proof-of-concept study presented in *Chapter 6* in which transtympanic sodium thiosulfate (STS) injections were applied to the middle ear are promising. In only 1 out of 8 patients treated with STS injections ototoxicity occurred. In this patient otoprotective activity of the STS application was observed. The non-treated ear in this patient suffered 15 dB more hearing loss at 8 kHz, as compared to the STS treated ear. Although these preliminary results are promising, the study needs to be completed by inclusion of 4 additional patients. Moreover, a randomized phase II trial is ultimately needed to assess whether the STS injections do result in a clinically relevant reduction in hearing loss. Both the proof-of-principle study and future phase II or III studies should focus on inclusion of patients who are to receive high-cisplatin dosages (e.g. 100 mg/m²) as given for example in the treatment of SCCHN, as the higher dosages are expected to result in more significant hearing loss [33].

Inclusion of patients for the study was difficult, as patients were unwilling to participate. It is expected, that this will improve in future trials, as patients are hesitant to participate in first in man studies. The allocation has improved during the trial, as patients can now be informed in more detail on side-effects and the method of application, based on the experience gained from the first 8 patients. It is therefore expected that this will not hamper the allocation in the future phase II trial. If the concept of otoprotection by local administration of STS will be demonstrated, it would be of even greater importance in children treated with cisplatin. The dosages administered are generally high and result in severe ototoxicity, which in some patients does lead to speech impairment and developmental difficulties [34]. Proving this concept in a pediatric trial is therefore an important future goal.

Pharmaco-economic evaluation of treatment costs in NSCLC:

Over time costs on oncolytic drugs have become the major cost-driver in NSCLC in the Netherlands, as was shown in *Chapter 7*. In the years after our study new anti-cancer agents (e.g. osimertinib, nivolumab and pembrolizumab) have been registered for the treatment of NSCLC. These novel agents significantly improve survival of patients. The costs associated are however significant with nivolumab costing about €134,000 per quality adjusted life year gained [35]. The minister of health has negotiated the price and the nivolumab treatment cost has decreased (to a publically undisclosed price). Costs are however likely to increase even further, as a result of: (1) more personalized cancer treatment, (2) novel agents that rapidly reach clinical practice, (3) an increasing life expectancy of cancer patients and (4) combination treatment with expensive drugs. An example of an expansive drug combination is found in the treatment of advanced melanoma in which dabrafenib and trametinib are combined [36]. It is to be expected, that more of these synergistic combinations will be identified and implemented in clinical practice resulting in further increases in treatment costs. In order to reduce costs attempts to optimize existing treatments should be made. For example, by novel routes of administration, as described in *Chapter 2* or by re-evaluation of existing anti-cancer drugs, of which the patent has expired, in more selected indications based on novel insights in tumor biology. On a political level attempts should be made to come to a more reasonable and more transparent pricing of new anti-cancer agents, as it remains unclear how pricing of drugs is done by the pharmaceutical companies.

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Appendix

Summary & Summary in Dutch

Acknowledgements

Author affiliations

Curriculum vitae

Overview of publications

Molecular structures

Summary

In the chapters included in this thesis several clinical pharmacological studies will be discussed. In *Chapters 2, 4 and 5* pharmacological properties of novel oral anti-cancer agents are outlined. In *Chapter 3* a method for the enumeration of circulating endothelial cells is described. In *Chapter 6* the first results of the proof-of-concept study that aims to prevent cisplatin induced ototoxicity by transtympanic administration of sodium thiosulfate are presented. Finally, in *Chapter 7* a pharmaco-economic analyses of the treatment costs of non-small cell lung cancer is discussed.

In *Chapter 1* the clinical pharmacological properties of the taxanes, docetaxel and paclitaxel are discussed. The working mechanism, the clinical application and the expected adverse events of treatment are highlighted. Some new potential applications of the taxanes are presented, for example low dose metronomic therapy, wherein the goal is to inhibit the growth of the tumor vasculature. Finally strategies to enable oral administration of the taxanes are presented.

In *Chapter 2* the clinical development of oral docetaxel and oral paclitaxel is discussed. The absorption of docetaxel and paclitaxel from the gastro-intestinal tract is hampered by their poor water-solubility and metabolism and excretion by CYP3A4 and the drug transporter P-glycoprotein (P-gp), respectively. Both CYP3A4 and P-gp are highly expressed in the gut-lumen and the liver. In order to improve the water-solubility of docetaxel and paclitaxel novel formulations were developed. By either spray- or freeze drying the particle size was decreased, which in combination with the addition of the selected excipients resulted in increased water-solubility. The final product was formulated in a capsule or tablet, denoted for docetaxel, ModraDoc001 capsule and ModraDoc006 tablet and for paclitaxel ModraPac001 capsule and ModraPac005 tablet.

In *Chapter 2.1* the effect of co-administration of oral docetaxel as ModraDoc001 in combination with inhibitors of the CYP3A4 enzyme and/or of the drug transporter P-gp, to boost the uptake of docetaxel from the gastro-intestinal tract, was investigated. ModraDoc001 was administered in combination with the boosters ritonavir, ketoconazole, clarithromycin and grapefruit juice. In this study the boosters ritonavir, ketoconazole and clarithromycin were able to increase the systemic exposure to oral docetaxel. No effect on the systemic exposure to oral docetaxel was observed in the combination with grapefruit juice. The results showed limited differences between the boosters (except for grapefruit juice) in their ability to increase the systemic exposure to docetaxel. The study proofed that oral administration of docetaxel as ModraDoc001 in combination with inhibitors of CYP3A4 and/or P-gp is feasible. Because of the limited side-effects and the relatively low-dose needed to boost uptake of docetaxel ritonavir was selected for the further development.

In *Chapter 2.2* the dose-escalation trial with oral docetaxel as either ModraDoc001 or ModraDoc006 in combination with the CYP3A4 and P-gp inhibitor ritonavir is presented. In this study, oral docetaxel and ritonavir were administered once weekly on day 1 of a weekly cycle. In total 67 patients were included in the trial. The maximal tolerable dose (MTD) was established as 60 mg ModraDoc001 or ModraDoc006 in combination with 100 mg ritonavir. Most common adverse events were nausea, vomiting, diarrhea and fatigue. These were also the most common dose-limiting toxicities (DLT). The observed exposure to docetaxel was comparable to that in trials with weekly iv docetaxel. In nine patients a partial response (i.e. decrease of $\geq 30\%$ in tumor volume (PR)) was observed, of which six were confirmed after a minimum of four weeks. Taking everything together this was found encouraging for further development of oral docetaxel in combination with ritonavir.

In *Chapter 2.3* a different administration schedule of oral docetaxel in combination with ritonavir was investigated. In this study docetaxel was administered twice on day 1 (bi-daily ((BID), morning/afternoon) of a weekly cycle in combination with BID 100 mg ritonavir. The MTD was established as 20/20 mg ModraDoc001 with BID 100 mg ritonavir and as 30/20 mg ModraDoc006 with BID 100 mg ritonavir. In this study diarrhea, nausea, vomiting and fatigue were also the most common adverse events and the most common DLT. Neutropenia, sensory neuropathy and hypersensitivity reactions were both in this study and in the study described in *Chapter 2.2* rare events, or did not occur at all. The variation in the plasma pharmacokinetics of docetaxel was lower in this study, as compared to *Chapter 2.2*. In five patients a PR in tumor volume was observed, of which two were confirmed after a minimum of four weeks. Further development in the BID once weekly schedule is preferred over the once weekly administration schedule, because of the lower variation in exposure to docetaxel.

In *Chapter 2.4* the study design of a planned phase II trial with oral docetaxel combined with ritonavir is presented. The aim of the study is to assess whether the combination of oral docetaxel combined with ritonavir has comparable anti-tumor activity in the treatment of advanced angiosarcoma, as compared to intravenous paclitaxel. Secondary aims include comparison of quality of life and adverse events between the two arms of the study. The study will be performed in collaboration with the European Organization for Research and Treatment of Cancer (EORTC). The study will be an European multi-center study.

In *Chapter 2.5* a study with a comparable design as in *Chapter 2.1* with oral paclitaxel is presented. In this study, an oral drinking solution of paclitaxel is co-administered with the boosters ritonavir, cyclosporin A, ketoconazole and clarithromycin. The results of this study show that cyclosporin A is the strongest booster, possibly because of its stronger P-gp inhibition, as compared to the other

three boosters. Ritonavir was found to be a good alternative for boosting with cyclosporin A. It will therefore be used as a booster drug in future studies with oral paclitaxel, since it has a better safety profile than cyclosporin A.

In *Chapter 2.6* a dose-escalation study with low dose metronomic (LDM) paclitaxel in combination with ritonavir is discussed. The aim of continuous low dose therapy is, apart from a direct anti-tumor effect, inhibition of the growth of the tumor vasculature by inhibition of growth of the tumor endothelial cells. In this study paclitaxel was administered as ModraPac001 capsule or ModraPac005 tablet (which replaced the capsule in the course of the study). Oral paclitaxel was administered BID in combination with 100 mg ritonavir. The most commonly observed adverse events were nausea, vomiting, fatigue and diarrhea. These also proved to be DLT. The administration schedule was found to be feasible and safe at or below the MTD. The MTD was determined as BID 20 mg ModraPac005 combined with BID 100 mg ritonavir. The anti-tumor activity of the schedule was limited with stable disease as best response to treatment. Further development will focus on combination treatment with other chemotherapeutic agents, for which at present no decision on the agent of first choice has been made yet.

In *Chapter 2.7* a pharmacokinetic (PK)-toxicodynamic (TOX) model was established to relate exposure to oral docetaxel to the probability of DLT in patients. An effect compartment was introduced to account for the harmful effect caused by docetaxel, whereby the amount of drug in the effect compartment was a significant predictor for the probability of a DLT. The PK-TOX model was used to evaluate different dose-regimens that were tested in the clinic. This model will further support the dose-escalation for future clinical trials.

In *Chapter 3* the pre-clinical and clinical development of an assay to quantitate circulating endothelial cells (CEC) in whole blood is described. The assay was developed, since CEC were considered a potential biomarker of LDM therapy with paclitaxel (as was applied in *Chapter 2.6*). By applying a sample enrichment using CD34 micromagnetic beads, all possible endothelial cells (CD34 positive) were preselected. CEC were then enumerated using flow-cytometry. Prior to enumeration cells were incubated with anti-bodies directed against CD14, CD15, CD34, CD45, CD146 and Hoechst, whereby CEC were defined as being all cells negative for CD14, CD15 and CD45 and positive for CD34, CD146 and Hoechst. By the use of the micromagnetic beads the assay became highly sensitive for the detection of CEC's. This was proved by the use of Human Umbilical Vein Endothelial Cells (HUVEC) as a surrogate cell for CEC. We were able to confirm the previous finding of elevated CEC numbers in cancer patients. An effect on CEC numbers after LDM paclitaxel was, however not observed.

Chapter 4.1 presents the results of the dose-escalation trial with the HDM2-p53 interaction inhibitor SAR405838. SAR405838 could safely be administered in both a daily and a once weekly schedule. The only toxicity found to be dose limiting in this study was thrombocytopenia. Other commonly observed toxicities were nausea and fatigue. The anti-tumor activity observed was limited to prolonged stable disease. The dose of once daily 300 mg SAR405838 was selected for the further development, which was pursued in liposarcoma and in combination studies.

In *Chapter 4.2* the dose-escalation combination trial of SAR405838 and pimasertib is described. The combination was based on a synergistic effect that was found in preclinical studies. SAR405838 and pimasertib were combined at different dosages. High grade adverse events (i.e. grade ≥ 3) were already observed at the lower dose-levels investigated. The lowest dose investigated of daily 200 mg SAR405838 and BID 45 mg pimasertib was ultimately defined as the MTD. Commonly observed adverse events were acneiform skin rash, thrombocytopenia, nausea and fatigue. DLT's observed were thrombocytopenia, lipase increase and inability to continue study medication as a result of toxicity. Although one partial response was observed and prolonged stable disease (≥ 6 months) was observed in seven patients, the phase II part of the study was not opened, because of the adverse events observed.

In *Chapter 5* the drug-drug interaction study of dovitinib and the CYP1A2 inhibitor fluvoxamine is described. During the first 3 weeks of the study dovitinib was administered, as monotherapy at a dose of 300 mg in a 5 days on / 2 days of schedule. In week 4 patients took besides dovitinib a 100 mg dose of fluvoxamine for 7 days. On days 5, 6, 7 and 8 of weeks 3 and 4 plasma samples were collected to study the potential interaction. In the study, a clinically relevant interaction was observed, whereby the plasma exposure to dovitinib increased 2.88 fold when it was co-administered with fluvoxamine. This interaction is clinically relevant since dovitinib already has moderate toxicity when administered alone at its MTD. The increase in exposure observed in this study would definitely result in severe dovitinib related toxicity, when administered at the MTD. Given the observed interaction dovitinib is therefore not to be co-administered with CYP1A2 inhibitors, if co-administration is needed a dose-reduction of dovitinib should be applied.

In *Chapter 6* the preliminary results of a proof of concept study in which sodium thiosulfate (STS) is administered transtympanically (through the ear drum) in order to prevent cisplatin induced ototoxicity. This study was performed in two arms, in arm A a placebo gel was administered to one ear while the other received a STS containing gel. In arm B the method of injection was changed slightly and administration of placebo was omitted, the STS gel was still only administered to one ear. In one of the eight patients included an otoprotective effect of the STS gel was observed. Up to

now this was the only patient in whom clinically relevant ototoxicity was observed. The inclusion of arm B is currently ongoing. The results of the additional patients are needed to assess whether transtympanically administered STS can truly prevent cisplatin induced ototoxicity.

In *Chapter 7* the results of a cost-analyses of the treatment of non-small cell lung cancer (NSCLC) in the Netherlands in the period 2006 to 2012 are presented. A comparison on the distribution of costs was performed by comparison to a study that was performed in the period of 2003 to 2005. In comparison to this period the total costs of the treatment of NSCLC declined. A change in the major contributor to costs was however observed. In the previous study hospitalization was the major cost driver, whereas in the current study this was oncolytic drug treatment. This result fits with the tendency wherein the duration of hospitalization declines and costs associated with anticancer treatment increase. This study underscores, the importance of regularly actualizing cost-of illness studies of a disease.

Nederlandse samenvatting

In dit proefschrift worden in de verschillende hoofdstukken klinisch farmacologische studies besproken. De *Hoofdstukken 2, 4 en 5* bespreken de eigenschappen van verschillende orale anti-kanker medicijnen. In *Hoofdstuk 3* wordt een methode voor het meten van circulerende endotheelcellen beschreven, welke een mogelijke biomarker vormen voor anti-kankertherapie. *Hoofdstuk 6* beschrijft een “proof-of-concept” studie waarin beoordeeld wordt of natriumthiosulfaat gehoorschade door cisplatine kan voorkomen. Tot slot wordt in *Hoofdstuk 7* een farmacoeconomische analyse van de kosten van het niet-kleincellig longkanker beschreven.

In *Hoofdstuk 1* worden de klinisch farmacologische eigenschappen van de taxanen, docetaxel en paclitaxel besproken. Het werkingsmechanisme, de klinische toepassing en de mogelijk te verwachten bijwerkingen worden toegelicht. Tevens worden mogelijke nieuwe toepassingen besproken, zoals laag gedoseerde metronomische chemotherapie, wat tot doel heeft de tumorvaatgroei te remmen. Ook worden strategieën besproken die de orale toediening van de taxanen mogelijk maken.

In *Hoofdstuk 2* wordt de ontwikkeling van oraal docetaxel en oraal paclitaxel besproken. De opname van docetaxel en paclitaxel vanuit de darm is beperkt door een beperkte oplosbaarheid in water en door afbraak en excretie door respectievelijk het enzym CYP3A4 en geneesmiddelen-transporters, zoals bijvoorbeeld P-glycoproteïne (P-gp). Deze komen beide in hoge mate voor in de darm en de lever. Om de wateroplosbaarheid van docetaxel en paclitaxel te verhogen werden nieuwe formuleringen ontwikkeld. Door middel van sproei- of vriesdrogen werd de deeltjesgrootte van docetaxel of paclitaxel verkleind, wat in combinatie met het toevoegen van hulpstoffen resulteert in verhoogde wateroplosbaarheid. Het eindproduct werd geformuleerd in een capsule of een tablet. Voor docetaxel zijn dit ModraDoc001 capsule en ModraDoc006 tablet en voor paclitaxel ModraPac001 capsule en ModraPac005 tablet.

In *Hoofdstuk 2.1* is gekeken naar het effect van het gezamenlijk toedienen van oraal docetaxel als ModraDoc001 capsule met remmers van of het CYP3A4 enzym en/of de drug transporter P-gp om de opname van docetaxel te bevorderen (boosten). ModraDoc001 werd toegediend in combinatie met ritonavir, ketoconazol, claritromycine en grapefruitsap (boosters). In de studie konden de boosters ritonavir, ketoconazol en claritromycine de opname van docetaxel verhogen. Er werd geen effect van grapefruitsap gevonden betreffende de opname. Tussen de andere boosters werden kleine verschillen gezien in de mate van bevordering van de docetaxel opname in het bloed. Deze studie toonde aan dat de orale behandeling met docetaxel in combinatie met een remmer van CYP3A4

en/of P-gp mogelijk is. Vanwege het gunstige bijwerkingen profiel en de relatief lage dosering die nodig is om de opname te boosten werd ritonavir geselecteerd voor de verdere ontwikkeling.

In *Hoofdstuk 2.2* wordt de dosisescalatie studie met oraal docetaxel als ModraDoc001 of ModraDoc006 in combinatie met de CYP3A4 en P-gp remming met ritonavir besproken. In deze studie werden oraal docetaxel en ritonavir 1 keer per week toegediend op dag 1 van de week. In totaal werden 67 patiënten in de studie geïnccludeerd. De maximaal veilige dosering werd vastgesteld op één keer per week 60 mg ModraDoc001 of ModraDoc006 in combinatie met 100 mg ritonavir. Veel voorkomende bijwerkingen waren misselijkheid, braken, diarree en vermoeidheid. Deze bijwerkingen waren ook de meest voorkomende dosis limiterende toxiciteit (DLT). De blootstelling aan docetaxel was vergelijkbaar met die bereikt in studies met wekelijks intraveneus docetaxel. Bij negen patiënten werd een partiële afname van het tumorvolume gezien. Verdere ontwikkeling van oraal docetaxel in combinatie met ritonavir wordt daarom nagestreefd.

In *Hoofdstuk 2.3* werd een ander toedieningsschema van oraal docetaxel in combinatie met ritonavir onderzocht. In deze studie werd op dag 1 van de week, 2 keer oraal docetaxel (ochtend/middag) met 2 keer 100 mg ritonavir toegediend. De maximale veilige dosis werd vastgesteld op 20/20 mg ModraDoc001 met twee keer 100 mg ritonavir en 30/20 mg ModraDoc006 met twee keer 100 mg ritonavir. Ook in deze studie waren diarree, misselijkheid, braken en vermoeidheid de belangrijkste bijwerkingen en ook de meest voorkomende DLT. Neutropenie, sensorische neuropathie en overgevoeligheidsreacties waren zowel in deze studie als die beschreven in *Hoofdstuk 2.2* zeldzaam of werden in het geheel niet gezien. De variatie in de farmacokinetiek (PK) was in deze studie kleiner dan in de studie beschreven in *Hoofdstuk 2.2*. Bij vijf patiënten werd een partiële afname van tumorvolume gezien. Verdere ontwikkeling in combinatie in dit schema wordt dan ook nagestreefd. Vanwege die kleinere variatie in blootstelling aan docetaxel in deze studie, heeft het twee keer daags toedienen de voorkeur boven 1 keer daags toedienen.

In *Hoofdstuk 2.4* wordt de studieopzet van een van de vervolgstudies met oraal docetaxel, gecombineerd met ritonavir, beschreven. Het doel van deze studie is om te beoordelen of de orale combinatie net zo actief is in de behandeling van uitgezaaid angiosaroom, als wekelijks intraveneus paclitaxel. Naast activiteit tegen de kanker wordt er in deze studie gekeken naar kwaliteit van leven en het optreden van bijwerkingen als secundaire eindpunten. De studie zal in samenwerking met de European Organization for Research and Treatment of Cancer (EORTC) worden uitgevoerd. De studie zal een Europese multicenter studie worden.

Hoofdstuk 2.5 beschrijft een studie met oraal paclitaxel, met een opzet die vergelijkbaar is met die van de studie beschreven in *Hoofdstuk 2.1*. In deze studie wordt oraal paclitaxel als een drankoplossing gecombineerd met een van de boostergeneesmiddelen: ritonavir, cyclosporine A, ketoconazol of claritromycine. Uit deze studie komt naar voren dat cyclosporine de sterkste booster is, mogelijk als gevolg van de sterkere remming van P-glycoproteïne, in vergelijking met de andere drie boosters. Ritonavir is in deze studie een goed alternatief gebleken voor cyclosporine A en zal ook gebruikt worden in andere studies als booster van paclitaxel, omdat het een beter veiligheidsprofiel heeft dan cyclosporine A.

In *Hoofdstuk 2.6* wordt de dosisescalatiestudie met lage dosis metronomisch paclitaxel in combinatie met ritonavir beschreven. Het doel van deze lage continue dosering is om naast een direct anti-tumor effect ook een anti-tumor effect te verkrijgen door het remmen van de vaatgroei in de tumor door remming van tumor endotheelcellen. In deze studie werd paclitaxel gegeven als de ModraPac001 capsule of de ModraPac005 tablet (deze verving de capsule in de loop van de studie). Oraal paclitaxel werd twee keer daags toegediend in combinatie met 100 mg ritonavir. De belangrijkste bijwerkingen waren misselijkheid, braken en diarree. Deze bijwerkingen bleken ook de belangrijkste DLT voor dit schema. Het toedienen van paclitaxel in zo'n schema bleek haalbaar en veilig. De maximale dosering werd vastgesteld op 2 keer daags 20 mg ModraPac005 met 2 keer 100 mg ritonavir. De anti-tumor activiteit van dit schema was echter tot op heden gering. Verdere ontwikkeling wordt daarom nagestreefd in een nader te bepalen combinatiebehandeling.

In *Hoofdstuk 2.7* werd een PK-toxicodynamisch (TOX) model ontwikkeld dat de blootstelling aan oraal docetaxel en de kans op een DLT bij patiënten beschrijft. Een effect compartiment werd geïntroduceerd om de schadelijke effecten van docetaxel te verklaren. De hoeveelheid docetaxel in het effect compartiment was een significante voorspeller van de kans op DLT. Het PK-TOX model werd hierna gebruikt om de doseerschema's, zoals beschreven in Hoofdstukken 2.2 en 2.3, te evalueren. Dit model zal de selectie van de dosis voor toekomstige studies ondersteunen.

In *Hoofdstuk 3* wordt de preklinische en klinische ontwikkeling van een assay voor het meten van circulerende endotheelcellen (CEC) in volbloed beschreven. De assay werd ontwikkeld omdat CEC een mogelijke biomarker zijn van metronome paclitaxel chemotherapie (zoals toegepast in *Hoofdstuk 2.6*). Middels een scheiding met CD34 micromagnetische beads, werden alle cellen die mogelijk CEC zijn geselecteerd. CEC werden dan geteld door middel van een flow-cytometer. Voorafgaande aan het tellen werden de cellen geïncubeerd met anti-lichamen gericht tegen CD14, CD15, CD34, CD45, CD146 en Hoechst, CEC werden gedefinieerd als alle cellen die negatief zijn voor CD14, CD15 en CD45, en positief voor CD34, CD146 en Hoechst. Door de scheiding middels

de micromagnetische beads werd de assay zeer gevoelig voor het meten van CEC. Dit werd onder andere aangetoond door gebruik te maken van "Human Umbilical Vein Endothelial Cells" (HUVEC), een veel gebruikte surrogaat cel voor endotheelcellen van andere origine. We bevestigden de eerdere bevinding dat CEC in verhoogde aantallen aanwezig zijn bij kankerpatiënten. Een effect op het aantal CEC in de patiënten die behandeld werden met metronoom paclitaxel kon echter nog niet worden aangetoond.

In *Hoofdstuk 4.1* wordt de multicenter dosisescalatie studie met de HDM2-p53 interactie inhibitor SAR405838 beschreven. SAR405838 kon veilig in zowel een dagelijks, als een één keer per week schema worden toegediend. De enige dosis-beperkende bijwerking die werd gezien was trombopenie. Andere veel voorkomende bijwerkingen waren misselijkheid en vermoeidheid. De anti-tumor activiteit was gering met langdurige stabiele ziekte als beste resultaat. De dagelijkse dosering van 300 mg SAR405838 werd geselecteerd voor de verdere ontwikkeling. Deze werd nagestreefd in combinatiebehandeling of in liposarcoom.

In *Hoofdstuk 4.2* wordt een dosisescalatie-vervolgstudie beschreven op de monotherapie studie met SAR405838. In deze studie werden SAR405838 en pimasertib gecombineerd. Deze combinatie werd gebaseerd op een synergistische werking die werd gezien in preklinische studies. SAR405838 en pimasertib werden in verschillende doseringen gecombineerd. De combinatie gaf veel bijwerkingen, zelfs op de laagste dosering, die werden geëvalueerd. Deze laagste dosering van dagelijks 200 mg SAR405838 met 2 keer daags 45 mg pimasertib werd gedefinieerd als de maximaal veilig dosering. Veel voorkomende bijwerkingen waren acneïforme-huiduitslag, trombopenie, misselijkheid en vermoeidheid. De dosis beperkende bijwerkingen waren trombopenie, lipaseverhoging en het moeten staken van de medicatie in de eerste drie weken vanwege matig ernstige bijwerkingen. Hoewel er bij 1 patiënt partiële afname van tumorvolume werd gezien en bij zeven patiënten langdurige ziekte stabilisatie, werd het fase II deel vanwege de gevonden bijwerkingen niet geopend.

In *Hoofdstuk 5* wordt de studie beschreven waarin de geneesmiddel-geneesmiddel interactie tussen dovitinib en de CYP1A2 inhibitor fluvoxamine werd onderzocht. Gedurende de eerste 3 weken werd dovitinib monotherapie gegeven in een 5 dagen op en 2 dagen af schema op een dosis van 300 mg. In week 4 namen patiënten naast dovitinib 100 mg fluvoxamine gedurende 7 dagen. Op dag 5, 6 en 7 van week 3 en week 4 werden plasma kinetiekmonsters afgenomen, om een mogelijke interactie te onderzoeken. In de studie werd een klinisch relevante interactie gevonden, waarbij de plasma blootstelling aan dovitinib 2.88 keer verhoogd werd door de co-administratie van fluvoxamine. Deze interactie is klinisch relevant omdat dovitinib al een redelijke toxiciteit heeft op de maximale dosering. De in deze studie gevonden toename zal daarom zeker extra bijwerkingen

veroorzaken. Vanwege de gevonden interactie moet dovitinib niet gecombineerd worden met CYP1A2 inhibitoren, mocht dit toch noodzakelijk zijn dan dient de dovitinib dosering verlaagd te worden.

In *Hoofdstuk 6* wordt de “proof-of-concept” studie beschreven waarin natriumthiosulfaat (STS) toegediend wordt via transtympane injectie (door het trommelvlies), om te beoordelen of dit gehoorschade als gevolg van cisplatine behandeling kan voorkomen. Deze studie werd uitgevoerd in twee armen, waarbij in arm A aan het ene oor placebo werd gegeven en aan het andere STS. In arm B werd de methode van injectie iets aangepast en werd niet langer placebo gebruikt, wel werd STS in 1 oor toegediend. In 1 van de acht geïncludeerde patiënten werd een beschermend effect gevonden van de STS toediening, deze patiënt was tot op heden ook de enige met klinisch significant gehoorverlies. De inclusie van patiënten in arm B is op dit moment nog gaande. De resultaten van de nog te includeren patiënten zullen moeten uitwijzen of transtympaan toegediend STS inderdaad de toxiciteit van cisplatine kan verminderen.

In *Hoofdstuk 7* wordt beschreven hoe de kosten van de behandeling van het niet-kleincellige longcarcinoom in Nederland in de periode van 2006 tot 2012 waren opgebouwd. De opbouw van de kosten in deze periode werd vergeleken met een eerdere studie, uitgevoerd in Nederland in de periode 2003-2005. In vergelijking met de eerdere studie namen de totale kosten van de behandeling van het niet-kleincellige longcarcinoom af. Wel werd er een verschil gezien in de belangrijkste kostenpost tussen de twee studies. De belangrijkste kostenpost in de studie van Pompen was de behandeling in het ziekenhuis, waar dit in de huidige studie de uitgaven aan anti-kanker therapie waren. Dit resultaat past in de tendens waarbij ziekenhuisopnames korter, en de kosten van medicamenteuze therapie alsmar hoger worden. De studie onderstreept dat het belangrijk is om ziektekosten-onderzoeken up-to-date te houden.

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Artur, samenwerken met jou was op momenten een uitdaging voor ons beide. Uiteindelijk was de samenwerking erg prettig en heeft het geresulteerd in een mooie assay voor het detecteren van CEC en jouw eerste publicatie als eerste auteur. Het heeft wat bloed, zweet en tranen gekost om het allemaal zelf op te schrijven, maar het resultaat mag er zijn. Helaas is uit de studie met metronoom ModraPac nog niet gebleken dat CEC een biomarker zijn voor deze vorm van behandeling.

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Binnen de afdeling farmacologie zijn er velen die ook van onschatbare waarde zijn geweest in de afgelopen jaren. De verpleging op de afdeling farmacologie eerst op 4C en nu op de 1A/CRU.

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Curriculum vitae



Vincent Anne de Weger werd geboren op 28 september 1987 te Utrecht. Hij groeide op in de Bilt. Na het behalen van zijn VWO diploma aan Het Nieuwe Lyceum in 2005 werd hij ingeloot voor de studie geneeskunde aan de Vrije Universiteit in Amsterdam. De studie geneeskunde rondde hij af in 2011. Tijdens zijn studie liep hij in het kader van het honoursprogramma geneeskunde een uitgebreide onderzoeksstage. Deze stage werd uitgevoerd op de afdeling pathologie van het VUmc. Hier deed hij onderzoek naar de invloed van het al of niet aanwezig zijn van microsatelliet-instabiliteit op de uitkomst van patiënten behandeld met “active specific immunotherapy”. Deze stage werd uitgevoerd onder begeleiding van prof. dr. Gerrit Meijer en dr. Erik Hooijberg. Na het afronden van zijn studie werkte hij van september 2011 tot en met februari 2013 in het Spaarne Ziekenhuis te Hoofddorp als arts niet in opleiding tot specialist (ANIOS) op de afdelingen interne/MDL/oncologie, cardiologie en longziekten. Op 1 maart 2013 startte hij zijn promotieonderzoek onder begeleiding van promotoren prof. dr. Jan Schellens en prof. dr. Jos Beijnen en copromotor Serena Marchetti in het Antoni van Leeuwenhoek / Nederlands kanker instituut (NKI-AVL). Hier onderzocht hij de klinisch farmacologische effecten van nieuwe orale antikanker medicijnen bij kanker patiënten. Tijdens zijn promotieonderzoek volgde hij de opleiding tot klinisch farmacoloog welke in 2017 werd afgerond. Per 1 september 2017 is hij gestart met zijn nieuwe functie als arts-assistent in opleiding tot internist (AIOS) bij de Noordwest ziekenhuisgroep te Alkmaar.

Vincent Anne de Weger was born in Utrecht, on September 28, 1987. He grew up in de Bilt. After receiving his VWO diploma at Het Nieuwe Lyceum in 2005, he started medical school at the Vrije Universiteit in Amsterdam. He finished medical school after six years in 2011. During his medical school he enrolled in the Honoursprogram, where he performed an extended scientific internship at the department of pathology of the VUmc. He investigated the effect of having a micro-satellite instable tumor, on the treatment outcome of patients, treated with active specific immunotherapy. The internship was performed under supervision of prof. dr. Gerrit Meijer and dr Erik Hooijberg. After completing medical school, he worked from September 2011 until February 2013 in the Spaarne Hospital in Hoofddorp, where he worked as a resident not in training on the internal medicine, gastroenterology, oncology, cardiology and pulmonology wards. On March 1, 2013 he started with his PhD thesis under supervision of promotor prof. dr. Jan Schellens and prof. dr. Jos Beijnen and copromotor Serena Marchetti at the Netherlands Cancer Institute. Here he investigated the clinical pharmacological aspects of novel oral anticancer agents in patients with cancer. Next to his PhD training he completed his training to become a clinical pharmacologist in 2017. As of September 1, 2017 he started as a resident in training to become a doctor in internal medicine at the Noordwest ziekenhuisgroep in Alkmaar.

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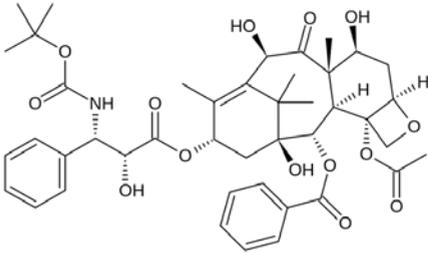
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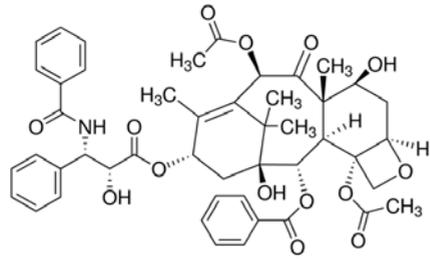
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Molecular structures

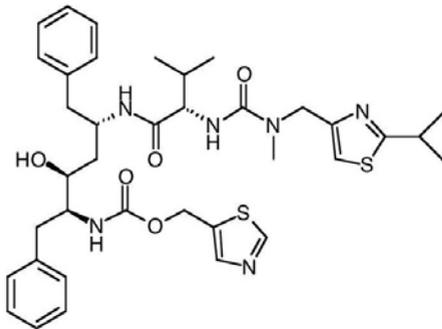
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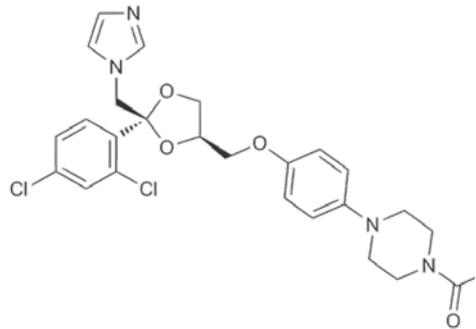
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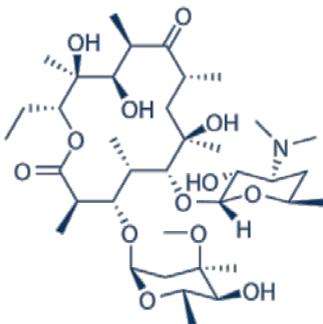
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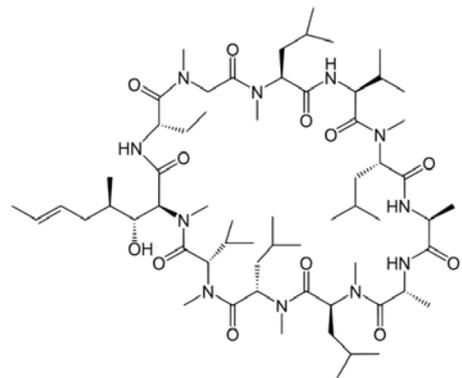
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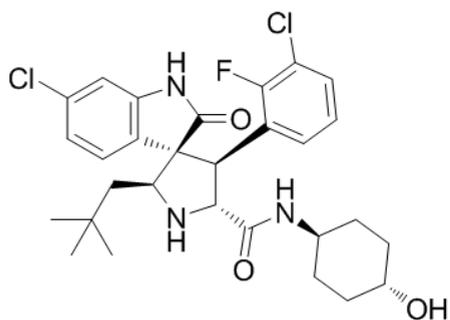
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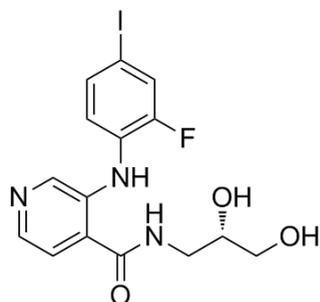
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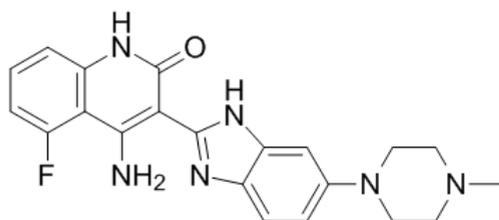
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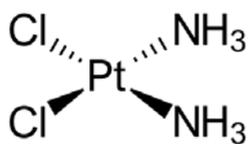
Pimasertib



Dovitinib



Cisplatin



Sodium thiosulphate

