Personalized Irinotecan Treatment:

The patient matters

Jessica van der Bol

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Geïndividualiseerde irinotecan behandeling: De patiënt prominent

Proefschrift

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PROMOTIECOMMISSIE

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Copromotor: Dr. A.H.J. Mathijssen

Ik dacht dat het leven veel exacter in elkaar stak,
maar het blijkt één grote improvisatie te zijn.
Harry Mulisch

Ter nagedachtenis aan Leo Biemans
22 juni 1959 - 26 juli 2001

Voor mama

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Chapter 1

Introduction

INTRODUCTION

In the early sixties of the last century, camptothecin (CPT) was isolated from the Chinese plant *Camptotheca acuminata* (Nyssaceae family),[1] and was found to be a very potent antitumor agent *in vitro*.[2] However, its clinical development was hindered by a relatively limited clinical activity and severe and unpredictable toxicities,[3-6] most problematic being hemorrhagic cystitis and enteritis. These turned out to be partially related to the poor hydrophilicity of the drug and the initial administration of camptothecin in the inactive carboxylate form.[7,8] Once the mechanism of action of camptothecin was discovered,[9] there was renewed interest in the drug. Efforts were made to develop water-soluble camptothecin analogues with improved antitumor activity and decreased toxicity. Irinotecan, also known as CPT-11, was developed as a water-soluble prodrug of SN-38, a very potent camptothecin analogue,[6,10] which has a 100-1000 fold higher cytotoxic activity *in vitro* than the parent compound.[11,12] Camptothecins, including irinotecan and SN-38, inhibit the enzyme topoisomerase-I by binding to it and forming a stable complex between topoisomerase-I and DNA. This induces single-strand breaks in chromosomal DNA, ultimately leading to cytotoxicity and apoptosis.[9,13]

Irinotecan has a highly complex metabolism, involving multiple metabolizing phase I and phase II enzymes and several drug transporters (**Figure 1**). Irinotecan itself is not the active substance, but needs to be hydrolyzed by carboxylesterases into its active metabolite SN-38.[14] These carboxylesterases are predominantly localized in the liver, but also in the lungs and in the mucosa of the gastrointestinal tract.[15] However, only a small fraction of irinotecan is directly converted into SN-38. Competing with the formation of SN-38 is the CYP3A-mediated oxidation of irinotecan into the inactive metabolites NPC and APC, and the structurally unresolved metabolite M4. NPC and APC both also can be converted into SN-38 by carboxylesterases; NPC has the same affinity as irinotecan, but APC is a very poor carboxylesterase-substrate.[16] SN-38 is mainly eliminated via glucuronidation into SN-38 glucuronide (SN-38G), which involves several UGT1A enzymes;[17] UGT1A1 being the most important.[18] After biliary excretion, SN-38G can be re-activated into SN-38 by *β*-glucuronidase-producing bacteria in the intestines. This reactivation is thought to have a causative role in irinotecan's intestinal toxicity.[19] In addition, several drug-transporting proteins are involved in the cellular uptake (Organic Anion Transporting Polypeptides; OATP1B1 and OATP1B3)[20,21] and the hepatobiliary and renal elimination of irinotecan and its metabolites (ATP Binding Cassette transporters; ABCB1 (P-glycoprotein), ABCC1 (MRP), ABCC2 (cMOAT), and ABCG2 (BCRP)).[22-27] To make it even more complex, both irinotecan and SN-38 exist in an active lactone form and an inactive carboxylate form. There is a pH-dependent equilibrium between the two; an acidic pH promotes the formation of the lactone form, while a physiological pH favors the carboxylate form.[28,29]
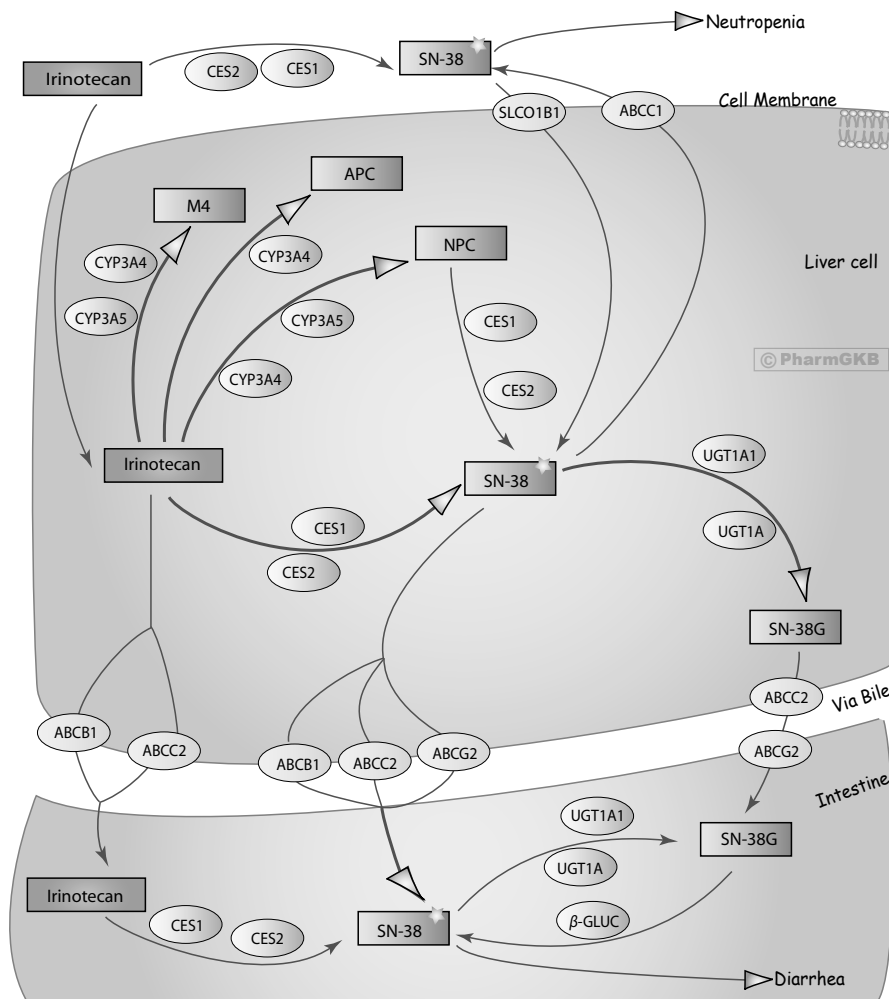
Figure 1. Metabolism of irinotecan

After intravenous infusion, irinotecan is distributed throughout the body. It is metabolized into the active metabolite SN-38 by carboxylesterases (CES), which are predominantly localized in the liver, but also in the lungs and the mucosa of the gastrointestinal tract. In addition, irinotecan is being oxidized by CYP3A enzymes into the inactive metabolites APC, M4, and NPC; the latter also being a substrate for CES-mediated conversion into SN-38. SN-38 is inactivated by UGT1A enzymes, UGT1A1 being the most important, into its glucuronide-conjugate SN-38G. After hepatobiliary excretion, SN-38G can be reactivated into SN-38 by β-glucuronidase (β-GLUC) producing bacteria. Several uptake (Solute Carrier Organic Anion (SLCO) transporter family) and efflux transporters (ATP-binding cassette (ABC) transporter family) are involved in the elimination of irinotecan.
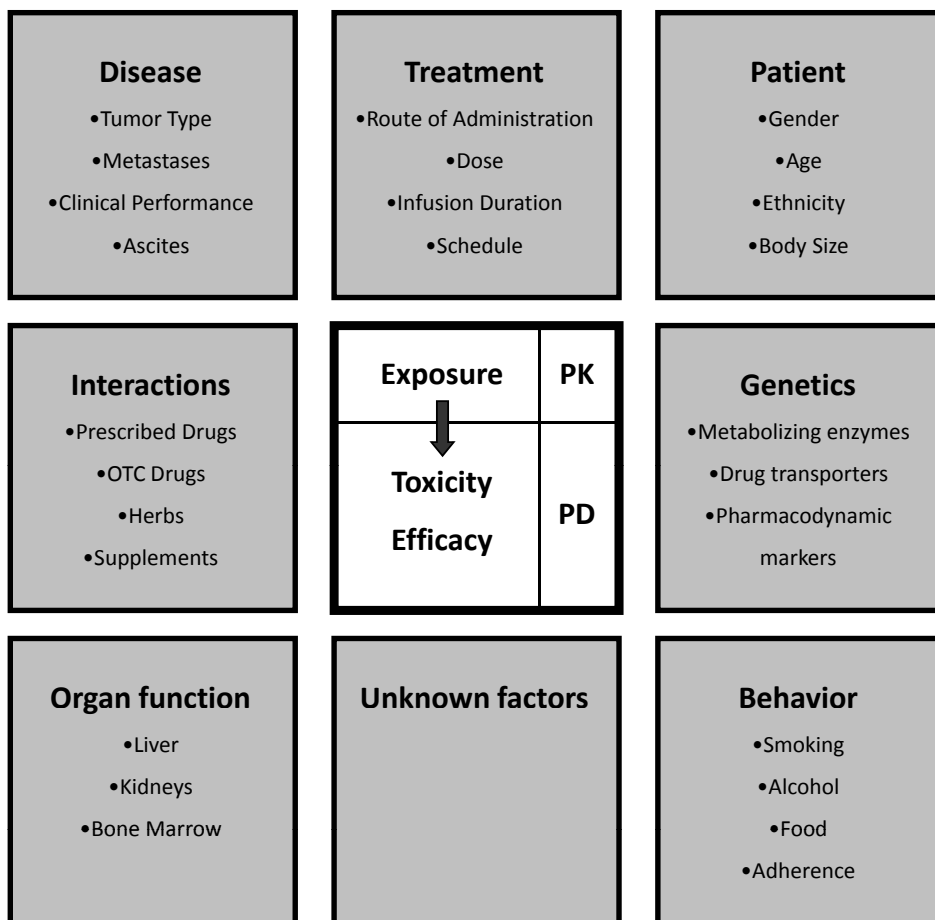
Early clinical studies with irinotecan were performed in the nineties and showed responses in patients with colorectal cancer, and several other solid and hematological malignancies, such as lung, breast, esophageal, head and neck, pancreatic, renal cell, cervical, and ovarian cancer, leukemia and lymphoma.[6,30] In 1996, irinotecan received accelerated approval in the USA for the treatment of metastatic colorectal cancer after failure of fluorouracil (5-FU)-based therapy. Two years later it was approved in the Netherlands. Currently, irinotecan is used in combination therapy and as single agent in the first-line and second-line treatment of colorectal cancer, as it prolongs life and improves the quality of life.[31-33]

Although irinotecan is an active drug, it is notorious in clinical practice because of its unpredictable and severe toxicities, mainly diarrhea and neutropenia. Many years of research have given some more insight in the pathophysiology and predictors of these toxicities.[34] However, until now there is no clear explanation for the interpatient variability in exposure and efficacy of irinotecan. Body surface area (BSA)-based dosing of irinotecan does not reduce this variability,[35] which makes BSA-based dosing useless in the case of irinotecan, as it is for many other anticancer drugs.[36] Although flat-fixed dosing seems a good alternative because it is simpler and safer (as no calculation errors can be made),[37] the interpatient variability in pharmacokinetics and toxicities remains the same. Therefore, a new dosing algorithm on the basis of patient characteristics that are known to influence the pharmacokinetics, toxicities and efficacy of irinotecan, is necessary to truly personalize irinotecan therapy.

In general, interpatient variability in drug exposure and efficacy can be explained by several factors, both inherited (genetic) and environmental (**Figure 2**). Since the start of the Human Genome Project in which all human genes and base pairs were analyzed,[38] much focus has been put on polymorphisms in metabolizing enzymes and drug transporters to explain the large interpatient variability that is seen with many anticancer drugs. In the case of irinotecan, much focus was put on the *UGT1A1**28 polymorphism, a promoter repeat in the TATA-box of the *UGT1A1* gene, that results in a reduced formation of the enzyme UGT1A1.[39,40] In addition, several other polymorphisms in the metabolic pathway of irinotecan and their role in the variability of pharmacokinetics and toxicities were investigated.[41-46] However, although *UGT1A1**28 and other polymorphisms do explain a part of the interpatient variability of the pharmacokinetics and toxicities of irinotecan, it is not the holy grail.[47,48]

As already mentioned, not only genetic but also environmental factors play a role in pharmacokinetic variability. In the case of irinotecan, the effect of several concomitant drugs and herbal products has already been investigated, such as combinations of irinotecan with ketoconazole, valproic acid, St John's wort, medical cannabis, and milk thistle.[49-53] However, the effect of other environmental factors, such as comorbidity and lifestyle is scarcely investigated and other drug-drug interactions could also play a role.

Disease	Treatment	Patient
•Tumor Type	•Route of Administration	•Gender
•Metastases	•Dose	•Age
•Clinical Performance	•Infusion Duration	•Ethnicity
•Ascites	•Schedule	•Body Size

Interactions	Exposure	PK	Genetics
•Prescribed Drugs	↓		•Metabolizing enzymes
•OTC Drugs			•Drug transporters
•Herbs	Toxicity	PD	•Pharmacodynamic
•Supplements	Efficacy		markers

Organ function	Unknown factors	Behavior
•Liver		•Smoking
•Kidneys		•Alcohol
•Bone Marrow		•Food
		•Adherence

Figure 2. Factors affecting the interpatient variability of drug therapy
Abbreviations: OTC, over the counter; PK, pharmacokinetics; PD, pharmacodynamics.

In addition to indirect ways of reducing toxicities by reducing the variability in pharmacokinetics, direct ways to decrease irinotecan's toxicities also have been explored, especially for diarrhea. Although nowadays diarrhea is manageable by using high-dose loperamide and antibiotics, prediction and more importantly prevention of the occurrence of diarrhea remains difficult. Strategies for reducing intestinal toxicity have mainly been aimed on preventing the reactivation of SN-38 by β-glucuronidase producing bacteria and the absorption of unbound intestinal SN-38. These include the administration of neomycin, cholestyramine/levofloxacin, activated charcoal, budesonide and compounds that promote intestinal alkalization.[54-60]

AIMS OF THE THESIS

This thesis describes research that was performed in order to add new tools into the toolbox of personalized irinotecan treatment. We set out to find new factors that explain the relatively large interpatient variability in pharmacokinetics and toxicity of irinotecan. Several factors were taken into consideration, such as life style factors (smoking) in **Chapter 2**, genetic factors (mannose-binding lectin polymorphisms) in **Chapter 3**; comorbidity (renal failure) in **Chapter 4** and co-medication in **Chapter 5** (omeprazole) and **Chapter 6** (strumazole). Finally, **Chapter 7** describes a new individualized dosing model for irinotecan based on the most predictive patient characteristics. The aim of this research was to gain knowledge with respect to interpatient variability in pharmacokinetics and toxicities of irinotecan, with the ultimate aim to personalize treatment for each single patient on the basis of his/her characteristics.

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Chapter 2

Cigarette smoking and irinotecan treatment: pharmacokinetic interaction and effects on neutropenia

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ABSTRACT

Purpose

Several constituents of cigarette smoke are known to interact with drug-metabolizing enzymes and potentially affect treatment outcome with substrate drugs. The purpose of this study was to determine the effects of cigarette smoking on the pharmacokinetics and adverse effects of irinotecan.

Patients and methods

A total of 190 patients (49 smokers, 141 non-smokers) treated with irinotecan (90-minute intravenous administration on a three-weekly schedule) were evaluated for pharmaco-kinetics. Complete toxicity data were available in a subset of 134 patients receiving 350 mg/m^2 or 600 mg flat-fixed dose irinotecan.

Results

In smokers, the dose-normalized area under the plasma concentration-time curve of irinotecan was significantly lower (median, 28.7 *versus* 33.9 ng×h/mL/mg; *P* = .001) compared with non-smokers. In addition, smokers showed an almost 40% lower exposure to SN-38 (median, 0.54 *versus* 0.87 ng×h/mL/mg; *P* = .001) and a higher relative extent of glucuronidation of SN-38 into SN-38G (median, 6.6 *versus* 4.5; *P* = .006). Smokers experienced considerably less hematologic toxicity. In particular, the incidence of grade 3 to 4 neutropenia was 6% in smokers versus 38% in non-smokers (odds ratio [OR], 0.10; 95% CI, 0.02 to 0.43; *P* = .001). There was no significant difference in incidence of delayed-onset diarrhea (6% *versus* 15%; OR, 0.34; 95% CI, 0.07 to 1.57; *P* = .149).

Conclusion

This study indicates that smoking significantly lowers both the exposure to irinotecan and treatment-induced neutropenia, indicating a potential risk of treatment failure. Although the underlying mechanism is not entirely clear, modulation of CYP3A and uridine diphosphate glucuronosyltransferase isoform 1A1 may be part of the explanation. The data suggest that additional investigation is warranted to determine whether smokers are at increased risk for treatment failure.

INTRODUCTION

Tobacco is the single largest preventable cause of cancer in the modern world.[1] It accounts for approximately 30% of all cancer deaths. In addition to the fact that it explains almost 90% of lung cancer deaths, it is linked to more than ten different cancer types, including cancer of the head and neck, esophagus, bladder, pancreas, cervix, kidney, stomach, colon, and rectum, and some leukemias, as well as to an earlier onset of cancer and to a worse prognosis.[2-4] Despite all antismoking campaigns, there are currently about 1.3 billion smokers worldwide and this number is still increasing.[5] Interestingly, little data are available on the prevalence of smoking in cancer patients. At the M.D. Anderson Cancer Center (Houston, TX), smoking rates of 30% among both male and female cancer patients were reported.[6] In addition, 25% of patients referred to the Canadian Ottawa Regional Cancer Center were smoking.[7] These numbers are in concordance with smoking prevalence in the general population in the Americas, with estimates of 24% to 32% among men and 18% to 21% among women, respectively.[5,8]

Cigarette smoke contains several constituents known to interact with drug-metabolizing enzymes. For example, polycyclic aromatic hydrocarbons (PAHs) induce CYP1A1 and CYP1A2,[9] both of which are isoforms of the cytochrome P-450 family (CYP) that is involved in the metabolism of almost all anticancer drugs,[10] thereby interfering with the pharmacokinetic profile of drugs metabolized by these CYPs. For instance, the oral clearance of erlotinib, an epidermal growth factor receptor tyrosine kinase inhibitor, was shown to be 24% faster in smokers compared with non-smokers,[11] and this may affect overall survival in non-small cell lung cancer.[12]

PAHs are also known to induce some isoforms of the uridine diphosphate glucuronosyltransferase (UGT) family,[9] which includes important enzymes involved in glucuronic acid conjugation. For example, increased rates of glucuronidation of propranolol and codeine have been reported in smokers.[13,14] In addition to PAHs, other cigarette constituents such as nicotine, carbon monoxide, and cadmium may also be involved in modulation of the expression and function of enzymes and drug transporters involved in drug elimination.[15,16]

Against this background, the purpose of this study was to explore the effect of smoking behavior on the pharmacokinetics and adverse effects of irinotecan (Campto; Pfizer, Capelle aan den IJssel, the Netherlands), a topoisomerase-I inhibitor registered for the first-line and second-line treatment of metastasized and/or inoperable colorectal cancer, which is known to be a substrate for several cytochrome P-450 and UGT1A isozymes (**Figure 1**) and drug transporters, in a large cohort of cancer patients.
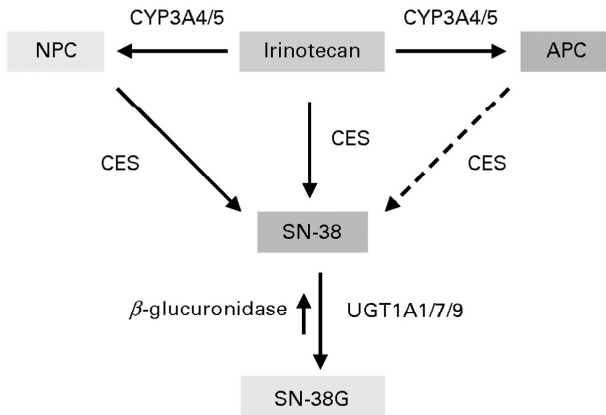
Figure 1. Metabolism of irinotecan
Irinotecan is mainly metabolized by carboxylesterases (CES) forming active SN-38 that is subsequently glucuronidized into SN-38G by uridine diphosphate glucuronosyltransferase (UGT1A). After hepatobiliary excretion, SN-38 is the subject of bacterial β-glucuronidase-mediated reactivation. Alternatively, irinotecan is inactivated by cytochrome P-450 3A (CYP3A) mediated oxidation into APC and NPC, which can be activated by carboxylesterases.

PATIENTS AND METHODS

Patients

A total of 202 patients, previously enrolled in nine different prospective clinical trials with irinotecan between 1996 and 2005 that involved pharmacokinetic analyses,[17-25] were included in this study. The common inclusion criteria were a histologically or cytologically confirmed diagnosis of any form of cancer that was believed to be sensitive to irinotecan or for which no other treatment options were available; age older than 18 years; Eastern Cooperative Oncology Group/WHO performance score less than 2; and adequate hematopoietic, hepatic, and renal functions. A specific exclusion criterion for the current analysis was the use of known CYP3A or UGT1A inducers or inhibitors. All trials were conducted according to the revised Declaration of Helsinki and were approved by the local medical ethical committee. Written informed consent was obtained from all patients.

Treatment

Patients received irinotecan once every three weeks as a 90-minute intravenous infusion at doses ranging from 175 to 350 mg/m^2 or a 600 mg flat-fixed dose. Patients received prophylactic antiemetics and atropine was administered if acute cholinergic syndrome occurred. For the treatment of irinotecan-induced delayed-onset diarrhea, patients received a treatment scheme with loperamide and, if necessary, antibiotics.

Smoking status

Patients were categorized as smokers or non-smokers based on information retrieved from medical files from patient interviews performed on the day before commencing treatment. Former smokers were classified as non-smokers. To use a safe washout period for possible enzyme induction, patients who reported they had stopped smoking within 4 weeks before treatment were excluded from analysis.

Pharmacokinetic analysis

Blood samples of 5 to 7 mL were collected for measurements of irinotecan, SN-38, and SN-38G at serial time points up to 500 hours after infusion. Samples were handled and analyzed by high-performance liquid chromatography as described elsewhere.[26-29] Individual pharmacokinetic parameters were derived as empirical Bayes estimates and were predicted using a previously developed population model,[30] and the POSTHOC option in the software package NONMEM version V (Globomax, Hanover, MD). Clearances were calculated as the dose divided by the area under the plasma concentration-time curve (AUC). Clearances of SN-38 and SN-38G are actually metabolic clearances (i.e., clearance divided by metabolic fraction, for which no assumption in each individual patient was made). Dose-normalized AUCs were calculated as AUC divided by dose.

Relative extent of conversion of irinotecan into SN-38 (percentage), calculated as the molar $AUC_{0\ to\ 100\ hours}$ ratio of SN-38 to irinotecan $\times 100\%$, and the relative extent of glucuronidation of SN-38 into SN-38G, defined as the molar $AUC_{0\ to\ 100\ hours}$ ratio of SN-38G to SN-38, were considered as well. Although these measures reflect carboxylesterase capacity and UGT1A capacity, respectively, both measures are actually surrogate measures.[31] Other factors, although less pronounced, may affect the measures as well, such as CYP3A-mediated inactivation and adenosine triphosphate binding cassette (ABC) drug transporter-mediated excretion.

*UGT1A1*28 genotyping*

UGT1A1 genotype analysis was performed for the presence of an additional (seventh) repeat in the promoter region of *UGT1A1* (i.e., *UGT1A1*28) in whole blood, as described elsewhere.[23] Patients homozygous for six repeats (wild type) were assigned as TA6/TA6, patients homozygous for seven repeats were assigned as TA7/TA7, and heterozygous patients were assigned as TA6/TA7.

CYP3A phenotyping

In a subset of 30 patients, midazolam and erythromycin were administered as phenotyping probes for CYP3A. Both tests (i.e., the midazolam clearance test and the erythromycin breath test) have been described in detail elsewhere.[23]

Pharmacodynamic analysis

Complete blood counts with differential, including white blood cell (WBC) count and absolute neutrophil count (ANC), and clinical chemistry data were determined at baseline and weekly during the three-week follow-up period. Pharmacodynamic relationships were investigated in the subgroup of patients who had received single agent irinotecan at the registered dose of 350 mg/m^2 or the 600 mg dose equivalent.[32] Leukopenia, neutropenia, and diarrhea were graded according to the National Cancer Institute Common Terminology Criteria for Adverse Events version 3.0, and were dichotomized further in no/mild (grade 0 to 2) and severe (grade 3 to 4) toxicity.[33] In addition, hematologic toxicity was evaluated using absolute nadir and percentage decrease at nadir from baseline, defined as: percentage decrease = (baseline value − nadir value)/baseline value x 100%.

Statistical analysis

Data are presented as median and range, unless stated otherwise. To compare continuous variables between smokers and non-smokers, the Mann-Whitney U test was used. Spearman's ρ correlation coefficient was used for relating two continuous variables. If two dichotomous variables were obtained, odds ratios were calculated and a χ^2-test was used to calculate a corresponding P-value. Based on a Bonferroni correction for five variables,[34] two-sided $P < .01$ were considered significant. All statistical tests were performed with SPSS version 14.0.0 (SPSS Inc, Chicago, IL).

RESULTS

Patients

A total of 190 patients (49 smokers and 141 non-smokers; **Table 1**) were assessable for analysis of the influence of smoking on the pharmacokinetic parameters of irinotecan. For 10 patients, smoking status could not be obtained with certainty. Two patients reported having quit smoking within 4 weeks before treatment and therefore were considered not eligible. Other former smokers quit smoking at least 2 months before their first irinotecan treatment and were thus considered as non-smokers. Except for age and bilirubin, none of the baseline demographic data, including *UGT1A1*28* genotype status, were significantly different between smokers and non-smokers (**Table 1**).

Smoking and irinotecan pharmacokinetics

Smokers showed about an 18% faster clearance of irinotecan than non-smokers (median, 34.8 *versus* 29.5 L/h; $P = .001$; **Table 2**). The systemic exposure to the active metabolite SN-38 was almost 40% lower in smokers (median dose-normalized $AUC_{0 \text{ to } 100 \text{ hours}}$, 0.54 *versus* 0.87 ng×h/mL/mg; $P < .001$), whereas no significant effect on the glucuronide

Table 1. Patient characteristics

Parameter	All patients		Non-smokers		Smokers		P^a
	N	%	N	%	N	%	
Total number of patients	190	100	141	74	49	26	
Sex							.584[b]
Male	110	58	80	57	30	61	
Female	80	42	61	43	19	39	
Age, years							.001
Median	54		56		49		
Range	24-75		24-75		27-70		
BSA (m²)							.594
Median	1.88		1.88		1.89		
Range	1.29-2.40		1.29-2.40		1.46-2.36		
Tumor type							.123[b]
Colorectal	78	41	66	47	12	24	
Lung	25	13	16	11	9	18	
(A)CUP	28	15	19	13	9	18	
Pancreas/biliary tract	18	9	12	9	6	12	
Esophageal/gastric	17	9	13	9	4	8	
Miscellaneous	24	13	15	11	9	18	
UGT1A1 genotype	128		93		35		.312[b]
TA6/TA6	64	50	43	46	21	60	
TA6/TA7	56	44	43	46	13	37	
TA7/TA7	8	6	7	7	1	3	
Baseline hematology							
Platelets (×10⁹/L)							.917
Median	296		296		297		
Range	99-966		117-966		99-586		
WBC (×10⁹/L)							.077
Median	7.9		7.8		8.4		
Range	2.8-27.0		2.8-27.0		4.4-15.7		
ANC (×10⁹/L)							.176
Median	5.5		5.4		6.1		
Range	1.5-24.0		1.5-24.0		3.0-12.1		
Hemoglobin (g/dL)							.412
Median	12.7		12.6		12.7		
Range	8.4-16.7		8.5-16.7		8.4-15.1		
Hematocrit (L/L)							.314
Median	0.38		0.38		0.38		
Range	0.27-0.50		0.27-0.50		0.27-0.46		

Table 1. continued

| Parameter	All patients		Non-smokers		Smokers		
	N	%	N	%	N	%	P^a
Baseline chemistry							
Albumin (g/L)							.754
Median	41		41		41		
Range	20-53		20-53		31-53		
Total bilirubin (μmol/L)							< .001
Median	8		9		7		
Range	1-26		3-26		1-18		
Alk phos (U/L)							.552
Median	108		109		106		
Range	26-925		26-925		29-722		
GGT (U/L)							.042
Median	57		61		44		
Range	7-1,437		7-1,437		10-1,364		
AST (U/L)							.049
Median	26		29		24		
Range	6-185		6-185		7-106		
ALT (U/L)							.109
Median	19		20		16		
Range	3-225		5-225		3-87		
Creatinine (μmol/L)							.477
Median	77		76		78		
Range	44-151		44-151		45-134		

Abbreviations: BSA, body surface area; (A)CUP, (adeno) carcinoma of unknown primary; UGT1A1, uridine diphosphate glucuronosyltransferase isoform 1A1; ANC, absolute neutrophil count; Alk phos, alkaline phosphatase; AST, aspartate aminotransferase; ALT, alanine aminotransferase; GGT, gamma-glutamyl transpeptidase; WBC, white blood cell count.
[a] Two-sided Mann-Whitney U test, unless stated otherwise.
[b] χ^2-test (variable categories *versus* smoking status).

conjugate of SN-38 (i.e., SN-38G) was observed (**Table 2**). Mean dose-normalized plasma concentration-time profiles of both irinotecan and SN-38 for smokers and non-smokers are shown in **Figure 2**. In addition, the relative extent of conversion of irinotecan into SN-38, and in particular, the relative extent of glucuronidation of SN-38 into SN-38G, were significantly different between both groups; the relative extent of conversion was lower (median, 2.9% *versus* 3.8%; $P < .001$) and the relative extent of glucuronidation was higher in smokers (median, 6.6 *versus* 4.5; $P = .006$; **Table 2**). No dose-effect relationship could be established between the pharmacokinetic parameters and the amount of smoked cigarettes (data not shown).

In the subgroup of patients not carrying a single *UGT1A1*28* allele (i.e., TA6/TA6 patients), results were comparable. In particular, systemic exposure to SN-38 was significantly lower in smokers (N = 21) compared with non-smokers (N = 43; median dose-normalized $AUC_{0\ to\ 100\ hours}$, 0.54 *versus* 0.82 ng×h/mL/mg; *P* < .001). Likewise, the relative extent of glucuronidation was higher in smokers (median, 6.75 *versus* 4.41; *P* = .006).
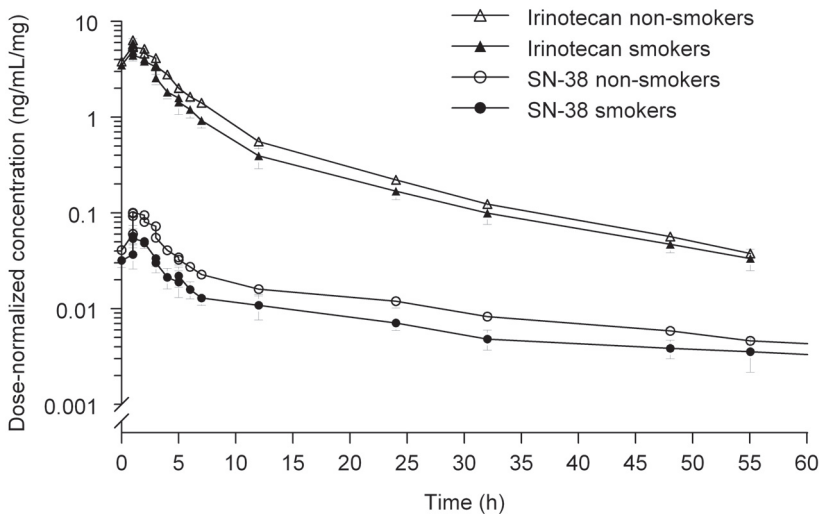
Figure 2. Mean dose-normalized plasma concentration-time curves (± 95% CI) of irinotecan and SN-38 in smokers and non-smokers (N = 190).

Smoking and CYP3A phenotype

Thirty patients (10 smokers and 20 non-smokers) were tested for CYP3A phenotype using the midazolam clearance test and the erythromycin breath test. Pharmacokinetic parameters in this group were representative of the entire population. Although smokers showed a somewhat lower exposure to both midazolam ($t_{4\ hours}$) and erythromycin, indicating higher CYP3A activity, no significant differences were found between both groups **(Table 3)**.

Smoking and irinotecan-related toxicity

In the subgroup of patients treated with the registered dose of irinotecan (350 mg/m^2 or a 600 mg flat-fixed dose), smokers (N = 35) experienced significantly less hematologic toxicity than non-smokers (N = 99). Specifically, the median WBC values decreased to 5.3 ×10^9/L in smokers and 3.0 ×10^9/L in non-smokers (*P* < .001), whereas the respective ANC nadir values were 3.3 ×10^9/L *versus* 1.6 ×10^9/L (*P* < .001). Incidences of grade 3 to 4 leukopenia (9% *versus* 32%; OR, 0.20; 95%CI, 0.06 to 0.69; *P* = .006) and neutropenia (6% *versus* 38%; OR, 0.10; 95% CI, 0.02 to 0.43; *P* < .001) were significantly lower in smokers

Table 2. Effect of smoking behavior on irinotecan pharmacokinetic parameters

Parameter	Number of patients		All patients		Non-smokers		Smokers		P^a
	Non-smokers	Smokers	Median	Range	Median	Range	Median	Range	
Irinotecan									
CL (L/h)	141	49	30.1	10.5-60.8	29.5	10.5-60.8	34.8	11.9-58.9	.001
$AUC_{0-100 \text{ hours}}$ (ng×h/mL/mg)[b]	141	49	33.2	16.4-95.5	33.9	16.4-95.5	28.7	16.9-83.7	.001
$AUC_{0-100 \text{ hours}}$ (mg×h/L)[c]	99	35	21.5	11.9-57.3	21.9	13.3-57.3	18.9	11.9-41.8	.003
SN-38									
CL_{Fm} (L/h)	141	48	331	69-1,683	312	69-1,087	364	164-1,683	.004
$AUC_{0-100 \text{ hours}}$ (ng×h/mL/mg)[b]	141	48	0.81	0.29-3.11	0.87	0.33-3.11	0.54	0.29-1.53	< .001
$AUC_{0-100 \text{ hours}}$ (ng×h/mL)[c]	99	35	494	200-1,868	550	200-1,868	334	210-733	< .001
SN-38G									
CL_{Fm} (L/h)	119	42	47.3	2.2-214	47.7	2.2-214	43.9	12.0-179	.266
$AUC_{0-100 \text{ hours}}$ (ng×h/mL/mg)[b]	119	42	5.6	1.2-56.7	5.8	1.2-56.7	4.9	1.4-20.5	.364
$AUC_{0-100 \text{ hours}}$ (ng×h/mL)[c]	93	34	3,055	831-31,168	3,159	862-31,168	2,841	831-9,859	.346
Metabolic conversion ratios									
REC (%)	141	48	3.5	1.1-9.2	3.8	1.1-8.1	2.9	1.6-9.2	< .001
REG	119	42	5.0	1.3-36.3	4.5	1.3-36.3	6.6	1.9-20.7	.006

Abbreviations: CL, clearance (dose divided by AUC); AUC, area under the plasma concentration-time curve; SN-38, active metabolite of irinotecan; CL_{Fm}, metabolic clearance (clearance divided by metabolic fraction); SN-38G, inactive metabolite of SN-38; $AUC_{0-100 \text{ hours}}$, area under plasma concentration-time curve from time 0 to 100 hours; REC, relative extent of conversion of irinotecan into SN-38; REG, relative extent of glucuronidation of SN-38 into SN-38G.

[a] Two-sided Mann-Whitney U test.

[b] Dose-normalized $AUC_{0-100 \text{ hours}}$ (i.e., $AUC_{0-100 \text{ hours}}$ divided by dose).

[c] $AUC_{0-100 \text{ hours}}$ of patients in the 350 mg/m² or 600 mg flat-fixed dose group.

Table 3. Effect of smoking behavior on CYP3A phenotype

	Non-smokers (N = 20)		Smokers (N = 10)		
Parameter	Median	Range	Median	Range	P^a
Erythromycin breath test					
CER_{20} (% dose/min)	0.018	0.006-0.045	0.016	0.008-0.032	.746
$AUC_{CER\,0-40}$ (% dose)	0.63	0.22-1.53	0.57	0.26-1.11	.779
$1/t_{max}$ (1/min)	0.064	0.041-0.097	0.066	0.045-0.111	.559
Midazolam clearance test					
$t_{4\,hours}$ (ng/ml)	4.05	1.19-12.71	3.51	1.81-9.54	.373
CL (mL/min)	650	261-877	556	332-1,012	.999

Abbreviations: CER_{20}, flux of exhaled radiolabeled CO_2 at time 20 minutes; $AUC_{CER\,0-40}$, area under the flux of exhaled radiolabeled CO_2-time curve from 0 to 40 minutes; $1/t_{max}$, reciprocal of time to peak concentration; $t_{4\,hours}$, midazolam concentration in the 4-hour sample; CL, clearance.
[a] Two-sided Mann Whitney *U* test.

compared with non-smokers (**Table 4**). The $AUC_{0\,to\,100\,hours}$ of both irinotecan and SN-38 were correlated with both the nadir of WBC ($\rho = -.434$ and $\rho = -.582$, respectively; $P <$ .001) and the nadir of ANC ($\rho = -.394$ and $\rho = -.593$, respectively; $P < .001$). Although the incidence of grade 3 to 4 diarrhea was 60% lower in smokers, no significant difference between smokers and non-smokers was found (6% *versus* 15%; OR, 0.34; 95% CI, 0.07 to 1.57; $P < .149$).

DISCUSSION

This study suggests that smoking significantly affects the pharmacokinetics and toxicity profile of irinotecan. The clearance of irinotecan was significantly faster in smokers. In addition, smokers showed a more extensive glucuronidation of SN-38, resulting in reduced systemic exposure to the active metabolite SN-38, which likely contributed to less severe hematologic toxicity. The data complement previous knowledge on the clinical pharmacology of irinotecan, and may have important practical implications for its optimal use. The incentive for this study was the recent finding that smoking might affect the partitioning of irinotecan in red blood cells *in vitro*.[35] In addition, because irinotecan is subject to a highly complex metabolic pathway, involving several phase I and II enzymes and drug transporters (**Figure 1**), an effect of individual constituents of cigarette smoke on these metabolic pathways was hypothesized.

The decreased exposure to irinotecan in smokers observed in this study may be explained by induction of CYPs. Although irinotecan is not metabolized by CYPs typically associated with drug interactions caused by smoking, such as CYP1A1 and CYP1A2,[36,37] it is known to be highly sensitive to CYP3A induction.[24] Previous studies have suggested that smoking may induce CYP3A. For example, the systemic exposure to quinine, a

Table 4. Effect of smoking behavior on irinotecan-induced toxicity in the 350 mg/m^2 or 600 mg flat-fixed dose group

Parameter	Number of patients		All patients		Non-smokers		Smokers		P^a
	Non-smokers	Smokers	Median	Range	Median	Range	Median	Range	
White blood cell count									
Nadir (×10^9/L)	99	35	3.3	0.1-11.9	3.0	0.1-11.9	5.3	0.9-10.3	<.001
Decrease (%)[b]	99	35	57	0-99	61	4-99	36	0-92	<.001
CTC grade 3-4	99	35							.006[c]
Number of patients			35		32		3		
%			26		32		9		
Absolute neutrophil count									
Nadir (×10^9/L)	96	35	1.9	0.03-10.3	1.6	0.03-10.3	3.3	0.5-7.1	<.001
Decrease (%)[b]	95	35	66	0-100	70	4-100	46	0-95	<.001
CTC grade 3-4	96	35							<.001[c]
Number of patients			39		37		2		
%			30		38		6		
Diarrhea									
CTC grade 3-4	99	35							.149[c]
Number of patients			17		15		2		
%			13		15		6		

Abbreviations: CTC, National Cancer Institute Common Terminology Criteria for Adverse Events version 3.0.

[a] Two-sided Mann-Whitney U test, unless stated otherwise.

[b] Percentage decrease nadir to baseline.

[c] χ^2-test (variable categories *versus* smoking status).

known CYP3A substrate, was found to be 44% lower in smokers compared with non-smokers.[38,39] In addition, in two *in vitro* studies, nicotine, the addictive constituent of cigarettes, caused induction of CYP3A transcription by activating the nuclear receptor NR1I2 (pregnane X receptor).[40,41] The possibility of CYP3A modulation by cigarette smoking is supported by the current finding that measures of exposure to the CYP3A probe drugs midazolam and erythromycin were lower in smokers, although this did not reach statistical significance, presumably due to the small sample size studied. It is notable that reduced systemic exposure in smokers has also been reported for the CYP3A4 phenotyping probe alprazolam.[42] However, other studies involving CYP3A substrates have not confirmed this influence of smoking,[43-46] suggesting that, if CYP3A is the causative regulator, this effect might be dependent on the substrate drug.

Alternatively, modulation of carboxylesterases could explain the effects on irinotecan clearance and the relative extent of conversion of irinotecan into SN-38. Indeed, the relative extent of conversion is lower in smokers, at first suggesting inhibition of carboxylesterases and less effective conversion of irinotecan into SN-38. However, conflicting with this hypothesis, smokers were found to have lower systemic concentrations of irinotecan. Apart from CYP3A modulation, this latter finding might be explained by induction of functional expression of carboxylesterases. In this case, the apparent lower extent of conversion of irinotecan into SN-38 should be attributed to a higher glucuronidation of SN-38 into SN-38G, compensating for the metabolism of additionally formed SN-38. Indeed, there is some *in vitro* research supporting the hypothesis that carboxylesterases might be modulated by certain PAHs found in cigarette smoke.[47] However, to date, there are insufficient data to draw any conclusions regarding the influence of smoking on carboxylesterase activity in relation to irinotecan therapy.

In addition to CYPs and carboxylesterases, cigarette smoke is also known to induce glucuronidation of certain drugs.[48,49] Furthermore, there is evidence that cigarette constituents can specifically induce UGT1A.[50,51] The higher relative extent of glucuronidation of SN-38 into SN-38G observed in smokers can be explained by induction of UGT1A1. To excrete the breakdown products of hemoglobin, bilirubin is glucuronidized by UGT1A1. In line with earlier published data,[52-63] somewhat lower baseline bilirubin concentrations in smokers were found in the current study (median, 7.0 *versus* 9.0 μmol/L; *P* < .001), indicating that smoking cigarettes may induce UGT1A1. This theory has also been proposed by others,[64] although the lower bilirubin concentration in smokers might also be explained by an effect on specific drug transporters, such as ABCC2 (canalicular multispecific organic anion transporter) and the organic anion transporting polypeptide 8. Given that the distribution of *UGT1A1**28, which is known to be related to both bilirubin levels and clearance of SN-38, did not differ between smokers and non-smokers, it is unlikely that the different bilirubin levels can be attributed to differences in *UGT1A1**28 status. The lower exposure to SN-38 in smokers is in line with the lower bilirubin level in

this group of patients and strongly suggests that cigarette smoke induces UGT1A1, indirectly lowering the risk of severe adverse effects and the chance of therapeutic benefit as a consequence of the lower SN-38 exposure.

Indeed, in this study, smokers had remarkably less hematologic toxicity. Although smokers are known to have higher WBC counts,[65-67] in this particular population the baseline WBC counts in smokers were only marginally higher ($P = .077$ and $P = .176$ for WBC and ANC, respectively; **Table 1**), suggesting that the higher nadirs and the smaller percentage decrease at nadir seen in smokers during irinotecan therapy cannot be attributed solely to the direct effects of their smoking habit on bone marrow function. Whether the therapeutic outcome was affected could not be investigated reliably in the present study because of the design of the conducted trials and the heterogeneity of the included patients. Induction of other UGT1A enzymes known to be capable of SN-38 glucuronidation, such as UGT1A7 and UGT1A9,[68,69] should not be disregarded. Whether the higher glucuronidation capacity in smokers is to be attributed to induction of UGT1A isoforms expressed in the liver or extrahepatically remains to be investigated. For example, Villard *et al*,[49] found that mice exposed to cigarette smoke had enhanced glucuronidation capacity in the liver and particularly in the lung.

In addition to these effects on phase I and II enzymes, induction of ABC transporters by smoking can result in faster elimination of irinotecan and its metabolites, and hence in decreased exposure.[68] In rats, increased expression of placental ABCB1 (P-glycoprotein) was observed after tobacco exposure,[70] indicating that cigarette smoke may influence irinotecan pharmacokinetics via modulation of ABC transporters. Furthermore, immunohistochemical analysis of non-small cell lung carcinomas in 94 patients revealed higher ABCB1 expression in smokers (58% *versus* 9%; $P < .001$).[71] However, no difference in placental expression of ABCB1 and ABCG2 (breast cancer resistance protein) was seen between smoking and non-smoking mothers.[72] In addition, no effect of nicotine metabolites on organic anion transport by ABCC2 (canalicular multispecific organic anion transporter) was found *in vitro*.[73] In summary, at present, there are insufficient data to make conclusions regarding the influence of smoking on ABC transporters.

Although additional investigation is required to determine the underlying mechanism of the current observations, they may have important clinical implications. Smoking before and during irinotecan treatment seems to have unfavorable effects. The data presented suggest that knowledge of smoking behavior before irinotecan treatment needs to be taken into consideration. Given that our analysis was conducted retrospectively in a heterogeneous patient population, recommendations regarding dose adjustments for smokers or smoking cessation during irinotecan treatment cannot be made at present. In addition, no data are available about the effect of smoking on outcome of irinotecan treatment. Furthermore, influence of smoking also should be investigated in the frequently used combination schemes with irinotecan.

In conclusion, this study suggests that smoking is associated with reduced systemic exposure to irinotecan and its active metabolite SN-38, and subsequently, less severe hematologic toxicity. Given that they both more or less depend on systemic irinotecan and SN-38 exposure, less hematologic toxicity indirectly may reflect a less favorable therapeutic outcome. Therefore, it should be stressed that the lower incidence and less severity of hematologic toxicity of irinotecan therapy in smoking patients is likely an unbalanced and unwanted adverse effect. Although the underlying actual mechanism is not completely clear, the effects on irinotecan pharmacokinetics probably can be ascribed to modulation of enzymes involved in the metabolism of irinotecan, in particular CYP3A and UGT1A1. Although results presented in this article indicate that therapeutic outcome of irinotecan might be affected negatively by smoking, whether smokers should quit smoking or should receive a higher dose (if smoking cessation is not an option) to achieve equal outcome to irinotecan treatment as non-smokers remains to be investigated.

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Chapter 3

Effects of mannose-binding lectin polymorphisms on irinotecan-induced febrile neutropenia

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ABSTRACT

Objective

Mannose-binding lectin (MBL) is important in the innate immune response. *MBL2* gene polymorphisms affect MBL expression, and genotypes yielding low MBL levels have been associated with an elevated risk for infections in hematological cancer patients undergoing chemotherapy. However, these reported associations are inconsistent, and data on patients with solid tumors are lacking. Here, we investigated the effects of *MBL2* genotypes on irinotecan-induced febrile neutropenia in patients with solid tumors.

Patients and methods

Irinotecan-treated patients were genotyped for the *MBL2* gene. Two promoter (-550 H/L and -221 X/Y) and three exon polymorphisms (52 A/D, 54 A/B, and 57 A/C) were determined, together with known risk factors for irinotecan-induced toxicity. Neutropenia and febrile neutropenia were recorded during the first course.

Results

Of the 133 patients, 28% experienced severe neutropenia and 10% experienced febrile neutropenia. No associations were found between exon polymorphisms and febrile neutropenia. However, patients with the H/H promoter genotype, associated with high MBL levels, experienced significantly more febrile neutropenia than patients with the H/L and L/L genotypes (20% *versus* 13% *versus* 5%). Moreover, patients with the HYA haplotype encountered significantly more febrile neutropenia than patients without this high MBL-producing haplotype (16% *versus* 4%). In the subgroup with wild-type exon polymorphisms (A/A), patients with the high MBL promoter phenotype had the highest incidence of febrile neutropenia, regardless of known risk factors.

Conclusion

Patients with high *MBL2* promoter genotypes and haplotypes seem more at risk for developing febrile neutropenia. If confirmed, these preliminary findings may contribute to more individualized approaches of irinotecan treatment.

INTRODUCTION

Mannose-binding lectin (MBL) is produced in the liver and plays an important role in the innate immune system.[1-3] As a member of the collectin family, MBL possesses a carbohydrate recognition domain, which recognizes and binds microbial surface carbohydrates.[4] A wide range of Gram-positive and Gram-negative bacteria, viruses, fungi, and protozoa can be bound by MBL, mediating opsonophagocytosis directly and indirectly by activation of the lectin complement pathway.[1,5] Especially when the adaptive immune system is immature or compromised, MBL becomes very important. In these instances, the innate immune response forms the principal defense against infection, thereby theoretically rendering MBL deficiency a serious risk factor for infection.[1] This seems particularly relevant when a patient is under immunosuppressive therapy or is receiving a bone marrow transplant and/or chemotherapy for hematological malignancies.[3,6-11] However, research on the effects of MBL on hematological malignancies has yielded conflicting results.[7,12-16]

Five different polymorphisms in the *MBL2* gene, located on chromosome 10, are related to serum MBL protein levels.[17-20] Two promoter polymorphisms, -550 H/L (rs11003125) and -221 X/Y (rs7096206), together form three haplotypes, which result in high (HY), intermediate (LY), and low (LX) MBL levels (**Table 1**).[18,21] The remaining three polymorphisms, also referred to as exon polymorphisms, are located on the first exon of the *MBL2* gene; codon 52 (Arg→Cys, rs5030737; also known as the *MBL2*-D variant allele), codon 54 (Gly→Asp, rs1800450; also known as the *MBL2*-B variant allele), and

Table 1. Overview of *MBL2* polymorphisms and haplotype-related expression of MBL

Genotype	Phenotype
Exon 1 polymorphism	
A/A	High MBL
A/O[a]	Intermediate MBL
O/O[a]	Low MBL
Promoter haplotype[b]	
HY	High MBL
LY	Intermediate MBL
LX	Low MBL
Promoter phenotype in A/A patients	
HY/HY and HY/LY	High MBL
LY/LY and HY/LX	Medium MBL
LY/LX and LX/LX	Low MBL

Abbreviations: MBL, mannose-binding lectin.
[a] 'O' refers to 'D' (52Arg→Cys), 'B' (54Gly→Asp), or 'C' (57Gly→Glu) *MBL2* variants.
[b] The HX genotype has only been described in three Sub-saharians and has not been demonstrated in whites.[39,40]

codon 57 (Gly→Glu, rs1800451; also known as the *MBL2*-C variant allele). Homozygous wild-type patients for each of these exon polymorphisms are referred to as A/A and have high circulating MBL levels.[22] Patients carrying one variant allele have intermediate MBL levels and are referred to as A/O, in which the "O" stands for one of the *MBL2* variants (D, B, or C). Patients carrying two variant alleles, whether two equal or two different variants, are referred to as O/O and have low functional MBL levels.[22] The occurrence of these variants is rather frequent, with approximately 40% of whites bearing at least one variant exon polymorphism resulting in reduced serum MBL levels.[21,23,24]

Irinotecan, a prodrug of the topoisomerase I inhibitor SN-38, is widely used in patients with colorectal cancer. It is typically known for its unpredictable toxicities, mainly consisting of (febrile) neutropenia and late-onset diarrhea. The occurrence of these side effects may lead to dose reductions or even treatment discontinuation, thereby attenuating the antitumor activity of irinotecan.[25,26] Several inherited and environmental factors affect the occurrence of these adverse effects. These include polymorphisms in genes encoding drug-metabolizing enzymes and transport proteins, lifestyle factors such as smoking habits, renal function, and co-medication.[27-31] However, despite the elucidation of these risk factors, there is an obvious need to get more insight into factors rendering patients at risk for developing irinotecan-induced toxicities, in particular, febrile neutropenia.

Therefore, together with the facts that data on the association of *MBL2* genotypes with the occurrence of febrile neutropenia in hematological cancer patients are inconsistent and data on such an association in solid cancer patients are lacking, we explored the effect of *MBL2* polymorphisms on febrile neutropenia in irinotecan-treated patients.

PATIENTS AND METHODS

Patients and treatment

Patients who were treated with single agent irinotecan once every three weeks over 90 minutes at a dose of 350 mg/m^2, its 600 mg flat-fixed (irrespective of BSA) dose equivalent,[32,33] or at a dose that was based on cytochrome P450 (CYP)3A4 phenotyping,[34] were studied during their first treatment course. Premedication consisted of dexamethasone and granisetron. In cases of acute cholinergic syndrome, atropine was administered subcutaneously. Delayed-type diarrhea was treated with loperamide and, if necessary, with antibiotics, at the discretion of the treating physician. Febrile neutropenia was treated with the broad-spectrum antibiotic imipenem.

All patients had participated in four prospective trials involving pharmacokinetic and pharmacodynamic analyses.[34-37] Those trials were approved by the institutional medical ethical boards of the participating medical centers and were performed in accordance

with the Declaration of Helsinki. All patients gave written informed consent for participation in those studies and for additional pharmacogenetic analyses. The most important inclusion criteria of these studies were: a histologically or cytologically confirmed diagnosis of any solid tumor that was believed to be sensitive to irinotecan treatment; age ≥ 18 years; a World Health Organization performance status score < 2; and adequate hematological, renal, and hepatic function. Additionally, the time between the last anticancer treatment and the start of irinotecan treatment had to be > 4 weeks. Furthermore, the use of any known CYP3A and/or P-glycoprotein inhibitor or inducer during the whole study period (starting two weeks before the first irinotecan administration) was not allowed. Patients with unresolved bowel obstruction or chronic colic disease were excluded from the study.

In one of the four studies, some of the patients were prophylactically treated with neomycin.[35] Because this aminoglycoside antibiotic is poorly absorbed,[38] and in this study no effects of neomycin prophylaxis on the systemic clearance of SN-38 and the incidence of serious neutropenia and leukopenia were noted,[35] no effect of neomycin co-treatment on febrile neutropenia was anticipated.

Toxicities

Before the start of irinotecan treatment, a complete medical history, physical examination, and hematological and chemical blood analyses were performed. During the three-week follow-up period after the first administration of irinotecan, patients were seen weekly at the outpatient clinic for follow-up, which included a physical examination and routine hematological, renal, and hepatic laboratory analyses. When patients were admitted to the hospital because of severe toxicities, laboratory tests were performed more frequently (at least thrice a week). Toxicities were graded according to the Common Terminology Criteria for Adverse Events version 3.0 of the National Cancer Institute. Febrile neutropenia was defined as a neutrophil count ≤ 1.0×10^9/mL in the presence of fever. Fever was defined as a temperature > 38.5°C on a single measurement or a temperature > 38.0°C on two separate occasions.[9,13]

Genotyping

DNA was obtained from whole blood as described previously,[37] and samples were genotyped for polymorphisms in the *MBL2* gene. Five common polymorphisms of the *MBL2* gene – two in the promoter region (-550 H/L and -221 X/Y) and three in the first exon (52 A/D, 54 A/B, and 57 A/C – were analyzed by allele-specific amplification reactions, according to Mulligan *et al*.[39] The polymerase chain reaction (PCR) amplification was performed in a 25-μL reaction volume, containing 15 ng genomic DNA, PCR Buffer II (Perkin Elmer, Waltham, MA), 1.5 mM $MgCl_2$, 0.2 mM of each of the deoxynucleotide triphosphates (Roche, Indianapolis, IN), 0.5 U Amplitaq Gold (Perkin Elmer), and 20 pmol

of each of the primers, as described elsewhere.[39] PCR products were analyzed on a 1% agarose gel with ethidium bromide, and results were archived using a Gel Doc GD 2000 system (Bio-Rad, Hercules, CA).

Construction of *MBL2* haplotypes and phenotypes

The two promoter polymorphisms, -550 H/L and -221 X/Y, were combined to form three promoter haplotypes: HY, LY, and LX,[12] which are related to high, intermediate, and low levels of MBL, respectively (**Table 1**).[18,21] The HX haplotype probably does not occur in whites because of linkage disequilibrium.[39,40] Combination of these haplotypes in patients having the wild-type exon genotype (A/A) yielded three *MBL2* promoter pheno-types: high (HY/HY and HY/LY), medium (HY/LX and LY/LY), and low (LY/LX and LX/LX).

Additional analyses

Pharmacokinetic analyses and parameters of irinotecan and the active metabolite SN-38, as well as the presence of *UGT1A1**28 and *UGT1A1**93 (-3156 G>A) alleles, which are known to influence irinotecan pharmacokinetics and toxicities by decreasing glucuroni-dation,[30,41] were determined as described previously.[37]

Statistical analyses

All exploratory analyses were performed anonymously and without awareness of clini-cal outcome to exclude any form of potential bias. Differences in the incidence of febrile neutropenia among exon polymorphisms (A/A, A/O, O/O), promoter polymorphisms (-550 H/H, H/L, L/L and -221 X/X, X/Y, Y/Y), and promoter phenotypes (high, medium, low) were analyzed using χ^2-tests for trends.

Associations between toxicities and the presence or absence of the HYA and LXA haplotypes were analyzed using χ^2-tests. In addition, odds ratios (ORs) with 95% con-fidence intervals (CIs) were calculated. Differences in patient characteristics among the MBL-phenotype groups and the promoter polymorphism and haplotype groups were analyzed using Kruskal-Wallis tests in cases of continuous variables and χ^2-tests (for trends) in cases of nominal variables.

Statistical calculations were performed using SPSS version 16.0 (SPSS Inc., Chicago, IL). *P*-values < .05 were considered significant.

RESULTS

Patient characteristics

One hundred thirty-three patients were successfully genotyped for *MBL2* and were included in the analyses. Thirteen patients were excluded from the analyses because there was no blood available for genotyping (N = 7), MBL genotype could not be obtained (N = 4), or no toxicity data were available (N = 2). The characteristics of these patients are shown in **Table 2**. Seventy-two patients (54%) were male and 61 patients (46%) were female. Ninety-seven percent of the patients were white. The most common tumor types were colorectal cancer (27%), upper gastrointestinal cancer (15%), and (adeno) carcinoma of unknown primary (15%). Nineteen patients (15%) had been prophylactically treated with neomycin, as mentioned before.[35]

Toxicities

Twenty-nine patients (22%) experienced fever during their first irinotecan course. Twenty patients (15%) had grade 3 neutropenia and 17 patients (13%) experienced grade 4 neutropenia. Febrile neutropenia was seen in 13 patients (10%) and lasted a median of 1.5 days (range, 1-4 days). In 11 patients with febrile neutropenia (85%), there were neither positive blood cultures nor any clinical signs of infection other than fever. In the only two positive blood cultures that were found, one showed *Escherichia coli* and the other showed *Pseudomonas aeruginosa*. No effect of co-treatment with neomycin on the incidence of febrile neutropenia was seen in this study. Of the 19 patients who received neomycin, 3 developed febrile neutropenia (16%), whereas in patients who were not treated with neomycin, 9 of 102 (8%) developed febrile neutropenia (*P* = .285).

MBL2 polymorphism frequencies

In four patients (2.9%), *MBL2* genotyping was not successful, and therefore those patients were excluded from the analyses. In line with recent publications,[8,14] 50 patients (38%) carried at least one exon 1 mutation. The allele frequency of the wild-type allele (A) was 78%, whereas the rest of the alleles were variably present, with allele frequencies of 5%, 15%, and 2% for the D, B, and C variants, respectively. The frequencies of the -550H and -550L alleles in the promoter were 32% and 68%, respectively, and the frequencies of the -221X and -221Y promoter alleles were 22% and 78%, respectively.

Combining the promoter and exon 1 polymorphisms, six haplotypes were found based on the genotyping results and available literature.[18,39] The most frequent haplotypes were LYA, HYA, and LXA, with haplotype frequencies of 20-30%, whereas LYC and HYD were found sporadically, with haplotype frequencies < 5%. The haplotype frequency of LYB was 15%. These frequencies were in accordance with previously reported frequencies in whites.[8,14,24,42,43]

Table 2. Patient characteristics

Characteristic[a]	N	% or range
Age (years)[b]	57	27-74
BSA (m²)[b]	1.90	1.35-2.50
Race		
White	129	97%
Other[c]	4	3%
Sex		
Male	72	54%
Female	61	46%
Tumor type		
Colorectal	36	27%
Upper gastrointestinal tract	20	15%
(A)CUP	20	15%
Lower gastrointestinal tract[d]	18	14%
Lung	15	11%
Sarcoma	8	6%
Other[e]	16	12%
*UGT1A1*28*[f]		
TA6/TA6 (wild-type)[g]	59	44%
TA6/TA7[h]	63	47%
TA7/TA7[i]	8	6%
*UGT1A1*93 (-3156G>A)*[f]		
G/G (wild-type)	64	49%
G/A	58	45%
A/A	8	6%

Abbreviations: (A)CUP, (adeno)carcinoma of unknown primary; BSA, body surface area.

[a] Data are presented as numbers with percentages, unless stated otherwise.

[b] Data are presented as median with range.

[c] Including Asian (N = 1) and Afro-Caribbean (N = 3).

[d] Including cancer of the bile tract (pancreatic cancer, cholangiocarcinoma, gall bladder cancer, ampulla of vater carcinoma) and small intestines, excluding colorectal cancer.

[e] Including breast cancer (N = 4), head and neck cancer (N = 3), cervical carcinoma (N = 3), ocular melanoma (N = 2), urothelial cell carcinoma (N = 2), ovarian carcinoma (N = 1), and medulloblastoma (N = 1).

[f] N = 130 (*UGT1A1*28* and *UGT1A1*93* status could not be obtained in three patients).

[g] All patients who were homozygous for the wild-type *28 allele were also homozygous for the wild-type *93 allele.

[h] Fifty-eight of 63 heterozygous *28 patients (TA6/TA7) were also heterozygous for the *93 variant allele. Five TA6/TA7 patients were homozygous for the wild-type *93 allele (G/G).

[i] All patients who were homozygous for the variant *28 allele were also homozygous for the variant *93 allele.

Table 3. *MBL2* polymorphisms and haplotypes in relation to irinotecan-induced toxicities

Polymorphisms and haplotypes	Febrile neutropenia[a]					Severe (grade 3 or 4) neutropenia[a]				
	Yes		No			Yes		No		
	N	%	N	%	*P*[b]	N	%	N	%	*P*[b]
Exon 1 polymorphism					.853					.175
A/A	7	8%	76	92%		25	30%	57	70%	
A/O	6	15%	35	85%		12	29%	30	71%	
O/O	0	0%	8	100%		0	0%	8	100%	
-550 promoter polymorphism (H/L)					.039					.437
H/H	3	20%	12	80%		6	40%	9	60%	
H/L	7	13%	46	87%		14	26%	39	74%	
L/L	3	5%	61	95%		17	27%	47	73%	
-221 promoter polymorphism (X/Y)					.079					.869
X/X	0	0%	10	100%		3	30%	7	70%	
X/Y	2	5%	37	95%		10	26%	29	74%	
Y/Y	11	13%	72	87%		24	29%	59	71%	
HYA haplotype					.019[c]					.396[c]
Present	10	16%	51	84%		19	32%	41	68%	
Absent	3	4%	68	96%		18	25%	54	75%	
LXA haplotype					.088[c]					.768[c]
Present	2	4%	47	96%		13	27%	36	73%	
Absent	11	13%	72	87%		24	29%	59	71%	
Promoter phenotype in exon 1 wildtype patients (A/A)[d]					.030					.687
High (HY/HY and HY/LY)	6	15%	33	85%		13	34%	25	66%	
Medium (LY/LY and HY/LX)	1	5%	20	95%		5	24%	16	76%	
Low (LY/LX and LX/LX)	0	0%	23	100%		7	30%	16	70%	

[a] N = 132 patients (data on febrile neutropenia missing in one patient, data on severe neutropenia missing in another patient).
[b] *P*-value result from χ^2-test for trend, unless stated otherwise.
[c] *P*-value result from χ^2-test.
[d] N = 83 patients.

MBL2 exon polymorphisms in relation to irinotecan-induced febrile neutropenia

Febrile neutropenia was not significantly associated with the presence of a certain exon polymorphism (**Table 3**). The incidences of febrile neutropenia were 8% (7 of 83 patients) in patients with the A/A genotype, 15% (6 of 41 patients) in patients with the A/O genotype, and 0% (0 of 8 patients) in the O/O patients (*P* = .853). In the subgroup of patients with severe (grade 3 or 4) neutropenia, no relation was found between the incidence of fever and the presence of a certain exon polymorphism.
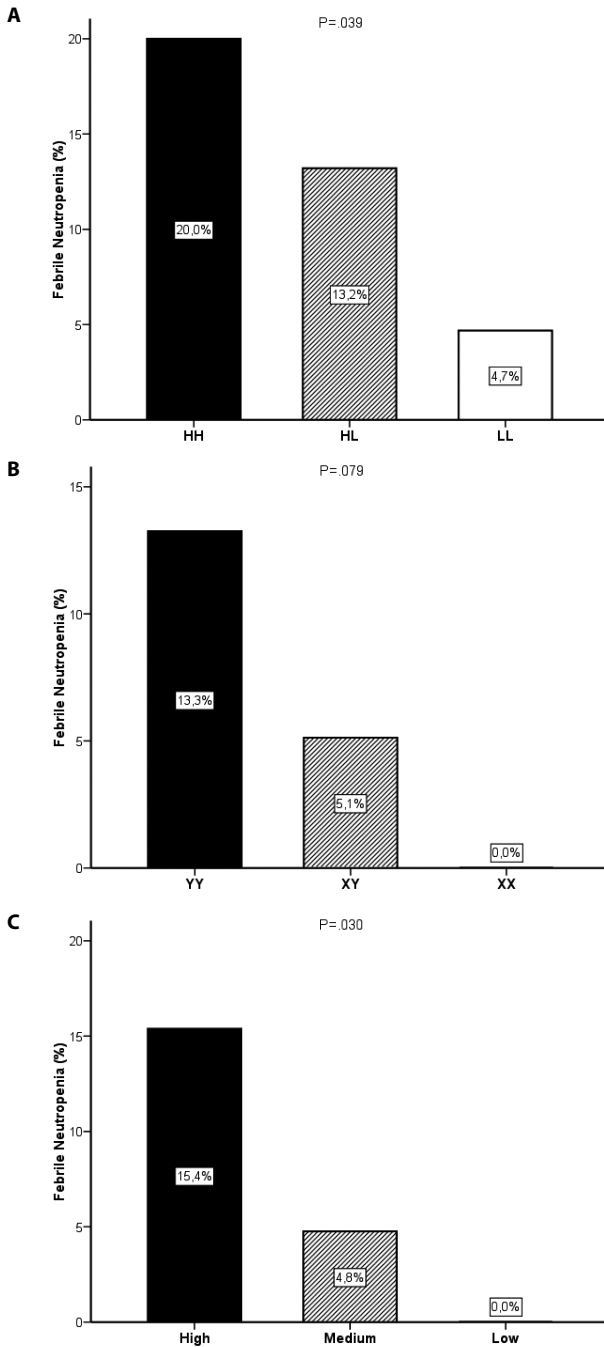
Figure 1. Bar graphs showing the effects of the *MBL2*-promoter -550H/L polymorphism **(A)**, *MBL2*-promoter -221X/Y polymorphism **(B)**, and *MBL2*-promoter phenotypes (high, HY/HY and HY/LY; medium, LY/LY and HY/LX; and low, LY/LX and LX/LX) in patients with the wild-type exon polymorphism (A/A) **(C)** on the incidence of febrile neutropenia in cancer patients treated with irinotecan.

MBL2 promoter polymorphisms in relation to irinotecan-induced febrile neutropenia

As illustrated in **Figure 1A** and **Table 3**, patients with the H/H genotype had a higher incidence of febrile neutropenia than patients with the H/L and L/L genotype (3 of 15 patients (20%) *versus* 7 of 53 patients (13%) *versus* 3 of 64 patients (5%); *P* = .039). A higher incidence of febrile neutropenia was also observed in patients carrying the Y/Y genotype than in those carrying the X/Y and X/X genotypes (11 of 83 patients (13%) *versus* 2 of 39 patients (5%) *versus* 0 of 10 patients (0%)) (**Figure 1B**), although this finding did not reach statistical significance (*P* = .079). In both analyses, the differences in the incidence of febrile neutropenia could not be explained by a different incidence of severe (grade 3 or 4) neutropenia in the groups (*P* = .437).

Similarly, in patients experiencing severe neutropenia, the risk for febrile neutropenia was likewise dependent on promoter genotype, illustrating the role of MBL in immune-compromised patients. Patients with the H/H and the H/L genotypes had higher incidences of febrile neutropenia than patients with the L/L genotype (50% *versus* 50% *versus* 18%; *P* = .066), and patients carrying the Y/Y genotype had a higher incidence of febrile neutropenia than patients carrying the X/Y and X/X genotypes (48% *versus* 20% *versus* 0%; *P* = .044).

MBL2 promoter haplotypes and phenotypes in relation to irinotecan-induced febrile neutropenia

In line with analyses of individual promoter polymorphisms, patients with at least one HYA haplotype had a four times higher incidence of febrile neutropenia than patients who did not have this high MBL level haplotype (16% *versus* 4%; OR, 4.4; 95% CI, 1.2-17.0; *P* = .019) (**Figure 2A**). In the subgroup of patients with a wild-type exon polymorphism (A/A), patients with the HYA haplotype also had a higher incidence of febrile neutropenia (N = 83; 15% *versus* 0%; OR, 1.2; 95% CI, 1.0-1.3; *P* = .018). Importantly, in patients with severe neutropenia (grade 3 or 4), the presence of at least one HYA haplotype resulted in a higher risk for developing fever during the neutropenic episode (N = 36; 53% *versus* 18%; OR, 5.2; 95% CI, 1.1-24.1; *P* = .029). This was also the case in the subgroup of patients with the wild type exon polymorphism who had severe neutropenia (N = 25; 47% *versus* 0%; OR, 1.9; 95% CI, 1.2-3.0; *P* = .011).

Although not significant, patients without the low MBL-producing haplotype LXA had a trend toward a more than three times higher risk for febrile neutropenia than patients with this haplotype (13% *versus* 4%; OR, 3.6; 95% CI, 0.8-16.9; *P* = .088) (**Figure 2B**).

In the subgroup of patients with the wild-type exon polymorphism (A/A), patients with the high *MBL2* promoter phenotype (HY/HY and HY/LY) had the highest incidence of febrile neutropenia, compared with the medium (LY/LY and HY/LX) and low (LY/LX and LX/LX) *MBL2* promoter phenotypes, as illustrated in **Figure 1C** (15% *versus* 5% *versus* 0%; *P* = .030). This could not be explained by a higher incidence of severe (grade 3 or 4) neutropenia in this group (34% *versus* 24% *versus* 30%; *P* = .687). Likewise, no effect
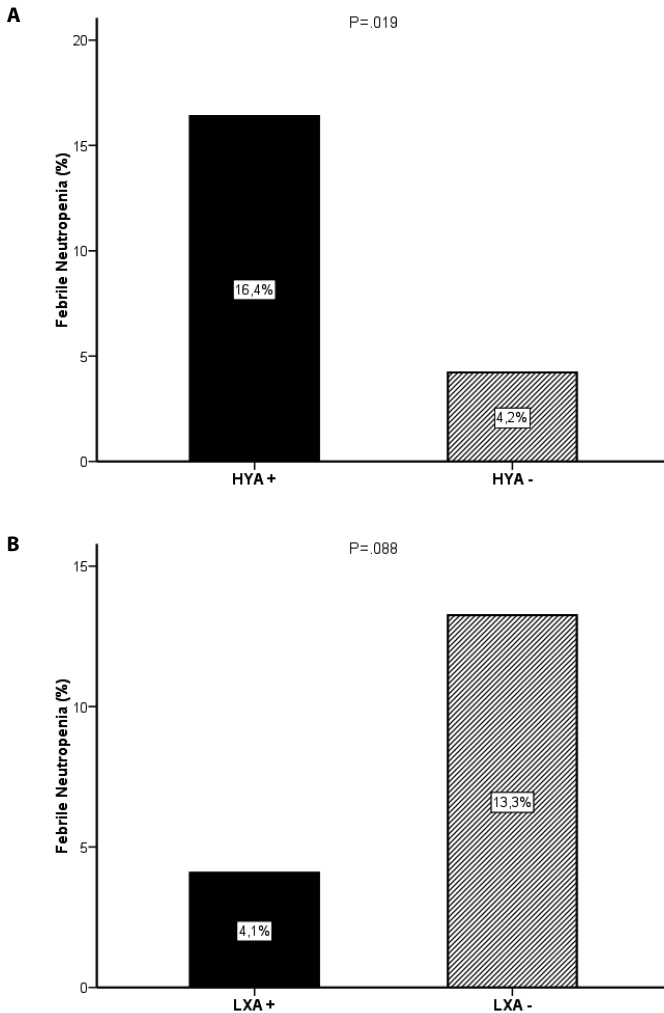
Figure 2. Bar graphs showing the negative effects of the presence of at least one *MBL2* HYA haplotype **(A)** and the absence of a *MBL2* LXA haplotype **(B)** on the incidence of febrile neutropenia in cancer patients treated with single agent irinotecan.

of other potential confounders such as age, exposure to SN-38, or the presence of the *UGT1A1**28 or *UGT1A1**93 alleles was found (**Tables 4-6**).

Also, in the subgroup of patients with the wild-type exon polymorphism who experienced severe neutropenia, a higher incidence of febrile neutropenia was observed in patients with the high *MBL2* promoter phenotypes (46% *versus* 20% *versus* 0%; *P* = .029).

Table 4. Distribution of other variables in patients with the -550 H/L *MBL2* promoter polymorphism

Variable[a]	H/H	H/L	L/L	*P*[b]
Irinotecan dose (mg)	680	600	600	.554
Irinotecan AUC_{0-100h} (mg×h/L)	21.4	20.5	21.9	.706
SN-38 AUC_{0-100h} (ng×h/mL)	451.5	488.3	482.9	.932
UGT1A1*28 genotyping[c]				.329[d]
No *28 allele	11 (19%)	19 (32%)	29 (49%)	
≥ 1 *28 allele	4 (6%)	33 (46%)	34 (48%)	
UGT1A1*93 genotyping[c]				.676[d]
No *93 allele	11 (17%)	20 (31%)	33 (52%)	
≥ 1 *93 allele	4 (6%)	32 (48%)	30 (45%)	

[a] Data are presented as medians, unless stated otherwise.
[b] Kruskal-Wallis test, unless stated otherwise.
[c] Data are presented as numbers with percentages in parentheses.
[d] Chi-square test for trend.

Table 5. Distribution of other variables in patients with and without the HYA haplotype

Variable[a]	HYA+	HYA-	*P*[b]
Irinotecan dose (mg)	620	600	.223
Irinotecan AUC_{0-100h} (mg×h/L)	20.5	21.9	.306
SN-38 AUC_{0-100h} (ng×h/mL)	483.9	449.2	.190
UGT1A1*28 genotyping[c]			.935[d]
No *28 allele	27 (46%)	32 (54%)	
≥ 1 *28 allele	33 (46%)	38 (54%)	
UGT1A1*93 genotyping[c]			.588[d]
No *93 allele	28 (44%)	36 (56%)	
≥ 1 *93 allele	32 (48%)	34 (52%)	

[a] Data are presented as medians, unless stated otherwise.
[b] Kruskal-Wallis test, unless stated otherwise.
[c] Data are presented as numbers with percentages in parentheses.
[d] Chi-square test.

DISCUSSION

This study explored the influence of *MBL2* polymorphisms on chemotherapy-induced neutropenic fever in adult cancer patients with solid tumors. Because the innate immune response, with MBL as a key factor, is thought to play a crucial role in the defense against infectious pathogens (in particular, in cases of neutropenia), we initially hypothesized that patients with functional genotypes associated with low MBL production would be more prone to develop neutropenic fever. In contrast, we found that patients with high MBL-producing promoter genotypes, haplotypes, and haplotype combinations of the *MBL2* gene suffer more often from febrile neutropenia after irinotecan chemotherapy

Tabel 6. Distribution of other variables in *MBL2* promoter phenotype groups in the exon polymorphism wild-type (A/A) patients (N=83)

Variable[a]	High	Medium	Low	*P*[b]
	HY/HY and HY/LY	LY/LY and HY/LX	LY/LX and LX/LX	
Irinotecan dose (mg)	620	600	610	.736
Irinotecan AUC$_{0-100h}$ (mg×h/L)	20.0	21.3	21.7	.901
SN-38 AUC$_{0-100h}$ (ng×h/mL)	498.7	424.7	492.6	.278
UGT1A1*28 genotyping[c]				.855[d]
No *28 allele	18 (47%)	9 (43%)	10 (45%)	
≥ 1 *28 allele	20 (53%)	12 (57%)	12 (55%)	
UGT1A1*93 genotyping[c]				.938[d]
No *93 allele	19 (50%)	9 (43%)	11 (50%)	
≥ 1 *93 allele	19 (50%)	12 (57%)	11 (50%)	

[a] Data are presented as medians, unless stated otherwise.
[b] Kruskal-Wallis test, unless stated otherwise.
[c] Data are presented as numbers with percentages in parentheses.
[d] Chi-square test for trend.

than patients with low MBL-producing genotypes. Also, in the group of patients who developed grade 3 or 4 neutropenia, the incidence of neutropenic fever was higher in patients with genotypes yielding high MBL levels.

In view of our results, we hypothesize that low MBL levels (as determined by genotype) might have a protective effect against the occurrence of fever in neutropenic patients because of insufficient activation of the complement system. A high frequency of unexplained fever during neutropenic episodes was shown previously.[44,45] Also, in our study population, almost no infectious causes could be identified in the patients developing febrile neutropenia. Therefore, it could be hypothesized that fever in (most) patients with neutropenia is induced by the neutropenic state as such, rather than by the presence of infectious pathogens. This might explain why patients with high MBL genotypes had a higher incidence of febrile neutropenia in our study, because higher MBL levels might induce the occurrence of an immunological response.[46] Another explanation for our findings is that transient neutropenia is commonly associated with subclinical infections. If true, those patients with genotypes yielding high MBL levels may be more prone to developing fever as a response to neutropenia than patients with genotypes yielding low MBL concentrations. However, these explanations are hypothetical and should be further investigated.

To the best of our knowledge, this is the first study addressing the association of MBL genotypes and neutropenic fever in adult patients with solid malignancies who are treated with chemotherapy. Previous research on the influence of *MBL2* polymorphisms and MBL serum levels on the incidence and duration of infectious toxicities has been conducted only in hematological and pediatric cancer patients, and yielded conflicting

results. In patients with genotypes producing low MBL levels, a longer duration of febrile neutropenia and a higher risk for major infections were reported in pediatric patients undergoing chemotherapy for (mainly) hematological malignancies and in adults after allogeneic hemopoietic stem cell transplantation.[7,9] The differences between these and our results may be explained by the, in general, much more myelosuppressive (or even myeloablative) effect of chemotherapy for hematological malignances than of chemotherapy administered to our population, apart from the fact that hematological malignancies themselves also cause myelosuppression,[47] and that the mode of action of MBL is influenced by the depth and duration of myelosuppression.

In line with our findings, Schlapbach *et al.* found that not only children with the lowest MBL levels (< 100 µg/L), but also children with the highest MBL levels (> 1,000 µg/L) had more frequent episodes of febrile neutropenia and were hospitalized longer than children with intermediate MBL levels after chemotherapeutic treatment for solid and hematological cancers.[10] They showed that high MBL levels may be less beneficial for patients, possibly as a result of greater complement activation and the stimulation of pro-inflammatory signals,[46] and that moderate MBL deficiency may be favorable, especially in patients who briefly receive immunosuppressive chemotherapy. This could explain why we only found an effect of promoter polymorphisms, which are known to have a less dramatic effect on MBL levels than exon polymorphisms, on the incidence of febrile neutropenia.[18] Another interesting analysis showed a trend toward more grade 4 neutropenic infections in multiple myeloma patients carrying the high MBL-producing wild-type exon polymorphism.[14] Interestingly, Klostergaard *et al.* found a significantly higher incidence of Gram-negative blood cultures in high MBL genotypes,[48] which could explain why we found a higher incidence of febrile neutropenia in high MBL genotypes and positive blood cultures of only Gram-negative bacteria (*Escherichia coli* and *Pseudomonas aeruginosa*) in our study.

In addition to these studies, several other studies could not reveal an association between *MBL2* genotype or serum MBL levels and infectious complications after chemotherapy.[12,13,16,49] The conflicting outcomes of these studies clearly underline that more research is required to unravel the exact relationship among *MBL2* genotypes, MBL serum levels, and the occurrence of neutropenic events in cancer patients treated with chemotherapy. We therefore feel that the initiation of studies on MBL replacement therapy, as has occurred in patients with multiple myeloma or children with chemotherapy-induced neutropenia, is premature.[50]

An important caveat of studies on the relationship between *MBL2* gene polymorphisms and neutropenic events (including ours) is the fact that in ill people, MBL levels are determined not only by genotype, but likely also by the disease itself in the context of an acute-phase response.[8,12,13] Moreover, our study population was relatively small, which resulted in a low absolute number of neutropenic events seen. In addition, sev-

eral tumor types were involved, which could have influenced outcomes. Because this study was exploratory in nature, no corrections for multiple analyses were performed. Therefore, our findings should be interpreted with caution and should be confirmed in larger (and more uniform) study populations, preferably in combination with MBL serum level measurements, to determine whether the known genotype/phenotype relations in healthy humans are comparable in cancer patients. If confirmed, the finding that patients having polymorphisms in the *MBL2* gene, associated with high MBL levels, have an elevated risk for developing irinotecan-induced febrile neutropenia is of importance. As a result, *MBL2* genotyping could have a meaningful place in clinical decision making and contribute to more personalized patient management.

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3

Chapter 4

Renal function as a predictor of irinotecan-induced neutropenia

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ABSTRACT

Although approximately half of the administered dose of irinotecan is recovered in urine, scarce data are available on the association of renal function with irinotecan pharmacokinetics and toxicity. Here, these relationships are investigated in 187 patients treated with irinotecan in a three-weekly schedule. No significant effects on irinotecan pharmacokinetics were found in these patients. However, in 131 patients treated with the registered dose, categorized renal function was related to hematological toxicity. The incidence of grade 3-4 neutropenia decreased as function of creatinine clearance, particularly in non-smoking patients ($P < .01$). Patients with lower creatinine clearances (35-66 mL/min) had a four times higher risk of grade 3-4 neutropenia (58% *versus* 14%; $P < .001$). This study suggests that pretreatment renal function values are associated with irinotecan-induced neutropenia. A confirmatory analysis is warranted to determine whether measures of renal function should be incorporated in future attempts toward individualized treatment with irinotecan.

INTRODUCTION

Irinotecan is an anticancer drug used in the treatment of various types of cancers. Added to fluorouracil, it has become (part of) the standard of care in colorectal cancer.[1,2] Like other anticancer drugs, a narrow therapeutic window characterizes this topoisomerase-I inhibitor, with delayed-type diarrhea and neutropenia being the main dose-limiting toxicities.[3-5] Indeed, occurrence of these toxicities has been directly related to the exposure to its active metabolite SN-38 (7-ethyl-10-hydroxycamptothecin). After the view emerged that dose-individualization based on body surface area (BSA) does not truly personalize irinotecan treatment,[6,7] numerous other potential determinants have been investigated.

The ultimate goal of these attempts is to predict the individual patient's drug clearance before administration of irinotecan to calculate a patient-tailored dose resulting in a controlled, predictable, and safe systemic exposure. For example, genetic polymorphisms in the uridine diphosphate glucuronosyltransferase (UGT) genes are known to result in a reduced detoxification capacity of SN-38 into its glucuronide conjugate (SN-38G) and thus in a higher systemic exposure to toxic SN-38. In particular, the homozygous presence of an additional, seventh, TA-repeat in the promoter region of *UGT1A1* (TA7; *UGT1A1*28) has been identified as a risk factor for therapy-induced neutropenia.[4,8]

Cytochrome P450 (CYP) 3A activity also relates to irinotecan interpatient variability. Apart from drug-drug interactions on the CYP3A level resulting in unintended toxicity or undertreatment,[9] a high correlation between the clearance of irinotecan and the CYP3A phenotyping probe midazolam has been demonstrated,[10] based on which a new dosing formula truly personalizing irinotecan treatment has been derived, which is currently being tested prospectively. Lifestyle behavior may affect irinotecan therapy as well, making it a factor that should be kept in mind while considering the application of or actually dosing irinotecan. Recently we showed that cigarette smoke constituents induce key enzymes and transporters involved in irinotecan metabolism, resulting in a significantly reduced exposure to SN-38 and a reduction in National Cancer Institute's Common Terminology Criteria (version 3.0) grade 3-4 neutropenia from 38 to 6%.[11]

For those cytotoxic agents for which renal excretion plays a major role in its elimination pathways, drug pharmacokinetics may be altered in patients with impaired renal function. Although recently questioned for patients with adequate renal function,[12] carboplatin, for example, is routinely tailored to a patient's renal function according to Calvert's formula. Based on this formula, carboplatin dosing is adjusted to an expected exposure given a patient's renal function, defined as the creatinine clearance, which is supposed to avoid excessive exposure and toxicity, as well as too low exposure related to reduced therapeutic benefit,[12,13] thus optimizing treatment outcome. Likewise, depending on the drug and the severity of renal impairment in patients with impaired renal

function, dose adjustment should be considered for anticancer drugs such as cisplatin, epirubicin, cyclophosphamide, etoposide, and capecitabine, among others.[14]

In mass balance studies on irinotecan more than half of the dose recovered was found in urine, with unchanged irinotecan, the inactive metabolite APC (7-ethyl-10-[4-*N*-(5 aminopentanoic acid)-1-piperidino]-carbonyloxy-camptothecin), and the glucuronide conjugate of SN-38 (**Figure 1**) as the main excretion products in urine.[15-17] Despite the significant renal involvement in the excretion of irinotecan, most attention has been focused on hepatic and intestinal factors explaining interpatient variability in pharmacokinetics and toxicity. In addition, data on the pharmacokinetics of irinotecan in renally impaired patients are scant.[14,18] A single previous study showed that renal impairment did not alter irinotecan pharmacokinetics, although the number of studied patients was very small.[18] Because irinotecan was administered at a reduced dose of 225 mg/m^2 to only nine patients with varying degrees of renal impairment (median creatinine clearance of 41 mL/min; range 21-60 mL/min), it was not possible to provide a dosing recommendation.

In this study, we retrospectively assessed in prospectively obtained data the associations of renal function with irinotecan pharmacokinetics and toxicity in a large cohort of cancer patients to investigate whether renal function can be a valuable addition in the toolbox to individualize irinotecan therapy.
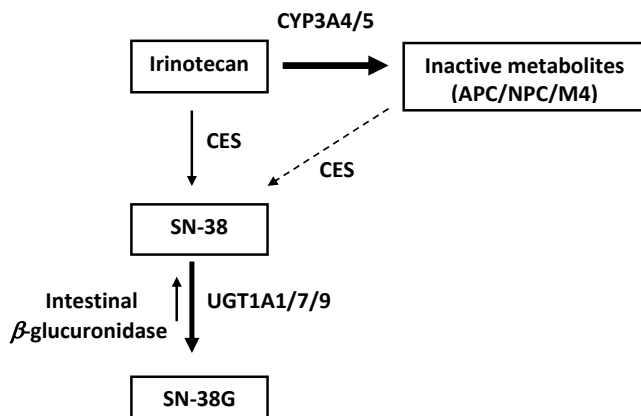
Figure 1. Metabolism of irinotecan
Irinotecan is metabolized into its active metabolite SN-38 by carboxylesterases (CES). SN-38 (7-ethyl-10-hydroxycamptothecin) is subsequently inactivated into its glucuronide conjugate, SN-38G, by uridine diphosphate glucuronosyltransferases (UGTs), especially by UGT1A1, 1A7, and 1A9, in decreasing order of importance. After hepatobiliary excretion, SN-38G can be reconverted into active SN-38 by β-glucuronidases, produced by intestinal bacteria. An alternative pathway of irinotecan detoxification is the CYP3A (cytochrome P450 isoforms 3A4 and 3A5)-mediated oxidation of irinotecan in APC (7-ethyl-10-[4-*N*-(5-aminopentanoic acid)-1-piperidino]-carbonyloxycamptothecin) and NPC (7-ethyl-10-[4-amino-1-piperidino]-carbonyloxycamptothecin). NPC, and possibly APC, can be converted into SN-38 by CES as well.

METHODS

Patients

Common inclusion criteria for the various trials, including pharmacokinetic analysis of irinotecan and its metabolites SN-38 and SN-38G, in which the patients participated have been described extensively elsewhere.[3,6,10,19-24] Depending on the specific study protocol, patients with serum creatinine levels on study entry of < 1.25 or 1.5 times the institutional upper limit of normal and/or a minimum creatinine clearance calculated according to Cockcroft-Gault of 60 mL/min were considered eligible. All trials were conducted according to the revised Declaration of Helsinki and were approved by the local medical ethical committee and written informed consent was obtained from all patients.

A specific exclusion criterion for this study was the use of agents known to interfere with the function and transcription of CYP3A or UGT1A. Other study-specific criteria included the availability of a baseline serum creatinine level, and the availability of smoking status, given the previously documented effects of smoking on irinotecan pharmacokinetics and neutropenia.[11] In those patients who had separately consented for a genetic procedure, *UGT1A1* genotype analysis was performed for the presence of an additional (seventh) repeat in its promoter region (i.e., *UGT1A1*28) as described previously.[10] Patients homozygous for six repeats (wild type) were assigned as TA6/TA6, patients homozygous for seven repeats (variant) as TA7/TA7, and heterozygous patients as TA6/TA7.

Treatment

Irinotecan (Campto/Camptosar; Pfizer, Capelle aan den IJssel, The Netherlands) was administered in a three-weekly schedule using a 90-minute infusion. Doses ranged from 175 to 350 mg/m^2 or 600 mg. One hundred and thirty-one patients were treated with 350 mg irinotecan per square meter BSA or its 600 mg flat-fixed dose equivalent. Prophylactic antiemetics, atropine (in case of an acute cholinergic syndrome), loperamide (at the earliest signs of delayed-onset diarrhea), and antibiotics (if loperamide did not control diarrhea) were administered as needed.

Pharmacokinetic analysis

Blood samples were collected, handled, and analyzed for irinotecan, SN-38, and SN-38G, as described previously in detail.[25-28] Individual pharmacokinetic parameters were derived as empirical Bayes estimates and were predicted using a three-compartment population model using the software package NONMEM version V (Globomax, Hanover, MD).[29] Clearances for irinotecan, SN-38, and SN-38G were calculated, and the area under the plasma concentration-time curves (AUC) were simulated up to 100 hours.

Pharmacodynamic analysis

Complete blood cell counts with differential, including white blood cell count and absolute neutrophil count, and clinical chemistry data were determined at baseline. For inclusion into the toxicity analyses, patients had to be treated with single agent irinotecan at the three-weekly registered dose of 350 mg/m^2 or its 600 mg flat-fixed dose equivalent. During a three-week follow-up period, patients were seen weekly in the outpatient clinic for toxicity assessment and blood analysis. Toxicities, in particular leukopenia, neutropenia, and diarrhea, were graded according to the National Cancer Institute's Common Terminology Criteria for Adverse Events version 3.0 and were further dichotomized in no/mild (grade 0-2) and severe (grade 3-4) toxicity. In addition, hematological toxicity was evaluated using absolute nadir and percentage decrease at nadir from baseline.

Renal function

The creatinine clearance was calculated according to the formula derived by Cockcroft-Gault (140 − (age in years)) × (weight in kg) × (1 − 0.15 × (gender; 0 if male, 1 if female))/ ((serum creatinine in mg/dL) × 72). In clinical practice, several classifications are used. The FDA, for example, divides renal function into five different groups.[30] Group 1 (normal function) has an estimated creatinine clearance > 80 mL/min, group 2 (mild impairment) between 50 and 80 mL/min, group 3 (moderate impairment) between 30 and 50 mL/ min, group 4 (severe impairment) < 30 mL/min, and group 5 (end stage renal disease) those requiring dialysis (**Table 1**).

Table 1. Distributions of estimated creatinine clearances over different classifications[a]

	Present study			FDA		
	Boundaries[b]	**N**	**%**	**Boundaries[b]**	**N**	**%**
Group 1	> 130	23	12.2%	> 80	128	68.4%
Group 2	98-130	65	34.8%	50-80	56	29.9%
Group 3	66-98	68	36.4%	30-50	3	1.6%
Group 4	35-66	31	16.6%	< 30[c]	0	0%

Abbreviations: FDA, US Food and Drug Administration; N, number of patients.
[a] Creatinine clearance calculated according to Cockcroft-Gault.
[b] Boundaries in mL/min.
[c] Those requiring dialysis are included in a fifth category.

Also other classification systems with somewhat other boundaries or normalized to BSA are used. If renal function is expected to play a role in predicting its pharmacokinetics and/or adverse effects, in clinical practice usually a cutoff value of 60 mL/min is used to determine whether a specific anticancer agent can be safely administered or not. Given the inclusion criteria of the studies in which the patients included in the present

analysis participated,[3,6,10,19-24] and the clinical irinotecan practice for safety applied lower boundaries of renal function, a limited number of patients in groups 2-5 was expected (**Table 1**). Therefore, to investigate the effects of renal function on irinotecan pharmacokinetics and toxicity adequately, an alternative classification, categorizing creatinine clearance in four different groups based on its mean and SD, as shown in **Table 2**, was used.

Table 2. Boundaries of estimated creatinine clearance used in this study[a]

	Lower bound	Upper bound	Boundaries (mL/min)	N
Group 1	mean + SD	—	> 130	23
Group 2	mean	mean + SD	98-130	65
Group 3	mean – SD	mean	66-98	68
Group 4	—	mean – SD	< 66	31

Abbreviations: N, number of patients; SD, standard deviation.
[a] Creatinine clearance calculated according to Cockcroft-Gault.

Statistical analysis

Data are presented as mean with SD in parentheses, unless stated otherwise. Pearson's correlation coefficient was calculated to evaluate potential relationships between continuous variables. In case of two categorical variables, the χ^2-test was performed. Multiple linear regression analysis was performed to exclude possible confounding effects of parameters related to creatinine clearance on the relationship between toxicity and categorized creatinine clearance. The Student's *t*-test or one-way ANOVA was used to compare differences in continuous variables among different categories of creatinine clearance, whereas the Jonckheere-Terpstra test was performed to investigate trends over the categorized groups. Correcting for multiple analyses, two-sided probability values of < .01 were considered as statistically significant. All tests were performed with SPSS version 14.0.0 (SPSS, Chicago, IL).

RESULTS

Patients

A total of 187 patients (**Table 3**) were evaluable for pharmacokinetic analysis of irinotecan, SN-38, and SN-38G. About half of patients were male and one in three was classified as a (current) smoker. The most common tumor types were colorectal cancer, (adeno)carcinoma of unknown primary, and lung cancer. One hundred and thirty-one patients were treated with the registered dose of irinotecan (350 mg/m^2 or 600 mg) and their data were available for toxicity analysis.

Table 3. Patient characteristics as function of creatinine clearance[a]

Parameter[b]	All patients	Group 1 (> 130 mL/min)	Group 2 (98-130 mL/min)	Group 3 (66-98 mL/min)	Group 4 (35-66 mL/min)	P[x]
Number of patients[d]	187 (100%)	23 (12%)	65 (35%)	68 (36%)	31 (17%)	
Gender[d]						.03
Male	109 (58%)	17 (74%)	43 (66%)	37 (54%)	12 (39%)	
Female	78 (42%)	6 (26%)	22 (34%)	31 (46%)	19 (61%)	
Smoking status[d]						.72
Current smoker	48 (26%)	7 (30%)	19 (29%)	15 (22%)	7 (23%)	
Non-smoker	139 (74%)	16 (70%)	46 (71%)	53 (78%)	24 (77%)	
Age (years)	54.0 (10.1)	46.0 (9.2)	52.1 (8.7)	55.4 (9.8)	61.0 (9.2)	<.001
Weight (kg)	75.1 (15.3)	86.6 (15.1)	81.1 (14.9)	71.1 (11.0)	63.0 (13.3)	<.001
Length (m)	1.73 (0.09)	1.79 (0.08)	1.75 (0.09)	1.71 (0.09)	1.68 (0.09)	<.001
BSA (m^2)	1.89 (0.22)	2.06 (0.19)	1.97 (0.21)	1.83 (0.17)	1.71 (0.21)	<.001
Tumor type[d]						.55
Colorectal	76 (41%)	5 (22%)	25 (39%)	33 (49%)	13 (42%)	
(A)CUP	28 (15%)	6 (26%)	10 (15%)	5 (7%)	7 (23%)	
Lung	25 (13%)	2 (9%)	10 (15%)	10 (15%)	3 (10%)	
Pancreas/biliary tract	18 (9%)	2 (9%)	8 (12%)	6 (9%)	2 (7%)	
Esophageal/gastric	17 (9%)	4 (17%)	5 (8%)	5 (7%)	3 (10%)	
Miscellaneous	23 (12%)	4 (17%)	7 (11%)	9 (13%)	3 (10%)	
UGT1A1 genotype[d]	125	22	46	37	20	.34
TA6/TA6	62 (50%)	13 (59%)	20 (43%)	22 (59%)	7 (35%)	
TA6/TA7	55 (44%)	7 (32%)	24 (52%)	12 (32%)	12 (60%)	
TA7/TA7	8 (6%)	2 (9%)	2 (8%)	3 (8%)	1 (6%)	

						p
Baseline hematology						
Platelets (×10⁹/L)	306 (105)	313 (124)	283 (98)	317 (111)	317 (92)	.34
White blood cell count (×10⁹/L)	8.3 (2.8)	8.4 (2.4)	8.1 (2.8)	8.2 (2.7)	8.8 (3.0)	.59
Absolute neutrophil count (×10⁹/L)	6.0 (2.4)	6.2 (2.0)	5.8 (2.7)	5.8 (2.3)	6.5 (2.5)	.50
Hemoglobin (mmol/L)	7.8 (1.1)	7.9 (1.1)	8.0 (1.0)	7.7 (1.0)	7.4 (1.1)	.04
Hematocrit (L/L)	0.38 (0.05)	0.38 (0.04)	0.39 (0.04)	0.38 (0.05)	0.36 (0.05)	.13
Baseline chemistry						
Albumin (g/l)	40.7 (4.8)	38.4 (5.9)	40.5 (4.7)	41.5 (4.5)	41.3 (4.3)	.05
Total bilirubin (µmol/L)	8.7 (4.0)	8.4 (3.7)	8.5 (3.9)	9.1 (4.3)	8.4 (3.9)	.75
Alkaline phosphatase (U/L)	152.0 (142.1)	189.6 (182.0)	156.1 (152.0)	139.5 (125.4)	143.4 (134.8)	.52
γ-glutamyl transpeptidase (U/L)	136.1 (224.8)	201.4 (364.3)	153.1 (238.5)	105.8 (125.8)	132.1 (261.0)	.38
ASAT (U/L)	35.9 (29.7)	47.6 (37.1)	38.6 (33.0)	31.8 (23.8)	31.1 (25.9)	.10
ALAT (U/L)	27.7 (28.7)	30.4 (26.5)	33.4 (38.3)	24.4 (20.9)	21.2 (18.9)	.16
Lactate dehydrogenase (U/L)	453.2 (347.8)	550.3 (475.1)	476.6 (370.5)	422.6 (294.1)	407.3 (312.6)	.46
Renal function						
Creatinine (µmol/L)	79.9 (20.5)	61.0 (11.7)	75.0 (17.7)	83.9 (18.2)	95.3 (22.3)	<.001
Creatinine clearance (mL/min)	98.0 (32.0)	158.6 (28.4)	111.4 (8.6)	82.6 (9.3)	58.4 (6.5)	<.001

Abbreviations: (A)CUP, (adeno)carcinoma of unknown primary; ALAT, alanine aminotransferase; ASAT, aspartate aminotransferase; BSA, body surface area; UGT1A1, uridine diphosphate glucuronosyltransferase isoform 1A1.

[a] Creatinine clearance calculated according to Cockcroft-Gault.

[b] All data are represented as mean with SD in parentheses, unless stated otherwise.

[c] For continuous variables probability values of the one-way ANOVA are reported, whereas in case of categorical values probabilities of the χ^2-test are reported.

[d] Numbers with percentages in parentheses.

Table 4. Irinotecan pharmacokinetics as function of creatinine clearance[a]

Parameter[b]	All patients	Group 1 (> 130 mL/min)	Group 2 (98-130 mL/min)	Group 3 (66-98 mL/min)	Group 4 (35-66 mL/min)	P[c]
Number of patients PK-group[d]	187	23 (12%)	65 (35%)	68 (36%)	31 (17%)	
Smokers	48	7 (15%)	19 (40%)	15 (31%)	7 (15%)	
Non-smokers	139	16 (12%)	46 (33%)	53 (38%)	24 (17%)	
Irinotecan						
CL (L/h)	31.4 (9.4)	31.0 (7.6)	33.6 (9.7)	30.9 (9.9)	28.5 (8.5)	.04
Irinotecan in smokers						
CL (L/h)	35.2 (10.0)	34.7 (3.5)	35.7 (9.4)	38.7 (10.7)	27.1 (11.5)	.44
Irinotecan in non-smokers						
CL (L/h)	30.1 (8.9)	29.3 (8.4)	32.7 (9.8)	28.7 (8.5)	28.9 (7.7)	.19
SN-38						
CL (L/h)	363 (181)	399 (137)	387 (239)	323 (109)	370 (188)	.05
SN-38 in smokers						
CL (L/h)	407 (221)	467 (109)	430 (319)	347 (74)	410 (194)	.15
SN-38 in non-smokers						
CL (L/h)	348 (163)	369 (140)	369 (198)	316 (116)	360 (189)	.23
SN-38G						
CL (L/h)	58.0 (41.1)	70.0 (32.4)	67.0 (46.6)	47.2 (37.2)	55.1 (38.9)	.01
SN-38G in smokers						
CL (L/h)	50.2 (35.0)	58.0 (18.2)	59.8 (48.8)	32.8 (12.2)	54.1 (29.5)	.10
SN-38G in non-smokers						
CL (L/h)	60.8 (42.8)	76.4 (37.0)	70.2 (46.0)	51.4 (40.9)	55.4 (41.2)	.02

Abbreviations: CL, clearance; SN-38, active metabolite of irinotecan; PK, pharmacokinetics; SN-38G, glucuronide conjugate of SN-38.

[a] Creatinine clearance calculated according to Cockcroft-Gault.

[b] All data are represented as mean with SD in parentheses.

[c] Probability values of the Jonckheere-Terpstra test are reported, indicating a trend over the separate groups (1>2>3>4, or the opposite).

[d] Numbers with percentages in parentheses.

Renal function

The mean baseline serum creatinine concentration was 80 μmol/L ($\pm$ 21) and ranged from 44 to 151 μmol/L. Creatinine clearance calculated according to Cockcroft-Gault was between 35 and 231 mL/min, and based on its mean (98 mL/min) and standard deviation (32 mL/min), renal function was categorized in four separate groups (**Table 2**). Group 1 consisted of 23 patients with the highest creatinine clearance (i.e., > 130 mL/min), group 2 included 65 patients with a creatinine clearance between 98 and 130 mL/min, group 3 had 68 patients with a creatinine clearance between 66 and 98 mL/min, and group 4 involved 31 patients with the lowest creatinine clearance (i.e., between 35 and 66 mL/min).

Distribution over the more conventional classification according to the US Food and Drug Administration (FDA) is shown in **Table 1**. Baseline characteristics of all four groups are shown in **Table 3**. Only variables directly related to creatinine clearance (i.e., age, weight, height, BSA, and serum creatinine) showed significant differences between the four groups (*P* < .01). Importantly, no significant difference of smoking prevalence (*P* = .72) or distribution of the variant *UGT1A1*28 allele (*P* = .84) were detected among the four groups.

Renal function and irinotecan pharmacokinetics

No significant correlation between renal function and the clearance of irinotecan (ρ = .14; *P* = .05), SN-38 (ρ = .12; *P* = .12), and SN-38G (ρ = .15; *P* = .06) was found (**Table 4**). Patients with lower creatinine clearance (between 35 and 66 mL/min) had a 13% lower irinotecan clearance (28.5 L/h *versus* 32.9 L/h; *P* = .02) than patients with a creatinine clearance over 98 mL/min. Clearances of SN-38 and SN-38G did not differ substantially (*P* > .17). Similarly, in the subgroup of patients treated at the recommended dosage, no significant association of creatinine clearance with irinotecan pharmacokinetics (data not shown) could be demonstrated, although the clearance of SN-38G in non-smokers tended to decrease with categorized creatinine clearance (*P* = .02), indicating that renal function might affect irinotecan excretion.

Renal function and irinotecan-related toxicity

In the subgroup of 131 patients treated at a dose of 350 mg/m^2 or 600 mg, renal function was significantly associated with the occurrence and severity of hematological toxicity, in particular with neutropenia (**Table 5**). Specifically, the incidence of grade 3-4 neutropenia increased when categorized creatinine clearance worsened. Multivariate analysis revealed that none of the demographic factors *a priori* known to be related to creatinine clearance was significantly associated with the occurrence of grade 3-4 neutropenia, whereas only creatinine clearance was retained in the model (*P* < .01). Likewise, controlling for these demographic factors, creatinine clearance was the only factor significantly related to the nadir in neutrophil count. Patients with a mild-to-moderate impaired renal

Table 5. Irinotecan adverse effects as function of creatinine clearance[a]

Parameter[b]	All patients	Group 1 (> 130 mL/min)	Group 2 (98-130 mL/min)	Group 3 (66-98 mL/min)	Group 4 (35-66 mL/min)	p[c]
White blood cell count						
Nadir (×10^9/L)	3.6 (2.2)	4.4 (2.3)	4.0 (2.3)	3.1 (1.9)	2.9 (1.8)	<.01
Decrease (%)[d]	55 (26)	46 (29)	51 (23)	58 (26)	66 (25)	<.01
CTC grade 3-4[e]	33/131 (25%)	5/22 (23%)	8/49 (16%)	12/42 (29%)	8/18 (44%)	.05
White blood cell count in smokers						
Nadir (×10^9/L)	5.2 (2.2)	6.0 (2.0)	5.0 (2.3)	5.7 (1.8)	3.5 (2.2)	.13
Decrease (%)[d]	40 (26)	22 (21)	44 (24)	36 (21)	62 (30)	.03
CTC grade 3-4[e]	3/34 (9%)	1/7 (14%)	1/15 (7%)	0/7 (0%)	1/5 (20%)	.93
White blood cell count in non-smokers						
Nadir (×10^9/L)	3.1 (1.9)	3.7 (2.1)	3.5 (2.2)	2.6 (1.4)	2.6 (1.7)	.04
Decrease (%)[d]	60 (24)	57 (25)	54 (22)	63 (24)	67 (23)	.10
CTC grade 3-4[e]	30/97 (31%)	4/15 (27%)	7/34 (21%)	12/35 (34%)	7/13 (54%)	.06
Absolute neutrophil count						
Nadir (×10^9/L)	2.1 (1.6)	4.4 (2.3)	2.4 (1.8)	1.6 (1.3)	1.6 (1.3)	<.01
Decrease (%)[d]	62 (25)	51 (28)	59 (23)	58 (26)	73 (23)	<.01
CTC grade 3-4[e]	37/128 (29%)	2/21 (10%)	11/48 (23%)	16/42 (38%)	8/17 (47%)	<.01
Absolute neutrophil count in smokers						
Nadir (×10^9/L)	3.3 (1.6)	4.1 (1.6)	3.2 (1.6)	3.4 (1.3)	2.0 (1.5)	.07
Decrease (%)[d]	47 (26)	28 (22)	49 (24)	47 (24)	68 (28)	.03
CTC grade 3-4[e]	2/34 (6%)	0/7 (0%)	1/15 (7%)	0/7 (0%)	1/5 (20%)	.35

					p
Absolute neutrophil count in non-smokers					
Nadir (×10⁹/L)	1.7 (1.4)	2.2 (1.1)	2.0 (1.8)	1.3 (0.9)	1.4 (1.2)	<.01
Decrease (%)[d]	68 (22)	63 (23)	63 (22)	72 (23)	75 (22)	.03
CTC grade 3-4[e]	35/94 (37%)	2/14 (14%)	10/33 (30%)	16/35 (46%)	7/12 (58%)	<.01
Diarrhea					
CTC grade 3-4[e]	16/131 (8%)	2/22 (9%)	6/49 (13%)	6/42 (14%)	2/18 (11%)	.72
Diarrhea in smokers					
CTC grade 3-4[e]	2/34 (6%)	0/7 (0%)	1/15 (7%)	1/7 (14%)	0/5 (0%)	.64
Diarrhea in non-smokers					
CTC grade 3-4[e]	14/97 (14%)	2/15 (13%)	5/34 (15%)	5/35 (14%)	2/13 (15%)	.92

Abbreviations: CTC, National Cancer Institute's Common Terminology Criteria for Adverse Events version 3.0.

[a] Creatinine clearance calculated according to Cockcroft-Gault.

[b] Evaluated in the 350 mg/m² or 600 mg flat-fixed dose group. All data are represented as mean with SD in parentheses, unless stated otherwise.

[c] Probability values of the Jonckheere-Terpstra test are reported, indicating a trend over the separate groups (1 > 2 > 3 > 4, or the opposite) .

[d] Percentage decrease nadir to baseline.

[e] Patients with grade 3 or 4 CTC toxicity over total number of patients in particular group with percentages in parentheses.

function (i.e., creatinine clearance between 35 and 66 mL/min) had a 2.5-times higher risk on grade 3-4 neutropenia (47.1% *versus* 18.8%) than patients with a creatinine clearance over 98 mL/min. To be more specific, the nadir of the absolute neutrophil count in the renally impaired group was one-third lower (1.6 *versus* 2.5 ×10^9/L). Similar results were found on using the FDA classification. Specifically, patients with a mildly impaired renal function (i.e., creatinine clearance between 50 and 80 mL/min) had a 2.2-times higher risk on grade 3-4 neutropenia (47.2% *versus* 21.7%; *P* < .01) than patients with a creatinine clearance over 80 mL/min.

Given the low incidence of neutropenia in smoking patients (6%; **Table 5**), the effects of renal function on toxicity were particularly noticeable in the non-smoking group (N = 97; **Figure 2**). In this group, patients with a lower creatinine clearance (i.e., > 1 SD below the average creatinine clearance) had a 4.1-times increased risk for grade 3-4 neutropenia than patients with a high creatinine clearance (i.e., > 1 SD above the average creatinine clearance). The incidence of grade 3-4 neutropenia rose from 14 to 58% (*P* < .001). In the entire group, a higher risk in the same order of magnitude was noted, whereas no effects on diarrhea were seen (data not shown).

Apart from hematological and gastrointestinal toxicity, also modest renal toxicity was encountered during treatment. In particular, creatinine clearance decreased from 103 to 95 mL/min (*P* < .001), reflecting an increase of serum creatinine from 75 to 85 μmol/L (*P* < .001). In a multiple regression analysis including age, baseline creatinine, gender, height, weight, BSA, and the exposure (area under the plasma concentration-time curve (AUC)) to irinotecan, SN-38, and SN-38G, the percentage increase of serum creatinine from baseline only related to the exposure to SN-38G (ρ = .37; *P* < .001).
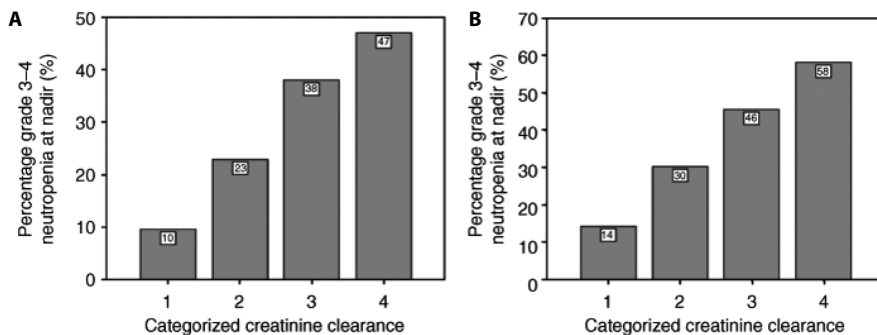
Figure 2. Effects of renal function on irinotecan-induced neutropenia
The percentage of grade 3-4 neutropenia, graded according to the National Cancer Institute's Common Terminology Criteria for Adverse Events version 3.0, is plotted against the creatinine clearance calculated according to Cockcroft-Gault in all patients (**A**) and in the non-smoking patients (**B**) available for toxicity analysis. Group 1 represents a creatinine clearance > 130 mL/min, group 2 a creatinine clearance between 98 and 130 mL/min, group 3 a creatinine clearance between 66 and 98 mL/min, and group 4 a creatinine clearance between 35 and 66 mL/min.

DISCUSSION

This study suggests that renal function is a predictor of the hematological toxicity profile of irinotecan. Patients with a lower pretreatment renal function, as determined by the Cockcroft-Gault formula, had a higher risk of severe neutropenia. Given the low incidence of neutropenia in smoking patients, this finding appears to be especially relevant to non-smoking patients. Compared to patients with a higher creatinine clearance (over 98 and over 130 mL/min), patients with a low creatinine clearance (35-66 mL/min) had a 2.5-times and 4.1-times higher risk of severe neutropenia, respectively. It has been suggested that this effect should be attributed to decreased drug excretion,[31,32] however, in the currently studied cohort of patients, exposure to irinotecan and SN-38 was not found to be significantly related to renal function. These data complement existing knowledge on the toxicity profile of irinotecan and may have important practical implications for its optimal use.

A limitation of this study is that only a small number of patients with a creatinine clearance < 60 mL/min could be included, since in clinical practice irinotecan is mostly administered to patients with adequate renal function, i.e., an estimated creatinine clearance over 60 mL/min. All clinical studies included in this analysis contained an exclusion criterion in this line.[3,6,10,19-24] Because of this skewed distribution, an alternative categorization of renal function, based on its mean and SD, was chosen instead of conventionally used categorizations. Using the categorization according to the FDA, it was only possible to compare patients with a creatinine clearance over 80 mL/min to patients with a mildly impaired renal function (i.e., creatinine clearance between 50 and 80 mL/min), which resulted in comparable findings. Despite its limitations, the nonconventional categorization was chosen deliberately to investigate the effects of renal function on irinotecan pharmacokinetics and toxicity in its full nature and to be able to extrapolate findings. Indeed, the risk of grade 3-4 neutropenia progressively increased with lower estimated creatinine clearances, strongly suggesting an effect of pretreatment renal function on irinotecan toxicity.

Although no significant relationship could be established between renal function and irinotecan clearance, a potential (partial) relationship cannot be completely ruled out. Especially not since patients with lower creatinine clearance (i.e., between 35 and 66 mL/min) had a somewhat lower (13%) clearance of irinotecan than patients with a higher creatinine clearance (i.e., over 98 mL/min), which is in line with earlier findings relating exposure to irinotecan to the severity of neutropenia.[4,5,8] However, given its borderline significance and the limited difference between both groups, this explanation cannot be the sole factor, if contributory at all. In addition, exposure to the active irinotecan metabolite SN-38, which is considered to predict the occurrence of neutropenia even stronger,[4,5,8] was comparable between both groups.

In the context of mechanisms associated with renal elimination of camptothecins, it is important to emphasize that both irinotecan and its metabolites are known to be excreted by a variety of anion-transporting polypeptide binding cassette (ABC) drug transporters, such as ABCB1 (P-glycoprotein), ABCG2 (BCRP), and ABCC2 (cMOAT; MRP2). Given the affinity of irinotecan for multiple transporters, an effect of these transporters on irinotecan clearance was hypothesized.[32] In our study, however, no significant relationships of renal function with the plasma pharmacokinetics of irinotecan, SN-38 or SN-38G were demonstrated, which was in line with an earlier study.[18] It should be pointed out that in this previous study, irinotecan was administered at a low dose of 225 mg/m^2 to only nine patients with a degree of renal impairment that was worse than that observed in the currently studied group with the lowest creatinine clearance.

The lack of a statistically significant relationship between renal function and plasma pharmacokinetics is potentially related, in part, to the possibility that the causative factor of impaired renal function is impaired tubular secretion, not filtration. Furthermore, the measures of systemic exposure were based on a composite chromatographic analysis that combines the total of lactone (active) and carboxylate (inactive) forms of this anticancer drug,[33] and urine data were not available to directly assess this possibility. Nonetheless, it is likely that the carboxylate form of camptothecins is more sensitive to the active secretion process that regulates the initial drug uptake from the circulation into the kidney.[34] Likewise, CPT-11 and SN-38 have been shown to be mainly excreted into bile in the carboxylate form.[35] Based on expression levels and localization, the organic anion transporters OAT1 and OAT3 are the most plausible candidates involved in this process, with the latter being presumably more important on the basis of recent information published for the related camptothecin, topotecan.[36]

Impaired renal function has also been associated with increased circulating concentrations of renal toxins that inhibit organic anion-transporting polypeptide-mediated uptake of drugs into the liver. This is one of the proposed mechanisms for decreased CYP3A function (and thus potentially higher exposure to SN-38 in case of irinotecan therapy), as estimated by the erythromycin breath test, in patients with impaired renal function.[37] However, since no effects on the plasma pharmacokinetics of irinotecan as function of renal function could be demonstrated, this is not likely to play a significant role.

Several UGT isoforms have been shown to catalyze the formation of acyl glucuronides, including UGT1A3, UGT1A9, and particularly UGT2B7.[38] Acyl glucuronides are potentially reactive intermediates, which undergo hydrolysis and intramolecular acyl migration, and also bind irreversibly to plasma proteins.[39-42] To date, formation of acyl glucuronides in the metabolic pathway of irinotecan has not been shown. However, given the presence of a lot of unidentified (minor) metabolites in urine, bile, and plasma, the formation of acyl glucuronides cannot be excluded on forehand. Although highly

speculative, a role for such toxic compounds might be considered in line with the relationships found here between baseline renal impairment and decreased renal function during treatment *versus* the exposure to the water soluble glucuronide conjugate of SN-38.

Typically, for drugs substantially eliminated by the kidneys, dose adjustments in patients with renal dysfunction are required either by dose reduction or by prolongation of the administration interval. Such recommendations should be based on prospective pharmacokinetic study data and the observed efficacy and toxicity of the drug. However for irinotecan, strong data on toxicity in this specific population are lacking.[18] Most reports are case reports describing renally impaired patients being treated with irinotecan and/or studying renally impaired patients being treated with lower irinotecan doses, making it difficult to assess whether full dosing of irinotecan in renal impaired patients is feasible. Since such data are currently scarce for irinotecan, its package label states to be cautious with its use in renally impaired patients without giving more precise dosing guidelines.

Because many factors affect irinotecan toxicity, tailored dosing of irinotecan remains difficult in the post-BSA era. Many of the possible determinants of irinotecan and SN-38 exposure have been investigated and numerous attempts have been made to predict the individual patient's clearance before irinotecan administration using this knowledge to calculate a dose ultimately resulting in a controlled, predictable, and safe exposure. Most attention has been focused on hepatic factors; in particular, the homozygous presence of the *UGT1A1**28 allele has been identified as a risk factor for the occurrence of irinotecan-induced toxicity.[4,8] The package label of irinotecan has been changed accordingly, incorporating a statement regarding dose adjustment for homozygous *UGT1A1**28 carriers. However, nonhepatic factors, such as renal function and lifestyle, should not be disregarded, particularly, since the group of homozygous *UGT1A1**28 carriers is relatively small,[8] whereas more patients are prone to irinotecan-induced toxicity because of lifestyle behavior (e.g., use of alcohol, herbal tea, and complementary and alternative medicine). Other subgroups of patients may be prone to toxicity because of the presence of other (relatively rare) genetic risk factors. Additionally, although strong relationships between toxicity and systemic exposure to SN-38 have been demonstrated, it cannot be excluded that neutropenia after irinotecan application is mediated through other pathways as well. For example, as stated in its package label, older age (> 65 years), prior radiotherapy, and a World Health Organization performance score over 2 have been identified as risk factors for the occurrence of irinotecan-induced toxicity. Additionally, in patients receiving low dose, protracted irinotecan, *UGT1A1**28 status correlated with SN-38 pharmacokinetics, but not with toxicity.[43]

Because delayed-onset diarrhea and neutropenia contribute to the risk of developing febrile neutropenia, many attempts have been undertaken to reduce their incidence

and severity. For example, withholding *UGT1A1*28* homozygous patients full-dosed irinotecan, the overall incidence of grade 4 neutropenia will decrease from 10 to 6%. Depending on the exact criteria used for defining febrile neutropenia and the population under study (e.g., pretreatment, tumor type, age, performance score, etc.) the incidence of febrile neutropenia of 1-15% in the three weekly schedule of 350 mg/m^2,[44-46] does not justify a primary prophylactic role of granulocyte colony-stimulating factors.[47] However, to avoid febrile neutropenia, infections, and (*a priori*) dose reductions in renally impaired patients, it may be worth investigating the clinical and economic benefits of prophylactic treatment with granulocyte colony-stimulating factors, like (pegylated) filgrastim (Neupogen/Neulasta; Amgen, Thousand Oaks, CA) in these patients. Particularly, given the fact that renal function does not appear to significantly affect the exposure to SN-38 and the occurrence and severity of delayed-onset diarrhea, which has recently been related to the detoxification of SN-38 in the intestinal mucosa,[29,48] a cautious use of irinotecan in renally impaired patients, as suggested in its package label, may result in suboptimal exposure to SN-38 and a subsequent lower chance on therapeutic efficacy,[49-51] if *a priori* dose reductions are applied.

In conclusion, this study suggests that lower creatinine clearance is associated with a fourfold increased risk of irinotecan-induced neutropenia (14% *versus* 58%), especially in non-smoking patients. Although the exact mechanism of this interaction remains to be established, the current data suggest that further investigation is warranted to determine whether renal function should be considered as an important factor in irinotecan dose-optimization and a valuable addition to the toolbox to individualize irinotecan therapy.

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Chapter 5

Effect of omeprazole on the pharmacokinetics and toxicities of irinotecan in cancer patients: A prospective cross-over drug-drug interaction study

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ABSTRACT

Background
Omeprazole is one of the most prescribed medications worldwide and within the class of proton pump inhibitors, it is most frequently associated with drug interactions. *In vitro* studies have shown that omeprazole can alter the function of metabolic enzymes and transporters that are involved in the metabolism of irinotecan, such as uridine diphosphate glucuronosyltransferase subfamily 1A1 (UGT1A1), cytochrome P-450 enzymes subfamily 3A (CYP3A) and the ATP-binding cassette drug transporter G2 (ABCG2). In this open-label cross-over study we investigated the effects of omeprazole on the pharmacokinetics and toxicities of irinotecan.

Methods
Fourteen patients were treated with single agent irinotecan (600 mg i.v., 90 minutes) followed 3 weeks later by a second cycle with concurrent use of omeprazole 40 mg once daily, which was started two weeks prior to the second cycle. Plasma samples were obtained up to 55 hours after infusion and analysed for irinotecan and its metabolites 7-ethyl-10-hydroxycampothecin (SN-38), SN-38-glucuronide (SN-38G), 7-ethyl-10-[4-(1-piperidino)-1-amino]-carbonyloxycamptothecin (NPC) and 7-ethyl-10-[4-N-(5-aminopentanoic acid)-1-piperidino]-carbonyloxycamptothecin (APC) by high-performance liquid chromatography (HPLC). Non-compartmental modelling was performed. Toxicities were monitored during both cycles. Paired statistical tests were performed with SPSS.

Results
The exposure to irinotecan and its metabolites was not significantly different between both cycles. Neither were there significant differences in the absolute nadir and percentage decrease of WBC and ANC, nor on the incidence and severity of neutropenia, febrile neutropenia, diarrhea, nausea and vomiting when irinotecan was combined with omeprazole.

Conclusion
Omeprazole 40 mg did not alter the pharmacokinetics and toxicities of irinotecan. This widely used drug can therefore be safely administered during a three-weekly single agent irinotecan schedule.

INTRODUCTION

Drug-drug interactions can cause serious adverse effects, especially in oncology, as a result of the narrow therapeutic window of chemotherapeutic agents. Small changes in the pharmacokinetics or pharmacodynamics of chemotherapy caused by another drug can result in significant changes in its toxicity or efficacy. Because cancer patients often experience disease-related and age-related organ failure, they frequently use several other drugs, which put them at risk for drug-drug interactions.[1]

Proton pump inhibitors (PPIs) act as potent blockers of the gastric acid pump without major side effects.[2] They belong to one of the most frequently prescribed medications in the United States.[3]

Omeprazole was the first registered proton pump inhibitor and is one of the most prescribed drugs worldwide.[4,5] Although widely used, being approved as over-the-counter product in several countries, and mostly designated as harmless, omeprazole is actually known to be involved in several drug-drug interactions,[6] which could potentially be dangerous when combined with drugs with a narrow therapeutic window, such as chemotherapeutic agents.

Several drug-drug interaction studies with omeprazole have been performed, mainly focusing on interactions on the level of hepatic cytochrome P450 (CYP) enzymes and alteration of the absorption of (oral) drugs via changes in gastric pH. Clinically, the most important drug-drug interaction of omeprazole is a 27-54% reduction in clearance of diazepam due to competitive inhibition of CYP2C19.[7,8] Next to this effect there are *in vivo* and *in vitro* results pointing to induction of UDP-glucuronosyltransferases,[9-11] induction[12,13] and inhibition of cytochrome P-450 enzymes subfamily 3A (CYP3A),[14,15] and inhibition of the ATP-binding cassette drug transporter B1 (ABCB1)[14,15] and ATP-binding cassette drug transporter G2 (ABCG2).[16,17] These metabolising enzymes and drug transporters play an important role in the disposition of the topoisomerase-I inhibitor irinotecan (Campto®, Pfizer), which is registered for the treatment of metastatic and/or inoperable colorectal cancer (**Figure 1**).

In vitro research of the combination of irinotecan and omeprazole showed an 85% reduction of 7-ethyl-10-[4-(1-piperidino)-1-amino]-carbonyloxycamptothecin (NPC) formation, one of the metabolites of irinotecan, which could potentially lead to increased levels of the active metabolite 7-ethyl-10-hydroxycampothecin (SN-38) and consequently more severe toxicity.[18] We performed comparable *in vitro* experiments and the results led us to initiate a clinical study to investigate the effect of omeprazole on the pharmacokinetics of irinotecan and toxicities in cancer patients.
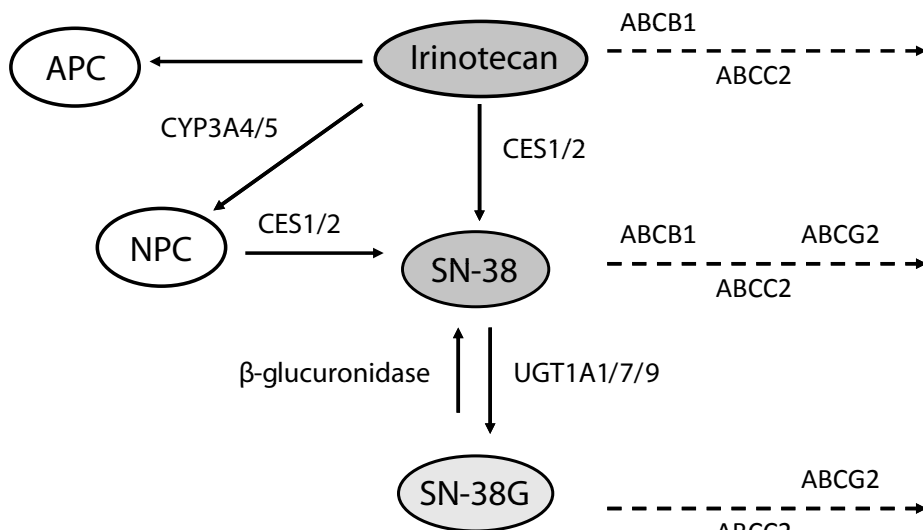
Figure 1. Metabolism of irinotecan

The prodrug irinotecan is metabolized into its active metabolite SN-38 by carboxylesterases type 1 and 2. The affinity for this reaction is low, since only a fraction of irinotecan is directly converted into SN-38. Competing with the formation of SN-38 is the oxidation of irinotecan into the inactive metabolites APC and NPC by CYP3A4 and CYP3A5, which both (partially) can be converted further into SN-38. To facilitate excretion, SN-38 is glucuronidated into its inactive metabolite SN-38-glucuronide (SN-38G) by several UGT1A isoforms; UGT1A1 being the most important. In the intestines, SN-38G can be deglucuronidated into SN-38 by *β*-glucuronidase-producing bacteria. Several drug transporters are involved in the elimination of irinotecan and its metabolites.

Abbreviations: ABCB1, ATP-binding cassette drug-transporter B1, also known as P-glycoprotein; ABCC2, ATP-binding cassette drug-transporter C2, also known as canalicular multispecific organic anion transporter (C-MOAT); ABCG2, ATP-binding cassette drug transporter G2, also known as Breast Cancer Resistance Protein (BCRP); APC, 7-ethyl-10-[4-N-(5-aminopentanoic acid)-1-piperidino]-carbonyloxycamptothecin, inactive metabolite of irinotecan; CES, carboxylesterase; CYP3A; cytochrome P-450 enzymes subfamily 3A; NPC, 7-ethyl-10-[4-(1-piperidino)-1-amino]-carbonyloxycamptothecin, inactive metabolite of irinotecan; SN-38, 7-ethyl-10-hydroxycampothecin, active metabolite of irinotecan; SN-38G, SN-38-glucuronide, inactive metabolite of SN-38; UGT1A, uridine diphosphate glucuronosyltransferase subfamily 1A.

MATERIALS AND METHODS

In vitro studies

In vitro experiments were performed to study the effect of omeprazole on the metabolism of irinotecan. Pooled human liver microsomes (Becton Dickinson Gentest) were incubated for 30 minutes with irinotecan (10 μM) in the presence or absence of omeprazole (25 μM) or fluconazole (25 μM; CYP3A inhibitor) based on an earlier de-

scribed method.[19] The experiments were performed on four separate occasions. In each experiment, microsomes (1 mg protein/mL) were incubated in triplicate. In another experiment, microsomes (0.8 mg/mL) were co-incubated for 30 minutes with SN-38 (5 μM) and omeprazole (25 μM) and ketoconazol (25 μM; uridine diphosphate glucuronosyl-transferase subfamily 1A (UGT1A) inhibitor) based on methods described.[20] Experiments were terminated by the addition of perchloric acid/methanol. Irinotecan and metabolite concentrations were analyzed based on validated assays.[21,22]

HCT116 (colorectal carcinoma) and Caco2 (colorectal adenocarcinoma) cells were cultured in Hepes-buffered RPMI 1640 medium supplemented with Glutamax™, 10% fetal bovine serum (Gibco), 100 U/mL penicillin and 100 μg/mL streptomycin at 37°C in a humidified atmosphere containing 5% CO_2. Cells were cultured for 24 hours in the presence of 25 μM omeprazole or 0.1% (v/v) DMSO as solute control. After 24 hours, total RNA was extracted using RNA-Bee (Tel-TEST Temco, Inc.). Relative UGT1A1 expression levels were measured by real time RT-PCR using Taqman Universal Master mix and Assay-On-Demand products from Applied Biosystems (UGT1A1 assay ID: Hs02511055-s1). The human glyceraldehyde-3-phosphate dehydrogenase (GAPDH assay ID: 4310884E; VIC/TAMRA) was used for normalization. Reactions were run on an ABI PRISM 7900 sequence detector system (Applied Biosystems) using the following cycling conditions: 50°C for 2 minutes, 95°C for 10 minutes followed by 40 cycles of 95°C for 15 seconds, and 60°C for 1 minute.

Patients

Nineteen patients were included in this open-label cross-over interaction study. Inclusion criteria were: (1) histological or cytological confirmed diagnosis of any form of (irresectable and/or metastatic) cancer, which was thought to be sensitive to irinotecan treatment; (2) age ≥ 18 years; (3) WHO performance score ≤ 1; and (4) adequate hematological, renal and hepatic function. Starting two weeks before irinotecan administration, patients were not allowed to use grapefruit, star fruit, dietary supplements, St. John's wort, herbal tea and herbals or any other known inhibitor and/or inducer of CYP3A and ABCB1. In addition, the use of proton pump inhibitors was prohibited. Specific exclusion criteria were: (1) any form of anticancer treatment within four weeks of start of irinotecan administration; (2) unresolved bowel obstruction or chronic colic disease; and (3) any form of illness that would prohibit the process of understanding and giving of informed consent. All patients gave written informed consent and the local institutional review board approved the clinical protocol, which was written in accordance with the declaration of Helsinki.

Treatment

All patients received their first cycle of irinotecan (Campto®, Pfizer) without and their second cycle with concomitant omeprazole (Losec®MUPS®, AstraZeneca). Fourteen

days before the start of the second cycle, patients started with omeprazole 40 mg once daily until the third day after the second administration. Irinotecan was administered intravenously over 90 minutes at a flat-fixed dose of 600 mg during both cycles.[23] All patients received a standard antiemetic regimen of intravenous granisetron (1 mg) and dexamethason (10 mg) 30 minutes before the administration of irinotecan and atropine (0.25 mg, subcutaneously) prior to irinotecan infusion, to prevent an acute cholinergic syndrome. For the treatment of irinotecan-induced diarrhea, patients received treatment with loperamide and, when necessary, antibiotics. A dose reduction of 25% was performed at the discretion of the physician, when necessary. Patients were asked to record side effects, the intake of any other drugs during both treatment cycles and the time of intake of omeprazole in a specific diary.

Pharmacokinetic analyses of irinotecan

Pharmacokinetic analyses of irinotecan and its main metabolites SN-38, SN-38G, APC and NPC were performed during both treatment cycles. Blood samples (5 mL; lithium-heparin) were collected prior to infusion, 30 minutes after the start of infusion, at the end of infusion, as well as 10, 20 and 30 minutes, and 1, 1.5, 2, 3, 4, 5, 6, 22.5, 30, 46.5 and 53.5 hours post-infusion. Samples were centrifuged for 10 minutes at 2,860 g (4°C) and plasma was stored at -80°C until analysis by validated reversed-phase high-performance liquid chromatography assays with fluorescence detection, as described elsewhere.[21,22,24] Pharmacokinetic parameters of irinotecan and its metabolites were calculated using weighted non-compartmental analyses with WinNonLin 5.2 (Pharsight Corp., Mountain View, CA).

Toxicities

During both cycles, patients were seen weekly at the outpatient clinic for physical examination, toxicity screening and laboratory tests. Leukopenia, neutropenia, diarrhea, nausea and vomiting were graded using the Common Terminology Criteria for Adverse Events (CTC) version 3.0,[25] and were also classified into severe (grade 3-4) and not severe (grade 0-2). In addition, leukopenia and neutropenia were evaluated as absolute nadir and as percentage decrease at nadir from baseline which was calculated as: percentage decrease = [baseline value – nadir value]/baseline value × 100%. Toxicity analyses were only performed in the group of patients who received two full dose cycles of irinotecan (600 mg; N = 12).

Genotyping

In all patients, *UGT1A1*-genotype analyses were performed for the *UGT1A1**28 ((TA)6→(TA)7) and *UGT1A1**93 (-3156G>A) polymorphisms as described.[26] In addition,

patients were screened for being an ultra-rapid metabolizer of CYP2C19 (*CYP2C19**17),[27] which may result in a sub-therapeutic exposure to omeprazole.[27,28]

Statistics

The primary objective of this study was to investigate the influence of omeprazole on the plasma pharmacokinetics of irinotecan and its metabolites in cancer patients. To detect a 25% difference in SN-38 AUC between the cycles with and without concomitant omeprazole with a two-sided significance level of 5% and a power (1-β) of 90%, a sample size of at least 14 patients was required. For the sample size calculation, data were used from patients who received two subsequent cycles of irinotecan at a flat-fixed dose of 600 mg.[26] Dose-reduced patients were excluded from this analysis. The secondary objective was to compare side effects, especially leukopenia and neutropenia, and late-onset diarrhea, in the presence and absence of omeprazole.

Data are presented as mean values with 95% confidence intervals, unless stated otherwise. To compare pharmacological parameters and nadir and percentage decrease of neutrophils and leukocytes between the cycle with and without omeprazole, paired *t*-tests were used. For the comparison of the CTC-graded toxicities between both cycles, Mc Nemar's test was used. Statistical tests were calculated two-sided and *P*-values of less than .05 were regarded as statistically significant. All statistical calculations were performed with SPSS version 15.0 (SPSS Inc., Chicago, IL).

RESULTS

In vitro experiments

As shown in **Figure 2**, co-incubation of human liver microsomes with irinotecan and omeprazole resulted in an 80% inhibition on NPC formation and a 75% inhibition on APC formation, which was comparable with results with the CYP3A inhibitor fluconazole (78% and 74% inhibition, respectively). Although *in vitro* no effect of omeprazole was seen on the formation of SN-38, the inhibition of both NPC and APC formation could potentially lead to higher SN-38 levels *in vivo*.

Also shown in **Figure 2**, co-incubation of human liver microsomes with SN-38 and omeprazole did not result in reduced formation of SN-38G, whereas the formation of SN-38G was reduced with 57% when SN-38 was co-incubated with the UGT1A1 inhibitor ketoconazole.

A 24-hour exposure of the colorectal carcinoma cell Lines HCT116 and Caco2 to 25 μM omeprazole resulted in a two-fold up-regulation of UGT1A1 mRNA levels as determined by quantitative RT-PCR.
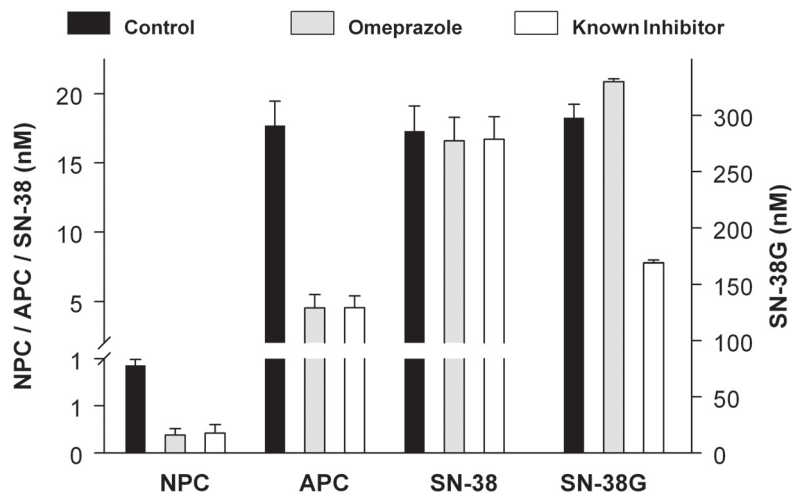
Figure 2. Omeprazole affects the metabolism of irinotecan *in vitro*
Effect of omeprazole (grey bars) and CYP3A inhibitor fluconazole (white bars) on the formation of NPC, APC and SN-38 during incubation of human liver microsomes with irinotecan and effect of omeprazole (grey bars) and UGT1A1 inhibitor ketoconazole (white bars) on the formation of SN-38G during incubation with SN-38. The black bars represent the formation of metabolites in the absence of a potential inhibitor. Depicted are the mean values of the formed metabolite + SD.
Abbreviations: APC, 7-ethyl-10-[4-N-(5-aminopentanoic acid)-1-piperidino]-carbonyloxycamptothecin, inactive metabolite of irinotecan; CYP3A, cytochrome P-450 enzymes subfamily 3A; NPC, 7-ethyl-10-[4-(1-piperidino)-1-amino]-carbonyloxycamptothecin, inactive metabolite of irinotecan; SD, standard deviation; SN-38, 7-ethyl-10-hydroxycampothecin, active metabolite of irinotecan; SN-38G, SN-38-glucuronide, inactive metabolite of SN-38; UGT1A1, uridine diphosphate glucuronosyltransferase subfamily 1A1.

Patients

Nineteen patients were included in the clinical study. Two patients did not start treatment after registration; one due to the diagnosis of a second malignancy, the other due to progressive liver failure. One patient did not receive a second administration of irinotecan because of severe toxicity during the first cycle (grade 4 diarrhea and hematological toxicity). One patient was not evaluable for pharmacokinetics due to ascites with possible third space pharmacokinetics. Another patient was not evaluable due to problems with pharmacokinetic sampling. Of the 14 evaluable patients for pharmacokinetics, two patients were not evaluable for toxicity analysis due to 25% dose reduction during their second cycle because of severe toxicity (grade 4 hematological toxicity plus grade 3 gastrointestinal toxicities and grade 3 hepatological toxicity, respectively). The pharmacokinetics of these dose-reduced cycles were extrapolated to full dose pharmacokinetics, since the pharmacokinetics of irinotecan and its metabolites are linear in this dose range.[29] Patient demographics are stated in **Table 1**.

Table 1. Patient characteristics[a]

Characteristics	N	%	Median	Range
Age (years)			65	26-74
BSA (m^2)			1.87	1.59-2.38
Sex				
Male	9	64%		
Female	5	36%		
Tumor type				
Colorectal	4	29%		
Pancreatic	4	29%		
(A)CUP	2	14%		
Miscellaneous[b]	4	29%		
Smoking status				
Smoker	1	7%		
Non-smoker	13	93%		
UGT1A1[*]28 genotype				
TA6/TA6 (wildtype)	7	50%		
TA6/TA7	7	50%		
TA7/TA7	0	0%		
UGT1A1[*]93 genotype				
GG (wildtype)	9	64%		
GA	5	36%		
AA	0	0%		
CYP2C19[*]17 genotype				
CC (wildtype)	5	36%		
CT	7	50%		
TT	2	14%		

Abbreviations: (A)CUP, (adeno)carcinoma of unknown primary; BSA, body surface area; *UGT1A1*[*]28, polymorphism for an additional (seventh) repeat in the TATA box of the promoter region of *UGT1A1* leading to reduced UGT1A1 formation; *UGT1A1*[*]93, polymorphism in the *UGT1A1* gene, also known as -3156G>A, resulting in less functional UGT1A1; *CYP2C19*[*]17, polymorphism in *CYP2C19* gene (-806C>T and -3402C>T), resulting in more functional CYP2C19 (ultra rapid metabolizer).
[a] N = 14, patients evaluable for two treatment cycles.
[b] Including primitive neuro-ectodermal tumour (1), cholangiocarcinoma (1), jejunal carcinoma (1) and breast cancer (1).

Irinotecan pharmacokinetics

As shown in **Table 2**, there was no significant difference in the area under the curve (AUC) and maximum concentration (C_{max}) of irinotecan ($P > .24$), SN-38 ($P > .63$), SN-38G ($P > .07$), APC ($P > .07$) and NPC ($P > .13$) between the cycles with and without omeprazole. Similar results were obtained when the two ultra-rapid metabolizers of CYP2C19 (*CYP2C19**17/*17) were left out of analysis ($P > .06$). **Figure 3** shows the time *versus* plas-

ma concentration curves of irinotecan and its metabolites as well as the intra-individual AUCs with and without concomitant omeprazole.

Table 2. Pharmacokinetics of irinotecan and its metabolites without (–) and with (+) concomitant use of omeprazole

Parameter[a]	Omeprazole (–)	Omeprazole (+)	Ratio[b]	P
Irinotecan				
AUC_{0-55h} (ng×h/mL)	24,498 (16,186-32,811)	23,472 (16,195-30,748)	0.97 (0.92-1.02)	.24
C_{max} (ng/mL)	3,700 (2,998-4,401)	3,585 (2,814-4,355)	0.97 (0.90-1.04)	.34
SN-38				
AUC_{0-55h} (ng×h/mL)	439 (346-533)	453 (354-551)	1.05 (0.92-1.19)	.63
C_{max} (ng/mL)	41.9 (29.9-53.9)	43.0 (31.7-54.3)	1.09 (0.87-1.31)	.81
SN-38G				
AUC_{0-55h} (ng×h/mL)	2,913 (1,874-3,953)	3,167 (1,963-4,371)	1.08 (0.96-1.19)	.15
C_{max} (ng/mL)	209 (155-264)	228 (165-291)	1.09 (1.00-1.19)	.07
APC				
AUC_{0-55h} (ng×h/mL)[c]	7,471 (4,944-9,998)	6,438 (5,016-7,859)	0.94 (0.80-1.07)	.15
C_{max} (ng/mL)[c]	587 (393-781)	476 (378-575)	0.90 (0.77-1.04)	.07
NPC				
AUC_{0-55h} (ng×h/mL)	189 (114-265)	154 (119-189)	0.92 (0.75-1.09)	.25
C_{max} (ng/mL)	19.9 (12.5-27.3)	15.0 (12.5-17.5)	0.89 (0.72-1.05)	.13

Abbreviations: APC, 7-ethyl-10-[4-N-(5-aminopentanoic acid)-1-piperidino]-carbonyloxycamptothecin, inactive metabolite of irinotecan; AUC_{0-55h}, area under the concentration-time curve from timepoint 0 to 55 hours; C_{max}, maximum concentration; NPC, 7-ethyl-10-[4-(1-piperidino)-1-amino]-carbonyloxycamptothecin, inactive metabolite of irinotecan; SN-38, 7-ethyl-10-hydroxycampothecin, active metabolite of irinotecan; SN-38G, SN-38-glucuronide, inactive metabolite of SN-38.
[a] Data presented as mean with 95% confidence interval in parentheses.
[b] Ratio of mean pharmacokinetic parameters of irinotecan with and without omeprazole [ratio = with omeprazole/without omeprazole].
[c] N = 13, data on pharmacokinetics of APC missing in one patient.

Toxicities

No statistical differences were seen in the absolute nadir and percentage decrease of leukocytes and neutrophils after irinotecan treatment with or without omeprazole (P > .34; **Table 3**). In addition, no differences were seen in the incidence of severe leukopenia and neutropenia (P = 1.0). Overall, the incidence of severe (grade 3-4) gastrointestinal toxicities was low in our study. Only 2 patients suffered from grade 3 or 4 diarrhea, nausea and vomiting.
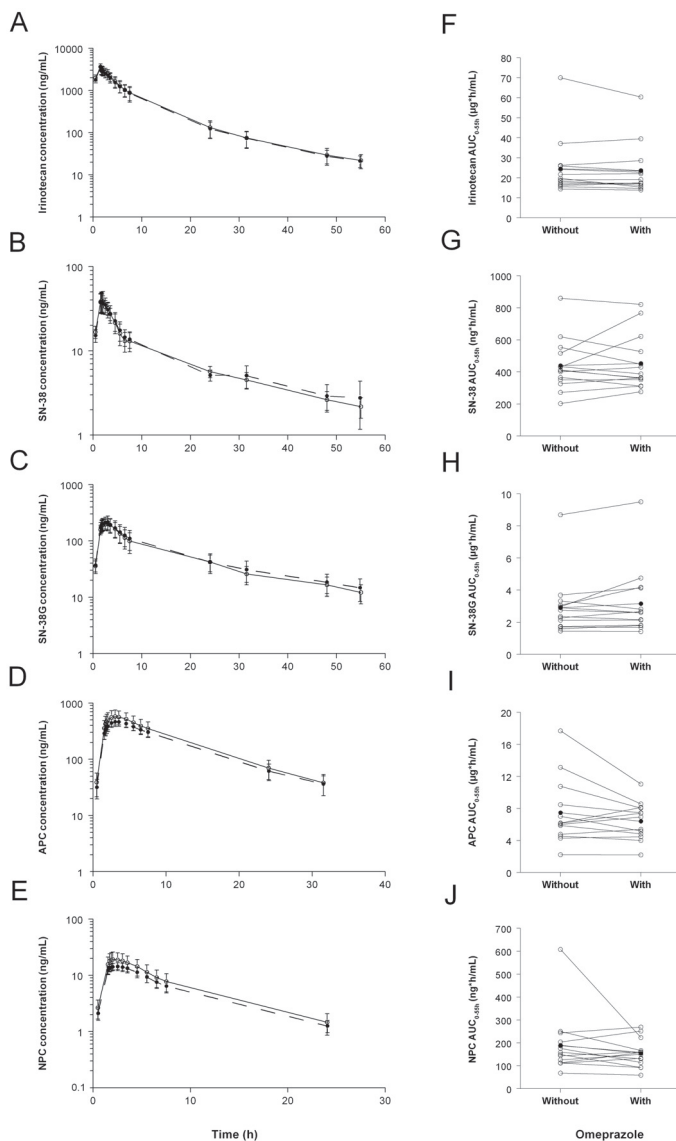
Figure 3. Pharmacokinetics of irinotecan with and without concomitant omeprazole

A-E. Mean (± 95% confidence interval) plasma concentration-time curves of irinotecan (**A**), SN-38 (**B**), SN-38G (**C**), APC (**D**) and NPC (**E**) in 14 cancer patients after intravenous infusion of 600 mg irinotecan, with (closed circles) and without (open circles) concomitant use of omeprazole 40 mg once daily.

F-J. Intraindividual (open circles) and mean (closed circle) area under the curve (AUC) of irinotecan (**F**), SN-38 (**G**), SN-38G (**H**), APC (**I**, N = 13) and NPC (**J**) of 14 cancer patients treated with irinotecan 600 mg intravenously with and without concomitant use of omeprazole 40 mg once daily.

Abbreviations: APC, 7-ethyl-10-[4-N-(5-aminopentanoic acid)-1-piperidino]-carbonyloxycamptothecin, inactive metabolite of irinotecan; NPC, 7-ethyl-10-[4-(1-piperidino)-1-amino]-carbonyloxycamptothecin, inactive metabolite of irinotecan; SN-38, 7-ethyl-10-hydroxycampothecin, active metabolite of irinotecan; SN-38G, SN-38-glucuronide, inactive metabolite of SN-38.

Table 3. Toxicities of irinotecan without (–) and with (+) concomitant use of omeprazole of patients who received two full-dose treatments (N = 12)[a]

Parameter	Omeprazole (–)	Omeprazole (+)	*P*
Leukocytes			
Nadir (×10⁹)	2.79 (2.00-3.59)	3.01 (2.07-3.95)	.46
Decrease (%)[b]	46.8 (31.3-62.4)	40.3 (17.6-63.0)	.34
Severe leukopenia (grade 3-4)[c]	5 (42%)	4 (33%)	1.00[d]
Neutrophils			
Nadir (×10⁹)	1.47 (0.86-2.08)	1.43 (0.88-1.98)	.87
Decrease (%)[b]	57.4 (41.8-73.1)	49.6 (25.3-74.0)	.35
Severe neutropenia (grade 3-4)[c]	4 (33%)	5 (42%)	1.00[d]

[a] Two patients were excluded from this analysis because of dose reduction during the second cycle due to severe toxicities during the first cycle.

[b] Percentage decrease compared with baseline = [baseline value – nadir value]/baseline value × 100%.

[c] Number of patients with percentage in parentheses.

[d] Mc Nemar test.

DISCUSSION

Here we investigated the possible drug-drug interaction between the proton pump inhibitor omeprazole and irinotecan. No effect of co-administration of omeprazole on the pharmacokinetics and toxicities of irinotecan and its metabolites was seen. Two patients in our study were characterised as CYP2C19 ultra-rapid metabolizers, which could have influenced our results as they could have had suboptimal levels of omeprazole. However, when these patients were excluded from analysis, there still was no significant influence of omeprazole on the pharmacokinetics and toxicities of irinotecan and its metabolites.

Since irinotecan has a complex disposition profile involving several drug-metabolizing enzymes and drug transporters, drug-drug interactions can occur at several levels. In recent years, several herbs and drugs were combined with irinotecan to investigate the possibility of a drug interaction, potentially explaining the occurrence of treatment failure or severe side effects, such as neutropenia and late-onset diarrhea. For example, a reduced exposure to irinotecan and its potent metabolite SN-38 was seen when irinotecan was combined with the CYP3A inducer phenytoin.[30,31] Concomitant smoking also resulted in reduced plasma concentrations of irinotecan and SN-38.[32] Reduced levels of SN-38 were seen when irinotecan was combined with valproic acid,[33] and with St. John's wort.[34] Higher levels of SN-38 were seen in combination with lopinavir/ritonavir and the combined CYP3A and UGT1A inhibitor ketoconazole,[20,35,36] and when irinotecan was combined with tacrolimus.[37] However, no effect was seen when irinotecan was combined with medicinal cannabis.[38]

We detected a modest two-fold increase in UGT1A1 mRNA levels when colorectal carcinoma cell lines were cultured with omeprazole for 24 hours. Similarly Donato *et*

al. reported a six-fold induction of UGT1A1 activity in HepG2 cells when they were cultured in the presence of 50 µM omeprazole for 72 hours.[9] This can be explained by the agonistic effect of omeprazole on the Ah-receptor,[39] which is known to be involved in transcription of the *UGT1A1* gene.[40] However, *in vivo* omeprazole had no significant inducing effect on the glucuronidation of SN-38, possibly because *in vivo* lower concentrations of the drug are present.

Our results complement outcomes of other drug-drug interaction studies with omeprazole and anticancer drugs. For example, no effect of omeprazole was seen on the pharmacokinetics of the CYP3A substrates imatinib and bortezomib.[41,42] However, the exposure to dasatinib was reduced in combination with omeprazole.[43] The mechanism for this effect could be CYP3A4 induction or reduced gastric acid secretion which influences the absorption of dasatinib. As irinotecan is administered intravenously, the latter cannot play a role in a possible interaction. And, in contrast with dasatinib, where only CYP3A4 is thought to play an important role in its metabolism,[44] irinotecan has multiple enzymes that are involved in its disposition.

A limitation of our study might be the fixed-sequence design instead of a randomised design. We chose this design to avoid a possible influence of the different sequences on the pharmacokinetics of irinotecan and to avoid treatment delay due to the two-weeks induction period for omeprazole. Although the sample size was large enough to detect a possible difference in pharmacokinetics according to the power analysis, this was a small study and the study was not powered to detect differences in toxicity outcome.

To conclude, our results indicate that omeprazole 40 mg once daily can be safely combined with a single agent irinotecan schedule, administered once every three weeks. Since other proton pump inhibitors have a different potential for drug-drug interactions,[45] effects of other proton pump inhibitors on the pharmacokinetics and toxicities of irinotecan should be further investigated, before they might be safely combined with irinotecan.

ACKNOWLEDGEMENTS

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Chapter 6

Effects of methimazole on the elimination of irinotecan

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ABSTRACT

Purpose
To study the possible pharmacokinetic and pharmacodynamic interactions between irinotecan and methimazole.

Methods
A patient treated for colorectal cancer with single agent irinotecan received methimazole co-medication for Graves' disease. Irinotecan pharmacokinetics and side effects were followed during a total of four courses (two courses with and two courses without methimazole).

Results
Plasma concentrations of the active irinotecan metabolite SN-38 and its inactive metabolite SN-38-glucuronide (SN-38G) were both higher (a mean increase of 14% and 67%, respectively) with methimazole co-medication, compared to irinotecan monotherapy. As a result, the mean SN-38 glucuronidation rate increased with 47% during concurrent treatment. Other possible confounding factors did not change over time. Specific adverse events due to methimazole co-treatment were not seen.

Conclusions
Additional *in vitro* experiments suggest that these results can be explained by induction of UGT1A1 by methimazole, leading to higher SN-38G concentrations. The prescribed combination of these drugs may lead to highly toxic intestinal SN-38 levels. We therefore advise physicians to be very careful in combining methimazole with regular irinotecan doses, especially in patients who are prone to irinotecan toxicity.

INTRODUCTION

Irinotecan is extensively used in the treatment of advanced colorectal cancer.[1] It is a prodrug of the active compound SN-38 and is metabolized by cytochrome P450 3A (CYP3A) into inactive metabolites (**Figure 1A**). In the liver, uridine diphosphate glucuronosyltransferase 1A (UGT1A) is capable of inactivating SN-38 to its glucuronide form SN-38G, which facilitates excretion from the circulation.[2] In the intestines, SN-38G can be transformed back into SN-38 by bacterial β-glucuronidase. The metabolism of irinotecan is complicated by several drug-transporting proteins involved in the elimination of the drug (i.e. ATP-binding cassette transporters).[3] Besides genetic alterations in the *UGT1A* gene, also environmental factors are known to influence its metabolism.[3-5] For instance, cigarette smoking may induce its metabolism, thereby decreasing the systemic exposure to SN-38.[6]

Here we report on a patient who used methimazole, while being treated with irinotecan chemotherapy. We hypothesized that methimazole could seriously influence irinotecan metabolism, as preclinical data suggest that methimazole inhibits CYP3A,[7] and induces certain UGT1A subtypes,[8] both being crucial enzyme systems involved in the elimination of irinotecan and its metabolites. By performing additional *in vitro* experiments, we tested our hypothesis.

MATERIALS AND METHODS

Our patient was a cigarette smoking 53-year old male, with locally advanced colorectal cancer. Six months prior to the start of irinotecan treatment, he was treated for hyperthyroidism due to Graves' disease using a combination of methimazole and levothyroxine. Other daily medicines were acetaminophen, fentanyl, pantoprazole, carbasalate calcium, metoclopramide, and dexamethasone. During two three-weekly courses of irinotecan, the patient took a daily dose of 30 mg methimazole. Two weeks after the second chemotherapy course, methimazole was stopped and the patient was treated with radioiodine (I-131) as a definitive treatment for Graves' hyperthyroidism. Without delay, the following courses of irinotecan were administered. After written informed consent, plasma pharmacokinetics of irinotecan and its metabolites were collected during a total of four courses. Patient-related factors (i.e., co-medication, smoking status), laboratory values and toxicities were closely monitored, *UGT1A1**28 genotype was determined, and active hormone 3,5,3'-triiodothyronine (T3), and thyroid stimulating hormone (TSH, thyrotropin) levels were determined to exclude possible confounding effects of varying thyroid status.

During and after the 90-minute intravenous infusions of irinotecan (350 mg/m^{2}), blood was drawn at specific time points.[6] Plasma concentrations of irinotecan, NPC, APC, SN-38, and SN-38G were determined using validated chromatography methods.[9,10] In-
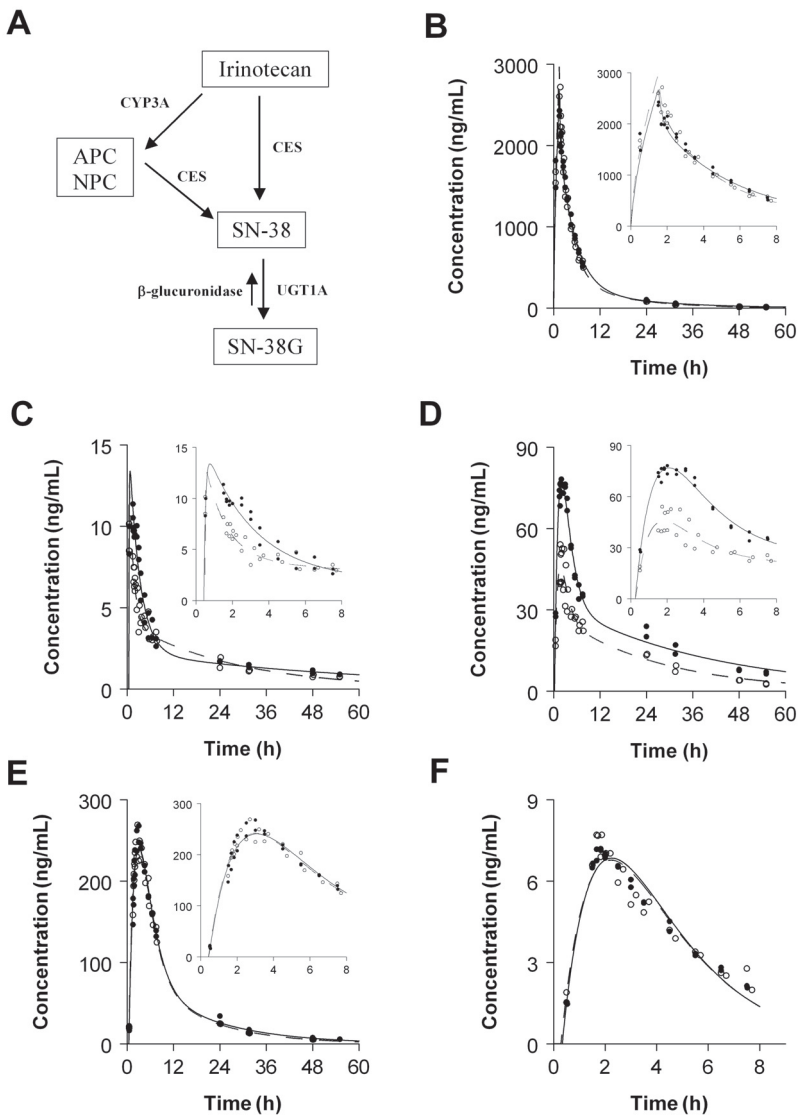
Figure 1. Pharmacokinetics of irinotecan and metabolites
A. Irinotecan is converted into its 1,000 times more active metabolite SN-38 by carboxylesterases (CES), which are predominately found in the liver. The affinity for this reaction is low, since only a fraction of irinotecan is directly converted into SN-38. Competing with the formation of SN-38 is the CYP3A-mediated inactivation of irinotecan into the metabolites APC and NPC. In turn, carboxylesterases also have the ability to convert both of these compounds into SN-38. SN-38 is glucuronidated by UGT1A into its inactive metabolite SN-38G. In the intestines, β-glucuronidase-producing bacteria can reverse this latter reaction, and thus reactivate SN-38, which causes the dose-limiting toxicity diarrhea.
B-F. Area under the plasma concentration-time curves for irinotecan (**B**), SN-38 (**C**), SN-38G (**D**), APC (**E**), and NPC (**F**) during all four chemotherapy courses. The open and closed symbols describe the two courses with methimazole and the two courses without methimazole co-treatment, respectively.

dividual plasma pharmacokinetics were estimated using non-compartmental analyses (WinNonLin 5.0, Pharsight, CA). At weekly outpatient visits, toxicities were scored.

Methods of *in vitro* experiments

In vitro experiments were performed to study the effects of methimazole on the metabolism of irinotecan and SN-38. Pooled human liver microsomes were incubated for 30 minutes with irinotecan (10 μM) in the presence or absence of methimazole (37.5 μM) or fluconazole (25 μM; CYP3A inhibitor) based on the method described by Slatter *et al*.[11]

The experiment was performed on four separate occasions. In each experiment, microsomal proteins (1 mg/mL) were incubated in triplicate. In addition, pooled human liver microsomes were incubated for 30 minutes with SN-38 (5 μM) in the presence or absence of methimazole (37.5 μM) or ketoconazole (25 μM; UGT1A inhibitor) based on the method described by Yong *et al*.[12] This experiment was performed on separate occasions, during which microsomal proteins (0.8 mg/mL) were incubated in triplicate. Experiments were terminated by the addition of a perchloric acid/methanol solution. Irinotecan and metabolite concentrations from these experiments were measured using the methods mentioned earlier.

HCT116 colorectal carcinoma cells were cultured in Glutamax™ containing Hepes-buffered RPMI 1640 medium supplemented with 10% fetal calf serum (Gibco BRL, Paisley, UK), 100 U/mL penicillin and 100 μg/mL streptomycin at 37°C in a humidified atmosphere containing 5% CO_2. HCT116 cultures of 60% confluence were cultured in the presence of methimazole (25, 50, and 100 μM) or 0.1% (v/v) MeOH as a solute control. After 5, 24, and 48 hours, total RNA was extracted using RNA-Bee (Tel-TEST Temco, Inc. Friendswood, TX). Relative UGT1A1 mRNA expression levels were measured by real-time RT-PCR using the Taqman Universal Master mix and Assay-On-Demand products from Applied Biosystems (UGT1A1 assay ID: Hs02511055-s1). The human glyceraldehyde-3-phosphate dehydrogenase (GAPDH assay ID: 4310884E; VIC/TAMRA) was used for normalization. Reactions were run on an ABI PRISM 7900 sequence detector system (Applied Biosystems, Foster City, CA) using the following cycling conditions: 50°C for two minutes, 95°C for ten minutes followed by 40 cycles of 95°C for 15 seconds, and 60°C for one minute.

Hek293 cells transfected with pcDNA3 (HEK293/neo) or wild-type BCRP/ABCG2-R482R (HEK293/R) were cultured under similar conditions as HCT116. To investigate whether ABCG2 activity is affected by methimazole, we analyzed the intracellular accumulation of mitoxantrone (a well known ABCG2 substrate) in the presence of methimazole. A cell suspension of 106 cells of HEK293/neo or HEK293/R in RPMI1640 medium without further supplements was incubated in the presence of 3 μM mitoxantrone at 37°C for 90 minutes with or without the addition of 10, 25, 50, 100 μM methimazole or 200 nM of the fumitremorgin C analog Ko-143 (ABCG2 inhibitor). The mitoxantrone accumulation (fluorescence intensity) was determined by flow cytometry as described previously.[13]

6

RESULTS

The area under the plasma concentration-time curve (AUC) of irinotecan did not differ between the courses with or without methimazole co-treatment (mean 16.1 µg×h/mL; **Figure 1B; Table 1**). However, plasma concentrations of both SN-38 (**Figure 1C**) and SN-38G (**Figure 1D**) were higher during the courses with methimazole. With methimazole, the mean SN-38 AUC was 14% higher than without methimazole (125 ng×h/mL *versus* 110 ng×h/mL). In the presence of methimazole, the mean SN-38G AUC was 67% higher (1,255 ng×h/mL *versus* 753 ng×h/mL). Consequently, a mean 47% increased relative extent of glucuronidation, which is the ratio of SN-38G over SN-38 (mean, 10.1 *versus* 6.85), was found during the combination treatment courses. The exposure to the CYP3A metabolites APC (mean 3,018 ng×h/mL; **Figure 1E**) and NPC (mean 32.3 ng×h/mL; **Figure 1F**) was not different between the courses with or without methimazole.

Table 1. Baseline characteristics, pharmacokinetics, chemistry, and toxicities during four treatment courses with irinotecan

Irinotecan course	1	2	3	4
Methimazole	Yes	Yes	No	No
TSH (0.4-4.3 mU/L)[a]	0.0	2.6	0.0	0.0
T3 (1.4-2.5 nmol/L)[a]	1.54	2.11	2.14	2.86
Dose irinotecan (mg)	660	660	660	660
$AUC_{(0-56h)}$ irinotecan (µg×h/mL)	16.6	16.0	15.8	16.0
$AUC_{(0-56h)}$ APC (ng×h/mL)	3,227	2,919	3,028	2,897
$AUC_{(0-8h)}$ NPC (ng×h/mL)	31.9	32.4	32.2	32.5
$AUC_{(0-56h)}$ SN-38 (ng×h/mL)	117	133	106	114
$AUC_{(0-56h)}$ SN-38G (ng×h/mL)	1,301	1,209	728	777
REG	11.1	9.09	6.87	6.82
Bilirubin (0-16 U/L)[a]	5	6	7	6
Alkaline phosphatase (0-119 U/L)[a]	189	182	176	152
γ-glutamyltransferase (0-49 U/L)[a]	60	42	34	33
AST (0-36 U/L)[a]	20	21	18	16
ALT (0-40 U/L)[a]	24	23	24	18
Neutrophil nadir (×10⁹/L)	4.7	4.1	4.1	4.0
Diarrhea (grade)[b]	1	0	0	0

Abbreviations: TSH, thyroid stimulating hormone; T3, active hormone 3,5,3'-triiodothyronine; AUC, area under the plasma concentration-time curve; SN-38, active metabolite of irinotecan; SN-38G, detoxified metabolite of SN-38; REG, relative extent of glucuronidation; AST, aspartate aminotransferase; ALT, alanine aminotransferase.

[a] Normal values between brackets. For TSH and T3, means of weekly values within a period of three weeks are shown.

[b] Grading according to National Cancer Institute Common Terminology Criteria for Adverse Events version 3.0.

Except for methimazole, co-medication did not change during the studied courses and also smoking behavior of the patient (15-20 cigarettes per day) did not change. The patient appeared to be wild-type (TA6/TA6) for *UGT1A1*28*, meaning that UGT1A1 function was not lowered based on this familiar genetic polymorphism.[3] Thyroid hormone levels varied during the assessed courses (**Table 1**), with a transient but clear hyperthyroidism during the fourth course. Nevertheless, a correlation between thyroid function and systemic chemotherapy levels could not be established. Irinotecan-related toxicity was mild during all courses. The absolute neutrophil count nadir was similar in all courses (mean 4.2 ×10^9/L).

In the *in vitro* experiments, simultaneous incubation of human liver microsomes with irinotecan and methimazole resulted in a slightly decreased formation of NPC and APC (both 16% reduced compared to incubation in the absence of methimazole; **Figure 2A**). Control experiments in which irinotecan was combined with fluconazole, a known potent CYP3A-inhibitor, resulted in a strong inhibition of NPC and APC formation (78 and 74% inhibition, respectively, compared to experiments in the absence of fluconazole; **Figure 2A**). No effect of methimazole on SN-38 formation was noticed. In addition, SN-38G formation did not decrease during the co-incubation of microsomes with methimazole and SN-38, while a reduction of 43% was observed during simultaneous incubation of SN-38 with the UGT1A1 inhibitor ketoconazole.

In order to determine whether methimazole is capable of inducing UGT1A1 expression, a HCT 116 colorectal carcinoma cell line was cultured in the presence of various concentrations of methimazole (25-100 µM) for ± 48 hours after which UGT1A1 mRNA levels were measured by quantitative RT-PCR. **Figure 2B** displays the results of the experiment showing a 15 to 18-fold induction of UGT1A1 mRNA levels by methimazole compared to untreated control cells. The lowest concentration of methimazole (25 µM) gave rise to the highest induction levels after 48 hours, whereas the higher concentrations (50 and 100 µM) already showed strongly elevated UGT1A1 levels after 24 hours that seem to return to normal in a concentration-dependent manner after a longer incubation period.

Our *in vitro* tests in which the transport of mitoxantrone by ABCG2 was studied showed that methimazole in concentrations up to 100 µM did not affect the efflux of mitoxantrone. In contrast, the known ABCG2 inhibitor Ko-143 reduced the efflux of mitoxantrone, thereby causing accumulation of the drug.
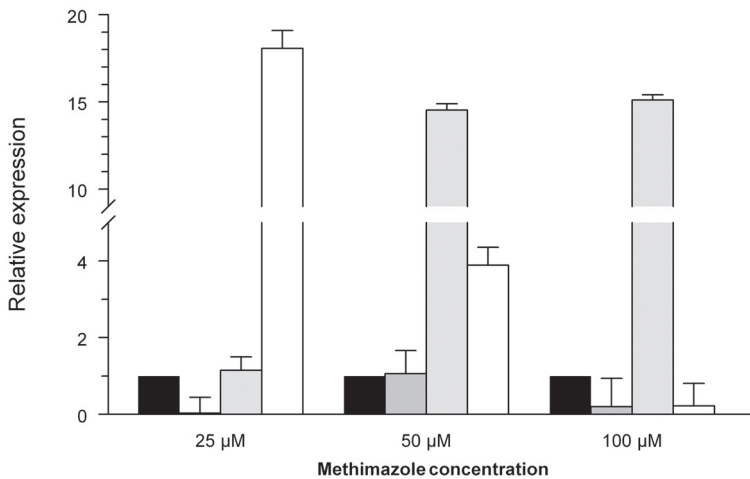
DISCUSSION

In the current case, factors known to significantly alter irinotecan pharmacokinetics did not change over time, except for the use of methimazole. As a relationship between thyroid status and plasma concentrations of chemotherapy was not found, a possible

A
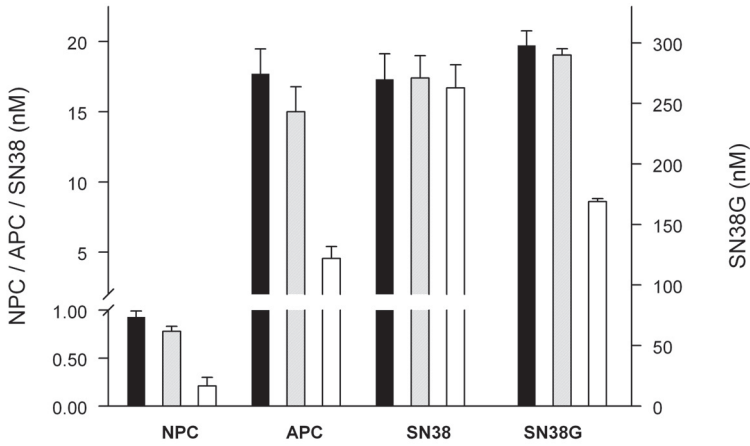
B

Figure 2. *In vitro* experiments

A. Effects of methimazole (grey bars) and fluconazole (white bars) on the formation of NPC, APC, and SN-38 during co-incubation with irinotecan, and effects of methimazole (grey bars) and ketoconazole (white bars) on the formation of SN-38G during co-incubation with SN-38. The black bars represent the formation of irinotecan metabolites in the absence of any (potential) inhibitor.

B. Methimazole induces UGT1A1 mRNA levels.

HCT116 cells were cultured in the presence or absence (control) of various concentrations of methimazole. At 5 (dark grey bars), 24 (light grey bars) and 48 (white bars) hours total RNA was isolated and used to measure UGT1A1 mRNA levels by quantitative RT-PCR (Taqman™). Depicted is the expression of UGT1A1 compared to the control UGT1A1 levels (black bars), which are arbitrarily set at 1. Error bars indicate 2 x SD (N = 3).

influence of thyroid hormone on UGT1A activity is probably very limited in our case.[14] Therefore, we assume that the intrapatient differences in pharmacokinetics found over the courses are mainly attributable to methimazole.

Irinotecan drug concentrations assessed in the courses without methimazole are within the low-normal range of literature data[6,15,16] possibly due to patients' smoking behavior. As SN-38 levels are only moderately increased, while SN-38G concentrations are much higher in the presence of methimazole, our clinical data support the preclinical literature describing combined effects of CYP3A inhibition and UGT1A induction by methimazole.[7,8] However, inhibition of CYP3A was not confirmed by lower systemic concentrations of NPC and APC in the courses with methimazole. In addition, our *in vitro* experiments could not determine an important effect of methimazole on CYP3A activity. Therefore, we have to conclude that the involvement of CYP3A is probably limited in the observed pharmacokinetic alterations during methimazole-exposed courses.

UGT1A induction in turn will lead to increased SN-38G levels, catalyzing SN-38 transformation into SN-38G. This is expressed by the higher glucuronidation rate during the combination treatment. Although the exact mechanism of irinotecan-induced delayed-onset diarrhea is unclear, there is consensus that intestinal toxicity is caused by local reactivation of SN-38 from SN-38G by β-glucuronidase-producing bacteria in the gut.[17,18] Therefore, higher SN-38G levels as a result of methimazole co-treatment could potentially lead to more intestinal toxicity, since higher systemic SN-38G concentrations could cause increased intestinal SN-38 levels, when SN-38G is excreted in the gut and is deglucuronidated by intestinal bacteria. This was also shown in a population pharmacokinetic model of irinotecan, in which the AUC of SN-38G was correlated with diarrhea scores ($P < .005$).[19] In addition, it was shown that co-treatment with the antibiotic neomycin reduces the fecal β-glucuronidase activity and fecal SN-38G/SN-38 ratio, thereby reducing the incidence of diarrhea.[20]

In our view, it is plausible that the effect of higher SN-38G levels in our patient did not result in more diarrhea, because this patient already had a low pharmacokinetic and toxicity profile, most likely because of his smoking behavior and wild type *UGT1A1*28* genotype.[6] However, in patients who are more prone to toxicity, higher SN-38G levels as a result of concomitant methimazole use, could potentially lead to severe diarrhea.

One cannot formally rule out the possibility that additional mechanisms contribute to the observed clinical phenotype. For instance, methimazole might interfere with ABC transporters involved in the efflux of the irinotecan metabolites, leading to accumulation of SN-38 and SN-38G. However, there is no literature available indicating that methimazole is a substrate or inhibitor of ABC transporters. Moreover, our experiments confirmed that therapeutic concentrations of methimazole do not affect ABCG2-mediated transport.

CONCLUSIONS

Although the exact explanation for our findings is subject for further study, the combination of irinotecan and methimazole might potentially lead to increased toxicity due to higher systemic SN-38 and SN-38G concentrations. Reactivation of high concentrations SN-38G by bacterial enzymes in the bowel is potentially dangerous in that respect and could lead to local toxicity. We therefore advise physicians to be careful in combining methimazole with regular irinotecan doses, especially in patients who are prone to irinotecan toxicity.

ACKNOWLEDGEMENTS

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Chapter 7

A CYP3A4 phenotype-based dosing algorithm for individualized treatment of irinotecan

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ABSTRACT

Purpose

Irinotecan, the prodrug of SN-38, is extensively metabolized by cytochrome P450 3A4 (CYP3A4). A randomized trial was done to assess the utility of an algorithm for individualized irinotecan dose calculation based on a priori CYP3A4 activity measurements by the midazolam clearance test.

Experimental design

Patients were randomized to receive irinotecan at a conventional dose level of 350 mg/m^2 (group A) or doses based on an equation consisting of midazolam clearance, γ-glutamyltransferase, and height (group B). Pharmacokinetics and toxicities were obtained during the first treatment course.

Results

Demographics of 40 evaluable cancer patients were balanced between both groups, including *UGT1A1*28* genotype and smoking status. The absolute dose of irinotecan ranged from 480 to 800 mg in group A and 380 to 1,060 mg in group B. The mean absolute dose and area under the curve of irinotecan and SN-38 were not significantly different in either group (P > .18). In group B, the interindividual variability in the area under the curve of irinotecan and SN-38 was reduced by 19% and 25%, respectively (P > .22). Compared with group A, the incidence of grades 3 to 4 neutropenia was > 4-fold lower in group B (45 *versus* 10%; P = .013). The incidence of grades 3 to 4 diarrhea was equal in both groups (10%).

Conclusions

Incorporation of CYP3A4 phenotyping in dose calculation resulted in an improved predictability of the pharmacokinetic and toxicity profile of irinotecan, thereby lowering the incidence of severe neutropenia. In combination with *UGT1A1*28* genotyping, CYP3A4 phenotype determination should be explored further as a strategy for the individualization of irinotecan treatment.

INTRODUCTION

In 1996, the topoisomerase-I inhibitor irinotecan received accelerated approval in the United States for the second-line treatment of patients with metastatic colorectal carcinoma. Currently, irinotecan is approved as a single agent as well as in combination with other drugs (i.e., fluorouracil, oxaliplatin, and bevacizumab) in different first-line and second-line regimens for the treatment of this disease.[1-4]

Several enzymes and drug transporters are involved in the elimination of irinotecan, including members of the cytochrome P450 3A (CYP3A) and uridine diphosphate glucuronosyltransferase 1A (UGT1A) families, both of which influence exposure to the active metabolite, SN-38 (**Figure 1**).[5] Because the expression and function of these proteins could be affected by numerous environmental and genetic factors, the pharmacokinetics of irinotecan and its metabolites vary greatly between patients.[5,6]
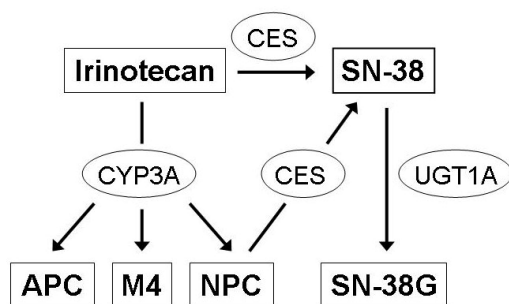

Figure 1. Metabolism of irinotecan
Irinotecan is converted into its 100 to 1,000 times more active metabolite SN-38 by human carboxylesterases type 1 and 2, which are predominately found in the liver. The affinity for this reaction is low, because only ~3% of irinotecan is converted into SN-38. SN-38 is glucuronidated by UGT1A into the inactive metabolite SN-38G. β-glucuronidase-producing bacteria can reverse this reaction in the intestines, reactivating SN-38, and causing the dose-limiting toxicity diarrhea. Competing with the formation of SN-38 is the CYP3A-mediated inactivation of irinotecan into the metabolites APC and NPC. Abbreviations: APC, 7-ethyl-10-[4-N-(5-aminopentanoic acid)-1-piperidino]-carbonyloxycamptothecin; CES, carboxylesterases; CYP3A, cytochrome P450 3A isoforms (3A4/3A5); M4, fourth unspecified metabolite of irinotecan; NPC, 7-ethyl-10-(4-amino-1-piperidino)-carbonyloxycamptothecin; SN-38, 7-ethyl-10-hydroxycamptothecin; SN-38G, glucuronide form of SN-38; UGT1A, uridine diphosphate glucuronosyltransferase 1A isoforms (1A1/1A7/1A9).

Like most other cytotoxic anticancer agents, irinotecan has a narrow therapeutic window. Therefore, the large interindividual pharmacokinetic variability may result in overtreatment with unacceptable side effects in some patients and in undertreatment with diminished therapeutic effects in others. The conventional dose calculation of irinotecan is based on an individual's body surface area (BSA), although this approach does not result in reduced pharmacokinetic variability (**Figure 2A**) compared with a flat-fixed dose.[7,8] New dosing strategies that take the pharmacologic profile of irinotecan in

the individual patient into account could potentially replace BSA-based dosing, if this would lead to a reduction in the pharmacokinetic variability. Ideally, this should prevent the occurrence of highly unpredictable severe toxicities associated with irinotecan administration, such as neutropenia and late-onset diarrhea, and should result in a maximal antitumor response in each patient.

Thus far, new dosing strategies have mainly focused on polymorphisms affecting the expression of enzymes involved in the metabolism of SN-38, such as the *UGT1A1*28* polymorphism.[9-11] It should be pointed out, however, that the expression of many enzymes and transporters of relevance to irinotecan, is also influenced by environmental factors, such as co-medication, complementary and alternative medicine, disease status, and lifestyle.[12] Therefore, dose-individualization strategies should not solely focus on inherited variables.
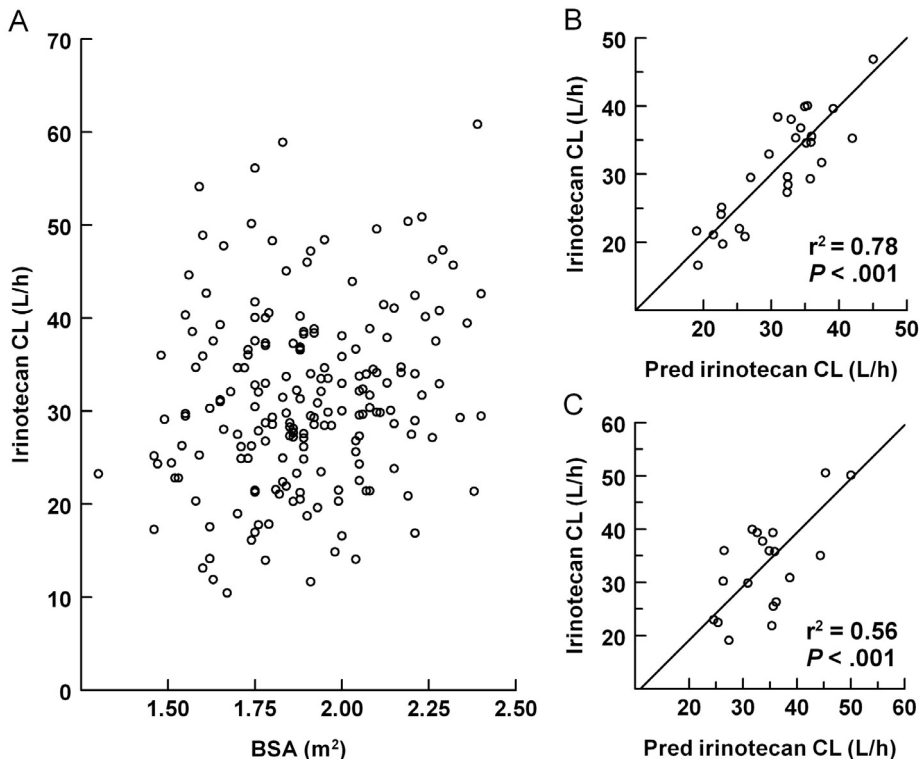
Figure 2. Development and validation of a new dosing equation for irinotecan
A. The relationship between BSA and irinotecan clearance in a cohort of 190 patients.[18] Conclusion: BSA-based dosing does not reduce the interindividual pharmacokinetic variability of irinotecan.
B-C. Correlation between the equation-based predicted clearance and the measured irinotecan clearance in the former study (**B**, N = 30),[17] and in the current study (**C**, N = 20).
Abbreviations: CL, clearance; N, number; pred, predicted.

From drug interaction studies involving enzyme inducing and enzyme inhibiting compounds, such as St. John's wort and ketoconazole, it is known that the CYP3A4 pathway plays a crucial role in the inactivation of irinotecan into a number of inactive, oxidative metabolites.[13,14] Because CYP3A4 activity can be influenced by many factors, and is largely variable between patients,[15] it has been proposed that *a priori* assessment of the functional activity of CYP3A4, for instance by using probe drugs such as midazolam or erythromycin,[16] may aid in irinotecan dose calculation. Previously, we found that CYP3A4 activity as determined by midazolam pharmacokinetics was highly correlated with irinotecan clearance.[17]

We developed a new dosing equation for irinotecan treatment by using linear regression analysis to identify clinical parameters that could predict irinotecan clearance. Here, we report on a randomized clinical trial in which we compared classic BSA-based dosing with individualized irinotecan dose calculation on the basis of an equation that incorporated an individual's CYP3A4 activity, height, and γ-glutamyltransferase.

MATERIALS AND METHODS

Patients

Patients were included according to the following inclusion criteria: (a) a histologically or cytologically confirmed diagnosis of any form of metastatic cancer which was thought to be sensitive to irinotecan treatment; (b) age ≥ 18 years; (c) a WHO performance score of < 2; and (d) adequate hematologic, renal, and hepatic function as determined within two weeks before inclusion and repeated one day prior to the start of treatment. An additional criterion was used for baseline γ-glutamyltransferase, (< 200 units/L, which is approximately five times the upper limit of normal) because it was one of the three parameters on which the dosing equation was based, and this was the upper limit that was seen in the earlier study upon which the equation was formulated.[17]

During the study period, starting three weeks before irinotecan administration, patients were not allowed to use grapefruit or grapefruit juice, St. John's wort or any other known inhibitor and/or inducer of CYP3A4. Use of temazepam was prohibited as well, because this compound was used as the internal standard for the midazolam assay. Other specific exclusion criteria included (a) any form of anticancer treatment within four weeks of the start of irinotecan administration, (b) unresolved bowel obstruction or chronic colic disease, and (c) any form of illness that would prohibit the process of understanding and giving of informed consent. All patients gave written informed consent and the local institutional review boards approved the clinical protocol, which was written in accordance with the declaration of Helsinki.

Treatment

Before the start of treatment, baseline toxicities were recorded, a physical examination took place, and bone marrow, renal, and hepatic function were obtained using routine laboratory analyses. Patients were randomized to either receive a BSA-based dose of 350 mg/m^2 or a dose calculated from an equation that took into account the height, baseline γ-glutamyltransferase, and midazolam clearance of the patient. This equation was derived from data obtained previously in 30 patients,[17] using regression analysis in Stata version 8.2 (Stata Corp.). All patient-related factors that could possibly affect irinotecan clearance were taken into account, and included age, height, weight, BSA, baseline blood cell counts, measures of liver and renal function, and genetic variation in genes encoding ATP-binding cassette drug transporters and drug-metabolizing enzymes involved in the elimination pathway of irinotecan.

The final equation for irinotecan clearance was: 0.0325 × midazolam clearance (mL/min) − 0.0396 × γ-glutamyltransferase (units/L) + 27.180 × height (m) − 31.926.

This equation explained ± 80% of the variability in irinotecan clearance (**Figure 2B**), whereas BSA explained only 21% (data not shown). A dose for each patient in the equation group was calculated by multiplying the predicted irinotecan clearance by 22.157 (μg×h/mL), which was the mean area under the curve of irinotecan observed previously, and this value was arbitrarily defined as the target measure of systemic exposure.[17] To eliminate age as a potential confounding variable, patients were stratified and matched according to age (≤ 55 *versus* > 55 years) within each treatment group and within each participating institution. Application of premedication and treatment of gastrointestinal toxicity were done as described previously.[18]

Pharmacologic studies

All patients underwent a midazolam clearance test seven or eight days prior to the start of irinotecan treatment. A midazolam dose of 0.025 mg/kg was injected i.v. as a bolus, followed by a running infusion of 0.9% saline over a 30-second period. Blood sample collection, analytic measurements of midazolam, irinotecan and SN-38 in plasma, and pharmacokinetic parameter calculations were done as described previously, with minor modification.[17] Following separate consent, DNA was obtained from all patients and analyzed for the *UGT1A1**28 variant using a LightCycler method,[19] with modifications.[17]

Toxicity evaluation

Patients were seen weekly at the outpatient clinic for follow-up, which included a physical examination and routine hematologic, renal, and hepatic laboratory analyses. All side effects, including leukopenia, neutropenia, and late-onset diarrhea were graded using the National Cancer Institute Common Terminology Criteria for Adverse Events version 3.0.[20]

Statistical considerations

Statistical calculations were done using SPSS version 14.0 (SPSS Inc.) and Stata version 8.2 (Stata Corp.). An estimation of the SEM of the interindividual variability was obtained as described.[21] Based on an estimated mean interindividual variability in irinotecan area under the curve of 30% with an SD of 22.6 in patients receiving a dose of 350 mg/m^2, a sample size of 20 patients per treatment group was necessary to detect a 50% lower variability with a two-sided $P = .05$ and a power of 80%. Differences in categorical data were analyzed using χ^2-test.

Differences in interindividual pharmacokinetics and all other continuous variables in both groups were calculated using a Student's *t*-test. Root mean square error, mean predictive error, and Pearson's correlation coefficient were used to relate two continuous variables.[22] All data are presented as means with SD or 95% confidence intervals in parentheses, unless stated otherwise. $P < .05$ values were considered significant.

RESULTS

Patient characteristics

Forty-five cancer patients were randomized between September 2005 and August 2007 to receive a BSA-based dose or an equation-based irinotecan dose, and received irinotecan treatment in accordance with the protocol. Of these, five patients were not evaluable for analysis; three due to technical problems with blood sampling, one was lost to follow-up, and one had an elevation of γ-glutamyltransferase induced by alcohol abuse at the start of treatment. The remaining 40 patients (22 males and 18 females) with a mean age of 58 (range, 27-70) were evaluable for analysis. No significant differences were seen in age, gender, smoking status, *UGT1A1*28 genotype, and number of previous chemotherapeutic treatments between both groups (**Table 1**).

Pharmacokinetics of midazolam

CYP3A4 activity, as determined by midazolam clearance, varied ± 6-fold (range, 203 1,257 mL/min), with a mean of 698 mL/min (95% confidence interval, 609-786 mL/min). These data are consistent with previously reported values.[17,23,24]

Pharmacokinetics of irinotecan

The mean absolute dose was not significantly different between either group (650 *versus* 698 mg; $P = .28$; **Figure 3A**). However, the range of doses was much broader in the group that received the dose calculated by the equation (480-800 *versus* 380-1,060 mg). If the patients from the equation group would have been dosed on BSA, this would have resulted in a slightly lower mean dose of 675 mg, and a range of 494 to 830 mg for this group.

Table 1. Patient characteristics

Characteristics[a]	BSA group (N = 20)	Equation group (N = 20)	P[b]
Sex			
Male	11 (50%)	11 (50%)	
Female	9 (50%)	9 (50%)	
Age (years)[c]	57 (27-70)	55 (27-69)	.60[d]
Height (m)[c]	1.74 (1.52-1.85)	1.75 (1.54-1.87)	.83[d]
γ-glutamyltransferase (units/L)[c]	76 (57)	61 (46)	.34[d]
Smoking status[e]			.63
No	16 (48%)	17 (52%)	
Yes	3 (60%)	2 (40%)	
Previous chemotherapy			.72
0-1	14 (48%)	15 (52%)	
≥ 2	6 (55%)	5 (45%)	
Tumortype			.18
Colorectal	3 (43%)	4 (57%)	
Gastric	2 (40%)	3 (60%)	
Pancreas	2 (50%)	2 (50%)	
Miscellaneous[f]	13 (54%)	11 (46%)	
UGT1A1*28 genotype[g]			.79
6/6	9 (56%)	7 (44%)	
6/7	8 (44%)	10 (56%)	
7/7	1 (50%)	1 (50%)	

[a] Data are presented as absolute numbers with percentages in parentheses, unless stated otherwise.
[b] P values result from χ^2-test, unless stated otherwise.
[c] Data presented as mean with range or SD in parentheses.
[d] P values resulting from two-sample t-tests.
[e] N = 38 (smoking status unknown in two patients).
[f] Including sarcoma (4), esophageal carcinoma (3), breast cancer (3), (adeno)carcinoma of unknown primary (2), jejunal carcinoma (2), small cell lung cancer (2), urothelial carcinoma (2), melanoma (2), cholangiocarcinoma (1), ovarian carcinoma (1), medulloblastoma (1), and head and neck cancer (1).
[g] N = 36 (genotype unknown in four patients).

There were no differences in the mean area under the curve of irinotecan and SN-38 between both groups of patients (P > .18; **Figure 3B and C**). In the group receiving the dose calculated by the equation, the interindividual variability (expressed by SD/mean) in the area under the curve was 19% lower for irinotecan (31% *versus* 25%; P = .35) and 25% lower for SN-38 (45% *versus* 34%; P = .22), whereas the mean interindividual variability in the administered dose was significantly higher in this group (11% *versus* 26%; P = .002).
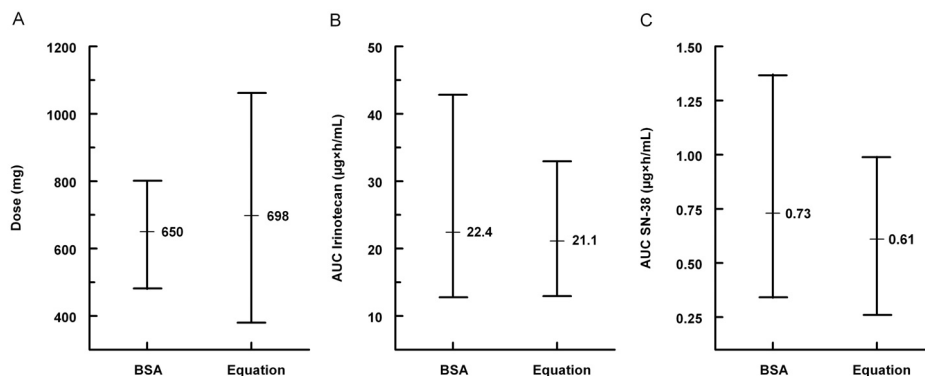
Figure 3. Interindividual variability in the dose and pharmacokinetics of irinotecan
Range, means, and interindividual variability of the administered dose (**A**), irinotecan area under the plasma concentration-time curve (**B**), and SN-38 area under the plasma concentration time-curve (**C**) in the BSA group (left) and the equation group (right).

The predicted clearance of irinotecan calculated by the equation was correlated with the observed clearance in the group receiving the dose calculated by the equation (root mean square error, 21.8%; mean predictive error, 4.08%, $R^2 = .56$; $P < .001$; **Figure 2C**).
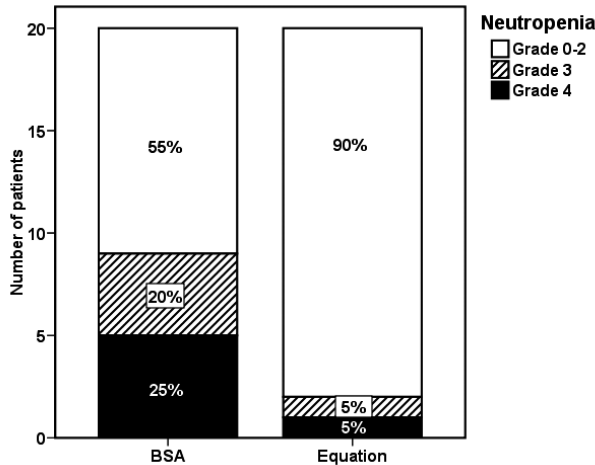
Toxicities of irinotecan

The incidence of grades 3 to 4 neutropenia was more than four times lower in the patients receiving the equation-based dose (45% *versus* 10%; $P = .013$; **Figure 4**). Similarly, the incidence of grades 3 to 4 leukopenia was three times lower in this group (45% *versus* 15%; $P = .038$). In the group receiving a BSA-based dose, three patients experienced febrile neutropenia (15%), whereas in the other group, none of the patients experienced this dose-reducing side effect. Although not reaching statistical significance, a lower mean nadir white blood cell count (66% *versus* 45%; $P = .10$) and nadir absolute neutrophil count (80% *versus* 55%; $P = .10$) were found in the group receiving an irinotecan dose determined by the equation. No differences were seen in the incidence of severe diarrhea (grades 3-4), which was 10% in both groups.

DISCUSSION

We developed a new dosing algorithm for irinotecan treatment, which was based on an individual's CYP3A4 activity, γ-glutamyltransferase, and height and was prospectively compared with BSA-based dosing in a randomized trial. We found that the predicted irinotecan clearance according to the equation was correlated (with little bias and good accuracy) with the measured clearance in the group that received the equation-based dose. As a result, the interindividual pharmacokinetic variability of irinotecan and its active metabolite SN-38 were 19% to 25% lower in the equation-based dosing group,

Figure 4. Toxicities of irinotecan
Bargraph showing that the incidence of severe (grades 3-4) neutropenia (according to the Common Terminology Criteria of Adverse Events) was higher in the BSA group (left) when compared with the equation group (right; P = .013).

whereas the dose range was substantially broader in this group. In line with the known association between irinotecan pharmacokinetics and drug-related toxicities,[5,7,18] a > 4-fold reduction of severe myelosuppression was observed in the equation-based dosing group as compared with the other group.

In our study, we excluded all patients who were using known CYP3A inducers and/ or inhibitors which could not be replaced by other medicines because of the potential hazard or treatment failure when giving these patients a (full) BSA-based dose. However, in clinical practice, patients who are taking this medication would probably benefit even more from dosing on the basis of our CYP3A phenotype-based equation.

It is noteworthy that two patients in the equation-based dosing group were treated with an absolute irinotecan dose of 1,060 mg. This is an extremely high dose because the standard dose for the three-weekly schedule was 350 mg/m^2, suggesting that only patients with a BSA of > 3.0 m^2 would have received a similar absolute dose, which is usually only the case for morbidly obese individuals. Even under these circumstances, most physicians would not prescribe such a high dose because doses are typically capped at a BSA of 2.0 m^2.[25] In the current study, both patients received a total of six courses of irinotecan at a dose of 1,060 mg in each course without experiencing any toxicity higher than grade 1. It is of interest to note that one of these patients was even heterozygous for the *UGT1A1*28* allele, and may therefore have been genetically predisposed to an increased risk of experiencing severe toxicity after irinotecan treatment.[26,27] This illustrates the feasibility of administering higher doses of irinotecan than the approved BSA-based dose in subgroups of patients that are at low risk for experiencing severe side

effects. The selection of such patients might also increase the potential clinical benefit of irinotecan, whereas the administration of lower doses, based on our equation, to patients who cannot tolerate standard doses might decrease the occurrence of potentially life-threatening adverse effects.

One could speculate that the response to treatment might be negatively affected by the use of a dosing algorithm that is associated with a reduced incidence of severe side effects. However, it should be pointed out that the systemic exposure to both irinotecan and SN-38 was equal in both groups, and that overall, the administered doses were even slightly higher in the equation-based dosing group, making this hypothesis unlikely. In addition, if the patients from the equation group were dosed on BSA, this would have resulted in a lower mean absolute dose. Our view is that by predicting irinotecan exposure in the individual patient, those patients who were at risk for severe toxicities based on their phenotypic profile had received a lower dose, whereas those patients with a more favorable profile had received a higher dose. The average administered dose and exposure to irinotecan in the whole group remained equal, but by administering a tailored dose to each individual, the overall toxicity in the group was reduced. Additional investigation into the influence of equation-based dosing on antitumor activity (response and survival) of irinotecan is required to support this theory.

In addition to its role in irinotecan dosing, CYP3A4 phenotyping may also be valuable in dose calculations of other anticancer drugs with narrow therapeutic windows that are extensively metabolized by CYP3A4, such as docetaxel,[28,29] sunitinib,[30] and gefitinib.[31] Indeed, Yamamoto *et al.* have shown the potential clinical effect of applying CYP3A4-phenotyping into the dose calculation of docetaxel.[32] The applied equation consisted of urinary concentrations of 6β-hydroxycortisol as a measure of CYP3A4 activity, α1-acid glycoprotein, transaminases and age, and resulted in a reduced interindividual pharmacokinetic variability when compared with BSA-based dosing. However, before these kinds of dose calculations could be clinically implemented, CYP3A4 phenotyping tests should become routinely available in daily clinical practice, and become less time-consuming and less invasive. Further refining of these tests is warranted, for example, by developing strategies for determining midazolam clearance that involve a reduced number of blood samples, and by specifically identifying an optimal CYP3A4 probe for each agent under investigation.[33]

The small population that was investigated in our study could have accounted for the fact that the 19% to 25% differences in interindividual pharmacokinetic variability were not statistically significant. Yet, these decreases may have clinical relevance. Importantly, the study showed a significant change in grades 3 to 4 hematologic toxicity in favor of the equation-based dose calculation. However, independent confirmation of our study results is necessary, as well as a demonstration that the antitumor response is equal in both groups or higher when the irinotecan dose is calculated according to the equation.

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Because irinotecan is often administered in combination regimens; at lower doses, and at a higher frequency than the regimen that was investigated in our study, it is also necessary to investigate the relevance of equation-based dose calculation in other irinotecan schemes before implementing this dosing strategy.

In conclusion, the current study supports the feasibility of using CYP3A4 activity, height, and baseline γ-glutamyltransferase measurements to individualize irinotecan dose calculation. The application of this methodology was associated with a reduced interindividual pharmacokinetic variability, although it was not statistically significant. The incidence of severe myelosuppression was significantly reduced in the equation-based dosing group compared with the BSA-based dosing strategy. In combination with *UGT1A1*28* genotyping, CYP3A4 phenotype determination should be explored further as a strategy to identify patients that are at risk for experiencing severe side effects following irinotecan administration.[34]

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Chapter 8

Summary and conclusion

SUMMARY

Although irinotecan can be very effective in the treatment of advanced colorectal cancer, it still is a notorious drug to some medical oncologists, due to its unpredictable side effects, mainly diarrhea and neutropenia. These side effects can be very severe and sometimes even lethal. Years of research have elucidated the complex metabolism of irinotecan, which involves several phase I and II enzymes and drug transporters (**Chapter 1**). In addition, many attempts have been made to explain and reduce the interpatient variability in the pharmacokinetics of irinotecan, thereby improving efficacy and reducing toxicity. Also, many efforts were made to directly decrease the intestinal toxicity. In this thesis, several pharmacological aspects of irinotecan treatment were investigated, with the ultimate goal to optimalize and personalize irinotecan therapy.

Despite the knowledge of the causative role of smoking to several types of cancer, little is known about the effects of smoking on the outcome of anticancer treatment. This is striking, given the fact that almost 30% of cancer patients still smoke during treatment. Several constituents of cigarette smoke are known to interact with certain drug-metabolizing enzymes, and may therefore influence the pharmacokinetics of anticancer drugs, potentially resulting in altered toxicity and efficacy of these drugs. In **Chapter 2**, the effects of smoking on the pharmacokinetics and toxicities of irinotecan were investigated in 190 cancer patients treated with single agent irinotecan. Twenty-six percent of the patients were smoking during their anticancer treatment. Patients who smoked during irinotecan treatment had a significantly lower exposure to irinotecan and in particular to the active metabolite SN-38. The area under the curve of SN-38 was 40% lower in smokers. The incidence and severity of leukopenia and neutropenia were also lower in smokers. The combination of a decreased exposure to irinotecan and the active metabolite SN-38, and less severe toxicity in smokers, suggests that patients who smoke during their irinotecan treatment have an increased risk of treatment failure.

While only a limited number of studies have been performed on the influence of lifestyle factors on anticancer treatment, a lot more attention has been paid to the role of polymorphisms in genes coding for metabolizing enzymes and drug transporters that are involved in the metabolism of chemotherapeutic drugs. However, genetic variation in genes coding for proteins that are linked to pharmacodynamic outcome could be of importance too. We investigated this for mannose binding lectin (MBL); a protein that plays an important role in the innate immune response. Polymorphisms in the *MBL2* gene affect MBL expression and patients with hematological malignancies having genotypes that are related to low MBL levels, have been associated with an increased risk of infections after chemotherapy. However, these results are inconsistent and data on patients with solid tumors are lacking. **Chapter 3** describes the effect of polymorphisms in the *MBL*2 gene on irinotecan-induced febrile neutropenia in 133 patients with solid tumors. Patients with high MBL promoter genotypes and haplotypes had a significantly

higher incidence of neutropenic fever. This could be explained by an increased activation of the complement system in patients with high MBL genotypes, as compared to patients with low MBL genotypes.

Although a substantial part of the administered dose of irinotecan is recovered in urine, little data is available on the association between renal function and the disposition of irinotecan. In **Chapter 4**, the effects of renal function on irinotecan pharmacokinetics and toxicities were investigated in 187 patients treated with single agent irinotecan. The creatinine clearance was calculated by the Cockcroft-Gault equation and patients were categorized according to renal function. No influence on irinotecan pharmacokinetics over the studied range of renal functions was seen. However, categorized renal function was related to hematological toxicity. The incidence of severe neutropenia (grade 3-4) was significantly higher in patients with decreased renal function, particularly in non-smoking patients. It is therefore recommended to take renal function into account in the dosing of irinotecan.

Next to lifestyle factors and comorbidity, the use of concomitant medication that influence metabolizing enzymes and drug transporters may play an important role in affecting drug therapy. It is known that drug-drug interactions are a major source of treatment failure by reducing efficacy and increasing toxicities. In the next chapters of this thesis, the influence of co-medication on the pharmacokinetics and toxicities of irinotecan was investigated.

Chapter 5 describes a drug-drug interaction study between irinotecan and the commonly prescribed proton pump inhibitor omeprazole. The *in vitro* study showed that omeprazole induces the enzyme UGT1A1 and reduces the formation of the inactive irinotecan metabolites, APC and NPC, in the same proportion as the known CYP3A inhibitor fluconazole. In the clinical study, fourteen patients were treated with two cycles of irinotecan, one with and one without omeprazole. *In vivo* no effects of omeprazole on the pharmacokinetics and toxicities of irinotecan were seen. A standard dose of omeprazole can therefore be safely administered in combination with a three-weekly schedule of single agent irinotecan.

In **Chapter 6** we investigated the effect of methimazole on the pharmacokinetics and toxicities of irinotecan in a patient who was treated with four cycles of irinotecan; two with and two without methimazole. Although no effect was seen on the pharmacokinetics of irinotecan itself, SN-38 exposure was higher in the cycles combined with methimazole. Strikingly, SN-38G exposure was almost doubled in the cycles with methimazole, suggesting an induction of UGT1A1, which was confirmed by additional *in vitro* studies. As higher intestinal SN-38G levels can be (re-)activated locally into SN-38, the combination of methimazole and irinotecan may result in increased intestinal toxicity.

Currently many factors are known to contribute to the large interpatient variability in the pharmacokinetics of irinotecan. However, body surface area (BSA)-based dosing

of irinotecan is still largely practised worldwide, despite the knowledge that BSA-based dosing does not result in a reduction of pharmacokinetic variability. To optimize and truly personalize irinotecan treatment, new dosing algorithms which incorporate factors that influence irinotecan pharmacokinetics, should be developed.

Therefore, in **Chapter 7** a new dosing equation based on multivariate analysis of pharmacokinetic data was developed to reduce the interpatient variability in pharmacokinetics and toxicities. All known factors possibly affecting irinotecan clearance were taken into account, including age, height, weight, BSA, baseline blood cell counts, liver and renal function, and genetic variations in the *UGT1A1* gene. The resulting dosing algorithm was based on three patient characteristics: height, baseline γ-glutamyltransferase and CYP3A activity, as determined by the clearance of the CYP3A probe midazolam (**Figure 1**). These three parameters together explained almost 80% of the variability in irinotecan clearance. This is extremely high in comparison to the variability explained by BSA, which is only 20%. The new dosing equation was prospectively compared with regular BSA-based dosing in a randomized study with 40 cancer patients. Although the range of doses administered to the patients in the 'equation-group' (380-1,060 mg) was much wider than in the BSA-group (480-800 mg), dosing with the new equation resulted in a lower interpatient variability of the exposure to irinotecan and SN-38, and a significantly lower incidence of severe neutropenia.
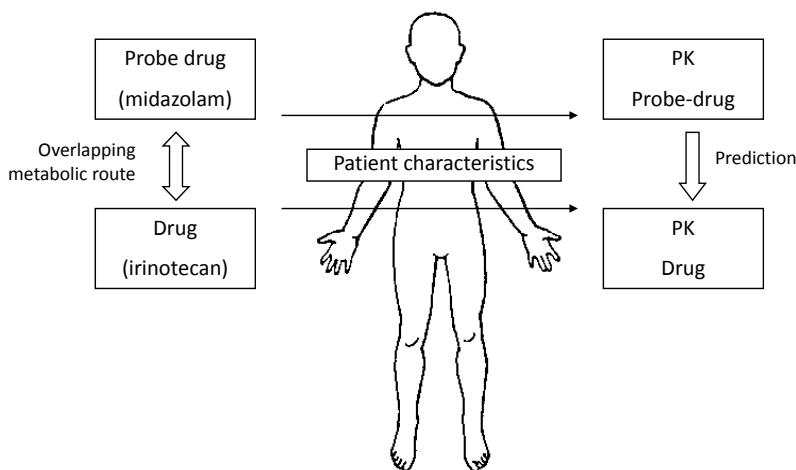
Figure 1. Phenotyping
By determining the pharmacokinetics (PK) of a drug that has an overlapping metabolic route with the drug of interest, one can predict the pharmacokinetics of this drug. It takes into account not only the genetic profile, but also other characteristics of the patient that influence the metabolism of a drug. The drug that is used to predict the pharmacokinetics of the other drug is called a probe drug. Just like irinotecan, midazolam is metabolized by CYP3A. By determining the pharmacokinetics of midazolam in the patient prior to irinotecan infusion one can predict the CYP3A activity of that patient and thereby predict the pharmacokinetics of irinotecan.

CONCLUSION

This thesis introduces new factors that influence irinotecan pharmacokinetics and toxicities. In addition, a new dosing strategy for individualized treatment of irinotecan was proposed on the basis of CYP3A phenotyping. Phenotyping has the potential to be helpful in individualized irinotecan dosing, as it takes into account several patient characteristics that influence the activity of metabolizing enzymes. However, it is quite labor-intensive and requires a good interplay between the clinic and well-equipped laboratories. For this reason, simplified strategies should be developed that require minimal blood sampling. In addition, pharmacokinetic optimization of irinotecan dosing, for instance by using therapeutic drug monitoring, could be an option.

In the era of personalized medicine, there still is some way to go for irinotecan. Although new factors can be added to the toolbox of individualized irinotecan treatment, many undiscovered factors remain. The most important question yet to be answered is how the interplay of all currently known factors relates to treatment outcome of irinotecan. Although it is a generally assumed that outcome is related to drug exposure, solid data on these relationships in the field of chemotherapy are lacking.

Additional small studies will likely not add useful information. To answer the remaining questions on interplay, research groups should collaborate more and exchange databases of irinotecan pharmacokinetic, pharmacodynamic, and pharmacogenetic studies and phase III trials, combine them to create large patient populations, and subsequently perform multivariate and meta-analyses. Finally, pharmacological research should be incorporated in large phase III and phase IV studies, preferably with limited sampling models to investigate and confirm exposure-outcome relationships.

8

Appendix

Samenvatting en conclusie

SAMENVATTING

Hoewel het geneesmiddel irinotecan goed werkzaam is bij de behandeling van uit-gezaaide dikke darmkanker (colorectaal carcinoom), is het impopulair bij sommige internisten-oncologen. Het medicijn veroorzaakt namelijk een aantal onvoorspelbare bijwerkingen, met name diarree en een tekort aan witte bloedcellen (leukopenie en neutropenie). Deze bijwerkingen kunnen zeer ernstig zijn en in enkele gevallen zelfs dodelijk.

Onderzoek naar de werking van geneesmiddelen, oftewel farmacologisch onderzoek, bestaat uit twee verschillende onderdelen: het bestuderen van enerzijds de farmacokinetiek en anderzijds de farmacodynamiek van een medicijn. De farmacokinetiek beschrijft hoe het medicijn door het lichaam wordt verwerkt en bestaat uit vier processen: opname van het geneesmiddel na toediening (absorptie), de verdeling over het lichaam (distributie), de omzetting in andere werkzame of niet-werkzame stoffen (metabolisme) en de uitscheiding van het medicijn via de nieren of darmen (eliminatie). Deze processen tezamen bepalen de blootstelling aan een medicijn gedurende een bepaalde periode. Door het meten van concentraties van het medicijn in het bloed van een patiënt op bepaalde tijdstippen, kan een grafiek worden gemaakt waarin de concentraties worden uitgezet in de tijd. De oppervlakte onder deze tijd-concentratie grafiek noemen we AUC (area under the curve) en is een maat voor de blootstelling aan het medicijn gedurende een bepaalde tijd. De andere tak van de farmacologie, de farmacodynamiek, beschrijft wat het medicijn met het lichaam doet en bestaat uit de werking (effectiviteit) en de bijwerkingen (toxiciteit). Chemotherapeutica (anti-kankermedicijnen) hebben een zogenoemde smalle therapeutische breedte. Dat wil zeggen dat het doseren van het medicijn zeer nauw luistert, omdat de dosis die nodig is om het middel werkzaam te laten zijn en de dosis waarbij bijwerkingen optreden heel dicht bij elkaar liggen. Als er te weinig van het medicijn wordt gegeven en er dus een te lage blootstelling is, is er onvoldoende werking; als er teveel wordt gegeven en er dus een te hoge blootstelling is, kunnen er (ernstige) bijwerkingen optreden.

Jaren van farmacologisch onderzoek hebben het complexe metabolisme van irinotecan grotendeels opgehelderd. Bij het metabolisme van irinotecan zijn verschillende eiwitten betrokken; metaboliserende enzymen die zorgen voor de omzetting van irinotecan in werkzame en niet-werkzame stoffen en transport-eiwitten die zorgen voor de opname en uitscheiding van irinotecan in en door verschillende organen (**Figuur 1**).

Irinotecan is een zogenoemde 'pro-drug', dat wil zeggen dat het in het lichaam moet worden omgezet in een andere stof voordat het werkzaam is. Deze werkzame stof van irinotecan heet SN-38.

Er is veel verschil in de werking en bijwerkingen van irinotecan tussen patiënten. Bij de ene patiënt werkt het medicijn heel goed en zijn er nauwelijks bijwerkingen, bij de andere patiënt werkt het medicijn nauwelijks of zijn er ernstige bijwerkingen. Naast

het onderzoek naar het metabolisme van irinotecan zijn er vele pogingen gedaan om de verschillen tussen patiënten (inter-patiënt variabiliteit) in de farmacokinetiek te verklaren en deze verschillen tussen patiënten te verminderen. Hiermee zou de werkzaamheid van het medicijn worden verbeterd en de bijwerkingen worden verminderd. Ook zijn er verschillende pogingen gedaan om de door irinotecan veroorzaakte diarree rechtstreeks te verminderen in plaats van op een indirecte manier door de verschillen in blootstelling te verminderen. In dit proefschrift zijn verschillende farmacologische aspecten van irinotecan behandeling onderzocht, met als uiteindelijk doel de behandeling met irinotecan te optimaliseren door voor iedere patiënt afzonderlijk een ideale dosering te bepalen die zorgt voor een optimale werking met minimale bijwerkingen.

Hoewel het algemeen bekend is dat roken een rol speelt in het veroorzaken van verschillende vormen van kanker, is er weinig bekend over het effect van roken op de werkzaamheid van een anti-kankerbehandeling met chemotherapie. Dit is opvallend, gezien het feit dat bijna 30% van de kankerpatiënten rookt tijdens de behandeling. Van verscheidene bestanddelen van sigarettenrook is bekend dat ze een invloed hebben op bepaalde metaboliserende enzymen en daarmee de farmacokinetiek van anti-kankermedicijnen mogelijk kunnen beïnvloeden. Door een verandering in de blootstelling aan het medicijn (door een veranderd metabolisme) kunnen er veranderingen optreden in de bijwerkingen en werkzaamheid. In **Hoofdstuk 2** is het effect van roken op de farmacokinetiek en bijwerkingen van irinotecan onderzocht bij 190 kankerpatiënten die werden behandeld met irinotecan. Zesentwintig procent van de patiënten rookte tijdens hun anti-kankerbehandeling met irinotecan. Rokende patiënten hadden een significant lagere blootstelling aan irinotecan en vooral aan de werkzame stof SN-38. De AUC van SN-38 was 40% lager bij de rokers. Daarnaast was het optreden en de ernst van leukopenie en neutropenie (een tekort aan witte bloedcellen) lager bij rokende patiënten. De combinatie van een lagere blootstelling aan irinotecan en de werkzame stof SN-38, en minder ernstige bijwerkingen bij rokers, suggereert dat patiënten die roken tijdens hun irinotecan-behandeling een verhoogd risico hebben op het falen van de behandeling.

Terwijl er slechts weinig onderzoek is gedaan naar de invloed van levensstijl op de werkzaamheid van anti-kankerbehandelingen, is er veel meer aandacht besteed aan de rol van variaties (polymorfismen) in genen die de aanmaak van metaboliserende enzymen en transport-eiwitten reguleren. Echter, variaties in andere genen, zoals genen die de aanmaak reguleren van eiwitten die te maken hebben met farmacodynamische factoren, zouden ook belangrijk kunnen zijn. We hebben dit onderzocht voor mannose-bindend lectine (MBL), een eiwit dat een belangrijke rol speelt in de aangeboren afweer-reactie. Polymorfismen in het *MBL2* gen beïnvloeden de aanmaak van het eiwit MBL. Sommige genotypen (bepaalde genetische variaties) leiden tot hogere concentraties van MBL in het bloed (hoge MBL spiegels) en andere genotypen tot lagere MBL spiegels. Patiënten met kanker van de bloedcellen (hematologische maligniteit) met genotypen
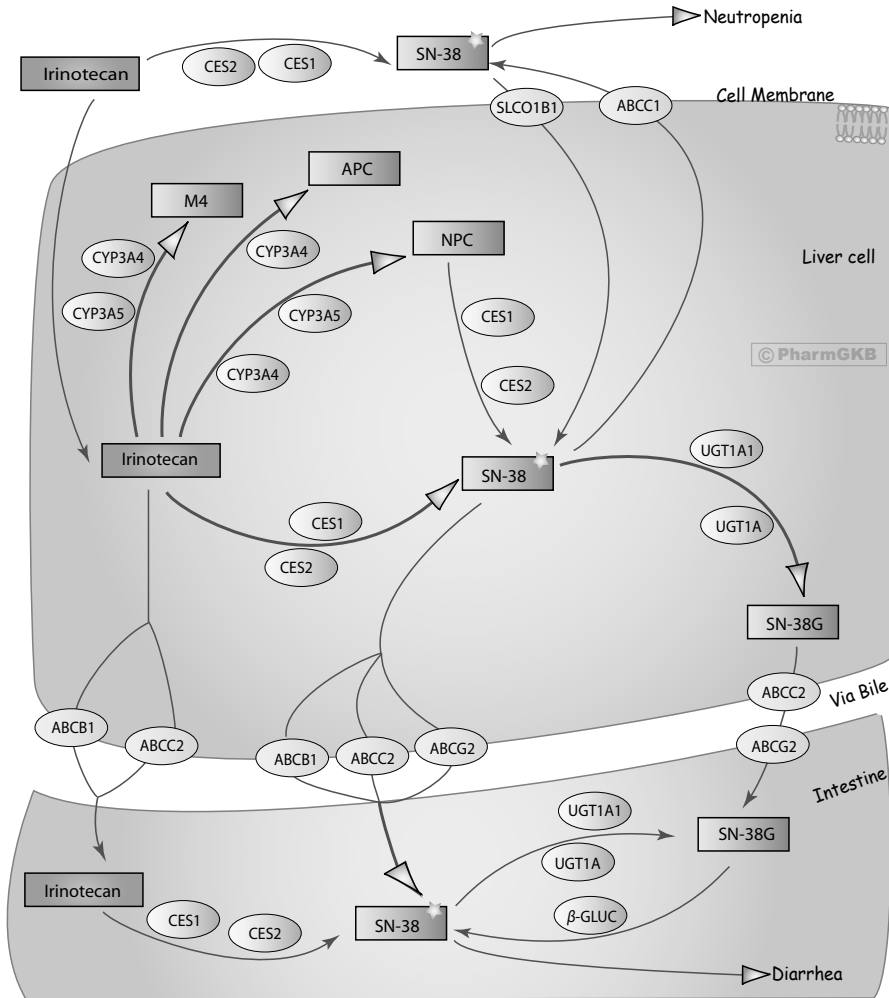
Figuur 1. Het metabolisme van irinotecan

Nadat irinotecan via het infuus is toegediend, wordt het verdeeld over het lichaam. Het wordt omgezet in de werkzame stof SN-38 door eiwitten genaamd carboxylesterases (CES), die voornamelijk aanwezig zijn in de lever, longen en darmen. Naast de omzetting van irinotecan in SN-38 kan irinotecan ook worden omgezet door CYP3A eiwitten in de niet-actieve stoffen APC en NPC. NPC kan vervolgens ook weer door CES worden omgezet in SN-38. SN-38 zelf kan worden geïnactiveerd door UGT1A eiwitten, waarvan UGT1A1 de belangrijkste is. Hierdoor wordt de inactieve stof SN-38G gevormd. Nadat SN-38G via de lever en galwegen is uitgescheiden in de darmen, kan het weer terug omgezet worden in SN-38 door bacteriën die het enzym *β*-glucuronidase (*β*-GLUC*)* maken. Er wordt verondersteld dat dit de oorzaak van de bijwerking diarree is, aangezien SN-38 schadelijk is voor de darmcellen. Naast alle genoemde metaboliserende enzymen, zijn er ook nog verschillende transport-eiwitten betrokken bij de uitscheiding van irinotecan. Deze transport-eiwitten spelen een rol in het opnemen (SLCO transport-eiwitten) en uitscheiden van irinotecan (ABC transport-eiwitten) en haar afbraakproducten in bepaalde organen van het lichaam, zoals de darmen en de nieren.

die zorgen voor lage MBL spiegels hebben mogelijk een verhoogd risico op infecties na chemotherapie. De in de literatuur beschreven resultaten zijn echter tegenstrijdig met elkaar en het is onbekend wat de invloed is van MBL op infecties bij patiënten die behandeld zijn met chemotherapie voor andere vormen van kanker. **Hoofdstuk 3** beschrijft het effect van genetische variaties in het *MBL2* gen op het ontstaan van neutropene koorts (koorts in combinatie met een zeer lage hoeveelheid witte bloedcellen) na irinotecan behandeling bij 133 kankerpatiënten. Patiënten met genotypen die zorgen voor hoge MBL spiegels hadden significant vaker neutropene koorts. Dit zou verklaard kunnen worden door een verhoogde activiteit van het immuunsysteem bij patiënten met genotypen die hoge MBL spiegels tot gevolg hebben, in vergelijking tot patiënten met genotypen die lage MBL spiegels geven.

Terwijl een groot deel van de toegediende dosis van irinotecan via de nieren wordt uitgescheiden in de urine, zijn er weinig gegevens bekend over de relatie tussen nierfunctie en de farmacokinetiek van irinotecan. In **Hoofdstuk 4** is het effect van de werkzaamheid van de nieren (nierfunctie) op de farmacokinetiek en bijwerkingen van irinotecan onderzocht bij 187 kankerpatiënten. De kreatinine klaring (een maat voor de nierfunctie) werd berekend aan de hand van de zogenoemde Cockcroft-Gault formule en patiënten werden in categorieën ingedeeld aan de hand van hun nierfunctie. Er werd in dit onderzoek geen invloed gezien van de nierfunctie op de farmacokinetiek van irinotecan. Echter, patiënten met een verminderde nierfunctie hadden significant vaker en meer ernstige neutropenie. Dit was voornamelijk het geval bij niet-rokende patiënten. Het is daarom te adviseren rekening te houden met de nierfunctie bij het bepalen van de toe te dienen irinotecan dosis.

Naast levensstijl en het voorkomen van andere ziekten (co-morbiditeit), kan het gelijktijdig gebruik van andere medicijnen die een invloed hebben op metaboliserende enzymen en transport-eiwitten een belangrijke rol spelen bij het effect van chemotherapie. Het is bekend dat medicijn-interacties een belangrijke oorzaak zijn van het falen van behandelingen door het veranderen van de blootstelling en daarmee potentieel het verminderen van de werkzaamheid en het vergroten van de bijwerkingen. In de hoofdstukken 5 en 6 van dit proefschrift is de invloed van co-medicatie op de farmacokinetiek en bijwerkingen van irinotecan onderzocht.

Hoofdstuk 5 beschrijft een medicatie-interactie onderzoek tussen irinotecan en de vaak voorgeschreven maagzuurremmer omeprazol. Laboratoriumonderzoek liet zien dat omeprazol zorgt voor een verhoogde aanmaak van het enzym UGT1A1 en dat omeprazol zorgt voor een verminderde vorming van de niet-werkzame afbraakproducten van irinotecan, APC en NPC, door het enzym CYP3A. In het klinische onderzoek werden 14 patiënten behandeld met 2 chemokuren irinotecan, één kuur met en één kuur zonder gelijktijdig gebruik van omeprazol. Er werd geen effect van omeprazol op de farmacoki-

netiek en bijwerkingen van irinotecan gezien. Een standaard dosering omeprazol kan daarom veilig gecombineerd worden met een drie-wekelijkse irinotecan behandeling.

In **Hoofdstuk 6** hebben we het effect van strumazol (een schildklierremmer) op de farmacokinetiek en bijwerkingen van irinotecan onderzocht bij een patiënt die behandeld werd met 4 kuren irinotecan, twee met en twee zonder strumazol. Er werd geen effect gezien van strumazol op de farmacokinetiek van irinotecan. De blootstelling aan de werkzame stof SN-38 was echter hoger in de kuren die gecombineerd waren met strumazol. Opvallend was dat de blootstelling aan SN-38G (het niet-werkzame afbraak-product van SN-38) bijna dubbel zo hoog was in de kuren met strumazol. Dit leek te worden veroorzaakt door een verhoogde aanmaak van het enzym UGT1A1 onder invloed van strumazol (UGT1A1 zorgt voor omzetting van SN-38 in SN-38G). Dit werd bevestigd in het aanvullende laboratoriumonderzoek. Aangezien hogere SN-38G spiegels in de darm lokaal ge(re)activeerd kunnen worden in SN-38, wat schadelijk is voor de darmen, is het mogelijk dat de combinatie van strumazol en irinotecan leidt tot een verhoogde kans op diarree.

Momenteel zijn er vele factoren bekend die bijdragen aan de grote variabiliteit in de farmacokinetiek van irinotecan tussen patiënten onderling (inter-patiënt variabiliteit). Het doseren van irinotecan gebeurt echter wereldwijd nog steeds op basis van de lichaamsoppervlakte (BSA) van een patiënt. Er werd gedacht dat een grotere of dikkere patiënt meer van het medicijn nodig heeft dan een kleinere of dunnere patiënt om hetzelfde effect te bereiken. Dit lijkt logisch, maar voor irinotecan en vele andere chemotherapeutica is inmiddels aangetoond dat doseren op basis van lichaamsoppervlakte de inter-patiënt variabiliteit in de farmacokinetiek niet verkleint en de bijwerkingen niet vermindert en dus geen goede manier is om op 'maat te doseren'. Daarnaast worden de meeste andere medicijnen, bijvoorbeeld paracetamol, ook in een vaste dosering gegeven. Voor irinotecan wordt daarom tegenwoordig geadviseerd om een vaste dosering van 600 mg te geven aan iedere patiënt in plaats van de geregistreerde dosis van 350 mg per m^2 lichaamsoppervlakte. Dit is makkelijker en veiliger omdat er geen berekeningsfouten gemaakt kunnen worden. Het is echter nog steeds géén op maat berekende dosis en het zorgt niet voor minder bijwerkingen.

Om de behandeling van irinotecan te optimaliseren en werkelijk te individualiseren (voor iedere patiënt een op maat berekende dosis op basis van de karakteristieken van die patiënt), dient er een nieuwe manier van doseren ontwikkeld te worden die rekening houdt met de factoren die de farmacokinetiek en bijwerkingen van irinotecan beïnvloeden.

In **Hoofdstuk 7** is een nieuwe doseringsformule ontwikkeld. Alle tot dusver bekende factoren die de farmacokinetiek van irinotecan zouden kunnen beïnvloeden werden meegenomen in de ontwikkeling van deze nieuwe formule, zoals leeftijd, lengte, gewicht, lichaamsoppervlakte, aantal bloedcellen, lever- en nierfunctie en genetische va-

riaties in het *UGT1A1* gen. Genetische variaties in het *UGT1A1* gen, waarvan *UGT1A1*28 de bekendste is, kunnen een invloed hebben op de farmacokinetiek en bijwerkingen van irinotecan, doordat er meer of minder van het eiwit UGT1A1 aangemaakt wordt, dat zorgt voor de afbraak van de werkzame stof SN-38 in de niet-werkzame stof SN-38G. Het resulterende doseringsmodel was gebaseerd op drie patiëntkarakteristieken: lengte, de waarde van γ-glutamyltransferase (een bepaald levereiwit dat iets zegt over de afvloed van gal in de galwegen) en de activiteit van het enzym CYP3A. De activiteit van het eiwit CYP3A werd berekend door middel van het bepalen van de omzetting en uitscheiding (klaring) van het medicijn midazolam, dat ook door CYP3A wordt afgebroken (CYP3A probe). Deze techniek noemen we fenotyperen (**Figuur 2**).

De drie patiëntkenmerken die uit het doseringmodel kwamen verklaarden tezamen bijna 80% van de variabiliteit in de uitscheiding van irinotecan. Dit is zeer hoog in vergelijking met de variabiliteit die verklaard wordt door lichaamsoppervlakte, die slechts ongeveer 20% is. Vervolgens werd de nieuwe doseringsformule vergeleken met doseren op basis van lichaamsoppervlakte in een gerandomiseerd onderzoek met 40 kankerpatiënten. Hoewel de spreiding van de doseringen die gegeven werden aan de patiënten in de formule-groep (380-1060 mg) veel breder was dan die in de lichaamsoppervlakte-
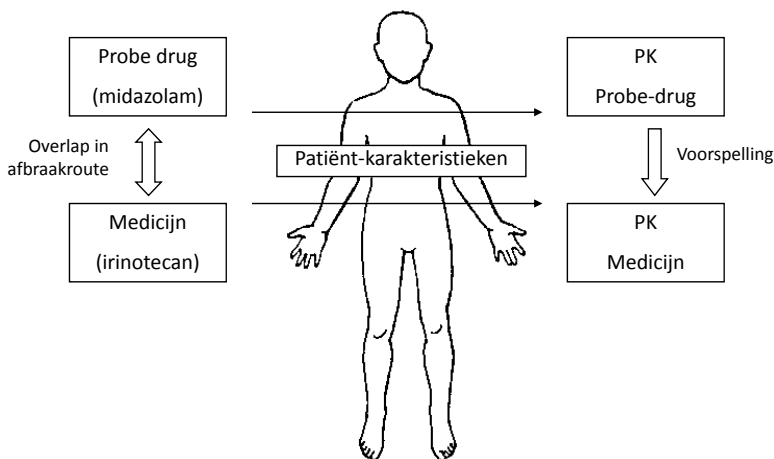
Figuur 2. Fenotyperen

Door middel van het meten van de farmacokinetiek (PK) van een medicijn dat op dezelfde manier wordt afgebroken en uitgescheiden als het medicijn waarin men geïnteresseerd is, kan men een voorspelling doen van de farmacokinetiek van het medicijn van interesse. Het medicijn dat gebruikt wordt om de farmacokinetiek van een ander medicijn te voorspellen, noemt men een 'probe drug'. Deze techniek noemt men fenotyperen. Hierbij wordt rekening gehouden met zowel genetische variaties (genotypering) als andere karakteristieken van de patiënt, die het metabolisme van een medicijn kunnen beïnvloeden. Het slaapmiddel midazolam wordt net als irinotecan afgebroken door het enzym CYP3A. Door voorafgaand aan de irinotecan kuur de farmacokinetiek van midazolam in de patiënt te bepalen, kan een voorspelling gedaan worden over de activiteit van het CYP3A eiwit bij die patiënt, waarmee een voorspelling van de farmacokinetiek van irinotecan kan gedaan worden.

groep (480-800 mg) resulteerde het doseren met de nieuwe doseringsformule in een lagere inter-patiënt variabiliteit in de blootstelling aan irinotecan en de werkzame stof SN-38 en in een significant minder voorkomen van ernstige neutropenie.

CONCLUSIE

Dit proefschrift introduceert nieuwe factoren die de farmacokinetiek en bijwerkingen van irinotecan beïnvloeden. Daarnaast is er een nieuwe doseringsstrategie voor het individualiseren van irinotecan behandeling ontwikkeld. Fenotyperen, oftewel de uitscheiding van een geneesmiddel voorspellen op basis van de uitscheiding van een ander geneesmiddel dat op de zelfde wijze wordt afgebroken en uitgescheiden, kan mogelijk behulpzaam zijn bij het individualiseren van het doseren van irinotecan, aangezien het rekening houdt met verschillende patiëntkarakteristieken die de werking van metaboliserende enzymen beïnvloeden. Het is echter behoorlijk arbeidsintensief en vereist een goede samenwerking tussen de kliniek en de laboratoria waar de metingen worden gedaan. Om deze reden zouden er simpelere strategieën ontwikkeld moeten worden waarbij slechts beperkte, of liever nog géén, bloedbuizen van de patiënt nodig zijn. Een andere optie zou het aanpassen van de dosering op basis van de berekende blootstelling kunnen zijn (het zogenoemde 'therapeutic drug monitoring').

In het huidige tijdperk van 'op maat behandelen' is nog een weg te gaan voor de behandeling met irinotecan. Hoewel nieuwe factoren toegevoegd kunnen worden aan de gereedschapskist van geïndividualiseerde irinotecan behandeling, blijven er nog vele niet-ontdekte factoren. Een belangrijke vraag die nog beantwoord moet worden is hoe de wisselwerking van alle momenteel bekende factoren van invloed is op de werkzaamheid van irinotecan. In het algemeen wordt aangenomen dat de werkzaamheid van chemotherapie gerelateerd is aan de blootstelling aan het medicijn, maar hier zijn geen harde bewijzen voor.

Nieuwe onderzoeken met kleine aantallen patiënten zullen waarschijnlijk geen waardevolle informatie toevoegen. Om uiteindelijk alle overgebleven vragen te kunnen beantwoorden, zullen onderzoeksgroepen meer moeten gaan samenwerken en hun patiëntengegevens uit farmacokinetische, farmacodynamische en farmacogenetische onderzoeken en fase III onderzoeken (waarin de effectiviteit van een geneesmiddel wordt onderzocht) moeten uitwisselen en combineren om grote groepen patiënten te creëren waarin relevante vraagstellingen kunnen worden onderzocht. Bovendien zou farmacologisch onderzoek ingebed moeten worden in fase III onderzoek en fase IV onderzoek (onderzoek dat wordt gedaan nadat het medicijn op de markt is gebracht), bij voorkeur op een manier waarbij een minimaal aantal bloedbuizen nodig is, om de relatie tussen blootstelling aan een medicijn en de werkzaamheid ervan te onderzoeken en te bevestigen.

Appendix

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YES, Irinote**CAN**!

Rotterdam, 20 april 2011.

Appendix

Curriculum Vitae
About the author

CURRICULUM VITAE

Jessica Margaretha van der Bol werd geboren op 27 juli 1981 in Wenen. Op haar vierde verhuisde ze naar Groningen, waar ze in 1999 cum laude haar VWO diploma haalde aan het Belcampo-college. Aansluitend ging ze geneeskunde studeren aan de Rijksuniversiteit Groningen. Tijdens de opleiding was ze betrokken bij de jaarvertegenwoordiging en de co-assistentenraad. Tevens werden extra vakken gevolgd bij de faculteiten Psychologie en Filosofie en had ze verscheidene bijbanen in verpleeg- en verzorgingshuizen.

In 2004 begon Jessica aan haar co-schappen; het eerste jaar in het St. Elisabeth Hospitaal te Curaçao, daarna in het Universitair Medisch Centrum Groningen (UMCG). Deze periode werd afgesloten met een oudste-coschap op de afdeling Interne Geneeskunde (Interne Oncologie en Nefrologie) van het Reinier de Graaf Gasthuis te Delft (supervisoren dr. C.M. Segeren, dr. E. Maartense en prof. dr. E.G.E. de Vries (UMCG)).

In 2006 begon Jessica met haar wetenschappelijke stage op de afdeling Interne Oncologie van het Daniel den Hoed Oncologisch Centrum, Erasmus MC te Rotterdam onder supervisie van dr. F.A. de Jong, prof. dr. J. Verweij en prof. dr. E.G.E. de Vries (UMCG). Datzelfde jaar volgde Jessica de Summer School 'Oncology for Medical Students' van de European Society of Oncology in Ioannina, Griekenland, waar ze de ESO Student Fellowship Award kreeg uitgereikt.

Na het behalen van haar artsexamen in 2006, werd Jessica aangenomen als AGIKO (assistent geneeskundige in opleiding tot klinisch onderzoeker) op de afdeling Interne Oncologie van het Erasmus MC (afdelingshoofd prof. dr. G. Stoter). Onder supervisie van dr. A.H.J Mathijssen en prof. dr. J. Verweij werd gestart met het onderzoek naar de farmacologie van irinotecan beschreven in dit proefschrift. In 2008 kreeg Jessica een Merit Award uitgereikt voor haar mondelinge presentatie op de Annual Meeting van de American Society of Clinical Oncology (ASCO).

Na twee jaar aan haar promotieonderzoek te hebben gewerkt, startte ze in 2009 als AIOS Interne Geneeskunde in het Maasstad Ziekenhuis te Rotterdam (opleiders prof. dr. J.L.C.M. van Saase en dr. M.A. van den Dorpel), waarna zij in het kader van haar AGIKO-constructie in 2010 weer terugkeerde naar het laboratorium Translationele Farmacologie van de afdeling Interne Oncologie in het Erasmus MC.

Tijdens het afronden van haar proefschrift werkte Jessica in verpleeghuis Laurens Stadzicht. Per augustus 2011 zal zij haar opleiding Interne Geneeskunde voortzetten in het Ikazia Ziekenhuis te Rotterdam (opleiders prof. dr. J.L.C.M. van Saase en dr. A.A.M. Zandbergen).

ABOUT THE AUTHOR

Jessica Margaretha van der Bol was born on July 27th 1981 in Vienna (Austria). She moved to Groningen when she was four years old. In 1999, she completed pre-university education at the Belcampo College in Groningen. She graduated with honors and was admitted to the Medical School of the University of Groningen/University Medical Center Groningen (UMCG). During her medical study, she was involved in committees for the improvement of education at the Medical School. She also attended additional courses at the Faculties of Psychology and Philosophy, and worked part time at several retirement and nursing homes.

In 2004, Jessica started her clinical internships. The first year, she worked at the St. Elisabeth Hospital, Curaçao (Dutch Antilles) and the second year at the University Medical Center Groningen (UMCG). This period was finished with a three-month elective internship at the Department of Internal Medicine (Medical Oncology and Nephrology) of the Reinier de Graaf Hospital in Delft (supervisors Dr. C.M. Segeren, Dr. E. Maartense and Prof. Dr. E.G.E. de Vries (UMCG)).

In 2006, Jessica started her scientific internship at the Department of Medical Oncology of the Daniel den Hoed Cancer Center, Erasmus MC in Rotterdam under the supervision of Dr. F.A. de Jong, Prof. Dr. J. Verweij and Prof. Dr. E.G.E. de Vries (UMCG). In the same year, she participated in the Summer School 'Oncology for Medical Students' of the European Society of Oncology in Ioannina (Greece), where she was awarded with the ESO Student Fellowship Award.

After obtaining her medical degree in 2006, Jessica started with a combined residency and PhD-training (AGIKO) at the Department of Medical Oncology of the Erasmus MC (chairman Prof. Dr. G. Stoter). Under the supervision of Dr. A.H.J. Mathijssen and Prof. Dr. J. Verweij, she started with her PhD research on the clinical pharmacology of irinotecan described in this thesis. In 2008, she was awarded with a Merit Award for her oral presentation at the Annual Meeting of the American Society of Clinical Oncology (ASCO).

After working for two years on her PhD thesis, she started her residency in Internal Medicine at the Maasstad Hospital, Rotterdam (supervisors Prof. Dr. J.L.C.M. van Saase and Dr. M.A. van den Dorpel). In 2010, she continued her PhD research at the Laboratory of Translational Pharmacology of the Department of Medical Oncology at the Erasmus MC in Rotterdam.

During the last phase of her PhD studies, Jessica worked at nursing home Laurens Stadzicht. In August 2011, she will continue her residency in Internal Medicine at the Ikazia Hospital in Rotterdam (supervisors Prof. Dr. J.L.C.M. van Saase and Dr. A.A.M. Zandbergen).

Appendix

Publications

ARTICLES

van der Bol JM, Mathijssen RH, Loos WJ, Friberg LE, van Schaik RH, de Jonge MJ, Planting AS, Verweij J, Sparreboom A, de Jong FA. Cigarette smoking and irinotecan treatment: pharmacokinetic interaction and effects on neutropenia. *J Clin Oncol 2007; 25(19): 2719-26.*

Mathijssen RH, de Jong FA, Loos WJ, van der Bol JM, Verweij J, Sparreboom A. Flat-fixed dosing versus body surface area based dosing of anticancer drugs in adults: does it make a difference? *Oncologist 2007; 12(8): 913-23.*

de Jong FA, van der Bol JM, Mathijssen RH, Loos WJ, Mathôt RA, Kitzen JJ, van den Bent MJ, Verweij J. Irinotecan chemotherapy during valproic acid treatment: pharmacokinetic interaction and hepatotoxicity. *Cancer Biol Ther 2007; 6(9): 1368-74.*

de Jong FA, van der Bol JM, Mathijssen RH, van Gelder T, Wiemer E, Sparreboom A, Verweij J. Renal function as a predictor of irinotecan-induced neutropenia. *Clin Pharmacol Ther 2008; 84(2): 254-62.*

van der Bol JM, Mathijssen RH, Creemers GJ, Planting AS, Loos WJ, Wiemer AC, Friberg LE, Verweij J, Sparreboom A, de Jong FA. A CYP3A4 phenotype-based dosing algorithm for individualized treatment of irinotecan. *Clin Cancer Res 2010; 16(2): 736-42.*

van der Bol JM, Visser TJ, Loos WJ, de Jong FA, Wiemer EA, van Aken MO, Planting AS, Schellens JH, Verweij J, Mathijssen RH. Effects of methimazole on the elimination of irinotecan. *Cancer Chemother Pharmacol 2011; 67(1): 231-6.*

van der Bol JM, de Jong FA, van Schaik RH, Sparreboom A, van Fessem MA, van de Geijn FE, van Daele PL, Verweij J, Sleijfer S, Mathijssen RH. Effects of mannose-binding lectin polymorphisms on irinotecan-induced febrile neutropenia. *Oncologist 2010; 15(10): 1063-72.*

Engels FK, Loos WJ, van der Bol JM, de Bruijn P, Mathijssen RH, Verweij J, Mathot RA. Therapeutic drug monitoring for the individualization of docetaxel dosing: a random-ized pharmacokinetic study. *Clin Cancer Res 2011; 17(2): 353-62.*

van der Bol JM, Loos WJ, de Jong FA, van Meerten E, Konings IR, Lam MH, de Bruijn P, Wiemer EA, Verweij J, Mathijssen RH. Effect of omeprazole on the pharmacokinetics and toxicities of irinotecan in cancer patients: A prospective cross-over drug-drug interaction study. *Eur J Cancer 2011; 47(6): 831-8.*

Schiavon G, Eechoute K, Mathijssen RH, de Bruijn P, van der Bol JM, Verweij J, Sleijfer S, Loos WJ. Biliary excretion of imatinib and its active metabolite CGP74588 during severe liver dysfunction. *J Clin Pharmacol 2011. In press.*

ABSTRACTS

de Jong FA, van der Bol JM, Verweij J. Cigarette smoking during irinotecan therapy: Effects on pharmacokinetics and neutropenia. 11th Annual MolMed Day, Erasmus MC Postgraduate School Molecular Medicine; # PIII - 47.

de Jong FA, van der Bol JM, Mathijssen RH, Loos WJ, Friberg LE, van Schaik RH, de Jonge MJ, Planting AS, Sparreboom A, Verweij J. Cigarette smoking during irinotecan therapy: Effects on pharmacokinetics and neutropenia. Proc Am Soc Clin Oncol (ASCO) Annual Meeting 2007; #2506.

van der Bol JM, Mathijssen RH, Verweij J, Planting ASTh, Creemers GM, Sparreboom A, de Jong FA. CYP3A phenotype-based individualized dosing of irinotecan to reduce the Interindividual variability in pharmacokinetics and toxicity: Results from a randomized trial. Proc Am Soc Clin Oncol (ASCO) Annual Meeting 2008; #2506.

van der Bol JM, de Jong FA, van Schaik RH, Sparreboom A, van de Geijn FE, van Fessem MA, Verweij J, Sleijfer S, Mathijssen RH. Effects of mannose-binding lectin polymorphisms on irinotecan chemotherapy-induced febrile neutropenia. Proc Am Soc Clin Pharmacology & Therapeutics (ASCPT) Annual Meeting 2009; OI-C-3.

Mathijssen RH, van der Bol JM, Visser TJ, de Jong FA, van Aken MO, Planting AS, Schellens JH, Verweij J. Effects of methimazole on the pharmacokinetics of irinotecan chemo-therapy. Meeting Dutch Society of Clinical Pharmacology & Biopharmacy (NVKF&B), March 2009.

van der Bol JM, Verweij J, de Jong FA, Loos WJ, Lam MH, van Meerten E, Mathijssen RH. Effects of omeprazole on the pharmacokinetics and toxicies of irinotecan in cancer patients: a prospective open-label cross-over drug-interaction study. Proc Am Soc Clin Oncol (ASCO) Annual Meeting 2009; #2502.

van der Bol JM, Verweij J, de Jong FA, Loos WJ, Lam MH, van Meerten E, Mathijssen RH. Effects of omeprazole on the pharmacokinetics and toxicies of irinotecan in cancer patients. Meeting Dutch Society of Clinical Pharmacology & Biopharmacy (NVKF&B), October 2009.

ORAL PRESENTATIONS

<u>van der Bol JM</u>
CYP3A phenotype-based individualized dosing of irinotecan to reduce the interindividual variability in pharmacokinetics and toxicity: Results from a randomized trial.
ASCO Annual Meeting 2008, Chicago, IL, June 2nd 2008.

<u>van der Bol JM</u>
Effects of mannose-binding lectin polymorphisms on irinotecan chemotherapy-induced febrile neutropenia.
ASCPT Annual Meeting 2009, Washington DC, March 19th 2009.

<u>van der Bol JM</u>
Effects of omeprazole on the pharmacokinetics and toxicties of irinotecan in cancer patients: a prospective open-label cross-over drug-interaction study.
ASCO Annual Meeting 2009, Orlando, FL, May 31st 2009.

Appendix

PhD portfolio

PHD PORTFOLIO

PhD training	Year	Workload	
		Hours	ECTS
General academic skills and research skills			
Classical Methods for Data Analysis, NIHES[a]	2007		6
Biomedical English Writing and Communication, Erasmus MC	2008		4
Basiscursus Regelgeving en Organisatie van Klinisch onderzoek (Good Clinical Practise), CPO[b]	2008		1
Methodology of Patient Orientated Research, CPO[b]	2008	8	
Medical Library Courses, Erasmus MC	2008	8	
In-depth courses			
Molecular Diagnostics, MolMed[c]	2007	16	
Acute Life Support, Care Training Group	2009	16	
Palliative Care in the Hospital, Leerhuis Palliatieve Zorg	2009	16	
Clinical Pharmacology Curriculum Review Course, ASCPT[d]	2009	8	
Communicating about Donation, NTS[e]	2009	16	
Research in Clinical Pharmacology, NVKF&B[f]	2010	16	
Oral presentations			
JNI Scientific Lab Meeting, Erasmus MC	2007	8	
Research Meeting Dept of Medical Oncology, Erasmus MC	2007+2008	16	
Annual Meeting ASCO[g]	2008+2009		2
Annual Meeting ASCPT[d]	2009		1
Conferences			
Molecular Medicine Day, MolMed[c]	2007	8	
Annual Meeting NVMO[h]	2008	16	
Annual Meeting IKR[i]	2008	20	
Annual Meeting ASCO[g]	2008+2009		2
Annual Meeting ASCPT[d]	2009		1
Seminars and workshops			
Talent Day, NWO[j]	2007	8	
Get-Out-of-your-Lab Days, MolMed[c]	2007	16	
Journal Club Pharmacogenetics, Erasmus MC	2007+2008		1
Phase I Meetings Dept of Medical Oncology, Erasmus MC	2007+2008		1
Multi-disciplinary Treatment in Oncology Courses, Erasmus MC	2007+2008		1
Research Meetings Dept of Medical Oncology, Erasmus MC	2007+2008+2010		1
PhD day, Erasmus MC	2008+2010	8	

PhD training	Year	Workload	
		Hours	**ECTS**
Translational Pharmacology Research Meetings, Dept of Medical Oncology, Erasmus MC	2010		2
Didactic skills			
Staff Training Clinical Research Unit, Dept of Medical Oncology, Erasmus MC	2007+2008		1
Supervising and teaching Interns, Maasstad Ziekenhuis	2009		1
Awards			
Merit Award ASCO[g]			
TOTAL		**204**	**25**

[a] Netherlands Institute for Health Sciences (NIHES)

[b] Consultation center for Patient Oriented research (CPO)

[c] Molecular Medicine Postgraduate School (MolMed)

[d] American Society for Clinical Pharmacology and Therapeutics (ASCPT)

[e] Dutch Transplant Foundation, Nederlandse Transplantatie Stichting (NTS)

[f] Dutch Society of Clinical Pharmacology & Biopharmacy, Nederlandse Vereniging voor Klinische Farmacologie & Biofarmacie (NVKF&B)

[g] American Society of Clinical Oncology (ASCO)

[h] Dutch Society for Medical Oncology, Nederlandse Vereniging voor Medische Oncologie (NVMO)

[i] Comprehensive Cancer Center Rotterdam, Integraal Kankercentrum Rotterdam (IKR)

[j] Netherlands Organisation for Scientific Research, Nederlandse Organisatie voor Wetenschappelijk Onderzoek (NWO)