Clinical pharmacology of novel anticancer drug formulations

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Clinical pharmacology of novel anticancer drug formulations

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toedieningsvormen van cytostatica
(met een samenvatting in het Nederlands)

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"Verba volant, scripta manent"

Caius Titus

"Simpel is het moeilijkst"

Johan Cruijff

Voor alle patiënten
en hun families

Preface

Cancer is a leading cause of death worldwide, accounting for 7.6 million deaths (around 13% of all deaths) in 2008 and an estimated 13.1 million deaths in 2030. Hence, major effort is put into unraveling the biological mechanisms underlying neoplastic diseases. Identification of novel mechanisms of unresponsiveness recently lead to the implementation of clinical trials testing the hypothesis that has been derived from preclinical studies whereby rational combinations of anticancer agents may prevent unresponsiveness of cancer. The majority of these new anticancer drugs are oral pharmaceutical formulations, consisting of new chemical entities, like molecular targeted agents and novel variants of existing drugs. The development of oral formulations is, however, often hampered by low and variable bioavailability.

The aim of this thesis was to investigate novel drug formulations of the existing drugs docetaxel, paclitaxel and gemcitabine in clinical studies. The results of the clinical pharmacological and oncological studies and acquired pharmacological knowledge will be used to determine the therapeutic value of these novel drug formulations.

Docetaxel and paclitaxel have played a major role in the treatment of various tumors as intravenous (IV) infusion over the past two decades and are still standard of practice. The main disadvantages of the current intravenous route of administration are the invasive procedures for the patient, the unfavorable, in particular allergic infusion reactions and the development of peripheral neuropathy, caused by the formulation vehicles. Besides, both taxanes can induce significant bone marrow suppression when applied at maximum tolerated dosing levels. Both have a limited oral bioavailability because of a poor solubility and high affinity for drug transporters and metabolizing enzymes present in the gut wall and liver. We were able to overcome the low oral bioavailability of both taxanes by the development of an oral formulation and by inhibiting metabolizing enzymes in gut wall and liver employing a low dose of ritonavir. Chapter 2.1 and 2.2 describe the feasibility, safety and pharmacokinetics of two administration schedules of a novel capsule formulation containing docetaxel (ModraDoc001) in combination with ritonavir. Further research of the dosage form lead to the development of a tablet formulation (ModraDoc003) and a combination tablet formulation, which contained both docetaxel and ritonavir (ModraDoc004). This development and evaluation in humans are described in chapter 2.3. One of the most frequently reported adverse events of oral docetaxel in these studies was diarrhea. Both clinical and preclinical data suggest intestinal toxicity by oral docetaxel as major etiology. The mechanism behind the development of intestinal toxicity in both mice and humans is discussed in chapter 2.4.

Oral administration enables more chronic treatment regimes, like "low-dose metronomic" regimes, chronic administration at relatively low, non-toxic dose-levels on a frequent schedule of administration, with no drug-free breaks. Based on preclinical studies, paclitaxel is considered to be an ideal drug to use for the concept of metronomic therapy, if repeated oral dosing would be possible. Chapter 3.1 describes an interim analysis of five dose-levels of the first dose escalation study of "low-dose metronomic" treatment with oral paclitaxel as ModraPac001 and ritonavir. To increase the knowledge of the working

mechanism of "low-dose metronomic" treatment with paclitaxel, several biomarkers are developed and implemented in the study (chapter 3.2).

Gemcitabine is a nucleoside analogue used as IV infusion in the first line treatment of patients with various solid tumors, including non-small cell lung and pancreatic cancer. After administration, gemcitabine needs to be actively transported into tumor cells to exert its anticancer activity. CO-101, also known as CP-4126, is gemcitabine linked with an inert moiety. This linkage gives CO-101 two advantages over gemcitabine. First, CO-101 could be given orally, which is described in chapter 4.1. And second, CO-101 could traverse cell membranes by passive diffusion, followed by intracellular conversion to gemcitabine. This would improve the clinical activity after intravenous administration of CO-101 compared to gemcitabine. A comparative pharmacokinetic study of two intravenous formulations of CO-101 is described in chapter 4.2.

Altogether, the results of the clinical studies and acquired pharmacological knowledge were used to determine the therapeutic value of these novel drug formulations. In the final chapter 5 conclusions are drawn and perspectives given for future research.

Contents

chapter 1

Oral anticancer drugs: mechanisms of low bioavailability and strategies for improvement

1

Oral anticancer drugs: mechanisms of low bioavailability and strategies for improvement

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Abstract

The use of oral anticancer drugs has increased during the last decade, because of patient preference, lower costs, proven efficacy, lack of infusion related inconveniences and the opportunity to develop chronic treatment regimens. Oral administration of anticancer drugs is, however, often hampered by limited bioavailability of the drug, which is associated with a wide variability. Since most anticancer drugs have a narrow therapeutic window and are dosed at or close to the maximum tolerated dose, a wide variability in bioavailability can have a negative impact on treatment outcome. This review discusses mechanisms of low bioavailability of oral anticancer drugs and strategies for improvement.

The extent of oral bioavailability depends on many factors, including release of the drug from the pharmaceutical dosage form, a drug's stability in the gastro-intestinal tract, factors affecting dissolution, the rate of passage through the gut wall and the pre-systemic metabolism in gut wall and liver. These factors are divided into pharmaceutical limitations, physiological endogenous limitations and patient specific limitations. There are several strategies to reduce or to overcome these limitations. First, pharmaceutical adjustment of the formulation or the physicochemical characteristics of the drug can improve the dissolution rate and absorption. Second, pharmacological interventions by combining the drug with inhibitors of transporter proteins and/or pre-systemic metabolizing enzymes can overcome the physiological endogenous limitations. Third, chemical modification of a drug by synthesis of a derivative, salt form or prodrug could enhance the bioavailability by improving the absorption and bypassing physiological endogenous limitations.

Although the bioavailability can be enhanced by various strategies, the development of novel oral products with low solubility or cell membrane permeability remains cumbersome and is often unsuccessful. Main reasons are unacceptable variation in bioavailability and high investment costs. Furthermore, novel oral anticancer drugs are frequently associated with toxic effects including unacceptable gastrointestinal adverse effects. Therefore, compliance is often suboptimal, which may negatively influence treatment outcome.

Introduction

Traditionally, most anticancer drugs are administered by intravenous infusion [1]. During the last decade, however, the use of oral anticancer drugs has increased, because of proven efficacy and patient preference for oral therapy [2–4]. Moreover, chronic oral dosing enables sustained inhibition of the target and can reduce dose related toxicities. Nowadays, the majority of new anticancer drugs either approved or in development are oral pharmaceutical formulations [5], consisting of new chemical entities like molecular targeted agents and novel variants of existing drugs. The development of oral formulations is, however, often hampered by low and variable bioavailability. This review will discuss mechanisms of low bioavailability of anticancer drugs and selected strategies for improvement.

Oral versus intravenous therapy

From a patient's point of view, oral anticancer therapy is preferable compared to intravenous therapy, because of the convenience of home treatment. A prerequisite is that the therapy is equally effective and that toxicities will enable chronic intake not interfering with daily activities [2,6,7]. Generally, an oral alternative is preferred if compliance, tolerability, convenience and efficacy are at least as good as the intravenous option.

Next to the patients' convenience, oral administration of anticancer drugs has several other benefits. Chemotherapy is the largest cost component of treatment and represents half of the total treatment costs per cancer patient [8]. Since in-patient administration of chemotherapy is on average twice as expensive as on an out-patient setting, oral chemotherapy can offer a considerable financial benefit [8]. In addition, oral administration enables chronic regimens to be explored like "metronomic chemotherapy", i.e. chronic administration of oncolytic drugs at relatively low, non-toxic doses on a frequent administration schedule with no drug-free breaks [9]. Other benefits are the lack of infusion related inconveniences, like formulation related infusion reactions [10,11], the development of phlebitis and central line related bacterial infections [12].

Relevance of variability

The absorption of oral anticancer drugs depends on a range of varying factors and is often low. Limited bioavailability is associated with a wide variability (both intra- as well as interpatient variability) [13]. Conventional anticancer drugs have a narrow therapeutic window and are dosed at or close to the maximum tolerated dose (MTD). Therefore, a wide variability can have a significant negative impact on treatment outcome. A relatively low uptake may result in reduced efficacy, whereas a relatively high uptake can lead to severe toxicities. Overall, high intra- and/or interpatient variability can be a limiting factor in the use of oral agents. In the development phase of an oral formulation the absolute bioavailability must be evaluated to submit a full new drug application to the authorities as well as the degree of exposure variation [14,15]. These data provide an estimate of the relative fraction of the orally administered dose that is absorbed, as well as its subsequent distribution and elimination. Although there are no general specifications, these data can be of importance to interpret the results in clinical studies.

Limitations in bioavailability

The extent of oral bioavailability depends on many factors, including release of the drug from the pharmaceutical dosage form, a drug's stability in the gastro-intestinal tract, factors affecting dissolution, the rate of passage through the gut wall and the pre-systemic metabolism in gut wall and liver (see figure 1) [16,17]. These factors are divided into pharmaceutical limitations, physiological endogenous limitations and patient specific limitations.

Pharmaceutical limitations

Pharmaceutical factors

After oral ingestion the formulation enters the acidic content of the stomach. The drug must be in a solute form before it can be absorbed and therefore the disintegration and dissolution are important for the absorption of solid dosage forms. The disintegration and dissolution of the drug are dependent on the formulation and the physicochemical characteristics of the drug. Excipients and formulation itself (e.g. solution, capsules, liquid-filled capsules, sustained-release tablets) determine the disintegration. The dissolution rate of the drug is given by the Noyes–Whitney equation [18,19] :

$$\frac{dm}{dt} = D * A * \frac{C_s - C_b}{s}$$

(dm/dt = dissolution rate (mg/min), D= diffusion coefficient (cm^2/min), A = the surface area of dissolving solid (cm^2), C_s = solubility of drug at saturation (mg/L), C_b = is the concentration of the drug already dissolved (mg/L), s = the diffusion layer thickness (cm)). The surface area of the drug is dependent on the formulation and the solubility of the drug is dependent on physicochemical factors.

Physicochemical factors

Most of the oral anticancer drugs are absorbed in the small intestine, due to the large surface area provided by epithelial folding and the villous structures. The epithelium is covered by absorptive cells, goblet cells, endocrine cells and calveolated cells. Secretions from these cells, bile and pancreas create a pH gradient from pH 2 in the stomach to pH 5-6 at the jejenum and finally to pH 7-8 at the ileocaecal valve [20–23]. This gradient influences the bioavailability in two ways. First, the absorption is dependent on the transit time and the solubility of the drug in absorption sections of the intestines. For some drugs the solubility depends on the pH of the section. The solubility of acidic drugs is inversely correlated to the pH of the solution, because they ionize at a high pH. Also the solubility of some tyrosine kinase inhibitors, like dasatinib, gefitinib and lapatinib, decreases dramatically above a certain pH [24–26]. Therefore, the pH gradient influences the absorption and consequently the bioavailability of these drugs. This is concurred with the drug-drug interaction with antacids. Simultaneous intake of antacids and dasatinib decreases the systemic exposure and the maximum plasma concentration of dasatinib by 55% and 58%, respectively [26]. Secondly, both the pH and the composition of the intestinal fluids can affect the stability of drugs by degradation [27,28]. The low and variable oral bioavailability of etoposide and chlorambucil are primarily caused by the poor chemical stability in gastric and intestinal fluids [29,30].

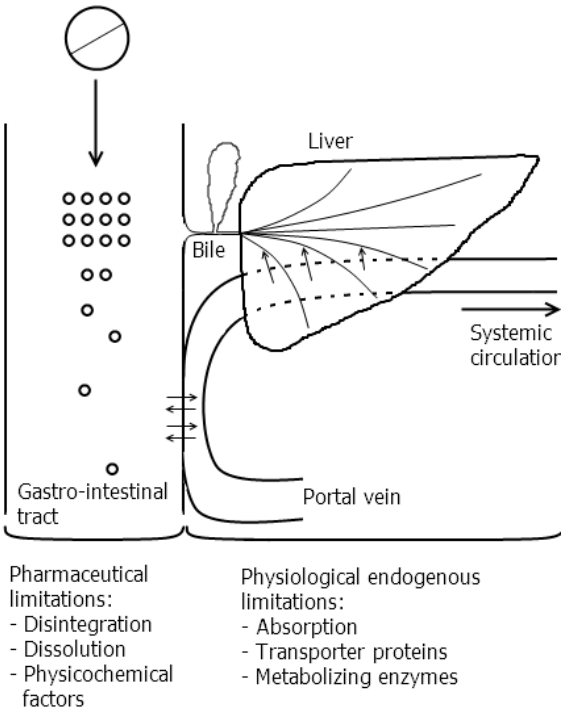

Figure 1 Systemic overview of the pharmaceutical and physiological endogenous limitations

Other physicochemical properties of the drugs influencing the solubility are the lipophilicity, (pseudo)polymorphism and salt form of a drug. Several classification systems have been developed to distinguish between good water-soluble and poorly water-soluble drugs and are used in drug development. Both the biopharmaceutics classification system (BCS) [31] and the development classification system (DCS) [32] categorize oral drug substances into four groups based on the solubility properties and the ability to penetrate the gastrointestinal tract (permeability). Traditionally, only drug candidates with a good solubility in water made it to the development phase. Since the pharmaceutical industry adjusted the "rational drug design" in the screening phase, also poorly water-soluble drug candidates are becoming more prevalent in drug research [33]. A benefit of poorly water-soluble drugs could be lipophilicity. Despite restricting the solubility, lipophilicity can provide an efficient interaction with the target receptor and gives a possibility to pass the lipidic domain of natural membranes [34].

Physiological endogenous limitations

During absorption, the uptake occurs at the epithelium of the gastrointestinal tract. Molecules pass these columnar epithelia transcellularly (passive, facilitated or carrier-mediated diffusion) and intercellularly and flow through the hepatic portal vein to the liver. Some lipophilic drugs can be absorbed via the lymphatic system, thereby avoiding the liver, although not many drugs have a sufficient lymph-to-blood flow ratio [35].

Physiological endogenous limitations can prevent the drugs from reaching the systemic circulation and limit the bioavailability. These limitations consist of expression and activity of several drug transporter proteins and metabolism enzymes in the gut wall and liver. A complete description of these proteins and enzymes is outside the scope of this review and extensively described in other reviews [36–41]. We will primarily discuss general principles of the activities of these enzymes and the implications for oral administration of anticancer drugs.

Effects of transporter proteins

ATP binding cassette (ABC) transporters are transmembrane proteins, responsible for transport of a wide variety of substrates across extra- and intracellular membranes. Most anticancer drugs are substrates for active drug efflux ABC transporters, which are abundantly expressed in the epithelial layer of the gut wall. These transporters are also expressed in tumors and tissues with an excretory function, such as the biliary canalicular membrane of hepatocytes, the epithelial layer of renal tubules, the placenta, the blood-testis barrier and blood-brain barrier for active clearence [42]. The three subfamilies of the ABC transporters, which are involved in the elimination of oral anticancer drugs are P-glycoprotein (P-gp; ABCB1), the multidrug resistance-associated proteins (MRPs; ABCCs) and the breast cancer resistance protein (BCRP; ABCG2) [39].

P-gp, expressed in the apical membrane of the epithelial layer of the gut wall restricts the uptake of several oral anticancer drug with an amphiphatic nature, like paclitaxel, docetaxel, etoposide, idarubicin, doxorubicin, irinotecan, topotecan, vinorelbine and almost all tyrosine kinase inhibitors[43–48]. This restriction can be substantial. Preclinical experiments in mice with a genetic knockout of genes responsible for P-gp, show an improvement of bioavailability of etoposide from 23% to 49% compared to controls[49].

The MRP family primarily but not exclusively transports hydrophobic anionic conjugates and extrudes hydrophobic uncharged drugs. MRP2 and MRP4 are located at the apical site of the epithelial membrane and can therefore contribute to the efflux of substrates [39]. Tissues that express MRP2 are the epithelium of the intestines, hepatocytes, renal tubules and the placenta. Substrates for the MRP2 transporter enzymes are mitoxantrone, doxorubicin, epirubicin, methotrexate, paclitaxel, docetaxel, irinotecan, vinblastine, vincristine, cisplatin and etoposide [36,39,50,51]. MRP4 is also expressed in tissues with an excreting function but its expression levels are much lower compared to P-gp and MRP2 and the contribution of MRP4 to drug transport appears to be limited [36]. Anticancer drugs with affinity for MRP4 are purine analogues and methotrexate [52,53].

BCRP is expressed in the small intestine, colon, liver, mammary gland, bladder, pancreas, brain, placenta, prostate and kidney [54–57]. This strategic and substantial tissue localization indicates that BCRP also plays an important role in absorption, distribution, and elimination of drugs that are BCRP substrates. BCRP emerged as an important multidrug resistance protein because it confers cross-resistance to several structurally unrelated classes of anticancer chemotherapeutic agents [38]. Anticancer drugs which are known substrates of BCRP are mitoxantrone, methotrexate, doxorubicin, erlotinib, lapatinib,

gefitinib, danusertib and topoisomerase I inhibitors, such as topotecan [58–63]. Preclinical experiments with knockout mice show that BCRP can give a 3-fold reduction of the maximum plasma concentration[64].

The localization and the substrate specificity of P-gp, BCRP and MRP2 overlap and thereby they form an efficient barrier against drug penetration. The tissues that express these transporters have a dynamic regulation of the amounts of protein both at transcriptional and post-transcriptional levels [36,38]. Expression levels vary significantly within and between patients during treatment [36,65,66], contributing to both intra- and interpatient variance in bioavailability of orally applied drugs[67].

Effects of metabolizing enzymes

Both the liver and the intestines contain enzymes with a high metabolizing capacity that can convert a significant proportion of a drug before it can enter the systemic circulation. Some metabolizing enzymes are present in both glands. Therefore, a differentiation of the relative importance of intestinal versus hepatic enzyme-mediated metabolism is difficult to make [37]. Absorption via the transcellular route through the intestinal enterocytes and uptake by the hepatocytes expose the drugs to intracellular cytochrome P450 (CYP) enzymes (phase I metabolism) and other conjugating enzymes (phase II metabolism). Because the formed metabolites and conjugates are often substrates for the efflux transporters, they can be extruded back into the gut. This suggests a functional interplay between the transporters and the metabolizing enzymes, which acts as a coordinated absorption barrier for orally applied drugs [41]. The fraction of the drug that bypasses the hepatocytes enters the systemic circulation.

CYP enzymes belong to a large superfamily of diverse groups of metabolizing enzymes. An important subfamily is CYP3A, which is involved in the oxidative metabolism of approximately half of the commercially available drugs. Because CYP3A is localized in both intestine and liver, it contributes to pre-systemic metabolism of many orally applied drugs [68]. There is an extensive overlap in substrate specificity and tissue expression between CYP3A and P-gp [69]. Similarly to the transport enzymes, the variation in expression of metabolic enzymes among humans is significant (e.g. 40-fold variation of CYP3A levels in both intestine and liver [70]) and a correlation between the activity of hepatic and intestinal enzymes is lacking [71,72]. Moreover, co-medication and dietary components can both induce and inhibit CYP3A [73,74].

Another important enzyme in the intestinal wall and liver, which is involved in the pre-systemic metabolism of oral anticancer drugs, is dihydropyrimidine dehydrogenase (DPD). Substrates for DPD have an erratic oral bioavailability due to the metabolic conversion by DPD. DPD is the rate-limiting step in the catabolism of fluorouracil and therefore one of the limitations of oral administration of this drug. This is illustrated with the wide variation and the low bioavailability of orally administered fluorouracil (28%, coefficient of variation (CV) is 89%) [75–78]. Interpatient variability is also related to polymorphisms in genes responsible for DPD activity and intrapatient variability is associated with the circadian rhythm of DPD activity throughout the day [79].

Nonlinear pharmacokinetics

A high absolute oral dose can also limit the bioavailability for drugs with a limited area for absorption in the gut by saturation of the transport system. Saturation of the transport proteins decreases the uptake of a drug, causing nonlinear pharmacokinetics[80]. A dose increase will not increase the exposure if the process has reached the maximal rate. An example of nonlinear oral pharmacokinetics is etoposide. The bioavailability following a 100-mg dose of oral etoposide is approximately 76%, which is significantly higher compared to the bioavailability following a 400-mg oral dose (48%). Also the coefficient of variation increased significantly: 29% versus 37%, respectively [81,82].

Patient specific limitations

Malabsorption and hepatic impairment

Cancer patients often develop gastrointestinal disorders and/or liver dysfunction. Underlying mechanisms can be surgical resections in the gastro-intestinal tract, hormonal secretions of the tumor or adverse events of chemotherapy [83]. Malabsorption and damage of the mucosa of the small intestines can reduce the absorption of orally applied anticancer drugs [48,84] Changes in hepatic (pre-systemic) metabolism and transport systems can alter the pharmacokinetics after oral intake [85]. Studies in patients with impaired hepatic function are important for characterizing the pharmacokinetics and safety if hepatic metabolism accounts for a substantial portion of the elimination of the drug and provide information that may help guide initial dosing [86,87].

Elderly patients

Aging influences absorption, metabolism and excretion of drugs [88]. Changes in the gastrointestinal physiology result in symptomatic gastrointestinal dysfunctions, like gastroesophageal reflux disease, primary dyspepsia, irritable bowel syndrome, primary constipation, maldigestion, and reduced absorption of nutrients, which may influence the absorption of drugs [89,90]. Furthermore, increasing age is associated with decline in hepatic blood flow and liver mass, thereby possibly affecting the hepatic metabolizing and transport systems [91]. These age-related changes have contradictory effects on absorption and metabolism and therefore the bioavailability of oral anticancer drugs could potentially be increased or decreased [88]. In addition, drug-drug interactions may arise in this patient population, because of often applied polypharmacy. These influences on the pharmacokinetics of oral drugs and adverse events are, however, often underexposed. Because the oral route of administration is often only preferred by physicians for patients in a palliative setting [4], both ineffective treatment and unacceptable side effects are the major source of therapy failure within this population.

Food effect

The presence of food may decrease, delay or even increase drug absorption. Even within a group of comparable drugs, like tyrosine kinase inhibitors this effect can be contradictory [92–95]. The matter can influence the absorption by acting as a physical barrier to the epithelium and by affecting transit times of different sections of the gut and by affecting the pH [23]. Also the composition of the food can bind to or react with the drugs.

Increased absorption can occur by increased drug transport via the lymphatic system. Co-administration of triglycerides enhances the lymphatic absorption and can therefore by-pass the first-pass effect [35]. Food can also increase the micellar solubilization as a result of increased bile output [92]. A complete overview of the underlying mechanisms and implications for oral anticancer drugs is described elsewhere [96].

Genotyping

Differences in expression and activity of transporter proteins and metabolism enzymes in the gut and liver may cause interpatient and intrapatient variability in bioavailability of orally applied drugs. A major factor responsible for this variability is variation in the genetic constitution among patients. Polymorphism in genes encoding for transporter proteins and metabolism enzymes may influence the absorption and elimination of the oral drug with a low permeability. For docetaxel for example, patients with a homozygous C1236T polymorphism in the ABCB1 gene (ABCB1*8) have a significantly reduced docetaxel clearance [97] and Baker et al demonstrated that the simultaneous presence of the CYP3A4*1 and CYP3A5*3 was associated with a relevantly increased docetaxel clearance [98]. The clinical implications of pharmacogenetic variability in phase I and II drug metabolism, drug transport and pharmacodynamics have been described in a four-part series on pharmacogenetics, with a special focus on opportunities for patient-tailored anticancer therapy [67,99–101].

Strategies to enhance the bioavailability

Pharmaceutical strategies

For poorly water-soluble drugs with a good permeability (BCS class 2), the dissolution rate is often the limiting step for absorption. Adjusting the formulation and/or the physicochemical characteristics of the drug is a possibility to increase the dissolution rate [34]. Particle size reduction and improved wetting increase the surface area and addition of co-solvents or changing the physical state of the drug (amorphous versus crystal state) can increase the saturation solubility. Drug administration as a solid dispersion or in a dissolved form could improve the absorption of these drugs.

Solid dispersion

A solid dispersion is a dispersion of one or more active ingredients in an inert carrier matrix at solid-state [102]. Most commercially available solid dispersions are amorphous glass solutions with hydrophilic polymeric carriers, in which the drug is molecularly dispersed. The dissolution rate is improved in several ways. Because of the amorphous state the drug possesses higher internal energy and therefore the solubility and the dissolution rate are higher compared to the crystalline state. If the conversion rate to the stable crystalline form is lower than the dissolution rate of the drug, the dissolution will be enhanced[103]. Conversion of the drug into a more stable physical state within the formulation before or during dissolution will lower the dissolution rate towards that of the stable crystalline form. As the hydrophilic carrier dissolves, the drug is released as very fine, colloidal particles. The huge increase in surface area also enhances the dissolution rate and therefore a supersaturated solution is formed [104], although there is a risk for rapid precipitation before the drug is absorbed. A solid dispersion can enhance the bioavailability on the

condition that the formulation is physicochemically stable. There are solid dispersions in development for camptothecin, taxanes and curcumin [105–109].

Dissolved form

Another possibility to overcome the pharmaceutical restrictions is to administer the drug directly in a dissolved form. Parallel to the development of an intravenous dosage form, the solubility can be increased by the use of co-solvents. An example of application of a dissolved form is that of vinorelbine. Vinorelbine is a semisynthetic anticancer agent of the vinca alkaloid group and initially developed as an intravenous formulation (Navelbine®). Several oral formulations of vinca alkoids have been developed, but the process was hampered by potential production hazards and variable absorption in vivo [110–112]. The development of various liquid-filled soft gelatin capsule formulations with glycerol, phosal 53 MCT and triglycerides as (co)solvents appeared to at least partly improve oral bioavailability to approximately 27% [111,113] and 33% - 38% [110,114]. Although these formulations showed a more than 2.5-fold increase in bioavailability [113], usage is limited because of low stability and high variation (CV are 37- 55%) [110,114]. More successful applications of drug-filled capsules in dissolved form are of etoposide and topotecan [115–117]. Also for paclitaxel a comparable dosage form is in development [118].

The main disadvantages of formulations with a drug in a dissolved form are the physicochemical instability and the risk of precipitation during dilution with water miscible solvents in the gastrointestinal fluids after ingestion. An alternative is a water immiscible type of solubilization, like self-emulsifying drug delivery systems (SEDDS) [34]. In SEDDS the drug is dissolved in a stable liquid isotropic mixture of oils and surfactants [119]. SEDDSs emulsify spontaneously to produce fine oil- in-water emulsions when diluted in the aqueous gastro-intestinal tract, which leads to rapid absorption and thus reduced variability [120,121]. Such formulation by co-absorption of excipients could also limit presystemic metabolism [122], although it is often not sufficient to bypass all restrictions[123]. Although several SEDDS are under development [122,124–129], no registered SEDDS formulation of anticancer drugs is known yet.

Other pharmaceutical strategies

Several other pharmaceutical approaches to enhance the oral bioavailability of anticancer drugs are being developed. These methods include the use of nano-suspensions [34], cyclodextrins[130,131], dendrimers [132,133], micelles [134], PEG based nanoparticles [135] and colloid dispersions [136]. Most of these approaches show an increase in solubility of the drug, an increase in residence time of the drug in close contact with the absorptive membrane or a combination of both. These methods are still in the preclinical development phase, therefore at present it is unclear whether they will reach commercialization and will become competitors of the existing formulations.

Pharmacological strategies

As described earlier, low permeability is related to a high interpatient variability, which is undesirable for agents with a narrow therapeutic window. Several attempts have been made to enhance the bioavailability by temporarily inhibiting the transport proteins in the gut wall and liver. Preclinical studies have shown increased intestinal absorption of a variety of anticancer drugs in mice with genetic knockout of transporter genes [137,138].

Consequently, proof of concept studies in mice were conducted with anticancer drugs in combination with inhibitors of metabolizing enzymes and transporter proteins, which showed 10-fold increases of systemic exposure of paclitaxel [139,140] and topotecan[141]. These results have led to many (pre)clinical studies with different inhibitors of P-gp [142–152] and BCRP [144,153–157] and have proved the possibility to enhance the bioavailability for taxanes, topoisomerase I and II inhibitors and anthracyclines in a clinical setting (table 1). Three phase II trials were conducted with paclitaxel in combination with ciclosporin [158–160]. Although these trials proved activity of the combination, the development of this combination was terminated, because both preclinical and clinical research had shown a satisfactory enhancement of the systemic exposure of taxanes by inhibition of intestinal CYP3A4 activity with a low dose of ritonavir [46,161]. A benefit of ritonavir is the long-term clinical experience with temporary inhibition of CYP3A4 to enhance the exposure of protease inhibitors [162–164]. Similarly a low dose of ritonavir was shown to enhance the oral pharmacokinetics of docetaxel with an apparent bioavailability of approximately 60% [108].

Also inhibition of other enzymes can enhance the bioavailability of orally applied drugs. Pharmacologic inactivation of DPD is a mechanism to enhance the absorption of orally applied fluorouracil . Preclinical studies have shown that the oral bioavailability is enhanced in combination with an inhibitor of DPD. Pretreatment with 5-Ethynyluracil increased the oral exposure of fluorouracil in rats 6-fold to a bioavailability of 100% and decreased the variation from 55% to 14% [165]. This bioavailability could also be obtained in patients using different DPD inhibitors, like gimeracil, uracil and eniluracil [75,79,166,167] and also the wide interindividual variability was reduced to 33%-40% [75,79]. An additional benefit of enzyme inhibition is reduction of toxicities caused by metabolites. DPD inhibition reduces the formation of the catabolic metabolite α-fluoro-β-alanine, which has been associated with development of neurotoxicity [168]. Also co-administration of another inhibitor for orotate phosphoribosyl transferase (OPRT) reduced the gastrointestestinal phosphorylation of fluorouracil to 5-fluorouridine-5'-monophosphate (FUMP) by 70% and thereby the severity of gastro-intestinal toxicities [169].

The clinical feasibility of temporary inhibition of these enzymes for orally applied fluorouracil has been determined in multicenter phase III colorectal cancer studies [170]. The combination of oral fluorouracil and eniluracil showed less antitumor benefit compared to the standard regimen of fluorouracil /leucovorin without eniluracil, but this is possibly caused by antagonism of fluorouracil by excess amounts of eniluracil [171]. The combination of a prodrug of fluorouracil and a DPD inhibitor, like tegafur-uracil (UFT) and tegafur-gimeracil-oteracil (S-1) is more successful and approved for the treatment of several tumor types [172,173].

Table 1 Clinical studies performed with oral anticancer drug in combination with inhibitors of transporter proteins and metabolizing enzymes to enhance the oral bioavailability.

Compound	Inhibitor	Restrictions	Effect	Apparent Bioavailability[a]	CV[a]	Reference
Paclitaxel						
	-	P-gp and CYP3A	-	4%[b]		[215]
	Ciclosporin	P-gp and CYP3A	Bioavailability increased	13-47% [b]		[158–160,215,216]
	Elacridar	P-gp	Bioavailability increased	30-50% [b]	50%	[217]
	Ritonavir	CYP3A	Exposure in same range as in combination with ciclosporin			[218]
	HM30181A	P-gp	Unknown			[219]
Docetaxel						
	-	P-gp and CYP3A	-	8%	75%	[220]
	Ciclosporin	P-gp and CYP3A	Bioavailability increased	90%	49%	[220,221]
	Ritonavir	CYP3A	Bioavailability increased	60% (capsules)	50%	[107,108,161,222]
				50-150% (drinking solution)		
	ONT-093	P-gp	Bioavailability increased	26%	31%	[223]
Etoposide						
	-	P-gp and CYP3A	-	47-76%	11%	[81,224]
	Ketoconazole	CYP3A	Exposure increased with 20%, variability increased 2-fold			[225]
	Grapefruit juice	CYP3A	Bioavailability reduced	50%	22%	[226]

Topotecan						
	-	P-gp and BCRP	-	42%	17%	[227]
	Elacridar	P-gp and BCRP	Bioavailability increased	97%	11%	[228,229]
	Ciclosporin	P-gp and BCRP	Exposure increased 2 - 2.5-fold			[230]
Idarubicin						
	-	P-gp	-	20-25%		[231]
	PSC-833	P-gp	No effect			[232]
	Ciclosporin	P-gp	Exposure increased 1.7 -fold, variation decreased 1.4-fold			[233]
	Ciclosporin + dexverapamil	P-gp	No effect			[233]
Fluorouracil						
	-	DPD	-	28%	89%	[78,234]
	Gimeracil	DPD	Exposure increased 3-fold after 16-fold lower dose			[235]
	Uracil	DPD	Bioavailability increased	115%	7%	[167]
	Eniluracil	DPD	Bioavailability increased	117 -122%	32%	[75]

[a] *Apparent bioavailability and the CV are given or calculated where applicable.*

[b] *Cremophor EL, a co-solvent of intravenous paclitaxel which is responsible for the non-linear pharmacokinetic behavior of intravenous paclitaxel, is not absorbed following oral administration. The apparent bioavailability should therefore be interpreted with caution [220].*

Enhancement of the permeability has also been reported with several pharmaceutical excipients. Surfactants as solutol and d-alpha-tocopheryl polyethylene glycol succinate derivatives are known inhibitors P-gp and are used for the enhancement of the solubility and uptake of taxanes in various preclinical studies [174]. Some dosage forms combine excipients, which enhance both the solubility and uptake of a drug and thereby combining the pharmaceutical and pharmacological strategies. There are even excipients with both characteristics, like cyclodextrins. Cyclodextrins are used as complexing carriers to increase the aqueous solubility of poorly soluble drugs [175], but can have also inhibitory effects on the activity of P-gp [176,177] and cytochrome P450 [178]. In combination with bioadhesive properties of poly(anhydride) nanoparticles, cyclodextrins can enhance the bioavailability of paclitaxel to 80% in rats (30-fold increase) [131]. However, clinical proof of this effect and mechanism is lacking.

Chemical strategies

Chemical modification of a drug by synthesis of a derivative, salt form or prodrug may enhance the bioavailability in several ways. Since derivatives are new chemical entities with their own pharmacokinetic characteristics, activity and affinity for enzymes, they are considered outside the scope of this review.

First, chemical modification of a drug to a salt form can improve the solubility. For example, the solubility of etoposide is < 0.1 mg/mL in water. Etoposide phosphate has a much higher solubility (20 mg/mL) and has been developed for intravenous administration to minimize the use of co-solvents [117,179]. After administration etoposide phosphate is converted into etoposide by alkaline phosphatase [82]. Both compounds are orally available, liquid-filled capsules containing etoposide and hard gelatin capsules containing etoposide phosphate. Etoposide phosphate is favored because of the mean increase of the bioavailability from 19% to 76% and the lack of toxicity of the solvents [82], although the interpatient variability for exposure has not been reduced (approximately 45% for both) and it does not offer a clinically relevant benefit over oral etoposide [180,181].

Second, chemical modification of a drug into a prodrug can bypass physicochemical limitations. DNA alkylating agent monomethyltriazenoimidazole carboxamide (MTIC) is highly unstable in the acidic gastrointestinal fluids [182]. Temozolomide is a prodrug of MTIC and stable at a low pH. At physiological pH, temozolomide is converted rapidly into MTIC by a nonenzymatic, chemical degradation process [183]. The bioavailability is almost complete and bioequivalent to intravenous administration [184,185]. Because of the stability of the prodrug, the production process is a noncomplex method of mixing and filling [186].

Third, chemical modification of a drug into a prodrug can change the affinity for metabolizing enzymes and thereby could reduce first-pass metabolism. Doxifluridine, capecitabine and tegafur are (pre-)prodrugs of fluorouracil which are not cleavable by DPD. After uptake, doxifluridine is converted in the liver or in the tumor to 5'-deoxy-5-fluorouridine (5'-DFUR) by cytidine deaminase and 5'-DFUR is activated intracellularly to fluorouracil by thymidine phosphorylase, an enzyme that is often expressed in tumor tissue [187]. Capecitabine is metabolized in the liver into doxifluridine by carboxylesterase

and tegafur is converted into fluorouracil by CYP enzymes [79,187–189]. Without pre-systemic catabolic metabolism by DPD, the bioavailability is increased and the wide interindividual variability is reduced (bioavailability of fluorouracil is 34-47%, 40-45% and 93-153% for doxifluridine, capecitabine and tegafur, respectively) [167,187,189]. These prodrugs can be combined with enzyme inhibitors (S-1, UFT and emitefur) as complementary strategy for reducing variability of oral chemotherapy [190,191]. Oral administration of (pre-)prodrugs of fluorouracil enables prolonged exposure by metronomic dosing and replaces the use of intravenous applied fluorouracil in several treatments [192,193].

Also for gemcitabine (dFdC), oral prodrugs with the aim to escape hepatic metabolism by deoxycytidine deaminase are being developed. After oral administration of gemcitabine itself, deoxycytidine deaminase converts gemcitabine directly into the inactive main metabolite 2′,2-difluorodeoxyuridine (dFdU) in the liver [194,195]. Pre-systemic conversion reduces the bioavailability of gemcitabine and the accompanying accumulation of dFdU contributes to severe liver toxicity [196]. Two prodrugs with reduced affinity for deoxycytidine deaminase have been developed by linking an inert moiety to gemcitabine (LY2334737 and CP-4126). By this linkage the site responsible for deamination to dFdU has been blocked, in an attempt to reduce the pre-systemic conversion. Despite the reduction of affinity seen in preclinical setting [128,197], phase I trials showed a poor systemic exposure (bioavailability around 10% compared to historical intravenous data [198]) and the exposure ratio dFdU/dFdC of LY2334737 (range 216-404) and CP-4126 (range 261-1268) suggests there is still a substantial pre-systemic metabolism (range after intravenous administration 31-201) [77,199].

For some prodrugs the extensive pre-systemic conversion could be in favor of oral administration. Irinotecan is a prodrug for 7-ethyl-10-hydroxy-camptothecin (SN-38), a potent topoisomerase I inhibitor. SN-38 is approximately 1,000-fold more active than irinotecan, but is insoluble in water. By adding a bis-piperidine, the compound becomes water-soluble and is converted into SN-38 by hepatic carboxylesterases [133]. Because the conversion takes place in the liver, relatively more SN-38 is formed after oral administration than after intravenous administration (oral/intravenous ratio of SN-38 is 0.50 with the same dose and a mean oral bioavailability of 24% [200]). Low bioavailability caused by the affinity for P-gp and CYP3A4, a high variability (CV 96%) and toxicities like diarrhea (partly due to the bis-piperidine) hampered further development of oral application of this prodrug [45,200–204].

Discussion

Oral anticancer drugs are an evolving alternative in the treatment of cancer patients. However, the development of oral anticancer drugs with low solubility or cell membrane permeability is often unsuccessful. This is due to a number of reasons. First, most anticancer drugs have a highly variable bioavailability, but a narrow therapeutic range. Many studies have been performed to reduce the pharmacokinetic variability, but various strategies as modification of gastric emptying time and gastric pH have not been successful [28]. Inhibition of drug metabolizing enzymes such as CYP3A4 and DPD has been shown to improve the bioavailability and, to a lesser degree, reduce interpatient variability. A disadvantage of this strategy is that cancer patients often require concomitant medication, including inhibitors and inducers of these enzymes. Unintentional drug-drug interactions can occur, which are a major cause of unwanted toxicity [205].

Second, a proper formulation development including production scale up is expensive for pharmaceutical companies, since they require time and additional investments [34]. Also novel drug delivery technologies require the development of new guidelines for quality requirements by regulatory authorities [206], through which the development or existing production processes has to be adapted. Companies aim to determine the efficacy of drugs as quickly as possible in the clinic; consequently there is little time for formulation development.

Third, among physicians the prescription of oral anticancer drugs is traditionally controversial. Some physicians may have a prejudice that intravenous administration is more effective and reliable than oral administration [1]. Also concerns for a limited and variable bioavailability and concerns about compliance are frequently expressed[207]. Compliance can influence the safety and efficacy of oral anticancer treatment dramatically. Both underdosing and overdosing can occur in an outpatient setting, since the administration is not monitored by hospital personnel. Reports on compliance among patients with cancer show rates between 16% and 100% [208]. A major cause for treatment discontinuation is the experience of adverse events of the treatment. Since the novel oral anticancer drugs comprise molecular-targeted agents and other drugs, which are associated with several toxic effects including unacceptable gastrointestinal adverse effects, compliance is often suboptimal [209]. Good education of the patient and an active management of adverse events are crucial to achieve optimal compliance and outcome of the treatment [48,210].

A recent breakthrough in cancer treatment is the use of oral molecular targeted therapies with tyrosine kinase inhibitors. Oral administration enables the opportunity to suppress the target continuously by chronic dosing. These drugs are mainly administered at a fixed dose, although most of these drugs show a wide variability in bioavailability and exposure[211]. Extreme variation has been demonstrated in exposures of for example axitinib (CV is 39-94%[212]), imatinib (CV is 10-78%[213]) and gefitinib (CV is 35-113%[214]). Main reasons for these variations are food–drug interactions and affinity for drug metabolizing enzymes and ABC transporters. Most tyrosine kinase inhibitors are metabolized by CYP3A in liver and intestine and are substrates for P-gp or BCRP [43,44,60,62]. Although the therapeutic window

for these drugs is wider compared to conventional cytotoxic anticancer drugs, variation in exposure of these drugs impacts both efficacy as well as toxicity[211]. In order to deal with the inter-individual pharmacokinetics and to achieve target exposures, several dose titration strategies are explored for dose individualization (therapeutic drug monitoring, toxicity-adjusted dosing and ramp-dosing), but almost no effort is put into enhancement of bioavailability to reduce the inter-individual variability in pharmacokinetics.

In conclusion, the use of oral anticancer drugs is emerging, because of the introduction of orally active targeted agents, the proven efficacy and convenience for patients, despite the main concerns of low compliance and variable bioavailability. Various pharmaceutical, pharmacological and chemical strategies have been developed and implemented to bypass the pharmaceutical and physiological endogenous limitations and enable oral application of anticancer drugs.

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chapter 2

Docetaxel

2.1

A phase I dose escalation study of once daily administration of weekly docetaxel (ModraDoc001) in combination with ritonavir

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Interim analysis (data were monitored, except for pharmacokinetics and radiological evaluations)

Abstract

Background ModraDoc001 is a novel oral formulation containing 10 mg docetaxel as a solid dispersion. Oral administration of docetaxel is feasible in combination with the CYP3A4 inhibitor ritonavir. The objective of this study was to determine the safety, maximum tolerated dose (MTD) and pharmacokinetics (PK) of weekly oral docetaxel (as ModraDoc001) in combination with ritonavir.

Methods Patients with advanced solid tumors, WHO PS ≤ 2, no concomitant use of P-glycoprotein or CYP3A modulating drugs, adequate bone marrow, liver and renal function were eligible for this study. Docetaxel and ritonavir (Norvir®) were simultaneously administered once weekly in a classical '3+3 cohort' dose escalation design. The MTD was defined as the highest dose resulting in <1/6 probability of causing a dose limiting toxicity in the first 4 weeks of treatment. The MTD cohort was expanded with evaluable six patients. Pharmacokinetics (PK) was established on days 1 and 8.

Results A total of 48 patients (58% male) were enrolled in six dose-levels (30/100, 40/100, 60/100, 80/100, 60/200 and 80/200 mg docetaxel/ritonavir). Common treatment related adverse events were diarrhea (68%), nausea (62%) and fatigue (62%), mostly CTC grade 1-2 (80%, 95% and 73% respectively). Seven patients experienced a dose limiting toxicity (DLT). The most common DLT was grade 3 diarrhea (n=6). The MTD was identified at 60 mg docetaxel and 200 mg ritonavir. Both drugs were rapidly absorbed after oral administration. The mean maximum docetaxel plasma concentration was reached after 2.2 hours (CV 33%) in combination with 100 mg ritonavir and after 3.6 hours (CV 48%) in combination with 200 mg ritonavir, independent of the docetaxel dose. The mean maximum plasma concentration and mean plasma exposure (AUC) of docetaxel increased less than proportionally with dose to 170 ng/mL (CV 62%) and 1.79 µg/mL*hr (CV 80%), respectively, in 17 patients at the MTD. A partial remission was seen in 4 patients, with advanced non-small cell lung cancer (NSCLC), gastric cancer, breast cancer or cancer of unknown primary origin and sustained stable disease in 18 patients, of whom 8 had NSCLC.

Conclusions At the MTD (once weekly 60 mg docetaxel and 200 mg ritonavir) ModraDoc001 is safe, well tolerated and shows encouraging antitumor activity. The recommended dose for future phase 2 studies is defined as 60 mg docetaxel and 100 mg ritonavir. The systemic exposure to docetaxel with both dose-levels is comparable to weekly intravenous administration of 35 mg/m^2 docetaxel.

Introduction

Docetaxel is a semi-synthetic analogue originating from the Taxus baccata and is currently used as an anticancer agent in several types of cancer, such as non-small cell lung cancer (NSCLC), breast, head and neck, prostate and ovarian cancer. Doses range from 60 to 100 mg/m^2 administered as a 1-hour infusion every 3 weeks [1]. The main disadvantages of the current intravenous route of administration are the invasive procedures for the patient and the adverse events, in particular allergic infusion reactions, caused by the formulation vehicle containing polysorbate 80 [2,3] (Taxotere®). These events are not to be expected after oral administration of docetaxel without this pharmaceutical vehicle. Other advantages of oral treatment of docetaxel are the patients' convenience of flexible home treatment and necessity of pre-medication with dexamethason. Moreover, an oral dosage form enables more easily chronic treatment regimes.

Docetaxel has limited oral bioavailability because of its poor aqueous solubility and high affinity for active ABC drug efflux transporters and cytochrome P450 3A drug metabolizing enzymes (CYP3A). As uptake in the gastro-intestinal tract requires dissolution, its poor aqueous solubility significantly complicates oral absorption of docetaxel as a solid dosage form. In addition, both P-glycoprotein (P-gp/ABCB1/MDR1) and CYP3A, which are abundantly expressed in the epithelial layer of the gut wall and in the liver, contribute to low absorption and significant pre-systemic metabolism of orally administered docetaxel [7–9]. Preclinical and clinical proof of concept studies have shown that temporarily inhibition of P-gp and/or CYP3A enhances systemic exposure to docetaxel, what could enable oral

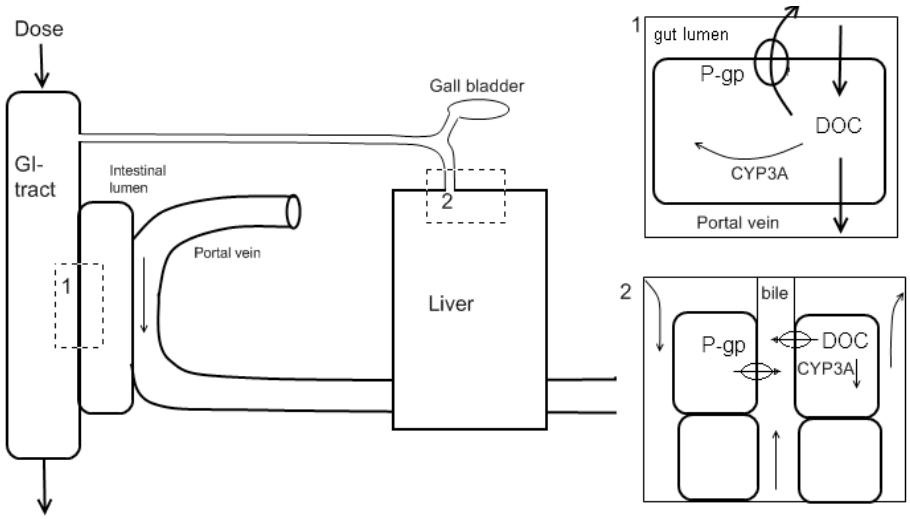

Figure 1 Docetaxel has limited oral bioavailability because of poor aqueous solubility and high affinity for active ABC drug efflux transporters and, cytochrome P450 3A drug metabolizing enzymes (CYP3A). Both P-glycoprotein (P-gp) and CYP3A, which are abundantly expressed in the epithelial layer of the gut wall (1) and in the liver (2), contribute to low absorption and significant pre-systemic metabolism of orally applied docetaxel. The systemic exposure upon oral docetaxel is increased when co-administered with an inhibitor of P-gp and/or CYP3A. (Abbreviations: DOC = docetaxel)

treatment with the drug [10–16]. However, inhibition of P-gp alone increases the exposure to oral docetaxel insufficiently [14]. Both preclinical and clinical research showed satisfactory enhancement of the systemic exposure to docetaxel with the CYP3A inhibitor ritonavir [15,16]. Recent studies revealed a mean apparent oral bioavailability of docetaxel of around 100% when give in combination with a low dose of ritonavir [16]. An additional benefit of ritonavir is the long term clinical experience with temporary inhibition of CYP3A4 to enhance the exposure to orally applied protease inhibitors in HIV-infected patients [17–19].

In this proof of concept studies, docetaxel was administered as a drinking solution employing the i.v. liquid formulation. This solution is, however, not suitable for regular clinical use, because of bad taste, poor dosing accuracy and limited stability after preparation. Therefore, ModraDoc001 10 mg capsules have been developed containing docetaxel as a solid dispersion without the use of polysorbate 80 [20].

In this dose escalation phase I study, patients with advanced solid tumors were treated with oral docetaxel in combination with ritonavir. The primary objective was to determine the maximum tolerated dose (MTD) of ModraDoc001 capsules in combination with ritonavir in patients in a weekly schedule. The secondary objectives were to determine the dose limiting toxicities (DLT), the safety, pharmacokinetics (PK) of docetaxel and ritonavir, and preliminary antitumor activity of the oral combination.

Patients and Methods

Study design and treatment schedule

This phase I study was an open-label, dose escalation study of oral docetaxel in combination with ritonavir. During the first four dose-levels, patients received during the first week 20 mg docetaxel i.v. (Taxotere®, Sanofi Aventis, France) administered over a 30 minute infusion in combination with an oral dose of 100 mg ritonavir (Norvir®; Abbott, Illinois, USA). In the second week and every subsequent week, patients received oral docetaxel and ritonavir (intake around the same time) until progressive disease or unacceptable toxicity. After the fourth dose-level, patients received only the oral formulation of docetaxel in combination with ritonavir. The medication was taken in fasting conditions in the morning, at least 2 hours after and at least 1 hour before food intake, since the effect of food on the pharmacokinetics of the drug was unknown. The study had a classic 3+3 dose escalation design [21]. Three patients were assigned to each dose-level. If one patient of the first three at a defined dose-level experienced a DLT, the number of patients treated at this dose-level was expanded to a maximum of six patients. The dose escalation continued if none of the additional patients experienced a DLT. The MTD level was expanded to at least six patients in any case. The docetaxel doses in the new dose-levels were based on safety evaluations and PK profiles observed at prior dose-levels.

The starting dose was 30 mg docetaxel by oral administration of the docetaxel i.v. formulation (Taxotere®, Sanofi Aventis, France) combined with 100 mg ritonavir. This dose combination was considered safe and was selected based on the standard weekly dose of i.v. docetaxel (35 mg/m^2) and the results of previous proof of concept studies [16]. Pre-treatment consisted during the first dose-level of 4 mg dexamethason 1 hour prior to, and

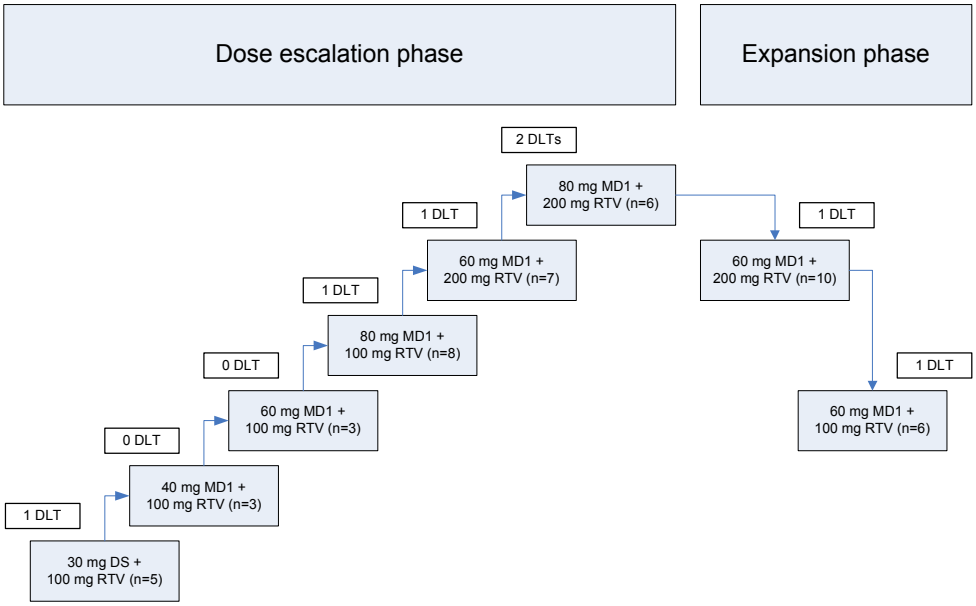

Figure 2 Study scheme (abbreviations: DS = drinking solution, MD1 = ModraDoc001 capsules, RTV = ritonavir, DLT = dose limiting toxicity)

12 and 24 hours after docetaxel intake. In addition, patients received 1 mg granisetron orally 1 hour prior to treatment. After the first dose-level, the dexamethasone pre-medication was omitted and the novel capsule formulation of docetaxel (ModraDoc001 10 mg capsules, Slotervaart Hospital, The Netherlands) became available for clinical use and replaced the drinking solution of the docetaxel i.v. formulation [20] (Figure 2). The ritonavir dose was increased to 200 mg after the fourth dose-level. Patients were considered evaluable for safety if they had received at least one dose of docetaxel. To determine the safety of a dose-level, patients were evaluable for safety if they had completed the first four treatment weeks or if a DLT occurred in this period. Otherwise, a patient was replaced. A DLT was defined as any of the following that occurred during the first four treatment weeks and was determined to be possibly, probably or definitely related to docetaxel: grade 3 or 4 non-haematological toxicity (other than untreated nausea, vomiting or diarrhea), grade 4 trombocytopenia, grade 4 neutropenia lasting for more than 7 consecutive days, grade 3 febrile neutropenia and inability to begin the next course of treatment within 3 weeks of scheduled dosing due to toxicity other than stated before.

Eligibility

Patients were eligible if they were diagnosed with a histological or cytological proof of cancer, if there were no standard treatment options available and if docetaxel treatment was considered appropriate. Other inclusion criteria were age ≥ 18 years, performance status of 0, 1 or 2 according to the WHO Performance Status (PS) scale, life expectancy longer than 3 months, and adequate bone marrow, hematological and biological functions

(neutrophil count of ≥ 1.5 * 10^9/L and platelets of ≥ 100 * 10^9/L; alanine transaminase (ALT) and aspartate transaminase (AST) ≤ 2.5 times the institutional upper limit of normal (ULN), bilirubin of ≤ 1.5 times the ULN; serum creatinine ≤ 1.5 times the ULN or creatinine clearance ≥ 50 mL/min by Cockcroft-Gault formula).

Patients with known alcoholism, drug addiction and/or psychotic disorders were considered not suitable for adequate follow up, and thus excluded. Patients were not allowed to concomitantly use P-gp and CYP3A modulating drugs, H_2-receptor antagonists or proton pump inhibitors. Other exclusion criteria were uncontrolled infectious disease, bowel obstructions that may influence drug absorption, neurologic disease, pre-existing neuropathy higher than grade 1, symptomatic cerebral or leptomeningeal metastases, pregnancy, breast feeding, refusal to use adequate contraception and previous anticancer therapy within 4 weeks prior to the first dose of oral docetaxel. The study protocol was approved by the local Medical Ethics Committee and all patients had to give written informed consent. The study was registered under identifier ISRCTN32770468 (ISRCTN register).

Study procedures

Pre-treatment evaluations included a complete medical history, physical examination (including vital signs and performance status), assessment of adverse events using the National Cancer Institute's Common Terminology Criteria for AEs (NCI-CTCAE v3.0) and concomitant medications, a pregnancy test in female patients, laboratory assessment of hematology, serum chemistry and urinalysis and a radiologic tumor assessment. Before each administration, assessment of adverse events and concomitant medication was repeated and hematology and serum chemistry were checked. A physical examination was performed at least every three weeks. Tumor response was evaluated every 6-8 weeks according to RECIST 1.0.

Pharmacokinetics

The PK of docetaxel and ritonavir was monitored at day 1 and 8 of treatment. Venous blood samples for the PK analysis were obtained through an indwelling peripheral intravenous catheter. PK of docetaxel after i.v. administration was evaluated pre-dose, at end of infusion and 0.25, 0.5, 1, 1.5, 2, 4, 7, 10, 24 and 48 hours after infusion. PK of docetaxel after oral administration was evaluated pre-dose, 0.25, 0.5, 0.75, 1, 1.5, 2, 4, 7, 10, 24 and 48 hours after dosing. The time points for PK sampling of ritonavir were predose, 0.5, 1, 2, 7 and 24 hours after administration (during the i.v. administration and at day 1 of the oral combination). Blood samples were collected in tubes containing lithium heparin as an anticoagulant. All samples were centrifuged within 1 hour at 1500 *g* for 10 minutes at 4°C and stored at –20°C.

Docetaxel was quantified in plasma by a high-performance liquid chromatography assay with tandem mass spectrometric detection (LC-MS/MS) as described by Kuppens et al. [22]. Ritonavir was quantified in plasma by a LC-MS/MS method as described by Hendrikx et al. [23]. For both assays labelled analogues were used as internal standards. Briefly, both compounds were extracted from 200 µL human plasma using tertiair butylmethylether.

Subsequently, the organic solvent was evaporated to dryness under a gentle stream of nitrogen and the residue was reconstituted in reconstitution solvent. Of each sample, 25 µL was injected onto a Zorbax Extend C18 column (150 x 2.1 mm ID; particle size 5 µm; Agilent Technologies, Amstelveen, The Netherlands). The mobile phase consisted of a mixture of 7:3 *v/v* methanol/10 mM ammonium hydroxide in water. Compounds were detected using positive ionization electrospray tandem mass spectrometry. The lower limit of quantification of the assay was 0.25 ng/mL for docetaxel and 2.0 ng/mL for ritonavir.

Data analysis

The individual non-compartmental PK parameters were determined using validated scripts in the software package R (version 2.15.0). 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}), apparent clearance (CL/F), distribution volume at steady state (Vss/F), the terminal elimination half-life ($t_{1/2}$), the area under the plasma concentration-time curve between t=0 and the time point of the last quantifiable data point (AUC_{0-48}) or, if possible, with extrapolation to infinity (AUC_{inf}) using the terminal rate constant. Furthermore, the apparent bioavailability (F) was calculated for patients who received 20 mg i.v docetaxel in combination with ritonavir (during the first course in the first four dose-levels). This was done using equation 1:

$$F(\%) = \frac{AUC_{inf}(oral)}{AUC_{inf}(i.v.)} * \frac{Dose\ (i.v.)}{Dose\ (oral)} * 100\% \qquad (1)$$

Within-subject variability (WSV) in AUC0-48, AUC_{inf} and C_{max} was calculated if a patient underwent PK assessments of the same regimen twice (after the fourth dose-level). This was calculated like equation 2 for AUC_{inf}:

$$WSV\ (\%) = \frac{1}{n} * \sum^{n} \left(\frac{\left| AUC_{inf}^{2nd\ cycle} - AUC_{inf}^{1st\ cycle} \right|}{AUC_{inf}^{1st\ cycle}} \right) * 100\% \qquad (2)$$

Where n represents the number of evaluable patients per dose-level who underwent PK assessments of the same regimen twice.

Results

Patient characteristics and disposition

A total of 48 patients was enrolled in the dose escalation study divided over six dose-levels (30/100, 40/100, 60/100, 80/100, 60/200 and 80/200 mg docetaxel and ritonavir, respectively). After establishing the MTD, dose-levels 60/200 (n=17) and 60/100 (n=9) were expanded with six evaluable patients for additional safety data to determine the recommended dose for future phase 2 studies. The median age was 59 years (range, 36-79 years) and 44% had a WHO performance status ≤1. Forty-two percent of the patients were female and 92% of the patients were Caucasian (Table 1). All enrolled patients received at least one dose of oral docetaxel, of which the majority (73%) completed the DLT evaluation period of 4 weeks. The median number of weeks on treatment for all patients in the six dose-levels was 3 (range 2-24), 6 (range 5-37), 6 (range 2-40), 16 (range 7-42), 14 (range 1-43) and 10 (range 1-26), respectively. One patient is currently ongoing in the study in dose-level 60/100 (>40 weeks on treatment). In total 41 patients (85 %) discontinued study treatment permanently due to progressive disease or clinical deterioration, the other patients discontinued treatment as a result of an adverse events and patient refusal.

Twenty-two patients received a dose reduction or delay of treatment for at least one week. The main reasons leading to dose reduction or delay were patient request, poor performance status and hospitalization for a serious adverse event (SAE). Most of the dose reductions and delays of treatment occurred in the dose-levels with 60 mg and 80 mg docetaxel in combination with 200 mg ritonavir (50 of 66 events).

Safety and tolerability

All patients included (n=48) were evaluated for treatment-related adverse events. Table 2 lists all adverse events that were possibly, probably or definitely related to the study drug with an incidence rate of at least 5%. Overall, ModraDoc001 in combination with ritonavir was well tolerated. The most common drug-related adverse events in all dose-levels were nausea (69 %), diarrhea (65%), fatigue (63%) and vomiting (44%), the majority being grade 1-2. Generally, these events started within 24 hours after intake and decreased during the treatment week. Diarrhea was treated by prompt loperamide treatment for grade >1. In most cases (>70%), patients recovered fully from diarrhea

(grade >1) after loperamide treatment and the mean duration of diarrhea was eight days for grade 2 diarrhea and three days for grade 3 diarrhea. There were 15 patients (31 %) with alopecia reported in this study, of which seven with grade 1. Other adverse events, which are often seen after i.v. administration of docetaxel [24], occurred less frequently after oral administration. In this study neutropenia was seen in seven patients (15%), leucocytopenia in two patients (both grade 2) and neuropathy in eight patients (17%). Allergic reactions and febrile neutropenia were not observed.

Seven patients experienced a DLT during the first four weeks after start of oral docetaxel in combination with ritonavir (Table 3). Six of these patients suffered from grade 3 diarrhea whether or not in combination with other adverse events. In the first dose-level (30/100), one patient experienced diarrhea as DLT while on treatment with drinking formulation.

Table 1 Patient demographics

Character	N	%
Total number of patients	48	100%
Sex		
Male	28	58%
Female	20	42%
Age		
Median (range)	59 (36-79)	
WHO performance status		
0	21	44%
1	22	46%
2	5	10%
Ethnic origin		
Caucasian	44	92%
Asian	2	4%
African Descent	2	4%
Primary tumor site		
NSCLC	22	46%
UCC	4	8%
Anal	3	6%
Ovary	3	6%
Primary unknown	3	6%
Other	13	28%
Stage of cancer		
Locally advanced	2	4%
Metastatic	46	96%
Prior Treatment		
Chemotherapy	47	98%
Radiotherapy	33	69%
Surgery	26	54%

(abbreviations: NSCLC = non-small cell lung cancer, UCC = urothelial cell carcinoma)

Table 2 adverse events with a possible, probable or definite relationship to oral docetaxel in combination with ritonavir (incidence rate of at least 5% in the total safety population (N=48))

	30/100 (n=5)			40/100 (n=3)			60/100 (n=9)			80/100 (n=8)			60/200 (n=17)			80/200 (n=6)			Total (n=48)		
Toxicity	**%**	**Gr 1-2**	**Gr 3-4**	**%**	**Gr 1-2**	**Gr 3-4**	**%**	**Gr 1-2**	**Gr 3-4**	**%**	**Gr 1-2**	**Gr 3-4**	**%**	**Gr 1-2**	**Gr 3-4**	**%**	**Gr 1-2**	**Gr 3-4**	**%**	**Gr 1-2**	**Gr 3-4**
Nausea	40%	2		33%	1		89%	7	1	75%	5	1	65%	11		83%	5		69%	31	2
Diarrhea	40%	1	1				67%	5	1	88%	6	1	65%	9	2	83%	3	2	65%	24	7
Fatigue	60%	3		33%	1		78%	5	2	63%	4	1	59%	7	3	67%	2	2	63%	22	8
Vomiting	20%	1					67%	5	1	63%	4	1	29%	4	1	50%	3		42%	17	3
Alopecia	20%	1					22%	2		25%	2		41%	7		33%	2		29%	14	0
Stomatitis /mucositis	20%	1					11%	1		25%	2		29%	5		50%	2	1	25%	11	1
Neuropathy							22%	2		13%	1		29%	4	1				17%	7	1
Pain abdominal							22%	2		13%	1		18%	3		33%	2		17%	8	0
Constipation							11%	1					18%	3		50%	3		15%	7	0
Neutrophils													24%	3	1	50%	2	1	15%	5	2
Nail changes										38%	3		18%	3					13%	6	0
Anorexia							33%	3					6%	1		17%		1	10%	4	1
Infection with normal ANC	20%	1								13%	1		6%	1		33%	2		10%	5	0
Weight loss							22%	2					18%	3					10%	5	0
Cough							11%	1					6%	1		33%	2		8%	4	0
Hemmorhage							11%	1					18%	3					8%	4	0
ALT													18%	1	2				6%	1	2
Dehydration													12%		2	17%		1	6%	0	3
Dry skin				33%	1								6%	1		17%	1		6%	3	0
Dyspneu										13%	1		12%	2					6%	3	0
Platelets													12%	1	1	17%	1		6%	2	1
Watery eye													18%	3					6%	3	0

Table 3 Dose limiting toxicities after administration of oral docetaxel in combination with ritonavir (every line represents one patient)

Dose-level	Dose limiting toxicity (CTCAE 3.0)
30/100	Grade 3 diarrhea
60/100	Grade 3 diarrhea, vomiting and nausea
80/100	Grade 3 diarrhea, vomiting and nausea
60/200	Grade 3 diarrhea, elevated AST and ALT
60/200	Grade 3 gastritis, ulcus duodeni, elevated AST and ALT
80/200	Grade 4 dehydration, grade 3 diarrhea and mucositis
80/200	Grade 4 neutropenia, grade 3 diarrhea, fatigue and anorexia

Despite of the DLT in the dose-level 30/100, dose escalation continued after these three evaluable patients since parallel to this dose escalation study fifteen patients received 30 mg docetaxel as drinking solution or ModraDoc001 and 100 mg ritonavir in several proof of principle studies and without experiencing a DLT. The tolerability of this dose was therefore considered acceptable and the safety of this dose was adequately described elsewhere [25]. In both dose-level 60/100 and 80/100, one patient suffered from grade 3 diarrhea, vomiting and nausea. In dose-level 60/200 two of the seventeen patients experienced a DLT. One patient had grade 3 diarrhea in combination with grade 3 elevated AST and ALT and one patient had grade 3 gastritis and ulcus duodeni, also in combination with grade 3 elevated AST and ALT. In the dose-level with the highest dose (80/200), two out of six patients experienced a DLT. One patient had grade 4 dehydration in combination with grade 3 diarrhea and mucositis and one patient had grade 4 neutropenia in combination with grade 3 diarrhea, fatigue and anorexia. Because of these DLTs, the MTD was set at 60 mg docetaxel and 200 mg ritonavir.

There were 61 SAEs reported by 35 patients. In total 25 events (41%) in 10 patients (29%) were considered to be possibly, probably or definitely related to the study drug. All other SAEs were considered to be either unrelated or unlikely to be related to the study drug. Most reported SAEs that were possibly, probably or definitely related to the study drug were diarrhea (28%), dehydration (12%), nausea (12%) and vomiting (12%). All these events resolved after discontinuation of study treatment, except for one patient. This patient was hospitalized elsewhere and went off study because of clinical deterioration and brain metastasis.

Pharmacokinetics

The PK of docetaxel was monitored during day 1 and 8 of the study. In four patients, docetaxel was measured only on the first day, because these patients went off study before the second PK assessment. Mean plasma concentration–time curves for oral docetaxel are shown in Figure 3 and the results of the non-compartmental PK analysis are shown in Table 4. The mean plasma concentration–time profile of docetaxel exhibited a profile of bi-exponential PK. The mean C_{max} of docetaxel was reached after 2.2 hours

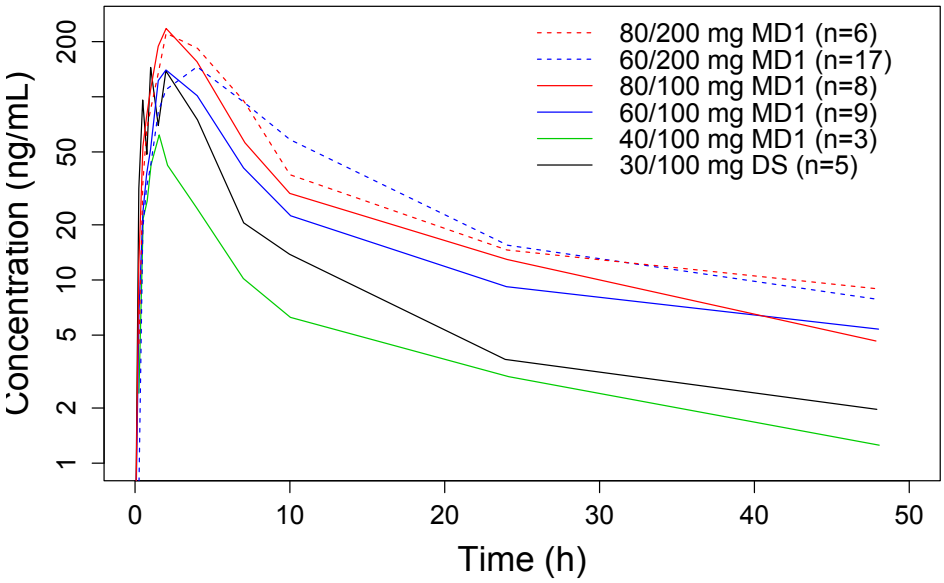

Figure 3 Mean plasma concentration-time curves of docetaxel in patients after administration of the drinking solution (DS, 30 mg) and ModraDoc001 10 mg capsules (MD1). Solid lines represent docetaxel in combination with 100 mg ritonavir and dashed lines represent docetaxel in combination with 200 mg ritonavir.

(CV 33%) in combination with 100 mg ritonavir and 3.6 hours (CV 48%) in combination with 200 mg ritonavir, independently of the docetaxel dose. The mean terminal half-life of docetaxel was 14.7 hours, independently of the docetaxel and ritonavir dose. The C_{max} and systemic exposure to docetaxel (expressed as AUC_{inf}) increased with increasing dose. However, these increases were less than dose proportional. The relationship between the AUC_{inf} and the docetaxel dose is shown in Figure 4. The increase of the ritonavir dose to 200 mg resulted in an increase of approximately 65% (60 mg docetaxel) and 28% (80 mg docetaxel) of the mean AUC_{inf} to docetaxel, without an increase of the mean C_{max}. The mean C_{max} and mean AUC_{inf} of docetaxel at the MTD was 170 ng/mL (CV 62%) and 1.79 µg/mL*hr (CV 80%), respectively, in 17 patients.

The PK of ritonavir was monitored during the i.v. administration of docetaxel and at day 1 of the oral combination. Mean plasma concentration–time curves of ritonavir are shown in Figure 5 and the results of the non-compartmental PK analysis in Table 5. After a dose of 100 mg ritonavir, the mean C_{max} of ritonavir was reached after 2.8 hours and the PK profile showed a monophasic decline. After a dose of 200 mg ritonavir, AUC_{inf} and the C_{max} of ritonavir were three fold higher compared to AUC_{inf} and the C_{max} after 100 mg ritonavir and the C_{max} was reached 1 hour later. The mean terminal half-life of ritonavir was 6.0 (CV 40%) hours after 100 mg ritonavir and 6.1 (CV 30%) hours after 200 mg ritonavir.

Table 4 Summary statistics for pharmacokinetic parameters of docetaxel after the first and second administration. The data are shown as mean values and coefficient of variation (%).

Dose-level (docetaxel/ ritonavir)	20/100	30/100	40/100	60/100[1]	60/100[2]	80/100	60/200[1]	60/200[2]	80/200[1]	80/200[2]
Dosage form	**i.v.**	**DS**	**MD1**	**MD1**	**MD1**	**MD1**	**MD1**	**MD1**	**MD1**	**MD1**
N	**19**	**5**	**3**	**9**	**6**	**8**	**17**	**14**	**6**	**5**
AUC_{0-48} (µg•h/mL)	0.54	0.83	0.31	1.00	1.10	1.48	1.63	1.37	1.78	1.68
CV (%)	48%	95%	49%	69%	101%	52%	78%	58%	46%	103%
AUC_{inf} (µg•h/mL)	0.61	1.02	0.33	1.08	1.33	1.57	1.79	1.52	2.01	2.12
CV (%)	46%	110%	44%	69%	1.13	50%	80%	61%	48%	114%
C_{max} (ng/mL)	477	161	63	177	163	264	170	181	226	224
CV (%)	44%	113%	79%	77%	64	46%	62%	65%	25%	78%
T_{max} (h)	EOI	1.35	1.32	2.56	2.67	2.19	4.01	2.53	2.34	2.8
CV (%)	-	35%	31%	43%	39%	34%	61%	43%	35%	39%
T_{half} (h)	20.5	12.7	15.8	15.1	17.1	12.2	13.0	14.8	16.3	20.4
CV (%)	51%	50%	25%	16%	20%	27%	25%	30%	21%	40%
F (%)	-	64%[a]	50%	70%[b]	-	62%	-	-	-	-
CV (%)	-	58%[a]	42%	47%[b]	-	28%	-	-	-	-
WSV AUC_{0-48} (%)	-	-	-	-	31%	-	-	26%	-	46%
WSV AUC_{inf} (%)	-	-	-	-	31%	-	-	30%	-	53%
WSV C_{max} (%)	-	-	-	-	70%	-	-	45%	-	56%

(abbreviations: i.v. = intravenous administration, DS = docetaxel drinking solution, MD1 = ModraDoc001 capsules, AUC_{0-48} = the area under the plasma concentration-time curve between t=0 and the time point of the last quantifiable data point (48 hours), AUC_{inf} = AUC with extrapolation to infinity, C_{max} = maximum observed plasma concentration, T_{max} = time to reach C_{max}, Thalf = the terminal elimination half-life, F = apparent bioavailability, WSV = within subject variation, N = number of evaluable patients per dose-level, EOI = end of infusion, [1] = First week, [2] = second week, [a] N=4, [b] N=3)

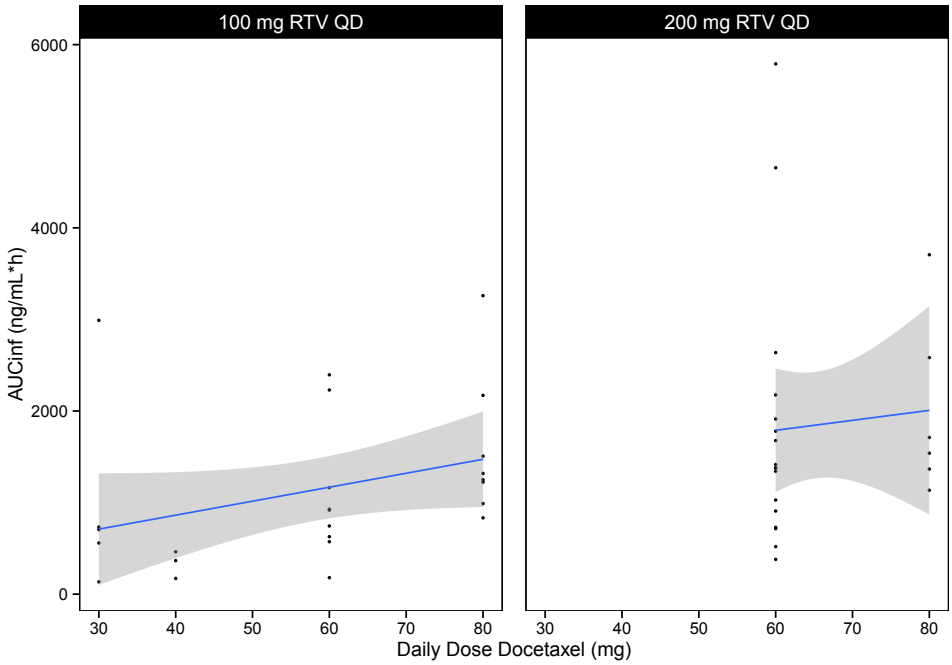

Figure 4 Dose- exposure curves of docetaxel in patients after administration of oral docetaxel (drinking solution for 30 mg and ModraDoc001 10 mg capsules for other doses) in combination with 100 mg ritonavir (left panel) or 200 mg ritonavir (right panel) in the first week of oral docetaxel. Lines are the linear regressions with 95% confidence interval.

Anti-tumor activity

The tumor evaluable population included only patients with measurable disease at baseline. Four patients had a partial response. These patients had NSCLC, gastric cancer, breast cancer, or cancer of unknown primary origin (CUP). Eighteen patients exhibited stable disease (SD) as their best overall response with a median duration of 16 weeks (range, 5-43 weeks), of which eight patients had NSCLC. There were no patients with an objective complete response.

Discussion

This report describes the first dose escalation study of oral docetaxel in combination with ritonavir. Six dose-levels were evaluated in this study, ranging from 30 mg to 80 mg docetaxel and 100 mg and 200 mg ritonavir. At the lowest dose-level, docetaxel was given as a drinking solution (i.v. liquid formulation). Moes et al demonstrated in parallel to this study no significant differences between the pharmacokinetic parameters of docetaxel after oral administration of the drinking solution and ModraDoc001 capsules in combination with 100 mg ritonavir [20].

With the exception of dose-level 80/200 mg (docetaxel/ritonavir), oral treatment with docetaxel and ritonavir was well tolerated. Most frequently reported treatment related adverse events were nausea, diarrhea and fatigue, independently of the dose of docetaxel.

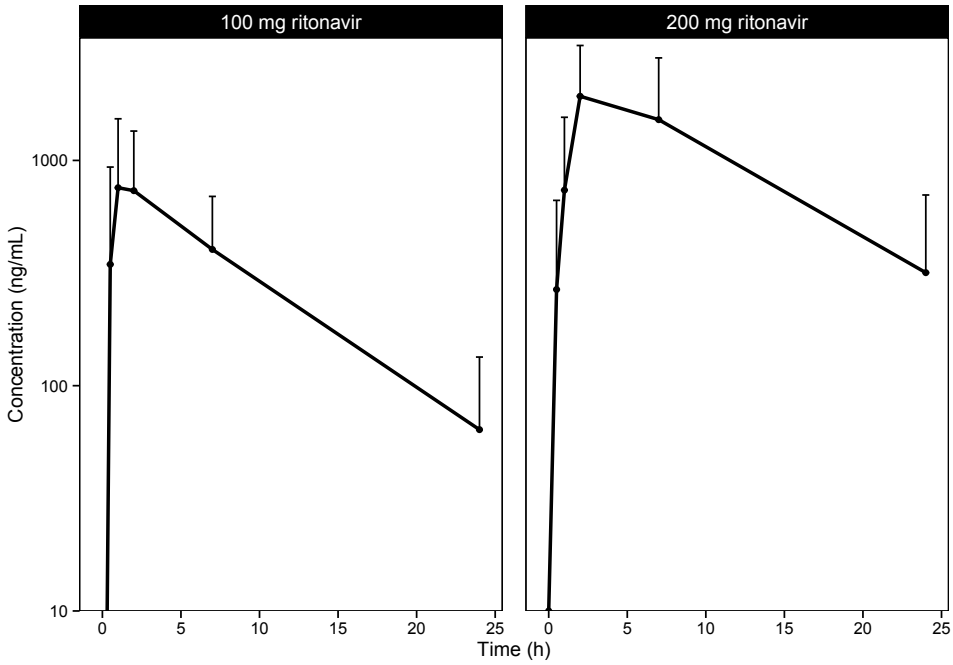

Figure 5 Plasma concentration-time curves of ritonavir in patients after administration of 100 mg (n=25) and 200 mg ritonavir (n=23). The data are shown as mean values (symbols) with SD (error bars).

These events, mostly CTC grade 1-2, occurred almost twice as often in this study compared to treatment with i.v. administration of docetaxel [24]. The most frequently reported DLT was diarrhea. Since oral administration of docetaxel has been explored only in few clinical studies, limited data is available regarding the pathophysiology of these events. Local high docetaxel concentrations in the human enterocyte might cause apoptosis of intestinal epithelial cells, and therefore cause mucositis [26,27]. It is also reported that ritonavir can induce apoptosis in human intestinal epithelial cells [28]. In both cases, the intestinal villi could be damaged leading to a reduced surface area for absorption resulting in diarrhea via secretory mechanisms [29,30], which is also seen after ritonavir treatment in HIV patients at low daily doses of 100-400 mg [30]. The incidence of mild and moderate diarrhea could therefore be increased due to both ritonavir and docetaxel. These events of diarrhea were reversible and treated by prompt management with loperamide.

The most commonly reported adverse drug reactions associated with i.v. treatment of docetaxel are alopecia, allergic reactions, malaise, anemia, leucocytopenia, neutropenia and febrile neutropenia [24]. In this study, we did not observe these adverse events as frequent as after i.v. treatment. Only 33% of the patients suffered from thinning of the hair (scored as alopecia grade 1), while during weekly administration of i.v. docetaxel, almost 60% of the patients loses the hair completely [31]. Concerning the hematologic adverse events, only two patients had severe neutropenia and none suffered from severe

Table 5, Summary statistics for pharmacokinetic parameters of ritonavir after the first and second administration. The data are shown as mean values and coefficient of variation (%).

Doselevel	**100 mg[1]**	**100 mg[2]**	**200 mg**
N	**25**	**11**	**23**
AUC_{0-24} (µg•h/mL)	6.86	7.74	23.1
CV(%)	67%	90%	75%
AUC_{inf} (µg•h/mL)	7.70	5.58*	26.9
CV(%)	72%	50%	88%
C_{max} (µg/mL)	0.99	0.99	2.60
CV(%)	68%	84%	58%
T_{max} (h)	2.76	3.45	4.13
CV(%)	74%	79%	113%
T_{half} (h)	6.0	6.7*	6.1
CV(%)	40%	60%	30%
WSV AUC (%)		27%	
WSV AUC_{inf} (%)		22%*	
WSV C_{max} (%)		47%	

*(abbreviations: AUC = the area under the plasma concentration-time curve between t=0 and the time point of the last quantifiable data point (24 hours), AUC_{inf} = AUC with extrapolation to infinity, C_{max} = maximum observed plasma concentration, T_{max} = time to reach C_{max}, T_{half} = the terminal elimination half-life, WSV = within subject variation, N = number of evaluable patients per dose-level, 1: First week, 2: second week, *N=9)*

leucocytopenia or anemia. Also the most life-threatening docetaxel toxicity febrile neutropenia was not seen in this study. Previous phase II/III studies indicated that severe myelosuppression was reduced by administering docetaxel once a week compared to the conventional treatment, which is given every three weeks [32]. In different studies with weekly administration of i.v. docetaxel, the incidence of severe neutropenia and leucocytopenia was 2-28% and 0-20%, respectively [31,33–37]. This reduction is most probably related to the reduced C_{max} of docetaxel at the end of the infusion period [34,38]. The C_{max} of oral docetaxel is lower than after infusion, which may explain the low incidence of myelosuppression in our study. Also the absence of polysorbate 80 in the oral formulation ModraDoc001 could contribute to this reduction, since polysorbate 80 is able to influence plasma binding of docetaxel by formation of a complex with serum proteins and/or a displacement interaction on the binding proteins [38,39].

This study showed that the combination of docetaxel with 100 mg ritonavir resulted in an apparent bioavailability of docetaxel of 62% (CV = 39%), independently of the docetaxel dose. Without any co-administration of oral ritonavir, the bioavailability of docetaxel was estimated to be about 8% (CV= 75%) [40]. Preclinical studies with transgenic mice indicated that intestinal CYP3A4 is more important for the first-pass metabolism than hepatic CYP3A4. By co-administration with ritonavir intestinal CYP3A4 was inhibited and

therefore the most limiting factor of the enhancement of systemic exposure of docetaxel [41]. Nevertheless, in the proof-of-concept study an apparent oral bioavailability of >100% of 100 mg docetaxel in combination with 100 mg of ritonavir was found indicating that ritonavir also inhibits the elimination of docetaxel [16], which was confirmed by a population PK analysis [42]. This latter analysis showed that the clearance of docetaxel was inversely correlated with the concentration of ritonavir and decreased almost instantly after administration of ritonavir. The results of this phase I study confirm this theory by showing an enhancement of the bioavailability. Doubling the dose of ritonavir increased the mean AUC_{inf} to docetaxel by 65% (60 mg docetaxel) and 28% (80 mg docetaxel), but did not influence the terminal half-life of docetaxel. Furthermore, the T_{max} increased by approximately 1 hour after 200 mg of ritonavir compared to 100 mg as a consequence of the delaying effect of ritonavir gastric emptying. [43,44]. Since the inhibition of CYP3A4 is correlated with the concentration of ritonavir, the inhibition of intestinal CYP3A4 may be more efficient and/or last longer with 200 mg ritonavir, but does not influence the rate of elimination.

The interpatient variability in AUC_{inf} was wide and seems to be higher for oral docetaxel than for docetaxel given as a infusion (CV, 30%-44% [38,45]). A plausible explanation can be that docetaxel precipitated before absorption, because of the limited volume in the intestinal tract. The variation in AUC_{inf} of docetaxel after 200 mg of ritonavir between patients is slightly higher than after 100 mg of ritonavir (Figure 4). This is mainly due to a few patients with an extremely high AUC_{inf} (>3 µg/mL*h) after co-administration of 200 mg ritonavir. These patients experienced severe toxicities and were taken off study. Based on these events and because most of the dose reductions and delays of treatment occurred at the dose levels with 200 mg ritonavir, the recommended dose for future phase 2 studies is defined as 60 mg docetaxel in combination with 100 mg ritonavir instead of the MTD. The AUC_{inf} at both dose-levels was comparable with the AUC_{inf} after weekly i.v. administration (35 mg/m^2) [38].

In conclusion, this study demonstrates that ModraDoc001 capsules can be safely administered to patients with a MTD of 60 mg in combination with 200 mg ritonavir in a weekly schedule. The most common drug-related adverse events were diarrhea, nausea and fatigue, the majority being grade 1-2. The best tumor response observed was partial remission, which was seen in 4 patients (CUP, NSCLC, gastric and breast cancer). This study strengthens the concept that docetaxel can be given orally and enables the possibilities for an oral alternative for standard docetaxel treatment and more frequent administration schedules.

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). J.J. Moes, B. Nuijen, J.H. Beijnen and J.H.M. Schellens have patents for oral taxane formulations. The other authors declare that they have no conflict of interest.

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2.2

A phase I dose escalation study of bi-daily once-weekly administration of oral docetaxel (ModraDoc001) in combination with ritonavir

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Interim analysis (data were monitored, except for pharmacokinetics and radiological evaluations)

Abstract

Background ModraDoc001 is a novel oral formulation containing 10 mg docetaxel as a solid dispersion. Oral administration of docetaxel is feasible in combination with the CYP3A4 inhibitor ritonavir. The objective of this study was to determine the safety, maximum tolerated dose (MTD) and pharmacokinetics (PK) of bi-daily (BID) once-weekly oral docetaxel (as ModraDoc001) in combination with ritonavir.

Methods Patients with advanced solid tumors, WHO PS ≤ 2, no concomitant use of P-glycoprotein or CYP3A modulating drugs, adequate bone marrow, liver and renal function were eligible for this study. Docetaxel and ritonavir (Norvir®) were simultaneously administered (BID) once-weekly in a classical '3+3 cohort' dose escalation design. The MTD was defined as the highest dose resulting in < 1/6 probability of causing a dose limiting toxicity in the first four weeks of treatment. Pharmacokinetics (PK) was determined on days 1 and 15.

Results A total of 17 patients (53% male) were enrolled in three dose-levels (20/100, 30/100 and 40/100 mg docetaxel and ritonavir BID, respectively). Common treatment related adverse events were fatigue (82%), diarrhea (65%), anorexia (47%) and nausea (47%), the majority being grade 1-2 of severity. Four patients experienced a dose limiting toxicity (DLT). The most common DLT was grade 3 nausea (n=2). The MTD was identified at 20 mg docetaxel and 100 mg ritonavir BID. Both drugs were rapidly absorbed after oral administration. The mean C_{max} of docetaxel was reached at 2.2 hours (CV 69%) after the first administration and at 2.9 hours (CV 16%) after the second administration, independently of the dose. The mean maximum plasma concentration and mean plasma exposure (AUC_{inf}) to docetaxel increased less than proportionally with dose to 78 ng/mL (CV 62%) and 0.77 µg•h/mL (CV 57%), respectively. Four of the patients treated had stable disease (SD) with median duration of 14 weeks. There were no patients with a partial or complete tumor response.

Conclusions At the MTD (BID administration of once-weekly 20 mg docetaxel and 100 mg ritonavir) ModraDoc001 is safe and well tolerated.

Introduction

Docetaxel is a semi-synthetic analogue of the Taxus baccata and is currently used as an anticancer agent in several types of cancer, such as non-small cell lung cancer (NSCLC), breast, head and neck, prostate and ovarian cancer. Doses range from 60 to 100 mg/m^2 administrated as a 1-hour infusion every 3 weeks [1]. The main disadvantages of the current route of administration are the invasive procedure for the patient and the adverse events, in particular allergic infusion reactions, caused by the formulation vehicle containing polysorbate 80 (Taxotere®) [2,3]. These events are not expected after oral administration of docetaxel as ModraDoc001 10 mg capsules, as the formulation contains docetaxel as a solid dispersion without polysorbate 80 [4]. Other advantages of oral treatment of docetaxel are the patients' convenience of flexible home treatment and necessity of pre-medication with dexamethason. Moreover, an oral dosage form enables more easily chronic treatment regimes.

Docetaxel has limited oral bioavailability because of its poor aqueous solubility and high affinity for active ABC drug efflux transporters and cytochrome P450 3A drug metabolizing enzymes (CYP3A). As uptake in the gastro-intestinal tract requires dissolution, the poor aqueous solubility significantly complicates oral absorption of docetaxel as a solid dosage form. In addition, both P-glycoprotein (P-gp/ABCB1/MDR1) and CYP3A, which are abundantly expressed in the epithelial layer of the gut wall and in the liver, contribute to low absorption and significant pre-systemic metabolism of orally administered docetaxel

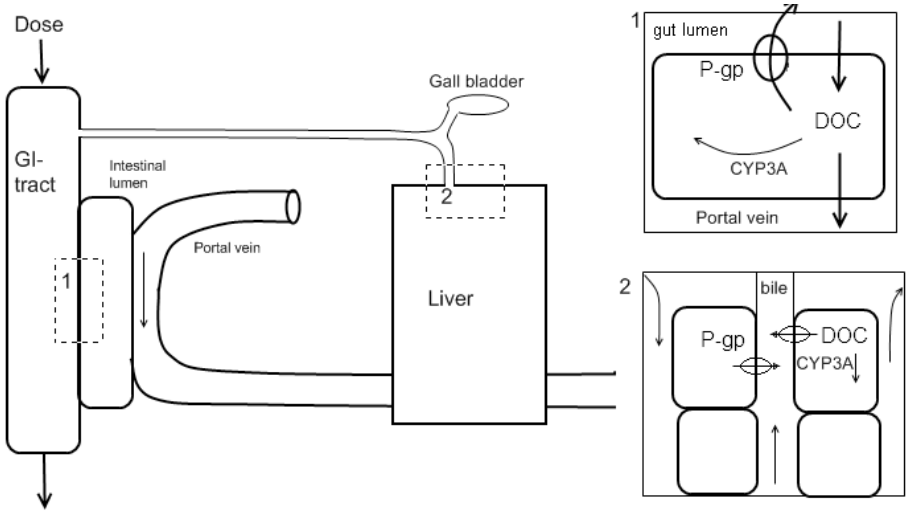

Figure 1 Docetaxel has limited oral bioavailability because of poor aqueous solubility and high affinity for active ABC drug efflux transporters and, cytochrome P450 3A drug metabolizing enzymes (CYP3A). Both P-glycoprotein (P-gp) and CYP3A, which are abundantly expressed in the epithelial layer of the gut wall (1) and in the liver (2), contribute to low absorption and significant pre-systemic metabolism of orally applied docetaxel [5–7]. The systemic exposure upon oral docetaxel is increased when co-administered with an inhibitor of P-gp and/or CYP3A. (Abbreviations: DOC = docetaxel)

[5–7]. Preclinical and clinical proof of concept studies have shown that inhibition of P-gp and/or CYP3A enhances systemic exposure to docetaxel, and this could enable oral administration of the drug [8–14]. However, inhibition of P-gp alone increases the exposure to oral docetaxel insufficiently [12]. Both preclinical and clinical research showed satisfactory enhancement of the systemic exposure to docetaxel by co-administration with the CYP3A inhibitor ritonavir [13,14]. Recent studies revealed a mean apparent oral bioavailability of docetaxel of around 100% when given in combination with a low dose of ritonavir [14]. An additional benefit of ritonavir is the longterm clinical experience with temporary inhibition of CYP3A4 to enhance the exposure to orally applied protease inhibitors [15–17]

At our institute, we performed a phase I dose escalation study to determine the feasibility of this concept with ModraDoc001 10 mg capsules in combination with oral ritonavir as booster drug [18]. The results of this once-daily (QD) once-weekly dosing schedule showed a non-linear relationship in the oral pharmacokinetics (PK) of docetaxel, which encouraged us to explore a twice daily (BID) once-weekly dosing schedule to further improve and prolong the systemic exposure to orally administered docetaxel. In the present dose escalation phase I study, patients with advanced solid tumors were treated with BID oral docetaxel in combination with ritonavir in a weekly schedule. The primary objective was to determine the maximum tolerated dose (MTD) of BID ModraDoc001 capsules in combination with ritonavir in patients in a weekly schedule. The secondary objectives were to determine the dose limiting toxicities (DLT), the safety, PK of docetaxel and ritonavir, and preliminary antitumor activity of the oral combination.

Patients and Methods

Study design and treatment schedule

This phase I study was an open-label, dose escalation study of oral docetaxel in combination with ritonavir. Patients received oral docetaxel (ModraDoc001 10 mg capsules, Slotervaart Hospital, The Netherlands) in combination with an oral 100 mg ritonavir tablet (Norvir®, Abbott, Illinois, USA) (intake around the same time) BID, once a week, until progressive disease or unacceptable toxicity. The medication was taken in fasting conditions, i.e. at least two hours after and at least one hour before food intake, since the effect of food on the pharmacokinetics of the drug was unknown. The period between both administrations was 7 to 12 hours. Pre-medication consisted of 1 mg granisetron orally 1 hour prior to treatment. After three weeks, pre-medication was optional. The study had a classic '3+3' dose escalation design [19]. Three patients were assigned to each dose-level. If one patient of the first three at a defined dose-level experienced a DLT, the number of patients treated at this dose-level was expanded to a maximum of six patients. The dose escalation continued if none of the additional patients experienced a DLT. The MTD level was expanded to at least six patients in any case. The docetaxel doses in the next dose-levels were based on safety evaluations and PK profiles observed at prior dose-levels. The starting dose was 40 mg docetaxel combined with 100 mg ritonavir BID. This daily dose of 80 mg was proven safe and tolerable in a comparable dose escalation study where ModraDoc001 10 mg capsules were administered in combination with 100 mg ritonavir once daily in a weekly schedule [18].

Patients were considered evaluable for safety if they had received at least one dose of docetaxel. Patients were considered evaluable for the safety of a dose level if they had completed the first four treatment weeks or if they experienced a DLT occurred in this period. A DLT was defined as any of the following events occurring during the first four treatment weeks and was determined to be possibly, probably or definitely related to docetaxel by the investigator: grade 3 or 4 non-hematological toxicity (other than untreated nausea, vomiting or diarrhea), grade 4 thrombocytopenia, grade 4 neutropenia lasting for more than seven consecutive days, grade 3 febrile neutropenia, or treatment delay of ≥3 weeks (or ≥1 week after protocol amendment) due to treatment related toxicity. Of note, Grade 3 fatigue was not considered a DLT if the patient had grade 1 fatigue at baseline.

Eligibility

Patients were eligible if they had a histological or cytological proof of cancer, if there were no standard treatment options available and if docetaxel treatment was considered appropriate. Other inclusion criteria were age ≥ 18 years, performance status of 0, 1 or 2 according to the WHO Performance Status (PS) scale, life expectancy longer than 3 months, and adequate bone marrow, liver and renal functions (neutrophil count ≥ 1.5 * 10^9/L, platelet count ≥ 100 * 10^9/L; alanine transaminase (ALT) and aspartate transaminase (AST) ≤ 2.5 times the institutional upper limit of normal (ULN), bilirubin of ≤ 1.5 times the ULN; serum creatinine ≤ 1.5 of the ULN or creatinine clearance ≥ 50 mL/min by Cockcroft-Gault formula).

Patients with known alcoholism, drug addiction and/or psychotic disorders were excluded. Patients were not allowed to concomitantly use P-gp and CYP3A modulating drugs, H_2-receptor antagonists or proton pump inhibitors. Other exclusion criteria were uncontrolled infectious disease, bowel obstructions able to influence drug absorption, neurologic disease, pre-existing neuropathy higher than grade 1, symptomatic cerebral or leptomeningeal metastases, pregnancy, breast feeding, refusal to use adequate contraception and previous anticancer therapy within 4 weeks prior to the first dose of oral docetaxel. The study protocol was approved by the local Medical Ethics Committee. All patients had to give written informed consent. The study was registered under identifier NCT01173913 (NIH register).

Study procedures

Pre-treatment evaluations consisted of a complete medical history including concomitant medications, physical examination (including vital signs and performance status), assessment of baseline symptoms , a pregnancy test in female patients, laboratory assessment of hematology, serum chemistry and urinalysis and a radiologic tumor assessment. Before each administration, assessment of adverse events using the National Cancer Institute's Common Terminology Criteria for AEs (NCI-CTCAE v3.0) and of concomitant medications was repeated and hematology and serum chemistry were checked. A physical examination was performed at least every three weeks. Tumor response was evaluated every 6-8 weeks according to RECIST version 1.0.

Pharmacokinetics

The PK of docetaxel and ritonavir were monitored at day 1 and 15 of treatment. Venous blood samples for PK analysis were obtained through an indwelling peripheral intravenous catheter. PK of docetaxel and ritonavir were evaluated pre-dose, 0.5, 1, 1.5, 2, 3, 4, 6, 7 (prior to the following daily administration), 7.5, 8, 8.5, 9, 10, 11, 13, 24 and 48 hrs after first administration of study drug. Blood samples were collected in tubes containing lithium heparin as an anticoagulant. All samples were centrifuged within 1 hour at 1500 g for 10 minutes at 4°C and plasma was stored at –20°C until analysis.

Docetaxel and ritonavir were quantified in plasma by a high-performance liquid chromatography assay with tandem mass spectrometric detection (LC-MS/MS) as described by Hendrikx et al. [20]. For the assay labelled analogues were used as internal standards. Briefly, both compounds were extracted from 200 µL human plasma using tertiair butylmethylether. Subsequently, the solution was evaporated to dryness under a gentle stream of nitrogen and the residue was reconstituted in methanol: water (1:1, v/v). Of each sample, 25 µL were injected onto a Zorbax Extend C18 column (150 x 2.1 mm ID; particle size 5 µm; Agilent Technologies, Amstelveen, The Netherlands). The mobile phase consisted of a mixture of 7:3 *v/v* methanol/10 mM ammonium hydroxide in water. Compounds were detected using positive ionization electrospray tandem mass spectrometry. The validated range of the assay was 0.5-500 ng/mL for docetaxel and 2.0-2000 ng/mL for ritonavir.

Data analysis

The individual non-compartmental PK parameters were determined using validated scripts in the software package R (version 2.15). 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}), apparent clearance (CL/F), distribution volume at steady state (Vss/F), the terminal elimination half-life ($t_{1/2}$), the area under the plasma concentration-time curve between t=0 and the time point of the last quantifiable data point (AUC0-48) or, if possible, with extrapolation to infinity (AUC_{inf}) using the terminal rate constant. The AUCs were estimated by the linear trapezoidal (absorption phase) and logarithmic trapezoidal rule (elimination phase). Within-subject variability (WSV) in $AUC_{0\text{-}48}$, AUC_{inf} and C_{max} were calculated if a patient underwent PK assessments of the same regimen twice. This was calculated like equation 1 for AUC_{inf}:

$$WSV\ (\%) = \frac{1}{n} * \sum^{n} \left(\frac{\left| AUC_{inf}^{2nd\ cycle} - AUC_{inf}^{1st\ cycle} \right|}{AUC_{inf}^{1st\ cycle}} \right) * 100\% \qquad (1)$$

where n represents the number of evaluable patients per dose-level who underwent PK assessments of the same regimen twice.

Table 1 Patient demographics

Character	N	%
Total number of patients	17	
Sex		
Male	9	53%
Female	8	47%
Age		
Median (range)	60 (41-77)	
WHO performance status		
0	8	47%
1	8	47%
2	1	6%
Ethnic origin		
Caucasian	16	94%
African Descent	1	6%
Primary tumor site		
NSCLC	11	65%
UCC	3	17%
Anal	1	6%
Ovary	1	6%
Colorectal	1	6%
Stage of cancer		
Metastatic	17	100%
Prior Treatment		
Chemotherapy	17	100%
Radiotherapy	9	53%
Surgery	7	41%

(Abbreviations: NSCLC = non-small cell lung cancer, UCC = urothelial cell carcinoma)

Results

Patient characteristics and disposition

A total of 17 patients divided over three dose-levels (20/100, 30/100 and 40/100 mg docetaxel and ritonavir BID respectively) was enrolled in the dose-escalation study. Dose-level 20/100 BID was expanded with three evaluable patients in order to obtain additional safety data. The median age was 60 years (range, 41-77 years) and 94% had a WHO performance status ≤1. In total 47% of the patients were female and 94% of the patients were Caucasian (Table 1). All enrolled patients received at least one dose of oral docetaxel, of which the majority (76%) completed the DLT evaluation period of 4 weeks without a DLT. The median number of weeks on treatment for all patients enrolled in the three dose-levels was 10 (range 7-19), 5 (range 1-7) and 6 (range 1-8), for dose-levels 20/100, 30/100 and 40/100 mg docetaxel and ritonavir BID respectively. Eleven patients (65%) discontinued study treatment permanently due to progressive disease, whereas the remaining six patients discontinued treatment as a result of an adverse event (especially fatigue, infection, mucositis and anorexia).

Six patients experienced a dose reduction or delay of treatment for at least one week. The main reasons leading to dose reduction or delay were patient request, poor performance status and adverse events (fatigue [n=2], nausea [n=1], mucositis [n=1], and elevated liver transaminases [n=2]). Dose reductions were required at dose-levels 20/100 and 40/100 BID. There were no clear differences in treatment delays between the dose-levels.

Safety and tolerability

All patients included (n=17) were evaluated for treatment-related adverse events. Table 2 lists all adverse events that were possibly, probably or definitely related to the study drug with an incidence rate of at least 10%. Overall, ModraDoc001 in combination with ritonavir was well tolerated. The most common drug-related adverse events in all dose-levels were fatigue (82%), diarrhea (65%), anorexia (47%) and nausea (47%), the majority being grade

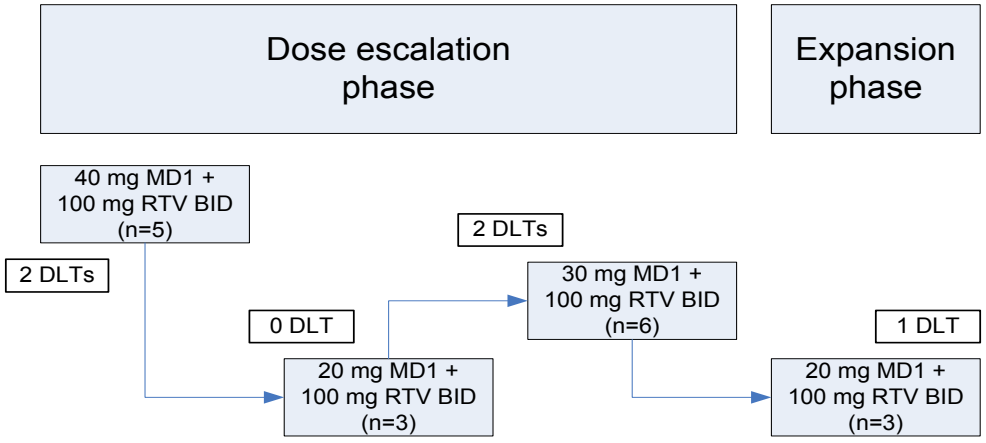

Figure 2 Study scheme (abbreviations: MD1 = ModraDoc001 capsules, RTV = ritonavir, DLT = dose limiting toxicity)

Table 2 Adverse events with a possible, probable or definite relationship to oral docetaxel in combination with ritonavir (incidence rate of at least 10% in the total safety population (N=17))

	20/100 (n=6)			30/100 (n=6)			40/100 (n=5)			Total (n=17)		
Toxicity	**%**	**Gr 1-2**	**Gr 3-4**	**%**	**Gr 1-2**	**Gr 3-4**	**%**	**Gr 1-2**	**Gr 3-4**	**%**	**Gr 1-2**	**Gr 3-4**
Fatigue	67%	4		83%	2	3	100%	4	1	82%	10	4
Diarrhea	50%	3		83%	4	1	60%	3		65%	10	1
Anorexia	0%			83%	4	1	60%	3		47%	7	1
Nausea	33%	1	1	67%	3	1	40%	2		47%	6	2
Vomiting	33%	2		50%	3		20%	1		35%	6	0
Pain (abdominal or stomach)	33%	2		17%	1		40%	2		29%	5	0
Weight loss	33%	2		33%	2		20%	1		29%	5	0
Dyspneu	17%	1		17%	1		40%	2		24%	4	0
Hemoglobin / anemia	0%			33%	2		40%	1	1	24%	3	1
Alopecia	17%	1		17%	1		20%	1		18%	3	0
AST	17%	1		33%	2		0%			18%	3	0
Neuropathy	17%	1		0%			40%	2		18%	3	0
Stomatitis / mucositis	0%			17%	1		40%	1	1	18%	2	1
ALT	17%		1	17%	1		0%			12%	1	1
Constipation	33%	2		0%			0%			12%	2	0
Dehydration	0%			17%		1	20%		1	12%	0	2
Heartburn	17%	1		0%			20%	1		12%	2	0
Leucocytopenia	0%			0%			40%	1	1	12%	1	1
Neutropenia	0%			0%			40%	1	1	12%	1	1
Pain (joint)	17%	1		17%	1		0%			12%	2	0
Pain (muscle)	33%	2		0%			0%			12%	2	0
Trombocytopenia	0%			17%	1		20%	1		12%	2	0

(Abbreviations: AST = aspartate transaminase , ALT = alanine transaminase, Gr = Grade)

1-2 as severity. Generally, these events started within 24 hours after drug intake and decreased during the treatment week. In case of grade ≥1 diarrhea loperamide was given. In most cases (>80%), patients recovered fully from diarrhea (grade >1) with loperamide treatment and the mean duration of diarrhea was five days for grade 2 diarrhea (6 events) and twelve days for grade 3 diarrhea (1 event). There were three patients (18%) with alopecia (grade 1) reported in this study. Other well-known adverse events seen after i.v. administration of docetaxel [21], occurred less frequently after oral administration. In this study neutropenia in combination with leucocytopenia was seen in two patients (12%).

Table 3 Dose limiting toxicities after administration of oral docetaxel in combination with ritonavir (every line represents one patient)

Dose-level	Dose limiting toxicity (CTCAE 3.0)	SAE
20/100	Grade 3 nausea, elevated ALT	No
30/100	Grade 3 anorexia, hemorrhage nose and upper gastrointestinal tract	Yes
30/100	Grade 3 diarrhea, nausea, dehydration	Yes
40/100	Grade 4 febrile neutropenia, grade 3 mucositis (oral cavity), dehydration, hyponatremia	Yes
40/100	Delay of > 1 week because of grade 3 fatigue and grade 2 neutropenia and leukopenia	No

(Abbreviations: ALT = alanine transaminase, SAE = serious adverse event)

One patient (6%) had grade 4 neutropenia and leucocytopenia requiring hospitalization and lasting 3 days. Another patient (6%) had grade 2 neutropenia and leucocytopenia during one weekly visit. Anemia was seen in four patients (24%). Allergic reactions and febrile neutropenia were not seen in this study.

In the first dose-level (40 mg docetaxel and 100 mg ritonavir BID), two patients had a DLT (Table 3). One patient was hospitalized five days after start treatment with grade 4 febrile neutropenie and grade 3 dehydration. These events recovered, but the patient was taken off study due to toxicity. The other patient could not receive the second and third cycles because of a poor condition (grade 3 fatigue and grade 2 neutropenia and leucocytopenia, all related to the study drug). The patient restarted in the fourth week with a dose reduction (20 mg docetaxel and 100 mg ritonavir BID). Because of these DLTs, the doses of the next levels were reduced to 20 mg docetaxel in combination with 100 mg ritonavir BID (equals to 40 mg docetaxel per week) and 30 mg docetaxel in combination with 100 mg ritonavir BID (equals to 60 mg docetaxel per week), respectively (see Figure 2). In the second dose-level (20 mg docetaxel and 100 mg ritonavir BID) one patient had a DLT which consisted of three days grade 3 nausea, followed by three days of grade 3 elevated ALT. This patient restarted treatment two weeks after the DLT event with a dose reduction (40 mg docetaxel and 100 mg ritonavir QD). In the dose-level with 30 mg docetaxel and 100 mg ritonavir BID, two patients had a DLT. These patients suffered of grade 3 hemorrhages in nose and upper gastrointestinal tract combined with grade 3 anorexia and a combination of grade 3 diarrhea, nausea and dehydration, respectively. Both patients were hospitalized and taken off study. Because two out of six patients experienced a DLT in this dose-level, the MTD was set at 20 mg docetaxel and 100 mg ritonavir BID.

There were ten serious adverse events (SAEs) reported in four patients. Besides the adverse events of the three patients who were hospitalized due to DLTs, no other SAEs were considered to be possibly, probably or definitely related to the study drug (Table 3). All SAEs related to treatment resolved after discontinuation of study treatment.

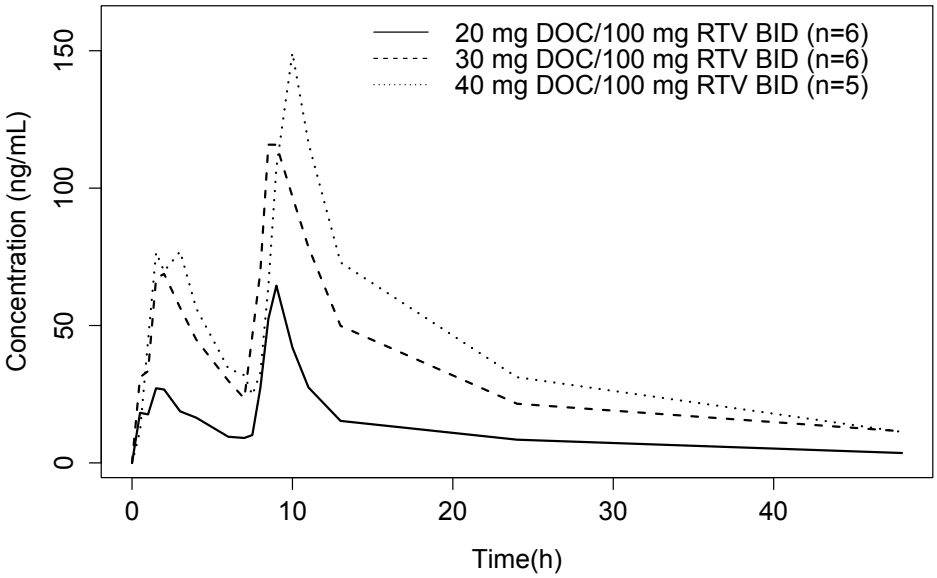

Figure 3 Mean plasma concentration-time curves of docetaxel in patients after administration of ModraDoc001 10 mg capsules. (Abbreviations: DOC = docetaxel, RTV = ritonavir)

Pharmacokinetics

The PK of docetaxel and ritonavir were monitored during day 1 and 15 of the study. In four patients, PK was measured only on the first day, because these patients went off study before the second PK assessment. One patient at dose-level 40 mg docetaxel and 100 mg ritonavir BID received a dose reduction before the second PK assessment. The information of the second PK assessment was therefore added to the PK characteristics of the third cycle of dose-level 20 mg docetaxel and 100 mg ritonavir BID.

Mean plasma concentration–time curves composed for oral docetaxel are shown in Figure 3 and the results of the non-compartmental PK analysis are shown in Table 4. Docetaxel exhibited a bi-exponential PK profile. The mean C_{max} of docetaxel was reached at 2.2 hours (CV 69%) after the first administration and at 2.9 hours (CV 16%) after the second administration, independently of the dose. The mean terminal half-life of docetaxel was 15.6 hours (CV 52%), also independently of the docetaxel dose. The C_{max} and systemic exposure (expressed as AUC_{inf}) to docetaxel increased with higher dose. The relation between the AUC_{inf} and the docetaxel dose is shown in Figure 4. The mean AUC_{inf} of docetaxel at the MTD (n=6) was 0.77 µg•h/mL (CV 57%).

The mean plasma concentration–time curve composed for ritonavir is shown in Figure 5 and the results of the non-compartmental PK analysis are shown in Table 4. The mean C_{max} of ritonavir was reached at 3.0 and 3.4 hours after the first and second dose of 100 mg ritonavir, respectively. The PK profile showed a monophasic decline. The C_{max} of ritonavir

Table 4 Summary statistics for pharmacokinetic parameters of docetaxel and ritonavir in cycle 1 and 3. The data are shown as mean values and coefficient of variation (%).

	Docetaxel						Ritonavir	
Dose-level (docetaxel/ritonavir)	**20/100**		**30/100**		**40/100**		**All**	
Cycle	1	3	1	3	1	3	1	3
N	6	6	6	4	5	3	17	13
AUC_{inf} (µg•h/mL)	0.77	0.8	1.78	2.02	2.04	0.67	24.22	28.63
CV (%)	57%	47%	59%	54%	96%	20%	66%	74%
$C_{max,1}$ (ng/mL)	36	55	76	112	102	22	836	1035
CV(%)	62%	62%	58%	40%	95%	56%	56%	95%
$T_{max,1}$ (h)	2.0	1.9	2.6	1.4	2.4	4.0	3.0	2.5
CV (%)	52%	58%	65%	46%	45%	50%	59%	53%
$C_{max,2}$ (ng/mL)	78	82	150	174	151	29	2152	2132
CV (%)	79%	60%	53%	17%	112%	58%	83%	65%
$T_{max,2}$ (h)	2.0	2.9	2.1	1.9	3.2	4.3	3.4	4.2
CV (%)	6%	16%	11%	3%	4%	13%	18%	21%
T_{half} (h)	13.1	12.4	16.9	17.8	13.5	25.0	9.8	11.5
CV (%)	14%	24%	30%	23%	33%	96%	49%	100%
N_{WSV}		5		4		3		13
WSV AUC_{inf} (%)		40%		58%		30%		48%
WSV $C_{max,1}$ (%)		41%		120%		37%		37%
WSV $C_{max,2}$ (%)		76%		19%		44%		41%

(Abbreviations: AUC_{inf} = the area under the plasma concentration-time curve with extrapolation to infinity, C_{max} = maximum observed plasma concentration, T_{max} = time to reach C_{max}, T_{half} = the terminal elimination half-life, N: number of evaluable patients per dose-level, WSV: within subject variation, NWSV: number of evaluable patients per dose-level that underwent PK assessments of the same regimen twice)

after the second administration is more than two-fold higher than the C_{max} of ritonavir after the first administration (Table 4).

Anti-tumor activity

All patients had measurable disease at baseline. Five patients were not evaluable for anti-tumor activity, because they went off study before the first radiological evaluation. Reasons leading to an early end of treatment were the previously described DLTs, infection and anorexia. Out of the remaining patients, four patients had stable disease (SD) and eight patients had progressive disease (PD) as their best overall response. The primary tumor types of the patients experiencing SD were NSCLC (n=3) and colorectal cancer (n=1). The median duration of SD was 14 weeks (range, 8-19 weeks). There were no patients with a complete or partial tumor response.

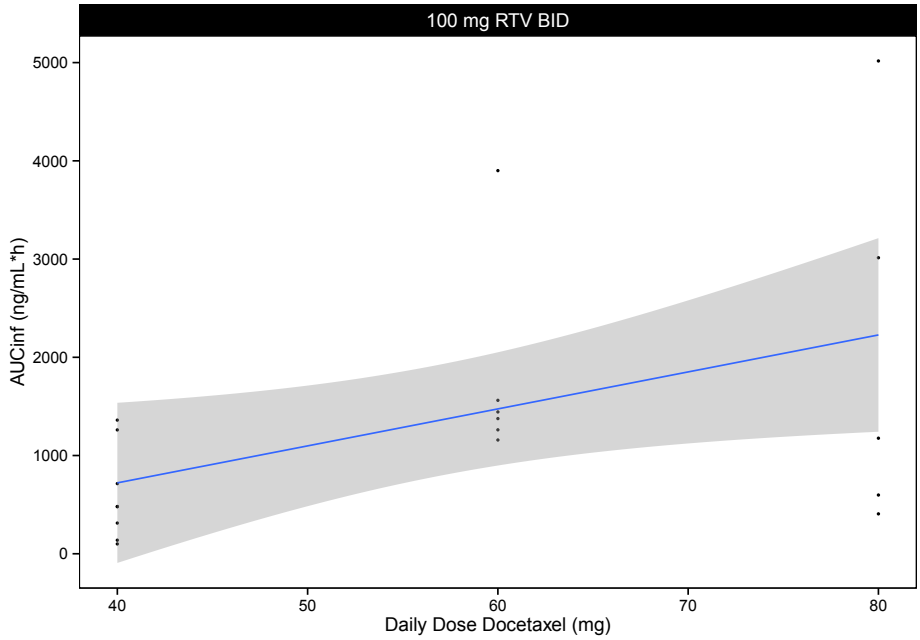

Figure 4 Dose-exposure curves of docetaxel in plasma in patients after administration of oral docetaxel (as ModraDoc001 10 mg capsules) in combination with 100 mg ritonavir once-weekly BID in the first week of treatment. The line represents the linear regression with 95% confidence interval.

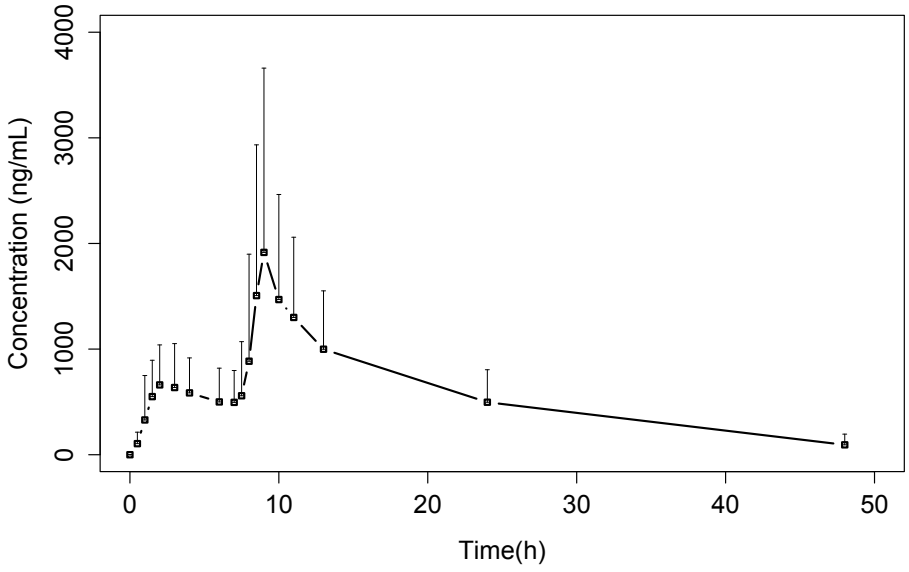

Figure 5 Plasma concentration-time curve of ritonavir in patients after administration of 100 mg ritonavir BID (n=17). The data are shown as mean values (symbols) with SD (error bars).

Discussion

This report describes a dose escalation study of once-weekly BID oral docetaxel with a novel capsule formulation denoted ModraDoc001, in combination with ritonavir. In this study, three dose-levels were evaluated, ranging from 20 mg to 40 mg docetaxel and 100 mg ritonavir administered BID in a weekly schedule.

For most patients, BID administration of weekly docetaxel as ModraDoc001 in combination with ritonavir was well tolerated. Most frequently reported treatment related adverse events were fatigue, nausea and diarrhea, independently of the administered dose of docetaxel. This toxicity profile is comparable to the toxicity profile that is seen with oral docetaxel and ritonavir in a QD once-weekly daily schedule [18]. Compared to treatment with i.v. administration of docetaxel [21], gastrointestinal disorders seem to occur more often during this study. For diarrhea, the increase in incidence can be a result of high local concentrations in the human enterocytes and/or related to the co-administered ritonavir. Ritonavir is known to induce apoptosis in human intestinal epithelial cells [22], and this could contribute to mild or moderate diarrhea. Non-infectious diarrhea was reported also in HIV patients treated with ritonavir [23]. These events of diarrhea were reversible by prompt management with loperamide. The incidence of commonly reported adverse drug reactions associated with i.v. treatment of docetaxel, like alopecia, allergic reactions, malaise, anemia, leucocytopenia, neutropenia and febrile neutropenia, seems to be lower after oral administration [21]. This is possibly due to the lower C_{max} of docetaxel compared to after i.v. treatment and to the route of administration [24,25]. Also the absence of polysorbate 80 in the oral formulation ModraDoc001 could contribute to this reduction [25,26].

A possible relation was observed between (high) AUC_{inf} and (severe) toxicity. Five patients had a high AUC_{inf} (>1.5 µg•h/mL) during the first or third cycle. Three of the five patients experienced a DLT during these cycles. The other patients with a DLT developed the DLT after the second cycle and therefore without the PK assessment. The remaining patients with a high docetaxel exposure had grade 1 diarrhea, fatigue and taste alteration as most severe related adverse events. One of these patients suffered from chronic urinary tract infection which could partly explain the high exposure as inflammatory reactions are known to reduce the expression of CYP3A [27,28].

PK analysis revealed that over the doses administered in this study, the mean AUC_{inf} increased with the dose. The interpatient variability in AUC_{inf} was wide, especially at the highest dose-level. The variability seems to be higher for oral docetaxel than for docetaxel given as a infusion (CV, 30%-44% [25,29]). A plausible explanation can be that docetaxel precipitated before absorption, because of the limited volume in the intestinal tract. The wide interpatient variability was also seen in other phase I studies conducted with docetaxel as ModraDoc001 capsules and ritonavir in a once-weekly QD schedule [18,30]. A comparison between both schedules for the AUC_{inf} to docetaxel is given in Table 5. After the same daily dose of docetaxel in combination with 100 mg ritonavir, fractionated administration resulted in a higher AUC_{inf} to docetaxel. This was expected, because the results of the dose escalation study with QD schedule showed a non-linearity in the oral PK of docetaxel in combination with 100 mg ritonavir. In this QD study the AUC_{inf} of

docetaxel was increased when co-administered with 200 mg ritonavir QD compared to 100 mg ritonavir QD, possibly due to longer and/or more efficient inhibition of intestinal CYP3A4. This increase was in the same range as the increase observed by BID dosing. Although based on different mechanisms, the mean AUC_{inf} after corresponding daily doses of docetaxel and ritonavir are comparable, regardless of whether the drug is given in a single or multiple dose regimen.

In conclusion, this study demonstrates that ModraDoc001 capsules can be safely administered to patients. The observed MTD was 20 mg docetaxel in combination with 100 mg ritonavir BID in a weekly schedule. The most common drug-related adverse events were nausea, diarrhea and fatigue, the majority being grade 1-2. This study strengthens the concept that docetaxel can be given orally and that fractionated administration is an appropriate option to further increase and prolong the systemic exposure to docetaxel.

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 study was registered under identifier NCT01173913 (NIH register). 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 have patents for oral taxane formulations. The other authors declare that they have no conflict of interest.

Table 5 Summary statistics for the exposure of docetaxel (as ModraDoc001 10 mg capsules) after different doses of ritonavir and different regimes.

Daily docetaxel dose (mg)	Daily ritonavir dose (mg)	Schedule	AUC_{inf} (µg•h/mL)	CV	Ref
40	200	BID	0.77	57%	
40	100	QD	0.33	44%	[18]
40	200	QD	0.73	30%	[30]
60	200	BID	1.78	59%	
60	100	QD	1.08	69%	[18]
60	200	QD	1.79	80%	[18]
80	200	BID	2.04	96%	
80	100	QD	1.57	50%	[18]
80	200	QD	2.00	48%	[18]

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Pharmacokinetic evaluation of three oral formulations of docetaxel boosted with ritonavir: two single drug formulations versus a fixed-dose combination tablet

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Abstract

The ability to deliver the potent anticancer agent docetaxel via the oral route may enable the development of promising new treatment regimens with reduced toxicity, increased efficacy, and increased patient convenience. Recently, we were able to overcome the low oral bioavailability of docetaxel by concomitant administration of the pharmacokinetic booster ritonavir and the design of an oral solid dispersion formulation of docetaxel (ModraDoc001 10 mg capsule).

Further research lead to the development of a docetaxel tablet (ModraDoc003 10 mg tablet) and a fixed-dose combination (FDC) tablet of docetaxel and ritonavir (ModraDoc004 10/50 mg tablet). In this clinical proof-of-concept study the exposure to docetaxel and ritonavir was compared between the single agent formulations and the FDC tablet. Six evaluable patients received 40 mg docetaxel and 200 mg of ritonavir once a week according to a cross-over design.

No significant differences were found in the exposure to docetaxel and ritonavir between the single agent formulations and the FDC tablet. There was however a tendency towards a higher exposure to docetaxel after the administration of the FDC tablet, which could be an effect of the simultaneous release of docetaxel and ritonavir in the gastrointestinal tract. The FDC tablet of docetaxel and ritonavir is a pharmaceutically and clinically feasibly option in the development of patient convenient oral anticancer therapy with docetaxel.

Introduction

Docetaxel is a highly effective anticancer agent used in the treatment of various solid tumors [1] . Due to its cytostatic mechanism of action docetaxel could be even more effective when administered chronically to patients [2, 3]. However, because of its low oral bioavailability, docetaxel is currently only available as an intravenous infusion formulation, which makes chronic administration impractical, patient unfriendly, and expensive. Hence, for the successful implementation of oral docetaxel chemotherapy an oral formulation of docetaxel is needed that overcomes the low oral bioavailability of docetaxel.

Due to its low solubility and low permeability docetaxel is classified as a class IV drug according to the biopharmaceutical classification system [4]; its oral bioavailability is estimated to be less than 10% [3, 5]. The very low permeability of docetaxel can be mainly attributed to extensive metabolism by CYP3A4 enzymes in the gut wall and liver, and partly to active excretion by P-glycoprotein pumps [6]. We have shown that the oral bioavailability of a docetaxel micellar solution could be increased by concomitant administration of the strong CYP3A4 inhibitor ritonavir [7].

Recently, we increased the oral bioavailability of docetaxel by combining the pharmacokinetic booster ritonavir with the ModraDoc001 10 mg capsule, a newly developed solid dispersion formulation of docetaxel [8]. Subsequent studies with this new formulation confirmed that CYP3A4 inhibition in the liver and gut wall was primarily responsible for the increase in docetaxel exposure; and showed that 200 mg ritonavir led to a higher exposure to docetaxel compared to 100 mg of ritonavir [9]. The ModraDoc001 10 mg capsule contains a freeze-dried solid dispersion powder of docetaxel, polyvinylpyrrolidone K30 (PVP-K30), and sodium lauryl sulphate (SLS), in a weight ratio of 1/9/1 w/w/w (ModraDoc001 SD powder) [8]. To date the ModraDoc001 10 mg capsule has been administered in combination with ritonavir to more than 40 patients in a phase I dose escalation study [10].

Special formulations such as micellar solutions and solid dispersions can increase the pharmaceutical availability of docetaxel. However, over time these formulations cannot prevent docetaxel precipitation or degradation in the gastrointestinal tract [8, 11, 12]. It is therefore essential that the pharmacokinetic booster ritonavir is present to promote rapid absorption of the dissolved docetaxel before the onset of degradation or precipitation [7]. To date we were not fully able to fulfill this prerequisite, given the available docetaxel and ritonavir formulations. Immediately after oral administration, the docetaxel micellar solution will be available for absorption while the hard capsule shell of the ritonavir formulation will first have to be penetrated before ritonavir can be released. Indeed, administration of the ritonavir formulation 60 minutes prior to administration of the docetaxel micellar solution showed a tendency towards a higher exposure to docetaxel compared to simultaneous administration of both formulations [7]. Moreover, both theory and practice show that the timing and site of the ritonavir release relative to the docetaxel release influence the absorption of docetaxel. Consequently, docetaxel and ritonavir have to be released at the same time at the same site to achieve optimal pharmacokinetic boosting of docetaxel.

Recently, the manufacturing of spray dried solid dispersion powder enabled us to develop two tablet formulations of docetaxel: the ModraDoc003 10 mg tablet and the ModraDoc004 10/50 mg tablet. The ModraDoc003 10 mg tablet is the equivalent of the ModraDoc001 10 mg capsule except for the spray drying of the intermediate product. The ModraDoc004 10/50 mg tablet is a fixed-dose combination (FDC) tablet and contains 10 mg of docetaxel and 50 mg of ritonavir.

The FDC tablet of docetaxel and ritonavir has potentially several advantages over single agent formulations. First of all, patient convenience and adherence is likely to improve due to the reduced number of dosage units and the simplified dosing schedule [13]. Secondly, the FDC tablet eliminates the possibility that docetaxel is administered without its pharmacokinetic booster ritonavir. Finally, simultaneous release of docetaxel and ritonavir could probably increase the exposure to docetaxel and decrease its variability.

The aim of this study was to evaluate the in vitro and in vivo performance of three oral formulations of docetaxel boosted with ritonavir: two single drug formulations of docetaxel, the ModraDoc001 10 mg capsule and the ModraDoc003 10 mg tablet, versus the FDC tablet of docetaxel and ritonavir, the ModraDoc004 10/50 mg tablet.

Patients, Materials and Methods

Materials

Docetaxel anhydrate was obtained from Scinopharm Taiwan (Tainan, Taiwan). Ritonavir was purchased from LGM Pharma (Boca Raton, FL, USA). Povinylpyrrolidone K30 (PVP-K30) was supplied by BASF (Ludwigshafen, Germany). Pharmacopoeial grade absolute ethanol, Tert-butanol (TBA), sodium lauryl sulphate (SLS) and dimethyl sulfoxide (DMSO) were purchased from VWR (Amsterdam, The Netherlands). Water for Injection (WfI) was obtained from B. Braun (Melsungen, Germany). Lactose 200M and colloidal silicon dioxide were supplied by Spruyt Hillen (IJsselstein, The Netherlands). Granulated lactose (modified lactose monohydrate, SUPERTAB) was obtained from DMV-Fonterra Excipients (Veghel, the Netherlands). Hard gelatin capsules were purchased from Capsugel (Bornem, Belgium). Polyoxyethylene 10-lauryl ether was obtained from Sigma Aldrich Chemie B.V. (Zwijndrecht, The Netherlands).

Preparation of docetaxel and ritonavir formulations

The ModraDoc001 10 mg capsule contained a freeze-dried solid dispersion powder: ModraDoc001 SD powder. ModraDoc001 SD powder consisted of docetaxel, PVP-K30 and SLS in a weight ratio of 1:9:1 *w/w/w*. Preparation of the ModraDoc001 SD powder was done by freeze-drying. All solid dispersion components were accurately weighed and dissolved in TBA/WfI mixtures (60:40 *v/v*); the concentration of docetaxel in TBA was 10 mg/mL. The resulting solution was transferred to stainless steel boxes (Gastronorm size 1/9), placed in a freeze-dryer (Lyovac GT4, GEA Lyophil GmbH, Hürth Germany) and freeze-dried according to a method described earlier [14]. The freezing phase started with a freezing ramp from ambient temperature to -35°C in 1 hour followed by a holding step of 2 hours at -35°C. Primary drying was performed at -35°C and 0.2 mbar for 45 hours. Secondary drying started with a heating ramp from -35°C to 25°C at 0.2 mbar in 15 hours

followed by a holding step at 25°C and 0.2 mbar for 3 hours. The ModraDoc001 10 mg capsule contained lactose monohydrate 200 M, colloidal silicon dioxide, and an amount of ModraDoc001 SD powder equivalent to 10 mg of docetaxel. ModraDoc001 SD powder and capsule excipients were accurately weighed and gently grinded with mortar and pestle. Encapsulation into size 0 hard gelatin capsules was performed on a manual capsulation apparatus (Feton International NV, Brussels, Belgium).

The ModraDoc003 10 mg tablet contained a spray dried solid dispersion powder: ModraDoc003 SD powder. ModraDoc003 SD powder consisted of docetaxel, PVP-K30 and SLS in a weight ratio of 1:9:1 *w/w/w*. Preparation of ModraDoc003 SD powder done by spray drying. All solid dispersion components were accurately weighed and dissolved in a 75/25 *v/v* ethanol/Wfl mixture. The resulting solution was spray dried with a B290 mini spray dryer connected to a B-296 dehumidifier and a B-295 inert loop (Büchi Labortechnik AG, Flawil, Switzerland). A standard nozzle with an inner tip diameter of 0.7 mm and an outer tip diameter of 1.5 mm was used. Inlet temperature was set at 150 °C, N2 gas flow rate was set at 35 arbitrary units, aspirator flow rate was set at 100%, and product feed rate was set at 30%. The ModraDoc003 10 mg tablet contained granulated lactose, colloidal silicon dioxide, magnesium stearate, and an amount of ModraDoc003 SD powder equivalent to 10 mg of docetaxel. ModraDoc003 SD powder and the tablet excipients were accurately weighed in a 3 L stainless steel bin and mixed in a Turbula mixer T10B operating at the highest mixing speed (Willy A Bachofen AG Maschinenfabrik, Muttenz, Switzerland). Tablet powder was manually compacted on an EK 0 eccentric press (Korsch AG, Berlin, Germany) equipped with 9 mm tooling.

The ModraDoc004 10/50 mg tablet contained a spray dried solid dispersion powder: ModraDoc004 SD powder. ModraDoc004 SD powder consisted of the active ingredients, PVP-K30 and SLS in a weight ratio of 1/9/1 *w/w/w*; the active ingredients consisted of docetaxel and ritonavir in a weight ratio of 1/5 *w/w*. Preparation of the ModraDoc004 SD powder was equivalent to the preparation of the ModraDoc003 SD powder. The ModraDoc004 10/50 mg tablet contained granulated lactose, colloidal silicon dioxide, magnesium stearate, and an amount of ModraDoc004 SD powder equivalent to 10 mg of docetaxel and 50 mg of ritonavir. Preparation of the ModraDoc004 10/50 mg tablet was equivalent to the ModraDoc003 10 mg tablet except for the use of 14 mm tooling.

Ritonavir (NORVIR) 100 mg soft gelatin capsules and ritonavir (NORVIR) 100 mg tablets originated from Abbott Laboratories (Abbott park, Illinois, USA) [15] .

In vitro evaluation of docetaxel and ritonavir formulations

All formulations used in the clinical study, i.e. ModraDoc001 10 mg capsules, ModraDoc003 10 mg tablets, ModraDoc004 10/50 mg tablets, ritonavir (NORVIR) 100 mg capsules, and ritonavir (NORVIR) 100 mg tablets, were subjected to a dissolution test adapted from the USP monograph of lopinavir and ritonavir tablets [16]. Briefly, 500 mL of a 37.7 g/L polyoxyethylene-10-lauryl ether solution in Wfl was heated to 37°C and transferred to a type 2 (paddle) dissolution apparatus (Erweka, Heusenstamm, Germany). The paddle was operated at 75 RPM and each formulation was tested in triplicate. Samples of 1.0

mL were withdrawn at 0 (baseline), 5, 10, 15, 30, 60, 120, 180, and 240 minutes after the dosage form was added to the medium. All samples were filtrated with a 0.45 µm filter (Millex HV, Millipore) and subsequently analyzed on an adapted RP-HPLC-UV system originally described by Huizing et al. [17]. Of each sample 20 µL was injected on an APEX octyl analytical HPLC column (150 x 4.6 mm ID; particle size 5 µm; Grace Discovery Sciences, Deerfield, IL, United States). Eluens was a mixture of 1:4:5 *v/v/v* methanol/acetonitrile/0.02M ammonium acetate buffer at pH 5; run time was 20 minutes at a flow of 1.0 mL/min. Docetaxel was detected at 227 nm and ritonavir was detected at 210 nm.

Patient population

Patients with a histological or cytological proof of cancer refractory to current therapies who might benefit from treatment with docetaxel were eligible for the study. Other eligibility criteria included: Age > 18 years; life expectancy > 3 months; no radio- or chemotherapy within the last 4 weeks prior to study entry, however limited radiation for pain reduction as palliative treatment was allowed. Patients had to have a World Health Organization (WHO) performance status < 2, and adequate hematological, renal and hepatic function. Patients were not eligible if they suffered from uncontrolled infectious disease, neurologic disease, bowel obstructions, or symptomatic brain metastases. Other exclusion criteria included concomitant use of known P-glycoprotein or CYP3A4 inhibitors, and chronic use of H_2-receptor antagonists or proton pump inhibitors. The medical ethics committee of the Netherlands Cancer Institute approved the study protocol and all patients gave written informed consent prior to start of the study. The study was registered in the NIH registry www.clinicaltrials.gov under identifier NCT01173913.

Study Design

This study was designed as an open label, pharmacokinetic proof of concept study. In the first three weeks of the study patients received all three docetaxel formulations according to a cross-over design. Upon entering the study, patients were randomly assigned to one of the six possible treatment sequences of the cross-over design. Pharmacokinetic monitoring was performed for all tested formulations during the first three weeks of the study. After completion of the pharmacokinetic part of the study treatment was continued until the patient no longer had clinical benefit, e.g. progressive disease, or if toxicity led to patient withdrawal.

In the first three weeks patients received once a week 40 mg docetaxel concomitantly with 200 mg ritonavir. After completion of the pharmacokinetic part of the study patients received 80 mg docetaxel in combination with 100 mg ritonavir once a week.

Drug composition and administration

Study drugs were administered on an empty stomach; patients fasted at least 2 hours before drug administration, and at least 1 hour after drug administration. Docetaxel was administered simultaneously with ritonavir; patients received approximately 150 mL of tap water after administration of the study drugs. In the first three weeks, pre-treatment consisted of 1 mg granisetron (p.o.) 1 hour prior to administration of the study drugs. Docetaxel was administered as ModraDoc004 10/50 mg tablet, as ModraDoc001 10 mg

capsule or as ModraDoc003 10 mg tablet. Ritonavir was administered as ModraDoc004 10/50 mg tablets, as ritonavir 100 mg capsule, or as ritonavir 100 mg tablet. After completion of the pharmacokinetic part of the study pre-treatment was not specified in the protocol and was provided on an individual basis. Docetaxel was administered as ModraDoc001 10 mg capsule; ritonavir was administered as ritonavir 100 mg capsule or as ritonavir 100 mg tablet

Safety
All observed toxicities were graded according to the National Cancer Institute Common Toxicity Criteria for Adverse Events (NCI CTCAE) version 3.0 [18].

Sample collection and analysis
Blood samples were drawn in lithium heparinized tubes at baseline, and at 0.5, 1, 1.5, 2, 3, 4, 6, 8, 10, and 24 hrs after administration of the study formulations. Samples were immediately placed on ice and were centrifuged within 1 hour at 1500 *g* for 10 minutes at 4°C. Plasma was stored at or below -20°C until analysis.

Docetaxel and ritonavir were quantified in plasma by use of high-performance liquid chromatography with tandem mass spectrometric detection (LC-MS/MS) with labeled analogues as internal standards as described by Hendrikx et al. [19]. Briefly, compounds were extracted from 200 µL human plasma using tertiair-butylmethylether; the solution was subsequently dried and the residue was reconstituted in a 1:1 *v/v* Wfl/methanol mixture. Of each sample 25 µL was injected onto a Zorbax Etend C18 column (150 x 2.1 mm ID; particle size 5 µm; Agilent Technologies, Amstelveen, The Netherlands) protected with an inline filter (0.5 µm). Eluens was a mixture of 7:3 *v/v* methanol/10 mM ammonium hydroxide in Wfl; run time was 9 minutes at a flow of 0.3 mL/min; column temperature was set at 35 °C, and autosampler temperature was maintained at 4 °C. Compounds were detected using positive ionization electrospray tandem mass spectrometry. The lower limit of quantification of the assay was 0.5 ng/mL for docetaxel and 2 ng/mL for ritonavir.

Pharmacokinetic and statistical analysis
The individual pharmacokinetic parameters were analyzed using descriptive non-compartmental pharmacokinetic methods and validated R scripts (R version 2.10.0) [20]. The areas under the plasma concentration-time curves to the last quantifiable sample point (AUC_{0-t}) were estimated by the linear trapezoidal (absorption phase) and logarithmic trapezoidal rule (elimination phase). The areas under the plasma concentration-time curves to infinite time (AUC_{0-Inf}) were calculated by extrapolation. The observed maximum plasma concentration (C_{max}), the time to the maximum plasma concentration (T_{max}), the elimination half-life ($t_{1/2}$), and mean residence time (MRT) were reported. Pharmacokinetic parameters were compared visually and statistically with paired t-tests on the natural-log transformed values of AUC_{0-Inf} and C_{max}. To support the design of future bioequivalence studies the bioequivalence ratios for C_{max} and AUC_{0-Inf} were calculated according to the current EMA guideline [21].

Results

In vitro performance of docetaxel and ritonavir formulations

Figure 1 presents the initial dissolution profiles of the docetaxel and ritonavir formulations. The release rate of docetaxel from the ModraDoc001 10 mg capsule was the highest (Q=75% ~ 10 minutes); the lowest release rate of docetaxel was from the ModraDoc004 10/50 mg tablet (Q=75% ~ 30 minutes). The release rate of ritonavir was lowest from the 100 mg tablet (Q=75% >90 minutes); the highest release rate of ritonavir was from the 100 mg capsule (Q=75% <20 minutes). The release rates of docetaxel and ritonavir from the ModraDoc004 10/50 mg tablet were equal; within 60 minutes both docetaxel and ritonavir were completely released from the ModraDoc004 10/50 mg tablet.

Patient characteristics

In total nine patients were included in the study, all of them had metastatic disease. Patient characteristics are listed in Table 1. One patient (patient 3) developed vomiting in the first week, 30 minutes after administration of the study drugs; the patient was therefore excluded from the pharmacokinetic part of the study and continued treatment with 80 mg of docetaxel and 100 mg of ritonavir. Two patients (patient 4 and 5) went off study due to neutropenia (patient 4) and progressive disease (patient 5) before completion of the pharmacokinetic part of the study. Six patients (patients: 1, 2, 6, 7, 8, and 9) completed the pharmacokinetic part of the study and were therefore evaluable.

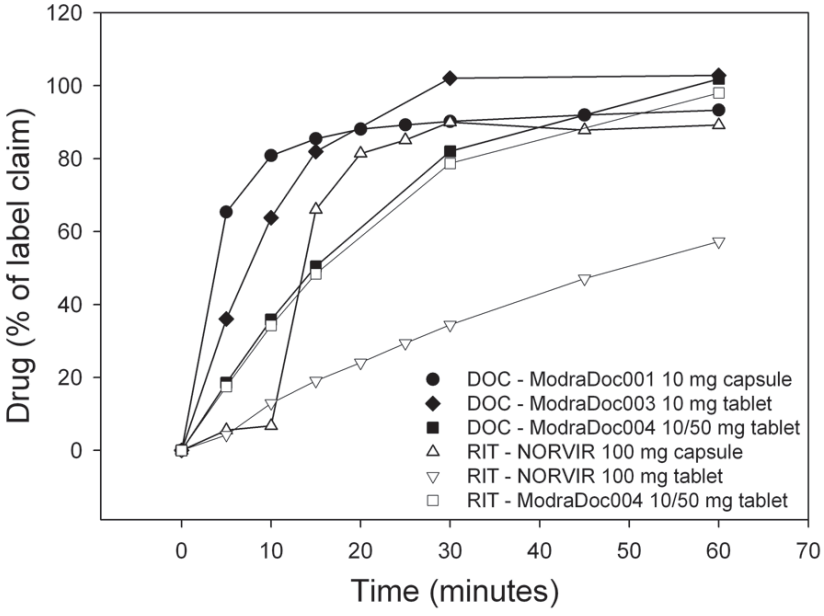

Figure 1 In vitro dissolution profiles of docetaxel (DOC; closed figures) and ritonavir (RIT; open figures). USP Type II (Paddle) dissolution apparatus, 500 mL 37.7 g/L polyoxyetyle-10-laurylether in WfI, 37°C, 75 RPM (n=3). Docetaxel release is highest from the ModraDoc001 10 mg capsule (Q=75% ~ 10 minutes) and lowest from the ModraDoc004 10/50 mg tablet (Q=75% ~30 minutes). Ritonavir release is highest from the ritonavir 100 mg capsule (Q=75% < 20 minutes) and lowest from the ritonavir 100 mg tablet (Q=75% > 90 minutes). The ModraDoc004 10/50 mg tablet has equal release rates for docetaxel and ritonavir.

Table 1 Patient characteristics

Parameter	N
Number of patients:	
Total (evaluable)	9 (6)
Sex:	
Male	5
Female	4
Age:	
median (range)	52 (47-72)
WHO status:	
0	3
1	4
2	2
Pathological diagnosis:	
NSCLC	4
UCC	2
Mamma	1
Ewing sarcoma	1
Oesophageal	1
Prior treatment:	
Chemotherapy	9
Radiotherapy	6
Surgery	4

(Abbreviations: NSCLC = non-small cell lung cancer, UCC = urothelial cell carcinoma)

Pharmacokinetic and statistical analysis

Figure 2 depicts the plasma pharmacokinetic profiles of docetaxel (Fig. 2a) and ritonavir (Fig. 2b.) after treatment with the docetaxel and ritonavir formulations. Table 2 lists for each treatment the characteristic pharmacokinetic parameters of docetaxel and ritonavir: T_{max}, C_{max}, $AUC_{0\text{-}Inf}$, $t_{1/2}$, and MRT. Paired t-tests revealed no significant differences in the pharmacokinetic parameters of docetaxel between the ModraDoc001 10 mg capsule, the ModraDoc003 10 mg tablet and the ModraDoc004 10/50 mg tablet, nor were there any significant differences in the pharmacokinetic parameters of ritonavir between the ritonavir 100 mg capsule and the ModraDoc004 10/50 mg tablet.

Table 3 lists the results of the bioequivalence evaluation of the tested docetaxel and ritonavir formulations. The analysis of variance revealed no significant period or sequence effects for any treatment. The point estimates for C_{max} and $AUC_{0\text{-}Inf}$ of docetaxel of the ModraDoc004 10/50 mg tablet were respectively 32% and 39% higher when compared

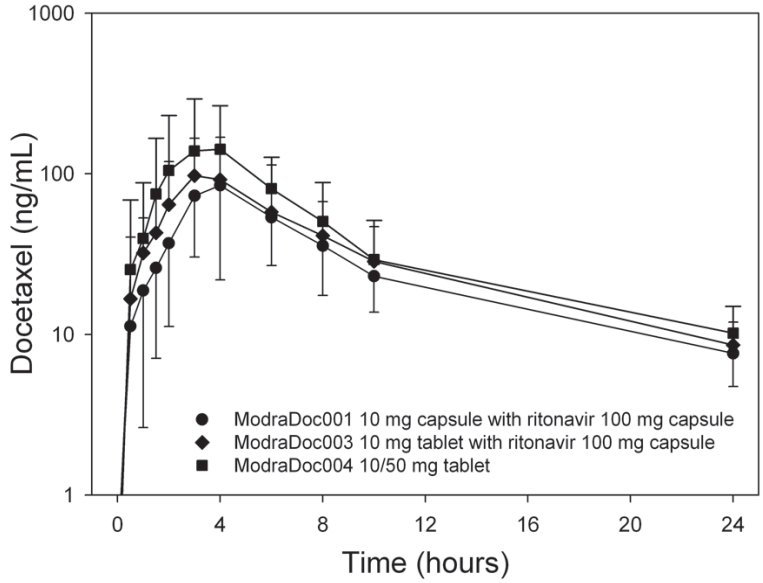

Figure 2a Log plasma concentration of docetaxel vs. time curves of 40 mg docetaxel (p.o.) administered concomitantly with 200 mg ritonavir (p.o.) (n=6, mean and SD).There is no significant difference in the exposure to docetaxel between the single drug formulations (ModraDoc001 10 mg capsule and ModraDoc003 10 mg tablet) and the fixed-dose combination tablet (ModraDoc004 10/50 mg tablet).

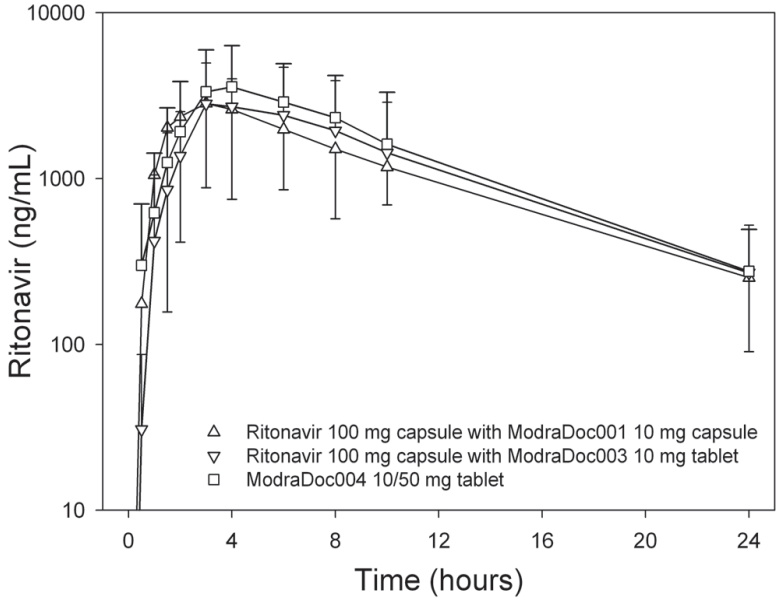

Figure 2b Log plasma concentration of ritonavir vs. time curves of 200 mg ritonavir (p.o.) administered concomitantly with 40 mg docetaxel (p.o.) (n=6, mean and SD). There is no significant difference in the exposure to ritonavir between the single drug formulation (ritonavir 100 mg capsule) and the fixed-dose combination tablet (ModraDoc004 10/50 mg tablet).

Table 2 Pharmacokinetic parameters of docetaxel and ritonavir formulations after oral administration

Docetaxel	**ModraDoc001 10 mg capsule[a]**	**ModraDoc003 10 mg tablet[a]**	**ModraDoc004 10/50 mg tablet[a]**
T_{max} (h)	4.2 ± 1.6 (38%)	5.3 ± 2.9 (55%)	3.7 ± 1.4 (37%)
C_{max} (ng/mL)	107 ± 50 (47%)	115 ± 74 (64%)	161 ± 143 (89%)
$AUC_{0\text{-}inf}$ (ng · h/mL)	731 ± 223 (30%)	882 ± 437 (50%)	1144 ± 864 (76%)
$t_{1/2}$ (h)	8.0 ± 2.3 (29%)	8.3 ± 3.4 (42%)	8.1 ± 3.3 (41%)
MRT (h)	11.8 ± 1.9 (16%)	12.6 ± 3.4 (27%)	11.4 ± 3.2 (28%)
Ritonavir	**Ritonavir 100 mg capsule[a,b]**	**Ritonavir 100 mg capsule[a]**	**ModraDoc004 10/50 mg tablet[a]**
T_{max} (h)	4.2 ± 2.9 (70%)	3.6 ± 1.5 (42%)	4.0 ± 1.1 (27%)
C_{max} (ng/mL)	3383 ± 1901 (56%)	3957 ± 2328 (59%)	3813 ± 2582 (68%)
$AUC_{0\text{-}inf}$ (ng · h/mL)	29122 ± 13761 (47%)	30488 ± 21971 (72%)	35815 ± 29385 (82%)
$t_{1/2}$ (h)	6.8 ± 3.1 (46%)	5.7 ± 1.1 (19%)	5.3 ± 1.3 (24%)
MRT (h)	11.3 ± 4.2 (37%)	10 ± 2.2 (22%)	9.9 ± 2.3 (23%)

[a] Values are means ± standard deviation and coefficients of variation (%) of 6 patients; [b] Two patients received ritonavir 100 mg tablets instead of ritonavir 100 mg capsules; T_{max}, time at which the maximum plasma concentration is reached; C_{max}, maximum plasma concentration; $AUC_{0\text{-}Inf}$, area under the plasma concentration time curve from 0h to infinite time; $t_{1/2}$, elimination half-life; MRT, mean residence time.

to the ModraDoc001 10 mg capsules, and 48% and 30% higher when compared to the ModraDoc003 10 mg tablet. The point estimate for C_{max} and $AUC_{0\text{-}Inf}$ of ritonavir were 4% and 14% higher for the ModraDoc004 10/50 mg tablet when compared to the ritonavir 100 mg capsule.

Safety evaluation

Oral docetaxel was overall well tolerated. The most common adverse event which are possibly, probably or definitely related to the study drug, were nausea (78%), diarrhea (78%) and fatigue (67%), the majority being grade 1-2. Two patients experienced a drug-related grade 3 toxicity. In both patients the adverse event was fatigue which occurred after completion of the pharmacokinetic part of the study, i.e. during treatment with 80 mg of docetaxel and 100 mg of ritonavir. One of the patients experiencing grade 3 fatigue had already experienced grade 1 fatigue before start of the study.

Discussion

To obtain information about the in vivo release rates of docetaxel and ritonavir all formulations were subjected to an in vitro dissolution test. The in vitro dissolution test revealed clear differences between the capsule and tablet formulations (Fig.1). The tablet formulations exhibited a gradual release rate of the active substance, which is a typical release profile for eroding tablets. In contrast, the capsule formulations initially had a very limited dissolution rate followed by a burst release of the active substance upon penetration of the capsule shell. During the burst release approximately 60% of the active substance was released within 5 minutes. The difference in capsule shell material is probably responsible for the difference in in vitro release rates of docetaxel and ritonavir from the ModraDoc001 10 mg capsule and the ritonavir 100 mg capsule (Fig.1).

Table 3 Statistical bioequivalence evaluation of docetaxel and ritonavir formulations

	ModraDoc003 10 mg tablet[3] vs. ModraDoc001 10 mg capsule[3] (docetaxel)	ModraDoc004 10/50 mg tablet[3] vs. ModraDoc001 10 mg capsule[3] (docetaxel)	ModraDoc004 10/50 mg tablet[3] vs. ModraDoc003 10 mg tablet[3] (docetaxel)	ModraDoc004 10/50 mg tablet[3] vs. Ritonavir 100 mg capsule[4] (ritonavir)
C_{max} [1,2]	0.89 (0.39 - 2.04)	1.32 (0.59 - 2.95)	1.48 (0.64 - 3.44)	1.04 (0.83 - 1.31)
$AUC_{0\text{-}Inf}$ [1,2]	1.07 (0.6 - 1.92)	1.39 (0.95 - 2.02)	1.30 (0.72 - 2.34)	1.14 (0.93 - 1.41)

[1]Values are the differences of the geometric mean and their 90% confidence intervals; [2]C_{max} and $AUC_{0\text{-}Inf}$ are considered bioequivalent when the 90% confidence interval of the difference of the geometric means falls within the 0.80 to 1.25 bioequivalence interval; [3]n=6; [4]Total n=12 of which n=10 ritonavir 100 mg capsules and n=2 ritonavir 100 mg tablets

More importantly, the in vitro release rates of docetaxel from the ModraDoc001 10 mg capsule and the ModraDoc003 10 mg tablet were higher compared to the in vitro release rates of ritonavir from the ritonavir 100 mg capsule and the ritonavir 100 mg tablet (Fig.1). This could indicate that in vivo docetaxel is released prior to ritonavir, which could lead to precipitation or degradation of docetaxel [8, 11, 12] resulting in a lower amount of docetaxel absorbed. In contrast, the ModraDoc004 10/50 mg tablet released docetaxel and ritonavir in vitro simultaneously (Fig.1); this is an indication that the ModraDoc004 10/50 mg tablet will release docetaxel and ritonavir in vivo at the same time as well.

Because the ritonavir 100 mg capsule ran out of stock before all patients had completed the pharmacokinetic part of the study, we were forced to use the ritonavir 100 mg tablet in combination with the ModraDoc001 10 mg capsule for patient 8 and 9. Unfortunately, use of the ritonavir 100 mg tablet under fasting conditions could significantly increase the $AUC_{0\text{-}Inf}$ (>40%) and C_{max} (>65%) of ritonavir compared to the use of ritonavir 100 mg capsule [22]. However, patient 8 and 9 did receive the ritonavir 100 mg capsule in combination with the ModraDoc003 10 mg tablet. This enabled us to assess the impact of changing the ritonavir formulation on the individual pharmacokinetic parameters of ritonavir. Because for both patients there were no significant differences in the $AUC_{0\text{-}Inf}$ and C_{max} of ritonavir between both ritonavir formulations, we included the results of the ritonavir 100 mg tablet and the ModraDoc001 10 mg capsule in the pharmacokinetic and statistical analysis.

For all formulations, the values of the pharmacokinetic parameters of docetaxel (Fig. 2a and Table 2) were comparable to the values established in the Phase I dose escalation study [10]. The exposures to ritonavir ($AUC_{0\text{-}Inf}$) after administration of the ritonavir 100 mg capsule (Fig. 2b and Table 2) were higher and more variable than the exposure to ritonavir reported in the Summary of Product Characteristics (SPC) of NORVIR [15]. The differences between the values reported in the SPC and our values could very well be due to the low number of patients in our study. Because ritonavir is primarily responsible for the oral bioavailability of docetaxel [7, 23] the high variability in the C_{max} and $AUC_{0\text{-}Inf}$ of docetaxel can to a large part be attributed to the high variability of the pharmacokinetic parameters of ritonavir (Table 2).

By calculating the exposure ratio of docetaxel and ritonavir the variability in the exposure to the boosted drug caused by the variability of the booster drug is removed. The ModraDoc001 10 mg capsule had a mean docetaxel/ritonavir exposure ratio of 0.028 ± 0.0074 (27%), the ModraDoc003 10 mg tablet had a mean docetaxel/ritonavir exposure ratio of 0.031 ± 0.0089 (29%), and the ModraDoc004 10/50 mg tablet had a mean docetaxel/ritonavir exposure ratio of 0.034 ± 0.0061 (18%). For all formulations the variability in the exposure ratio is considerably lower than the variability in the exposures to docetaxel. This result strengthens the hypothesis that most of the exposure variability of docetaxel is caused by ritonavir. Furthermore, the tendency towards a higher and less variable docetaxel/ritonavir exposure ratio for the ModraDoc004 10/50 mg tablet might indicate that the FDC tablet is the most effective formulation.

Although not significant, the exposures to docetaxel and ritonavir were higher after administration of the ModraDoc004 10/50 mg tablet compared to the other two treatment regimens (Figure 2 and Table 2). This difference was primarily caused by patient no. 2 who had a remarkably high exposure to docetaxel ($AUC_{0\text{-}Inf}$ 2881 ng · h/mL) and ritonavir ($AUC_{0\text{-}Inf}$ 94165 ng · h/mL) compared to the other patients. These high exposures coincided with the occurrence of pneumonia in patient no. 2 and the subsequent treatment with amoxicillin and clavulanic acid. It is not likely that amoxicillin or clavulanic acid caused the increased exposure, because there are no indications that these drugs act on the CYP3A4 enzyme system or on P-glycoprotein pumps [24, 25]. However, there is strong evidence that patients with an acute inflammatory reaction have reduced expression of the CYP3A4 enzyme system [26, 27]. Moreover, the average exposure to ritonavir in patient no. 2 was considerable higher compared to the average exposure to ritonavir in the other: 72390 ± 21913 ng · h/mL vs. 23692 ± 8189 ng · h/mL. Furthermore, excluding patient no. 2 from the dataset would lower the mean exposure and inter-patient variability of docetaxel and ritonavir and would decrease the differences in exposure between the formulations. In addition to this, without patient no. 2 the exposures to ritonavir for both the ritonavir 100 mg capsule and the ModraDoc004 10/50 mg tablet would be in line with the exposure to ritonavir reported in the SPC of NORVIR [15]. In conclusion, most likely the occurrence of pneumonia in patient no. 2 led to a decreased activity of the patients CYP3A4 enzyme system which caused a decreased clearance of ritonavir and finally resulted in an increased exposure to ritonavir and docetaxel.

As this study was not designed to assess the bioequivalence of the docetaxel and ritonavir formulations it was expected that the low number of patients would cause the 90% confidence intervals to be outside the bioequivalence limits (Table 3). The higher point estimates for the C_{max} and $AUC_{0\text{-}Inf}$ of ritonavir of the ModraDoc004 10/50 mg tablet are probably a combined effect of the solid dispersion formulation and administration of the formulations under fasting conditions. Bioequivalence studies between solid dispersion and liquid filled capsule formulations of ritonavir (NORVIR and KALETRA) have shown an increased exposure to ritonavir after administration of solid dispersion formulations, especially under fasting conditions [22, 28]. The higher point estimates for the C_{max} and $AUC_{0\text{-}Inf}$ of docetaxel of the ModraDoc004 10/50 mg tablet might be the result of the higher exposure to ritonavir and the simultaneous release of docetaxel and ritonavir throughout the gastrointestinal tract. Excluding patient no. 2 from the bioequivalence calculations decreased the differences between the docetaxel and ritonavir formulations, although the point estimate for the $AUC_{0\text{-}Inf}$ of docetaxel of the ModraDoc004 10/50 mg tablet remained 19% higher compared to the ModraDoc001 10 mg capsule and the ModraDoc003 10 mg tablet.

Conclusions

In this study we have shown that the single drug formulations of docetaxel, the ModraDoc003 10 mg tablet and the ModraDoc001 10 mg capsule, gave a comparable exposure to docetaxel after oral administration in combination with ritonavir. Furthermore we have shown that the FDC tablet of docetaxel and ritonavir, the ModraDoc004 10/50 mg tablet, gave exposures to docetaxel and ritonavir comparable to single drug formulations.

In addition to this, we found a tendency towards a higher and less variable docetaxel/ritonavir exposure ratio for the FDC tablet; this is probably the result of a simultaneous release of docetaxel and ritonavir in the gastrointestinal tract. We have now presented three promising oral formulations of docetaxel to be further investigated in clinical phase I, II and III trials.

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2.4

Relationship between systemic exposure to docetaxel and severe intestinal toxicity after oral docetaxel administration in mice and patients

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Abstract

Oral administration of docetaxel in combination with CYP3A4 and P-glycoprotein boosters is used in clinical trials to improve oral bioavailability of docetaxel. The most common and dose-limiting toxicity of oral docetaxel was diarrhea. This study combined preclinical and clinical data and focused on incidence, severity and cause of oral docetaxel induced diarrhea.

We examined intestinal toxicity in mice lacking Cyp3a and mice lacking both Cyp3a and P-glycoprotein after oral and intraperitoneal administration of docetaxel. Data were compared with results from clinical studies conducted in humans who received docetaxel orally.

Intestinal toxicity in mice was similar after oral and intraperitoneal administration of docetaxel and included severe degeneration of large and small intestinal mucosa. This indicated that intestinal toxicity by docetaxel was caused by systemic exposure to docetaxel rather than by a direct local effect of docetaxel. In human, severity and onset of diarrhea was used as parameter for intestinal toxicity. Plasma exposure to docetaxel was higher in patients that suffered from diarrhea than in patients without diarrhea. Higher plasma exposure tended to be associated with increased severity of diarrhea. The administered dose and maximal observed plasma concentrations were not higher in patients with diarrhea than in patients without diarrhea.

Our data indicate that the onset of severe diarrhea after oral co-administration of docetaxel in humans is probably caused by the level of docetaxel in the systemic blood circulation. Severe diarrhea after oral docetaxel is reversible and is not related to the route of administration of docetaxel.

Introduction

Docetaxel (Taxotere®) is a semi-synthetic derivative of a paclitaxel analogue originally obtained from the Taxus baccata and currently used as an anticancer agent in several types of cancer, such as non-small cell lung cancer (NSCLC), breast, gastric, prostate and head and neck cancer [1]. Docetaxel undergoes complex detoxification, involving both ABC drug transporters and drug-metabolizing enzymes, which results in low bioavailability after oral administration [2]. In vivo, both P-glycoprotein (P-gp/ABCB1/MDR1) and Cytochrome P450 (CYP) 3A are involved in the pharmacokinetics (PK) of docetaxel by decreasing exposure to docetaxel [3,4]. Due to the poor water solubility of docetaxel and its handling by P-gp and CYP3A, the oral bioavailability of docetaxel is limited and several studies have explored the usefulness of enhancers in combination with oral formulations to increase the bioavailability [5]. One of the applied boosting agents is the CYP3A inhibitor ritonavir (Norvir®). Low doses of ritonavir are widely used as a booster to increase the bioavailability of protease inhibitors in HIV therapy [6]. Similarly, a low dose of ritonavir was shown to enhance the systemic exposure to oral docetaxel resulting in an apparent bioavailability of approximately 60% [7].

These results encouraged us to perform a phase I dose escalation study to determine the feasibility of this concept with oral docetaxel in combination with ritonavir [8]. This study started with docetaxel as a drinking solution and during the study denoted ModraDoc001 10 mg capsules, containing docetaxel as a solid dispersion without the use of polysorbate 80, were introduced [9]. Oral docetaxel in combination with either 100 mg or 200 mg ritonavir, however, resulted in a modified toxicity profile of docetaxel compared to its intravenous administration. The most common and dose-limiting toxicity of oral docetaxel was diarrhea, while the most common treatment-related adverse events after intravenous administration of docetaxel were alopecia, anemia, leucocytopenia and neutropenia [8,10]. This increase of intestinal toxicity after oral administration of docetaxel was also seen in another dose escalation study with oral docetaxel and ritonavir and in several proof of principle studies with oral docetaxel boosted by one of each of the CYP3A inhibitors ritonavir, ketoconazole, grapefruit juice and clarithromycin [11].

Similarly, severe intestinal toxicity in mice was observed after oral administration of 10 mg/kg docetaxel in mice lacking murine P-gp (Mdr1a/b P-gp) and Cyp3a [12]. Pathological examination of these mice revealed degeneration and necrosis of the intestinal mucosa three days after docetaxel administration.

Damage to the intestinal mucosa can lead to an imbalance between absorption and secretion of fluids leading to diarrhea. This damage could be an effect of mitotic arrest of intestinal crypt cells caused by exposure to chemotherapeutic agents in the systemic circulation as observed after administration of 5-flourouracil [13], or it can be a direct local effect of intestinal luminal drug on the intestinal or colonic epithelium as it is believed to be the case after irinotecan administration [14,15]. In the case of the applied oral docetaxel formulation, both clinical and preclinical data suggest intestinal toxicity by oral docetaxel as major etiology. Therefore it is important to understand the mechanism behind the development of this toxicity and possible measures to prevent it.

In this study, we examined mice lacking Cyp3a and mice lacking both Cyp3a and Mdr1a/b P-gp, in order to mimic the clinical conditions wherein Cyp3a and possibly P-gp are inhibited. Clinical data of two phase I trials with oral docetaxel were selected to investigate the severity and the duration of intestinal toxicity after oral administration of the drug. Our study was aimed to elucidate 1) whether the intestinal toxicity caused by docetaxel is a direct local effect or related to the docetaxel concentration in the systemic circulation, and 2) whether the intestinal toxicity is related to the amount of docetaxel present in the lumen of the gastrointestinal tract. The data obtained from the mouse experiments were compared to the data derived from clinical studies with orally administered docetaxel.

Materials and Methods

Drugs and chemicals

Docetaxel and ritonavir for mice studies were purchased from Sequoia Research Products (Oxford, UK). Drug-free lithium-heparinized human plasma was obtained from Bioreclamation LLC (New York, NY, USA). All other chemicals were of analytical grade and obtained from commercial sources.

In patient studies, docetaxel was administered to patients as drinking solution (i.v. formulation, Taxotere®, Rhone-Poulenc Rorer/Aventis) or capsules (ModraDoc001 10 mg capsules, Department of Pharmacy & Pharmacology, Slotervaart Hospital/The Netherlands Cancer Institute). CYP3A inhibitors employed were ritonavir (Norvir®; Abbott, Illinois, USA), ketoconazole (Nizoral®; Janssen-Cilag, Tilburg, The Netherlands), grapefruit juice (Coolbest® pink grapefruit juice: Royal Friesland Foods N.V. Meppel, The Netherlands) and clarithromycin (Klacid®; Abbott, Illinois, USA).

Animals

Mice were housed and handled according to institutional guidelines complying with Dutch legislation. Mice were kept in a temperature-controlled environment with a 12-hr light / 12-hr dark cycle and received a standard diet (AM-II, Hope Farms, Woerden, The Netherlands) and acidified water ad libitum. Strains used in this study were Cyp3a knockout (Cyp3a$^{-/-}$) [16] and combined Cyp3a and Mdr1a/b P-gp knockout mice (Cyp3a/Mdr1a/b$^{-/-}$) [12]. All strains had a >99% FVB genetic background. In all experiments, male mice of 8-14 weeks of age were used. For dose-finding experiments 4-5 mice per group were used and toxicity was determined in 6-9 mice per group.

Docetaxel plasma pharmacokinetics for mice studies

Prior to the experiments, stock solutions containing 1, 3, 6, 9 and 36 mg/mL docetaxel in ethanol:polysorbate 80 (1:1, *v/v*) were prepared and stored at -20°C. On the day of the experiments stock solutions were diluted with water to obtain solutions containing various concentrations of docetaxel in ethanol:polysorbate 80:water (1:1:10, *v/v*). Animals were fasted 2 hours before oral drug administration to minimize variation in absorption. Docetaxel was administered orally or intraperitoneally at various doses using a total volume of 10 µL per kg of body weight. Oral administration was performed by gavage into the stomach using a blunt-ended needle. Intraperitoneal administration was performed by injection into the peritoneal cavity. Multiple blood samples (~50 µL) were collected

from the tail vein at 15 and 30 minutes and 1, 2, 4, 8 and 24 hours after administration, using heparinized capillary tubes (Oxford Labware, St. Louis, MO). Blood samples were centrifuged at ambient temperature at 8,000 *g* for 5 minutes and subsequently plasma was collected. All samples were stored at -20°C until analysis.

Histological analysis for mice studies

Three days after oral or intraperitoneal administration of docetaxel to mice, total body necropsy was performed and tissues and organs were fixed in EAF fixative (ethanol/acetic acid/formaldehyde/saline at 40:5:10:45 v/v) and embedded in paraffin. Sections were cut at 2 µm from the paraffin blocks and stained with hematoxylin and eosin (HE) according to standard procedures. The sections were reviewed with a Zeiss Axioskop2 Plus microscope (Carl Zeiss Microscopy, Jena, Germany) equipped with Plan-Apochroma and Plan-Neofluar objectives. Images were captured with a Zeiss AxioCam HRc digital camera and processed with AxioVision 4 software (both from Carl Zeiss Vision, Munich, Germany).

Clinical trials

The PK data in humans were obtained from two clinical studies which have been extensively described elsewhere [8,11]. Briefly, the first study was a phase I study with weekly once daily oral docetaxel in combination with different CYP3A inhibitors. This study included 73 patients in several cohorts, including a dose escalation cohort with ritonavir and proof of principle cohorts with ritonavir, ketoconazole, grapefruit juice and clarithromycin. In the dose escalating cohort of the study docetaxel was administered as drinking solution in the first dose level (30 mg docetaxel, n=5) and as ModraDoc001 capsules (n=43) in the other dose levels. The once weekly doses of the other dose levels were 40, 60 and 80 mg docetaxel in combination with 100 mg or 200 mg ritonavir. Patients received the treatment until progressive disease or until unacceptable toxicity despite dose reduction. In additional cohorts, a total of 25 patients received 30 mg docetaxel as ModraDoc001 capsules in combination with 100 mg ritonavir or another CYP3A inhibitor in a cross-over design (ketoconazole, grapefruit juice and claritromycin). Patients continued in the subsequent weeks with 30 mg or 40 mg docetaxel as drinking solution (n=6) or as ModraDoc001 capsules (n=17) in combination with 100 mg RTV until progressive disease or adverse events were observed, that required dose modifications or discontinuation of therapy.

The second study was a dose escalation study with oral docetaxel (as ModraDoc001 capsules) in combination with ritonavir with a comparable design as the dose escalation cohort in the first study (chapter 2.2). In this second study both docetaxel and ritonavir were given once a week in a bi-daily schedule. This study included 17 patients treated at three dose levels. The once weekly doses were 40, 60 and 80 mg docetaxel as ModraDoc001 capsules and 200 mg ritonavir. The demographic and baseline characteristics of the patients and treatment schedules of the different studies and cohorts are described in table 1.

In all studies patients were fasted two hours before and one hour after oral drug administration to minimize variation in absorption. Optional pre-treatment consisted of 1

mg granisetron orally one hour prior to treatment. The adverse events were determined using the National Cancer Institute's Common Terminology Criteria for AEs criteria (NCI-CTCAE v3.0). All clinical studies were approved by the Medical Ethics Committee of the Netherlands Cancer Institute and written informed consent was obtained from all patients prior to study entry. The studies were registered under identifier NCT01173913 (NIH register) and under identifier ISRCTN32770468 (ISRCTN register).

The PK of docetaxel were monitored according to various schedules in the different cohorts and studies during the first 24 or 48 hours (table 1). Blood samples were collected in heparinized tubes and centrifuged at 4°C at 1500 *g* for 10 minutes. Subsequently, plasma was collected and stored at -20°C until the time of analysis.

Patients

The clinical studies had similar inclusion and exclusion criteria. Patients were eligible if they had a histological or cytological proof of cancer, if there were no standard treatment options available and if docetaxel treatment was considered appropriate. Other inclusion criteria were age ≥ 18 years, performance status of 0, 1 or 2 according to the WHO Performance Status (PS) scale, life expectancy longer than 3 months, and adequate bone marrow, hematological and biological functions (neutrophil count of ≥ 1.5 * 10^9/L and platelets of ≥ 100 * 10^9/L; alanine transaminase (ALT) and aspartate transaminase (AST) ≤ 2.5 times institutional upper limit of normal (ULN), bilirubin of ≤ 1.5 times the ULN; serum creatinine ≤ 1.5 times the ULN or creatinine clearance ≥ 50 mL/min by Cockcroft-Gault formula).

Patients with known alcoholism, drug addiction and/or psychotic disorders were considered not suitable for adequate follow up, and thus excluded. Patients were not allowed to concomitantly use P-gp and CYP3A modulating drugs, H_2-receptor antagonists or proton pump inhibitors. Other exclusion criteria were uncontrolled infectious disease, bowel obstructions that might influence drug absorption, neurologic disease, pre-existing neuropathy higher than grade 1, symptomatic cerebral or leptomeningeal metastases, pregnancy, breastfeeding, refusal to use adequate contraception and previous anticancer therapy within 4 weeks prior to the first dose of oral docetaxel.

Drug analysis

Previously developed LC-MS/MS assays were used to quantify docetaxel in plasma samples of mice and humans [17,18]. D9-labelled docetaxel was used as internal standard for docetaxel. Mouse plasma samples of 20 µL were diluted with 180 µL of drug-free human plasma prior to sample pre-treatment. Human plasma was used for dilution of the samples as the concentrations in the undiluted mouse plasma were outside the calibration range and also to mimic the calibration standards that were prepared in human plasma. Sample

Table 1 Patient demographics and study details of two clinical studies used for PK data in humans. The first study was a phase I study with weekly once daily (QD) oral docetaxel in combination with different CYP3A inhibitors, including a dose escalation cohort with ritonavir and proof of principle cohorts with ritonavir, ketoconazole, grapefruit juice and clarithromycin. The second study was a dose escalation study with weekly bi-daily (BID) oral docetaxel in combination with ritonavir. (*= treatment in subsequent weeks after the cross-over phase)

	QD dose escalation		Proof of concept cohorts		BID dose escalation		Total	
Character	**N**	**%**	**N**	**%**	**N**	**%**	**N**	**%**
Number of patients	48		25		17		90	
Sex								
Male - female	28-20		15-10		9-8		52-38	
Age								
Median (range)	59 (36-79)		61 (46-74)		66 (41-77)		60 (36-79)	
WHO status								
0	21	44%	8	32%	8	47%	37	41%
1	22	46%	12	48%	8	47%	42	47%
2	5	10%	5	20%	1	6%	11	12%
Ethnic origin								
Caucasian	44	92%	25	100%	16	94%	85	94%
Asian	2	4%	0	0%	0	0%	2	2%
African Descent	2	4%	0	0%	1	6%	3	3%
Tumor characteristics								
NSCLC	22	46%	6	24%	11	65%	39	43%
UCC	4	8%	6	24%	3	18%	13	14%
Ovary	3	6%	3	12%	1	6%	7	8%
Primary unknown	3	6%	3	12%	0	0%	6	7%
Other	16	34%	7	28%	2	12%	25	28%
Stage of cancer								
Locally advanced	2	4%	0	0%	0	0%	2	2%
Metastatic	46	96%	25	400%	17	100%	88	98%
Prior Treatment								
Chemotherapy	47	98%	25	100%	17	100%	89	99%
Radiotherapy	33	69%	11	44%	9	53%	53	59%
Surgery	26	54%	18	72%	7	41%	51	57%
Dosage form	Drinking solution (n=5) ModraDoc001 (n=43)		Drinking solution (n=6) ModraDoc001 (n=19)*		ModraDoc001 (n=17)			
Daily docetaxel dose	30, 40, 60, 80 mg		30, 40 mg		40, 60, 80 mg			
Daily ritonavir dose	100, 200 mg		100 mg*		200 mg			
Schedule	QD		QD		BID			
PK assessments	Week 1 and 2		Week 1, 2 and 3		Week 1 and 3			
References	[8]		[11]		Chapter 2.2			

pre-treatment of human plasma and diluted mouse plasma was started by adding a small volume of internal standard working solution to the samples. Subsequently, the samples were mixed briefly, tertiary-butyl methyl ether was added and the samples were shaken for 10 minutes at 1250 rpm. The samples were centrifuged at 23,000 g, snap-frozen and the organic layer was collected. After evaporation of the organic layer, the samples were reconstituted with 100 µl reconstitution solvent and an aliquot was injected into the LC-MS/MS system.

Pharmacokinetic calculations and statistical analysis

Pharmacokinetic parameters in mice, including the area under plasma concentration-time curves (AUCs), were calculated using the software package PK Solutions 2.0.2 (SUMMIT, Research Services, Ashland, OH, USA). The individual pharmacokinetic parameters of the patients were analyzed using descriptive non-compartmental pharmacokinetic methods and validated R scripts (R version 2.13.1). The AUCs were estimated by the linear trapezoidal (absorption phase) and logarithmic trapezoidal rule (elimination phase). The areas under the plasma concentration-time curves to infinite time (AUC_{inf}) were calculated by extrapolation. All PK data of the animal and human studies are presented as mean ± SD.

For statistical testing in animal experiments, one-way ANOVA was used when multiple groups were compared and the Bonferroni post-hoc correction was used to accommodate multiple testing. The two-sided unpaired Student's t test was used when treatments or differences between two groups were compared. Data that did not show normal distribution were log-transformed to normalize the distribution of the datasets for statistical comparison. During all statistical analyses in animal experiments, differences in group sizes were considered in the calculations. The Mann-Whitney-Wilcoxon test is used for statistical testing in clinical studies. Differences were considered statistically significant when $P < 0.05$.

Results

Dose finding of docetaxel in mice

In our experiments, the plasma AUC in Cyp3a/Mdr1a/b$^{-/-}$ mice was 27-fold higher than in Cyp3a$^{-/-}$ mice after an oral dose of 10 mg/kg docetaxel (figure 1). Previously, severe toxicity (including intestinal toxicity) was observed three days after single oral administration of 10 mg/kg docetaxel to Cyp3a/Mdr1a/b$^{-/-}$ mice, but no toxicity was observed after administration of the same dose to Cyp3a$^{-/-}$ mice [12]. This difference in toxicity might be caused by differences in docetaxel exposure in enterocytes and plasma between the two strains. To differentiate between the amount of docetaxel present in the intestine and the amount of docetaxel that is absorbed and reaches the systemic circulation within the different strains, various doses of orally administered docetaxel were given, plasma concentrations were measured, and AUCs were calculated. It was observed that an oral dose higher than 60 mg/kg docetaxel in Cyp3a$^{-/-}$ mice did not result in a further increase in AUC compared to a dose of 60 mg/kg (data not shown). This is most likely due to the limited water solubility of docetaxel. Because of the limited volume in the intestinal tract, docetaxel could precipitate and therefore not be absorbed efficiently from the intestinal lumen.

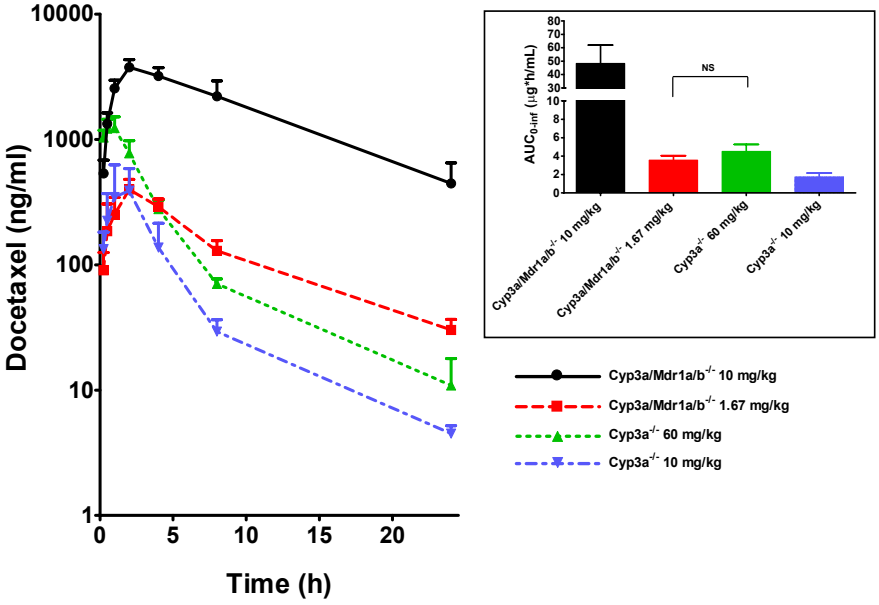

Figure 1 Plasma concentration-time curves obtained after oral administration of docetaxel to Cyp3a$^{-/-}$ and Cyp3a/Mdr1a/b$^{-/-}$ mice. Values represent the mean ± SD. Inset shows the area under the plasma concentration-time curves extrapolated from zero to infinity (AUC_{inf}). All AUCsinf differ mutually significantly ($P < 0.001$) as calculated with ANOVA of the Log-transformed data with Bonferroni's post-hoc test, unless otherwise specified (NS).

A dose of 10 mg/kg orally administered docetaxel in Cyp3a/Mdr1a/b$^{-/-}$ mice resulted in the highest AUC and the highest maximal plasma concentration (C_{max}) used in these experiments (figure 1). In Cyp3a$^{-/-}$ mice, the highest observed AUC was after administration of 60 mg/kg docetaxel. This AUC was comparable to the AUC after administration of 1.67 mg/kg in Cyp3a/Mdr1a/b$^{-/-}$ mice, although the C_{max} was lower in the latter group. Administration of 10 mg/kg in Cyp3a$^{-/-}$ mice resulted in a similar C_{max} as observed after a dose of 1.67 mg/kg in Cyp3a/Mdr1a/b$^{-/-}$ mice . Although the observed C_{max} was similar, administration of 10 mg/kg docetaxel in Cyp3a$^{-/-}$ mice resulted in the lowest AUC used in these experiments. The difference in shape of the plasma concentration-time curve between Cyp3a$^{-/-}$ and Cyp3a/Mdr1a/b$^{-/-}$ mice is caused by the Mdr1a/b P-gp effect on the elimination of docetaxel [4].

Various doses of intraperitoneally administered docetaxel were also tested in both strains. For each strain, we aimed for an AUC that was similar to the highest AUC as obtained after oral administration. This resulted in an intraperitoneal dose of 12 mg/kg docetaxel used for Cyp3a/Mdr1a/b$^{-/-}$ mice and an intraperitoneal dose of 5 mg/kg docetaxel used for Cyp3a$^{-/-}$ mice (figure 2). The difference in oral over intraperitoneal dose ratio between the strains illustrates the impact of intestinal Mdr1a/b P-gp in reducing oral bioavailability of docetaxel.

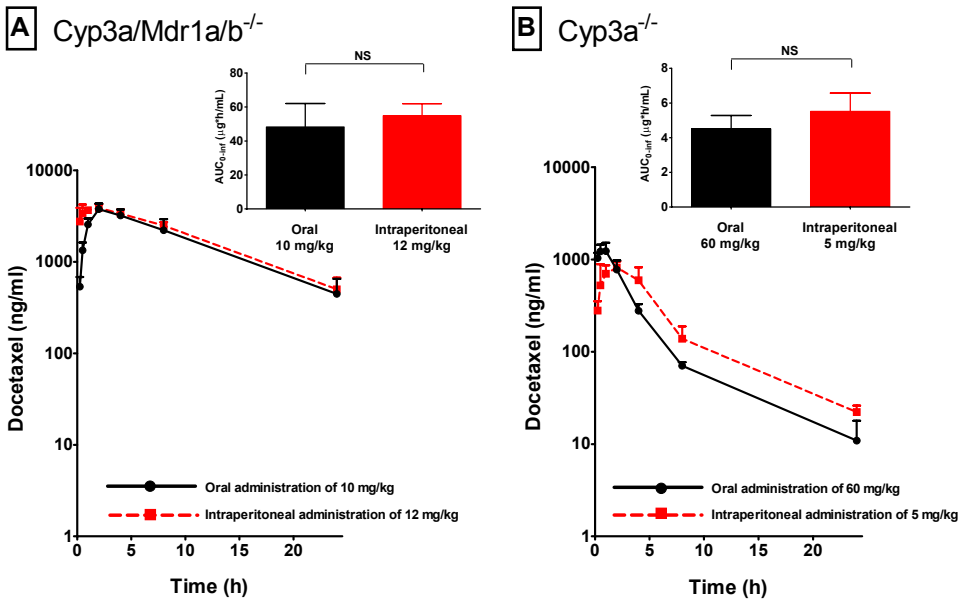

Figure 2 Plasma concentration-time curves after oral or intraperitoneal administration of docetaxel to Cyp3a/Mdr1a/b$^{-/-}$ (panel A) and Cyp3a$^{-/-}$ (panel B) mice. Values represent the mean ± SD. Insets show the area under the plasma concentration-time curves extrapolated from zero to infinity (AUC_{inf}). AUCsinf between oral and intraperitoneal administration do not differ significantly per strain as calculated with a two-sided unpaired Student's t-test.

Toxicity after oral and intraperitoneal administration of docetaxel in mice

For toxicity experiments, docetaxel was administered orally once at doses of 1.67 and 10 mg/kg in Cyp3a/Mdr1a/b$^{-/-}$ mice and at doses of 10 and 60 mg/kg in Cyp3a$^{-/-}$ mice. Plasma AUCs were similar after administration of 1.67 mg/kg docetaxel to Cyp3a/Mdr1a/b$^{-/-}$ mice and 60 mg/kg docetaxel to Cyp3a$^{-/-}$ mice (figure 1). Intraperitoneally administered doses were 12 mg/kg in Cyp3a/Mdr1a/b$^{-/-}$ mice and 5 mg/kg in Cyp3a$^{-/-}$ mice. This resulted in similar plasma AUCs as an oral dose of 10 mg/kg in Cyp3a/Mdr1a/b$^{-/-}$ mice and 60 mg/kg in Cyp3a-.- mice, respectively (figure 1 and 2). The different dosages and administration routes were used to allow comparison of docetaxel toxicity between the strains at similar plasma levels. Pathological examination performed 72 hours after oral administration of 10 mg/kg docetaxel to Cyp3a/Mdr1a/b$^{-/-}$ mice revealed a significant reduction of hematopoietic cells in spleen and bone marrow (see table 2 and figure 3), which did not occur after a low dose of docetaxel (1.67 mg/kg). The intestinal toxicity observed at 10 mg/kg was severe degeneration of the large and small intestinal mucosa with depletion of the crypts and inflammatory infiltrations. This toxicity was found in all mice in this group and was similar to previously observed toxicity after administration of the same dose of oral docetaxel in this strain[12]. After oral administration of the same dose (10 mg/kg docetaxel) to Cyp3a$^{-/-}$ mice no signs of severe toxicity were observed, but the mean AUC in these mice was almost 28-fold lower than in Cyp3a/Mdr1a/b$^{-/-}$ mice after the same dose. Even at the maximum achievable AUC in Cyp3a$^{-/-}$ mice after an oral dose of 60 mg/kg

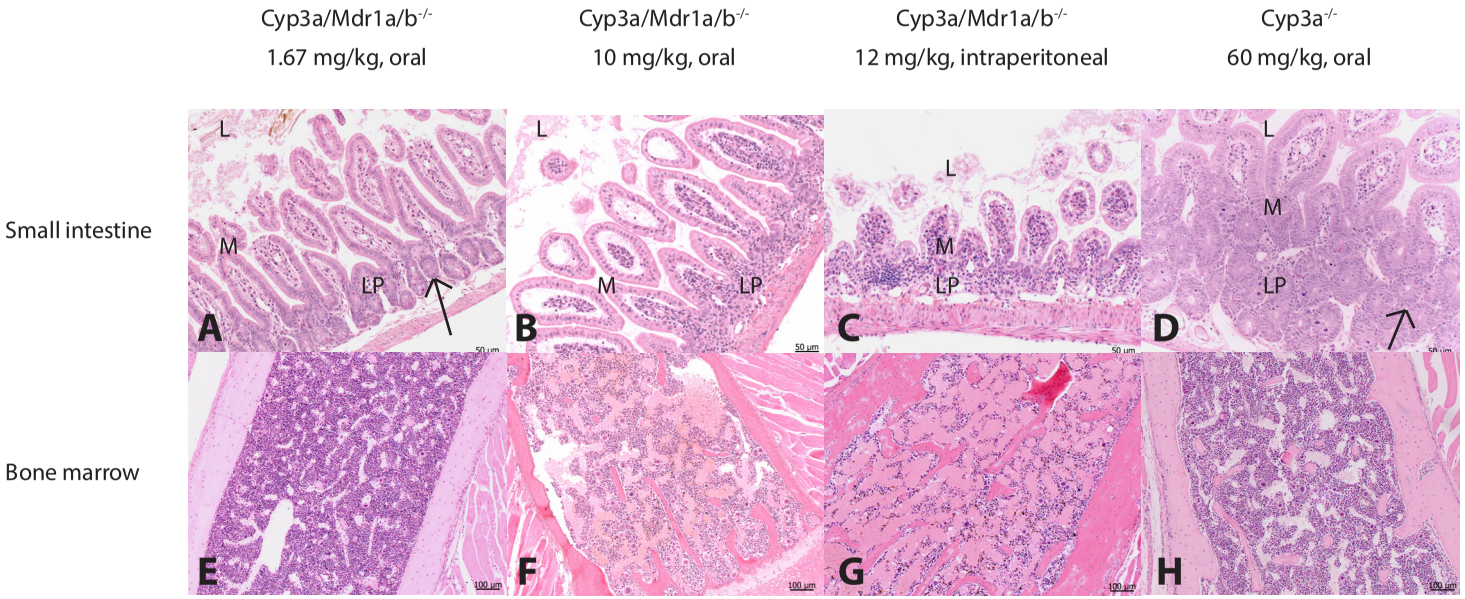

Figure 3 Microphotograph of a typical HE section of the ileum (upper panels, original magnification 20x) and bone marrow (lower panels, original magnification 10x) of Cyp3a/Mdr1a/b$^{-/-}$ and Cyp3a$^{-/-}$ mice after single oral or intraperitoneal administration of docetaxel. Mice were sacrificed for pathological examination 72 hours after docetaxel administration. The Cyp3a/Mdr1a/b$^{-/-}$ mice showed no toxicity after oral administration of 1.67 mg/kg (panel A and E), but showed severe toxicity after oral administration of 10 mg/kg (B and F) or intraperitoneal administration of 12 mg/kg (C and G). The severe toxicity observed in Cyp3a/Mdr1a/b$^{-/-}$ mice was depletion of crypts in small intestine (B and C) and depletion of hematopoietic cells in bone marrows (F and G). Cyp3a$^{-/-}$ mice showed an increase in mitosis and apoptosis in intestinal mucosa, but no changes in bone marrow after oral administration of 60 mg/kg (D and H). Abbreviations: L: intestinal lumen; M: mucosa; LP: lamina propria. Arrows indicate deep crypts.

docetaxel, only mild toxicity in the intestinal cells and spermatogenic cells was observed (table 2). However, the maximum AUC in Cyp3a$^{-/-}$ mice was still 10.7-fold lower than the AUC in Cyp3a/Mdr1a/b$^{-/-}$ mice after oral administration of 10 mg/kg. The observed toxicity in Cyp3a$^{-/-}$ mice after a dose of 60 mg/kg docetaxel was characterized as increased mitosis and apoptosis of cells in the mucosa of the small intestine in four out of nine mice and necrosis of spermatogenic cells in three out of nine mice. Administration of a low oral dose of 1.67 mg/kg docetaxel in Cyp3a/Mdr1a/b$^{-/-}$ mice did not result in signs of toxicity. Lesions and testicular degeneration observed in one testis of one out of six mice were likely to be FVB-strain background pathology. The mean AUC in these mice was comparable to the mean AUC after a dose of 10 mg/kg docetaxel in Cyp3a$^{-/-}$ mice.

In contrast to the diarrhea often observed in patients receiving oral docetaxel, in none of the mice of both strains diarrhea was observed after oral administration of docetaxel. In Cyp3a/Mdr1a/b$^{-/-}$ mice, a loss of body weight was observed after administration of 10 mg/kg docetaxel. The average body weight of these mice was decreased to 88% of the initial body weight in three days. The body weight after three days of all other mouse groups was 99-105% of the initial body weight.

After intraperitoneal administration of 5 mg/kg docetaxel in Cyp3a$^{-/-}$ mice, similar mild toxicity was observed as after oral administration of 60 mg/kg docetaxel to these mice (see table 2). Plasma AUCs and C_{max} values were similar under these conditions (figure 2B). Changes in the mucosa of the small intestine were observed in four out of six mice. Incidentally, depletion of hematopoietic cells in bone marrow and reduced hematopoietic activity in spleen or testicular degeneration were observed in one out of six mice. Strikingly, in Cyp3a/Mdr1a/b$^{-/-}$ mice, toxicity was also similar after intraperitoneal administration of 12 mg/kg docetaxel and oral administration of 10 mg/kg (table 2). Again, plasma AUCs and C_{max} values were similar under these conditions (figure 2A). The toxicity included severe degeneration of intestinal mucosa and depletion of the crypts combined with inflammatory infiltrations. In all mice of both strains, no diarrhea was observed after intraperitoneal administration of docetaxel. The mean body weight after three days was 87% and 95% of the initial body weight in Cyp3a/Mdr1a/b$^{-/-}$ and Cyp3a$^{-/-}$ mice, respectively.

Toxicity after oral administration of docetaxel in humans

To quantify the intestinal toxicity in humans, two phase I studies were selected. By combining these studies, we could compare the diarrhea at multiple daily dose-levels of docetaxel and increase the number of evaluable patients. The results of the studies could be combined, since the inclusion and exclusion criteria of these studies were identical. In the studies the range of the daily doses of docetaxel was 20-80 mg, and the PK parameters of docetaxel were determined in all patients. Therefore, the results in mice could also be compared to the results in patients. In humans the intestinal toxicity was measured by the severity of diarrhea and the number of patients with diarrhea, since no pathologic observations of the intestines were performed during the study.

Out of 90 patients, 12 patients (13%) had suffered from diarrhea, considered unrelated to the study treatment, and 49 patients (54%) had suffered from 150 events of diarrhea

Table 2 Overview of toxicity observed after various doses of docetaxel, administered orally or intraperitoneally to Cyp3a/Mdr1a/b$^{-/-}$ and Cyp3a$^{-/-}$ mice.

	Cyp3a/Mdr1a/b$^{-/-}$ mice	**Cyp3a$^{-/-}$ mice**
IP dose	**Dose:** 12 mg/kg	**Dose:** 5 mg/kg
	AUC_{inf}: 54.9 ± 7.1 µg/mL*h	**AUC_{inf}:** 5.5 ± 1.0 µg/mL*h
	C_{max}: 3880 ± 356 ng/mL	**C_{max}:** 817 ± 137 ng/mL
	Toxicity: severe toxicity.	**Toxicity:** little toxicity.
	Observations: Depletion of crypts in mucosa of intestine and colon, intestinal inflammation, edema in mucosa of colon, depletion of hematopoietic cells in bone marrow, reduced hematopoietic activity in spleen	**Observations:** Increase in mitosis and apoptosis in intestinal mucosa, incidental depletion of hematopoietic cells in bone marrow, incidentally reduced hematopoietic activity in spleen and incidental testicular degeneration.
High oral dose	**Dose:** 10 mg/kg	**Dose:** 60 mg/kg
	AUC_{inf}: 48.3 ± 13.8 µg/mL*h	**AUC_{inf}:** 4.5 ± 0.8 µg/mL*h
	C_{max}: 3766 ± 572 ng/mL	**C_{max}:** 1234 ± 281 ng/mL
	Toxicity: severe toxicity.	**Toxicity:** little toxicity.
	Observations: Depletion of crypts in mucosa of intestine and colon, intestinal inflammation, edema in mucosa of colon, depletion of hematopoietic cells in bone marrow, reduced hematopoietic activity in spleen	**Observations:** Increase in mitosis and apoptosis in intestinal mucosa and necrosis of spermatogenetic cells.
Low oral dose	**Dose:** 1.67 mg/kg	**Dose:** 10 mg/kg
	AUC_{inf}: 3.6 ± 0.5 µg/mL*h	**AUC_{inf}:** 1.7 ± 0.4 µg/mL*h
	C_{max}: 400 ± 80 ng/mL	**C_{max}:** 391 ± 196 ng/mL
	Toxicity: no toxicity.	**Toxicity:** no toxicity.
	Observations: Lesions in testis and testicular degeneration incidentally observed (likely to be FVB-strain background pathology)	**Observations:** No abnormalities detected

considered related to study drug. Patients with an event of unrelated diarrhea were excluded from the analyses. Life-threatening or disabling diarrhea (grade 4) was not seen during the studies. Most of the related events of diarrhea (89%) lasted not more than one week and for most patients (63%) the worst event of diarrhea started within three weeks after the start of treatment. In total 40% of the patients (n=31) had diarrhea up to grade 1, and 13% of the patients (n=10) reported grade 2 diarrhea. For most of this last group of patients (80%) grade 2 diarrhea started during the first two weeks of treatment. In case of grade 1- 2 diarrhea, patients were advised to use loperamide, except on the day of administration since loperamide is metabolized via CYP3A4 [19,20]. No dose interruption neither reduction was implemented. The mean duration of grade 2 diarrhea was eight

days (range, 1-21 days) and in most cases (70%) patients recovered fully from diarrhea after loperamide treatment.

Eight patients (10%) suffered from diarrhea up to grade 3. Grade 3 diarrhea started in all patients during the first two weeks of treatment and one patient had a second event in the sixth week. Seven patients were directly hospitalized and therefore this grade 3 diarrhea was labeled as serious adverse event. During an event of grade 3 diarrhea, loperamide was given and docetaxel was withheld by protocol until recovery to < grade 1 and restarted at a lower dose. The mean duration of grade 3 diarrhea was four days (range, 1-12 days) and in most cases (75%) patients recovered fully from diarrhea after this period. Three patients discontinued treatment, the others continued with a dose reduction.

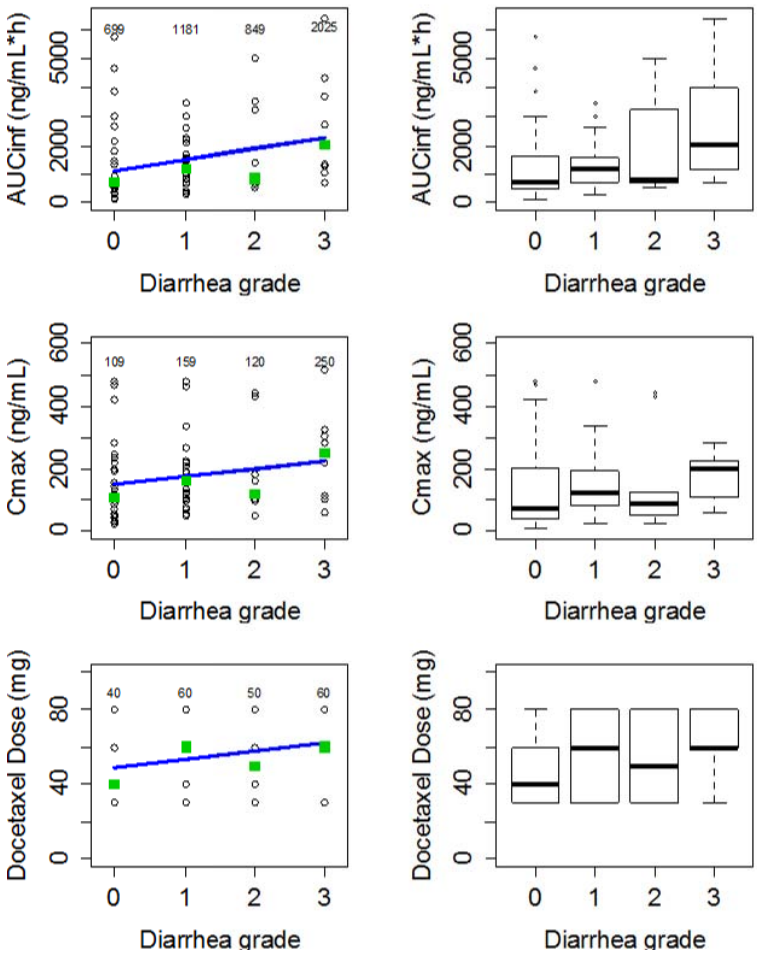

Figure 4 Relationship between the severity of diarrhea after oral administration of docetaxel and AUC of docetaxel, C_{max} and the daily dose given as events and boxplot. Grade 4 diarrhea is not seen in the clinical studies. Text and squares are the median values; line is the linear regression.

Most of the moderate and severe events of diarrhea (grade 2 and 3) occurred during the first three weeks of treatment and therefore the PK characteristics of docetaxel related to these events were known. The highest grade of the diarrhea observed per patient was coupled to the corresponding AUC_{inf}, C_{max} and daily dose. For the diarrhea events without corresponding PK characteristics, the maximum observed AUC_{inf} and the corresponding C_{max} of that patient were used.

In figure 4 the severity of the diarrhea events is plotted against the AUC_{inf}, the corresponding C_{max} and the daily dose. The AUC_{inf} of the patients who suffered from any grade of diarrhea was significantly higher than the AUC_{inf} of patients without diarrhea (Mann-Whitney-Wilcoxon Test, p=0.04). This difference might be underestimated, since the follow up ended within a week after start of treatment in two out of six patients without diarrhea and with an AUC_{inf} above 2 µg/mL*h. These patients died because of progression of disease and therefore events of diarrhea could have been missed in these patients. Another patient was hospitalized in the third week of treatment. This patient had no diarrhea, but other signs of intestinal toxicity, which were considered related to oral docetaxel (grade 3 gastritis and grade 3 duodenal ulcer). There was a tendency towards association of the severity of diarrhea with the AUC_{inf}.

In patients with diarrhea, C_{max} was not statistically significantly higher than in patients without diarrhea. Also the daily administered dose was not higher in patients with diarrhea than in patients without diarrhea. Moreover, it was observed that the median administered daily dose was similar in all patients with diarrhea of any grade. There was no tendency towards association of the administered daily dose or the C_{max} with the severity of diarrhea.

Discussion

A limitation in the treatment with most oral anticancer drugs is the development of gastrointestinal disorders. For several orally administered anticancer drugs, development of diarrhea is the major cause for treatment discontinuation and its severity sometimes represents a dose-limiting toxic event [21]. During development of a novel oral formulation of docetaxel significant diarrhea was encountered. This led to the execution of preclinical studies to unravel the mechanism of this toxicity.

We used mice lacking Cyp3a with and without intact Mdr1a/b P-gp expression to investigate the cause of the intestinal toxicity as observed in patients after oral administration of docetaxel. The Cyp3a deficient mice are used to reflect the co-administration of docetaxel with the CYP3A inhibitor ritonavir in humans. Although human CYP3A has no clear direct murine orthologues [22], there is a broad functional overlap between human CYP3A and murine Cyp3a for the metabolism of docetaxel [16,23]. Human MDR1 function is covered by murine Mdr1a and Mdr1b [24]. Despite these limitations associated with extrapolation of preclinical data, mice lacking Cyp3a with and without functional Mdr1a/b expression might be used as a model for oral co-administration of docetaxel and ritonavir in humans[25].

In our study, Cyp3a/Mdr1a/b$^{-/-}$ mice and Cyp3a $^{-/-}$ mice received high and low doses of oral docetaxel. Each strain also received an intraperitoneal dose which resulted in comparable plasma exposures as the high oral doses. This enabled us to discriminate between local versus systemic exposure in relation to toxicity. Severe intestinal and bone marrow toxicity was observed after the high oral dose in Cyp3a/Mdr1a/b$^{-/-}$ mice (10 mg/kg docetaxel). However, also after intraperitoneal administration of a dose of 12 mg/kg, similarly severe intestinal and bone marrow toxicity was observed in this strain. By intraperitoneal administration of docetaxel, the intestinal uptake step is circumvented. Since both administration routes of docetaxel resulted in comparable plasma AUCs, it is likely that the observed intestinal toxicity is caused by docetaxel in the systemic circulation rather than by a direct effect on the intestinal mucosal cells of docetaxel during absorption from the gut lumen. After both routes of administration, depletion of cells in the deep crypts of the intestine was observed at histological investigation. These crypt cells are not directly involved in drug absorption. The depletion of the deep crypt cells supports the hypothesis that intestinal toxicity is caused by systemic exposure to docetaxel, since similar findings (mitotic arrest and apoptosis in crypts of the mucosa) have been reported in the literature with other systemically applied anticancer drugs. For instance, after intraperitoneal administration of cisplatin, Allan et al observed decreased crypt cell production rates [26], leading to reduced height of the villi and loss of mucosal function.

The toxicity data in humans showed that patients with diarrhea had a higher AUC_{inf} than patients without diarrhea. On the basis of indirect comparison, the overall incidence of treatment-related diarrhea of any grade after i.v. treatment was two-fold lower than at the highest tolerable dose-levels of oral docetaxel in two dose escalation studies [27–33] (table 3). However, the incidence of grade 3/4 diarrhea after oral docetaxel administration was in the same range as after i.v. treatment, indicating that severe diarrhea is most probably caused by docetaxel in the systemic circulation rather than by local exposure in the intestinal tract. This hypothesis is supported by the observation that plasma AUCs in patients after oral administration of these doses of docetaxel were comparable to those after i.v. administration of standard doses of docetaxel [8,34,35]. The higher incidence of mild and moderate diarrhea (grade 1 and 2) after oral administration of docetaxel versus after i.v administration explains the observed two-fold higher overall incidence of diarrhea of any grade after oral administration of docetaxel.

The lack of intestinal toxicity after oral administration of 60 mg/kg docetaxel to Cyp3a$^{-/-}$ mice shows that the absolute amount of docetaxel present in the intestinal lumen is not directly related to the development of toxicity. In Cyp3a/Mdr1a/b$^{-/-}$ mice, an orally administered dose of only 10 mg/kg docetaxel already resulted in severe toxicity including intestinal toxicity. This indicates that docetaxel must be absorbed to cause intestinal toxicity. In Cyp3a$^{-/-}$ mice this absorption is blocked by Mdr1a/b P-gp. In patients, the severity of diarrhea does not appear to be related to the orally administered dose (i.e. amount of docetaxel present in the gastrointestinal tract). Therefore, incomplete absorption of an oral formulation of docetaxel most likely does not increase the risk of severe diarrhea, or of other types of intestinal toxicity.

Our mice data show that a high AUC of docetaxel in the systemic blood circulation is responsible for degeneration of the intestinal mucosa and depletion of the crypts combined with inflammatory infiltrations. Despite the severe changes in the intestinal mucosa, no diarrhea was observed. Based on the body weight loss in mice with severe toxicity, it is possible that the mice did not develop diarrhea, because the mice stopped eating and drinking early after the development of toxicities in the gastrointestinal tract. It is also possible that diarrhea would have developed after the three days used in our study as observed for 5-FU treatment by Wu et al [36]. In humans, death of colonic crypt cells can result in a cascade of effects whereby immature crypt cells release more secretory compounds and thereby cause diarrhea [37]. The damaged colonic crypts are also not able to absorb chloride, the driving force of water absorption in the colon. Degeneration of intestinal mucosa and inflammatory infiltrations can also lead to inflammatory diarrhea [38,39]. This is also seen during colonoscopy and in colon biopsies of patients who had

Table 3 Incidence and severity of diarrhea in various published trials in humans after intravenous administration of docetaxel compared to incidence and severity of diarrhea in humans after oral administration of docetaxel. (abbreviation: NSCLC = non-small cell lung cancer, gr = grade refers to the severity of diarrhea, Ref = reference)

Regime	Dose	Tumor	N	Overall	gr 1 -2	gr 3 - 4	Ref
Intravenous docetaxel, every 3 weeks	80 mg/m^2	Breast	56	30%	30%	0%	[27]
	75 mg/m^2	NSCLC	110	21%	18%	3%	[28]
	75 mg/m^2	Breast	54	37%	26%	11%	[29]
	75 mg/m^2	Prostate	176	46%	44%	2%	[30]
Intravenous docetaxel, every 2 weeks	50 mg/m^2	Prostate	170	37%	36%	1%	[30]
Intravenous docetaxel, every week	33.3 mg/m^2	NSCLC	110	26%	23%	3%	[28]
	30 mg/m^2	Breast	48	45%	27%	8%	[29]
	40 mg/m^2	Breast	20	>30%*	>25%*	5%	[31]
	36 mg/m^2	NSCLC	30	>24%*	>10%*	14%	[32]
	35 mg/m^2	NSCLC	36	9%	3%	6%	[33]
Oral docetaxel**		***	46	62%	56%	6%	

** Grade 1 toxicity was not reported*

*** Daily doses of 60/100, 80/100, 40/200 and 60/200 mg docetaxel and ritonavir, respectively (every week)*

**** Different tumor types, patients were eligible if they were diagnosed with a histological or cytological proof of cancer, if there were no standard curative or palliative treatment options available and if docetaxel treatment was appropriate for further treatment.*

developed docetaxel-induced pseudo-membranous colitis [40,41]. Therefore, it is likely that the onset of severe diarrhea in humans after docetaxel treatment is caused by malfunction of the intestinal tract due to similar structural changes of the intestinal mucosa as observed in mice.

Although these results indicate that severe toxicity in the intestine is caused by the amount of docetaxel in the systemic circulation and not by a direct local effect, the increase of mild and moderate diarrhea (grade 1 and 2) after oral administration of docetaxel is not explained. Unlike severe diarrhea, the incidence of mild and moderate diarrhea in patients after oral co-administration of docetaxel and ritonavir was two-fold higher compared to i.v. treatment with docetaxel. These events of mild and moderate diarrhea after oral administration often occur during the evening after treatment, but also some days later. Since oral administration of docetaxel is only explored in a few clinical studies, limited data is available regarding the pathophysiology of these mild and moderate toxicities. Short-term locally high docetaxel concentrations in the human enterocyte might cause apoptosis of intestinal epithelial cells, although apoptosis of epithelial cells is not observed in our mouse experiments. Cell death in the epithelial cells can cause synthesis of inflammatory cytokines, which eventually can cause mucositis [38,39]. It is also reported that ritonavir can induce apoptosis in human intestinal epithelial cells and thereby decrease barrier function of the epithelial layer [42]. The loss of epithelial cells might cause the observed onset of diarrhea, which is also seen after ritonavir treatment of HIV patients at low doses of 100-400 mg a day [43]. Since the patients in our study received the other CYP3A inhibitors for only one week in the proof of principle cohorts, we could not distinguish between the contribution of docetaxel and ritonavir in the onset of mild and moderate diarrhea. After single oral administration of docetaxel or ritonavir, the intestinal villi could be damaged. This damage could lead to a reduced surface area for absorption resulting in diarrhea via secretory mechanisms [37,43]. The higher incidence of mild and moderate diarrhea after oral co-administration of docetaxel and ritonavir than after i.v. administration of docetaxel can therefore be caused by both ritonavir and docetaxel. These events can be treated by prompt management with loperamide, but should be carefully monitored by the treating physicians [37].

In conclusion, our data indicate that diarrhea upon oral docetaxel administration is not directly related to the amount of docetaxel present in the lumen of the gastrointestinal tract. Moreover, in contrast to mild diarrhea the onset of severe diarrhea after oral co-administration of docetaxel and ritonavir in humans is probably caused by the level of docetaxel in the systemic blood circulation, is reversible and is not related to the route of administration of docetaxel.

Potential conflict of interest

The research group of A.H. Schinkel receives revenue from commercial distribution of some of the mouse strains used in this study. J.H. Beijnen and J.H.M. Schellens have received a grant for translational research (ZonMw code 40-41200-98-004) and are inventors of patents for oral taxane formulations. The other authors declare that they have no conflict of interest.

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chapter 3

Paclitaxel

3.1

A phase I dose escalation study of low dose metronomic chemotherapy with oral paclitaxel (ModraPac001) in combination with ritonavir

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Interim analysis (data were monitored, except for pharmacokinetics and radiological evaluations)

Abstract

Background ModraPac001 is a novel oral capsule formulation containing paclitaxel as a solid dispersion. Oral administration of paclitaxel is feasible in combination with a low dose of the CYP3A4 inhibitor ritonavir enabling low-dose metronomic (LDM) treatment with paclitaxel. The objectives of this study were to determine the feasibility, safety, maximum tolerated dose (MTD) and pharmacokinetics (PK) of continuous bi-daily (BID) administration of LDM paclitaxel as ModraPac001 in combination with ritonavir.

Methods Patients with advanced solid tumors, WHO PS ≤ 2, no concomitant use of P-glycoprotein or CYP3A modulating drugs, adequate bone marrow function and liver and renal function were enrolled. Paclitaxel and ritonavir (Norvir®) were simultaneously administered (BID) in a classical '3+3 cohort' dose escalation design. The MTD was defined as the highest dose resulting in < 1/6 probability of causing a dose limiting toxicity in the first three weeks of treatment. PK was determined on days 1, 2, 8 and 22.

Results A total of 17 patients (76% males) was enrolled in five dose-levels (BID 2.5 mg, 5 mg, 7.5 mg, 10 mg and 15 mg paclitaxel in combination with 100 mg ritonavir, respectively). Common treatment related adverse events were fatigue (53%), diarrhea (29%), nausea (24%) and neuropathy (24%), most being grade 1. None of the patients experienced a dose limiting toxicity. Both drugs were rapidly absorbed after oral administration. The mean C_{max} of paclitaxel was reached at 1.7 hours (CV 40%) after the first administration and at 2.0 hours (CV 6%) after the second administration, independently of the dose. The mean maximum plasma concentration and mean plasma exposure (AUC_{inf}) of paclitaxel increased linear with dose to 19.8 ng/mL (CV 11%) and 198.1 ng/mL*hr (CV 16%), respectively, at 15 mg paclitaxel in combination with 100 mg ritonavir BID. Seven patients had stable disease (SD) with a median duration of ten weeks. There were no patients with an objective tumor response.

Conclusions This is the first study demonstrating that LDM treatment with oral paclitaxel is feasible. The study is still ongoing at the time this report was written and therefore, the MTD was not reached yet. The preliminary results of this study further strengthen the possibility that paclitaxel can be given as a LDM treatment regime.

Introduction

Most of the cytotoxic anticancer drugs are DNA damaging agents or microtubule inhibitors mainly targeting rapidly dividing cells. These drugs do not specifically target tumor cells, but rather interfere with cell division. As a result, most of these agents also damage normal dividing cells, like dividing endothelial cells (EC), which are present in the expanding blood vasculature of the tumor [1]. This effect on the tumor vasculature is observed in various in vitro and in vivo assays and targeting angiogenesis has been considered as a potential opportunity in the treatment of several malignancies [1,2]. Current treatment regimens with cytotoxic drugs usually consist of intermittent treatment with dose-intensive chemotherapy applied at the maximum tolerated dose (MTD). These high doses require a treatment-free period to permit recovery of normal host cells, like rapidly dividing hematopoietic progenitor cells. Similarly, the damage to the vasculature of the tumors has been largely repaired before the next administration and therefore the effect on angiogenesis is only transient [3–6].

An alternative approach is the use of low-dose metronomic chemotherapy (LDM), consisting of chronic administration of cytotoxic drugs at relatively low dose-levels in a continuous schedule of administration [5–7]. Unlike dose-dense therapy, the main targets of the LDM are the dividing EC of the expanding vasculature of a tumor, instead of the proliferating tumor cells. Since the therapeutic effects rely on anti-angiogenic activity, the (cumulative) dose administered may be lower than with dose-dense chemotherapy [5,6]. By targeting the tumor vascular endothelium, LDM is expected to induce tumor vascular damage, which indirectly could result in tumor cell death.

Paclitaxel is an alkaloid ester derived from the Pacific yew (Taxus brevifolia). The drug is commonly used as first line therapy for various tumor types. It functions as a mitotic spindle poison through high-affinity binding to microtubules with enhancement of tubulin polymerization, arresting cells in mitosis [8]. Almost twenty years ago, it was found that paclitaxel has strong anti-angiogenic activity [9,10]. Even at low non-toxic concentrations (<10 nM) proliferation, migration and differentiation of EC were inhibited, while these levels of exposure had no effect on other cell types, such as tumor cells [11,12]. Next to this direct effect on the tumor endothelium, Bocci et al. showed a selective inhibition of human EC proliferation only after a prolonged exposure of ultra-low concentrations of paclitaxel [12]. This delayed effect revealed a second possible, indirect mechanism of action of LDM therapy. The precise molecular and cellular pharmacological mode of action explaining the indirect effect is not yet fully elucidated, but the potential key mediator of the indirect effect appears to be thrombospondin-1 (TSP-1) [13]. TSP-1 is a potent endogenous inhibitor of angiogenesis and acts primarily by binding to EC expressing the CD36 receptor. This interaction blocks the proliferation and selectively induces apoptosis of the EC [14]. Furthermore, TSP-1 can bind to VEGF and displace VEGF from endothelial cells [15], thereby switching the tumor microenvironment to an anti-angiogenic state, inhibiting neovascularization, tumor progression and metastasis. Several animal studies conducted in rats and mice showed that LDM therapy with paclitaxel suppressed different tumor types and metastasis [16–18]. These studies also demonstrated an upregulation of TSP-1 during LDM with paclitaxel, which is not seen when paclitaxel was administered at MTD [16,17].

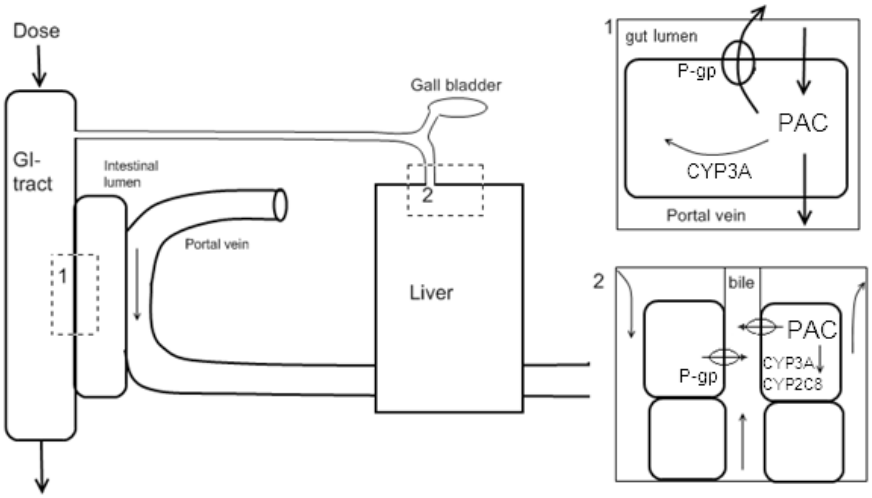

Figure 1 Paclitaxel has limited oral bioavailability because of poor aqueous solubility and high affinity for active ABC drug efflux transporters and, cytochrome P450 3A (CYP3A) drug metabolizing enzymes. Both P-glycoprotein (P-gp) and CYP3A, which are abundantly expressed in the epithelial layer of the gut wall (1) and in the liver (2), contribute to low absorption and significant pre-systemic metabolism of orally applied paclitaxel. Moreover, CYP2C8 metabolizes paclitaxel to the predominant metabolite 6α-hydroxypaclitaxel in the liver (2). The systemic exposure upon oral paclitaxel is increased when co-administered with an inhibitor of P-gp and/or CYP3A. (Abbreviations: PAC = paclitaxel, GI = gastrointestinal)

Oral administration of paclitaxel is crucial for the clinical development of LDM chemotherapy with paclitaxel. Paclitaxel has limited oral bioavailability because of poor aqueous solubility and high affinity for active ABC drug efflux transporters (P-gp/ABCB1/MDR1), cytochrome P450 3A (CYP3A) and 2C8 drug metabolizing enzymes present in the epithelial layer of the gut wall and/or the liver (Figure 1) [19]. The systemic exposure upon oral administration of paclitaxel is increased approximately 8 fold when co-administered with 15 mg/kg oral cyclosporine (CsA), an inhibitor of P-gp and CYP3A [20]. Subsequently, the oral paclitaxel-CsA combination has been proven feasible and active in three phase II trials conducted in cancer patients where the combination was administered at MTD in a weekly schedule [21–23]. Recent studies showed a comparable enhancement of exposure when paclitaxel was given in combination with a low dose of the CYP3A4 inhibitor ritonavir [24].In such studies, paclitaxel was administered as a drinking solution, employing the i.v. liquid formulation. This solution is, however, not suitable for regular clinical use especially in a LDM treatment, because of bad taste, poor dosing accuracy and limited storage stability after preperation. Therefore, ModraPac001 10 mg capsules have been developed containing paclitaxel as a solid dispersion without the use of Cremophor® EL [25].

In this dose escalation phase I study, patients with advanced solid tumors were treated with LDM oral paclitaxel in combination with ritonavir. The primary objective was to determine the safety and feasibility of continuous bi-daily (BID) administration of ModraPac001 capsules in combination with ritonavir. Secondary objectives were to determine the MTD, the dose limiting toxicities (DLT), the pharmacokinetics (PK) of paclitaxel and ritonavir,

and the preliminary antitumor activity of the oral combination. Since chronic drug administration without major adverse events is essential to obtain an anti-angiogenic effect, the recommended dose (RD) was defined as the dose-level below the MTD.

Patients and Methods

Study design and treatment schedule

This phase I study was an open-label, dose escalation study of oral paclitaxel in combination with ritonavir. Patients received continuously paclitaxel bid (ModraPac001 2.5 mg, 5 mg and 10 mg capsules, Slotervaart Hospital, The Netherlands) in combination with an oral 100 mg ritonavir tablet (Norvir®; Abbott, Illinois, USA) with at least 7, but not more than 12 hours dose interval (intake around the same time). Treatment was continued until progressive disease, unacceptable toxicity despite dose reduction and patient refusal. The medication was taken in fasting conditions, i.e., at least 1.5 hours after and at least 1 hour before food intake, since the effect of food on the pharmacokinetics of the drug was unknown. Weekly physical examination, blood hematology and blood chemistry parameters guided the safety of the treatment. Three treatment weeks were counted as one cycle.

The study had a classic 3+3 dose escalation design [26]. Three patients were assigned to each dose-level. If a patient of the first three at a defined dose-level experienced a dose limiting toxicity (DLT), the number of patients treated at this dose-level was expanded to a maximum of six patients. The dose escalation continued if none of the additional patients experienced a DLT. The safety of this schedule was determined by assessing the MTD, defined as the highest safe BID dose of oral paclitaxel at which maximally one out of six patients developed a DLT. The paclitaxel doses administered in escalating cohorts of patients were determined on the basis of on safety evaluations and PK profiles observed at prior dose-levels, according to a pre-specified dose escalation schema.

The starting dose was 2.5 mg paclitaxel combined with 100 mg ritonavir BID. This dose combination was considered safe and was selected based on the results of previous proof of concept studies [24,25] and because it was expected to result in plasma concentrations far below the threshold of 42.7 µg/L (0.05 µmol/L), which is associated with peripheral neurotoxicity and neutropenia [27,28]. No pre-medication with corticosteroids nor anti-emetics was given in view of the low daily doses administered and the absence of Cremophor® EL in the oral formulation of paclitaxel. Patients were considered evaluable for safety if they had received at least one administration of paclitaxel. To determine the safety of a dose-level, patients were considered evaluable if they had completed one cycle of three weeks or if a DLT occurred in this time period. A DLT was defined as any of the following events that occurred during the first cycle and that was determined to be possibly, probably or definitely related to paclitaxel by the investigator: National Cancer Institute's Common Terminology Criteria for AEs (NCI-CTCAE v4.02) grade 3 or 4 non-hematological toxicity (other than untreated nausea, vomiting or diarrhea), grade 4 thrombocytopenia, grade 4 anemia, grade 4 neutropenia lasting for more than 5 consecutive days, grade 3 or higher febrile neutropenia, grade ≥ 2 peripheral sensory or motor neuropathy and treatment delay for more than 7 days due to drug related toxicity.

Eligibility
Patients were eligible if they were diagnosed with a histological or cytological proof of cancer, if there were no standard curative or palliative treatment options available and if paclitaxel treatment was considered appropriate. Patients had to be at least 18 years of age and have a performance status of 0, 1 or 2 according to the WHO Performance Status (PS) scale. Life expectancy longer than 3 months and adequate bone marrow, hematological and biological functions (neutrophil count of ≥ 1.5 * 10^9/L and platelets of ≥ 100 * 10^9/L; alanine transaminase (ALT) and aspartate transaminase (AST) ≤ 2.5 times institutional upper limit of normal (ULN), bilirubin of ≤ 1.5 times the ULN; serum creatinine ≤ 1.5 times the ULN or creatinine clearance ≥ 50 mL/min by Cockcroft-Gault formula) were required.

Patients with known alcoholism, drug addiction and/or psychiatric disorders were considered not suitable for adequate follow up, and thus excluded. Patients were not allowed to concomitantly use P-gp and CYP3A modulating drugs. Other exclusion criteria were uncontrolled infectious disease, bowel obstructions that may influence drug absorption, neurologic disease, pre-existing neuropathy higher than grade 1, symptomatic cerebral or leptomeningeal metastases, pregnancy, breast feeding, refusal to use adequate contraception and previous anticancer therapy within 4 weeks prior to the first dose of oral paclitaxel. The study protocol was approved by the local Medical Ethics Committee and all patients had to give written informed consent. The study was registered under identifier NTR3632 (NTR register).

Study procedures
Pre-treatment evaluation included a complete medical history with concomitant medications, physical examination (including vital signs and performance status), assessment of adverse events according to the NCI-CTCAE v4.02, a pregnancy test, laboratory assessment of hematology, serum chemistry and urinalysis and a radiological evaluation to document the extent of malignant disease. Every week, assessment of adverse events and concomitant medication was repeated, as well as hematology and serum chemistry. A physical examination was performed at least every three weeks. Tumor status was evaluated at baseline and response was assessed at least every two cycles according to RECIST 1.1 [29].

Pharmacokinetics
The PK of paclitaxel and ritonavir were monitored on day 1, 2, 8 and 22 (day 1 of the second cycle) of treatment. Venous blood samples for the PK analysis were obtained through an indwelling peripheral intravenous cannula on the first day. The time points for paclitaxel and ritonavir measurements were pre-dose, 1, 1.5, 2, 2.5, 3, 4, 6, 7 (prior to the subsequent drug administration), 8, 8.5, 9, 9.5, 10, 11, 13 and 24 hours (prior to the subsequent drug administration) after first administration. On day 8 and 22 a blood sample was obtained prior to study drug administration. Blood samples were collected in tubes containing lithium heparin as an anticoagulant. All samples were centrifuged within 1 hour at 1500 *g* for 10 minutes at 4°C and stored at –20°C until analysis.

Paclitaxel and ritonavir were quantified in plasma by a high-performance liquid chromatography assay with tandem mass spectrometric detection (LC-MS/MS) as described by Hendrikx et al. [30]. For both analyses stable isotopically labeled analogues were used as internal standards. Briefly, both analytes were extracted from 200 µL human plasma using tertiairy butylmethylether. Subsequently, the solution was evaporated to dryness under a gentle stream of nitrogen and the residue was reconstituted in methanol:water (1:1, v/v). Of each sample, 25 µL were injected onto a Zorbax Extend C18 column (150 x 2.1 mm ID; particle size 5 µm; Agilent Technologies, Amstelveen, The Netherlands). The mobile phase consisted of a mixture of 7:3 *v/v* methanol/10 mM ammonium hydroxide in water. Analytes were detected using positive ionization electrospray tandem mass spectrometry. The lower limit of quantification of the assay was 0.5 ng/mL for paclitaxel and 2.0 ng/mL for ritonavir.

Data analysis

The individual non-compartmental PK parameters were determined using validated scripts in the software package R (version 2.15). 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}), apparent clearance (CL/F), distribution volume at steady state (Vss/F), the terminal elimination half-life ($t_{1/2}$), the area under the plasma concentration-time curve between t=0 and the time point of the last quantifiable data point (AUC) or, if possible, with extrapolation to infinity (AUC_{inf}) using the terminal rate constant.

Results

Patient characteristics and disposition

A total of 17 patients were enrolled in the dose escalation study divided over five dose-levels (2.5/100 BID, 5/100 BID, 7.5/100 BID, 10/100 BID and 15/100 mg BID paclitaxel and ritonavir, respectively). The median age was 67 years (range: 40-81 years) and 94% had a WHO performance status ≤1. Twenty-four percent of the patients were female and all patients were Caucasian (Table 1). All enrolled patients received at least one dose of oral paclitaxel, of which the majority (88%) completed the DLT evaluation period of one cycle of three weeks. The median number of weeks on treatment for all patients in the five dose-levels was 9 (range 1-13), 7 (range 1-24), 8 (range 4-10), 8 (range 6-8), and 5 (range 3-7), respectively. In total 13 patients (76%) discontinued study treatment permanently due to progressive disease or clinical deterioration, the other patients discontinued treatment as a result of patient refusal (n=2), a non related adverse event (broncho-pulmonary hemorrhage, n=1) or a treatment interruption of more than 3 weeks (due to chronic renal failure not related to study drug, n=1).

Seven patients had eleven interruptions of treatment, most of which lasted only a few days. Two events only lasted for more than two weeks (increased creatinine levels). The reasons leading to delay of drug administration were an unrelated adverse event (n=5), a missed dose (two logistic events and two times by vomiting after intake) and a pre-planned surgery (n=2). There were no clear differences in treatment delays between the dose-levels. No dose reductions were reported.

Table 1 Patient demographics

Character	N	%
Total number of patients	17	100%
Sex		
Male	13	76%
Female	4	24%
Age (years)		
Median (range)	59 (36-79)	
WHO performance status		
0	8	47%
1	8	47%
2	1	6%
Ethnic origin		
Caucasian	17	100%
Primary tumor site		
NSCLC	7	41%
UCC	2	12%
Other	8	47%
Tumor Stage		
Locally advanced	1	6%
Metastatic	16	94%
Prior Treatment		
Chemotherapy	16	94%
Radiotherapy	9	53%
Surgery	11	65%

(abbreviations: NSCLC = non-small cell lung cancer, UCC = urothelial cell carcinoma)

Safety and tolerability

All patients were evaluated for treatment-related adverse events. Table 2 lists all adverse events that were possibly, probably or definitely related to the study drug with all CTCAE grades. Overall, ModraPac001 in combination with ritonavir was well tolerated. Importantly, during these dose-levels, none of the patients experienced a DLT during the first cycle of oral paclitaxel in combination with ritonavir. The most common drug-related adverse events in all dose-levels were fatigue (53%), diarrhea (29%), nausea (24%) and peripheral sensory neuropathy (24%), most being grade 1. Only one patient reported an event of grade 3 fatigue. This patient had grade 2 fatigue during the last week of study treatment. The treatment was ended, because of disease progression and one week after the last study dose, fatigue deteriorated to grade 3. All patients with possibly related grade 1 peripheral sensory neuropathy had received a platinum based regimen as previous

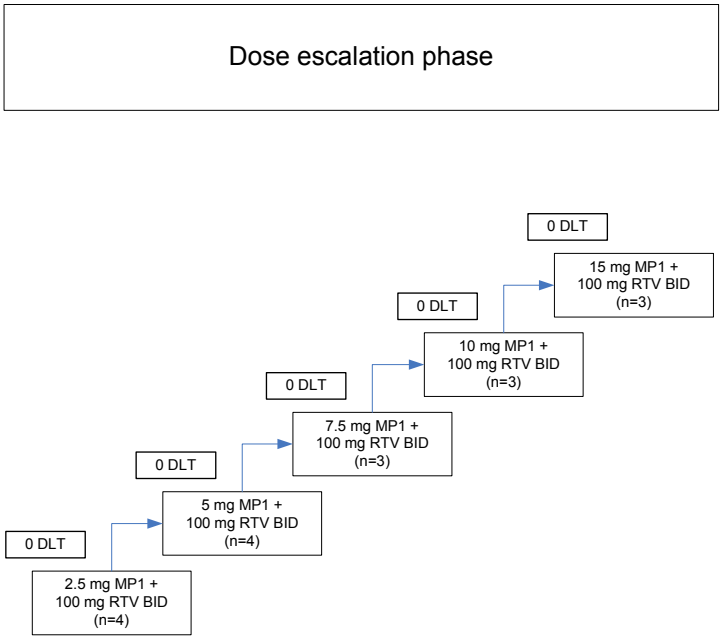

Figure 2 Study escalation scheme (abbreviations: MP1 = ModraPac001, RTV = ritonavir, DLT = dose limiting toxicity)

therapy and three of the four cases had already grade 1 peripheral sensory neuropathy at baseline. Only one hematologic adverse event of grade 2 anemia was considered clinically significant and possibly related to the study treatment.

There were six SAEs reported by three patients. All SAEs were considered to be either unrelated or unlikely to be related to the study drug. All events resolved, except for one patient. This patient died within 30 days after the last dose of ModraPac001 due to progression of disease.

Pharmacokinetics

The PK of paclitaxel and ritonavir were monitored on the first day of the study. Mean plasma concentration–time curves of the first day of oral paclitaxel are shown in Figure 3 and the results of the non-compartmental PK analysis are shown in Table 3. The mean plasma concentration–time profile of paclitaxel exhibited a profile of bi-exponential PK. PK data at the 2.5 mg dose level was limited, because a significant number of the plasma samples contained a concentration below the lower limit of quantification of the paclitaxel assay [30]. The mean C_{max} of paclitaxel was reached at 1.7 hours (CV 40%) after the first administration and at 2.0 hours (CV 6%) after the second administration, independently of the dose. The mean terminal half-life of paclitaxel was 6.4 hours (CV 36%), also independently of the paclitaxel dose. The C_{max} and systemic exposure (expressed as AUC_{inf}) to paclitaxel increased with higher dose. The relationship between AUC_{inf} and the paclitaxel dose is shown in Figure 4. The mean AUC_{inf} and mean C_{max} of paclitaxel at the highest dose-level were 198.1 ng/mL*hr (CV 16%) and 19.8 ng/mL (CV 11%), respectively.

Table 2 Adverse events with a possible, probable or definite relationship to oral paclitaxel in combination with ritonavir (N=17)

Dose-level (paclitaxel/ ritonavir)	2.5/100 BID (N=4)				5/100 BID (N=4)				7.5/100 BID (N=3)				10/100 BID (N=3)				15/200 BID (N=3)				Total (N=17)			
	%	G1	G2	G3	%	G1	G2	G3	%	G1	G2	G3	%	G1	G2	G3	%	G1	G2	G3	%	G1	G2	G3
Fatigue	50%	1	1		50%	1		1	33%	1			100%	3			33%		1		53%	6	2	1
Diarrhea	25%	1			50%	2			33%	1			33%	1							29%	5		
Nausea									67%	2			33%	1			33%		1		24%	3	1	
Neuropathy					75%	3							33%	1							24%	4		
Weight loss					25%		1		67%	2											18%	2	1	
Dry skin					25%	1							33%	1							12%	2		
Anemia									33%		1										6%		1	
Anorexia																	33%	1			6%	1		
Erythema													33%	1							6%	1		
Flatulence																	33%	1			6%	1		
Muscle cramps													33%	1							6%	1		
Myalgia													33%	1							6%	1		
Pyrosis					25%	1															6%	1		
Vomiting																	33%	1			6%	1		

(Abbreviations: N: number of evaluable patients, G= CTCAE grade)

Table 3 Summary statistics for pharmacokinetic parameters of paclitaxel and ritonavir on the first day. The data are shown as mean values and coefficient of variation (%).

	Paclitaxel					Ritonavir
Dose-level (paclitaxel/ ritonavir)	**2.5/100 BID**	**5/100 BID**	**7.5/100 BID**	**10/100 BID**	**15/100 BID**	**All**
N	**4**	**4**	**3**	**3**	**3**	**17**
AUC_{inf} (ng•h/mL)	26.8	48.7	86.1	93.8	198.1	$26.2•10^{3\#}$
CV (%)	87%	44%	33%	41%	16%	73%
$C_{max,1}$ (ng/mL)	2.5	4.4	8.1	9.1	18.4	$1.0•10^{3}$
CV (%)	50%	16%	33%	27%	25%	43%
$T_{max,1}$ (h)	1.4	1.8	1.7	1.7	2.0	2.1
CV (%)	35%	37%	46%	46%	43%	31%
$C_{max,2}$ (ng/mL)	1.6	3.9	8.2	7.8	19.8	$1.7•10^{3}$
CV (%)	70%	61%	41%	29%	11%	52%
$T_{max,2}$ (h)	1.8	2.4	1.5	2.2	2.0	2.8
CV (%)	9%	3%	6%	3%	6%	12%
Thalf (h)	4.8	6.4	6.7	6.8	7.6	$8.9^{\#}$
CV (%)	57%	58%	32%	15%	14%	39%

(Abbreviations: N: number of evaluable patients, AUC_{inf} = the area under the plasma concentration-time curve with extrapolation to infinity, C_{max} = maximum observed plasma concentration, T_{max} = time to reach C_{max}, Thalf = the terminal elimination half-life, #: N=15)

The mean plasma concentration–time curve of ritonavir is shown in Figure 5 and the results of the non-compartmental PK analysis are shown in Table 3. The mean C_{max} of ritonavir was reached after 2.1 hours (CV 31%) and 2.8 hours (CV 12%) after the first and second dose of 100 mg ritonavir, respectively. The PK profile showed a monophasic decline. The C_{max} of ritonavir after the second administration is almost two-fold higher than the C_{max} of ritonavir after the first administration, which is probably due to saturable metabolism [31].

The mean concentration of paclitaxel and ritonavir prior to administration on day 2 and 8 of the first cycle and day 1 of the second cycle per dose-level are shown in Table 4. No relevant differences were observed in C_{min} of paclitaxel on subsequent days of treatment.

Anti-tumor activity

All patients had measurable disease prior to start of treatment. Three patients were not evaluable for anti-tumor activity, because they went off study before the first radiological evaluation. Reasons leading to end of treatment were an adverse event (broncho pulmonary hemorrhage), clinical deterioration, and patient refusal. Seven of the other patients had stable disease (SD) and seven patients had progressive disease (PD) as their best overall response. The median duration of SD was 10 weeks and one patient had a

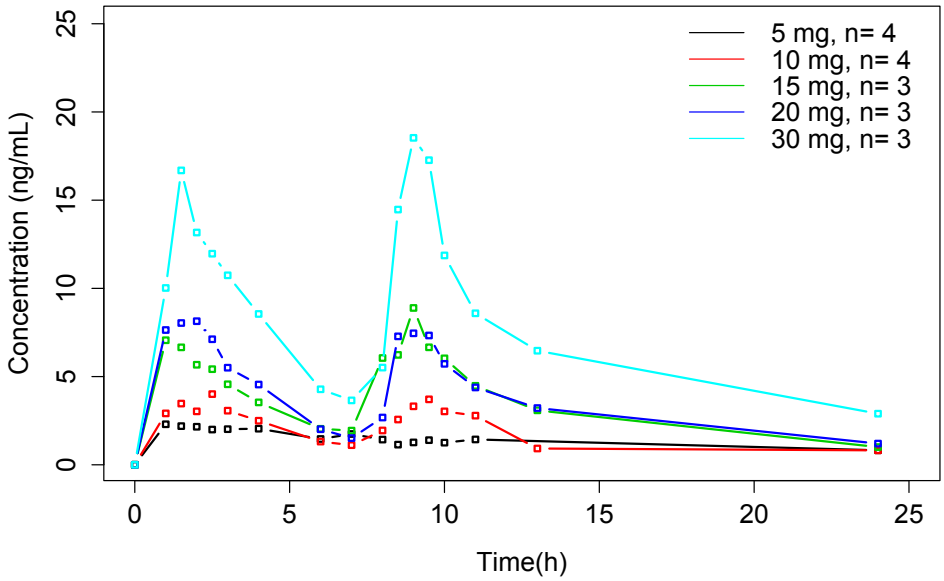

Figure 3 Mean plasma concentration-time curves of paclitaxel in patients after administration of ModraPac001 capsules.

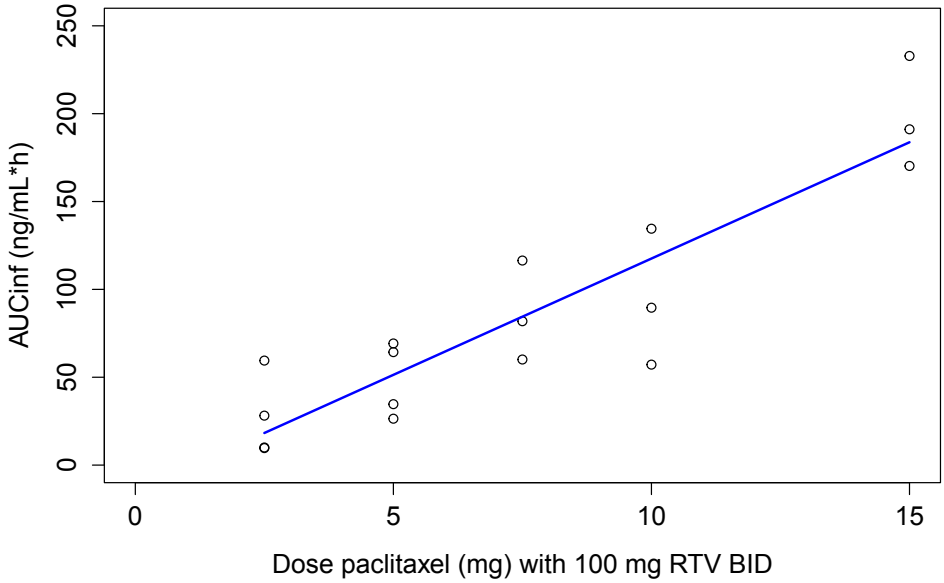

Figure 4 Dose-exposure plots of paclitaxel in patients after administration of oral paclitaxel (ModraPac001 capsules) in combination with 100 mg ritonavir on the first day of oral paclitaxel. The line represents the linear regression. (Abbreviations: AUC_{inf} = the area under the plasma concentration-time curve with extrapolation to infinity)

progression free survival of 24 weeks. The primairy tumor type of this patient was NSCLC. There were no patients with an objective tumor response.

Discussion

This report describes an interim analysis of the first dose escalation study of LDM chemotherapy with oral paclitaxel in combination with ritonavir. The study was still ongoing at the time this report was written and therefore, the MTD could not yet be established. Up to now, five dose-levels were evaluated in this study, ranging from 2.5 mg to 15 mg paclitaxel in combination with 100 mg ritonavir BID.

During these dose-levels, LDM oral treatment with oral paclitaxel and ritonavir was well tolerated. Most frequently reported treatment related adverse events were fatigue, diarrhea, nausea and peripheral neuropathy, most being grade 1, which are independent of the dose. No alopecia was reported and none of the patients suffered from a DLT. The most commonly reported adverse drug reactions associated with i.v. treatment of paclitaxel are alopecia, bone marrow suppression, peripheral neuropathy, myalgia, hypersensitivity reactions and injection site reactions [32,33]. This i.v. toxicity profile is not seen in the study population, possibly due to the lower exposures of paclitaxel compared to i.v. treatment and the route of administration. Neutropenia, which has been associated in the literature with the duration of plasma concentrations above a threshold of 42.7 µg/L (0.05 µmol/L), was not seen is this study, most likely because this threshold is about 2,000-fold higher than the mean C_{max} observed with the highest dose-level administered

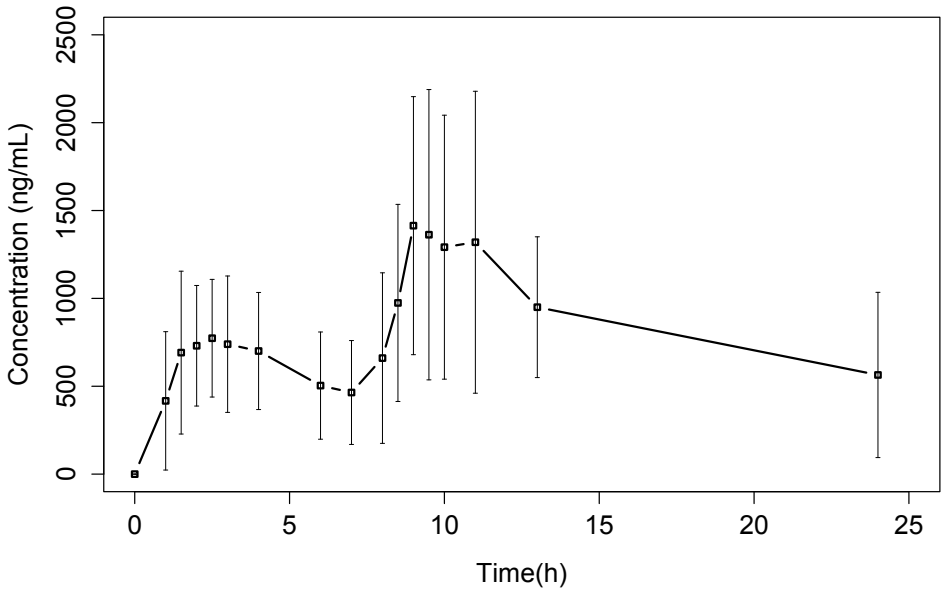

Figure 5 Plasma concentration-time curve of ritonavir in patients after administration of 100 mg ritonavir BID (n=17). Data are shown as mean values (symbols) with SD (error bars).

Table 4 Mean paclitaxel and ritonavir concentration prior to administration on day 2 and day 8 of the first cycle and day 1 of the second cycle.

Drug	Dose-level	N	Day 2		Day 8		Day 22	
			C_{min}	CV	C_{min}	CV	C_{min}	CV
Paclitaxel	2.5/100 mg BID	4	0.5	87%	0.5[1]	79%	0.7[2]	80%
	5/100 mg BID	4	0.6	51%	0.7[1]	51%	0.9[1]	61%
	7.5/100 mg BID	3	1.0	24%	1.7	13%	2.1	21%
	10/100 mg BID	3	1.2	42%	1.6	25%	1.4	3%
	15/100 mg BID	3	2.9	33%	4.7[2]	58%	2.9	49%
Ritonavir	All	17	529.8	92%	398.8[3]	91%	464.4[3]	90%

The data are shown as mean values and coefficient of variation (%). C_{min} is given in ng/mL. (Abbreviations: N: number of evaluable patients, C_{min} = Plasma concentration prior to administration, [1]: N= 3, [2]: N=2, [3]: N=14).

in our study [27]. The same association applies to the incidence and severity of peripheral neuropathy [28], which is only seen in four patients (grade 1). Because these patients had received platinum based chemotherapy prior to this study, they had an increased neuropathy risk [34].

Gastrointestinal disorders, like nausea, vomiting, diarrhea and mucosal inflammation are very commonly observed after paclitaxel treatment [32]. These adverse events are important, since they frequently lead to treatment discontinuation and consequently to decreased cancer control [35]. In this study none of the patients discontinued treatment because of gastrointestinal disorders. Because paclitaxel is co-administered with ritonavir, the incidence of mild and moderate diarrhea could be increased. Ritonavir may induce apoptosis in human intestinal epithelial cells, which could lead to a reduced surface area for absorption and therefore induce diarrhea via a secretory mechanism [36–38]. This is also seen after ritonavir treatment in HIV patients at low daily doses of 100-400 mg [38]. The incidence of mild diarrhea could therefore be the result of both ritonavir and paclitaxel.

PK analysis of paclitaxel showed a linear increase of the AUC_{inf} with increasing dose. The PK parameters of paclitaxel in this study are consistent with the results obtained in the previously published proof-of-principle study, in which the mean C_{max} of paclitaxel was 42 ng/mL (CV 16%) at 1.9 hours (CV 21%), after a dose of 30 mg paclitaxel, using ModraPac001 capsules in combination with 100 mg ritonavir [25].

The PK parameters of ritonavir in this study are comparable to the results observed with 100 mg ritonavir BID administered in combination with docetaxel (ref. chapter 3.2). Based on the boosting experiences with protease inhibitors during HIV treatment, plasma concentrations of ritonavir are generally highest during the first few days after multiple doses. In a continuous BID regime, the minimal concentration is expected to decrease over time and steady state of ritonavir to be achieved generally over a 10- to 14-day period [39,40]. This effect is less pronounced for AUC and C_{max} [39,40]. Importantly, long-term usage of continuous ritonavir is often associated with mild induction of P-gp and CYP3A,

yet net inhibition of CYP3A still predominates [41]. As such, population PK analysis may help to evaluate the influence of steady state ritonavir on the absorption and elimination processes of oral paclitaxel.

Currently, there is only one published clinical study with LDM paclitaxel [42]. In such phase II pilot study, paclitaxel was given as a continuous infusion in combination with oral celecoxib in patients with metastatic melanoma. Although three of the twenty heavily pretreated patients in the published study had stable disease during treatment, central line-related complications made this regime not feasible. Additionally, the presence of Cremophor® EL could reduce the antiangiogenic effect of paclitaxel [43,44], supporting the necessity of an oral dosage form for LDM treatment with paclitaxel.

It is a limitation that validated biomarkers are lacking for determination of the effect of LDM treatment with oral paclitaxel on angiogenesis, the main target of this treatment [45–47]. A biomarker predicting the response to LDM treatment, to define an optimal biological dose of oral paclitaxel, and to establish therapeutic activity would be helpful. Several candidate biomarkers are currently under investigation, and if validated, could be implemented during further development of oral LDM treatment [45].

In conclusion, this is the first study demonstrating that LDM with oral paclitaxel in combination with ritonavir is feasible. ModraPac001 capsules containing paclitaxel can be safely administered to patients in combination with 100 mg ritonavir in a continuous BID schedule. The most common drug-related adverse events were fatigue and diarrhea of mild to moderate severity. This study further strengthens the hypothesis that paclitaxel can be given as a LDM treatment regime.

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 register identifier is NTR3632 (NTR register). 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 have patents for oral taxane formulations.

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3.2

Biomarkers for monitoring the activity of low dose metronomic therapy with oral paclitaxel

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Interim analysis up to the 15/100 mg BID dose-level

Abstract

Low-dose metronomic therapy (LDM) is chronic administration of anticancer drugs at relatively low, non-toxic doses on a frequent schedule of administration. The main target of LDM therapy is dividing endothelial cells (EC) of the expanding vasculature of a tumor, instead of the proliferating tumor cells. Preclinical studies showed that LDM therapy with paclitaxel has a 'direct' effect, whereby EC are intrinsically sensitive to paclitaxel and an 'indirect' effect, whereby thrombospondin-1 (TSP-1) acts as an endogenous inhibitor of angiogenesis. Both mechanisms are considered to inhibit tumor angiogenesis and vasculogenesis, leading to a reduction in tumor neovascularization. In this interim analysis we validated novel assays for the following biomarkers: circulating levels of TSP-1 and gene expression of TSP-1 and VEGF-A in PBMCs. These biomarkers were evaluated in healthy volunteers and during an ongoing phase I study with oral LDM treatment with paclitaxel in combination with ritonavir. In healthy volunteers, it was shown that the procedures were easy to implement and had an acceptable variability within humans. Unfortunately, differences in the biomarkers between the responders and non-responders to LDM treatment with paclitaxel were, thus far, not observed. Whether this is a result of the mode of action of paclitaxel or the feasibility of these biomarkers warrants further progress of the clinical study.

Introduction

In classical dose-intensive oncolytic therapy it is believed that the highest tolerable dose-intensity (MTD, dose-dense) will result in the highest anti-tumor activity. An alternative is "low-dose metronomic therapy (LDM)", consisting of chronic administration of anticancer drugs at relatively low, non-toxic doses on a frequent schedule of administration, with no drug-free breaks [1–3]. Unlike dose-dense therapy, the main target is dividing endothelial cells (EC) of the expanding vasculature of a tumor, instead of the proliferating tumor cells. Since the therapeutic effects rely on its anti-angiogenic activity, the (cumulative) dose is significantly lower than with MTD-based chemotherapy [1,2]. However, a significant drawback is the empiricism in monitoring the therapeutic activity early during the course of treatment and in establishing the optimal biologic dose (OBD) [2,4]. Up to now a validated biomarker for angiogenesis is still lacking [5–7]. Therefore, a large number of questions regarding treatments targeting angiogenesis remain unanswered.

Paclitaxel is an alkaloid ester derived from the Pacific yew (Taxus brevifolia). The drug is commonly used as first line therapy for various tumors and functions as a mitotic spindle poison through high-affinity binding to microtubules with enhancement of tubulin polymerization, arresting cells in mitosis[8]. Almost twenty years ago, it was found that paclitaxel has strong antiangiogenic activity [9,10]. Even at low non-toxic concentrations (<10 nM) proliferation, migration and differentiation of EC were inhibited, while these concentrations had no effect on other human cell types, such as tumor cells [11,12]. Next to this direct effect on the tumor endothelium, Bocci et al. showed a selective inhibition of human EC proliferation only after a prolonged exposure of ultra-low concentrations of paclitaxel [12]. This delayed effect revealed a second possible, indirect working mechanism of LDM therapy. The precise molecular and cellular pharmacological mode of action explaining the indirect effect is not yet fully elucidated, but the potential key mediator of the indirect effect is thrombospondin-1 (TSP-1) [13]. TSP-1 is a large multimeric, multidomain glycoprotein that functions during development and tissue remodeling. As a potent endogenous inhibitor of angiogenesis, TSP-1 acts primarily by binding to EC expressing the CD36 receptor. This interaction blocks the proliferation and induces apoptosis selectively of the EC [14]. Furthermore, TSP-1 can bind to vascular endothelial growth factor (VEGF)

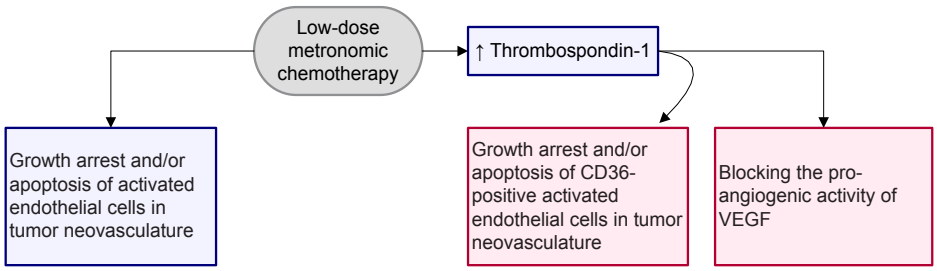

Figure 1 Possible mechanisms of the anti-angiogenic basis of low-dose metronomic treatment. A 'direct' effect, whereby endothelial cells are intrinsically sensitive to low-dose metronomic treatment and an 'indirect' effect (right), whereby thrombospondin-1 acts as an endogenous inhibitor of angiogenesis. Both mechanisms are considered to inhibit tumor angiogenesis and vasculogenesis, leading to a reduction in tumor neovascularization in the absence of significant side effects such as myelosuppression, hair loss, and nausea or vomiting (based on ref [2]).

and displace VEGF from EC [15], thereby switching the tumor microenvironment to an anti-angiogenic milieu, inhibiting neovascularization, tumor progression and metastasis. Several animal studies (rats and mice) showed that LDM therapy with paclitaxel suppresses different tumors and metastasis [16–18]. These studies also demonstrated an upregulation of TSP-1, which is not seen when paclitaxel was administered at MTD [16,17]. Moreover, small clinical studies showed that gene expression and plasma concentration of TSP-1 could be increased by LDM treatment with other drugs [19,20].

We performed a phase I dose escalation study to determine the feasibility of LDM treatment with paclitaxel as ModraPac001 capsules in combination with oral ritonavir as booster drug. The study was still ongoing at the time this report was written. A secondary objective of this study was to determine the usefulness and feasibility of the following exploratory biomarkers: circulating levels of EC and their bone marrow derived progenitor cells, circulating levels of TSP-1 and gene expression of TSP-1 and VEGF-A in peripheral blood mononuclear cells (PBMCs).

The aims of this interim analysis were: 1) to perform a fit-for-purpose validation of the assays for circulating levels of TSP-1 and gene expression of TSP-1 and VEGF-A in PBMCs, 2) to evaluate these biomarkers over time in healthy volunteers, and 3) to determine the usefulness and feasibility of the biomarkers in a clinical study with LDM treatment with paclitaxel and ritonavir.

Material and methods

Circulating levels of TSP-1

In the systemic circulation, TSP-1 is scavenged in platelets [21,22]. Therefore it is not possible to measure the TSP-1 levels directly in whole blood. Plasma levels of TSP-1 were measured by collecting platelet rich plasma (PRP) and platelet poor plasma (PPP). Before analysis, we activated and disrupted the platelets of PRP to release TSP-1 and to measure the content of TSP-1 in platelets. Several ways for activation and disruption were investigated during development of the assay, including disruption by sonication, activation by thrombin-receptor activating peptide (TRAP-6, Bio-Connect), and freezing at -20oC and in liquid nitrogen. To prevent activation of the platelets during handling, we used citrate tubes containing citrate, theophylline, adenosine and dipyridamole as anticoagulants (CTAD tubes, Becton Dickinson) [23]. The concentration of TSP-1 was measured with an enzyme-linked immunoassay (Thrombospondin-1 Immunoassay Kit, Quantikine, cat. nr: DTSP10, R&D Systems). The optical density was quantified using a microplate reader (Spectrophotometer EL808, Biotek) set at 450nm and the wavelength correction was set at 540nm. This assay was validated by the manufacturer for the determination of TSP-1 in separator tubes, plasma EDTA tubes, saliva and human milk, but not for tubes containing citrate as an anticoagulant. Therefore extra fit-for-purpose validation tests were executed using samples with spiked TSP-1 concentrations and samples with unknown TSP-1 concentrations. The precision was calculated with samples with unknown concentrations, provided by three healthy volunteers, to include the release of the TSP-1. To define the intra-assay, inter-assay and inter-day precision, two independent runs were employed in triplicate on two independent days. To calculate the accuracy, both Calibrator Diluent RD5-

33 (provided by the immunoassay kit) and PPP were spiked in triplicate with reconstituted recombinant TSP-1 in three independent runs, since PRP samples were diluted 100 times in Calibrator Diluent RD5-33 before analysis. There was no high quality grade human TSP-1 protein available to prepare spiked validation samples. Therefore the accuracy was calculated using the standard TSP-1 protein solution provided by the immunoassay kit in the range of 7.81-500 ng/mL.

PRP and PPP were prepared by collecting 4.5 mL whole blood in two CTAD tubes. The tubes were spun for 15 minutes at 120*g* in a 4°C pre-cooled centrifuge for PRP and 30 minutes at 3000*g* in a 4°C pre-cooled centrifuge for PPP [23,24]. The middle fraction of plasma (650 µl) was collected and stored at -20°C till analysis. On the day of analysis, PPP and PRP samples were thawed to room temperature and refrozen in liquid nitrogen for 1 minute to activate/disrupt the platelets. Before analysis, PPP was diluted 10 times in Calibrator Diluent RD5-33 and PRP was diluted 100 times in Calibrator Diluent RD5-33. The circulating levels of TSP-1 as ng/mL plasma were corrected for platelet counts and calculated like equation 1:

$$[TSP] = \left(\overline{[PRP] - [PPP]}\right) * \frac{[platelets]_{WB}}{[platelets]_{PRP}} \qquad (1)$$

([TSP] = circulating levels of TSP-1 within platelets (ng/mL plasma), [PRP] = concentration TSP-1 in PRP (ng/mL), [PPP] = concentration TSP-1 in PPP, [platelets]WB = concentration platelets in whole blood (109 platelets/L), [platelets]PRP = concentration platelets in PRP (109 platelets/L))

TSP-1 and VEGF-A gene expression

Gene expression of TSP-1 and VEGF-A in human PBMCs was determined using pre-validated TaqMan® probe-based assays (Applied Biosystems) normalized to housekeeping genes peptidylprolyl isomerase B (PPIB) and 2,4-dienoyl CoA reductase 1 (DECR1). These genes are stably expressed in human PBMCs [25,26]. These pre-validated TaqMan® probe-based assays (TSP-1 (THBS-1) Hs00962908_m1, VEGF-A Hs00900055_m1, PPIB Hs00168719_m1 and DECR1 Hs00154728_m1) were not yet validated for the procedures described in the clinical study with low dose metronomic paclitaxel. Therefore, fit-for-purpose validation tests were executed including linearity testing of cDNA synthesis and an establishment of overall precision of the assay including the RNA isolation and cDNA synthesis (reverse transcription reaction). A sample containing 400 ng of Reference RNA (Stratagene) was serially diluted in triplicate in the range 400-25 ng (final input) and then reverse transcribed to cDNA for linearity testing. For precision, PBMCs isolated from whole blood of five volunteers were stored at -80°C for at least 24 hours. To define the intra-sample, inter-sample, intra-assay and inter-day precision we measured the gene expression in triplicate during three independent runs per day on three independent days.

Gene expression assay sample preparation and analysis was executed as follows: PBMCs were prepared by collecting 8 mL of venous human blood in one CPT tube (Becton Dickinson) and centrifuged at room temperature at 1600*g* for 25 min. The cell suspension was transferred into a sterile RNase-free 50 mL tube and washed with sterile RNase-free

PBS at 1000 g for 10min. The supernatant was removed and the PBMCs were directly lysed in 1.5mL of RNA-BEE RNA isolation reagent (Bio-Connect) and stored in -80oC till RNA isolation procedure, according to RNA-BEE RNA isolation protocol. On the day of gene expression analysis, samples were thawed to room temperature, RNA was isolated and 400ng of RNA was reverse transcribed using 1 µl Superscript II Reverse Transcriptase (Invitrogen), 4 µl 5X first-strand buffer, 500ng random primers (Invitrogen), 10mM dNTP (each) (Qiagen) in final reaction volume of 20µl. The cDNA synthesis was exerted in an X thermo cycler (BioRad PTC-200): 25°C 10min, 42°C 60min, 70°C 15min, 10min cooling-down to 4°C. The resulting cDNA was amplified at standard thermal cycling conditions set in 7500 Fast Real-time PCR system (Applied Biosystems). The input data of TSP-1 and VEGF-A were normalized to the geometric average of control housekeeping genes, PPIB and DECR1, using the comparative concentration threshold (Ct) method [27]. In short, the Ct value of the housekeeping gene was subtracted from the Ct value of the gene of interest in each sample (ΔCt). Then the value of the calibrator, Stratagene QPCR Human Reference Total RNA (Agilent Technologies, Inc) was subtracted (ΔΔCt) and the linearized values were calculated (2-(ΔΔCt)). Every run included a sample with known TSP-1 and VEGF-A expression as quality control.

Variability in volunteers

For interpretation of the results of the pharmacodynamic biomarkers during treatment, the variability of the biomarkers was determined in eight healthy volunteers. The biomarkers were monitored at day 1, 8 and 22 after signing informed consent. At every time-point, blood samples were drawn for circulating levels of TSP-1 in platelets and gene expression of TSP-1 and VEGF-A. The samples were processed as previously described and stored at –20°C (PPP, PRP) and –80°C (PBMCs) until analysis. The data are presented as the percentage of the TSP-1 pre-dose plasma levels. The validated assays were executed after collection of all samples.

Clinical study with LDM oral paclitaxel

An interim analysis of the phase I dose escalation study conducted with LDM oral paclitaxel in combination with ritonavir is extensively described elsewhere (chapter 3.1). Every day, patients received BID paclitaxel (ModraPac001 2.5 mg, 5 mg and 10 mg capsules, Slotervaart Hospital, The Netherlands) in combination with an oral 100 mg ritonavir tablet (Norvir®; Abbott, Illinois, USA) with at least 7, but not more than 12 hours dose interval until progressive disease or until unacceptable toxicity despite dose reduction. The study protocol was approved by the local Medical Ethics Committee and all patients had to give written informed consent. The study was registered under identifier NTR3632 (NTR register).

To monitor the activity, samples for PPP, PRP and PBMCs were taken at baseline and after 24 hours, 7 days, 21 days of treatment and every 6 weeks of the treatment prior to the first administration of that day. The samples were processed as previously described and stored at –20°C (PPP, PRP) and –80°C (PBMCs) until analysis. To compare the course of the biomarkers, the concentrations and gene expressions were calculated as a percentage of the pre-dose concentration and gene expression of individual patients.

Patients

Patients were eligible if they had a histological or cytological proof of cancer, if there were no standard treatment options available and if paclitaxel treatment was considered appropriate. Other inclusion criteria were age ≥ 18 years, performance status of 0, 1 or 2 according to the WHO Performance Status (PS) scale, life expectancy longer than 3 months, and adequate bone marrow, hepatic and renal functions (neutrophil count ≥ 1.5 * 10^9/L, platelet count ≥ 100 * 10^9/L; alanine transaminase (ALT) and aspartate transaminase (AST) ≤ 2.5 times institutional upper limit of normal (ULN), bilirubin of ≤ 1.5 times the ULN; serum creatinine ≤ 1.5 times the ULN or creatinine clearance ≥ 50 mL/min by Cockcroft-Gault formula).

Patients with known alcoholism, drug addiction and/or psychiatric disorders were considered not suitable for adequate follow up, and thus excluded. Patients were not allowed to concomitantly use P-glycoprotein (P-gp) and CYP3A modulating drugs. Other exclusion criteria were uncontrolled infectious disease, bowel obstructions that might influence drug absorption, neurologic disease, pre-existing neuropathy higher than grade 1, symptomatic cerebral or leptomeningeal metastases, pregnancy, breast feeding, refusal to use adequate contraception and previous anticancer therapy within 4 weeks prior to the first dose of oral paclitaxel.

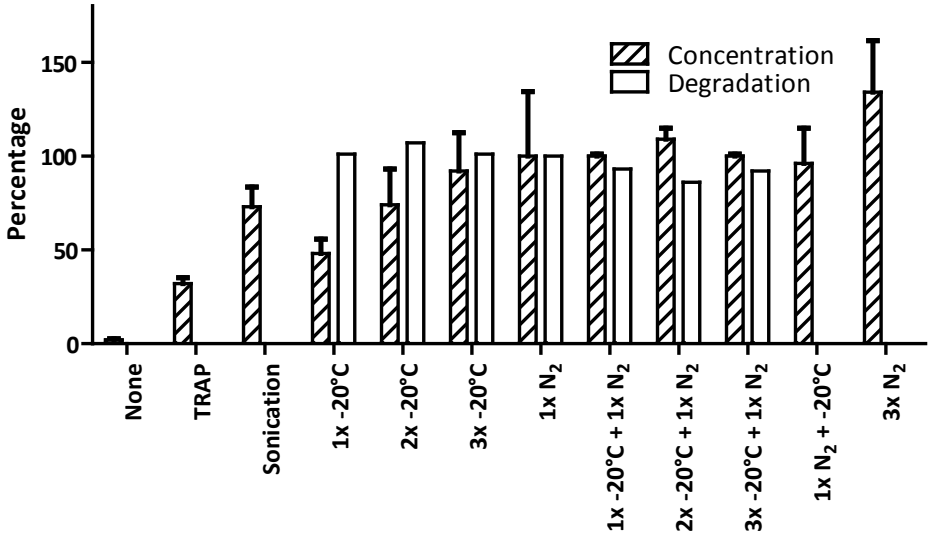

Figure 2 Impact of different activation and disruption methods to PRP (platelet rich plasma, blue bars) and recombinant thrombospondin-s (TSP-1) in PPP (platelet poor plasma, red bars). The methods are activation by TRAP (thrombin-receptor activating peptide), sonication, 1 time frozen at -20°C, 2 times frozen at -20°C, three times frozen at -20°C, 1 minute frozen in liquid nitrogen (N_2), 1 time frozen at -20°C and 1 minute frozen in N_2, 2 times frozen at -20°C and 1 minute frozen in N_2, 3 times frozen at -20°C and 1 minute frozen in N_2, 1 minute frozen in N_2 and 1 time at -20°C and the last method, 3 times frozen for 1 minute in N_2. The data are presented as concentration TSP-1 relative to the concentration after 1 time frozen in -20°C and 1 minute frozen in N_2 (hatched bars) and degradation of TSP-1 relative to the input of recombinant TSP-1 shown as mean values (open bars) with standard deviations (error bars).

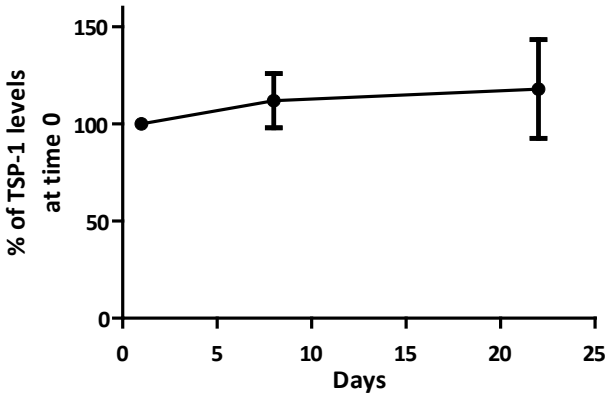

Figure 3 The circulating TSP-1 levels in healthy volunteers. The data are presented as percentage of the concentration at day 1 of each individual volunteer (n=8) and shown as mean values (symbols) with standard deviations (error bars). (abbreviation: TSP-1 = thrombospondin-1)

Results

Several ways for activation and disruption of platelets were investigated during development of the assay, including disruption by sonication, activation by TRAP and freezing at -20°C and in liquid nitrogen. Figure 2 shows that most of TSP-1 was released by storage at -20°C and refreezing the sample for 1 minute in liquid nitrogen. Storage of PRP in -20°C had no influence on the results of the analysis and the method proved to be constant, because of the refreezing step in liquid nitrogen. Additional freezing/thawing step seemed to release more TSP-1, which is most likely related to an increase of degradation.

After nine runs performed over two days with frozen samples of three volunteers (all male, age range 28-32), coefficients of variation (CV) were found to be 1.3%-6.5% (intra-assay precision), 1.3-4.2% (inter-day precision), and 1.1 – 3.5% (overall inter-assay precision), respectively. The accuracy was calculated after three runs with spiked validation samples. The ranges of the intra- and inter-assay inaccuracy of TSP-1 in Calibrator Diluent RD5-33 were 1%-15% and 2-7%, respectively, in the range of 500 - 31.3 ng/mL TSP-1. The mean recovery was 93%-106% for 500 - 125 ng/mL TSP-1.

To measure gene expression of TSP-1 and VEGF-A using total RNA from human PBMCs, linear course of cDNA synthesis, comparable amplification efficiencies of genes of interest and the housekeeping genes, as well as stable expression of housekeeping genes in PBMCs are crucial. The linearity of cDNA synthesis showed a correlation coefficient of ≥ 0.99 for all genes and the amplification efficiency were as follows: VEGF-A (92.0%), TSP-1 (89.6%), PPIB (89.6%) and DECR1 (85.4%). The intra-sample precision of frozen samples of five volunteers (all male, age range 25-32) was less than 1.8% for all genes and the average intra-sample precision of three independent runs per day was less than 1.4% for all genes. The range of the intra-assay precision of three independent runs on one day for TSP-1 normalized to the housekeeping genes was 0.9%-9.5% and the range of the inter-day

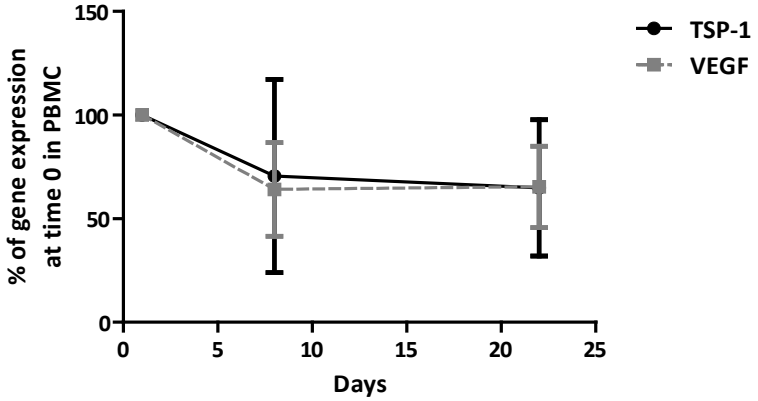

Figure 4 Gene expression of TSP-1 and VEGF-A in PBMCs of healthy volunteers. The data are presented as percentage of the 2-(ΔΔCt) at day 1 of each individual volunteer (n=8) and shown as mean values (symbols) with standard deviations (error bars). (abbreviations: TSP-1 = thrombospondin-1, VEGF = vascular endothelial growth factor, PBMC = peripheral blood mononuclear cells)

precision was 4.1-15.9%. For normalized VEGF-A the ranges for intra-assay precision and inter-day precision were 0.3%-11.5% and 6.2%-13.2%, respectively.

Variability in volunteers

The circulating TSP-1 levels and gene expression of TSP-1 and VEGF-A were monitored at day 1, 8 and 22 in eight healthy volunteers (three females and five males, age range 25-30). The mean levels of circulating TSP-1 as the percentage of the TSP-1 pre-dose levels are shown in Figure 3. These levels remained constant over 21 days. The mean variation among the volunteers was 11% and the mean level of TSP-1 platelet content was 9.1 µg/mL plasma (CV 29%). The mean levels of gene expression of TSP-1 and VEGF-A as the percentage of the pre-dose gene expression are shown in Figure 4. The mean variation among the volunteers was 44% for TSP-1 and 33% for VEGF-A.

Biomarkers during LDM treatment

To evaluate the use of the biomarkers in a clinical setting, the circulating TSP-1 levels and gene expression of TSP-1 and VEGF-A in PBMCs were monitored in a phase I dose escalation study performed with LDM oral paclitaxel in combination with ritonavir. The study was still ongoing at the time this report was written, therefore the samples of 17 patients of the first five dose-levels (2.5/100 BID, 5/100 BID, 7.5/100 BID, 10/100 BID and 15/100 mg BID paclitaxel and ritonavir, respectively) were measured.

To interpret the biomarkers in relation to the tumor response, we defined patients (N=7) with at least stable disease (SD) as their best overall response as responders to LDM treatment. The median duration of SD was 10 weeks and one patient had a progression free survival (PFS) of 24 weeks. Seven patients had progressive disease (PD) at the first radiological evaluation and three patients were not evaluable for anti-tumor activity, because they went off study before the first radiological evaluation. The results of the non-evaluable patients were excluded for analysis.

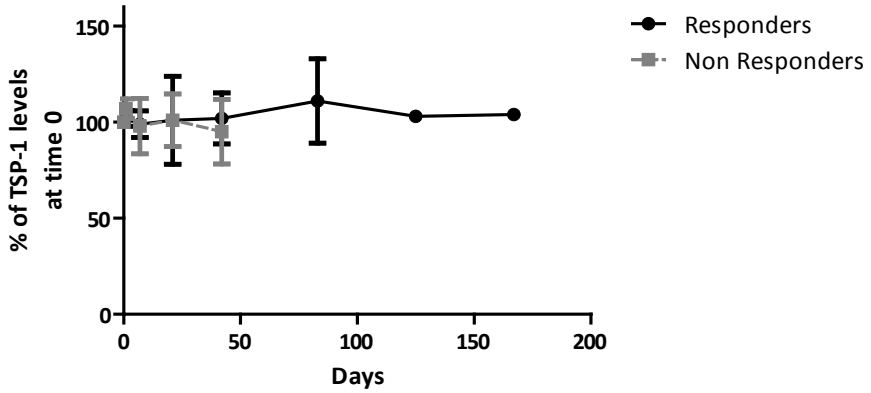

Figure 5 The circulating TSP-1 levels in patients on LDM treatment with oral paclitaxel and ritonavir. The data are presented as percentage of the concentration at day 1 of each individual patient and shown as mean values (symbols) with standard deviations (error bars). (abbreviation: TSP-1 = thrombospondin-1)

Figure 5 shows the results of the circulating TSP-1 levels during treatment. No further data were obtained of the non-responders as they discontinued therapy after 6 weeks, i.e. the moment of first radiological evaluation. The mean levels during treatment were similar to baseline, without any significant difference between responding and non-responding patients. Compared to the results of the individual patients, there was one patient with a markedly increase of TSP-1 levels to 143% compared to baseline after 83 days of treatment. Prior to progression, TSP-1 levels started to decline slowly towards the baseline value (Figure 8). This patient had a PFS of 24 weeks.

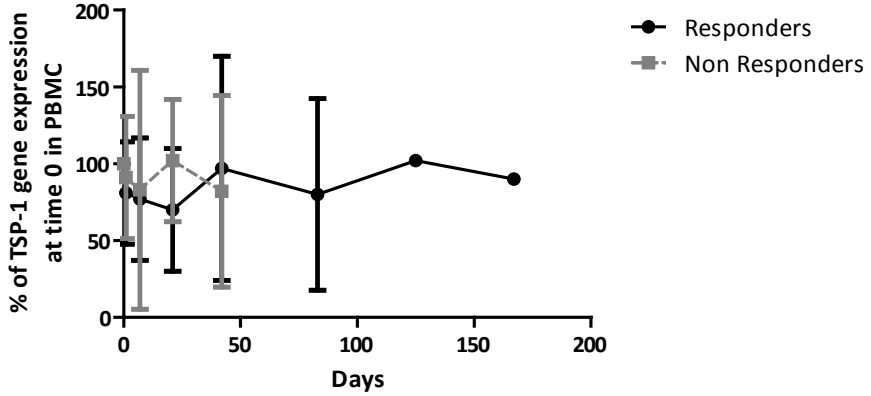

Figure 6 The gene expression values of TSP-1 measured in PBMCs in patients on LDM treatment with oral paclitaxel and ritonavir. The data are presented as percentage of the 2-(ΔΔCt) at day 1 of each individual patient and shown as mean values (symbols) with standard deviations (error bars). (abbreviations: TSP-1 = thrombospondin-1, PBMC = peripheral blood mononuclear cells, LDM = low-dose metronomic)

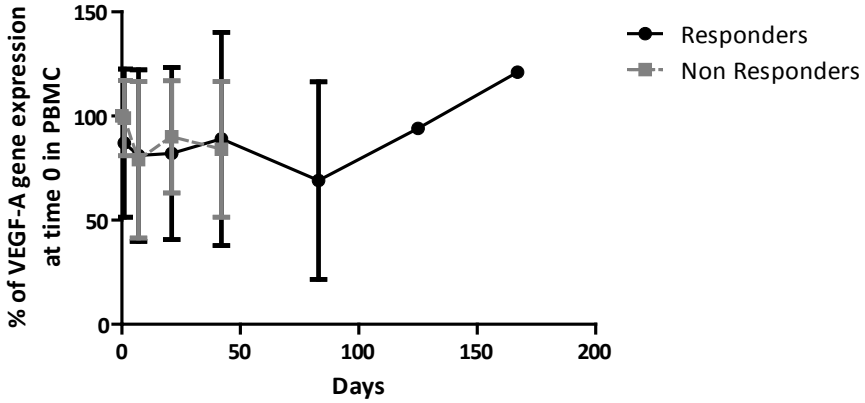

Figure 7 Gene expression levels of VEGF-A and in PBMCs in patients on LDM treatment with oral paclitaxel and ritonavir. The data are presented as percentage of the 2-(ΔΔCt) at day 1 of each individual patient and shown as mean values (symbols) with standard deviations (error bars). (abbreviations: VEGF = vascular endothelial growth factor, PBMC = peripheral blood mononuclear cells, LDM = low-dose metronomic)

Gene expression of TSP-1 and VEGF-A in PBMCs during treatment are shown in Figures 6 and 7. For both biomarkers no significant differences were noticed between the responders and the non-responders. The mean TSP-1 gene expression did not change significantly during treatment. For the individual non-responders no trend was seen during treatment. For the responders however, four patients had a decrease in TSP-1 gene expression to values corresponding to about 50% of the baseline value after 7 days. In contrast, TSP-1 gene expression increased by at least 50% for the other three patients including the patient with a PFS of 24 weeks (Figure 8). VEGF-A gene expressions of individual patients were similar to baseline, although with high variability.

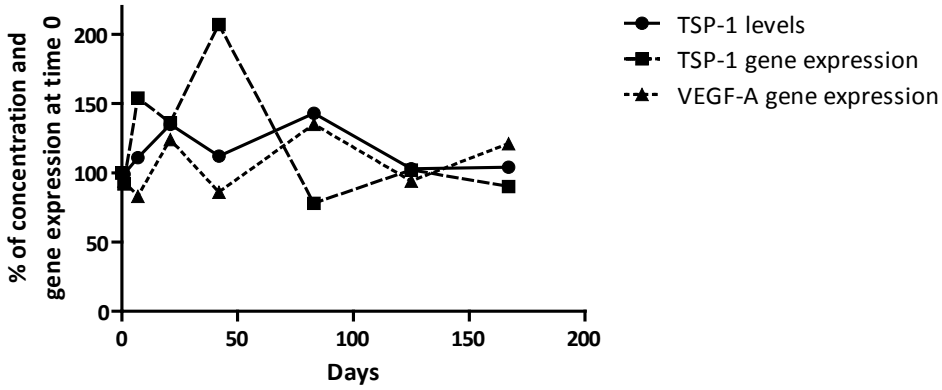

Figure 8 Circulating TSP-1 levels and gene expression levels of TSP-1 and VEGF-A in PBMCs of a patient who had a progression free survival of 24 weeks on LDM treatment with oral paclitaxel and ritonavir. The data are presented as percentage of TSP-1 levels and the 2-(ΔΔCt) at day 1. (abbreviations: TSP-1 = thrombospondin-1, VEGF = vascular endothelial growth factor, PBMC = peripheral blood mononuclear cells, LDM = low-dose metronomic)

Discussion

In this interim analysis, we performed a fit-for-purpose validation of the assays for circulating levels of TSP-1 and gene expression of TSP-1 and VEGF-A in PBMCs. The accuracy of the measurement of both biomarkers was difficult to calculate, since there are no existing high quality grade reference standards or surrogates. To compare the course of the biomarkers over time, we presented the concentrations and gene expression levels as a percentage of the pre-dose concentration and gene expression level. Therefore precision, defined by intra-assay, inter-assay and inter-day precision was the main focus of the fit-for-purpose validation of the assays of the biomarkers.

Several assays for measuring plasma TSP levels have been described, either as radioimmunoassays or enzyme immunoassays [23]. In the systemic circulation, most of TSP-1 is scavenged in a subset of platelet α-granules and is released in response to strong agonists [21,22]. The plasma concentration of TSP-1 is very low (around 180 ng/mL), but can locally significantly be increased when TSP-1 is released from the α-granules of activated platelets. Thus, measurement of plasma levels may not show an increase in circulating TSP-1 levels during treatment. Others tried to measure platelet content of TSP-1 by activating the platelets by clotting and subtract the plasma levels from the serum levels [24,28,29]. Since TSP-1 is involved in clotting, it is unknown whether all TSP-1 is released and available in the serum. Therefore, we developed a validated assay whereby TSP-1 is released in plasma without degradation and normalized for platelet counts. The results of the volunteer study show that the method was easy to implement and that the variation over time was low among untreated human healthy volunteers (14%).

During the clinical study with LDM paclitaxel, there was no significant difference between responders and non-responders to the treatment of both circulating TSP-1 levels and the gene expression levels of TSP-1 and VEGF-A in PBMCs. The mean levels and gene expression levels during treatment were similar to baseline values. The variability of the gene expression in treated patients was high, which was comparable with the results obtained in the study with healthy volunteers. Wide variability was also seen in other clinical studies in which gene expression of TSP-1 and VEGF-A in PBMCs was explored [19,20].

There are three possible explanations for the lack of difference in plasma TSP-1 levels between the responders and non-responders to treatment. First, the level of upregulation of TSP-1 induced by LDM paclitaxel is too low compared with the variability in the assay and endogenous variability in TSP-1 expression in humans. The clinical study with LDM paclitaxel was still ongoing at the time this report was written, and only the first seventeen patients of the first five dose-levels were included in this interim analysis. At the end of this study, the use and feasibility of the biomarkers will be evaluated again to explore upregulation of TSP-1 induced by LDM paclitaxel. Second, to interpret the biomarker levels in relation to the pharmacological response, we defined responders and non-responders based on the first radiological evaluation after 6 weeks of treatment. This period may have been too short to discriminate between responders and non-responders as the median duration of SD was only 10 weeks. Because this treatment regimen would be considered to be cytostatic, rather than cytotoxic, the activity of treatment could only

be confirmed during a later stage of treatment, which is also seen with LDM paclitaxel applied as a continuous infusion [30]. Only one patient had SD of more than three months. This was the only patient with a marked increase of TSP-1 levels to 143% and one of the three patients with an increase of TSP-1 gene expression to 150% of baseline value. The last possible explanation is that the activity of LDM treatment is mainly based on a direct effect of paclitaxel on the EC in humans and that there is no effect mediated by TSP-1.

In conclusion, this study showed that the assays for circulating levels of TSP-1 and gene expression of TSP-1 and VEGF-A in PBMCs were validated for their purpose. Evaluation of the biomarkers in healthy volunteers over a period of three weeks showed that the biomarkers were easy to implement and had an acceptable variability within humans. Differences in the biomarker levels between the patients defined as responders and non-responders, based on presence or absence of stable disease after 6 weeks of LDM treatment with paclitaxel were, thus far, not observed. Further studies are needed to better define the role of TSP-1 as a biomarker of LDM treatment with paclitaxel.

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chapter 4

Gemcitabine elaidate

4.1

Phase I study of oral CP-4126, a gemcitabine derivative, in patients with advanced solid tumors

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Abstract

CP-4126 is a gemcitabine (2',2'-difluorodeoxycytidine; dFdC) 5' elaidic acid ester. The purpose of this dose-escalating study was to assess safety, pharmacokinetics (PK) and preliminary antitumor activity of the oral formulation and to determine the recommended dose (RD) for phase II studies. The study had a two-step design: a non-randomized dose-escalating step I with oral CP-4126 alone, followed by a randomized, cross-over step II that compared oral CP-4126 with dFdC i.v.. CP-4126 was given on days 1,8,15 in a 4-week schedule with increasing doses until the RD was established. 26 patients with different solid tumours were enrolled in step I at seven dose levels (100 – 3000 mg/day). The most frequent drug-related AEs were fatigue and dysgeusia, the majority being grade 1-2. One patient experienced a dose limiting toxicity after one dose of CP-4126 at 1300 mg/day (ASAT grade 3). PK of CP-4126 could not be determined. The metabolites dFdC and dFdU obeyed dose-dependent pharmacokinetics. Exposures to dFdC were about ten-fold lower compared to exposures after comparable doses of dFdC i.v.. Nine patients reached stable disease as best response, whereby in one patient with vaginal carcinoma a 25% reduction of tumor volume was reached. This study demonstrates that CP-4126 can be safely administered orally to patients up to 3000 mg/day in a d1,8,15 q4w schedule with a tolerable safety profile. CP-4126 acts as a prodrug for dFdC when given orally, but because of the poor absorption and the rapid pre-systemic metabolism the study was terminated early and no RD could be determined.

Introduction

CP-4126 is a gemcitabine (2',2'-difluorodeoxycytidine; dFdC) 5' elaidic acid ester. dFdC is a nucleoside analogue used in the treatment of patients with various solid tumors, including non-small cell lung cancer and pancreatic carcinoma [1]. dFdC is currently formulated as an i.v. solution and usually administered as 30-minute infusion at a dose of 1,000 to 1,250 mg/m2 on days 1 and 8 of a 21-day or days 1, 8, and 15 of a 28-day cycle. dFdC is a hydrophilic compound, which needs to be actively transported into cells by human equilibrative and concentrative nucleoside transporters (hENT and hCNT) to exert its anticancer activity with hENT1 being the primary transporter [2]. A mechanism of resistance to dFdC is a decreasing transport activity of nucleosides across the cell membrane [2–4].

When dFdC has been transported into the cell, it is phosphorylated by deoxycytidine kinase (dCK) to its active diphosphate and triphosphate forms (respectively dFdCDP and dFdCTP) [3,5]. dFdCDP slows the synthesis and repair of DNA by inhibition of ribonucleotide reductase [6], which also subsequently leads to an increase in dCK activity. The other active form, dFdCTP, competes with deoxycytidinetriphosphate for incorporation into DNA, thereby inhibiting DNA polymerase and preventing the activity of DNA repair enzymes [7] (see figure 1). At high concentrations, it also directly inhibits deoxycytidine monophosphate deaminase, resulting in decrease in gemcitabine catabolism [8]. The main metabolite of dFdC, 2',2-difluorodeoxyuridine (dFdU), is formed by deoxycytidine deaminase, which is present at high levels in plasma, red blood cells and liver [9,10].

Besides the general advantages of an intravenous-to-oral switch in anticancer chemotherapy [11], oral gemcitabine has a potential to be applied on a low-dose metronomic treatment regime [12,13]. Several preclinical studies have shown that gemcitabine has anti-tumor and anti-angiogenic properties when used chronically and continuously at low-daily dose [14,15].

There are two previously published clinical studies with an oral dosage form of gemcitabine, one of which with oral administration of gemcitabine itself [16]. This study was terminated because of high presystemic conversion of dFdC to dFdU and accompanying accumulation of dFdU, which most likely contributed to severe liver toxicity. The second dosage form tested orally was the prodrug LY2334737, which was designed to overcome the pre-systemic deamination of gemcitabine during first pass metabolism [17]. LY2334737 has valproic acid bound to the metabolically unstable amine group and is therefore considered not to be a substrate for deaminase [18].

CP-4126 was originally developed as an intravenous formulation. It was synthesized in order to improve the clinical activity of gemcitabine [19]. An elaidic fatty acid (trans-9-octadecenoic fatty acid) was esterified at the 5'position of dFdC, which gives CP-4126 two advantages over dFdC. The first advantage is that unlike dFdC, CP-4126 can traverse cell membranes by passive diffusion, followed by intracellular conversion to gemcitabine by esterases. This means that uptake of CP-4126 in the cell is independent of nucleoside transporters and thereby relatively independent of multidrug resistance mechanisms mediated by down regulation of hENT and hCNT expression [20]. Since hENT can operate

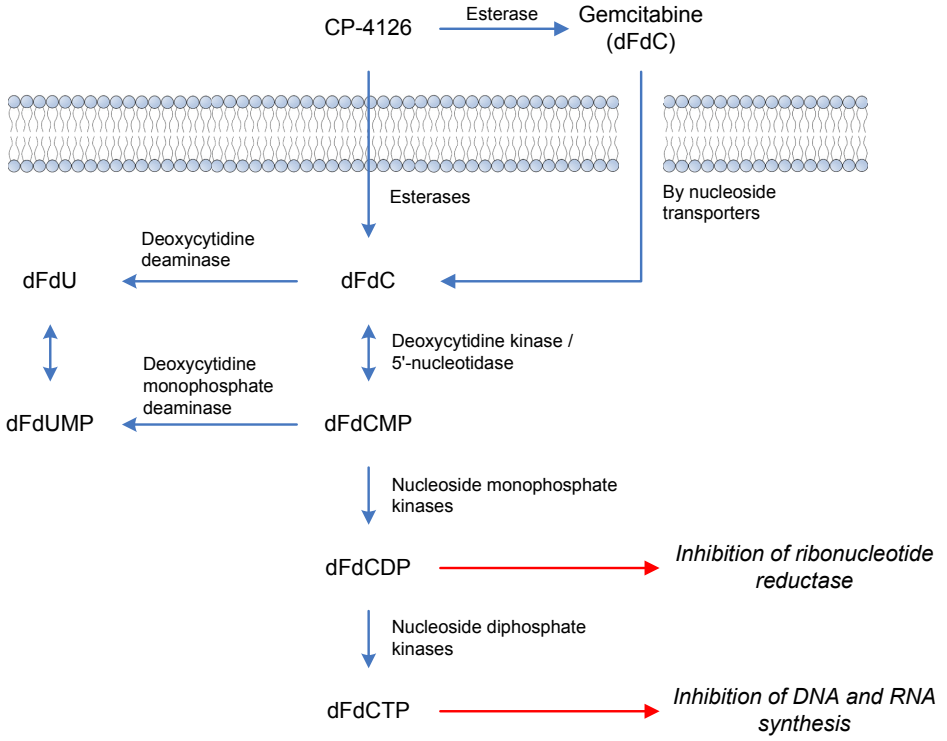

Figure 1 Cellular uptake, activation and deactivation of CP-4126 (dFdC = gemcitabine, dFdCMP = gemcitabine monophosphate, dFdCDP = gemcitabine diphosphate, dFdCTP = gemcitabine triphosphate, dFdU = 2',2-difluorodeoxyuridine, dFdDUMP = 2',2-difluorodeoxyuridine monophosphate)

as export as well as uptake transporters, the clinical activity could be improved by cellular accumulation and prolonged retention of the active metabolites [21]. Secondly, like LY2334737, CP-4126 appears not to be a substrate for deaminase [20], although the fatty acid is bound to the 5'position of dFdC and not to the metabolically unstable amine group. In a preclinical study in dogs, CP-4126 could be given orally, where it acts as a prodrug for gemcitabine with a high apparent systemic availability of dFdC [20]. Because of these potential advantages, the aim of this study was to explore whether oral CP-4126 is a prodrug of dFdC and whether it is possible to achieve pharmacologically significant systemic exposure to dFdC after oral administration of CP-4126.

In this dose escalation phase I study, patients with advanced solid tumors were treated with oral CP-4126. The primary objective was to determine the recommended dose for phase II studies (RD) of oral CP-4126. The secondary objectives were to determine the safety, pharmacokinetics (PK) of CP-4126, dFdC and dFdU, and preliminary antitumor activity of the oral formulation.

Patients and Methods

Eligibility

Patients with histologically or cytologically confirmed locally advanced or metastatic solid tumors, for whom there remained no known effective treatment, were eligible. Patients with symptomatic brain metastases were excluded. Patients needed to be 18 years of age or older and have a performance status 0 – 2 according to the Eastern Cooperative Oncology Group (ECOG) Performance Status (PS) Scale. The life expectancy needed to be be longer than 3 months and the bone marrow, haematological and biological functions had to be adequate (neutrophil count of ≥ 1.5 * 10^9/L, platelets of ≥ 100 * 10^9/L, and hemoglobin of ≥ 10 g/dL; alanine transaminase (ALT) and aspartate transaminase (AST) ≤ 2.5 times institutional upper limit of normal (ULN) (if liver metastases, AST/ALT ≤ 5 times ULN and alkaline phosphatase ≤ 2.5 times ULN), bilirubin of ≤ 1.5 times the ULN (liver metastases: ≤ 2 times the ULN); serum creatinine ≤ 1.5 of the ULN). Main exclusion criteria were current peripheral neuropathy of grade 1 or higher, mucositis of the upper digestive tract, or if the patient had received previous anticancer therapy (chemotherapy, hormonal therapy or immunotherapy) within 30 days prior to the first dose of oral CP-4126 (6 weeks for mitomycin C and BCNU (= carmustine) and CCNU (=lomustine)). Also radiotherapy to more than 30% of bone marrow or single dose radiation up to 8 Gy, less than one week prior to the study treatment or to the upper GI tract was an exclusion criterion. The study protocol was approved by the local Medical Ethics Committee and all patients had to give written informed consent.

Drug formulation

For the purpose of oral administration, CP-4126 was solubilised in a lipid-based formulation and encapsulated in non-gelatine hard-shell capsules for oral application. The capsules were provided by Clavis Pharma in two strengths: 100 mg and 250 mg.

Study design and treatment schedule

This phase I study was a first-in-human, non-randomized, multicentric, open-label, dose escalation study of oral CP-4126 as monotherapy. CP-4126 capsules were administered once a day on days 1,8,15 in a 4-week schedule (d1,8,15 q4w). CP-4126 capsules were taken in fasting conditions (i.e., in the morning, at least 2hrs after and at least 1hr before food intake) since the effect of food on the pharmacokinetics of the drug was unknown. The starting dose was 100 mg. Three to six patients were enrolled at each dose-level (DL), according to predefined dose escalation decision rules. The doses were increased based on safety evaluations per dose-level. The dose limiting toxicities (DLTs) were defined as any of the following events occurring during the first treatment cycle and related to study treatment: non-hematologic AE of CTCAE grade ≥3 (excluding alopecia), nausea and vomiting (only after adequate systemic antiemetic medication), neutropenia CTCAE grade 4 (i.e., neutrophils <0.5 x 10^9/L, lasting ≥5 days), febrile neutropenia, (i.e., neutrophils < 1.0 x 10^9/L with fever at least 38.5 °C), thrombocytopenia CTCAE grade 4 and treatment delay >1 week/cycle due to treatment related toxicity. The RD was defined as the highest DL where ≤ 1 of 6 patients experience a DLT.

Safety evaluation and treatment adjustments
Pre-treatment evaluation included a complete medical history, physical examination, ECG, vital signs, performance status, assessment of adverse events using CTCAE v3.0, the use of concomitant medications, pregnancy test, laboratory assessment of hematology, serum chemistry and urinalysis and a tumor assessment. Before each administration a physical examination, assessment of adverse events and notation of concomitant medication were repeated and hematology and serum chemistry were checked.

The next treatment cycle was allowed to be delayed for a maximum of one week (maximum duration of five weeks per cycle), and at least five out of the six dose administrations had to be given during the previous two cycles. In cases where subsequent treatment cycles were delayed for more than one week or if the patient had received less than five dose administrations within the previous two cycles the patient was withdrawn from the study. If the patient was obtaining clinical benefit and was able to continue treatment at a dose-level decided upon by the investigator, in agreement with the sponsor and the Project Medical Advisor (PMA), the patient was allowed to continue if in his/her best interest. If a patient had experienced a DLT, further treatment was allowed at a lower dose if the patient fulfilled the inclusion/exclusion criteria, the investigator expected clinical benefit from further treatment, and the investigator, the PMA and the sponsor were in agreement. Patients treated according to an adjusted treatment and/or modified dose were evaluated for safety and efficacy at this DL, but any DLTs that developed at the new DL were not added to the total number of DLTs at that dose-level or to the study.

Pharmacokinetics
The pharmacokinetics of CP-4126, dFdC and dFdU were monitored on Day 1 of the first cycle of the study. The sampling times were pre-treatment, 0.5, 1, 1.5, 2, 3, 4, 8 and 24 hrs. Blood samples were collected for study drug analysis in tubes containing the enzyme inhibitors potassium fluoride and tetrahydrouridine in addition to lithium heparin as anticoagulant. Plasma samples were measured by York Bioanalytical Solutions using a validated high-performance liquid chromatography tandem mass spectrometry (HPLC/MS/MS) method to determine the plasma concentrations of CP-4126, dFdC and dFdU. For the determination of CO-1.01, the analog CP-4055 was used as internal standard and ARA-U was used for the determination of dFdC and dFdU. Sample pre-treatment was done by protein precipitation with acetonitrile. A volume of 100 µL human plasma was processed. After mixing, samples were centrifuged and a part of the clear supernatant was diluted and submitted for the CP-4126 LC-MS/MS analysis. The other part was dried under a gentle stream of nitrogen. Residues were reconstituted with ammonium acetate and submitted for the dFdC/dFdU LC-MS/MS analysis. Detection was performed in positive ion mode on a tandem MS with a Turbo Ion Spray Interface (MDS Sciex API 3000). The transitions for CP-4126 were selected from m/z 528 to 264 and for the internal standard from *m/z* 508 to 112. The transitions for dFdC and dFdU were selected from m/z 264 to 112 and 265 to 113, respectively and for the internal standard from m/z 245 to 115. PK parameters, including C_{max}, T_{max}, AUC, and $t_{1/2}$ were calculated using WinNonlin Pro version 5 (Pharsight Corporation, USA).

Anti-tumor activity

Tumor measurements were recorded using RECISTv1.1 [22]. Responses based on other markers, for example prostate-specific antigen (PSA), were also recorded.

Table 1 Patient demographics

Character	N	%
Total number of evaluable patients	26	
Sex		
Male - female	8-18	
Age		
Median (range)	62 (42-72)	
ECOG performance status		
0	6	23%
1	17	65%
2	3	12%
Ethnic origin		
Caucasian	26	100%
Pathological diagnosis		
Colorectal	8	31%
Pancreas	5	19%
Breast	3	12%
Cholangiocarcinoma	2	8%
Other	8	31%
Stage of cancer		
Metastatic	24	92%
Prior Treatment		
Chemotherapy	26	100%
Radiotherapy	11	42%
Surgery	19	73%

Results

Patient characteristics and disposition

A total of 26 patients were enrolled in the dose-escalation study divided over seven dose-levels (100, 200, 400, 800, 1300, 2000 and 3000 mg/day). The median age was 62 years (range, 42-72 years) and 88% had an ECOG performance status ≤1. Almost 70% were females and all patients were Caucasians (table 1). All enrolled patients received at least one dose of CP-4126, of which the majority (88%) completed at least one cycle. The median number of cycles entered on treatment for all patients in the seven dose-levels was 2 (range 1-4), 2, 2 (range 1-2), 2 (range 2-5), 2.5 (range 1-8), 4 (range 2-4), 1.5 (range 1-6) respectively. In total 21 patients (81%) discontinued study treatment permanently due to progressive disease, the other patients discontinued treatment as a result of one or more adverse events (all ≥ grade 3, one event at the 1300 mg level was fatal). None of the patients discontinued due to toxicity related to CP-4126. Reductions were equally distributed among all dose-levels and there were no clear differences in dose delays and interruptions between the dose-levels.

The RD was not reached, as the study was stopped because of the poor absorption and subsequently low blood levels of the molecule.

Table 2 Adverse events (incidence rate of at least 5% in the total safety population) with a possible, probable or definite relationship to CP-4126. Grade ≥ 3 events are set between parentheses. The percentages are based on the number of patients in the safety population (n=26)

	100 mg	200 mg	400 mg	800 mg	1300 mg	2000 mg	3000 mg	All grades		Grade ≥ 3	
	n = 3	n = 4	n = 3	n = 3	n = 6	n = 3	n = 4	n	%	n	%
At least one adverse event	1 (0)	1 (1)	2 (0)	3 (1)	4 (2)	3 (1)	2 (0)	16	(62%)	5	(19%)
Fatigue		1 (1)	1 (0)	1 (0)	4 (1)	2 (1)	1 (0)	10	(38%)	3	(12%)
Dysgeusia				2 (0)	1 (0)	1 (0)	1 (0)	5	(19%)	0	(0%)
Liver dysfunction (AST/ALT/γGT)				2 (1)	1 (1)			3	(12%)	2	(8%)
Nausea		1 (1)				2 (0)		3	(12%)	1	(4%)
Decreased appetite					2 (0)	1 (0)		3	(12%)	0	(0%)
Influenza like Illness					1 (0)	1 (0)	1 (0)	3	(12%)	0	(0%)
Vomiting		1 (0)				1 (0)		2	(8%)	0	(0%)
Pyrexia					2 (0)			2	(8%)	0	(0%)
Dyspnoea					1 (0)	1 (0)		2	(8%)	0	(0%)

Safety and tolerability

All patients treated (n=26) were evaluated for treatment-related adverse events. Table 2 lists all adverse events which are possibly, probably or definitely related to the study drug with an incidence rate of at least 5%. Oral CP-4126 at the dose levels studied was overall well tolerated. The most common drug-related adverse event was fatigue (38%), the majority being grade 1-2. Also dysgeusia (19%) and gastrointestinal disorders (mainly nausea and vomiting, 23%) were often related to the study drug. There were no adverse events of neutropenia or thrombocytopenia reported during this study. One patient experienced a DLT after one dose of CP-4126 at 1300 mg/day. The aspartate transaminase (AST) in this patient reached more than five times the upper limit of normal (CTCAE grade 3). Four patients experienced fatal adverse events of which two of the deaths occurred within 30 days after the last dose of CP-4126. All fatal adverse events were considered unrelated to the study drug. In three cases this was due to disease progression and in one case the patient developed a fatal event of dyspnoea approximately 2 months after starting treatment which also was considered unrelated to study drug. The death occurred approximately 35 days later.

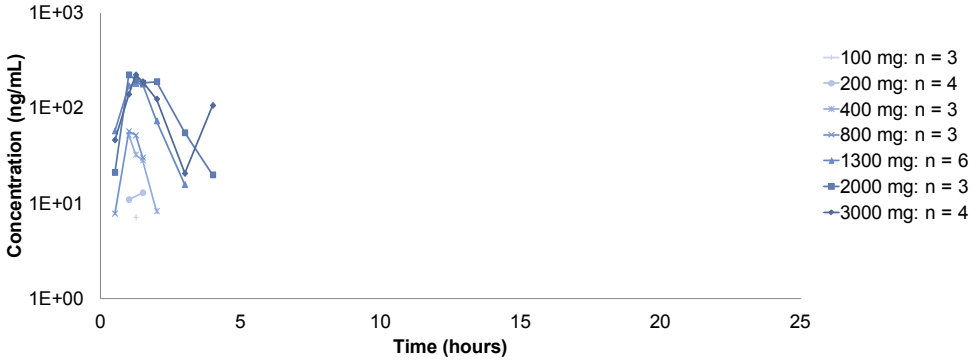

Figure 2 Mean plasma concentration-time curve of dFdC in patients treated with CP-4126 by oral capsule administration on day 1 of treatment

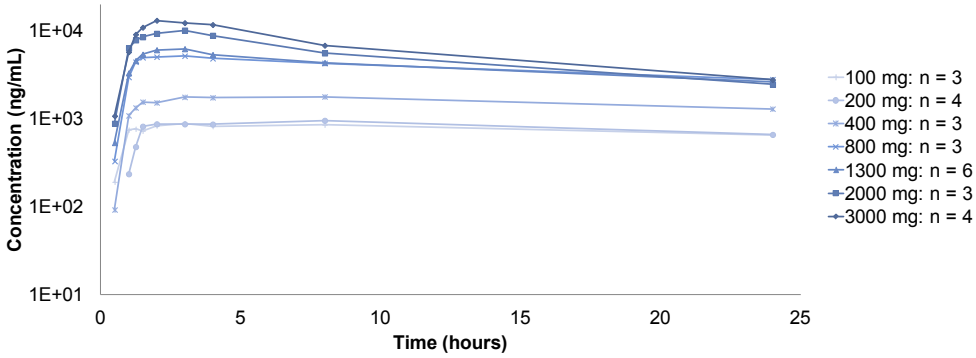

Figure 3 Mean plasma concentration-time curve of dFdU in patients treated with CP-4126 by oral capsule administration on day 1 of treatment

Table 3 PK parameters of dFdC and dFdU per dose-level (the geometric mean and coefficient of variation are given)

Dose	N	dFdC						dFdU					
		C_{max} (ng/mL)		T_{max} (h)		AUC_t (ng·h/mL)		C_{max} (ng/mL)		T_{max} (h)		AUC_t (ng·h/mL)	
100 mg	3	30.1	(51%)	1.17	(62%)	–	–	1,060	(32%)	6.31	(77%)	18,400	(9%)
200 mg	4	32.9	(96%)	1.07	(41%)	33.3	–	1,170	(70%)	5.14	(66%)	18,900	(54%)
400 mg	3	52.2	(93%)	1	–	73.2	–	2,060	(11%)	4.18	(81%)	37,100	(8%)
800 mg	3	67.6	(40%)	1.33	(44%)	72	(64%)	6,540	(27%)	1.92	(49%)	91,300	(18%)
1300 mg	6	232	(66%)	1.6	(49%)	272	(77%)	7,070	(30%)	2.48	(43%)	95,400	(32%)
2000 mg	3	273	(39%)	1.49	(34%)	423	(61%)	11,500	(26%)	2.42	(41%)	122,000	(3%)
3000 mg	4	345	(52%)	2.04	(69%)	578	(69%)	15,000	(31%)	2.34	(53%)	151,000	(27%)

C_{max} = Maximum measured plasma concentration, T_{max} = Time to C_{max}, AUC_t = Area under the plasma concentration time curve until last data point

Pharmacokinetic results
Pharmacokinetic blood samples were drawn from all patients on the first day of the study. PK profiles composed for dFdC and dFdU are shown in figure 2 and 3. Plasma concentrations of CP-4126 were only quantifiable in a few patients at the highest dose-levels around 2 hours (range 1-4 hr) post dose. There was no consistent relationship between the C_{max} and the dose.

The observed C_{max} and T_{max} and calculated AUCt values for dFdC and dFdU per dose-level are presented in table 3. The increase in systemic exposure of dFdC was proportional to the dose increase of CP-4126 (200 to 3000 mg). Also the systemic exposure of dFdU increased with increasing dose. However, these increases were less than the proportionate dose increase. For the majority of patients C_{max} of dFdC and dFdU were reached at 1.4 hours (range 0.5-4.1 hr) and at 3.4 hours (range 1-10.5 hr) after dosing, respectively, independent of the dose. The mean terminal half-life of dFdC and dFdU could only be determined in the highest dose-levels and were respectively ~0.5 hr and ~9.0 hr.

Anti-tumor activity
Anti-tumor activity was a secondary endpoint for this study. No complete or partial responses were achieved. Nine patients reached stable disease as best response (35%), whereby in one patient a 25% reduction of tumor volume from baseline was reached (vaginal carcinoma). This patient had progression-free survival of more than 7 months.

Discussion
This report describes the first study of an oral formulation of CP-4126, an elaidic acid ester of gemcitabine. In this study the tolerability, safety and pharmacokinetics of CP-4126 were explored. Seven dose-levels were evaluated in this study, ranging from 100 mg to 3000 mg. The treatment was overall well tolerated. Most reported adverse events were fatigue (38%) and dysgeusia (19%). The most commonly reported adverse drug reactions associated with i.v. treatment of gemcitabine are nausea with or without vomiting, elevated liver transaminases (AST/ALT) and alkaline phosphatase, all reported in approximately 60% of patients [23]. This profile is not seen in this population at the dose levels studied, probably because of the lower exposures of dFdC compared to the exposures after i.v. treatment of gemcitabine.

Plasma concentrations of CP-4126 were only quantifiable in 16 of the 260 samples from 26 patients, without a consistent relationship between the dose of CP-4126 and the maximum concentrations or the exposures to CP-4126. The low amount of CP-4126 detected in plasma corresponds with PK data in dogs and indicates a rapid pre-systemic metabolism of this parent drug after oral administration [20]. This is probably due to the amount of carboxylesterases in liver and intestines, because these esterases are most likely responsible for the conversion of CP-4126 outside as well as inside the cell [24]. dFdC and dFdU concentrations were detectable in all patients. For both metabolites, the C_{max} and exposure increased generally less than dose-proportionally with the dose, suggesting a capacity-limited absorption of CP-4126.

The exposures to dFdC (and dFdU) are significantly lower compared to the exposures after comparable doses of gemcitabine applied intravenously (on molar basis) [25,26]. Although the comparison is made with patients from other studies, the exposure of dFdC seems to be about ten-fold lower after oral administration (apparent systemic availability of dFdC is 5%-10%). For dFdU the AUC is relatively higher, but still only 20%-78% in comparison with gemcitabine i.v.. These lower exposures after oral administration indicate low absorption of CP-4126 in humans. In dogs, however, oral CP-4126 acts as a prodrug for gemcitabine and the exposures of dFdC and dFdU were in the same range as the exposures after i.v. administration of CP-4126 (the apparent systemic availability is in the range of 92%-169%, depending on the dose) [20]. In dogs, the half-life of dFdC after oral administration of CP-4126 was longer than the half-life after i.v. administration of dFdC (unpublished data). In humans, the terminal half-life of dFdC after oral administration of CP-4126 is equal to the half-life after i.v. administration dFdC [25] and significantly shorter compared to the half-life after i.v. administration of CP-4126 (0.5 hr versus 9 hr [27]). These PK results indicate that a substantial amount of parent drug which is absorbed in the intestines is directly metabolized to dFdC and in consequence to dFdU. Due to the high levels of deoxycytidine deaminase in the liver, the relatively high concentrations of dFdU after the rapid hydrolysis of the elaidic acid of CP-4126 are expected. These results show a large pre-systemic metabolism of both CP-4126 and dFdC after oral administration.

Due to the low exposure of CP-4126 and gemcitabine, once weekly administration of CP-4126 is not feasible as a possible alternative for gemcitabine i.v.. A more chronic administration of CP-4126 could be an option as seen in preclinical studies [20]. Comparable treatment regimens are investigated with alternative oral dosage forms of gemcitabine in two other studies [16,18]. In one study, oral gemcitabine was dosed every other day and the highest dose administered was 20 mg (equivalent to 40 mg CP-4126), before the study was terminated because of severe liver toxicity by dFdU accumulation. In another study, the prodrug LY2334737 was given once daily and the highest dose was 50 mg (equivalent to 70 mg CP-4126). A direct comparison with oral CP-4126 is difficult because of the difference in doses and treatment regimens, but the difference in the exposure ratio dFdU/dFdC between LY2334737 (range 216-404) and CP-4126 (range 261-1268) confirms the rapid pre-systemic metabolism of CP-4126 and dFdC (range after i.v. administration of dFdC is 31-201 [25]). Due to the long terminal half-life, dFdU accumulates during the first weeks after the metronomic treatment regimens. Although a clear causal relationship is lacking, the hepatotoxicity after chronic oral administration of both gemcitabine as LY2334737 is linked to the accumulation of dFdU and consequently the accumulation of phosphorylated dFdU (via deamination of gemcitabine monophosphate to dFdUMP and via phosphorylation of dFdU) in the liver, which can be incorporated into DNA and RNA [28]. The expected accumulation of dFdU by pre-systemic metabolism after more chronic administration of oral CP-4126 would make metronomic treatment regimens unsuitable.

In conclusion, this study demonstrates that CP-4126 can be safely administered orally to patients up to 3000 mg/day in a d1,8,15 q4w schedule. The best response was stable disease, which was seen in nine patients (35%) and one patient had a progression-free survival of over 7 months. The most reported related toxicities were fatigue and dysgeusia.

CP-4126 acts as a prodrug for gemcitabine when given orally, but because of the poor absorption, the rapid pre-systemic metabolism and consequently the amount of capsules the patient had to take (12 capsules at the highest dose), the study was terminated before the RD had been determined. Due to these findings, the development of oral CP-4126 was stopped and further research is focusing on the i.v. variant.

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 ClinicalTrials.gov identifier is NCT00778128. W. Rasch, T. Bergeland and P.-A. Hals are employed at Clavis Pharma. W. Rasch owns stock in Clavis Pharma. The other authors declare that they have no conflict of interest.

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4.2

A phase I comparative pharmacokinetic and cardiac safety study of two intravenous formulations of CO-101 in patients with advanced solid tumors

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Abstract

CO-101 (gemcitabine-elaidate) is a lipid-drug conjugate of gemcitabine. Primary objective was to compare pharmacokinetic (PK) profiles of two i.v. formulations of CO-101 (which differed in drug/solubilizer concentration ratio) for bioequivalence. Secondary endpoints included toxicity, efficacy and effects on QT interval. This study had a two-stage design with sample size re-estimation with a planned interim analysis after 12 patients. Urine and venous blood samples were collected for PK analysis. 11 evaluable patients were enrolled in stage 1. Given the low within-patient variability (14.7%), the study was adequately powered (81%). AUC0-inf and C_{max} of CO-101 after administration of the test formulation were respectively 28% and 23% lower than after the reference formulation. Geometric least square mean ratio of the AUCs was 72% (90% CI: 64%-81%). Mean plasma and urine PK parameters of the two metabolites of CO-101 were comparable. Four patients achieved disease control lasting > 5 months. CO-101 was well tolerated, in addition no effect on QT/QTc interval was observed. Based on the AUC0-inf and C_{max} of CO-101, the two formulations were not bioequivalent. This 2-stage adaptive design is an efficient design for comparative PK studies in patients when intra-patient PK variability is not known at the start of the study.

Introduction

CO-101 (also known as CP-4126) is a gemcitabine (2′,2′-difluorodeoxycytidine; dFdC) elaidic acid ester, developed to improve the clinical activity of dFdC [1]. dFdC is a nucleoside analogue and used in the treatment of patients with various solid tumors, including non-small cell lung cancer and pancreatic carcinoma [2]. dFdC is an hydrophilic compound, that needs to be transported into the cells by human equilibrative and concentrative nucleoside transporters (hENT and hCNT) to exert its anticancer activity with hENT1 being the primary transporter [3]. A growing body of literature has shown that tumors with low levels of hENT1 respond poorly to nucleoside analogues [4,5]. Moreover, a mechanism of resistance to dFdC is a decreased transport activity of nucleosides over the cell membrane [3,6,7]. By the esterification of the 5′ position of dFdC with an elaidic fatty acid (trans-9-octadecenoic fatty acid), CO-101 can traverse cell membranes by passive diffusion, followed by intracellular conversion to dFdC (figure 1). This means that uptake of CO-101 in the cell is independent of nucleoside transporters and might be more effective in patients with low levels of hENT1 in tumors [8–10]. Since hENT1 can operate as uptake as well as export transporter, the clinical activity could be improved by cellular accumulation and prolonged retention of the active metabolites [11].

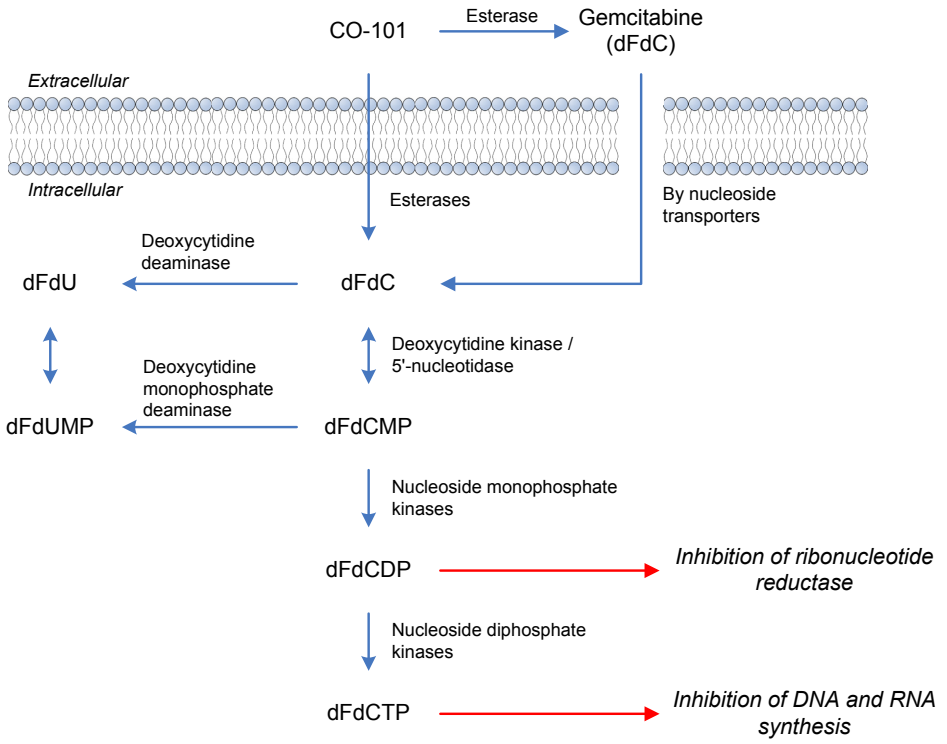

Figure 1 Cellular uptake, activation and deactivation of CO-101. (dFdC = gemcitabine, dFdCMP = gemcitabine monophosphate, dFdCDP = gemcitabine diphosphate, dFdCTP = gemcitabine triphosphate, dFdU = 2′,2-difluorodeoxyuridine, dFdDUMP = 2′,2-difluorodeoxyuridine monophosphate)

Within the cell, CO-101 is hydrolysed to dFdC which is phosphorylated by deoxycytidine kinase (dCK) to its active diphosphate and triphosphate forms (dFdCDP and dFdCTP, respectively) [6,12]. dFdCDP slows the synthesis and repair of DNA by inhibition of ribonucleotide reductase [13], which also subsequently leads to an increase in dCK activity. The other active form, dFdCTP, competes with deoxycytidinetriphosphate for incorporation into DNA, thereby inhibiting DNA polymerase and preventing the detection and repair of DNA repair enzymes [14] (see figure 1). The main metabolite of dFdC, 2',2-difluorodeoxyuridine (dFdU), is formed by deoxycytidine deaminase, which is present at high levels in plasma, red blood cells and in the liver [15,16].

At the start of this study, two phase II studies with CO-101 were ongoing for patients with advanced pancreatic cancer [17,18]. The pharmaceutical formulation of CO-101 used in these clinical studies contains 15 mg/mL of gemcitabine elaidate solubilized in purified phospholipids. Recently, another formulation of CO-101 has been developed, which contains 30 mg/mL of gemcitabine elaidate solubilized in purified phospholipids. The ratio of drug and solubilizer concentration may have an impact on the systemic pharmacokinetics (PK) of the drug. Therefore, a bioequivalence study was necessary before introduction of the novel formulation. The composition and concentration of all other excipients were the same between the formulations. The aim of this study was to compare PK bioequivalence of both formulations of CO-101 (15 mg/mL, reference formulation and 30 mg/mL, test formulation). Effects on QT/QTc intervals, overall tolerability, toxicity and efficacy of CO-101 were also determined.

Patients and Methods

Study design and treatment schedule

This study used a multicenter, two-stage, open-label, randomized, two treatment period by two sequence crossover design. Patients received 1250 mg/m^2/day CO-101 given in a day 1, 8, and 15 q4w schedule by a 30-min infusion. Two different formulations of CO-101 were used in this study. Formulation A (15 mg/mL) is the reference clinical formulation [17,18] and formulation B (30 mg/mL) is the test formulation. In both formulations C0-1.01 was solubilized in purified phospholipids. Patients received different formulations of CO-101 on day 1 and day 8. From day 15, treatment could continue with formulation A until tumor progression or intolerable toxicity (figure 2) in a treatment extension period. The study protocol was approved by the local Medical Ethics Committee and all patients had to give written informed consent. The ClinicalTrials.gov identifier is NCT01392976.

A two-stage sequential design with sample size re-estimation was implemented, since there was little knowledge about the within-patient variability for either formulation of CO-101. The adaptive sample size sequential method as described by Potvin[19] was used and is accepted by regulatory agencies (Center for Drug Evaluation and Research of the US Food and Drug Administration and the European Medicines Agency) [20]. This design included a planned interim analysis after 12 patients (stage 1) using the observed intra-patient PK variability to either stop early in case of sufficient power or to calculate the number of additional patients in stage 2. Based on the intra-patient variability of exposure in a first- in-man dose escalation study of 16.5% [21], twelve patients would provide 80%

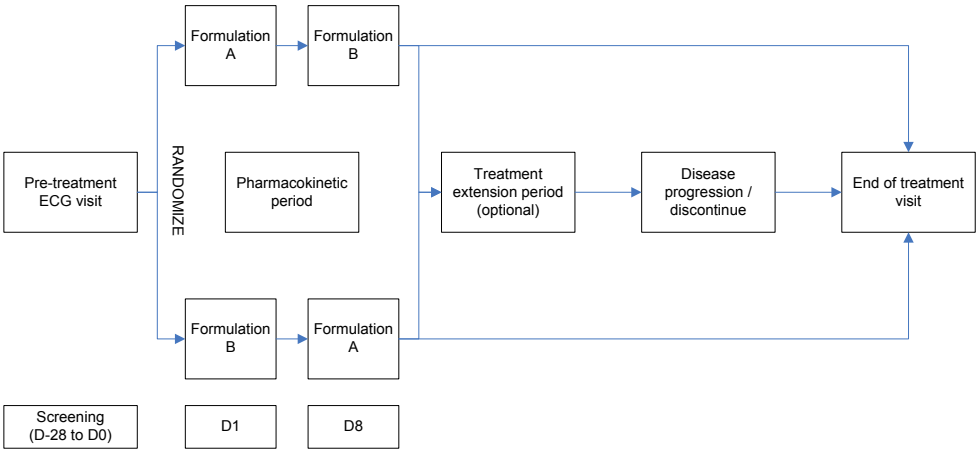

Figure 2 Study Scheme

power to show that the 90% confidence interval (CI) of the geometric least square (LS) mean ratio (test/reference) of area under the concentration time curves (AUC0-inf, exposure) will fall in the range of 0.80 to 1.25 with an intra-patient variability of 15% and an expected difference of 5% between the two treatment groups. If the primary objective was not met with 80% power based on the interim analyses, the total number of patients was calculated for stage 2 to ensure that the primary objective would be achieved with 80% power and a maximum of 36 patients.

Eligibility

Patients were eligible if they were diagnosed with a histologically confirmed solid tumor malignancy that was metastatic or unresectable, if there were no standard curative or palliative treatment options available and if CO-101 treatment was considered appropriate. Patients with symptomatic brain metastases were excluded. The patients were 18 years of age or older and had a performance status 0 – 1 according to the Eastern Cooperative Oncology Group (ECOG) performance status (PS) scale. The life expectancy was longer than 3 months and the bone marrow, haematological and biological functions were adequate (neutrophil count of ≥ 1.5 * 10^9/L, platelets of ≥ 100 * 10^9/L, and hemoglobin of ≥ 9 g/dL; alanine transaminase (ALT) and aspartate transaminase (AST) ≤ 3 times institutional upper limit of normal (ULN) (if liver metastases, AST/ALT ≤ 5 times ULN), bilirubin of ≤ 2 times of the ULN, albumin > 3 g/dL; serum creatinine ≤ 1.5 of the ULN). Patients with clinically significant abnormal 12-lead ECG or QTc F >450 msec (males) or > 470 msec (females), PR > 240 msec, or a QRS > 10 msec, an implantable pacemaker or defibrillator, a family history of long QT syndrome or concomitant treatment with any medication known to produce QT prolongation were excluded. Other exclusion criteria were allergy to dFdC or eggs, pregnancy, breastfeeding, refusal to use adequate contraception and previous anticancer therapy (radiation, surgical procedures, chemotherapy, hormonal therapy or immunotherapy) within 14 days prior to the first dose of oral CO-101.

Study procedures
Pre-treatment evaluation included a complete medical history, physical examination (including vital signs and performance status), evaluation of adverse events using CTCAE v4.0, the use of concomitant medications, pregnancy test, laboratory assessment of hematology, serum chemistry and urinalysis and determination of tumor status. Before each administration a physical examination, assessment of adverse events and notation of concomitant medication were repeated and hematology and serum chemistry were checked. Tumor response was calculated from tumor measurements performed at screening and at day 22 (±1 week) of even-numbered cycles (i.e., Cycle 2, Cycle 4, Cycle 6, etc) and was interpreted with RECIST 1.1 criteria [22].

Pharmacokinetics
The pharmacokinetics of CO-101, dFdC and dFdU were monitored during day 1 and 8 of the first cycle of the study. Venous blood samples for the PK analysis of CO-101 were withdrawn through an peripheral intravenous cannula before infusion and 0.5 (end of infusion), 0.67, 0.83, 1, 2, 4, 6, 8, 10, 24 and 48 hours after start of the infusion. Blood samples were collected for study drug analysis in tubes containing the enzyme inhibitors potassium fluoride and tetrahydrouridine in addition to lithium heparin as anticoagulant. Urine was collected for PK of dFdC and dFdU in the following periods: pre-dose (–2 to 0 hour) and 0 to 8, 8 to 16, 16 to 24, 24 to 34, 34 to 42, and 42 to 48 hours after dosing. All samples were processed on ice and stored at –70°C.

CO-101, dFdC and dFdU concentrations in human plasma and dFdC and dFdU concentrations in urine were determined using HPLC-MS/MS assays validated by the Department of Pharmacy & Pharmacology, Slotervaart Hospital/The Netherlands Cancer Institute. Each analytical run consisted of at least two calibration curves which were injected at the beginning and the end of the run. QC samples were included in two sets of three (low, medium, high) and interspersed throughout the analytical run with the study samples. For the determination of CO-101, the analogue CP-4055 was used as internal standard (Ferro Pfanstiehl). Stable isotope labelled internal standards (Toronto Research Chemicals Inc) were used for the LC-MS/MS determination of dFdC and dFdU. Sample pre-treatment of plasma involved protein precipitation with acetonitrile. A volume of 100 µL human plasma was processed. After mixing, samples were centrifuged and a part of the clear supernatant was diluted and submitted for the CO-101 LC-MS/MS analysis and the remaining was dried under a gentle stream of nitrogen. For urine samples, a volume of 100 µL was processed. After adding internal standard solution and mixing, samples were centrifuged and all the clear supernatant was dried under a gentle stream of nitrogen. Both residues were reconstituted and submitted for the dFdC/dFdU LC-MS/MS analysis. Detection was performed in positive ion mode on a Quadrupole MS/MS detector with a Turbo Ion Spray Interface (API4000, ABSciex, Foster City, USA). The transitions *m/z* for CO-101 were from *m/z* 528 -> 112 and for the internal standard from *m/z* 508 -> 112. The validated concentration range is from 0.5 to 1,000 ng/mL. The transitions for dFdC were from *m/z* 264 -> 112 and for the internal standard *m/z* 267 -> 115. The validated concentration range was from 0.5 to 1,000 ng/mL. The transitions for dFdU were selected as *m/z* 265 -> 113 and for the internal standard from *m/z* 268 -> 116. The validated

concentration range is from 25 to 50,000 ng/mL.

Pharmacodynamics

Pharmacodynamic effects on QT/QTc intervals were monitored by continuous electrocardiography (ECG) using a digital Holter device and by 12-lead ECG. Continuous measurements were conducted during a pretreatment ECG visit and during treatment day 1 and 8. The pretreatment ECG visit was performed within 5 days prior to day 1, cycle 1. Triplicate ECGs were extracted from the Holter data 10 minutes before each PK sample time (or equivalent time during pretreatment ECG visit). In addition, triplicate 12-lead ECGs were taken pre-dose and immediately after dosing on days 1 and 8 and at equivalent time points during the pretreatment ECG visit. Other triplicate 12-lead ECGs were taken prior to each PK sample 24 and 48 hours post-dose. The pretreatment and on-treatment pharmacodynamic 12-lead ECGs and the continuous Holter ECG recordings were analyzed at a central ECG laboratory (Cardiocore).

Results

Patient characteristics and disposition

24 patients were screened and 17 eligible patients were enrolled into stage 1, to ensure that 12 would be evaluable for PK. The median age of the patients was 58 years (range: 40-73 years) and nine (53%) had an ECOG performance status of 0. All patients were Caucasians and nine (53%) patients were males. Both groups were well balanced in terms of patient demographics (table 1). All 17 patients entered the treatment extension period. In total 14 patients (82%) discontinued study treatment permanently due to progressive disease and two patients withdrew consent. One patient is currently ongoing in the study.

Pharmacokinetic results

The pharmacokinetics of CO-101, dFdC and dFdU were monitored during day 1 and 8 of the first cycle of the study. Six patients were not suitable for the PK-evaluable population, because of infusion duration deviations (n=2), blood sampling deviations at the end of infusion (n=2) and two patients did not receive both formulations A and B during the pharmacokinetic period, owing to a prescription error (n=1) and an adverse event of elevated liver transaminases (n=1). Of the total 17 patients enrolled in stage 1, this left 11 patients for the PK-evaluable population. Mean plasma concentration–time curves composed for CO-101, dFdC and dFdU after both formulations are depicted in figure 3 and the results of the non-compartmental pharmacokinetic analysis are shown in table 2. There was no effect of treatment order.

The mean plasma concentration–time profile of CO-101 exhibited bi-exponential kinetics with maximum concentration levels observed at the end of the infusion period, a rapid decline over the next 1–2 hours followed by a slower rate of decrease in CO-101 plasma concentration after 2 hours post dose. The arithmetic mean maximum plasma concentration (C_{max}) following the infusion of formulation B (30 mg/mL) was approximately 30% lower than the arithmetic mean C_{max} after infusion of formulation A (15 mg/mL) (112 µg/mL (CV=32.9%) and 145.936 µg/mL (33.8%), respectively). Within 2 hours, i.e., 1.5 hour post termination of infusion, the levels of CO-101 were below 1,000 ng/mL.

Table 1 Patient demographics

	A,B N=8	B,A N=9	Total N=17
Gender			
male (%)	6 (75.0)	3 (33.3)	9 (52.9)
Age			
median (range)	57.5 (46–73)	64.0 (40–73)	58.0 (40–73)
BSA			
median (range)	1.9 (1.5–2.3)	1.9 (1.6–2.1)	1.9 (1.5–2.3)
ECOG at baseline, N (%)			
0	5 (62.5)	4 (44.4)	9 (52.9)
1	3 (37.5)	5 (55.6)	8 (47.1)
Primary tumor location, N (%)			
Bladder	2 (25.0)		2 (11.8)
Cholangiocarcinoma	1 (12.5)	1 (11.1)	2 (11.8)
Esophagus	1 (12.5)	1 (11.1)	2 (11.8)
Pancreas	1 (12.5)	1 (11.1)	2 (11.8)
Stomach	1 (12.5)	1 (11.1)	2 (11.8)
Other	2 (25)	5 (55.5)	7 (41.2)
Metastatic disease, N (%)	8 (100)	9 (100)	17 (100)
Prior treatment			
Chemotherapy, median (range)	2.0 (0–10)	2.0 (0–6)	2.0 (0–10)
Radiotherapy, N (%)	5 (62.5)	4 (44.4)	9 (52.9)
Both formulations received, N (%)	7 (87.5)	8 (88.9)	15 (88.2)
Evaluable population			
PK	7 (87.5)	4 (44.4)	11 (64.7)
ECG	7 (87.5)	8 (88.9)	15 (88.2)
Safety	8 (100)	9 (100)	17 (100)
Measurable disease	5 (62.5)	7 (77.8)	12 (70.6)

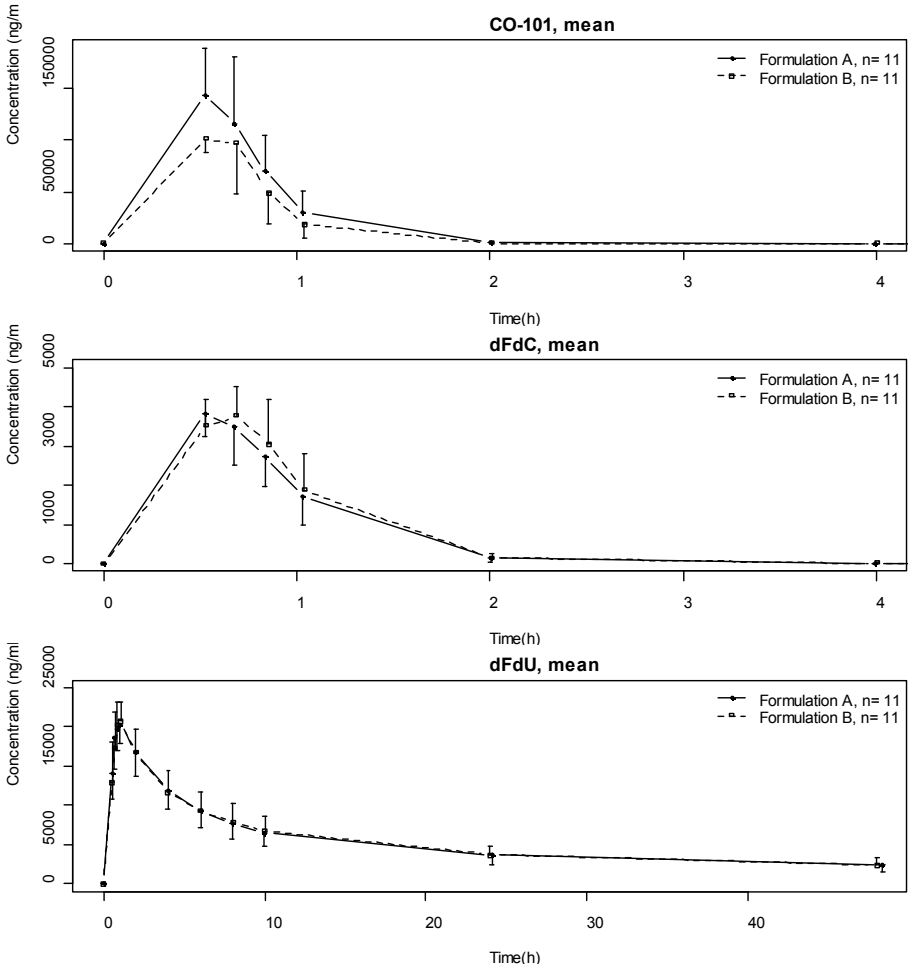

Figure 3 Plasma concentration-time curves of CO-101, dFdC and dFdU in patients after administration of formulation A (15 mg/mL) and formulation B (30 mg/mL) of CO-101. The data are shown as mean values (symbols) with SD (error bars).

The plasma concentrations of dFdC generally peaked shortly after the infusion period and declined in a bi-phasic manner for both formulations . In some patients, T_{max} of dFdC after formulation B was reached at a later time point (median 0.67 hours) compared with formulation A (median 0.57 hours). The arithmetic mean plasma C_{max} of dFdC of both formulations were comparable (4.10 µg/mL (18.3%) and 3.96 µg/mL (19.0%), respectively). The concentrations of dFdC declined to approximately 150 ng/mL within 2 hours post dose for both formulations.

The plasma concentrations of dFdU peaked at a later time point than dFdC, generally 0.5 hours after the end of the infusion period. The mean dFdU plasma C_{max} following the infusion of formulation A and formulation B were 21.2 µg/mL (13.4%) and 22.0 ng/mL (14.4%), respectively (table 2). The plasma concentrations of dFdU exhibited a slower

Table 2 Summary of pharmacokinetic parameters (PK population, N=11)

	CO-101		dFdC		dFdU	
Parameter	**A**	**B**	**A**	**B**	**A**	**B**
AUC_{0-48h} (µg·h/mL)	87.1	62.4	3.24	3.36	252	253
(% CV)	(41.6)	(25.6)	(25.4)	(22.6)	(22.5)	(25.5)
AUC_{0-inf} (µg·h/mL	87.1	62.4	3.26	3.38	355	353
(% CV)	(41.6)	(25.6)	(25.4)	(22.6)	(30.4)	(32.0)
C_{max} (µg/mL)	145	112	3.96	4.10	21.2	22.0
(% CV)	(33.8)	(32.9)	(19.0)	(18.3)	(13.4)	(14.4)
T_{max} (h, median)	0.52	0.58	0.57	0.67	0.98	1.00
(min–max)	(0.50–0.67)	(0.50–0.68)	(0.50–0.67)	(0.50–0.83)	(0.67–2.03)	(0.50–2.03)
$t_{1/2}$ (h)	4.44	5.06	10.03	8.83	28.35	27.7
(% CV)	(49.3)	(69.1)	(31.6)	(37.7)	(31.6)	(32.9)
V_{ss} (L)	12.54	16.09	—	—	—	—
(% CV)	(27.7)	(19.3)				

Values are given as arithmetic mean; A, CO-101 Formulation A (15 mg/mL); B, CO-101 Formulation B (30 mg/mL); CV, coefficient of variation; min, minimum; max, maximum; N=11 for all variables

decrease over time compared with CO-101 and dFdC. The plasma concentration–time profile of dFdU was similar after both formulations.

Statistical analysis of pharmacokinetic parameters

The results of the statistical comparison of the PK of CO-101 in formulation A and formulation B are summarized in table 3. For the primary endpoint equivalence of AUC0–inf, the 90% CI of geometric LS mean ratio of the two formulations did not fall within the acceptance interval of 80%–125%. This also applied for AUC_{0-48h} and C_{max} of CO-101. For the AUC0–inf, AUC0–48h and C_{max} of both metabolites of CO-101, the 90% CI of the geometric LS mean ratio of the two formulations did fall within the acceptance interval of 80%–125%.

Urinary excretion of dFdC and dFdU

Urinary excretion of metabolite dFdC mainly occurred during the first 8 hours post dose, while the excretion of dFdU lasted throughout the first 24 h. The total excretion of dFdC and dFdU in urine over 48 hours was comparable for formulation A (0.9–2.4% and 49.3–86.6%, respectively) and formulation B (0.8–2.5% and 39.6–76.1%, respectively).

Table 3 Statistical comparison of formulation B (30 mg/mL) and formulation A (15 mg/mL) for the PK parameters AUC0–inf, AUC0–t, and C_{max} (PK population, N=11)

	Variable	Geometric LS mean Formulation B	Geometric LS mean Formulation A	Ratio of geometric LS means	90% CI
CO-101	**AUC_{0-inf} (µg·h/mL)**	62.8	86.8	0.72	0.64, 0.82
	AUC_{0-48h} (µg·h/mL)	62.8	86.8	0.72	0.64, 0.82
	C_{max} (µg/mL)	110	147	0.75	0.68, 0.83
dFdC	**AUC_{0-inf} (µg·h/mL)**	3.43	3.29	1.04	0.92, 1.18
	AUC_{0-48h} (µg·h/mL)	3.42	3.28	1.04	0.92, 1.18
	C_{max} (µg/mL)	4.14	3.98	1.04	0.93, 1.17
dFdU	**AUC_{0-inf} (µg·h/mL)**	347	349	0.99	0.95, 1.04
	AUC_{0-48h} (µg·h/mL)	249	250	0.99	0.95, 1.04
	C_{max} (µg/mL)	22.0	21.1	1.04	0.99, 1.10

LS, least squares; CI, confidence interval

Pharmacodynamics

No abnormal ECG morphology was seen for any patient following either formulation. The mean change from pre-dose baseline in QTcF is illustrated in figure 4. The two formulations had a similar effect on QTcF and the largest observed mean increase from baseline in QTcF was less than 2.5 msec. No patient had an increase from baseline in QTcF >30 msec or a QTcF >450 msec at any time during the study.

Safety and tolerability

All patients treated (n=17) were evaluated for treatment-related adverse events. Table 4 lists all adverse events which were possibly, probably or definitely related to the study drug with an incidence rate of at least 10% with all CTCAE grades. Overall, CO-101 was well tolerated. The most common drug-related adverse events were nausea (64.7%), fatigue (52.9%), thrombocytopenia (52.9%) and pyrexia (47.1%) the majority being grade 1-2. Six (35.3%) patients experienced treatment-related grade 3 toxicity with two patients each having neutropenia, increased liver transaminases and fatigue and one patient having a grade 3 pain in extremity. The main treatment-related adverse event leading to dose reduction of the study drug in the treatment extension period was thrombocytopenia,

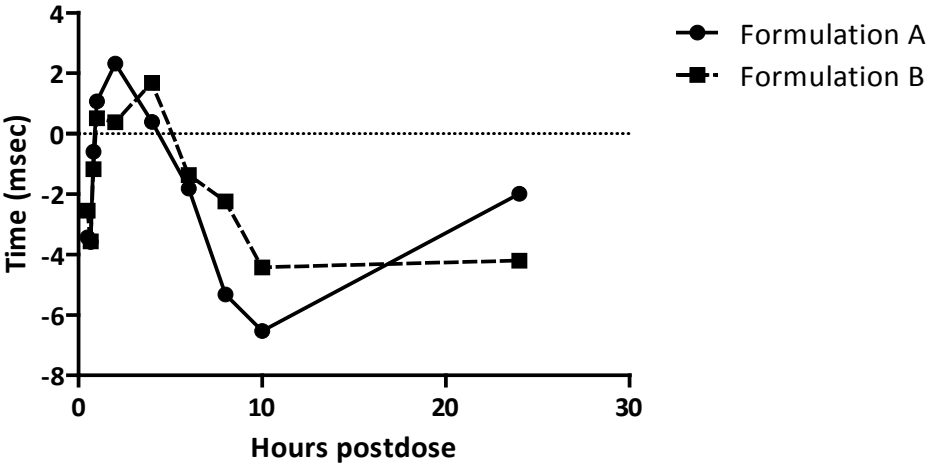

Figure 4, Mean change from predose baseline QTcF for both formulations

which occurred in six (35.3%) of the patients. Despite the administration of phospholipids during the infusion, plasma concentrations of total cholesterol, LDL cholesterol, HDL cholesterol, and triglycerides remained unchanged during the course of the study.

There were eight patients reported with treatment-emergent serious adverse events (SAEs). One event of pyrexia and one event of anemia were considered to be possibly/probably related to the study drug. All other SAEs were considered to be either unrelated or unlikely related to the study drug. One of these SAEs was the death of a patient 20 days after the last dose of study drug due to disease progression.

Anti-tumor activity

Anti-tumor activity was an exploratory objective of this study for patients who entered the treatment extension period of the study (N=17). The tumor evaluable population (n=12), included only patients with measurable disease at baseline. In this group, 6 (50%) of patients had stable disease (SD) and 6 (50%) of patients had progressive disease (PD) as their best overall response. One patient without measurable disease at baseline exhibited SD based on non-target lesions. Four of the seven patients had SD lasting more than 5 months (urothelial cell carcinoma (2 patients), leiomyosarcoma and malignant mesothelioma), including three patients who had progressed on prior gemcitabine therapy (monotherapy (n=1) and in combination with cisplatin (n=2)). There were no patients with an objective tumor response (complete or partial response).

Table 4 Adverse events with a possible, probable or definite relationship to CO-101 (incidence rate of at least 10% in the total safety population (N=17))

System organ class MedDRA preferred term	Grade 1-2 n	Grade 3 n	Overall N=17
Blood and lymphatic disorders			
Thrombocytopenia	9		53%
Neutropenia	4	2	35%
Anemia	3		18%
Gastrointestinal disorders			
Nausea	11		65%
Vomiting	7		41%
Stomatitis	5		29%
Diarrhea	3		18%
Constipation	2		12%
General disorders and administration site conditions			
Fatigue	7	2	53%
Pyrexia	8		47%
Infections and infestations			
Nasopharyngitis	2		12%
Investigations			
Alanine aminotransferase increased	5	2	41%
Aspartate aminotransferase increased	3	2	29%
Metabolism and nutritional disorders			
Decreased appetite	4		24%
Skin and subcutaneous tissue disorders			
Alopecia	2		12%
Rash	2		12%

n = number of patients with event

Discussion

This report describes a crossover study with two formulations of CO-101, an elaidic acid ester of dFdC. The planning of bioequivalence studies requires an a priori specification of the effect size for the determination of power and an assumption about the variance. The assumed variance can be either too small or too large, leading, respectively, to studies that are underpowered or overly large. For CO-101 there was little information on the variance within subjects and therefore a 2-stage adaptive sequential design with sample size re-estimation was performed [19]. At the end of stage 1, eleven patients were evaluable for the interim analysis of the PK characteristics. The last patient, who was included before the study went on hold for the interim analysis, was not evaluable for PK analyses because of a blood sampling deviation at the end of infusion. Nevertheless the interim analysis was completed. For $AUC_{0\text{-}inf}$ the intra-subject variance in this study was 14.7%. The power using this variance with eleven subjects was 80.9%. Stage 1 was therefore adequately powered to draw a conclusion on the primary endpoint, and continuation to stage 2 was not required.

For the primary endpoint equivalence of AUC_{0-inf}, the ratio of the geometric LS means of CO-101 exposure (measured as AUC_{0-inf}) was approximately 0.72 (formulation B : formulation A). The 90% CI of the geometric LS mean ratio of the two formulations does not fall within the acceptance interval of 80%–125%, and therefore CO-101 bioequivalence of the two formulations cannot be concluded. This conclusion was further supported by the data for AUC_{0-t} and C_{max}.

The difference in exposure to CO-101 after administration of both formulations is observed within the first 45 minutes after the start of infusion. CO-101 is an amphiphilic compound with an elaidic acid ester as lipophilic group and a polar, uncharged group. Due to the low solubility of CO-101 in both aqueous and oil media, the substance is solubilized in purified egg phosphatidylcholine. Since the concentration of CO-101 is the main difference between the formulations used in this study, the administered mass of phospholipids of formulation A is double the administered mass of phospholipids of formulation B provided by the administration of the same dose. This difference may alter the tissue distribution kinetics which can explain the results, as seen with other excipients like the influence of Cremophor EL on PK of paclitaxel [23]. It is possible that with formulation A more CO-101 is associated with phospholipids and lipoproteins which are abundantly present in the systemic circulation. After administration of formulation B, which contains less phospholipids relative to CO-101, the distribution of CO-101 is driven further toward tissues, including red blood cells, and moves out of the plasma.

The plasma concentrations of dFdC generally peaked within 10 minutes after the completion of dosing, suggesting rapid hydrolysis of CO-101 into dFdC. dFdC is highly hydrophilic compared to CO-101 and is therefore soluble in the plasma independent of the amount of formulation phospholipids. Therefore, the exposure measured as AUC0–t and AUC0–inf, and C_{max} of dFdC and its metabolite dFdU were similar for both formulations.

The mean PK values of CO-101 and dFdU in this study were comparable to those previously reported for CO-101 [21]. However, dFdC values were substantially lower (66%) compared to earlier studies. Even in the presence of inhibitors, the conversion of CO-101 and dFdC continues in blood after blood sampling at room temperature. In our study, a more robust procedure to cool the samples immediately after the blood sampling was implemented. The differences in absolute values of dFdC between studies may therefore be attributed to differences in blood sample processing. The average 48-hour urinary excretion of dFdC and dFdU were consistent with data reported for gemcitabine which indicates that very little dFdC is excreted unchanged, and that most of the administered dose was excreted as dFdU over 24 hours.

CO-101 was overall well tolerated. The most common drug-related adverse events were nausea (64.7%), fatigue (52.9%), thrombocytopenia (52.9%) and pyrexia (47.1%) the majority being grade 1-2. The safety profile of CO-101 in this study was consistent with the results of the phase 1 study with CO-101 and with the expected profile of the active metabolite dFdC [21,24]. Only the incidence of elevated liver transaminases is lower compared to gemcitabine, but this could probably be caused by the small number of patients in this study.

In conclusion, this study demonstrates that the reference clinical formulation and the test formulation are not bioequivalent. Furthermore, this treatment regime had no effect on the QT/QTc interval and had no effect on cardiac repolarization in patients with solid tumors and was overall well tolerated. The most common drug-related adverse events were comparable with the adverse events of the active metabolite, dFdC. The best response was stable disease, which was seen in seven patients and four of them had SD lasting more than 5 months, including three patients who had progressed on prior gemcitabine therapy.

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chapter 5

Conclusions and perspectives

Conclusions

New anticancer drugs consist of new chemical entities, like molecular targeted agents and novel variants of existing drugs. These existing drugs are often drugs with documented significant anti-tumor activity, which are already in use as first or second line treatment for several malignancies. Despite their proven anti-tumor activity, the registered drug formulations or mode of administration could be responsible for severe side effects, or lead to suboptimal dosing schedules.

The aim of this thesis was to investigate novel drug formulations of the existing drugs docetaxel, paclitaxel and gemcitabine in clinical studies. The results and acquired pharmacological knowledge will be used to evaluate the therapeutic potential of these novel drug formulations.

Taxanes

Docetaxel and paclitaxel have played a major role in the treatment of various tumors as intravenous (IV) infusion over the past two decades and are still standard of practice. Adverse events such as unpredictable hypersensitivity reactions and fluid retention have been attributed, in part, to the current drug formulation of both taxanes, containing Cremohor® EL (in the case of paclitaxel) and polysorbate 80 (in the case of docetaxel). The IV infusion limits flexibility of dosing regimes. Hence, there is a great interest in a better tolerated formulation and preferably an oral dosage form. Both drugs have limited oral bioavailability because of poor aqueous solubility and high affinity for active P-glycoprotein efflux transporters and cytochrome P450 3A drug metabolizing enzymes (CYP3A) [1]. As uptake in the gastro-intestinal tract requires dissolution, poor aqueous solubility significantly complicates oral absorption of both taxanes as a solid dosage form. Therefore, ModraDoc001 and ModraPac001 mg capsules have been developed, containing docetaxel and paclitaxel, respectively, as a solid dispersion [2,3]. Proof of concept studies showed that co-administration with the CYP3A inhibitor ritonavir enhances the systemic exposures to both oral docetaxel and oral paclitaxel, which enables oral treatment opportunities [1].

Docetaxel

In this theses, a dose escalation study demonstrated that once-daily (QD) once-weekly dosing of docetaxel as ModraDoc001 and ritonavir was safe and feasible (chapter 2.1). The maximum tolerated dose (MTD) was 60 mg docetaxel in combination with 200 mg ritonavir and the recommended dose (RD) was 60 mg docetaxel in combination with 100 mg ritonavir. The exposure (AUC_{inf}) after 60 mg oral docetaxel in combination with 100 or 200 mg ritonavir was comparable with the AUC_{inf} after weekly IV administration of 35 mg/m2. However, the variability seemed to be higher for oral docetaxel than for docetaxel given as IV infusion. The pharmacokinetic (PK) results revealed non-linearity in the oral PK of docetaxel, which encouraged us to explore a twice daily (BID) once-weekly dosing schedule to further improve and prolong the systemic exposure to orally administered docetaxel (chapter 2.2). This study showed that fractionated administration is an appropriate option to further increase and prolong the systemic exposure to docetaxel. The MTD of this study was 20 mg docetaxel in combination with 100 mg ritonavir BID, given once-weekly.

The incidence of severe adverse drug reactions associated with IV treatment of docetaxel, like hypersensitivity reactions, fluid retention and bone marrow suppression seemed to be lower in the studies with ModraDoc001. Consequently, high doses of dexamethason and the use of granulocyte colony stimulating factor were not required. In contrast, the most frequently reported adverse events after oral docetaxel was diarrhea, mostly of low grade severity (NCICTC grade 1-2) and rapidly reversible. Development of other oral anticancer drugs, including molecular-targeted agents, showed that gastrointestinal disorders, like diarrhea can frequently lead to treatment discontinuation and consequently decreased tumor control. Therefore, preclinical and clinical data were combined to understand the relationship between the development of diarrhea and oral administration of docetaxel and ritonavir (chapter 2.4). Severe diarrhea is most probably caused by the level of docetaxel in the systemic blood circulation and unrelated to the route of administration of docetaxel. The increase in incidence of mild diarrhea, however, is not fully understood. Since oral administration of docetaxel has been explored only in few clinical studies, limited data is available regarding the pathophysiology of these events. Moreover, ritonavir is known to induce apoptosis in human intestinal epithelial cells, what could contribute to the development of mild diarrhea during treatment [4]. These events of diarrhea were reversible and treated by prompt management with loperamide. As a result, most events of diarrhea lasted not more than one week and did not lead to structural treatment discontinuation.

ModraDoc001 consists of a solid dispersion which was filled semi-automatically into capsules. This production process is labor intensive and therefore not attractive for future scale-up. Therefore, further research led to the development of two novel tablet formulations, ModraDoc003 and ModraDoc004. Both are spray dried solid dispersions of docetaxel pressed in tablets of 10 mg docetaxel. The distinction between the two is that 50 mg ritonavir is included in the co-formulation of ModraDoc004 10/50 mg tablets. In chapter 2.3, it is shown that there are no significant differences in the exposure to docetaxel after administration of 40 mg docetaxel and 200 mg ritonavir as ModraDoc001 10 mg capsules, ModraDoc003 10 mg tablets and ModraDoc004 10/50 mg tablets. The fixed-dose combination tablet of docetaxel and ritonavir is a pharmaceutically and clinically feasibly future option in the development of patient convenient oral anticancer therapy with docetaxel.

Paclitaxel

Based on preclinical studies, paclitaxel is considered to be an ideal drug to use for the concept of "low-dose metronomic (LDM)" treatment, i.e. chronic administration of oncolytic drugs at relatively low, non-toxic doses on a frequent administration schedule with no drug-free breaks [5]. The main target of LDM treatment is dividing endothelial cells (EC) of the expanding vasculature of a tumor, instead of the proliferating tumor cells. LDM treatment with paclitaxel has a direct effect on the activated EC and an indirect effect on angiogenesis mediated by thrombospondin-1 (TSP-1) [6]. By using ModraPac001 capsules co-administered with ritonavir we were the first to demonstrate the feasibility of LDM treatment with continuous BID administration of oral paclitaxel in humans in chapter 3.1. The study is still ongoing at the time this thesis was written, but the safety profile and PK

of paclitaxel observed thus far strengthen the hypothesis that paclitaxel can be given as a LDM treatment regime.

In parallel to this study, we investigated in chapter 3.2 the feasibility of the following exploratory biomarkers: circulating levels of TSP-1 and gene expression of TSP-1 and vascular endothelial growth factor-A (VEGF-A) in peripheral blood mononuclear cells (PBMCs). Evaluation of these biomarkers in healthy volunteers showed that the procedures were easy to implement and had an acceptable variability in humans. Unfortunately, differences in the biomarkers between the responders and non-responders to LDM treatment with paclitaxel were, thus far, not observed. Whether this is a result of the mode of action of paclitaxel, or lack of sensitivity or appropriateness of these biomarkers warrants further clinical studies. Furthermore, additional patients with longer lasting stable disease and tumor regression need to be studied to demonstrate whether TSP-1 might be a good biomarker for monitoring of LDM with oral paclitaxel.

Gemcitabine elaidate

Gemcitabine is a nucleoside analogue used as IV infusion in the first line treatment of patients with various solid tumors, including non-small cell lung and pancreatic cancer. Gemcitabine needs to be actively transported into tumor cells by nucleoside transporters to exert its anticancer activity. Therefore, patients with low levels of nucleoside transporters may not respond to gemcitabine treatment. Gemcitabine elaidate (CO-101; CP-4126), an amphiphilic, unsaturated fatty acid ester derivative of gemcitabine can cross cell membranes by passive diffusion, followed by intracellular conversion to gemcitabine. The uptake of CO-101 in the cell is therefore independent of nucleoside transporters, contrary to gemcitabine. On the other hand, due to its amphiphilic character, CO-101 has a low solubility in both aqueous and oil media for infusion. As a result the solvent of the IV dosage form influences the tissue distribution kinetics, which is limiting in the development of novel dosage forms (chapter 4.2).

Preclinical studies in dogs showed that CO-101 acted as an oral prodrug with a high apparent systemic availability of gemcitabine. Oral administration of gemcitabine itself led to severe liver toxicity, most likely because of high presystemic conversion of gemcitabine by deaminase to 2',2-difluorodeoxyuridine (dFdU), which is preferentially taken up by the liver [7]. In chapter 4.1 it was shown that CO-101 acted also as a prodrug for gemcitabine when given orally to humans. Unfortunately, CO-101 was poorly absorbed and rapidly pre-systemically metabolized to its toxic metabolite dFdU. Therefore further research was focused on the IV variant.

Perspectives

This thesis presents the development and application of different novel drug formulations of existing chemotherapeutics. Not all of the tested novel drug formulations were found to be an improvement of existing therapy. Uptake of CO-101 in the cell is independent of nucleoside transporters and might be more effective in patients with low levels of hENT1 in tumors. Unfortunately, the LEAP (Low hENT1 and Adenocarcinoma of the Pancreas) study of CO-101 versus gemcitabine in metastatic pancreatic cancer showed that there was no difference in overall survival between the two arms in either the primary analysis of the patient population with low levels of nucleoside transporters, or in the overall population. Median survival in each arm was approximately six months with a hazard ratio of 0.99, which is entirely consistent with the survival results from other gemcitabine studies in metastatic pancreatic cancer. As a consequence, esterification of gemcitabine with an elaidic fatty acid has proven to have no added value and all development of CO-101 was stopped [8].

The oral formulations of docetaxel and paclitaxel, at the other hand, have more potential. Besides improved patients' convenience, oral administration has several other potential benefits. Adverse events attributed to the registered formulation, as hypersensitivity reactions and fluid retention were not seen after oral administration. Moreover, oral administration enabled chronic regimens with paclitaxel and docetaxel and has the potential for out-patient use of this treatment. Since in-patient administration of chemotherapy is on average twice as expensive as on an out-patient setting, this could offer a considerable cost reduction [9].

The development of oral formulations of docetaxel and paclitaxel is still ongoing. Based on the results obtained with the capsules and the different tablet formulations, the pharmaceutical composition of the tablet formulation was optimized for an automatic production process to produce 10 mg tablets that were denoted ModraDoc005. At this moment, ModraDoc005 tablets are tested in the clinic in a QD and BID once-weekly dosing schedule to determine the recommended dose of docetaxel and ritonavir for future phase II studies. Also an adapted mass-balance study with ModraDoc005 is planned to provide essential knowledge on the absorption, metabolism and excretion of docetaxel after administration of different doses. Based on the results of these studies, a phase II program will be initiated with the recommended dosing schedule. Protocols for maintenance treatment and second line treatment of patients with non-small cell lung cancer are already written. Because of the convenience, efficacy and low toxicity, oral docetaxel may also provide a potential alternative for heavily pre-treated or elderly patients, for whom intensive chemotherapy is no longer feasible.

In line with docetaxel, paclitaxel tablets will be developed, produced and tested in the clinical study with LDM paclitaxel. Scale-up of the production process is necessary for finishing this study and exploring potentially additive or even synergistic combinations with other drugs. Combinations with LDM therapy could potentiate anti-angiogenic and apoptotic effects on both proliferating endothelial cells and tumor cells. To define these combinations it is imperative to understand the mechanism of action of LDM paclitaxel

and the effects on the tumor vasculature. Several preclinical studies show that LDM paclitaxel inhibits neovascularization and tumor progression due to upregulation of TSP-1. The leaky and non-physiological structure of tumor vasculature developed by an imbalance of pro-angiogenic and anti-angiogenic growth factors may therefore not expand and possibly even normalize. This could improve the efficacy of a concomitant cytotoxic drug.

It is a limitation that validated biomarkers are lacking thus far for determination of the effect of LDM treatment on angiogenesis in humans. In animal studies, selected biomarkers have been established enabling assessment of an optimal biological dose and therapeutic activity of LDM treatment. Unfortunately, most biomarkers rely on quantification of formation of new vessels. Such surrogate markers are difficult to translate to experiments in humans. Instead, clinical opportunities are often limited to measurement of circulating levels of various angiogenic biomarkers and functional imaging. Despite the preclinically validated so-called soluble biomarkers and imaging, including contrast enhanced CT of MRI, validated biomarkers to determine the effect of LDM therapy in the clinic are lacking. Also, biomarkers to identify specific populations that may benefit are lacking. Although selection of patients based on biomarkers in early-phase trials may delay the recruitment of patients, it is necessary to define patient populations or cancer types with high angiogenic potential that might benefit from LDM therapy.

In this thesis the assay validation is described for circulating levels of TSP-1 in platelets and gene expression of TSP-1 and vascular endothelial growth factor-A (VEGF-A) in PBMCs. The choice for these biomarkers was mainly based on preclinical studies. Monitoring the activity of LDM therapy by measuring the platelet content of TSP-1 is an innovative approach and as far as we know the study with LDM paclitaxel is the first clinical trial in which the usefulness of this biomarker is explored. Additional studies are needed, for example in patients treated with intermittent high dose IV paclitaxel and other chemotherapy as well as with LDM treatment with other drugs to determine the feasibility, robustness and validity of this biomarker.

One of the most important growth factors involved in angiogenesis is VEGF. Extraordinary high levels of VEGF are present in the microenvironment of tumors, due to hypoxia-induced up regulation of VEGF production. Monitoring VEGF levels is used in several studies as a biomarker of angiogenesis, since VEGF stimulates proliferation and migration of EC and enhances the vascular permeability of tumor vessels. Although in some studies circulating VEGF levels were shown to be associated with tumor progression and poor prognosis, to date, there is no good evidence that these levels measured by ELISA can function as predictive biomarkers for anti-angiogenic drugs. However, VEGF could possibly be useful in a different mode. At low sub-therapeutic levels of radiolabeled bevacizumab, a humanized monoclonal antibody of VEGF, one can visualize non-invasively the uptake of bevacizumab into the tumor microenvironment. Since the uptake of bevacizumab is correlated to the amount of VEGF in the tumor microenvironment, the changes over time of the local levels of VEGF could be measured. In this way, bevacizumab kinetics could provide an insight into the local VEGF status and might thereby serve as a biomarker to guide anti-angiogenic therapy [10].

Dynamic contrast enhanced imaging could also be a potential biomarker. Because of the leaky and non-physiological tumor vasculature, blood volume and blood vessel permeability are locally increased. This could result in increased extravascular leakage of blood into interstitial spaces. With dynamic contrast enhanced imaging the biodistribution of intravenously injected tracers in tissues can be visualized and analyzed. The leaky blood vessels with high blood flow result in a higher uptake of the contrast agent in tumor tissue in comparison to normal tissues. In this way the changes in the angiogenic parameters (blood flow, blood volume, perfusion and permeability) could be measured.

Levels of circulating endothelial cells (CECs) and progenitor cells (CEPs) might also serve as a useful biomarker in the study, for which reason these parameters are being measured in the ongoing study with LDM paclitaxel. CECs and CEPs can be the key to angiogenesis and vasculogenesis [11]. These cells appear to proliferate, migrate, adhere and survive and shed from activated vasculature in response to tissue ischemia induced by high concentrations of chemotherapeutics. In preclinical studies evidence is found that CECs act as a biomarker for angiogenesis and anti-angiogenic drug activity. As a result CECs are commonly used to determine the optimal biological dose of anti-angiogenic drugs in mice. However, controversy exists about the identification of CECs in humans and therefore the appropriateness of this biomarker is still under investigation.

Current and future studies are focused on testing and validating biomarkers to measure the activity of LDM therapy, as well as to identify the patient population that may benefit. Unfortunately, the development of valid biomarkers is slow and does not always run in parallel with the development of new anticancer drugs. The development of predictive biomarkers and biomarkers for determination of the effect of treatment is considered to the success of new anticancer drugs and novel variants of existing drugs, especially when applied as LDM therapy.

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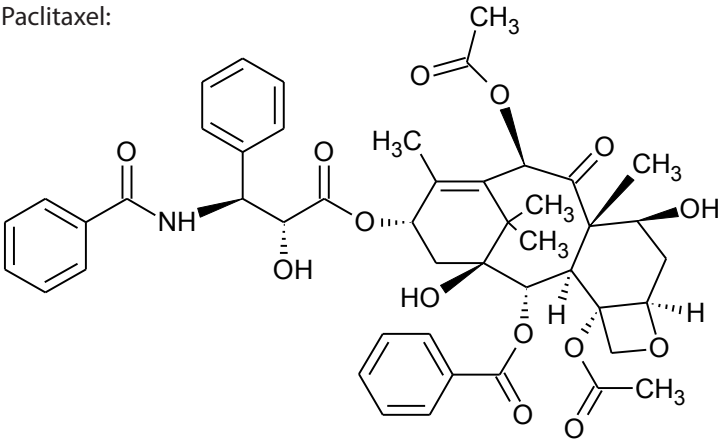
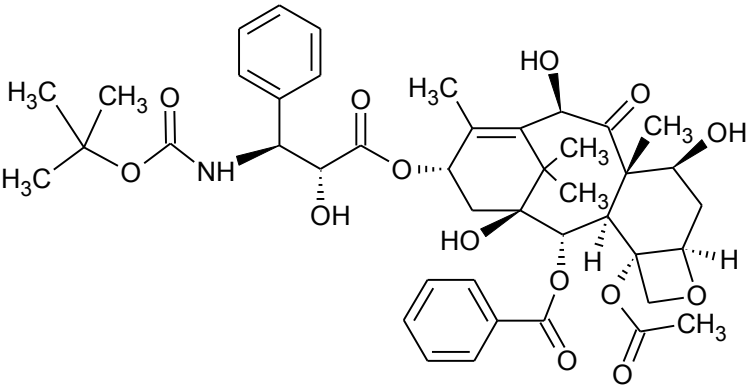
Chemical structures of investigated molecules

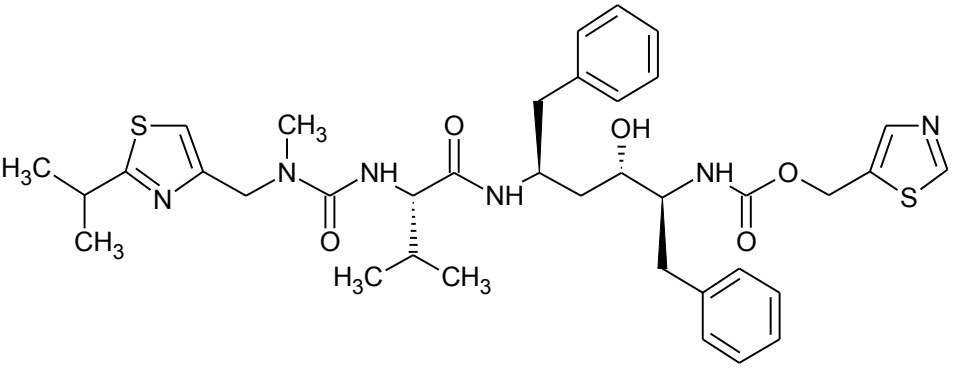
Paclitaxel:



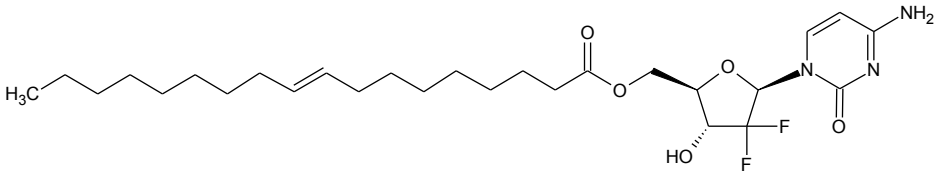
Docetaxel:



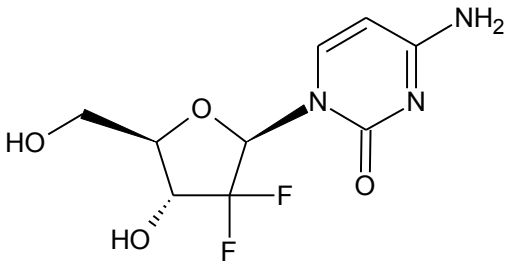
Ritonavir:



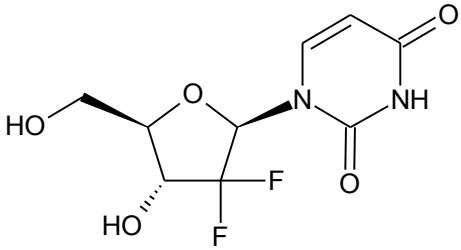
Gemcitabine elaidate (CP-4126, CO-101):



Gemcitabine (dFdC):



2′,2-difluorodeoxyuridine (dFdU):



Summary

Studies outlined in this thesis describe the impact of drug formulations on pharmacology of anticancer drugs. It consists of four chapters and starts with a review describing the mechanisms of low oral bioavailability of anticancer drugs and strategies for improvement of the bioavailability. The majority of new anticancer drugs are oral pharmaceutical formulations, consisting of new chemical entities, like molecular targeted agents and novel variants of existing drugs. The development of oral formulations is, however, often hampered by low and variable bioavailability. Since most anticancer drugs have a narrow therapeutic window and are dosed at or close to the maximum tolerated dose, a wide variability in bioavailability can have a negative impact on treatment outcome. The extent of oral bioavailability depends on many factors, including release of the drug from the pharmaceutical dosage form, a drug's stability in the gastro-intestinal tract, factors affecting dissolution, the rate of passage through the gut wall and the pre-systemic metabolism in gut wall and liver. There are several strategies to reduce or to overcome these limitations. First, pharmaceutical adjustment of the formulation or the physicochemical characteristics of the drug can improve the dissolution rate and absorption. Second, pharmacological interventions by combining the drug with inhibitors of transporter proteins and/or pre-systemic metabolizing enzymes can overcome the physiological endogenous limitations. Third, chemical modification of a drug by synthesis of a derivative, salt form or prodrug could enhance the bioavailability by improving the absorption and bypassing physiological endogenous limitations.

In the three other chapters of this thesis, these strategies are applied to docetaxel, paclitaxel and gemcitabine. These existing drugs are anticancer drugs with documented significant anti-tumor activity and are already in use as first or second line treatment for several malignancies. Despite their proven anti-tumor activity, the registered drug formulations or mode of administration could be responsible for severe side effects, or lead to suboptimal dosing schedules. The aim of this thesis was therefore to investigate novel drug formulations of docetaxel, paclitaxel and gemcitabine in clinical studies.

Docetaxel

Up to today, docetaxel plays a major role in the treatment of various tumors as intravenous (IV) infusion. Adverse events such as unpredictable hypersensitivity reactions and fluid retention have been attributed, in part, to the current drug formulation, containing polysorbate 80. The IV infusion limits flexibility of dosing regimes. Hence, there is a great interest in a better tolerated formulation and preferably an oral dosage form. In this thesis, a dose escalation study demonstrated that once-daily (QD) once-weekly dosing of oral docetaxel, denoted ModraDoc001, in combination with ritonavir was safe and feasible (chapter 2.1). The maximum tolerated dose (MTD) was 60 mg docetaxel in combination with 200 mg ritonavir and the recommended dose (RD) was 60 mg docetaxel in combination with 100 mg ritonavir. The exposure (AUC_{inf}) after 60 mg oral docetaxel in combination with 100 or 200 mg ritonavir was comparable with the AUC_{inf} after weekly IV administration of 35 mg/m^2. However, the variability seemed to be higher for oral docetaxel than for docetaxel given as IV infusion. The pharmacokinetic (PK) results revealed non-linearity in the oral PK of docetaxel, which encouraged us to explore a twice daily (BID)

once-weekly dosing schedule to further improve and prolong the systemic exposure to orally administered docetaxel (chapter 2.2). This study showed that fractionated administration is an appropriate option to further increase and prolong the systemic exposure to docetaxel. The MTD of this study was 20 mg docetaxel in combination with 100 mg ritonavir BID, given once-weekly.

The incidence of severe adverse drug reactions associated with IV treatment of docetaxel, like hypersensitivity reactions, fluid retention and bone marrow suppression seemed to be lower in the studies with oral ModraDoc001. Consequently, co-administration of dexamethason and the use of granulocyte colony stimulating factor were not required. In contrast, the most frequently reported adverse events after oral docetaxel was diarrhea, mostly of low grade severity (NCICTC grade 1-2) and rapidly reversible, which on the basis of indirect comparison appears to be more frequent than after IV administration. Therefore, preclinical and clinical data were combined to better understand the relationship between the development of diarrhea and oral administration of docetaxel and ritonavir (chapter 2.4). Severe diarrhea is most likely caused by the level of docetaxel in the systemic blood circulation and unrelated to the route of administration of docetaxel. The increase in incidence of mild diarrhea, however, is not fully understood. Ritonavir is known to induce apoptosis in human intestinal epithelial cells, which could contribute to the development of mild diarrhea during treatment. The events of diarrhea were reversible and treated by prompt management with loperamide. As a result, most events of diarrhea lasted not more than one week and did not lead to frequent treatment discontinuation.

ModraDoc001 consists of a solid dispersion which was filled semi-automatically into capsules. This production process is labor intensive and therefore not attractive for future scale-up. Therefore, further research led to the development of two novel tablet formulations, ModraDoc003 and ModraDoc004. Both are spray dried solid dispersions of docetaxel pressed in tablets of 10 mg docetaxel. The distinction between the two is that 50 mg ritonavir is included in the co-formulation of ModraDoc004 10/50 mg tablets. In chapter 2.3, it is shown in a crossover study that there are no significant differences in the exposure to docetaxel after administration of 40 mg docetaxel and 200 mg ritonavir as ModraDoc001 10 mg capsules, ModraDoc003 10 mg tablets and ModraDoc004 10/50 mg tablets. Both tablet formulations are pharmaceutically and clinically feasibly future options in the development of patient convenient oral anticancer therapy with docetaxel.

Paclitaxel

In parallel with the development of an oral formulation of docetaxel, ModraPac001 capsules were developed for paclitaxel. Based on preclinical studies, paclitaxel is considered to be an ideal drug to use for the concept of "low-dose metronomic (LDM)" treatment, i.e. chronic administration of oncolytic drugs at relatively low, non-toxic doses on a frequent administration schedule with no drug-free breaks. The main target of LDM treatment is the pool of dividing endothelial cells (EC) of the expanding vasculature of a tumor, instead of the proliferating tumor cells. LDM treatment with paclitaxel has a direct effect on the activated EC and an indirect effect on angiogenesis mediated by thrombospondin-1 (TSP-1). By using ModraPac001 capsules co-administered with ritonavir we were the first

to demonstrate the feasibility of LDM treatment with continuous BID administration of oral paclitaxel in humans (chapter 3.1). The study is still ongoing at the time this thesis was written, but the safety profile and PK of paclitaxel observed thus far strengthen the hypothesis that paclitaxel can be given as a LDM treatment regime.

In parallel to this study, we investigated in chapter 3.2 in a small and preliminary analysis the feasibility of the following exploratory biomarkers: circulating levels of TSP-1 and gene expression of TSP-1 and vascular endothelial growth factor-A (VEGF-A) in peripheral blood mononuclear cells (PBMCs). Evaluation of these biomarkers in healthy volunteers showed that the procedures were easy to implement and had an acceptable variability in humans. Unfortunately, differences in the biomarkers between the responders and non-responders to LDM treatment with paclitaxel were, thus far, not observed. However, a much larger patient cohort is needed for more definitive conclusions.

Gemcitabine elaidate

Gemcitabine is a nucleoside analogue used as IV infusion in the first line treatment of patients with various solid tumors, including non-small cell lung and pancreatic cancer. Gemcitabine needs to be actively transported into tumor cells by nucleoside transporters to exert its anticancer activity. Gemcitabine elaidate (CO-101; CP-4126), an amphiphilic, unsaturated fatty acid ester derivative of gemcitabine can cross cell membranes by passive diffusion, followed by intracellular conversion to gemcitabine. The uptake of CO-101 in the cell is therefore independent of nucleoside transporters, contrary to gemcitabine. In chapter 4.2 we compared PK bioequivalence of a novel IV formulation with the reference formulation of CO-101 in a crossover study. Due to its amphiphilic character, CO-101 has a low solubility in both aqueous and oil media for infusion and as a result phospholipids were chosen as solvent of both IV dosage forms. Since the novel formulation contained less phospholipids relative to the reference formulation, the tissue distribution kinetics of CO-101 differed after infusion of both formulations. Therefore bioequivalence of the two formulations could not be demonstrated.

Preclinical studies in dogs showed that CO-101 acted as an oral prodrug with a high apparent systemic availability of gemcitabine. Oral administration of gemcitabine itself led to severe liver toxicity, most likely because of high pre-systemic conversion of gemcitabine by deaminase to 2′,2-difluorodeoxyuridine (dFdU), which is preferentially taken up by the liver. In chapter 4.1 it was shown that CO-101 acted also as a prodrug for gemcitabine when given orally to humans. Unfortunately, CO-101 was poorly absorbed and rapidly pre-systemically metabolized to its toxic metabolite dFdU. Therefore further research focused on the IV variant.

In conclusion, the results of this thesis show that drug formulations can have an impact on pharmacology of anticancer drugs. These results are used for further development of novel dosage forms of docetaxel, paclitaxel and gemcitabine, but are also relevant for the development of new drugs.

Nederlandse samenvatting

De studies in dit proefschrift beschrijven de invloed van de toedieningsvormen en geneesmiddelformuleringen op de farmacologie van cytostatica. Het proefschrift bestaat uit vier hoofdstukken en begint met een review over de mechanismen waarom veel cytostatica een lage orale biologische beschikbaarheid hebben en de strategieën om de biologische beschikbaarheid te verbeteren. De meeste nieuwe cytostatica zijn orale formuleringen, bestaande uit nieuwe chemische entiteiten of nieuwe varianten van bestaande medicijnen. De ontwikkeling van orale formuleringen wordt echter vaak beperkt door lage en variabele biologische beschikbaarheid. Aangezien de meeste cytostatica een smalle therapeutische breedte hebben en vaak op of dicht bij de maximaal getolereerde dosis gedoseerd worden, kan een grote variabiliteit in biologische beschikbaarheid een negatieve invloed hebben op de resultaten van de behandeling. De biologische beschikbaarheid is afhankelijk van vele factoren, zoals afgifte van het geneesmiddel uit de farmaceutische doseringsvorm, de stabiliteit van een geneesmiddel in het maag-darmkanaal, de absorptie en het pre-systemisch metabolisme in de darmwand en de lever. Er zijn verschillende strategieën om deze factoren te beïnvloeden. Ten eerste kan een farmaceutische aanpassing van de formulering de oplossnelheid en absorptie verbeteren. Ten tweede kan een farmacologische interventie de fysiologische endogene beperkingen reduceren door het combineren van het geneesmiddel met remmers van transporteiwitten en / of pre-systemisch metaboliserende enzymen. Ten derde kan een chemische modificatie van een geneesmiddel door synthese van een derivaat, een zout of een prodrug de biologische beschikbaarheid verbeteren.

In de drie overige delen van het proefschrift worden deze drie strategieën toegepast op docetaxel, paclitaxel en gemcitabine. Deze cytostatica zijn als intraveneuze (IV) formulering geregistreerd voor aanvullende en/of eerstelijns en tweedelijns behandeling van verschillende tumor soorten. Ondanks hun bewezen anti-tumor activiteit, is de geregistreerde formulering of de manier van toediening deels verantwoordelijk voor suboptimale doseringsschema's of voor ernstige bijwerkingen. Het doel van dit proefschrift is daarom om nieuwe toedieningsvormen en formuleringen van docetaxel, paclitaxel en gemcitabine te onderzoeken in verschillende klinische studies.

Docetaxel

Docetaxel speelt als IV toediening een grote rol in de behandeling van verschillende tumoren. Bijwerkingen zoals onvoorspelbare overgevoeligheidsreacties en vochtophoping worden deels toegeschreven aan de formulering, die onder andere polysorbaat 80 bevat. Omdat IV toediening ook nauwelijks flexibiliteit geeft voor verschillende doseerschema's, is er grote interesse voor een orale formulering, die door patiënten beter verdragen wordt. In dit proefschrift zijn twee dosisescalatie studies beschreven met een orale formulering van docetaxel, ModraDoc001 capsules in combinatie met ritonavir. Docetaxel heeft een lage biologische beschikbaarheid, vanwege een slechte oplosbaarheid in water en een hoge affiniteit voor actieve ABC transport eiwitten en cytochroom P450 3A metaboliserende enzymen (CYP3A) in de darm en in de lever. Een lage dosis ritonavir remt tijdelijk de werking van de CYP3A enzymen, waardoor de biologische beschikbaarheid van docetaxel toeneemt en orale behandeling mogelijk wordt. De ModraDoc001 capsules bevatten

docetaxel als solid dispersion om de oplosbaarheid te verbeteren. Hoofdstuk 2.1 laat zien dat deze combinatie toepasbaar en veilig is bij wekelijkse orale toediening. De maximaal tolereerbare dosis (MTD) is vastgesteld op 60 mg docetaxel in combinatie met 200 mg ritonavir en de aangeraden dosering voor verdere vervolgstudies is vastgesteld op 60 mg docetaxel in combinatie met 100 mg ritonavir. De blootstelling (AUC_{inf}) aan docetaxel na 60 mg docetaxel in combinatie met 100 mg en 200 mg ritonavir was vergelijkbaar met de blootstelling aan docetaxel na wekelijkse IV toediening van 35 mg/m^2 docetaxel. De interindividuele variabiliteit na orale toediening lijkt echter wel wat groter te zijn in vergelijking met IV toediening. Omdat uit de farmacokinetische (PK) analyse blijkt dat de blootstelling aan docetaxel niet lineair toeneemt met de dosis, is een studie met een tweemaal daags doseringsregime (één keer per week) gestart om de systemische blootstelling aan docetaxel te verhogen. De resultaten uit hoofdstuk 2.2 lieten zien dat een gefractioneerd doseringsschema een goede optie is om dit doel te bereiken. De MTD van deze studie was tweemaal daags 20 mg docetaxel in combinatie met 100 mg ritonavir in een wekelijks regime.

Het aantal patiënten met ernstige bijwerkingen die geassocieerd worden met IV behandeling met docetaxel, zoals overgevoeligheidsreacties, vochtophoping en beenmergsuppressie, lijkt kleiner te zijn na orale behandeling met ModraDoc001 capsules. Behandeling met dexamethason en het gebruik van granulocytkoloniestimulerende factoren waren daarom niet nodig. De bijwerking diarree, in de meeste gevallen niet ernstig (NCICTC graad 1-2) en snel reversibel, leken daarentegen vaker voor te komen in vergelijking met IV behandeling. Om de relatie tussen de ontwikkeling van diarree en de orale toediening van docetaxel en ritonavir beter te begrijpen, zijn preklinische en klinische data gecombineerd en geanalyseerd (hoofdstuk 2.4). Ernstige diarree wordt hoogstwaarschijnlijk veroorzaakt door de hoeveelheid docetaxel in het bloed en is niet gerelateerd aan de toedieningsweg van docetaxel. De oorzaak van de toename van milde diarree is daarentegen niet geheel duidelijk. Zowel docetaxel als ritonavir kunnen de oorzaak zijn van milde diarree, aangezien ritonavir ook apoptose van intestinale epitheelcellen kan induceren. Tijdens de studies was diarree reversibel en goed te behandelen met loperamide. Diarree duurde daarom meestal niet langer dan een week en leidde niet structureel tot het staken van de behandeling.

ModraDoc001 capsules zijn capsules, die semi-automatisch gevuld worden met een solid dispersion van docetaxel. Dit productieproces is arbeidsintensief en daarom niet aantrekkelijk om op te schalen voor grote productie. Dit leidde tot de ontwikkeling van twee nieuwe tabletformuleringen, ModraDoc003 en ModraDoc004. Beide tabletformuleringen bevatten 10 mg docetaxel en bestaan uit een gesproeidroogde solid dispersion. Het verschil tussen de beide formuleringen is een toevoeging van 50 mg ritonavir aan de formulering bij de ModraDoc004 10/50 mg tabletten. Uit de crossover studie van hoofdstuk 2.3 blijkt dat er geen significante verschillen aantoonbaar zijn tussen de blootstellingen aan docetaxel na toediening van 40 mg docetaxel en 200 mg ritonavir als ModraDoc001 10 mg capsules met ritonavir, ModraDoc003 10 mg tabletten met ritonavir en ModraDoc004 10/50 mg tabletten. Beide tabletformuleringen zijn farmaceutisch en klinisch gezien potentiële opties voor de ontwikkeling van een patiëntvriendelijke behandeling met oraal docetaxel.

Paclitaxel
Parallel aan de ontwikkeling van een orale toedieningsvorm van docetaxel zijn ModraPac001 capsules ontwikkeld voor paclitaxel. Op basis van preklinische studies wordt paclitaxel beschouwd als ideaal cytostaticum voor een laaggedoseerde metronome (LDM) behandeling. Hierbij wordt een geneesmiddel frequent ingenomen met een relatief lage, niet toxische dosis zonder geneesmiddelvrije periodes. De LDM behandeling richt zich vooral op het remmen van de delende endotheelcellen van de vaatcellen in de tumor in plaats van de woekerende tumorcellen. LDM therapie met paclitaxel heeft naast een direct effect ook een indirect effect op geactiveerde endotheelcellen, door middel van het eiwit thrombospondine 1 (TSP-1). Door het gebruik van ModraPac001 capsules in combinatie met ritonavir, zijn wij de eerste onderzoeksgroep die de haalbaarheid van een LDM behandeling met tweemaal daags paclitaxel kunnen onderzoeken in een klinische studie (hoofdstuk 3.1). De studie is nog bezig op het moment van schrijven van dit proefschrift, maar de veiligheid en PK lijken tot nu toe te bevestigen dat paclitaxel op deze manier gegeven kan worden als LDM behandeling.

Naast deze fase I studie, hebben we in hoofdstuk 3.2 een voorlopige evaluatie gedaan van de toepasbaarheid van de volgende exploratieve biomarkers: (1) circulerende concentraties van TSP-1 en (2) genexpressie van TSP-1 in perifere bloed mononucleaire cellen (PBMC's) en (3) vasculaire endotheliale groeifactor-A (VEGF-A) in PBMC's. De evaluatie van deze biomarkers in gezonde vrijwilligers liet zien dat de procedures om de biomarkers af te nemen eenvoudig toe te passen waren en dat de interindividuele variatie binnen acceptabele grenzen was. Tot nu toe is er tijdens de studie geen verschil gezien in de uitkomsten van de biomarkers tussen de patiënten die na zes weken een stabiele ziekte hadden en de patiënten die na zes weken progressief waren. Niettemin is er een groter cohort van patiënten noodzakelijk voor een definitieve evaluatie van de biomarkers.

Gemcitabine elaidate
Gemcitabine is een nucleoside analoog, dat gebruikt wordt als IV toediening in de eerstelijns behandeling van patiënten met verschillende tumoren, inclusief niet-kleincellig longkanker en alvleesklierkanker. Gemcitabine moet actief in de tumorcel worden getransporteerd door middel van nucleoside transport eiwitten om actief te kunnen zijn. Gemcitabine elaidate (CO-101; CP-4126), een amfifilisch, onverzadigde vetzuur esterderivaat van gemcitabine, kan de celmembraam passeren door middel van passieve diffusie. Binnen de cel wordt CO-101 omgezet naar gemcitabine. De opname van CO-101 is daarom onafhankelijk van de transporteiwitten in tegenstelling tot gemcitabine. In hoofdstuk 4.2 wordt een crossover PK bioequivalentie studie beschreven, waarin een nieuwe IV formulering wordt vergeleken met de referentie formulering van CO-101. Beide formuleringen bevatten fosfolipiden als oplosmiddel, omdat CO-101 zowel hydrofobe als hydrofiele groepen bevat. Het grootste verschil tussen beide formuleringen was het volume van het oplosmiddel. Omdat de fosfolipiden invloed hebben op de distributiekinetiek van CO-101, kon er geen bioequivalentie worden aangetoond tussen beide formuleringen.

Preklinische studies in honden toonden aan dat CO-101 werkte als orale prodrug voor gemcitabine. In deze studies had gemcitabine een hoge biologische beschikbaarheid. Orale toediening van gemcitabine zelf leidde in eerdere studies tot ernstige levertoxiciteit, hoogstwaarschijnlijk veroorzaakt door een hoge pre-systemische omzetting tot 2',2-difluorodeoxyuridine (dFdU) in de lever. In hoofdstuk 4.1 wordt aangetoond dat CO-101 na orale toediening in mensen ook een prodrug is voor gemcitabine. Doordat CO-101 slecht geabsorbeerd werd en snel omgezet werd tot dFdU, is de studie stopgezet en werd de ontwikkeling van CO-101 voortgezet als IV formulering.

Concluderend, de resultaten in dit proefschrift laten zien dat de toedieningsvormen en formuleringen de farmacologie van verschillende cytostatica kunnen beïnvloeden. Deze resultaten worden gebruikt voor de verdere ontwikkeling van de beschreven cytostatica, maar zijn ook relevant voor de ontwikkeling van nieuwe geneesmiddelen.

Dankwoord

Het boek is af! Vier en een half jaar werk met een kaft van Annemiki erom. Maar onderzoek doe je niet alleen. Zonder de hulp van een aantal personen zou dit proefschrift er niet zijn geweest. Deze mensen wil ik daarom graag op deze manier bedanken.

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Verder ben ik iedereen van de afdeling klinische farmacologie veel dank verschuldigd. Ik heb altijd met veel plezier rondgelopen op 4C. Zonder de hulp en de inzet van de artsen, nurse practitioners, (kinetiek)verpleegkundigen, de planning en het secretariaat zijn er geen klinische studies mogelijk. Serena Marchetti wil ik als principal investigator van de Modra studies extra bedanken. Ik vind het knap hoe je je staande weet te houden met die enorme werkdruk van patiëntenzorg en klinische studies. Ik vind dat je geweldig werk verzet en ben je dankbaar voor de feedback over de protocollen en manuscripten en de efficiënte afhandeling van de CRF's.

I would like to thank the people from Clavis and Clovis for our collaboration. A special thanks for Wenche Rasch and Trygve Bergeland from Clavis Pharma and Lindsey Rolfe, Paramjit Kaur and Danya Simpson from Clovis Oncology for all the support during the clinical studies with gemcitabine elaidate.

Artur, mooie vent en m'n steun en toeverlaat op het lab van het NKI. Misschien wel de meest bijzondere samenwerking die ik ooit heb gehad. Het was altijd even spannend in wat voor bui je was 's ochtends, maar ik heb ontzettend veel van je geleerd over de verschillende technieken die we gebruikt hebben tijdens onze zoektocht naar de biomarkers. Door je harde werk de laatste weken, hebben we toch mooi de trombospondine assay en genexpressie assay kunnen valideren en de data van patiënten en vrijwilligers kunnen rapporteren. Niets mooier dan je smsje om half twaalf 's avonds dat alle data was uitgewerkt, zodat we het stuk een week later af hadden. Helaas hebben we de methode voor circulerende endotheel cellen nog niet echt kunnen vinden. Ik hoop dat je deze alsnog vindt en kan implementeren in de MOP studie.

Ook ben ik alle medewerkers van de wetenschappelijke administratie dankbaar voor hun medewerking in de data management van de Modra studies. Vooral Yvonne Groot heeft

veel werk verzet bij het monitoren van de data van de DOW, BOM en MOP studie. Het op de juiste manier documenteren van "alle" gegevens bracht af en toe wat discussie, maar ik denk dat we samen met Serena als efficiënte machine hebben gewerkt bij het vastleggen van de klinische data.

Jolanda, de personal assistant van Jan en misschien wel een beetje de moeder van de onderzoekers. Je werkte als perfecte brug tussen ons en Jan en het was altijd gezellig als ik op het wachtstoeltje zat voor afspraken met Jan of Serena. Daarbij ben ik je extra dankbaar voor je hulp bij de inschrijving van de opleiding klinische farmacologie.

Natuurlijk ben ik ook veel dank verschuldigd aan alle collega's in de keet en op het NKI met wie ik al dan niet heb samengewerkt. Jullie konden moeilijk om mijn stemvolume heen, dus dank dat jullie mij niet ingesloten hebben in een geluidsdicht kamertje. Het gaat te ver om iedereen te noemen, maar een paar mensen ben ik extra dankbaar: Jeroen voor het meten van alle PK samples, de discussies en onze samenwerking met de muizenstudies, Johannes en Stijn voor het samenwerken bij de Modra studies, Coen voor zijn kennis in pharmacometrics, het aanleren van R en het aanhoren van zo ongeveer alles wat ik op dat moment kwijt moest, Nynke voor de deal "review voor bloed", David voor z'n slimme inbreng bij het klinische inwerken en z'n wat minder slimme inbreng bij voetbaldiscussies, Bas voor de lasagne dates, Johannes en Jelte voor de altijd zinnige discussies bij de lunch en natuurlijk iedereen die meegedaan heeft aan mijn vrijwilligersstudie.

Ook buiten het werk zijn er een aantal mensen die ik moet bedanken. Juud, de boys van de mannenavond, club, Jitske, Jeroen, het voetbalteam van het NKI en vele anderen, dank voor de noodzakelijke relativering en ontspanning de laatste jaren!

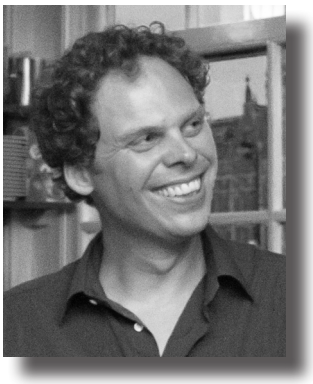
En dan de paranimfen.. Geert, we zijn samen begonnen bij het NKI en hebben min of meer hetzelfde traject bewandeld. Mooi om samen met jou te starten aan een onbekend avontuur, ervaringen te delen, leuke en moelijkere periodes te beleven en natuurlijk het samen af te sluiten met een mooie roadtrip. Ik denk dan ook met plezier terug aan onze wekelijkse avondjes aan de Haarlemmerstraat of de Kwakersstraat, de "hardlopen-biefstuk-voetbal"-combinatie en vele andere hoogtepuntjes (van het fietsen van de Ventoux tot het bijpraten aan de bar). En Job, sinds m'n 2e zijn we elkaars tegenpolen met veel overeenkomsten. Ik blijf het mooi vinden hoe we over alles kunnen discussiëren, maar ook van elkaar blijven leren. Onze brommertrips door Zuidoost-Azië blijf ik ook echt als een hoogtepunt zien. Boys, ik ben vereerd dat jullie 18 september achter me staan!

Ten slotte mijn ouders, van wie ik bijna alles heb geleerd. We hebben vijf jaar geleden veel gesproken over de beslissing om te beginnen aan dit project en ik ben zo ontzettend blij dat ik deze keuze met jullie heb gemaakt. Ik ben jullie enorm dankbaar dat jullie me altijd hebben gesteund en dat jullie het mogelijk maken, dat Job en ik kunnen doen wat we willen en ambiëren.

Allen, bedankt!
Rik

&

Curriculum Vitae



Rik Stuurman werd geboren op 9 april 1981 te Emmeloord in de Noordoostpolder. Na het behalen van zijn VWO diploma aan het Zuyderzee College in 1999 is hij begonnen met de studies Farmacie en Farmaceutische Wetenschappen aan de Rijksuniversiteit Groningen. Tijdens deze studies heeft hij een jaar onderzoek gedaan bij de werkgroep geneesmidelvormen en biofarmacie en bij de ziekenhuisapotheek van het Universitair Medisch Centrum Groningen onder leiding van Prof. dr. H.W. Frijlink en dr. R.C.A. Schellekens. Het doel van dit onderzoek was de ontwikkeling van een orale toedieningsvorm met colon-specifieke afgifte ten behoeve van onderzoek naar de etiologie van lactose intolerantie. In 2006 behaalde hij zijn apothekersdiploma, waarna hij naar Ghana vertrok om daar als apotheker vrijwilligerswerk te gaan doen voor de NGO Health Acces Network. Bij terugkomst begon hij aan zijn laatste stage bij Sandoz GmbH te Kundl in Oostenrijk, waar werd meegewerkt aan de ontwikkeling van een nieuwe formulering van een cefalosporine. In december 2006 werd het doctoraal Farmaceutische Wetenschappen behaald, waarna hij in februari 2007 startte als Trainee Pharmacist bij Organon/Schering-Plough te Oss. Hij was onder andere verantwoordelijk voor de kwalificatie en validatie van een nieuwe productie plant voor parenteralia en de NuvaRing®, voor de vergunningen van Schering-Plough, voor een pre-formuleringsstudie van een fase I product en voor de kwaliteit van de tabletproductie als productieapotheker. In januari 2009 maakte hij de overstap naar Amsterdam en begon hij als onderzoeker in opleiding aan het onderzoek dat beschreven staat in dit proefschrift. Het onderzoek werd uitgevoerd op de afdeling klinische farmacologie aan het Nederlands Kanker Instituut – Antoni van Leeuwenhoek ziekenhuis (NKI-AvL) en de afdeling farmacie en farmacologie in het Slotervaartziekenhuis, beiden te Amsterdam. Het onderzoek met als titel "Clinical pharmacology of novel anticancer drug formulations" stond onder leiding van Prof. dr. J.H.M. Schellens en Prof. dr. J.H. Beijnen. Naast dit onderzoek is hij in opleiding tot klinisch farmacoloog.

Curriculum Vitae

Rik Stuurman was born in Emmeloord on April 9, 1981. After he received his VWO exam at the Zuyderzee College in 1999, he started studying Pharmacy and Pharmaceutical Sciences at the Rijksuniversiteit Groningen. During these studies he completed a scientific research project of a year at the department dosage forms and biopharmacy and the hospital pharmacy of University Medical Centre Groningen under the supervision of Prof. dr. H.W. Frijlink and dr. R.C.A. Schellekens. The objective of this research project was the development of an oral dosage form with colon-specific release. In 2006 he obtained his pharmacist degree and left for volunteer work at the NGO Health Acces Network in Ghana. When he returned he started with his final internship at Sandoz GmbH in Kundl, Austria, where he cooperated with the development of a new formulation of a cephalosporin. In December 2006 he obtained his Master of Science degree of Pharmaceutical Sciences and in February 2007 he started as a Trainee Pharmacist bij Organon/Schering-Plough in Oss. He was responsible for the validation and qualification of a new production plant for parenteral dosage forms and the Nuvaring®, for the production licenses of Schering-Plough, for a pre-formulationstudy of a phase I product and for the quality control of the tablet production as a production pharmacist. In January 2009 he moved to Amsterdam and started his PhD project described in this thesis. The research was performed at the Division of Clinical Pharmacology of the Netherlands Cancer Institute, Amsterdam and Department of Pharmacy & Pharmacology, Slotervaart Hospital / The Netherlands Cancer Institute, Amsterdam. This research project was supervised by Prof. dr. J.H.M. Schellens and Prof. dr. J.H. Beijnen. Next to his PhD project he started the study Clinical Pharmacology.

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