Personalized Medicine in Cancer of the Gastro-Intestinal Tract:

A pharmacokinetic and pharmacogenetic approach

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Gepersonaliseerde behandeling bij kanker van de tractus digestivus:
Een farmacokinetische en farmacogenetische benadering

Proefschrift

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Binnen de perken zijn de mogelijkheden even onbeperkt als daarbuiten
Jules Deelder

CONTENT

1

GENERAL INTRODUCTION AND OUTLINE OF THE THESIS

PERSONALIZED ANTI-CANCER TREATMENT

This thesis describes pharmacokinetic and pharmacogenetic studies on four anti-cancer drugs that are commonly used in the systemic treatment of cancer in the gastrointestinal tract. The overarching aim of these studies is to truly 'personalize' anti-cancer treatment in daily clinical practice. Personalized medicine attempts to identify the right treatment, at the right time, and in the right dose for each individual patient. In oncology, this could be based on different aspects of the tumor itself, but patient characteristics are at least equally important and therefore the latter aspect will be emphasized in this thesis.

For most chemotherapeutic agents it is assumed that systemic exposure (expressed as pharmacokinetic parameters like area under the time concentration curve and drug clearance), is correlated with treatment response, resulting in a therapeutic window balancing between toxicity on one hand and undertreatment on the other.[1] In general, there is a large inter-individual variety in treatment efficacy and drug-related toxicity, which could be related to differences in systemic exposure (amongst other factors). Systemic exposure to anticancer drugs is determined by many patient-related factors such as patient characteristics (e.g. gender, age, size), organ function, life-style (e.g. smoking, us of certain foods and alternative agents), co-medication, and genetic factors concerning drug transporters or metabolizing enzymes (i.e. pharmacogenetics).[1] Ideally, all these factors should be incorporated in novel dosing strategies to reach a personalized dose for every patient. Although recent advances have been made, this is still far from daily clinical care, unfortunately.

Traditionally, dosing of chemotherapy is based on the patient's body surface area (BSA). This dosing-strategy aims to minimize inter-individual variability in exposure as a result of differences in body composition, thereby trying to achieve more similar exposure across patients, resulting in a maximal efficacy and limited toxicity.[2] However, many researchers have concluded that for the majority of anticancer agents there is no clear relationship between individual exposure and a BSA-based dose.[3-9] In fact, this is not surprising since there is no solid ground for several BSA-formula's proposed. From the first equations to quantify human body surface by Marcus Vitruvius Pollio (85-20 BC) till the Mosteller derivative used nowadays, they all represent a huge oversimplification of the human body and should only be used to adjust dosing if BSA is actually demonstrated to influence the inter-individual pharmacokinetic variability.[7, 10] This is also the case for the newer (oral) agents which are usually flat dosed, or dosed on kg weight.[7]

Therefore, it is important to investigate which other factors influence exposure and treatment effect of a specific drug and adjust dosing recommendations based on that knowledge to come to a true personalized dose.

PART I: FLUOROPYRIMIDINES

Fluoropyrimidines are a group of classic chemotherapeutic agents including the intravenous administered 5-fluorouracil (5-FU) and orally administered capecitabine and tegafur, which act as pro-drugs for 5-FU. Fluoropyrimidines are widely used in the treatment of colorectal, pancreatic, gastric and breast cancer amongst other solid tumor types. Capecitabine is nowadays more favored than 5-FU, as it has equal effectiveness and is more user friendly compared to 5-FU, resulting from its oral formulation. Depending on the different types of treatment regimens, capecitabine is given either as monotherapy, in combination with other chemotherapeutic agents, or it is combined with radiotherapy. Like other chemotherapeutic agents, capecitabine is registered in a BSA-based dose to reduce inter-individual variability in its pharmacokinetics. However, Baker et al. demonstrated that inter-individual variability in the clearance of capecitabine, expressed as coefficient of variation, is even increased from 31.3% to 36.5% when BSA was taken into account. Therefore, the rationale to use a BSA-based dosing strategy for capecitabine is not valid.[6] An alternative dosing strategy could be fixed-dosing, which means that the dose is not adjusted for body size, and every patient receives the same dose despite one's body size measures. In **Chapter 2** the safety and effectiveness of a fixed-dosed dose of capecitabine is described in a large cohort of patients with different tumor types, and compared with BSA-based dosed patients in a comparable cohort of patients.

Fluoropyrimidines are mainly metabolized by the enzyme dihydopyrimidine dehydrogenase (DPD) which converts more than 80% of 5-FU into the inactive metabolite dihydrofluorouracil, which is converted further into other inactive metabolites and eventually excreted via urine. Around 20% of 5-FU is also directly excreted via urine. As a result, only a small proportion (1 to 5%) of 5-FU is converted into the active metabolites fluorodeoxyuridine monophosphate (FdUMP), fluorodeoxyuridine triphosphate (FdUTP) and fluorouridine triphosphate (FUTP). The cytotoxic effects of 5-FU result from the inhibition of the enzyme thymidylate synthetase by FdUMP, and the incorporation of FdUTP in DNA and FUTP in RNA.[11] However, when DPD activity is reduced, 5-FU clearance is significantly reduced and the amount of 5-FU which is converted in these active metabolites will increase, followed by a largely increased risk of severe

or even fatal treatment related toxicity.[12, 13] A reduced DPD activity can be the result of polymorphisms in the *DPYD* gene, which encodes for the DPD enzyme. *DPYD* is a large gene with many variants described, which not all have functional consequences. Based on a meta-analysis by Meulendijks et al., four DPYD variants are considered clinically relevant (*DPYD**2A, c.1679T>G, c.2846A>T, and c.1236G>A) in Caucasian patients.[14] It has already been demonstrated that prospective genotyping for the *DPYD**2A variant and dose-reductions in heterozygote *DPYD**2A carriers improves treatment safety and is cost-effective.[15] However, for the other variants this is currently unknown. In **Chapter 3** the results of a large prospective trial, performed in 17 Dutch centers, on personalized fluoropyrimidine dosing based on these four *DPYD* variants are described. Furthermore, a cost-analysis of this study cohort is described in **Chapter 4**.

In addition to *DPYD* genotyping, DPD deficiency can also be identified using different phenotyping tests that measure the DPD activity (in)directly. Several phenotyping methods are currently described, of which DPD activity measurement in peripheral blood monocytes is the most direct one.[16] More indirect phenotyping tests are related to the measurement of the endogenous DPD substrate uracil or its product dihydrouracil.[16] In **Chapter 5**, different phenotyping tests are prospectively evaluated for their additional value to identify DPD deficiency and patients at risk for severe fluoropyrimidine-induced toxicity. Finally, although it is rare, one patient can carry multiple *DPYD* variants (i.e. homozygote or compound heterozygote variant carriers), which makes it difficult to predict the DPD enzyme activity and the optimal dose. In **Chapter 6**, several cases of patients with multiple variants and their phenotyping results, who were treated with personalized fluoropyrimidine treatment are described.

PART II: IRINOTECAN

Since the clinical introduction in 1998, the camptothecin derivative irinotecan is widely used in the treatment of solid tumors including colorectal and pancreatic cancer.[17] Irinotecan belongs to the class of topoisomerase-I inhibitors; inhibition of this enzyme involved in DNA replication induces DNA damage and eventually cell death.[18] Irinotecan is a prodrug of SN-38, which is 100-1,000 fold more active compared to irinotecan itself.[19] Several phase I and phase II enzymes including CYP3A4 and UGT1A, are involved in the highly complex irinotecan metabolism which makes it prone to environmental and genetic influences.[17] These factors will partly explain the large inter-individual variability in irinotecan pharmacokinetics. Although many years of research gave more insight in these factors, the full story about differences in

irinotecan exposure is not yet unraveled. In **Chapter 7** an overview of current evidence on irinotecan pharmacokinetics, pharmacodynamics, and pharmacogenetics is given. Irinotecan treatment is characterized by several dose-limiting toxicities such as severe neutropenia and diarrhea in up to a quarter of patients.[20, 21] Several interventions to reduce treatment related toxicities have been investigated including dietary adjustments. Preclinical studies in animals have demonstrated that by fasting before irinotecan treatment, toxicity can be reduced while preserving the anti-tumor effects.[22, 23] After 72 hours of fasting, mice experienced significantly less side effects of irinotecan chemotherapy, intra-tumoral SN-38 concentrations tended to be higher, and concentrations in both plasma and healthy liver were significantly lower.[23] The mechanisms behind the protective effects of dietary restriction are not completely understood and are actively being studied. One of the theories about the difference in response to dietary restriction between cancer and normal cells is that by starvation in normal cells the growth factor pathways (i.e. AKT, RAS, proto-oncogenes) can be down regulated as response to reduction in growth factors such as IGF-I, in contrast to cancer cells where oncogenic mutations will lead to continuous growth in the absence of growth factors.[24] However, it is currently unknown if fasting or short-term dietary restriction might help to reduce toxicity in patients treated with chemotherapy. A small case-series of 10 patients who had voluntarily fasted prior and/or after different chemotherapy regimens suggested that subjective wellbeing was improved without affecting the anti-cancer effects.[25] Furthermore, a randomized pilot study in 13 patients demonstrated that 24 hours fasting before chemotherapy resulted in reduced hematological toxicity.[26] Based on the preclinical evidence, we hypothesized that fasting might help to reduce toxicity in patients treated with irinotecan, due to lower irinotecan concentrations in healthy tissues and plasma, while preserving its intra-tumoral concentrations. Therefore, in a prospective pharmacokinetic crossover study we studied the effects of a short-term dietary restriction regimen in cancer patients with liver metastases treated with irinotecan as described in **Chapter 8**.

PART III: REGORAFENIB

Compared to the other (chemotherapeutic) agents in this thesis, regorafenib is a relatively new drug and not a classic chemotherapeutic agent. Regorafenib is an oral multi-kinase inhibitor that targets angiogenic, stromal and oncogenic receptor tyrosine kinases (e.g. VEGFR, KIT, BRAF, PDGFR and FGFR).[27] Regorafenib is currently worldwide used in the treatment of colorectal cancer (except for the Netherlands), gastro-intestinal stromal tumors, and hepatocellular carcinoma.[28-30] After oral administration,

regorafenib is rapidly absorbed, with a maximum concentration reached at 3-4 hours.[31, 32] Most tyrosine kinase inhibitors (TKIs) exhibit pH-dependent solubility, which makes them prone for drug-drug interactions with acid suppressive agents like proton pump inhibitors.[33] Up to one third of all cancer patients concomitantly also uses acid-suppressive therapy, both as prophylaxis for gastro-intestinal bleeding and as treatment for gastresophageal reflux disease.[34, 35] For many TKIs, a pharmacokinetic interaction with an acid-suppressive agent has already been demonstrated, for example erlotinib combined with omeprazole resulted in 46% decrease in systemic exposure.[33] Those drug-drug interactions could have serious clinical consequences, because when exposure is decreased, treatment efficacy could potentially decrease also.[31] However, for regorafenib there is no evidence of a possible drug-drug interaction with acid-reducing drugs. Therefore, in **Chapter 9** we describe a prospective cross-over study on the potential pharmacokinetic interaction between the proton pump inhibitor esomeprazole and regorafenib, with special interest in the influence of timing of esomeprazole intake relative to that of regorafenib.

Furthermore, in more than half of all patients, treatment with regorafenib is associated with severe and dose-limiting toxicities such as hypertension and hand foot skin reactions which not always outweigh treatment benefit.[36] Therefore, there is an urgent need for biomarkers predictive for response to identify specific patients who will, and who will not, benefit from regorafenib treatment. Multiple studies demonstrated that the detection of circulating tumor DNA (ctDNA) could be a powerful tool to monitor and understand the response to anti-cancer agents.[37] In metastatic colorectal cancer patients, high amounts of circulating cell free DNA (cfDNA) and ctDNA before initiation of treatment are correlated with shorter overall survival.[38] However, most of these studies only measured cfDNA and ctDNA at baseline and not during treatment. When looking at changes in the amount of ctDNA during treatment, two small studies demonstrated that an early and sustained decline in ctDNA during regorafenib treatment is correlated with an improved survival.[39, 40] Furthermore, an increase in ctDNA concentration after 14 days of regorafenib treatment is associated with a significantly decreased median progression free survival and overall survival.[41] We hypothesized that dynamic changes in cfDNA/ctDNA early during the treatment with regorafenib may be related to drug exposure and toxicity. In **Chapter 10** we describe an explorative analysis on early cfDNA/ctDNA dynamic changes and correlation with regorafenib pharmacodynamics in metastatic colorectal patients.

PART IV: CARBOPLATIN / PACLITAXEL

The combination of carboplatin and paclitaxel is used as treatment regimen in several solid cancers including esophageal, ovarium, and lung cancer. In this thesis, treatment with carboplatin and paclitaxel (with or without radiotherapy) is only discussed in relation to (gastro)esophageal cancer. Esophageal cancer is the 8th most common cancer worldwide, the incidence is still rising and mortality is high.[42-44] Although the prognosis of esophageal cancer has improved over the last decades, prognosis still remains poor with an overall 5-year survival of 20%.[43, 45] This survival improvement seems to be limited to patients with early stage (gastro)esophageal cancer, which might be caused by the recent advances in the treatment of resectable (gastro)esophageal cancer by introduction of neoadjuvant chemoradiotherapy according to the CROSS-regimen.[46, 47] However, almost half of all patients have already non-resectable (gastro) esophageal cancer at diagnosis (i.e. locally advanced tumors or distant metastasis).[48] For patients with locally advanced disease, systemic treatment can be considered in an attempt to downstage the tumor (i.e. induction treatment), which can be followed by surgery or chemoradiotherapy in case of a good response. For patients with distant metastases, palliative chemotherapy can be considered. Palliative systemic treatment improves survival compared to best supportive care, although survival benefit is limited and toxicity of treatment should not outweigh the treatment benefit.[45, 49] Many different induction or palliative treatment regimens are described, which are often fluoropyrimidine- or platinum-based doublet or triplet combination regimens.[45, 49, 50] A study of the Netherlands Cancer Registry demonstrated that already in the Netherlands up to 69 different palliative treatment regimens are administered in metastatic (gastro) esophageal cancer patients.[51] This clearly demonstrates that optimal palliative treatment in esophageal cancer is not well defined, and the same is probably true for induction chemotherapy.

Fifteen years ago, our research group performed a phase-1 study of weekly paclitaxel and carboplatin as palliative treatment for patients with metastatic esophageal cancer resulting in a recommended dose of carboplatin targeted at an area under the curve (AUC) of 4 and paclitaxel of 100 mg/m^2.[52] This weekly regimen was very tolerable and effective with an over-all response rate of 54%. Therefore, this regimen was implemented as standard of care for all patients with advanced or metastatic (gastro)esophageal cancer at the Erasmus University Medical Center, Rotterdam, The Netherlands. **Chapter 11** describes the efficacy and toxicity of this weekly carboplatin and paclitaxel regimen as induction or palliative treatment option in a real-world treatment setting.

Paclitaxel is also characterized by a large inter-individual variability in exposure, which will be in part explained by patient related factors.[53] Although a dose-response relation has been suggested for paclitaxel, it is currently unknown if treatment outcome is related to the variation in paclitaxel exposure.[1, 53] Therefore, in **Chapter 12** the association between systemic paclitaxel exposure and treatment outcome in patients with esophageal cancer was described. This was done in patients in several clinical settings (e.g. neo-adjuvant chemoradiotherapy, induction and palliative chemotherapy).

Hopefully the readers of this thesis will appreciate the clinical studies that are reported. It is my wish that the results of these studies will further contribute to personalized medicine in patients treated for cancer of the gastro-intestinal tract, and that this may boost efficacy and reduce toxicity.

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PART I

FLUOROPYRIMIDINES

2

COMPARISON OF TOXICITY AND EFFECTIVENESS BETWEEN FIXED-DOSE AND BODY SURFACE AREA-BASED DOSE CAPECITABINE

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Femke M. de Man, G.D. Marijn Veerman, Esther Oomen-de Hoop, Maarten J. Deenen, Didier Meulendijks, Caroline M.P.W. Mandigers, Marcel Soesan, Jan H.M. Schellens, Esther van Meerten, Teun van Gelder, and Ron H.J. Mathijssen

ABSTRACT

BACKGROUND

Capecitabine is generally dosed based on body-surface area (BSA). This dosing strategy has several limitations; however, evidence for alternative strategies is lacking. Therefore, we analyzed the toxicity and effectiveness of fixed-dose capecitabine and compared this strategy with BSA-based dose of capecitabine in a large set of patients.

METHODS

Patients treated with fixed-dose capecitabine between 2003 and 2015 were studied. A comparable group of patients, dosed based on BSA, was chosen as a control cohort. A total of two combined scores were used: capecitabine-specific toxicity (diarrhea, National Cancer Institute Common Toxicity Criteria grade ≥3, hand-foot syndrome ≥2, or neutropenia ≥2), and clinically relevant events due to toxicity, that is, hospital admission, dose reduction, or discontinuation. Per treatment regimen, patients were divided into three BSA groups based on BSA quartiles corrected for sex. Toxicity scores were compared by a Chi-square test between cohorts, and within cohorts using BSA groups. Progression-free survival (PFS) was estimated by the Kaplan-Meier method.

RESULTS

A total of 2,319 patients was included (fixed dosed n=1,126 and BSA-based dose n=1,193). Overall, four regimens were evaluated: capecitabine-radiotherapy (n=1,178), capecitabine-oxaliplatin (n=519), capecitabine-triplet (n=181) and capecitabine monotherapy (n=441). The incidence of capecitabine-specific toxicity and clinically relevant events was comparable between fixed-dose and BSA-based dose patients, while a small difference (7.1%) in absolute dose was found. Both cohorts showed only a higher incidence of both toxicity scores in the lowest BSA group of the capecitabine-radiotherapy group ($P<0.05$). Subgroups of the fixed-dose cohort analyzed for PFS, showed no differences between BSA groups.

CONCLUSIONS

Fixed-dose capecitabine is comparably well tolerated and effective as BSA-based dosing and could be considered as a reasonable alternative for BSA-based dosing.

INTRODUCTION

Capecitabine is an oral prodrug for the cytotoxic agent 5-fluorouracil (5-FU) and is widely used in the treatment of colorectal cancer and other solid tumors (e.g. breast and gastric cancer).[1-4] Capecitabine has equal effectiveness and shows, in general, a more favorable toxicity profile compared with intravenous 5-FU, except for the incidence of hand-foot-syndrome (HFS).[5] Depending on the different types of treatment regimens, capecitabine is given either as monotherapy, in combination with other cytotoxic agents, or it is combined with radiotherapy. Worldwide, dosing of capecitabine for the individual patient is based on the patient's body surface area (BSA). However, both effectiveness and toxicity depend on the individual exposure to capecitabine and therefore the rationale for dosing based on solely height and weight has been questioned for decades.[6-13]

BSA-guided dosing of anticancer agents aims to minimize inter-individual variability in exposure as a result of differences in body composition, thereby trying to achieve more similar exposure across patients, resulting in a maximal effect and limited toxicity.[14] However, this dosing strategy has several drawbacks. Firstly, there is limited evidence for the base of the BSA-formula since the first formula to calculate BSA (by Du Bois and Du Bois, more than a century ago) was based on only nine individuals.[15] Still, it forms the backbone for all (other) BSA-formulas, of which the Mosteller derivative is currently the most frequently used.[16] Secondly, BSA-dosing encounters increased costs and a larger chance of calculation errors compared with fixed dosing.[6] Thirdly and most importantly, although BSA dosing was intended to reduce the inter-individual variability in drug exposure, many researchers have concluded that for the majority of anticancer agents there is no clear relationship between an individual's exposure and a BSA-based dose.[7-13] Indeed, Baker et al. demonstrated by modeling that inter-individual variability in clearance of capecitabine expressed as coefficient of variation (CV) was even increased when BSA was taken into account (31.3% versus 36.5%).[10] In other words, there is fair skepticism regarding the question whether this dosing strategy really contributes to reducing inter-individual pharmacokinetic and consequent pharmacodynamic variability of anticancer agents.[11, 13]

BSA-based dosing is for many anticancer agents not evidence based, and especially for frequently used drugs such as capecitabine, there is a need for alternative dosing strategies to standardize the dose.[17] Fixed dosing means that the dose is not adjusted for body size, so that every adult patient with the same malignancy receives the same (fixed) dose. A major benefit of dose standardization by fixed dosing is that it will lead to less prescribing errors and a reduction in preparation and storage costs.[18-20] Fixed

dosing is already implemented in the majority of newly developed oral anticancer drugs.[21] However, unless there is more evidence that a fixed dose can safely be applied without compromising effectiveness, then conventional chemotherapy regimens will remain to be dosed based on BSA according to the registration studies, even though BSA-guided dosing is in many cases not evidence based.

To the best of our knowledge, there are no published studies with a sufficiently large sample size evaluating the outcomes of a fixed dose of capecitabine. In 2003, the Erasmus University Medical Center, Rotterdam, the Netherlands, implemented a fixed dose of capecitabine in different treatment regimens, as there was no evidence that BSA-based dosing was better. This resulted in a unique 'real-life' cohort of patients treated with a fixed dose of capecitabine, with a long follow-up period. Therefore, the aim of our present study was to evaluate the toxicity and effectiveness of fixed-dose capecitabine in several treatment schedules in this cohort of patients. Additionally, we compared this cohort with another large cohort of Dutch cancer patients, in which patients were dosed based on BSA and treated in the same time period, in order to determine whether fixed-dose capecitabine is as equally well tolerated and effective as BSA-based dosing of capecitabine.

METHODS

The cohorts for this analysis were obtained from the Erasmus University Medical Center, Rotterdam, The Netherlands for the fixed-dose cohort, and from the Netherlands Cancer Institute, Antoni van Leeuwenhoek, Amsterdam; Slotervaart Hospital, Amsterdam; and Canisius Wilhelmina Hospital, Nijmegen, all in the Netherlands for the BSA-based dose cohort, respectively.

The primary study endpoint was the incidence of treatment-related toxicity in a fixed-dose cohort compared with a BSA-based dose cohort. Secondary endpoints included the comparison of the absolute amount of capecitabine administered in the fixed-dose cohort compared to BSA-dosing strategies, incidence of toxicity between BSA groups within both cohorts, and the effectiveness of fixed-dose capecitabine compared between BSA groups in terms of disease-free survival (DFS) in (neo)adjuvant care and progression-free survival (PFS) in palliative care.

PATIENTS AND TREATMENTS

All patients treated with capecitabine between 2003 and 2015 at the Erasmus University

Medical Center were identified by the hospital pharmacy based on drug-dispensing data and evaluated for inclusion in the fixed-dose cohort. As fixed-dose capecitabine is considered routine clinical care at the Erasmus University Medical Center, no ethics approval or informed consent was required to retrospectively collect and analyze these patient data for research purposes. Patients in the BSA-based dose cohort were prospectively included in a previously conducted trial in three large hospitals in the Netherlands (ClinicalTrials.gov identifier: NCT00838370), this trial was approved by the medical ethical committees of all participating hospitals.[22] All patients of the BSA-based dose cohort provided informed consent for the prospective trial, including consent for additional analyses outside the subject of this trial. Patients were excluded from both cohorts when they had a World Health Organization (WHO) performance status of 3 or 4, when they were previously treated with fluoropyrimidines, when they were treated in an experimental treatment setting outside standard-of care; or when limited data on important parameters required for the current analysis were available (i.e. length, weight, toxicity evaluation).

In both cohorts, patients were divided into four groups based on treatment regimen: capecitabine monotherapy (CAPE MONO), capecitabine combined with radiotherapy (CAPE+RT), capecitabine combined with oxaliplatin (CAPOX), and capecitabine triplet therapy (CAPE TRIPLET). The CAPE TRIPLET group consisted of capecitabine combined with epirubicin and cisplatin or oxaliplatin (ECC/EOX) in both cohorts, and in the BSA-based dose cohort also patients treated with capecitabine with docetaxel and oxaliplatin (DOC) were included. Capecitabine was administered as fixed daily dose (divided over two doses daily) of 3,000 mg for CAPE+RT; 3,500 mg for CAPOX and ECC/EOX; 3,500 mg or 4,000 mg for CAPE MONO. Detailed descriptions of included treatment types are given in **Supplementary Table 1**.

DATA

All data for the fixed-dose cohort were retrospectively collected from the electronic health records. For the BSA-based dose cohort all data was prospectively collected in a previously conducted trial by Deenen et al [22]. Data on patient demographics (i.e. length, weight, WHO performance status) had to be known within one month before the start of capecitabine. BSA was calculated per patient using the Mosteller formula [16]. Renal function was expressed as estimated glomerular filtration rate (eGFR). For the fixed-dose cohort the eGFR was calculated according to the Cockcroft Gault formula[23], and for the BSA-based dose cohort according to the MDRD-formula.[24] Toxicity was defined as all possible capecitabine-related adverse events and laboratory abnormalities occurring during treatment with capecitabine until one month after end of treatment or until the start of a new treatment, whichever occurred first. Toxicity was

graded according to the Common Terminology Criteria for Adverse Events (CTC-AE) version 4.03.[25] Overall, two combined scores were created to evaluate severe toxicity and clinically relevant events due to toxicity. Capecitabine-specific toxicity was defined as toxicity grade for diarrhea ≥ 3, HFS ≥ 2, or neutropenia ≥ 2. Clinically relevant events consisted of hospital admission, dose reduction, or discontinuation caused by possible capecitabine-related adverse events. Data on DFS in (neo)adjuvant treated patients or PFS in palliative treated patients was collected to asses effectiveness of fixed-dose capecitabine. DFS was defined as time till disease recurrence. PFS was defined as time till disease progression or death from any cause. Disease recurrence or progression had to be pathologically proven or by imaging evaluated according to the response evaluation criteria in solid tumors (RECIST) 1.1.[26]

STATISTICAL ANALYSIS

Demographic characteristics were compared between the two cohorts by using the Chi-square test for categorical variables, and an unpaired T-test or Mann-Whitney U test for continuous variables. Per treatment, toxicity was compared between the fixed-dose and BSA-based dose cohort using the Chi-square or Fisher's exact test. In the fixed-dose cohort, patients were divided in three groups based on BSA quartiles per sex and treatment: lowest 25%, middle 50% and highest 25%. In the BSA-based dose cohort, patients were divided in the same treatment groups based on the BSA limits per sex obtained from the fixed-dose cohort. Toxicity was compared between the three BSA groups within both cohorts using the Chi-square test for trend.

For regimens were BSA was found to be predictive for toxicity, other relationships between known risk factors from literature and toxicity were studied within both cohorts using univariate and multivariate binary logistic regression analysis, where the assumption of linearity was checked for each continuous risk factor. Significant risk factors with $P < 0.05$ detected in the univariate analysis of the fixed-dose cohort, were included in the multivariate analysis of both cohorts. In the fixed-dose cohort; the mean given fixed daily capecitabine dose was compared to a calculated ('fictional') mean daily capecitabine dose based on patient's BSA and according to clinical guidelines per treatment type, by using a paired sample T-test.

Survival analysis was only performed in the fixed-dose cohort in separate groups per tumor type, indication and treatment regimen (i.e. CAPE+RT for locally advanced colorectal cancer (laCRC), CAPOX for metastatic colorectal cancer (mCRC), and ECC/EOX for gastric cancer). For the BSA-based dose cohort, this analysis could not be performed because these data were not collected. Survival analysis between three BSA groups was done by the Kaplan-Meier method. Only, treatment regimens per indication

with at least 20 events were included in this analysis.
P-values < 0.05 were considered statistically significant. All statistical analyses were performed using SPSS (version 24.0.0.1).

RESULTS

PATIENT AND TREATMENT CHARACTERISTICS

A total of 3,583 patients were screened for inclusion of whom 1,264 patients were excluded, mainly because of previous fluoropyrimidine treatment (**Figure 1**). This resulted in a total of 2,319 patients enrolled in the analysis of whom 1,126 patients were included in the fixed-dose cohort and 1,193 patients in the BSA-based dose cohort (**Figure 1**). Patient characteristics for both cohorts per treatment group are described in **Table 1**. Overall, more male patients were included in the fixed-dose cohort (61%) than in the BSA-based dose cohort (48%; P < 0.001). The mean age was comparable in most treatment groups, but patients treated with capecitabine monotherapy were slightly older in the fixed-dose cohort (65 versus 61 years, P = 0.019). The majority of patients were from Caucasian origin (91%), but fewer in the fixed-dose cohort (85%) compared with the BSA-based dose cohort (96%) (P <0.001). The BSA of patients was normally distributed per sex and treatment. The mean BSA of patients was comparable in most treatment groups, but in the CAPE+RT group the BSA was slightly higher in the fixed-dose cohort compared to the BSA-based dose cohort (1.94 m^2 and 1.91 m^2, respectively, P = 0.013).

Overall, the most common tumor type was colorectal cancer (75%), and capecitabine combined with radiotherapy was the most often used treatment regimen in both cohorts. The median capecitabine daily dose was 3,000 mg in the fixed-dose cohort, and 3,500 mg in the BSA-based dose cohort. Only in the CAPE MONO group, was no significant difference in the median capecitabine daily dose identified between both cohorts. Overall, the mean given fixed dose capecitabine was 7.2% lower than calculated dose based on BSA (P < 0.001); the results detailed per treatment are shown in **Supplementary Table 2**.

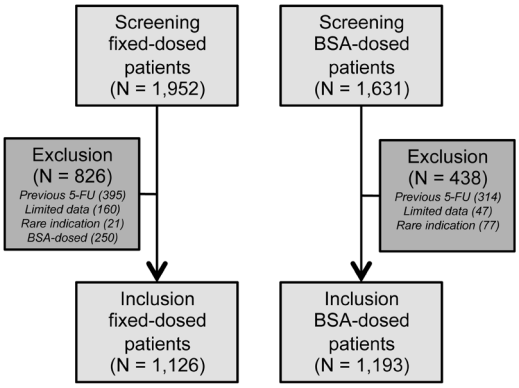

Figure 1. STROBE diagram of included patients

Abbreviations: 5-FU = 5-fluorouracil; BSA = body surface area;
STROBE = strengthening the reporting of observational studies in epidemiology

Table 1. Patient characteristics *[Table on the next page]*

P-values < 0.05 are considered statistically significant and are depicted in bold.

[a] BSA was calculated according to the Mosteller formula[16]

[b] eGFR was calculated according to the Cockcroft-Gault formula in the fixed-dose cohort [23], and calculated according to the CKD-EPI formula in the BSA-based dose cohort [24]

[c] The administered treatment regimens are described in more detail in supplementary table 1

[d] Total daily capecitabine dose at start of first cycle

Abbreviations: BC = breast cancer; BSA = body surface area; CAPE = capecitabine; CAPOX = capecitabine combined with oxaliplatin; CRC = colorectal cancer; ECC = capecitabine combined with epirubicin and cisplatin; eGFR = estimated glomerular filtration rate; EOX = capecitabine combined with epirubicin and oxaliplatin; GC = gastric cancer; IQR = interquartile range; mono = monotherapy; SD = standard deviation; RT = radiotherapy

TOXICITY BETWEEN THE FIXED-DOSE AND BSA-BASED DOSE COHORT

No differences in the incidence of capecitabine-specific toxicity or clinically relevant events could be identified between the fixed-dose and BSA-based dose cohort per treatment group (**Table 2**). Only in the CAPE MONO and CAPE TRIPLET group, were some minor differences in the single toxicity incidences identified. In the fixed-dose patients of the CAPE MONO group, a lower incidence of HFS ≥ 2 (22% versus 33%, P = 0.026) and a higher incidence of neutropenia ≥ 2 (14% versus 6%, P = 0.005) was observed than in the BSA-based dose patients (**Table 2**). Fixed-dose patients of the CAPE TRIPLET group had a higher incidence of neutropenia ≥ 2 (82% versus 61%, P = 0.003), and more discontinuation of treatment due to toxicity (37% versus 23%, P = 0.043) compared to the BSA-based dose patients (**Table 2**). Importantly, no difference in toxicity or clinically relevant events could be identified when toxicity was compared between the lowest BSA quartile of the fixed-dose and BSA-based dose cohort per treatment, indicating that patients with a low BSA did not receive too much capecitabine in the fixed-dose cohort (**Supplementary Table 3**).

Table 1. Patient characteristics

CHARACTERISTIC	CAPE + RT[c]			CAPOX[c]			CAPE MONO[c]			CAPE TRIPLET[c]		
	FIXED N=769	BSA N=409	*P-value*	FIXED N=189	BSA N=330	*P-value*	FIXED N=97	BSA N=344	*P-value*	FIXED N=71	BSA N=110	*P-value*
Sex			***0.006***			*0.148*			***<0.001***			
Male	494 (64%)	229 (56%)		97 (51%)	191 (58%)		39 (40%)	76 (22%)		54 (76%)	75 (68)	
Female	275 (36%)	180 (44%)		92 (49%)	139 (42%)		58 (60%)	268 (78%)		17 (24%)	35 (32%)	
Age (years)			***0.041***			*0.109*			***0.019***			
Mean [SD]	63 [10.6]	62 [9.9]		59 [11.8]	60 [10.0]		65 [14.3]	61 [11.4]		60 [9.5]	60 [9.7]	
Ethnic origin			***<0.001***			***<0.001***			***<0.001***			***<0.001***
Caucasian	682 (89%)	393 (96%)		159 (84%)	318 (96%)		69 (71%)	334 (97%)		50 (70%)	103 (94%)	
African	13 (2%)	6 (2%)		9 (5%)	6 (2%)		5 (5%)	6 (2%)		2 (3%)	4 (4%)	
Other	74 (10%)	10 (2%)		21 (11%)	6 (2%)		23 (24%)	4 (2%)		19 (27%)	3 (3%)	
BSA (m^2) [a]			***0.013***			*0.677*			*0.900*			*0.527*
Mean [SD]	1.94 [0.22]	1.91 [0.21]		1.90 [0.20]	1.91 [0.23]		1.81 [0.17]	1.81 [0.20]		1.91 [0.21]	1.94 [0.19]	
eGFR (mL/min)[b]			***<0.001***			***<0.001***			***<0.001***			*0.144*
Median [IQR]	83 [73-90]	88 [75-103]		86 [76-90]	89 [76-103]		81 [67-90]	86 [73-103]		85 [72-90]	85 [75-95]	
Unknown (N)	4	104		1	172		1	122		0		
Tumor type			***<0.001***			*0.140*			***<0.001***			-
Non-metastatic CRC	759 (99%)	340 (83%)		54 (29%)	122 (37%)		13 (13%)	22 (6%)		0	0	
Metastatic CRC	10 (1%)	24 (6%)		125 (66%)	187 (57%)		34 (35%)	32 (9%)		0	0	
Non-metastatic BC	0	5 (1%)		0	0		44 (45%)	25 (7%)		0	0	
Metastatic BC	0	1 (0%)		0	2 (1%)		0	183 (53%)		0	0	
GC	0	8 (2%)		0	0		3 (3%)	33 (10%)		71 (100%)	110 (100%)	
Other	0	31 (8%)		10 (5%)	19 (6%)		3 (3%)	50 (15%)		0	0	
Capecitabine daily dose (mg) [d]			***<0.001***			***<0.001***			*0.247*			***<0.001***
Median [IQR]	3,000	3,150 [2,800-3,300]		3,500	3,800 [3,500-4,000]		4,000 [3,500-4,000]	3,500 [3,150-4,000]		3,500	3,800 [3,500-4,000]	
Number of treatment cycles			***<0.001***			*0.067*			*0.984*			*0.649*
Median [IQR]	1 [1-1]	1 [1-1]		6 [3-8]	6 [4-8]		4 [3-8]	4 [2-9]		3 [3-6]	3 [3-6]	

Table 2. Toxicity compared between fixed-dose and BSA-based dose patients per treatment regimen

TREATMENT		TOXICITY							
		Diarrhea ≥ 3 (%)	HFS ≥ 2 (%)	Neutropenia ≥ 2 (%)	Cape-specific toxicity (%)[a]	Dose reduction (%)	Stop (%)	Hospital admission (%)	Clinically relevant events (%)[b]
CAPE+RT*	FIXED N=769	75 (9.8)	17 (2.2)	17 (2.2)	95 (12.4)	9 (1.2)	106 (13.8)	68 (8.8)	127 (16.5)
	BSA N=409	39 (9.5)	15 (3.7)	6 (1.5)	52 (12.7)	9 (2.2)	50 (12.2)	29 (7.1)	59 (14.4)
	P-value	*0.904*	*0.143*	*0.380*	*0.859*	*0.162*	*0.452*	*0.298*	*0.349*
CAPOX*	FIXED N=189	17 (9.0)	25 (13.2)	48 (25.4)	78 (41.3)	43 (22.8)	43 (22.8)	34 (18.0)	82 (43.4)
	BSA N=330	41 (12.4)	56 (17.0)	82 (24.8)	146 (44.2)	97 (29.4)	64 (19.4)	39 (11.8)	141 (42.7)
	P-value	*0.233*	*0.258*	*0.890*	*0.511*	*0.101*	*0.372*	*0.053*	*0.884*
CAPE MONO*	FIXED N=97	1 (1.0)	21 (21.6)	14 (14.4)	34 (35.1)	17 (17.5)	16 (16.5)	6 (6.2)	31 (32.0)
	BSA N=344	17 (4.9)	115 (33.4)	20 (5.8)	140 (40.7)	87 (25.3)	58 (16.9)	24 (7.0)	128 (37.2)
	P-value	*0.141*	***0.026***	***0.005***	*0.315*	*0.112*	*0.932*	*0.785*	*0.342*
CAPE TRIPLET*	FIXED N=71	5 (7.0)	11 (15.5)	58 (81.7)	59 (83.1)	12 (16.9)	26 (36.6)	19 (26.8)	35 (49.3)
	BSA N=110	12 (10.9)	15 (13.6)	67 (60.9)	78 (70.9)	26 (23.6)	25 (22.7)	21 (19.1)	53 (48.2)
	P-value	*0.444*	*0.728*	***0.003***	*0.062*	*0.277*	***0.043***	*0.225*	*0.884*

P-values < 0.05 are considered statistically significant and are depicted in bold. * The administered treatment regimens are described in more detail in supplementary table 1

[a] Capecitabine-specific toxicity was defined as at least one of the following toxicity scores: diarrhea ≥ 3, HFS ≥ 2, neutropenia ≥ 2

[b] Clinically relevant events was defined as at least one of the following events due to toxicity: dose reduction, stop with capecitabine, hospital admission

Abbreviations: BSA = body surface area; CAPE = capecitabine; CAPOX = capecitabine combined with oxaliplatin; HFS = hand-foot syndrome; mono = monotherapy;
RT = radiotherapy

TOXICITY COMPARED BETWEEN BSA GROUPS WITHIN BOTH COHORTS

No differences could be identified for CAPOX, CAPE MONO and CAPE TRIPLET when the incidence of capecitabine-specific toxicity and clinically relevant events was compared between the low, middle and high BSA group per treatment and cohort. However, only in the CAPE+RT group a significant difference in capecitabine-specific and clinically relevant events could be identified between BSA groups within the fixed-dose cohort (P = 0.009 and P = 0.013, respectively) and within the BSA-based dose cohort (P = 0.022 and P = 0.035, respectively; **Table 3**), demonstrating a higher risk of

toxicity in the lowest BSA quartile of patients from the CAPE+RT group of both cohorts.

Table 3. Toxicity compared between BSA groups within the fixed-dose cohort and the BSA-based dose cohort

TOXICITY per treatment	FIXED-dose cohort[a]				BSA-based dose cohort[b]			
	Low BSA	Middle BSA	High BSA	*P-value*	Low BSA	Middle BSA	High BSA	*P-value*
CAPE + RT*	N=204	N=378	N=187		N=117	N=219	N=73	
Cape-specific toxicity (%)[c]	33 (16.2)	48 (12.7)	14 (7.5)	***0.009***	20 (17.1)	28 (12.8)	4 (5.5)	***0.022***
Clinically relevant events (%)[d]	45 (22.1)	58 (15.3)	24 (12.8)	***0.013***	21 (17.9)	34 (15.5)	4 (5.5)	***0.035***
CAPOX*	N=48	N=96	N=45		N=84	N=158	N=88	
Cape-specific toxicity (%)[c]	18 (37.5)	39 (40.6)	21 (46.7)	*0.373*	33 (39.3)	73 (46.2)	40 (45.5)	*0.423*
Clinically relevant events (%)[d]	25 (52.1)	36 (37.5)	21 (46.7)	*0.573*	32 (38.1)	72 (45.6)	37 (42.0)	*0.612*
CAPE MONO*	N=25	N=49	N=23		N=97	N=173	N=74	
Cape-specific toxicity (%)[c]	9 (36)	15 (30.6)	10 (43.5)	*0.609*	41 (42.3)	75 (43.4)	24 (32.4)	*0.233*
Clinically relevant events (%)[d]	11 (44.0)	15 (30.6)	5 (21.7)	*0.099*	38 (39.2)	64 (37.0)	26 (35.1)	*0.585*
CAPE TRIPLET*	N=17	N=37	N=17		N=12	N=68	N=30	
Cape-specific toxicity (%)[c]	13 (76.5)	31 (83.8)	15 (88.2)	*0.363*	11 (91.7)	45 (66.2)	22 (73.3)	*0.536*
Clinically relevant events (%)[d]	11 (64.7)	18 (48.6)	6 (35.3)	*0.089*	6 (50.0)	33 (48.5)	14 (46.7)	*0.830*

P-values < 0.05 are considered statistically significant and are depicted in bold.

* The administered treatment regimens are described in more detail in supplementary table 1

a BSA groups were based on the lowest 25%, middle 50% and highest 25% BSA per sex and treatment in the fixed-dose cohort

b BSA groups within the BSA-based dose cohort were based on BSA-distribution and limits set in the fixed-dose cohort per sex and treatment

c Capecitabine-specific toxicity was defined as at least one of the following toxicity scores: diarrhea ≥ 3, HFS ≥ 2, neutropenia ≥ 2

d Clinically relevant events was defined as at least one of the following events due to toxicity: dose reduction, stop with capecitabine, hospital admission

Abbreviations: BSA = body surface area; CAPE = capecitabine; CAPOX = capecitabine combined with oxaliplatin; HFS = hand-foot syndrome; mono = monotherapy; RT = radiotherapy

RISK FACTORS FOR TOXICITY IN PATIENTS TREATED WITH CAPE+RT

Only in the CAPE+RT group, an increased toxicity risk was demonstrated in the low BSA group in both cohorts. Therefore, in this group additional analyses for other risk factors than BSA were performed. Univariate regression analysis, demonstrated that BSA was predictive for toxicity in the CAPE+RT group of both cohorts (**Table 4**). Sex, age, and kidney function were also significantly related to toxicity in the fixed-dose cohort, but not in the BSA-based dose cohort. After correction for these factors in a multivariate model, BSA remained significantly predictive for capecitabine-specific toxicity in the fixed-dose patients (OR = 0.25, 95%CI = 0.07-0.86, P = 0.028) and BSA-

based dose patients (OR = 0.09, 95% CI = 0.01-0.74, P = 0.025). Interestingly, fixed-dose women treated with CAPE+RT (and the same diagnosis) had a doubling of the toxicity risk compared with men for both capecitabine-specific toxicity (OR = 2.02, 95%CI = 1.22-3.37, P = 0.007) and clinically relevant events (OR = 2.12, 95%CI = 1.35-3.32, P = 0.001; **Table 4**).

EFFECTIVENESS

Overall, for mCRC patients treated with CAPOX, the median PFS was 8.6 months (95% CI 6.9-10.3 months) and for patients with gastric cancer treated with ECC/EOX, the median PFS was 24.6 months (95% CI 6.1-43.0 months). For patients with laCRC treated with CAPE+RT, the median DFS was not reached; the five-year survival probability was 0.56. No statistical differences between BSA groups in PFS for CAPOX for mCRC or ECC/EOX for gastric cancer, nor for the DFS with CAPE+RT for laCRC, could be identified (**Figure 2**). These results indicate that, in the fixed-dose regimens evaluated, there was no inadequate dosing of patients. Other fixed-dose treatment regimens could not be evaluated for survival due to too low number of events per treatment and indication.

Table 4. Risk factors for toxicity in patients treated with capecitabine and radiotherapy within the fixed-dose cohort and the BSA-based dose cohort

	Univariate analysis						Multivariate analysis					
	FIXED-dose cohort CAPE+RT (N=765)			BSA-based dose cohort CAPE+RT (N=305)			FIXED-dose cohort CAPE+RT (N=765)			BSA-based dose cohort CAPE+RT (N=305)		
RISK FACTOR	OR	95% CI	*P-value*	OR	95% CI	*P-value*	OR	95% CI	*P-value*	OR	95% CI	*P-value*
BSA												
Cape-specific toxicity[a]	0.10	0.03-0.30	***<0.001***	0.15	0.03-0.87	***0.034***	0.25	0.07-0.86	***0.028***	0.09	0.01-0.74	***0.025***
Clinically relevant events[b]	0.10	0.04-0.25	***<0.001***	0.17	0.03-0.91	***0.038***	0.23	0.08-0.70	***0.009***	0.20	0.03-1.50	*0.114*
SEX (F versus M)[c]												
Cape-specific toxicity[a]	2.67	1.72-4.14	***<0.001***	1.01	0.50-2.03	*0.981*	2.02	1.22-3.37	***0.007***	0.61	0.27-1.41	*0.250*
Clinically relevant events[b]	2.87	1.94-4.23	***<0.001***	1.30	0.67-2.54	*0.434*	2.12	1.35-3.32	***0.001***	0.94	0.43-2.08	*0.885*
AGE												
Cape-specific toxicity[a]	1.05	1.03-1.08	***<0.001***	0.99	0.96-1.03	*0.636*	1.04	1.02-1.07	***0.002***	0.99	0.96-1.03	*0.699*
Clinically relevant events[b]	1.03	1.01-1.05	***0.003***	0.99	0.95-1.02	*0.375*	1.02	1.00-1.04	*0.092*	0.99	0.96-1.02	*0.564*
eGFR												
Cape-specific toxicity[a]	0.98	0.96-0.99	***0.002***	1.01	0.99-1.02	*0.344*	0.99	0.97-1.01	*0.160*	1.00	0.99-1.02	*0.878*
Clinically relevant events[b]	0.98	0.96-0.99	***0.001***	1.01	1.00-1.03	*0.115*	0.98	0.97-1.00	***0.026***	1.01	0.99-1.02	*0.361*

P-values < 0.05 are considered statistically significant and are depicted in bold.

[a] Capecitabine-specific toxicity was defined as at least one of the following toxicity scores: diarrhea ≥ 3, HFS ≥ 2, neutropenia ≥ 2

[b] Clinically relevant events was defined as at least one of the following events due to toxicity: dose reduction, stop with capecitabine, hospital admission

Abbreviations: BSA = body surface area; CAPE = capecitabine; eGFR = estimated glomerular filtration rate; F = female; HFS = hand-foot syndrome; M = male; RT = radiotherapy

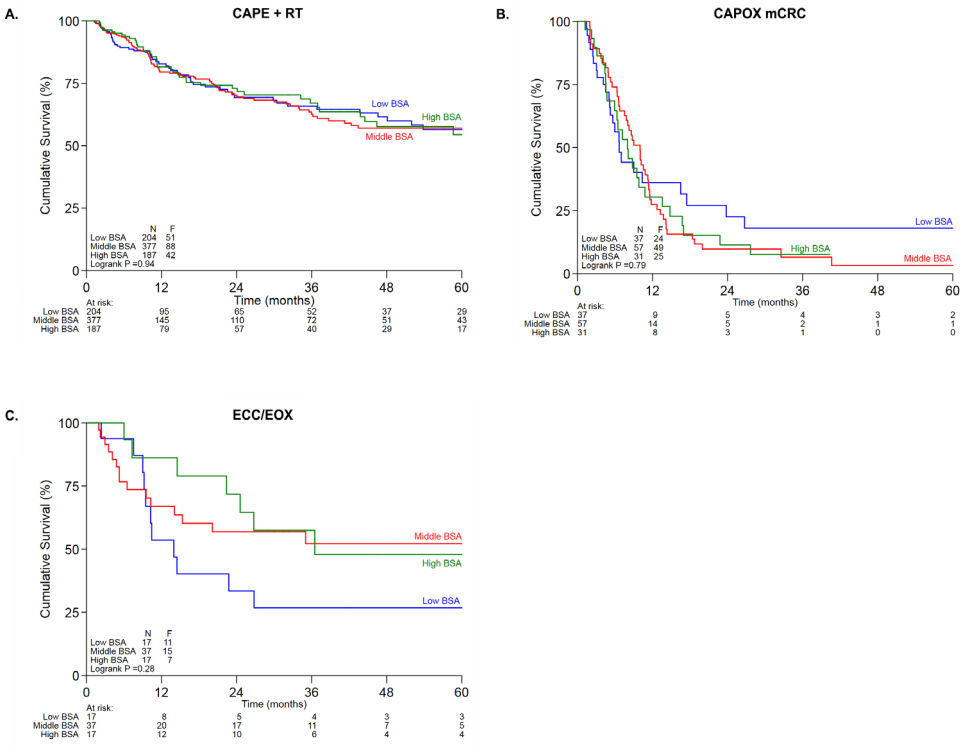

Figure 2. Survival compared between BSA groups within the fixed-dose cohort

Disease free survival in CAPE+RT for laCRC (Figure 2A.). Progression free survival in CAPOX for mCRC (Figure 2B.) and in ECC/EOX for gastric cancer (Figure 2C.).

Abbreviations: BSA = body surface area; CAPE = capecitabine; CAPOX = capecitabine combined with oxaliplatin; ECC = capecitabine combined with epirubicin and cisplatin; EOX = capecitabine combined with epirubicin and oxaliplatin; F = number of events; laCRC = locally advanced colorectal cancer; mCRC = metastatic colorectal cancer; N = number of patiens; RT = radiotherapy

DISCUSSION

This relatively large cohort study demonstrates that a fixed dose of capecitabine is as comparably well tolerated as dosing based on BSA in several treatment regimens (i.e. CAPOX, CAPE TRIPLET and CAPE MONO). Only in the CAPE+RT group, was a low BSA predictive for capecitabine-specific toxicity and clinically relevant events. In addition, our data suggest that a fixed dose of capecitabine was equally effective compared with dosing based on BSA. Therefore, we demonstrated that this fixed dosing strategy of capecitabine is feasible in a large 'real life' population with common treatment regimens.

Beforehand, the observed association between BSA and toxicity when capecitabine was combined with radiotherapy was not expected. It is remarkable that an increased incidence of capecitabine-specific and clinically relevant events was not only found in the low BSA group in the fixed-dose cohort, but also in the low BSA group in the BSA-based dose cohort (**Table 3**). The fact that also in the latter group a higher risk of toxicity was observed in the lowest BSA quartile of patients, suggests that this effect is likely to be caused by the interaction of the two treatment modalities. When capecitabine is combined with radiotherapy, the absolute dose of capecitabine used is lower compared to the other regimens, because it is used as radiosensitizer. The enzyme thymidine phosphorylase in the tumor tissue is responsible for the final metabolic step in the conversion of capecitabine into 5-FU. This conversion is boosted by radiotherapy and therefore mostly local effects of 5-FU will be seen.[27] Occurrence of diarrhea during RT could be explained by (at least) two reasons. The first reason is that there is a clear relationship between radiated small bowel volume and the incidence of diarrhea in chemoradiotherapy for rectal tumors, and possibly, this is also related to BSA, since hypothetically a higher small bowel volume is exposed to radiotherapy in patients with a low BSA.[28, 29] Another reason is a possible relation with rectal irritation by the tumor itself.[30] Finally, in the CAPE+RT group of both cohorts, diarrhea was the most frequent severe adverse event with an incidence of 10%. As a result, this finding might be biased because of diarrhea being the major side-effect of radiotherapy. Unfortunately, no studies have been performed on the mechanism of toxicity related to capecitabine combined with radiotherapy. Further research should therefore be conducted on the potential effects of radiotherapy and BSA on toxicity of this combination treatment.

Several factors are known to influence the risk of toxicity caused by capecitabine. Older age, female sex and decreased renal function have been related to the risk of toxicity.[31-33] In our multivariate analysis in the CAPE+RT group of fixed-dose patients, we have also confirmed an increased risk of toxicity with female sex, but we could not clearly confirm the role of age and renal function. This could potentially be explained

by the limited range of these two factors. In addition, in the BSA-based dose cohort all these risk factors could not be confirmed in the CAPE+RT group.

Secondly, another factor that strongly influences the risk of toxicity is genetic variation in capecitabine metabolism. The enzyme dihydropyrimidine dehydrogenase (DPD) is largely responsible for the inactivation of 5-FU, and with a decreased activity of this enzyme related to polymorphisms in the *DPYD* gene, the risk of severe toxicity largely increases.[34, 35] Only recently, genotyping of the four most common *DPYD* polymorphisms associated with DPD deficiency has been implemented as routine screening clinical care in The Netherlands prior to start of treatment with capecitabine. In patients carrying one of these polymorphisms, dose-adjustments are made according to the gene-activity score.[36, 37] Unfortunately, we have no knowledge about the genotype of the patients in the fixed dose cohort because they were treated before the implementation of upfront genotyping. Prevalence of partial DPD deficiency is around 3-5%. Therefore, we have to assume that a small group of patients in our cohort had indeed a partial DPD deficiency. The *DPYD* genotype is known for all the patients from the BSA-based dose cohort, and the mutant patients received a dose reduction; we do not think that this will influence our results. As all patients treated with a fixed dose in the mentioned time period were included in our analysis, we assume that the genetic distribution is comparable in the fixed-dose group of patients. However, these patients could not have received a dose reduction in case of DPD deficiency and therefore this could lead to a small increase in toxicity risk in the fixed-dose group.

Besides toxicity, we also have investigated effectiveness of given treatments. We hypothesized that if a fixed dose would (positively or negatively) influence effectiveness of the treatment a survival difference should occur between the patients with a low and a high BSA value per treatment and indication. Of interest, we have found no statistical differences in BSA subgroups in progression-free survival for CAPOX for mCRC or ECC/EOX for gastric cancer, nor for the disease-free survival for CAPE+RT for laCRC. In addition, the observed progression-free survival for CAPOX for mCRC and ECC/EOX for gastric cancer was comparable to literature (8.6 and 24.6 months versus 8.0 and 19.2 months, respectively).[38, 39] Although we have found no major differences in effectiveness in all subgroups analyzed, not all regimens could be evaluated due to small sample sizes and therefore we have to interpret these results with caution.

Our study has some limitations that need to be mentioned. Firstly, the retrospective nature of our data collection makes it difficult or even impossible to obtain toxicity and effectiveness data in a standardized fashion. However, we have evaluated combined toxicity scores, which consisted of severe capecitabine-specific toxicities or clinically

relevant events due to toxicity. In general, these scores are well documented because of the large impact on the patient and treatment decisions. Moreover, patients were excluded when their patient file was not available or when visits were poorly documented. In the survival analysis there was a frequent loss to follow-up and censoring of patients. Nevertheless, we found comparable survival probabilities for all subgroups as described in the literature. In addition, survival outcomes between different BSA groups were not different. However, the survival analysis was performed without a control-group as in the BSA-based dose cohort survival data were not collected. Another limitation, is the risk of confounding by hospital, treatment indication and dosing strategy. Although the fixed-dose cohort patients were included in a single hospital, we cannot argue that this might have influenced our results, because no major differences with the BSA-based dose cohort were identified.

Our study evaluated a large population of cancer patients treated with frequently used treatment regimens, thereby representing daily clinical care. In addition, we confirmed our results in another large and comparable cohort of patients. Although BSA-based dosing has been the standard choice in oncology for decades, there is little evidence for this approach. Therefore, there is a need for evidence that a fixed dose could be a well-tolerated alternative strategy for already existing anticancer agents such as capecitabine. Earlier, three small prospective studies demonstrated that a fixed-dose capecitabine is feasible.[40-42] However, these studies have not been translated into daily clinical care, probably because of their limited sample sizes. Sharma et al. showed that a fixed dose of capecitabine monotherapy in advanced colorectal cancer was safe and effective in a cohort of 55 patients.[40] Also, capecitabine in a fixed dose as monotherapy and in combination with vinorelbine was shown to be safe and effective in metastatic breast cancer patients.[41, 42] Additionally, a small retrospective study demonstrated that even a low fixed dose of 1,000 mg twice daily for 14 days might have effectivity in metastatic breast cancer patients.[43] In a study by Rudek et al., a large interpatient variability in capecitabine pharmacokinetics was shown without any influence of BSA, which also favors the fixed dosing strategy.[42] Furthermore, it would be interesting to investigate whether a fixed dosing strategy for capecitabine would lead to fewer prescribing mistakes and possibly to reducing costs, as has been demonstrated for some other drugs.[18-20]

In conclusion, we have shown that a fixed dose of capecitabine is equally well-tolerated as a BSA-based dose of capecitabine in several treatment regimens. Also, we have no data indicating that a fixed dose of capecitabine is less effective than a BSA-based dose. Our results indicate that fixed dosing of capecitabine is a reasonable and practical alternative for BSA-based dosing. Therefore, we would recommend implementing

fixed dosing in future clinical studies and we have found no arguments why it could not be used in daily clinical care.

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SUPPLEMENTARY INFORMATION

Supplementary Table 1. Detailed description administered treatment regimens

Treatment	Schedule	Fixed dose (mg/day)*	BSA-based dose (mg/ m²/day/)*
CAPE + RT			
Capecitabine	Continuous**	3,000	1,650
CAPOX			
Capecitabine	D1-14 (Q3W)	3,500	2,000
Oxaliplatin	D1 (Q3W)		130
MONO			
Capecitabine	D1-14 (Q3W)	3,500 (BC) 4,000 (CRC)	2,000
TRIPLET			
ECC			
Capecitabine	D1-14 (Q3W)	3,500	2,000
Epirubicin	D1 (Q3W)		50
Cisplatin	D1 (Q3W)		60
EOX			
Capecitabine	D1-21 (Q3W)	3,500	2,000
Epirubicin	D1 (Q3W)		50
Oxaliplatin	D1 (Q3W)		130
DOC			
Capecitabine	D1-14 (Q3W)	***	1,700
Docetaxel	D1 (Q3W)		50
Oxaliplatin	D1 (Q3W)		100

* Capecitabine total daily dose was divided over two daily doses according to standard of care

** For the fixed-dose cohort was capecitabine administered continuously the whole period of radiotherapy (also on non-radiotherapy days), for the BSA-based dose cohort was capecitabine administered continuous only on days of radiotherapy

*** The DOC-regimen was not administered in the fixed-dose cohort

Abbreviations: BC = breast cancer; BSA = body surface area; CAPE = capecitabine; CAPOX = capecitabine combined with oxaliplatin; CRC = colorectal cancer; D = day; HFS = hand-foot syndrome; mono = monotherapy; RT = radiotherapy; Q3W = 3-weekly schedule

Supplementary Table 2. Fixed daily dose compared to calculated BSA-based daily dose

Treatment	Mean fixed dose (mg/day)	Mean BSA-based dose (mg/day)	Difference (%)	Difference 95% CI (%)	*P-value*
CAPE + RT*	3,000mg	3,202mg	6.3	3.7-7.1	***<0.001***
CAPOX*	3,500mg	3,796mg	7.8	6.3-9.4	***<0.001***
CAPE MONO*	3,701mg	4,128mg	10.3	7.5-13.1	***<0.001***
CAPE TRIPLET*	3,500mg	3,839mg	8.8	6.3-11.4	***<0.001***
Total	3,175mg	3,421mg	7.2	6.5-7.9	***<0.001***

P-values < 0.05 are considered statistically significant and are depicted in bold.

* The administered treatment regimens are described in more detail in supplementary table 1

Abbreviations: BSA = body surface area; CAPE = capecitabine; CAPOX = capecitabine combined with oxaliplatin; HFS = hand-foot syndrome; mono = monotherapy; RT = radiotherapy

Supplementary Table 3. Toxicity compared between lowest BSA quartile of the fixed-dose cohort and BSA-based dose cohort per treatment regimen

TREATMENT		TOXICITY							
		Diarrhea ≥ 3 (%)	HFS ≥ 2 (%)	Neutropenia ≥ 2 (%)	**Cape-specific toxicity (%)[a]**	Dose reduction (%)	Stop (%)	Hospital admission (%)	**Clinically relevant events (%)[b]**
CAPE+RT*	**FIXED N=204**	26 (12.7)	6 (2.9)	8 (3.9)	33 (16.2)	2 (1.0)	37 (18.1)	33 (16.2)	45 (22.1)
	BSA N=117	13 (11.1)	7 (6.0)	1 (0.9)	20 (17.1)	4 (3.4)	17 (14.5)	11 (9.4)	21 (17.9)
	P-value	*0.666*	*0.183*	*0.163*	*0.831*	*0.196*	*0.406*	*0.089*	*0.381*
CAPOX*	**FIXED N=48**	7 (14.6)	4 (8.3)	8 (16.7)	18 (37.5)	15 (31.3)	12 (25.0)	9 (18.8)	25 (52.1)
	BSA N=84	11 (13.1)	9 (10.7)	23 (27.4)	33 (39.3)	18 (21.4)	20 (23.8)	12 (14.3)	32 (38.1)
	P-value	*0.811*	*0.768*	*0.162*	*0.839*	*0.210*	*0.878*	*0.500*	*0.119*
CAPE MONO*	**FIXED N=25**	0	7 (28.0)	4 (16.0)	9 (36.0)	7 (28.0)	6 (24.0)	2 (8.0)	11 (44.0)
	BSA N=97	9 (9.3)	34 (35.1)	5 (5.2)	41 (42.3)	26 (26.8)	20 (20.6)	9 (9.3)	38 (39.2)
	P-value	*0.201*	*0.506*	*0.084*	*0.570*	*0.904*	*0.713*	*1.000*	*0.661*
CAPE TRIPLET*	**FIXED N=17**	1 (5.9)	2 (11.8)	13 (76.5)	13 (76.5)	3 (17.6)	9 (52.9)	6 (32.3)	11 (64.7)
	BSA N=12	1 (8.3)	0	10 (83.3)	11 (91.7)	3 (25.0)	3 (25.0)	3 (25.0)	6 (50.0)
	P-value	*1.000*	*0.498*	*0.653*	*0.286*	*0.669*	*0.251*	*1.000*	*0.428*

[a] Capecitabine-specific toxicity was defined as at least one of the following toxicity scores: diarrhea ≥ 3, HFS ≥ 2, neutropenia ≥ 2

[b] Clinically relevant events was defined as at least one of the following events due to toxicity: dose reduction, stop with capecitabine, hospital admission

* The administered treatment regimens are described in more detail in Apendix table 1

Abbreviations: BSA = body surface area; CAPE = capecitabine; CAPOX = capecitabine combined with oxaliplatin; HFS = hand-foot syndrome; mono = monotherapy; RT = radiotherapy

3

DPYD GENOTYPE-GUIDED DOSE INDIVIDUALISATION OF FLUOROPYRIMIDINE THERAPY IN PATIENTS WITH CANCER: A PROSPECTIVE SAFETY ANALYSIS

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ABSTRACT

BACKGROUND

Fluoropyrimidine treatment can result in severe toxicity in up to 30% of patients and is often the result of reduced activity of the key metabolic enzyme dihydropyrimidine dehydrogenase (DPD), mostly caused by genetic *DPYD* variants. In a prospective clinical trial, we investigated whether upfront screening for four *DPYD* variants and *DPYD*-guided dose individualization can reduce fluoropyrimidine-induced toxicity.

METHODS

Prospective genotyping for *DPYD**2A, c.2846A>T, c.1679T>G, and c.1236G>A was performed in adult cancer patients for which fluoropyrimidine-based chemotherapy was considered in their best interest. All patients about to start with a fluoropyrimidine regimen (capecitabine or 5-fluorouracil as single agent or in combination with other chemotherapeutic agents and/or radiotherapy) could be included in the study. Heterozygous *DPYD* variant allele carriers received an initial dose reduction of 25% (c.2846A>T, c.1236G>A) or 50% (*DPYD**2A, c.1679T>G), *DPYD* wild-type patients were treated according to standard of care. The primary endpoint of the study was the incidence of severe (CTC-AE grade≥3) overall fluoropyrimidine-related toxicity. This toxicity incidence was compared between *DPYD* variant allele carriers and *DPYD* wild-type patients in the study in an intention-to-treat analysis, and relative risks for severe toxicity were compared between the current study and a historical cohort of *DPYD* variant allele carriers treated with full dose fluoropyrimidine-based therapy (derived from a previously published meta-analysis). This trial is registered under clinicaltrials.gov identifier NCT02324452 and is completed.

RESULTS

In total, 1103 evaluable patients were enrolled, of whom 85 *DPYD* variant carriers (7.7%). Overall grade ≥ 3 toxicity was higher in *DPYD* variant carriers than in wild-type patients (39% versus 23%, P = 0.0013). The relative risk (RR) for grade ≥ 3 toxicity was 1.31 (95% confidence interval [95%CI]: 0.63–2.73) for genotype-guided dosing versus 2.87 (95%CI: 2.14–3.86) in the historical cohort for *DPYD**2A, no toxicity versus 4.30 (95%CI: 2.10–8.80) in c.1679T>G, 2.00 (95%CI:1.19–3.34) versus 3.11 (95%CI: 2.25–4.28) for c.2846A>T, and 1.69 (95%CI: 1.18–2.42) versus 1.72 (95%CI: 1.22–2.42) for c.1236G>A.

CONCLUSIONS

Upfront *DPYD* genotyping was feasible in routine clinical practice, and improved patient safety of fluoropyrimidine treatment. For *DPYD**2A and c.1679T>G carriers, a 50% initial

dose reduction seems adequate. For c.1236G>A and c.2846A>T carriers, a larger dose reduction of 50% (instead of 25%) needs to be investigated. As fluoropyrimidines are among the most commonly used anticancer agents, the findings of this study are of high clinical importance, as they endorse implementing *DPYD* genotype-guided dosing as the new standard of care.

FUNDING
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INTRODUCTION

Fluoropyrimidine anticancer drugs, including 5-fluorouracil (5-FU) and its oral prodrug capecitabine, have been widely used for over sixty years in the treatment of different solid tumor types, such as colorectal, breast, and gastric cancer. Although these drugs are relatively well tolerated, up to 30% of patients experience severe treatment-related toxicity, including diarrhea, mucositis, myelosuppression, and hand-foot syndrome.[1–3] In addition, severe fluoropyrimidine-related toxicity can lead to treatment-related death in up to 1% of patients.[4,5] The occurrence of these severe side-effects can lead to treatment discontinuation and toxicity-related hospitalization, which in addition puts a heavy burden on health-care costs.

Fluoropyrimidine-related toxicity is often caused by reduced activity of the enzyme dihydropyrimidine dehydrogenase (DPD), the main metabolic enzyme for fluoropyrimidine inactivation.[6,7] A partial DPD deficiency (e.g. a ~50% reduced DPD activity compared to normal) is present in 3-5% of the Western population. These DPD deficient patients have a highly increased risk of developing severe treatment-related toxicity when treated with a standard dose of fluoropyrimidines.[8–10] Complete DPD deficiency is much rarer, with an estimated prevalence of 0.01-0.1%.[8,11,12] DPD deficiency is most often caused by genetic variants in *DPYD*, the gene encoding DPD. The four *DPYD* variants currently considered most clinically relevant and with convincingly demonstrated association with severe toxicity are *DPYD**2A (rs3918290, c.1905+1G>A, IVS14+1G>A), c.2846A>T (rs67376798, D949V), c.1679T>G (rs55886062, *DPYD**13, I560S), and c.1236G>A (rs56038477, E412E, in haplotype B3).[10,13,14] For these variants, available evidence suggests that heterozygous carriers of these variants have an average reduction in DPD enzyme activity of approximately 25% (c.2846A>T,

c.1236G>A) to 50% (*DPYD**2A, c.1679T>G).[14]

Prospective *DPYD* genotyping and dose reduction in heterozygous *DPYD* variant allele carriers is a promising strategy for preventing severe and potentially fatal fluoropyrimidine-related toxicity without affecting treatment efficacy. In a previous study prospective genotyping and dose-individualization for one *DPYD* variant, *DPYD**2A, in a cohort of 1631 patients showed that severe fluoropyrimidine-related toxicity could be decreased from 73% in *DPYD**2A carriers receiving a standard fluoropyrimidine dose (N = 48) to 28% by genotype-guided dosing, i.e. *DPYD**2A carriers receiving a 50% dose reduction (N = 18, $P<0.001$).[15] This study showed that by reducing the fluoropyrimidine dose by 50% in *DPYD**2A variant allele carriers, severe toxicity was reduced to a frequency (28%) comparable to that in *DPYD**2A wild-type patients treated with a standard fluoropyrimidine dose (23%).

It is expected that patient safety can be further improved by expanding the number of prospectively tested *DPYD* variants beyond *DPYD**2A alone. The objective of the current study was to assess the impact on patient safety of prospective screening for the four most relevant *DPYD* variants and subsequent *DPYD* genotype-guided dose individualization in daily clinical care.

PATIENTS AND METHODS

STUDY DESIGN AND PARTICIPANTS

This study was a prospective multicenter clinical trial in which 17 hospitals in the Netherlands participated. The study was approved by the institutional review board of The Netherlands Cancer Institute, Amsterdam, the Netherlands, and approval from the board of directors of each individual hospital was obtained for all participating centers. All patients provided written informed consent before enrollment in the study. Additional informed consent was obtained for *DPYD* variant allele carriers who participated in pharmacokinetic and DPD enzyme activity measurements.

The study population consisted of adult cancer patients ($\geq$18 years) intended to start with a fluoropyrimidine-based anticancer therapy, either as single agent or in combination with other chemotherapeutic agents and/or radiotherapy. Patients with all tumor types for which fluoropyrimidine-based therapy was considered in their best interest could be included. Prior chemotherapy was allowed, except for prior use of fluoropyrimidines. Patients had to have a WHO performance status of 0, 1 or 2, a

life expectancy of at least 12 weeks, and acceptable safety laboratory values. There were no restrictions on comorbidities, except for diseases expected to interfere with study or the patient's safety. Full inclusion and exclusion criteria can be found in the **Supplementary Methods**.

PROCEDURES

Patients were genotyped before start of fluoropyrimidine therapy for the previously mentioned four *DPYD* variants. Heterozygous *DPYD* variant allele carriers received an initial dose reduction of either 25% (for c.2846A>T and c.1236G>A) or 50% (for *DPYD**2A and c.1679T>G), in line with current recommendations from Dutch and international pharmacogenomic guidelines.[13,16] To achieve a maximal safe exposure, dose escalation was allowed after the first two cycles provided that treatment was well tolerated, and the decision to escalate was left to the discretion of the treating physician. The dose of other anticancer agents or radiotherapy were left unchanged at start of treatment. Homozygous or compound heterozygous *DPYD* variant allele carriers were excluded from the study and could be treated with personalized regimens outside this protocol.[17] Non-carriers of the above mentioned *DPYD* variants are considered wild-type patients in this study and were treated according to existing standard of care.

Toxicity was graded by participating centers according to the National Cancer Institute Common Terminology Criteria for Adverse Events (CTC-AE),[18] and severe toxicity was defined as grade three or higher. Patients were followed for toxicity during the entire treatment period and until toxicity was resolved. Toxicity scored by the treating physician or qualified nurse practitioner as possibly, probably or definitely related to fluoropyrimidine-treatment was considered treatment-related toxicity (definitions ini **Supplementary Methods**). Toxicity-related hospitalization and treatment discontinuation due to adverse events were also investigated. Standard laboratory assessments were performed prior to start of treatment and each new cycle according to routine clinical care, for evaluation of treatment safety.

Genotyping for the four *DPYD* variants *DPYD**2A, c.2846A>T, c.1679T>G and c.1236G>A was performed before start of treatment. Genotyping was performed in a clinical laboratory of the local hospital or in one of the other participating centers of this trial. Validated assays were used and all laboratories participated in a Dutch national proficiency testing program for all four *DPYD* variants.[19] In *DPYD* variant allele carriers who provided written informed consent for additional tests, plasma levels of capecitabine, 5-FU, and their metabolites were determined at the first day of a capecitabine/5-FU cycle (preferably the first cycle) to assess the pharmacokinetic profile in these patients. A validated ultra-performance liquid chromatography tandem

mass-spectrometry (UPLC-MS/MS) method was used (details in **Supplementary Methods**). Results of pharmacokinetic parameters, including the area under the plasma concentration-time curve (AUC) and half-life ($t_{1/2}$) were calculated using non-compartmental analysis, and compared to control values derived from literature.[20] DPD enzyme activity in peripheral blood mononuclear cells (PBMCs) was determined in a pretreatment sample in the *DPYD* variant allele carriers and compared to DPD enzyme activity measured in wild-type patients in this study, using a validated assay.[21]

OUTCOMES

The primary endpoint of the study was the frequency of severe overall fluoropyrimidine-related toxicity across the entire treatment duration. A comparison was made between the incidence of severe toxicity in *DPYD* variant allele carriers treated with reduced dose and in wild-type patients treated with standard dose in this study. In addition to this, the relative risk for severe toxicity of these *DPYD* variant allele carriers treated with reduced dose compared to non-carriers in the study was calculated. A comparison between this calculated relative risk and a similarly calculated relative risk for *DPYD* variant allele carriers treated with full dose in a historical cohort derived from a previously published meta-analysis[10] was made. Secondary endpoints included pharmacokinetics of capecitabine and 5-FU in *DPYD* variant allele carriers and measurements of DPD enzyme activity. Another secondary endpoint was a cost-analysis on individualized dosing based on upfront *DPYD* genotyping, of which results will be reported separately.

STATISTICAL ANALYSIS

The sample size was based on a one stage A'Hern (phase II) design[22] and calculated under the assumption that overall fluoropyrimidine-related severe toxicity could be reduced from 60% (in *DPYD* variant allele carriers receiving standard dose)[10,15] to 20% by individualized dosing in *DPYD* variant allele carriers. This resulted in a required sample size of 11 variant carriers. To reach this number of variant carriers, we used a single *DPYD* variant (c.2846A>T, assumed variant frequency of 1%) to calculate the total sample size, resulting in a total expected sample size of 1100 evaluable patients. Detailed information on the sample size calculation can be found in the **Supplementary Methods**. Patients were considered evaluable when meeting the inclusion and exclusion criteria, and if they received at least one fluoropyrimidine drug administration.

Associations between dichotomous outcomes, e.g. occurrence of severe toxicity or hospitalization, and genotype status were tested using χ^2 or Fisher's exact test (Fisher's exact test was chosen when the smallest cell count was 5 or lower; for this test the double one-tailed exact probability was reported). Baseline characteristics between *DPYD* variant allele carriers and wild-type patients in the study were compared using

either χ^2 test, Fisher's exact test or Kruskal-Wallis rank sum test depending on the type of variable. DPD enzyme activity was compared between carriers of individual *DPYD* variants and wild-type patients using Student's *t*-tests. P-values <0.05 were considered statistically significant. Statistical analyses on an intention-to-treat population were performed using SPSS (version 22.0.) and R (version 3.1.2). This study is registered with ClinicalTrials.gov, number NCT02324452.

RESULTS

Between April 30th, 2015 and December 21st, 2017 a total of 1181 patients intended to start fluoropyrimidine-based treatment were enrolled in this study. In total, 78 patients were considered non-evaluable (**Figure 1**), as they retrospectively were identified as not meeting the inclusion criteria (N = 48), did not start fluoropyrimidine-based treatment (N = 26), or were homozygous or compound heterozygous *DPYD* variant allele carriers (N = 4). This resulted in a total of 1103 evaluable patients, of whom 85 were heterozygous *DPYD* variant allele carriers (7.7%).

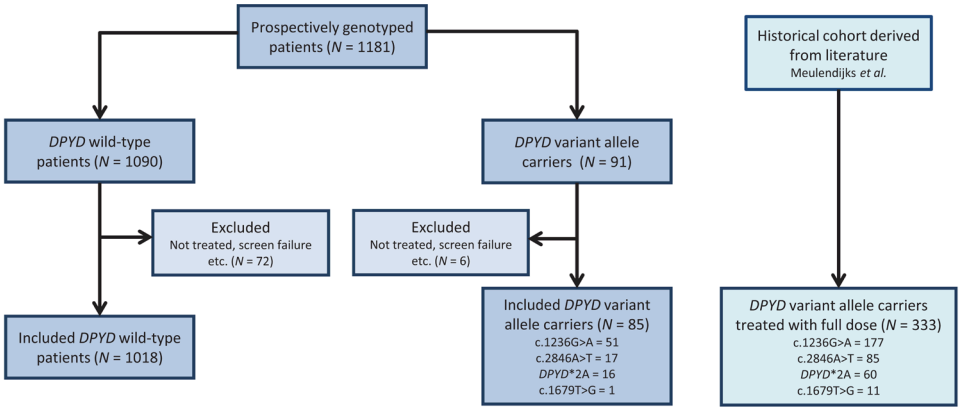

Figure 1. Trial profile

On the left our study, on the right the previous conducted meta-analysis by Meulendijks et al.

Abbreviations: *DPYD* : gene encoding dihydropyrimidine dehydrogenase; N: number

Baseline characteristics of *DPYD* variant allele carriers and *DPYD* wild-type patients are described in **Table 1 and Supplementary Table 1**. The most common tumor type was colorectal cancer (64%). In total, 83% of patients were treated with a capecitabine-based regimen.

Mean relative dose intensities for each patient group are presented in **Table 2**. In general, dose recommendations as described in the study protocol were followed by the treating physicians, which resulted in mean dose intensities in the first cycle of 74%, 73%, 51%, and 50% for c.1236G>A, c.2846A>T, *DPYD**2A and c.1679T>G, respectively. The performed dose reductions were therefore in line with the pre-specified dose reductions of 25% (for c.1236G>A and c.2846A>T) or 50% (for *DPYD**2A and c.1679T>G) However, for four patients carrying *DPYD* variants, dose reductions were not applied at start of treatment (details in **Supplementary Methods**). One of these patients, (c.2846A>T carrier) was treated by mistake with a full capecitabine dose for the first two cycles, which resulted in fatal fluoropyrimidine-related toxicity. Although dosing recommendations were not followed in these four patients, all results were included in the analysis (intention-to-treat analysis).

Doses were escalated during treatment in 11 out of 85 *DPYD* variant allele carriers (13%). In five of these patients (two *DPYD**2A and three c.1236G>A carriers) the higher dose was not well tolerated, leading to a dose reduction. Also, one patient (c.2846A>T carrier) discontinued treatment after the dose escalation due to toxicity. Five patients (one c.2846A>T, one c.1236G>A, one c.1679T>G, and two *DPYD**2A carriers) were able to continue treatment with the escalated dose.

The median follow-up period (similar to the entire treatment duration or when toxicity was resolved) was 71 days (interquartile range [IQR]: 36-161 days). For wild-type patients median follow-up was 69 days (IQR 36-161 days) and for *DPYD* variant allele carriers 90 days (IQR 35-168 days).

Frequencies of severe toxicity for *DPYD* variant allele carriers who received genotype-guided dosing and wild-type patients who received standard dosing are depicted in **Table 2**. A total of 33 out of 85 (39%) *DPYD* variant allele carriers experienced severe (grade≥3) fluoropyrimidine-related toxicity, which was significantly higher than the frequency in wild-type patients (23%), P=0.0013. The incidence of grade ≥4 toxicity was low but was comparable between both groups as well (4 out of 85 (5%) for *DPYD* variant allele carriers vs 29 out of 1018 3% for wild-type patients, P=0.49, **Table 2**).

The percentage of toxicity in *DPYD* variant allele carriers was mainly driven by the two most common variants, who also had higher toxicity frequencies. In total, 20 out of 51 c.1236G>A carriers experienced severe toxicity (39%) and eight out of 17 c.2846A>T carriers (47%). For *DPYD**2A carriers, five out of 16 patients (31%) experienced severe toxicity. The single c.1679T>G carrier, who did receive reduced-dose treatment, tolerated the treatment well and did not experience severe treatment-related toxicity

over the course of treatment (three cycles).

For 16 out of 85 *DPYD* variant allele carriers (19%) fluoropyrimidine-related toxicity resulted in hospitalization, compared to 140 out of 1018 wild-type patients (14%), *P*=0.26. Median duration of hospitalization was five days for both *DPYD* variant allele carriers and wild-type patients (IQR 3-7 days, and 3-10 days, respectively). For 15 out of 85 *DPYD* variant allele carriers (18%) fluoropyrimidine treatment was stopped due to fluoropyrimidine-related toxicity, compared to 175 out of 1018 wild-type patients (17%), which was comparable between both groups (*P*=1.0).

As described above, one c.2846A>T carrier experienced fatal fluoropyrimidine-related toxicity, but the intended dose reductions were not applied for this patient. When disregarding this patient for the critical protocol violation, no treatment-related death occurred in *DPYD* variant allele carriers. In the wild-type cohort, three patients died due to fluoropyrimidine-related toxicity (0.3%), which is comparable to literature.[4,5]

Table 1. Demographic and clinical characteristics of patients

Characteristic	*DPYD* variant allele carriers N = 85	Wild-type patients N = 1018	Total N = 1103	*P-value* [a]
Sex				
Male	48 (56%)	545 (54%)	593 (54%)	*0.68*
Female	37 (44%)	473 (46%)	510 (46%)	
Age				
Median [IQR]	63 [54-71]	64 [56-71]	64 [56-71]	*0.61*
Ethnic origin				
Caucasian	84 (99%)	964 (95%)	1048 (95%)	*0.61*
African	0	19 (2%)	19 (2%)	
Asian	1 (1%)	23 (2%)	24 (2%)	
Other [b]	0	12 (1%)	12 (1%)	
Tumor type				
Non-metastatic CRC	32 (38%)	440 (43%)	472 (43%)	*0.48*
Metastatic CRC	24 (28%)	208 (20%)	232 (21%)	
BC	10 (12%)	131 (13%)	141 (13%)	
GC	6 (7%)	57 (6%)	63 (6%)	
Other [c]	13 (15%)	182 (18%)	195 (18%)	
Type of treatment regimen				
CAPE mono	14 (16%)	191 (19%)	205 (19%)	*0.40*
CAPE + RT	18 (21%)	246 (24%)	264 (24%)	
CAPOX	31 (36%)	343 (34%)	374 (34%)	
CAPE other	5 (6%)	67 (7%)	72 (7%)	
5-FU mono	1 (1%)	1 (0%)	2 (0%)	
5-FU + RT	6 (7%)	57 (6%)	63 (6%)	
FOLFOX	5 (6%)	38 (4%)	43 (4%)	
5-FU other	5 (6%)	75 (7%)	80 (7%)	
BSA				
Median [IQR]	1.9 [1.8-2.1]	1.9 [1.8-2.1]	1.9 [1.8-2.1]	*0.60*
WHO performance status				
0	39 (46%)	515 (51%)	554 (50%)	*0.68*
1	36 (42%)	412 (40%)	448 (41%)	
2	4 (5%)	38 (4%)	42 (4%)	
NS [d]	6 (7%)	53 (5%)	59 (5%)	
Number of treatment cycles				
Median [IQR]	4 [1-8]	3 [1-8]	3 [1-8]	*0.97*
***DPYD* status**				
Wild-type	0	1018 (100%)	1018 (92%)	*NA*
c.1236G>A heterozygous	51 (60%)	0	51 (5%)	
c.2846A>T heterozygous	17 (20%)	0	17 (2%)	
*DPYD**2A heterozygous	16 (19%)	0	16 (1%)	
c.1679T>G heterozygous	1 (1%)	0	1	

[a] P-value comparing *DPYD* variant allele carriers to *DPYD* wild-type patients. A Kruskal-Wallis rank sum test was used for age, BSA, and number of treatment cycles, a Fisher's exact test was used for ethnic origin and WHO performance status and a χ^2 test for sex, tumor type, and treatment regimen.

[b] Other ethnic origins included Hispanic descent, mixed-racial parentage and unknown ethnic origin.

[c] Other tumor types included anal cancer, esophageal cancer, head and neck cancer, pancreas cancer, bladder cancer, unknown primary tumor, vulva carcinoma, and several rare tumor types.

[d] WHO performance status was not specified for these patients, but was either 0, 1, or 2, as this was required by the inclusion criteria of the study.

Abbreviations: 5-FU mono: 5-fluorouracil monotherapy; 5-FU other: 5-fluorouracil combined with other anticancer drugs (excluding the FOLFOX regimen); 5-FU + RT: 5-fluorouracil combined with radiotherapy (with or without mitomycin); BC: breast cancer; BSA: body surface area; CAPE mono: capecitabine monotherapy (with or without bevacizumab); CAPOX: capecitabine combined with oxaliplatin (with or without bevacizumab); CAPE other: capecitabine combined with other anticancer drugs; CAPE + RT: capecitabine combined with radiotherapy (with or without mitomycin); CRC: colorectal cancer; *DPYD*: gene encoding dihydropyrimidine dehydrogenase; FOLFOX: 5-fluorouracil combined with oxaliplatin and leucovorin (with or without bevacizumab); GC: gastric cancer; IQR: interquartile range; NA: not applicable; NS: not specified.

Table 2. Treatment outcome of patients included in this study

Type of event	*DPYD* variant allele carriers	Wild-type patients	*P-value*	c.1236G>A	c.2846A>T	*DPYD**2A	c.1679T>G
	N = 85	N = 1018		N = 51	N = 17	N = 16	N = 1
Relative dose intensity whole treatment: mean [range] [c]	69.1% [36.7% - 96.6%]	94.1% [48.8% - 127.6%]	*NA*	73.6% [50.9% - 96.6%]	71.6% [48.8% - 96.2%]	52..9% [36.7% - 74.1%]	54.2%
Relative dose intensity first cycle: mean [range] [c]	69.3% [24.8% - 96.2%]	96.3% [37.2% - 127.6%]	*NA*	74.0% [50.9% - 87.5%]	73.4% [55.3% - 96.2%]	51.1% [24.8% - 81.5%]	50.0%
Overall grade≥3 toxicity [d]	33 (39%)	231 (23%)	***0.0013*** [a]	20 (39%)	8 (47%)	5 (31%)	0
Grade≥3 gastrointestinal toxicity	17 (20%)	86 (8%)	***0.00089*** [a]	11 (22%)	4 (24%)	2 (13%)	0
Grade≥3 hematological toxicity	13 (15%)	65 (6%)	***0.0043*** [a]	7 (14%)	4 (24%)	2 (13%)	0
Grade 3 hand-foot syndrome [e]	1 (1%)	36 (4%)	*0.41* [b]	0	1 (6%)	0	0
Grade≥3 cardiac toxicity	1 (1%)	9 (1%)	*1.0* [b]	1 (2%)	0	0	0
Grade≥3 other treatment-related toxicity	9 (11%)	78 (8%)	*0.45* [a]	7 (14%)	1 (6%)	1 (6%)	0
Overall grade ≥4 toxicity [d]	4 (5%)	29 (3%)	*0.49* [b]	3 (6%)	1 (6%)	0	0
Grade≥4 gastrointestinal toxicity	1 (1%)	8 (1%)	*1.0* [b]	1 (2%)	0	0	0
Grade≥4 hematological toxicity	1 (1%)	12 (1%)	*1.0* [b]	1 (2%)	0	0	0
Grade≥4 cardiac toxicity	0	1 (0%)	*NA*	0	0	0	0
Grade≥4 other treatment-related toxicity	3 (4%)	9 (1%)	*0.12* [b]	2 (4%)	1 (6%)	0	0
Fluoropyrimidine-related hospitalization	16 (19%)	140 (14%)	*0.26* [a]	10 (20%)	4 (24%)	2 (13%)	0
Stop of fluoropyrimidines due to adverse events	15 (18%)	175 (17%)	*1.0* [a]	8 (16%)	3 (18%)	4 (25%)	0
Fluoropyrimidine-related death	1 (1%) **f**	3 (0%)	*0.55* [b]	0	1 (6%) [f]	0	0

[a] P-value determined with χ^2 test, with Yates' continuity correction. Values in bold are statistically significant (P < 0.05).

[b] P-value determined with Fisher's exact test with one-sided probability (with the P-value multiplied by two). Values in bold are statistically significant (P < 0.05).

[c] The relative dose intensity is calculated as the given dose in mg/m^2 divided by the standard dose in mg/m^2 given for the indication and treatment schedule which was applicable for the patient. The relative dose intensity was calculated for the first cycle alone and for the entire treatment duration.

[d] Overall toxicity includes all toxicities evaluated as possibly, probably or definitely related to fluoropyrimidine-treatment.

[e] Defined as palmar-plantar erythrodysesthesia syndrome by the Common Terminology Criteria for Adverse Events (CTC-AE) version 4.03.[18]

[f] This patient (c.2846A>T carrier) was wrongly treated with a full capecitabine dose for two cycles, which

resulted in fatal fluoropyrimidine-related toxicity.
Abbreviations: *DPYD*: gene encoding dihydropyrimidine dehydrogenase; NA: not applicable.

As another primary comparison, the relative risk for severe toxicity of *DPYD* variant allele carriers with genotype-guided dosing was compared with the corresponding relative risk for severe toxicity of *DPYD* variant allele carriers from a historical cohort of a previously performed meta-analysis.[10] *DPYD* variant allele carriers described in the meta-analysis were not identified prior to start of treatment and were therefore treated with a full dose. Relative risks for severe toxicity for each *DPYD* variant obtained in the meta-analysis[10] are described in **Table 3** (incidences of toxicity can be found in **Supplementary Table 2**) and were compared to calculated relative risks in the current study. This analysis showed that genotype-guided dosing reduced the relative risk for severe toxicity in *DPYD**2A carriers from 2.87 (95% confidence interval [95%CI]: 2..14-3.86)[10] when treated with full dose to 1.31 (95%CI: 0.63-2.73) when treated with individualized dose, thus showing a clinically relevant reduction of toxicity risk.

Interestingly, for c.1236G>A and c.2846A>T, a reduction in toxicity risk comparable to that of *DPYD* wild-type patients could not be demonstrated. The risk for c.1236G>A in the historical cohort was 1.72 (95%CI: 1.22-2.42),[10] and in our study it was 1.69 (95%CI: 1.18-2.42), showing that the toxicity risk was still increased even when applying a 25% dose reduction. For c.2846A>T, the risk of severe toxicity determined in the meta-analysis was 3.11 (95%CI: 2.25-4.28),[10] which was decreased to 2.00 (95%CI: 1.19-3.34) after 25% dose reduction. However, this risk was still higher compared to non-carriers of this variant.

For the c.1679T>G variant no relative risk could be calculated, as only one patient with this variant was included.

A total of 26 *DPYD* variant allele carriers (of which 16 c.1236G>A carriers, five c.2846A>T carriers, four *DPYD**2A carriers and one c.1679T>G carrier) treated with a reduced fluoropyrimidine dose gave informed consent to draw blood for pharmacokinetic analysis. Mean AUC values of the *DPYD* variant allele carriers and control values are depicted in **Figure 2**. Mean exposure to capecitabine and all metabolites, including 5-FU, was comparable between patients dosed based on *DPYD* genotype and control values,[20] suggesting that mean drug exposure of all combined *DPYD* variant allele carriers treated with a reduced dose was adequate. However, in line with toxicity data, AUC values for 5-FU were markedly higher for c.1236G>A carriers and especially for c.2846A>T carriers, compared to *DPYD**2A and c.1679T>G carriers as shown in **Supplementary Table 3**.

Table 3. Relative risk for severe toxicity of *DPYD* variant carriers compared to a historical cohort

DPYD variant	*DPYD* variant carriers treated with reduced dose (this study)	*DPYD* variant carriers treated with full dose (meta-analysis)
	Relative risk overall grade≥3 toxicity (95%CI) [a]	Relative risk overall grade≥3 toxicity (95%CI) [b]
c.1236G>A	1.69 (1.18 – 2.42)	1.72 (1.22 – 2.42)
c.2846A>T	2.00 (1.19 – 3.34)	3.11 (2.25 – 4.28)
*DPYD**2A	1.31 (0.63 – 2.73)	2.87 (2.14 – 3.86)
c.1679T>G	NA [c]	4.30 (2.10 – 8.80)

[a] Relative risk for overall grade≥3 fluoropyrimidine-related toxicity compared to non-carriers of this variant as described in Table 2.

[b] Relative risk for overall grade≥3 fluoropyrimidine-related toxicity compared to non-carriers of this variant, as determined in a random-effects meta-analysis by Meulendijks *et al.*[10] Unadjusted relative risks for the meta-analysis are depicted, as the relative risk in the current study was also calculated as an unadjusted value (as patient numbers were low).

[c] Relative risk cannot be calculated as only one patient who carried c.1679T>G was present. This patient did not experience severe toxicity.

Abbreviations: 95%CI: 95% confidence interval; NA: not applicable.

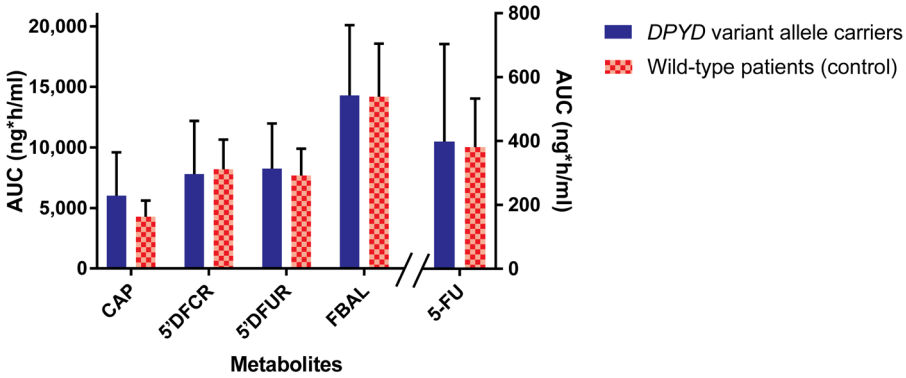

Figure 2. Pharmacokinetics of *DPYD*-guided capecitabine dosing

Depicted are the mean AUCs of capecitabine, and the metabolites 5'DFCR, 5'DFUR, 5-FU and FBAL of the *DPYD* variant allele carriers treated with *DPYD*-genotype guided dose (blue) and control values from wild-type patients from a published study (red).[20] Error bars represent the standard deviation.

Abbreviations: 5'DFCR: 5-deoxy-5-fluorocytidine; 5'DFUR: 5-deoxy-5-fluorouridine; 5-FU: 5-fluorouracil; AUC: area under the plasma concentration-time curve; CAP: capecitabine; FBAL: fluoro-β-alanine

In 56 *DPYD* variant allele carriers and 82 wild-type patients (participating in a subgroup of the study where DPD phenotyping tests were investigated), pretreatment DPD enzyme activity was determined (**Figure 3**). Mean DPD activity (with standard deviation) in *DPYD*

wild-type patients was 9.4 (3.6) nmol/(mg*h), similar to as previously published.[23] For the c.1236G>A variant (N = 35), the mean DPD activity was 7.5 (2.8) nmol/(mg*h) (i.e. a 20% reduction compared to wild-type). The mean DPD activity for c.2846A>T (N = 12) was 6.2 (1.9) nmol/(mg*h) (34% reduction), and for *DPYD**2A (N = 8) 5.2 (0.6) nmol/(mg*h) (45% reduction). The single patient carrying c.1679T>G had a DPD enzyme activity of 3.8 nmol/(mg*h) (60% reduction). For c.1236G>A, c.2846A>T, and *DPYD**2A, the mean DPD enzyme activity was significantly lower than the mean for wild-type patients. Statistical analysis was not possible for c.1679T>G. No correlation between DPD enzyme activity and the occurrence of severe fluoropyrimidine-related toxicity in *DPYD* variant allele carrying patients was seen (**Figure 3 and Supplementary Table 4**).

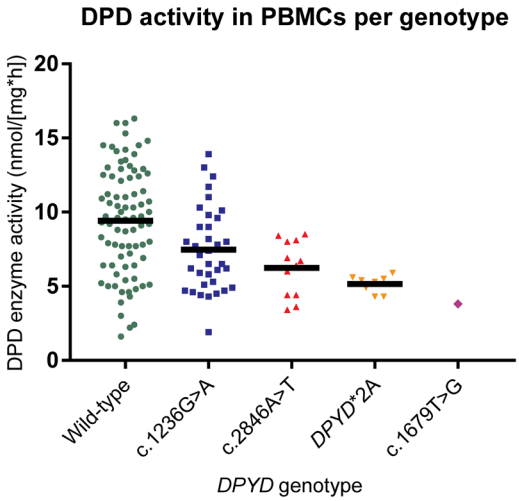

Figure 3. DPD enzyme activity in *DPYD* variant allele carriers and wild-type patients.
Wild-type patients were wild-type for the four *DPYD* variants that were prospectively tested. Mean DPD enzyme activity was statistically significantly lower than wild-type (mean 9.4 (3.6) nmol/[mg*h]) for the *DPYD* variants as determined by a *t*-test: c.1236G>A (7.5 (2.8) nmol/[mg*h], P = 0.0050), c.2846A>T (6.2 (1.9) nmol/[mg*h], P = 0.0034), and *DPYD**2A (5.2 (0.6) nmol/[mg*h], P = 0.0012). As only one patient carried c.1679T>G, no statistical test could be performed for this variant. However, the single measurement in this patient was in the range of DPD deficiency (3.8 nmol/[mg*h]). Patients with grade ≥3 fluoropyrimidine-related toxicity are depicted by closed triangles, patients without grade <3 toxicity by open circles; wild-type patients are treated with standard fluoropyrimidine doses, *DPYD* variant allele carriers with initially reduced doses according to protocol. PBMCs: peripheral blood mononuclear cells.

DISCUSSION

This is, to our knowledge, the first prospective study to investigate the effect on fluoropyrimidine-related toxicity by dose individualization based on four *DPYD* variants. Our results demonstrate that genotype-guided dosing is feasible in clinical practice. Dose individualization markedly decreased the risk of severe toxicity for *DPYD**2A carriers, was safe in the single c.1679T>G carrier, and moderately decreased the toxicity risk in c.2846A>T carriers. For c.1236G>A carriers, a 25% dose reduction was not enough to decrease severe treatment-related toxicity. This shows that *DPYD* genotype-guided dose-individualization is able to improve patient safety, as toxicity risk was reduced for three of the four variants in our study. Although sample sizes of variant allele carriers were modest and not all reductions in toxicity risk were statistically significant, these findings imply high clinical relevance. Also, implementation of *DPYD* genotype-guided dosing resulted in similar frequencies of toxicity-related hospitalization and discontinuation of treatment due to fluoropyrimidine-related toxicity for wild-type patients and *DPYD* variant allele carriers.

Interestingly, for *DPYD**2A carriers, the frequency of severe toxicity found in this study was 31%; drastically lower than the frequency in the historical cohort (72%). DPD enzyme activity measurements in this study showed that activity for *DPYD**2A carriers was approximately 50% reduced compared to wild-type patients, which endorses the dose recommendation of 50% for this variant.

As only one carrier of the rare c.1679T>G variant was identified in our current study, this made statistical comparisons impossible. However, while a relative risk for severe toxicity of 4.30 has been reported in literature, we showed that this patient did not experience severe toxicity in a completed treatment with 50% reduced dose. The DPD enzyme activity was about 50% decreased as well in this patient, which is in line with expectations based on previous studies.[24]

For carriers of the c.1236G>A and c.2846A>T variant, risk of severe toxicity remained relatively high despite dose individualization based on our dosing recommendations (25% reduction). In this study, 39% of the c.1236G>A carriers experienced severe toxicity and 47% of the c.2846A>T carriers. For these two variants, an initial dose reduction of 25% was applied in this study, because these variants are considered to have a less deleterious effect on DPD activity than the non-functional variants *DPYD**2A and c.1679T>G.[14,16] However, the Clinical Pharmacogenetics Implementation Consortium (CPIC) mentions that evidence is limited regarding the optimal degree of dose reduction for the decreased function variants c.1236G>A and c.2846A>T, and

a 25% dosing recommendation is mainly based on one small retrospective study.[25] Therefore, they advise a 25%-50% dose reduction in heterozygous c.1236G>A and c.2846A>T carriers.[13] Our current results suggest that applying 25% dose reduction might be insufficient for some patients, as toxicity risk was increased for carriers of c.1236G>A and c.2846A>T, compared to wild-type patients. In line with these findings, our pharmacokinetic analyses showed that exposure to 5-FU was markedly higher in c.2846A>T carriers than in *DPYD* wild-type controls. Exposure to 5-FU in the variant allele carriers was at least equal to levels observed in wild-type patients receiving standard dose, which is circumstantial evidence that the applied genotype-guided dose-reduction will not result in under-treatment. However, these pharmacokinetic results need to be interpreted with caution for some reasons. In patients with reduced DPD activity, 5-FU metabolism is affected, with 5-FU being the third metabolite derived from the parent compound capecitabine, which limits the interpretation of 5-FU exposure. Furthermore, pharmacokinetics of capecitabine and its metabolites exhibit a high inter-individual variability in exposure, even in wild-type patients, and are therefore difficult to interpret. In addition, based on the limited number of patients with a *DPYD* variant of whom we also obtained pharmacokinetic data (**Supplementary Table 3**) firm conclusions on the basis of pharmacokinetic measurements alone cannot be drawn.

The mean DPD enzyme activity for c.1236G>A was approximately 20% reduced, but a large variation in DPD activity was found (**Figure 3**), which suggests that a proportion of patients needs a larger dose reduction, while other patients might even tolerate a full dose. This is also in line with the large variation in pharmacokinetic exposure seen in c.1236G>A carriers. Individual dose titration is important to ensure an adequate and safe dose for all patients. Therefore, we recommend a more cautious initial dose reduction of 50%, followed by close monitoring and individual dose titration.

The mean value for c.2846A>T DPD enzyme activity was approximately 35% reduced compared to normal. These DPD activity measurements show that 25% dose reduction might not be sufficient for most of the patients, and this could be an explanation for the higher toxicity risk in this patient group. A more cautious initial dose reduction of 50% should be considered in these patients as well.

In this study, initially reduced doses were escalated in 11 out of 85 (13%) *DPYD* variant allele carriers, although only five patients were able to tolerate this escalated dose. In *DPYD* wild-type patients dose escalations are uncommon in clinical practice (3% in our study, mostly patients who started with an initially reduced dose as a precaution measure).

Our study was performed in a daily clinical care setting in general regional hospitals and a few academic centers, demonstrating the feasibility of implementation of upfront *DPYD* screening. In order to make *DPYD*-guided dosing feasible in all hospitals, it is important that the turn-around time for *DPYD* genotyping is short to prevent a delay in the start of treatment. Participating laboratories in our study had a turn-around time of a few days to a maximum of a week.

A limitation of this study is that a historical cohort of *DPYD* variant allele carriers treated with full dose was used as control, and no direct comparison was made with a control cohort within the study. Inherently to this chosen design, differences between the study populations could have influenced the observed toxicity outcomes. However, this study design was chosen as a randomized clinical trial is considered unethical in this context, since it is known that *DPYD* variant allele carriers are at increased risk of severe toxicity when treated with a full dose of fluoropyrimidines.[26] A previously performed clinical study was stopped prematurely as a patient in the arm without dose individualization died due to treatment-related toxicity.[27]

This study focused on toxicity and did not evaluate survival or other effectiveness outcomes, as this was considered not feasible due to the large variation in tumor types and treatment regimens. We did, however, perform pharmacokinetic measurements, which suggest that applied dose reductions in *DPYD* variant allele carriers did not result in under-dosing.

The four *DPYD* variants investigated in this study are especially relevant to Caucasian populations. For ethnicities other than Caucasians, more research on the frequency and clinical relevance of these and other *DPYD* variants is recommended.[28] In our current study, homozygous and compound heterozygous *DPYD* variant allele carriers were not included and were treated with individualized fluoropyrimidine dosing or alternative treatment outside this study.[17] However, for this group of patients *DPYD* genotype-guided dosing is of even greater importance than for heterozygous *DPYD* variant allele carriers, as these patients in general have less remaining DPD activity or even complete absence of DPD activity, and a full fluoropyrimidine dose, when not identified as DPD deficient patients, is therefore likely to be fatal.

Although our study revealed that the applied approach of genotype-guided adaptive dosing significantly reduced severe fluoropyrimidine-induced toxicity and prevented treatment related death, additional methods should be explored and prospectively tested to further reduce treatment related toxicity not only in poor metabolizers, but also in *DPYD* wild-type patients.

In conclusion, we showed safety of patients treated with fluoropyrimidines was improved by dose individualization based on *DPYD* genotype. Dose reduction of 50% in heterozygous *DPYD**2A and c.1679T>G carriers reduced toxicity risk markedly. The applied dose reductions of 25% in heterozygous c.1236G>A and c.2846A>T carriers appear to be insufficient to lower the risk of fluoropyrimidine-related toxicity to the background risk in wild-type patients. A larger initial dose reduction of 50% for c.2846A>T and c.1236G>A carriers with subsequent individual dose titrations should therefore be considered.

Acknowledgments

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SUPPLEMENTARY INFORMATION SUPPLEMENTARY METHODS

INCLUSION AND EXCLUSION CRITERIA

Patients with a pathologically confirmed malignancy for which treatment with a fluoropyrimidine drug was considered to be in the patient's best interest could be included in this study. Eligible patients were 18 years or older and were willing to undergo blood sampling for the purpose of this study (pharmacogenetic and phenotyping analysis). Patients had to have a WHO performance status of 0, 1 or 2, a life expectancy of at least 12 weeks, and acceptable safety laboratory values (neutrophil count of $\geq$1.5 x 10^9/L, platelet count of $\geq$100 x 10^9/L, hepatic function as defined by serum bilirubin $\leq$1.5 x upper limit of normal (ULN), alanine aminotransferase (ALAT), and aspartate aminotransferase (ASAT) $\leq$2.5 x ULN, or in case of liver metastases ALAT and ASAT$\leq$5 x ULN, renal function as defined by serum creatinine $\leq$1.5 x ULN, or creatinine clearance $\geq$60 ml/min (by Cockcroft-Gault formula).

Exclusion criteria were prior treatment with fluoropyrimidines, patients with known substance abuse, psychotic disorders, and/or other diseases expected to interfere with study or the patient's safety, women who were pregnant or breast feeding, man and women who refused to use reliable contraceptive methods throughout the study, and patients with a homozygous polymorphic *DPYD* genotype or compound heterozygous *DPYD* genotype.

TOXICITY ASSESSMENTS

For causality assessment of toxicity the following definitions were used:

- Possible: the event follows a reasonable temporal sequence from the time of drug administration, but could have been produced by other factors such as the patient's clinical state, other therapeutic interventions or concomitant drugs.
- Probable: the event follows a reasonable temporal sequence from the time of drug administration, and follows a known response pattern to the study drug. The toxicity cannot be reasonably explained by other factors such as the patient's clinical state, therapeutic interventions or concomitant drugs.
- Definite: the event follows a reasonable temporal sequence from the time of drug administration, and follows a known response pattern to the study drug, cannot be reasonably explained by other factors such as the patient's condition, therapeutic interventions or concomitant drugs; AND occurs immediately following study drug administration, improves on stopping the drug, or reappears on re-exposure.

SAMPLE SIZE CALCULATION

A sample size calculation was made based on the primary aim of the study, which was to determine whether fluoropyrimidine-related severe toxicity can be reduced by individualized dosing in *DPYD* variant allele carriers compared to standard dosing in these patients. Using a one stage A'Hern (phase II) design and a null hypothesis of a probability of toxicity of 60% (the estimated severe treatment-related toxicity probability if *DPYD* variant allele carriers received standard dose)[1,2] and an alternative hypothesis of 20% (estimated toxicity probability of *DPYD* variant allele carriers receiving individualized dose), a sample size of 11 *DPYD* variant allele carriers would give a one-sided type I error probability α of 2.93% and power of 83.9%. It was decided that the frequency of c.2846A>T carriers (approximately 1.0%)[3] would determine the total number of patients required in the study. These patients would then arise from an expected minimum population of 1100 treated patients. To account for a proportion of patients not evaluable for the study, the target accrual was set at 1250 patients. Given the very low allele frequency of the c.1679T>G variant, it was considered not feasible to power this study for this particular variant. The estimated frequency of c.1236G>A is 3% and of *DPYD**2A 1%, which means that the calculated sample size would be adequate for those individual variants, or when analyzing all four variants together (estimated frequency of 5%).

PHARMACOKINETIC ANALYSES

For pharmacokinetic analyses, peripheral blood was collected on the first day of treatment. Blood was collected in lithium heparin tubes at nine different time points up to eight hours after capecitabine intake (pre-dose, 0.25, 0.5, 1, 2, 3, 4, 6, and 8 hours after capecitabine intake). Samples were centrifuged immediately after the blood was drawn and plasma was stored at -80°C until analysis.

Capecitabine and the metabolites 5'-deoxy-5-fluorocytidine (5'DFCR), 5'-deoxy-5-fluorourdine (5'DFUR), 5-fluorouracil (5-FU), and fluoro-β-alanine (FBAL) were quantified in plasma samples using a validated ultra-performance liquid chromatography (UPLC)-tandem mass spectrometry (MS/MS) method. Lower limit of quantifications were 25 ng/ml for capecitabine, 10 ng/ml for 5'DFCR, 5'DFUR and 5-FU, and 50 ng/ml for FBAL. Stable isotopes were used as internal standard for all analytes. To a sample volume of 300 µl of plasma, 900 µl of methanol-acetonitrile (50:50 v/v) was added to precipitate the plasma proteins. Samples were vortex-mixed for 10 s, shaken for 10 min at 1,250 rpm and centrifuged at 14,000 rpm for 10 minutes. The clear supernatants were dried under a stream of nitrogen at 40°C and reconstituted in 100 µl of 0.1% formic acid in water. An Acquity UPLC® HSS T3 column (150 x 2.1 mm ID, 1.8 µlm particles) was used for chromatographic separation, at a flow rate of 300 µl/min and a gradient of

0.1% formic acid in water (mobile phase A) and 0.1% formic acid in acetonitrile (mobile phase B). The following gradient was applied: 100% A from 0-2.5 min, an increase from 0% to 90% B from 2.5-7.5 min, and 100% A from 7.5-9 min. For detection an API5500 triple quadruple mass spectrometer (Sciex) equipped with a turbo ionspray interphase was used, using optimized mass transitions *m/z* 360.0 → 243.9 for capecitabine, 244.9 → 128.8 for 5'DFUR, 128.9 → 42.1 for 5-FU, and 105.9 → 85.9 for FBAL.

Pharmacokinetic parameters were calculated using non-compartmental analysis and the calculated area under the plasma concentration-time curve (AUC) and half-life ($t_{1/2}$) were compared with pharmacokinetic data described in literature,[4] measured at the same laboratory as the current study.

DATA SHARING STATEMENT

Data collected in the study, including individual participant data, will not be made available to others, except to researchers involved in the study. However, upon request, data sharing for additional research is possible and will be supported. Requests will be judged on scientific and clinical rationale and may need to be reviewed by an authorized institutional review board (IRB) prior to data sharing. The study protocol of this study is publicly available (as online supplement available with this publication).

SUPPLEMENTARY RESULTS

DETAILED INFORMATION OF DPYD VARIANT ALLELE CARRIERS NOT TREATED ACCORDING TO DOSING RECOMMENDATIONS

For four patients dosing recommendations were not followed according to protocol. One patient carrying *DPYD**2A started with a full dose as genotyping results were not awaited before start of treatment. After one week of treatment the *DPYD* genotyping result became available and the dose was reduced to 50%. The patient did not experience severe treatment-related toxicity in this course. However, from the third cycle onwards the dose was quickly titrated upwards (75% in the third cycle and 90% in the fourth cycle), hereafter treatment-related toxicity (anorexia grade 2, fatigue grade 3) occurred and the dose was reduced again. A second patient (*DPYD**2A carrier) also started with a full dose as genotyping results were not awaited before starting treatment. As results were known the following day, the patient had only taken a full dose for one day, which did not result in severe toxicity. The patient was treated with a 50% dose from the second day onwards. A third patient carrying c.2846A>T, used a full dose for four days, but continued with a 50% dose after an interruption of 5 days. The

overall dose intensity of this cycle was approximately 55% and no toxicity occurred. The fourth patient (c.2846A>T carrier) was wrongly treated with a full dose for two cycles due to miscommunication with the patient. The patient experienced severe diarrhea, pancytopenia and sepsis, and passed away.

PHARMACOKINETIC ANALYSES

A total of 26 *DPYD* variant allele carriers treated with reduced dose of capecitabine was included in the analysis. Pharmacokinetic results are shown in Supplementary Table 3. In 24 out 26 patients (92%) pharmacokinetic sampling was performed at day 1 of cycle 1. In two patients this was done at day 1 of another cycle, after a resting period of one week without capecitabine intake.

Of five patients who were treated with 5-FU, pharmacokinetic blood samplings was performed as well, but results were considered unreliable, most likely as drawing of blood was not done correctly. Results of the 5-FU treated patients are therefore not included in the analysis.

Supplementary Table 1. Demographic and clinical characteristics of *DPYD* variant allele carriers

Characteristic	*DPYD* variant allele carriers	c.1236G>A	c.2846A>T	*DPYD**2A	c.1679T>G
	N = 85	*N* = 51	*N* = 17	*N* = 16	*N* = 1
Sex					
Male	48 (56%)	26 (51%)	11 (65%)	10 (63%)	1 (100%)
Female	37 (44%)	25 (49%)	6 (35%)	6 (38%)	0
Age					
Median [IQR]	63 [54-71]	62 [52-71]	62 [53-72]	64 [58-70]	70
Ethnic origin					
Caucasian	84 (99%)	51 (100%)	17 (100%)	15 (94%)	1 (100%)
African	0	0	0	0	0
Asian	1 (1%)	0	0	1 (6%)	0
Other [a]	0	0	0	0	0
Tumor type					
Non-metastatic CRC	32 (38%)	15 (29%)	7 (40%)	9 (56%)	1 (100%)
Metastatic CRC	24 (28%)	17 (33%)	4 (24%)	3 (19%)	0
BC	10 (12%)	5 (10%)	3 (18%)	2 (13%)	0
GC	6 (7%)	4 (8%)	1 (6%)	1 (6%)	0
Other [b]	13 (15%)	10 (20%)	2 (12%)	1 (6%)	0
Type of treatment regimen					
CAPE mono	14 (16%)	8 (16%)	4 (24%)	2 (13%)	0
CAPE + RT	18 (21%)	8 (16%)	5 (29%)	5 (31%)	0
CAPOX	31 (36%)	19 (37%)	5 (29%)	6 (38%)	1 (100%)
CAPE other	5 (6%)	3 (6%)	1 (6%)	1 (6%)	0
5-FU mono	1 (1%)	0	0	1 (6%)	0
5-FU + RT	6 (7%)	6 (12%)	0	0	0
FOLFOX	5 (6%)	2 (4%)	2 (12%)	1 (6%)	0
5-FU other	5 (6%)	5 (10%)	0	0	0
BSA					
Median [IQR]	1.9 [1.8-2.1]	1.9 [1.7-2.1]	2.0 [1.7-2.1]	2.0 [1.5-2.5]	2.1
WHO performance status					
0	39 (46%)	26 (51%)	8 (47%)	4 (25%)	1 (100%)
1	36 (42%)	18 (35%)	9 (53%)	9 (56%)	0
2	4 (5%	3 (6%)	0	1 (6%)	0
NS [c]	6 (7%)	4 (8%)	0	2 (13%)	0
Number of treatment cycles					
Median [IQR]	4 [1-8]	4 [2-8]	3 [1-7]	3 [1-7]	3

[a] Other ethnic origins included Hispanic descent, mixed-racial parentage and unknown ethnic origin.

[b] Other tumor types included anal cancer, esophageal cancer, head and neck cancer, pancreas cancer, bladder cancer, unknown primary tumor, vulva carcinoma, and several rare tumor types.

[c] WHO performance status was not specified for these patients, but was either 0,1, or 2, as this was required by the inclusion criteria of the study.

Abbreviations: 5-FU mono: 5-fluorouracil monotherapy; 5-FU other: 5-fluorouracil combined with other anticancer drugs (excluding the FOLFOX regimen); 5-FU + RT: 5-fluorouracil combined with radiotherapy (with or without mitomycin); BC: breast cancer; BSA: body surface area; CAPE mono: capecitabine monotherapy (with or without bevacizumab); CAPOX: capecitabine combined with oxaliplatin (with or without bevacizumab); CAPE other: capecitabine combined with other anticancer drugs; CAPE + RT: capecitabine combined with radiotherapy (with or without mitomycin); CRC: colorectal cancer; *DPYD*: gene encoding dihydropyrimidine dehydrogenase; FOLFOX: 5-fluorouracil combined with oxaliplatin and leucovorin (with or without bevacizumab); GC: gastric cancer; IQR interquartile range; NS: not specified.

Supplementary Table 2. Incidences of severe toxicity in *DPYD* variant allele carriers in this study and the historical cohort

DPYD variant	*DPYD* variant carriers treated with reduced dose (this study)	*DPYD* variant carriers treated with full dose (meta-analysis)
	N of patients with overall grade≥3 toxicity / total *N* of patients with this variant (%)	*N* of patients with overall grade≥3 toxicity / total *N* of patients with this variant (%)
c.1236G>A	20 / 51 (49%)	65 / 177 (37%)
c.2846A>T	8 / 17 (47%)	53 / 85 (62%)
*DPYD**2A	5 / 16 (31%)	43 / 60 (72%)
c.1679T>G	0 / 1 (0%)	6 / 11 (55%)

Supplementary Table 3. Pharmacokinetic parameters of capecitabine and metabolites in *DPYD* variant allele carriers and controls

Metabolite	Mean $AUC_{0-\bullet}$ (ng*h/ml) [CV%] [a]		Mean $T_{1/2}$ (h) [CV%]	
	DPYD variant allele carriers (N=26)	Wild-type control patients (N=23) [b]	DPYD variant allele carriers (N=26)	Wild-type control patients (N=23) [b]
Capecitabine	6007 [60%]	4281 [31%]	0.73 [49%]	0.76 [55%]
5'DFCR	7792 [56%]	8192 [30%]	0.83 [43%]	1.0 [45%]
5'DFUR	8243 [45%]	7673 [29%]	0.85 [38%]	0.9 [34%]
5-FU	398 [77%]	381 [40%]	0.92 [112%]	1.0 [57%]
FBAL	14295 [41%]	14177 [31%]	2.2 [133%]	2.6 [33%]

Metabolite	Mean $AUC_{0-\bullet}$ (ng*h/ml) [CV%] [a]				Mean $T_{1/2}$ (h) [CV%]			
	c.1236G>A (N=16)	c.2846A>T (N=5)	DPYD*2A (N=4)	c.1679T>G (N=1)	c.1236G>A (N=16)	c.2846A>T (N=5)	DPYD*2A (N=4)	c.1679T>G (N=1)
Capecitabine	6579 [65%]	5944 [26%]	4460 [51%]	3350	0.77 [48%]	0.68 [50%]	0.66 [61%]	0.53
5'DFCR	9162 [45%]	8320 [50%]	2552 [25%]	4185	0.84 [51%]	0.88 [28%]	0.83 [21%]	0.51
5'DFUR	9319 [41%]	8150 [46%]	4824 [17%]	5161	0.84 [38%]	0.73 [41%]	0.84 [19%]	1.6
5-FU	346 [49%]	765 [64%]	197 [54%]	219	1.1 [120%]	0.75 [47%]	0.54 [20%]	0.82
FBAL	16217 [30%]	15627 [36%]	6244 [18%]	9082	2.9 [78%]	2.5 [14%]	2.2 [15%]	2.9

[a] Note that for all metabolites the AUC is calculated until infinity ($AUC_{0-\bullet}$, extrapolated from the last time point). Only for FBAL, the metabolite with the longest half-life, this resulted in a difference between the AUC until last time point (AUC_{0-t}) and $AUC_{0-\bullet}$, with the $AUC_{0-\bullet}$ being on average 26% higher than AUC_{0-t}. For the metabolites capecitabine, 5'DFCR, 5'DFUR and 5-FU the difference between AUC_{0-t} and $AUC_{0-\bullet}$ was respectively 0.5%, 0.8%, 1%, and 4%.

[b] Control values are derived from Deenen *et al.*[4] for patients with advanced cancer of the stomach or gastresophageal junction after administration of capecitabine 850 mg/m2 (dose level 2 of the study).

Abbreviations: 5'DFCR: 5'-deoxy-5-fluorocytidine; 5'DFUR: 5'-deoxy-5-fluorouridine; 5-FU: 5-fluorouracil; AUC: area under the plasma concentration-time curve; CV%: coefficient of variation; FBAL: fluoro-β-alanine; $T_{1/2}$: half-life.

Supplementary Table 4. DPD enzyme activity in patients with and without severe toxicity

DPYD genotype	Patients without severe toxicity [a]		Patients with severe toxicity [a]		*P-value* [b]
	Mean activity (SD)	N of patients	Mean activity (SD)	N of patients	
Wild-type	9.6 (3.6)	67	8.7 (3.7)	15	*0.36*
c.1236G>A	7.6 (3.0)	22	7.3 (2.6)	13	*0.79*
c.2846A>T	6.8 (1.9)	6	5.7 (1.8)	6	*0.33*
*DPYD**2A	4.9 (0.7)	5	5.5 (1.1)	3	*0.22*
c.1679T>G	NA	1	NA	0	*NA*

[a] Severe toxicity is defined as CTC-AE grade 3 or higher.

[b] *P* value determined with *t*-test.

Abbreviations: CTC-AE: National Cancer Institute Common Terminology Criteria for Adverse Events; NA: not applicable.

Supplementary Table 5. Overview of participating centers in this study

Center	Principal investigator	Number of eligible patients included
Erasmus Medical Center, Rotterdam, the Netherlands	Prof. Ron H.J. Mathijssen, MD	264
The Netherlands Cancer Institute, Amsterdam, the Netherlands	Prof. Jan H.M. Schellens, MD	210
Catharina Hospital, Eindhoven, the Netherlands	Geert-Jan Creemers, MD	118
Leiden University Medical Center, Leiden, the Netherlands	Prof. Hans Gelderblom, MD	93
Hospital Gelderse Vallei, Ede, the Netherlands	Arnold Baars, MD	88
Reinier de Graaf Hospital, Delft, the Netherlands	Vincent O. Dezentjé, MD / Annelie J.E. Vulink, MD [a]	79
Haaglanden Medical Center, the Hague, the Netherlands	Frank J.F. Jeurissen, MD	46
Deventer Hospital, Deventer, the Netherlands	Alexander L.T. Imholz, MD	41
Haga Hospital, the Hague, the Netherlands	Prof. Johanna E.A. Portielje, MD / Danny Houtsma, MD [a]	35
Maastricht University Medical Center, Maastricht, the Netherlands	Rob L.H. Jansen, MD	28
Franciscus Gasthuis and Vlietland, Rotterdam, the Netherlands	Paul Hamberg, MD	24
Amphia Hospital, Breda, the Netherlands	Albert J. ten Tije, MD	20
Bravis Hospital, Roosendaal, the Netherlands	Helga J. Droogendijk, MD	17
University Medical Center, Utrecht, the Netherlands	Prof. Miriam Koopman, MD	14
Wilhelmina Hospital, Assen, the Netherlands	Peter Nieboer, MD	13
Laurentius Hospital, Roermond, the Netherlands	Marlène H.W. van de Poel, MD	9
Canisius-Wilhelmina Hospital, the Netherlands	Caroline M.P.W. Mandigers, MD	4

[a] In these centers the principal investigator was switched during the study.

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4

A COST ANALYSIS OF UPFRONT *DPYD* GENOTYPE-GUIDED DOSE INDIVIDUALISATION IN FLUOROPYRIMIDINE-BASED ANTICANCER THERAPY

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** Authors contributed equally*

ABSTRACT

BACKGROUND

Fluoropyrimidine therapy including capecitabine or 5-fluorouracil can result in severe treatment-related toxicity in up to 30% of patients. Toxicity is often related to reduced activity of dihydropyrimidine dehydrogenase (DPD), the main metabolic fluoropyrimidine enzyme, primarily caused by genetic *DPYD* polymorphisms. In a large prospective study, it was concluded that upfront *DPYD*-guided dose individualization is able to improve safety of fluoropyrimidine-based therapy. In our current analysis, we evaluated whether this strategy is cost-saving.

METHODS

A cost-minimization analysis from a health care payer perspective was performed as part of the prospective clinical trial (NCT02324452) in which patients prior to start of fluoropyrimidine-based therapy were screened for the *DPYD* variants *DPYD**2A, c.2846A>T, c.1679T>G, and c.1236G>A, and received an initial dose reduction of 25% (c.2846A>T, c.1236G>A) or 50% (*DPYD**2A, c.1679T>G). Data on treatment, toxicity, hospitalization and other toxicity-related interventions were collected. The model compared prospective screening for these *DPYD* variants with no *DPYD* screening. One-way and probabilistic sensitivity analyses were also performed.

RESULTS

Expected total costs of the screening strategy were €2,599 per patient, compared to €2,650 for non-screening, resulting in a net cost-saving of €51 per patient. Results of the probabilistic sensitivity and one-way sensitivity analysis demonstrated that the screening strategy was very likely to be cost-saving or worst case cost-neutral.

CONCLUSIONS

Upfront *DPYD*-guided dose individualization, improving patient safety, is cost-saving or cost-neutral, but is not expected to yield additional costs. These results endorse implementing *DPYD* screening before start of fluoropyrimidine treatment as standard of care.

INTRODUCTION

The class of fluoropyrimidine anticancer drugs includes 5-fluorouracil (5-FU) and its oral prodrug capecitabine. These drugs are used by approximately two million patients yearly worldwide,[1] and are the cornerstone of chemotherapeutic treatment for several solid tumor types, including colorectal, breast, gastric and head- and neck cancer. While fluoropyrimidine drugs are highly valuable treatment options, severe and potential fatal fluoropyrimidine-related toxicity remains a major clinical limitation. Around 15-30% of the patients develop severe treatment-related toxicity,[2,3] usually associated with interruption or discontinuation of therapy and often hospitalization, resulting in increased health care costs.

During the last decades it has become clear that safety of patients treated with fluoropyrimidine-based anticancer therapy is strongly affected by inter-individual variability in the enzyme dihydropyrimidine dehydrogenase (DPD), which is the main metabolic enzyme of fluoropyrimidines. The DPD enzyme is present in the liver and inactivates over 80% of 5-FU.[4] DPD enzyme activity varies widely between patients, with an estimated 3 to 8% of the population having a reduced DPD activity.[5,6] DPD deficiency results in reduced 5-FU clearance, and as a direct consequence, highly increased risk of severe treatment-related toxicity when DPD-deficient patients are treated with standard doses of a fluoropyrimidine drug.[7]

DPD deficiency can be caused by genetic polymorphisms in *DPYD*, the gene encoding DPD. Currently, four *DPYD* variants are considered as being clinically relevant and dosing recommendations are provided for these variants: *DPYD**2A, c.1679T>G, c.2846A>T and c.1236G>A).[8,9] Upfront genotyping followed by a fluoropyrimidine dose reduction in carriers in any of these four variants has proven a useful strategy to improve patient safety.[10,11] However, this strategy has not yet been universally implemented in daily clinical care.

One of the potential barriers that can make physicians reluctant to implement upfront *DPYD* screening as a routine test, is uncertainty on the cost-effectiveness of a *DPYD* screening strategy.[12] Deenen *et al.* previously showed that upfront screening for one *DPYD* variant, *DPYD**2A, is cost-saving, as average total medical costs in the screening arm were €2,772 per patient and therefore lower than the non-screening arm, for which the average total medical costs were €2,817 per patient. This shows that the reduction in toxicity-related costs outweighs the screening costs.[10] In our current study, we aimed to investigate the medical costs associated with upfront screening for the four *DPYD* variants currently considered clinically relevant and dose individualization

in heterozygous carriers of a *DPYD* variant, therefore evaluating the net cost effects of this expanded *DPYD* genotyping strategy.

PATIENTS AND METHODS

STUDY DESIGN AND PARTICIPANTS

The cost-analysis was performed as part of a recently published clinical trial.[11] This was a multicenter study in which seventeen hospitals in the Netherlands participated (NCT02324452). Study approval was obtained by the institutional review board of The Netherlands Cancer Institute, Amsterdam, the Netherlands, and approval from the board of directors of each individual hospital was obtained for all participating centers. All patients provided written informed consent before inclusion in the study.

The study population consisted of patients treated with a fluoropyrimidine-based anticancer therapy, either as single agent or in combination with other chemotherapeutic agents and/or radiotherapy. Prior chemotherapy was allowed, except for prior use of fluoropyrimidines. Before start of fluoropyrimidine therapy, patients were genotyped for four *DPYD* variants (*DPYD**2A, c.1679T>G, c.2846A>T and c.1236G>A). Heterozygous *DPYD* variant allele carriers received an initial dose reduction of either 25% (for c.2846A>T and c.1236G>A) or 50% (for *DPYD**2A and c.1679T>G), in line with current recommendations from Dutch and international pharmacogenomic guidelines.[9,13] To achieve maximal safe exposure, dose escalation was allowed after the first two cycles, provided that treatment was well tolerated and was left at the discretion of the physician. The dose of other chemotherapeutic agents or radiotherapy was left unchanged at the start of treatment. Homozygous or compound heterozygous *DPYD* variant allele carriers were not included in the study. Non-carriers of the above mentioned *DPYD* variants were considered wild-type patients in this study, and were treated according to existing standard of care.

Toxicity was graded by participating centers according to the National Cancer Institute Common Terminology Criteria for Adverse Events (CTC-AE),[14] and severe toxicity was defined as grade three or higher. Patients were followed for toxicity during the entire treatment period. Toxicity defined as possibly, probably or definitely related to fluoropyrimidine-treatment was considered treatment-related toxicity. Toxicity-related hospitalization and treatment discontinuation due to adverse events were also investigated.

The primary end point of the prospective study was the frequency of severe overall fluoropyrimidine-related toxicity across the entire treatment duration. A comparison was made between *DPYD* variant allele carriers treated with reduced dose and wild-type patients treated with standard dose in this study, and also with *DPYD* variant allele carriers treated with full dose in a historical cohort derived from a previously published meta-analysis.[8] Secondary endpoints of the prospective study included a cost-analysis of individualized dosing based on upfront genotypic assessment, and pharmacokinetics of capecitabine and 5-FU in *DPYD* variant allele carriers.

COST-ANALYSIS

To compare the prospective screening for four *DPYD* variants (screening strategy) with no *DPYD* screening (non-screening strategy), a cost-analysis model was composed. This analysis consisted of a cost-minimization analysis using a decision analytical model from a health care payer perspective.

A previously published model by Deenen *et al.*[10] was used and updated with data from the current study and current prices. Estimated parameters incorporated in the model were derived from data of the present trial and relevant data from literature.[15,16] Interventions for treatment-related toxicity were prospectively collected for all patients during the trial. An overview of the decision tree is depicted in **Figure 1**.

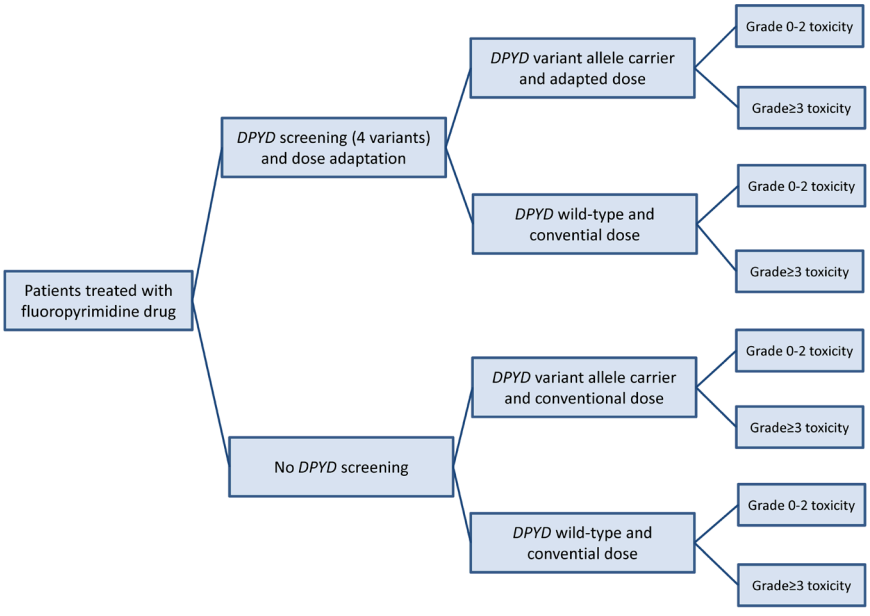

Figure 1. Decision tree for cost-analysis

In the model, a comparison between the screening strategy (prospective screening for four *DPYD* variants and dose adjustments in heterozygous *DPYD* variant allele carriers) and the non-screening strategy was made. Expected differences in costs of both strategies were calculated.

Costs included were restricted to direct medical costs only and included costs for genotyping, fluoropyrimidine drug therapy including visits to the medical doctor and day care, costs for treatment of adverse events (e.g. extra medication, extra doctor visits, extra assessments), and costs for hospitalization due to adverse events. Costs for other anticancer drugs than the fluoropyrimidine drugs were not included in the model, as they were expected to be equal in both arms. Cost-saving was calculated as the difference between the net direct costs of the *DPYD* screening strategy versus the non-screening strategy.

To examine the effects on variations in parameter values, one-way and probabilistic sensitivity analyses were performed. In the one-way sensitivity analysis, each parameter was varied individually at ±20% of the baseline value. In the probabilistic sensitivity analysis, all parameters were varied simultaneously by running 1000 simulations (Monte Carlo). Since the parameter values of the wild-type patients for both the screening and the non-screening arm are identical, these parameters remained fixed in the probabilistic sensitivity analysis.

RESULTS

PATIENT CHARACTERISTICS AND TOXICITY INCIDENCE

The study was open for inclusion between April 30th, 2015 and December 21st, 2017. In this period, a total of 1103 evaluable patients were enrolled in this study, of whom 85 heterozygous *DPYD* variant allele carriers (7.7%) and 1018 wild-type patients (92.3%). The group of *DPYD* variant allele carriers included 51 c.1236G>A carriers, seventeen c.2846A>T carriers, sixteen *DPYD**2A carriers and one c.1679T>G carrier. Details on patient characteristics, treatment and toxicity incidence are published separately.[11] In short, 33 out of 85 *DPYD* variant allele carriers (39%) experienced grade ≥3 treatment-related toxicity, while this was significantly lower in the group of wild-type patients with 231 out of 1018 patients (23%) experiencing severe toxicity (P=0.001). Compared to the historical cohort of *DPYD* variant allele carriers treated with full dose, *DPYD* genotype-guided dosing markedly decreased the risk of severe fluoropyrimidine-related toxicity for three out of four variants (*DPYD**2A, c.1679T>G and c.2846A>T;

Figure 2). No reduction in severe treatment-related toxicity was shown for c.1236G>A.

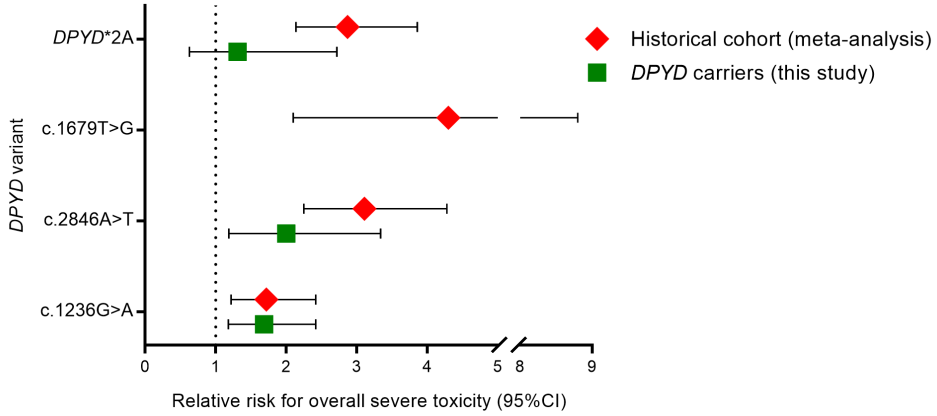

Figure 2. Relative risk for severe treatment-related toxicity of *DPYD* variant allele carriers receiving dose-reduction (this study) and *DPYD* variant allele carriers treated with full dose (historical cohort)

The relative risk for overall grade ≥3 fluoropyrimidine-related toxicity compared to non-carriers of this variant was calculated with data from this study[11] and for the historical cohort with data derived from a previously published random-effects meta-analysis.[8] Unadjusted relative risks for the meta-analysis are depicted, as the relative risk in the current study was also calculated as an unadjusted value. For c.1679T>G no relative risk could be calculated in this study, as only one patient who carried c.1679T>G was present. This patient did not experience severe toxicity.

Abbreviations: 95%CI: 95% confidence interval.

COST-ANALYSIS

All parameter estimates used in the model are provided in **Table 1**. In the cost-analysis the expected total costs for the screening strategy were €2,599 per patient, compared to €2,650 per patient for the non-screening strategy, resulting in a net cost-saving of €51 per patient treated.

Results of the one-way sensitivity analysis are depicted in **Figure 3**, demonstrating that the frequency of the *DPYD* variant allele genotype had the largest influence on outcome of the cost-analysis, followed by the risk of hospitalization at the nursing ward for *DPYD* variant allele carrier receiving standard dose, and *DPYD* genotyping costs. However, in all cases, the cost-saving remained positive.

Results of the simulations for the probabilistic sensitivity analysis are depicted in **Figure 4**. Average cost-savings from the simulation in the probabilistic sensitivity analysis were

€52 per patient (95%-interval range -€38 to €176). Average gain in safety was 0.89% (95%-interval range -0.04% to 1.79%). This gain in safety represents the difference between the proportion of patients treated without severe toxicity (both wild-type patients and *DPYD* variant allele carriers taken together) in the screening strategy and the non-screening strategy.

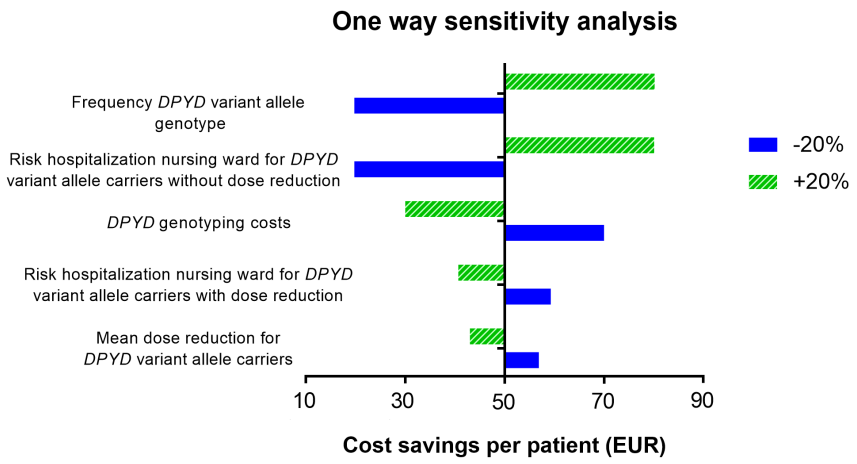

Figure 3. One-way sensitivity analysis of upfront *DPYD* genotyping versus non-screening

All parameters were individually varied by ±20% (-20% depicted in blue, +20% depicted in green), effects of which cost-savings are indicated by horizontal bars. The vertical line indicates the baseline costs savings of €50.

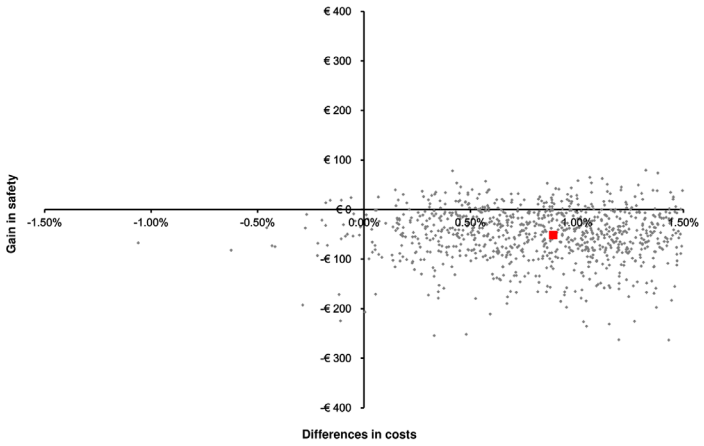

Figure 4. Probabilistic sensitivity analysis of the cost-analysis

For this sensitivity analysis, all parameters were varied simultaneously by running 1000 Monte Carlo simulations. The red square indicates the observed values.

Table 1. Cost and probability parameters used in the cost-analysis

Probabilities and other parameters				
Variable	**Baseline value**	**Standard error[a]**	**Sensitivity range[b]**	**Reference**
Frequency *DPYD* genotype				
DPYD wild-type	0.9229	0.0080	fixed	This study[11]
DPYD variant allele carrier	0.0771	0.0080	0.0617 – 0.0925	This study[11]
Risk severe toxicity				
DPYD wild-type	0.2269	fixed	fixed	This study[11]
DPYD variant allele carrier, reduced dose	0.3882	0.0526	0.3106 – 0.4658	This study[11]
DPYD variant allele carrier, standard dose	0.5015	0.0274	0.4012 – 0.6018	Meta-analysis[8]
DPYD wild-type				
Hospitalization nursing ward	0.1356	fixed	fixed	This study[11]
Mean duration (days)	7.9855	fixed	fixed	This study[11]
Hospitalization ICU	0.0088	fixed	fixed	This study[11]
Mean duration (days)	3.1111	fixed	fixed	This study[11]
DPYD variant allele carrier, reduced dose				
Hospitalization nursing ward	0.1647	0.0400	0.1318 – 0.1976	This study[11]
Mean duration (days)	5.7857	1.3350	4.6286 – 6.9428	This study[11]
Hospitalization ICU	0.0235	0.0163	0.0188 – 0.0282	This study[11]
Mean duration (days)	1.0000	0.1000	0.8000 – 1.2000	This study[11]
DPYD variant allele carrier, standard dose				
Hospitalization nursing ward	0.2350	0.0422	0.1880 – 0.2820	Analysis on previous study[10,20]
Mean duration (days)	13.1000	3.0000	10.4800 – 15.7200	Analysis on previous study[10,20]
Hospitalization ICU	0.0310	0.0172	0.0248 – 0.0372	Analysis on previous study[10,20]
Mean duration (days)	7.0000	3.0000	5.6000 – 8.4000	Analysis on previous study[10,20]
Mean number of cycles				
Capecitabine	5.0208	0.1567	4.0166 – 6.0250	This study[11]
5-FU	5.0426	0.3639	4.0341 – 6.0511	This study[11]
Type of fluoropyrimidine drug				
Capecitabine	0.83	fixed	fixed	This study[11]
5-FU	0.17	fixed	fixed	This study[11]
Mean dose intensity for *DPYD* variant allele carriers	0.6910	0.0124	0.5528 – 0.8292	This study[11]
Cost parameters (expressed in €)				
Variable	**Baseline value**	**Standard error[a]**	**Sensitivity range[b]**	**Reference**
DPYD genotyping costs	100	Fixed	80-120	This study[11]
Hospitalization nursing ward (per day)	636	Fixed	Fixed	Guideline[15]
Hospitalization ICU (per day)	2,015	Fixed	Fixed	Guideline[15]
Additional costs for interventions related to toxicity (expect hospitalization)				
Grade 0-2	86	fixed	fixed	This study[11]
Grade ≥3	234	fixed	fixed	This study[11]
Treatment costs capecitabine (per cycle)				
Capecitabine medication	144.06	30	fixed	This study[11] / Price info drugs[16]
Medical doctor visit	132	Fixed	fixed	Guideline[15]
Treatment costs 5-FU per cycle				
5-FU medication + pharmacy preparation	59.29	20	fixed	This study/Price info drugs[16]
Administration at day care	276	fixed	fixed	Guideline[15]
Medical doctor visit	132	fixed	fixed	Guideline[15]

[a] The standard error was calculated on data of this study, or otherwise estimated for parameters not derived from this study. The standard error is used for the probabilistic sensitivity analysis.

[b] The sensitivity range is calculated by varying the baseline value ±20%. The sensitivity range is used for the one way sensitivity analysis.
Abbreviations: 5-FU: 5-fluorouracil; *DPYD*: gene encoding dihydropyrimidine dehydrogenase; ICU: intensive care unit.

DISCUSSION

The cost-analysis performed in this study showed that prospective *DPYD* screening for these four variants and dose individualization is cost-saving. This confirms that upfront *DPYD* screening does not result in an increase in healthcare costs, while it can significantly improve patient safety and prevent toxicity-related deaths, as shown previously.[11] Results of the probabilistic sensitivity analysis and one-way sensitivity demonstrated that, even when varying parameters in the model, the screening strategy is unlikely to result in an increase in costs.

However, the net saving for the screening strategy in our cost-analysis was with €51 relatively small. One of the determinants for this finding is that in our clinical study patients carrying a *DPYD* variant were still at increased risk of developing severe treatment-related toxicity, compared to wild-type patients (39% versus 23%, P=0.001).[11] The higher incidence of toxicity in *DPYD* variant allele carriers was mainly driven by carriers of the variants c.1236G>A and c.2846A>T. For these two variants a 25% dose reduction was applied in the study, which was concluded to be probably insufficient to reduce the incidence of toxicity to the background incidence in wild-type patients. Our results are in line with four previous studies investigating costs of *DPYD* genotyping and toxicity.[10,17] Deenen *et al.* previously confirmed that upfront screening for one *DPYD* variant (*DPYD**2A) is cost-saving.[10] Another study, by Cortejoso *et al.* investigated screening for three variants (*DPYD**2A, c.2846A>T, c.1679T>G) and compared genotyping costs and costs for treating severe neutropenia in a retrospective analysis. Occurrence of severe neutropenia resulted in average costs for treatment for this side effect of €3,044 per patient (drug and hospitalization costs). Genotyping costs for the three *DPYD* variants were only €6.40 per patient (approximately sixteen times less expensive than in our study). The authors calculated that *DPYD* genotyping would be cost-effective, provided that at least 2.1 cases of severe neutropenia per 1000 treated patients are prevented by upfront genotyping for the three variants.[17] This was, however, not validated in a prospective setting.

The third study, by Murphy *et al.*, investigated the cost implications for reactive *DPYD* screening (i.e. screening patients for *DPYD* variants after experiencing severe toxicity) versus prospective screening.[18] In a period of three years, all patients experiencing

severe (grade ≥3) fluoropyrimidine-related toxicity in an Irish hospital were screened for four *DPYD* variants (*DPYD**2A, c.2846A>T, c.1679T>G and c.1601G>A). Genotyping costs if prospective *DPYD* screening for all patients would have been performed were calculated. Total costs of hospitalization for five *DPYD* variant allele carriers (identified after experiencing severe toxicity) were €232,061, while prospectively testing would have cost in total €23,718 for the 134 included patients (€177 per patient), showing that hospitalization costs are significantly higher than costs for prospective *DPYD* screening.[18] The main difference between their study and our study was that the study by Murphy *et al.* did not collect data on the prospective *DPYD* screening strategy, but only on reactive *DPYD* screening.

The fourth study was a retrospective study as well, performed by Toffoli *et al*.[19] Toxicity-related costs on 550 colorectal cancer patients were investigated and genotyping for the same four variants as in our study was performed, but this was done retrospectively and not used for dose adjustments. This showed that average costs for treatment of toxicity were higher in *DPYD* variant allele carriers (€2,972) than in non carriers (€825), $P<0.0001$.[19]

To conclude, in addition to the important finding that upfront *DPYD* genotype-guided dose individualization is able to markedly increase patient safety, this study now confirms that this upfront *DPYD* screening strategy does not result in an increase in direct medical costs. This further endorses that *DPYD* genotyping should be implemented as routine clinical care.

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6

TREATMENT ALGORITHM FOR HOMOZYGOUS OR COMPOUND HETEROZYGOUS *DPYD* VARIANT ALLELE CARRIERS WITH LOW-DOSE CAPECITABINE

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INTRODUCTION

Fluoropyrimidines, including 5-fluorouracil (5-FU) and its oral prodrug capecitabine, are widely used in the treatment of several types of cancer. The enzyme dihydropyrimidine dehydrogenase (DPD) is responsible for over 80% of 5-FU conversion into inactive metabolites.[1] Pathogenic single nucleotide polymorphisms (SNPs) in *DPYD,* the gene encoding DPD, can result in decreased function of DPD and are associated with a strongly increased risk of severe and potentially fatal fluoropyrimidine-induced toxicity.[2] Pretreatment screening for *DPYD* SNPs and reduction of the starting fluoropyrimidine dose in *DPYD* variant carriers has significantly improved patient safety.[3] In a recent perspective, we recommended reduction of the starting fluoropyrimidine dose in heterozygous carriers of one of four clinically relevant *DPYD* variants by 25% (c.2846A>T or c.1236G>A/haplotypeB3) or 50% (*DPYD**2A or c.1679T>G).[4]

However, for homozygous *DPYD* variant carriers, or for patients who carry multiple variants simultaneously, no dosing guidelines are available yet, because experimental data about the magnitude of the effect of these genotypes on DPD activity are scarce. In the Clinical Pharmacogenetics Implementation Consortium (CPIC) guideline, fluoropyrimidine-treatment in homozygous *DPYD* variant carriers is discouraged, which implies that a potentially effective anticancer therapy is withheld from these patients.[5]

In this article, six unique patients with a homozygous or compound heterozygous *DPYD* variant allele genotype who were treated with tailored fluoropyrimidine treatment are described. For three patients, pharmacokinetics, DPD-phenotyping and clinical course are included. Data about the other three homozygous *DPYD* variant carriers are provided in the Supplementary Information.

METHODS

Detailed methods are in the Supplementary Information. Before the start of fluoropyrimidine treatment, genotyping for four *DPYD* SNPs (*DPYD**2A, c.2846A>T, c.1679T>G, c.1236G>A/haplotypeB3) was performed as part of routine clinical care. Written informed consent for additional sample collection and use of clinical data was obtained for all patients. Analyses were part of individual patient care, so institutional review board approval was not applicable.

Genotyping results showed a homozygous or compound heterozygous *DPYD* genotype,

and the functional effects of these genotypes were uncertain. Therefore pretreatment DPD activity was measured in peripheral blood mononuclear cells (PBMCs). DPD activity was used to reach an individualized dose, in which the percentage of remaining DPD activity was used as guideline for the starting dose (expressed as percentage of the originally planned dose).

Pharmacokinetic analyses in three of six patients were performed to investigate whether applied dose reductions were adequate. Pretreatment plasma uracil, the endogenous DPD substrate, and dihydrouracil levels were quantified; results were unknown before the start of treatment.

RESULTS

CLINICAL COURSE OF TREATMENT

Patient characteristics are listed in **Table 1**.

Patient 1. A male patient with metastatic colorectal carcinoma was scheduled for palliative chemotherapy (capecitabine, oxaliplatin and bevacizumab). *DPYD* screening showed that the patient was homozygous for *DPYD**2A; measurement of DPD activity in PBMCs of this patient indicated absence of DPD activity. Therefore, it was decided to drastically reduce the capecitabine dose from 2,300 mg (1,000 mg/m^2) twice daily to 150 mg twice daily (6.5% of planned dose) and to start with capecitabine monotherapy. Seven days after the start of treatment, the patient experienced severe toxicity (grade 3 diarrhea, grade 3 oral mucositis, and grade 4 neutropenia). Capecitabine was discontinued immediately. The adverse events resolved within 1 week (neutropenia) to 2 months (diarrhea, mucositis).

After a 2-month period without any anticancer therapy, the patient had fully recovered, and monotherapy with capecitabine was restarted. On the basis of the severe toxicity and the pharmacokinetic results of the first cycle, the dose was further reduced to 150 mg once every 5 days (ie, 0.65% of originally planned dose). This was tolerated well for 1 month, but the patient then experienced diarrhea (grade 2), after which capecitabine was stopped for 3 weeks. The capecitabine schedule was then adjusted again to introduce a rest period of 5 days after every two intakes (every third intake was skipped). This schedule was tolerated well; thus, it was decided to add bevacizumab and oxaliplatin. This addition was well tolerated and resulted in stable disease as the best treatment response.

Patient 2. This female patient with locally advanced colorectal carcinoma had a planned treatment that consisted of neoadjuvant chemotherapy (capecitabine 825 mg/m^2 twice daily, or 1,500 mg) combined with radiotherapy (5-week schedule). Pretreatment *DPYD*-screening revealed that the patient was homozygous for c.2846A>T; DPD activity was reduced to 29%. It was decided to reduce the capecitabine dose to 500 mg once daily, (ie, 17% of planned dose –slightly lower than recommended dose of 29% on the basis of DPD activity, as decided by physician and patient). DPD activity was not immediately known, so chemotherapy started on day 7 of the radiotherapy schedule. Treatment was completed and tolerated well without occurrence of severe toxicity. After treatment, surgery was performed.

Patient 3. A male patient with metastatic colorectal cancer had a treatment plan to start capecitabine and oxaliplatin. The patient carried both c.2846A>T and c.1236G>A variants heterozygously. DPD activity was reduced to 45%. Remaining DPD activity was more than 50% reduced, so it was considered likely that this patient was a compound heterozygous carrier (variants present on different alleles). In the first cycle, capecitabine was reduced to 1,800 mg daily (51% of planned daily dose of 3,500 mg; 1,750 mg/m^2), which was tolerated without toxicity. When the dose in cycle 2 was increased to 71% of planned dose (2,500 mg), grade 3 thrombocytopenia occurred. Therefore the dose was reduced again, to 57% of the planned dose (2,000 mg). This dose was continued during the third cycle. However, the patient developed grade 2 thrombocytopenia after 8 days, and the daily capecitabine dose was adjusted to 1,000 mg for the rest of the cycle. Platelets increased again until normal values were reached. After three cycles, disease progression was established, and capecitabine treatment was discontinued.

PHARMACOKINETIC RESULTS

In all three patients, additional pharmacokinetic measurements were performed (**Figure 1**). For patient 1, only levels of capecitabine and of the metabolites 5'-deoxy-5-fluorocytidine, 5'-deoxy-5-fluorouridine and 5-FU could be quantified; other 5-FU metabolites were not detectable. For patients 2 and 3, all metabolites were quantifiable. Results of noncompartmental analysis of the pharmacokinetic results in plasma are shown in **Table 2** and include values normalized to control values.[6] In patient 1, 5-FU exposure was highly increased: the mean area under the plasma concentration-time curve (AUC) of 5-FU was 4,024 ng*h/ml, which is 10 times higher than in other pharmacokinetic studies with capecitabine.[6–9] These results were used for the decision to lower the dose 10-fold in the second cycle.

Baseline uracil and dihydrouracil levels are listed in **Table 1**. Results of urine analysis for patient 1 are shown in **Supplementary Figure 1**.

Table 1. Clinical characteristics of patients 1 through 6

Characteristic	Patient 1	Patient 2	Patient 3	Patient 4	Patient 5	Patient 6
DPYD genotype	homozygous *DPYD**2A	homozygous c.2846A>T	heterozygous for c.2846A>T & c.1236G>A	homozygous c.2846A>T	homozygous c.1236G>A	homozygous c.1236G>A
Sex	Male	Female	Male	Female	Female	Female
Age (years)	47	52	61	69	75	58
Body surface area (m^2)	2.3	1.8	2.0	1.5	1.8	1.5
Ethnicity	White	White	White	White	White	White
Tumor type	CRC (metastatic)	CRC (locally advanced)	CRC (metastatic)	CRC (local)	Vulva carcinoma (locally advanced)	Breast cancer (local)
WHO performance status	0	0	1	0	1	0
Baseline DPD activity in PBMCs (nmol/(mg*h), % of reference activity	<LLOQ [a] (0%)	2.9 [b] (29%)	4.5 [b] (45%)	0.97 [a] (10%)	7.8 [b] (79%)	4.2 [b] (42%)
Baseline uracil level (ng/ml) [c]	1,920	28.7	35.6	ND	14.5	26.8
Baseline dihydrouracil level (ng/ml) [c]	<LLOQ	71.7	114	ND	104	164
Treated with fluoropyrimidines [d]	Yes: C1 6.5% of planned dose (severe toxicity), then 10-fold reduction to 0.65% of planned dose	Yes: 17% of planned dose	Yes: C1 51% of planned dose, C2 72%, C3 57%	No: disease free after surgery	Yes: C1 75% of planned dose; C2 100%	Yes: 50% of planned dose (5-FU)

[a] Measured according to method by Pluim et al.[25] Reference DPD activity: 9.6 ± 2.2 nmol/(mg*h).[25]

[b] Measured according to method by Van Kuilenburg et al.[26] Reference DPD activity: 9.9 ± 2.8 nmol/(mg*h).[14]

[c] Reference baseline uracil level: median 8.32 ng/ml, range 3.2 to 38.2 ng/ml, reference baseline dihydrouracil level: median 91.9 ng/ml, range 31.9 to 189.0 ng/ml. Both were determined in a cohort of 550 patients.[27]

[d] The amount of dose reduction and clinical course for patients 1, 2, and 3 are described in detail in the main article. Details for patients 4, 5, and 6 are described in the Supplementary Results. Abbreviations: C1: cycle 1; C2: cycle 2; C3: cycle 3; CRC: colorectal carcinoma; DPD: dihydropyrimidine dehydrogenase; LLOQ: lower limit of quantification; ND: not determined; PBMCs: peripheral blood mononuclear cells; 5-FU: 5-fluorouracil.

Table 2. Pharmacokinetic parameters of capecitabine and metabolites in plasma of patients 1 through 3

Metabolite	$AUC_{0\text{-last time point}}$ (ng*h/ml)[a]				
	Patient 1[b]	Patient 1 normalized[f]	Patient 2[c]	Patient 2 normalized[f]	Patient 3[d]
Capecitabine	296 (195 – 387)	3,871	1,186	3,626	3,357
5'-dFCR	1,310 (960 – 1,495)	17,131	2,445	7,476	3,198
5'-dFUR	1,257 (1,224 – 1,308)	16,438	4,139	12,655	2,912
5-FU	4,024 (3,795 – 4,351)	52,622	1,079	3,299	407
5-FU relative exposure[g]	*1.1*	-	*1.4*	-	*1.1*
FUH_2	<LLOQ	<LLOQ	1,112	3,303	1,318
FUPA	<LLOQ	<LLOQ	476	1,414	740
FBAL	<LLOQ	<LLOQ	4,106	12,195	6,979

[a] For patient 1, the mean values of three intakes (C1D1, C2D1, and C2D16) are reported, including range. The ingested dose was the same (150 mg) for all three days. For patients 2 and 3, the values of the first intake are reported.

[b] Patient 1 was a homozygous *DPYD**2A variant allele carrier. Results depicted are after an intake of 150 mg capecitabine (65 mg/m^2).

[c] Patient 2 was a homozygous c.2846A>T variant allele carrier. Results depicted are after an intake of 500 mg capecitabine (278 mg/m^2).

[d] Patient 3 was a heterozygous carrier of both c.2846A>T and c.1236G>A. Results depicted are after an intake of 800 mg (400 mg/m^2).

[e] Control values are derived from Deenen et al.[6] and are the mean values and CV% for patients with advanced cancer of the stomach or gastresophageal junction (*N*=22), after administration of 850 mg/m^2 capecitabine (dose level 2 of the study). Capecitabine and metabolite control values are measured using the same assay as for patients 1, 2, and 3.

[f] Normalized AUC values (normalized AUC = AUC * (850 mg/m^2 / administered dose in mg/m^2)

[g] For the 5-FU AUC, the relative exposure is depicted, corrected for the dosing interval. 5-FU relative exposure =5-FU AUC / (factor * 5-FU AUC from Deenen et al.[6]). Factor patient 1 = 10 (as dosing 1x in the 5 days), factor patient 2 = 2 (as dosing once daily), factor patient 3 = 1 (as dosing twice daily).

Abbreviations: 5'-dFCR: 5'-deoxy-5-fluorocytidine; 5'-dFUR: 5'-deoxy-5-fluorouridine; AUC: area under the curve; CV%: coefficient of variation; C1D1: cycle 1 day 1; C2D1: cycle 2 day 1; C2D16: cycle 2 day 16; FBAL: fluoro-β-alanine; FUH_2: dihydro-5-fluorouracil; FUPA: α-fluoro-ureidopropionic acid; LLOQ: lower limit of quantification; ND: not determined; $T_{1/2}$: half-life; 5-FU: 5-fluorouracil.

		$T_{1/2}$ (h) [a]			
Patient 3 normalized [f]	Mean (CV%) control value [e]	Patient 1 [b]	Patient 2 [c]	Patient 3 [d]	Mean (CV%) control value [e]
7,134	4,281 (31%)	0.41 (0.24 – 0.61)	0.51	0.41	0.76 (55%)
6,796	8,192 (30%)	0.83 (0.70 – 1.04)	0.84	0.44	1.0 (35%)
6,188	7,673 (29%)	0.99 (0.76 – 1.26)	0.55	0.55	0.9 (34%)
865	381 (40%)	5.31 (4.91 – 6.07)	0.39	0.57	1.0 (57%)
-	*1 (reference)*	-	-	-	-
2,718	ND	<LLOQ	0.59	0.72	ND
1,526	ND	<LLOQ	2.01	1.84	ND
14,394	14,177 (31%)	<LLOQ	2.12	2.49	2.6 (33%)

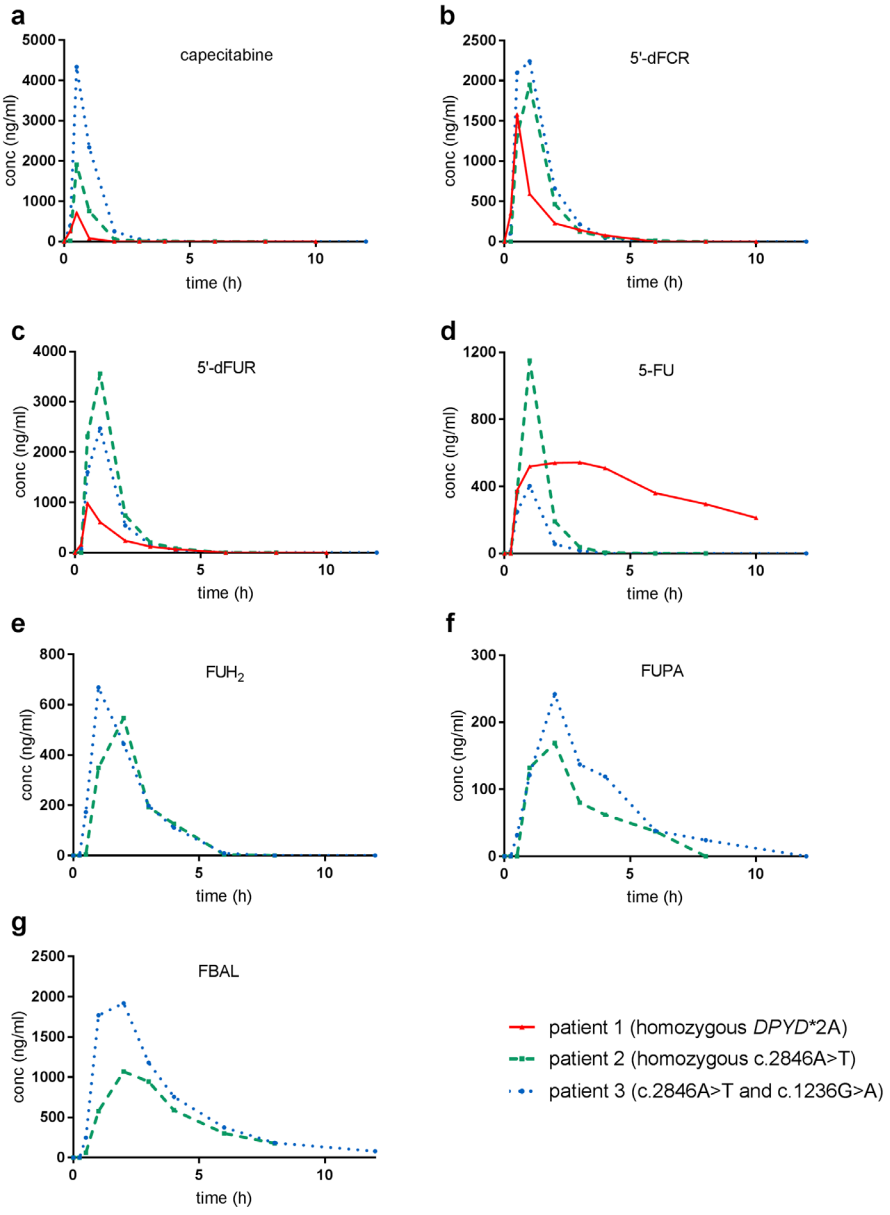

Figure 1. Pharmacokinetic results in plasma of patients 1, 2, and 3

Results of plasma levels of capecitabine (A) and the metabolites 5'-deoxy-5-fluorocytidine (5'-dFCR, B), 5'-deoxy-5-fluorouridine (5'-dFUR, C), 5-fluorouracil (5-FU, D), dihydro-5-fluorouracil (FUH_2, E), α-fluoro-ureidopropionic acid (FUPA, F) and fluoro-β-alanine (FBAL, G). For all three patients, the results after the first intake of capecitabine are depicted.

DISCUSSION

To our knowledge, this is the first report to describe prospectively identified patients, who are homozygous or compound heterozygous for *DPYD* variants, who could be treated safely with fluoropyrimidines. Multiple occurrences of fatal or life-threatening toxicity after fluoropyrimidine treatment have been described and, retrospectively, the patients who experienced these toxicities were identified as homozygous *DPYD* variant carriers who had complete DPD deficiency.[10–15]

Pretreatment identification of the patient homozygous for *DPYD**2A with complete DPD deficiency saved this patient from receipt of a full fluoropyrimidine dose, which most likely would have been fatal. We hypothesized that a dose of 5 to 10% would be well tolerated by this patient, because this percentage is usually excreted unchanged in urine.[16] However, this dose still resulted in severe toxicity. We showed, though, that treatment with an extremely low dose of capecitabine (0.65% of standard dose) was safe and feasible. Baseline uracil levels were extremely high, which confirmed the expected absent enzyme activity. Also, pharmacokinetic results showed that 5-FU could not be metabolized further, because the 5-FU half-life and exposure were highly increased. Urine results of this patient differed from results described elsewhere. In patients who are not DPD deficient, FBAL is the major urinary metabolite,[9,17] whereas this metabolite was not present in the urine analyzed in this paper. Evidence about pharmacokinetic-based dosing for capecitabine is limited. However, for 5-FU, dosing based on plasma levels is described more extensively.[18,19]

The two patients who had a homozygous c.2846A>T genotype and a c.2846A>T/c.1236G>A genotype had a partial remaining DPD activity. Pharmacokinetic results showed that administration of a moderately reduced dose of capecitabine resulted in adequate exposure.

A variation in retained DPD activity in the two carriers of the homozygous c.1236G>A variant was determined. This is in contrast to results by Meulendijks et al. in which DPD activity was reduced approximately 50% in two patients.[20] The c.1236G>A variant is part of haplotype B3, of which the intronic variant c.1129-5923C>G is expected to be responsible for the effect on DPD activity.[21] Nie et al. showed that this intronic variant resulted in a 35% reduction of DPD enzyme function.[22]

Because patients only underwent genotyping for four *DPYD* variants, the effects of additional deleterious *DPYD* variants cannot be ruled out. For example, *MIR27A* polymorphisms could play a role in variation of DPD activity, because these

polymorphisms reduce DPD activity.[23,24]

In conclusion, we showed that fluoropyrimidine treatment in homozygous or compound heterozygous *DPYD* variant allele carriers is feasible and that therapy does not have to be withheld. Additional DPD phenotyping tests, such as measurement of DPD activity in PBMCs, are recommended to compose an individualized treatment. After an initial dose reduction, tolerability in patients should be monitored closely, and the dose should be individually titrated according to tolerance.

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SUPPLEMENTARY INFORMATION
SUPPLEMENTARY METHODS

PATIENTS

Patients were treated at three different institutes in the Netherlands (the Netherlands Cancer Institute, Amsterdam; Erasmus Medical Center, Rotterdam; Fransciscus Gasthuis & Vlietland, Rotterdam). Toxicity was scored according to the Common Terminology Criteria for Adverse Events (CTC-AE) version 4.03.

DPYD GENOTYPING

Genomic DNA was isolated from peripheral blood cells and screening for the *DPYD* variants *DPYD**2A (IVS14+1G>A, rs3918290), c.2846A>T (rs67376798), c.1679T>G (*DPYD**13, rs55886062), and c.1236G>A (rs56038477) was performed using standard operating procedures in two different institutes (the Netherlands Cancer Institute, Amsterdam and Erasmus Medical Center, Rotterdam). This screening was performed before treatment as part of routine clinical care. In the Netherlands Cancer Institute, screening for *DPYD* variants was performed with the Roche LightCycler® 480II platform (Roche Diagnostics, Almere, the Netherlands) by using commercially available probes and primers (TIB Molbiol, Berlin, Germany), and results were confirmed by direct sequencing. At the Erasmus Medical Center, each sample was genotyped on two different platforms –Taqman (with predefined Drug metabolizing Enzyme (DME) assays) and PCR-restriction fragment length polymorphism (RFLP) assays –to allow checks for potential wrong genotyping. Details about the assays are included in **Supplementary Table 1**. Wild type, heterozygous, and no template controls were included in each run. Both laboratories participated during the study in the Dutch national quality control program for *DPYD* proficiency testing (SKML), in which all four *DPYD* variants were included.

DPD ACTIVITY IN PBMCs

Dihydropyrimidine dehydrogenase (DPD) activity was measured in peripheral blood mononuclear cells (PBMCs), isolated from a baseline (pretreatment) peripheral blood sample. One of two comparable validated methods by Van Kuilenburg et al. or Pluim et al. was used; both used radio-labeled thymine (^{14}C-labeled thymine or ^{3}H-labeled thymine) as a substrate and consisting of high performance liquid chromatography (HPLC) with online radioisotope detection and with liquid scintillation counting.[1,2] Reference values for both assays were highly comparable, respectively 9.9 $\pm$ 2.8 nmol/(mg*h) for the method of Van Kuilenburg et al.[2] and 9.6 $\pm$ 2.2 nmol/(mg*h) for the method of Pluim et al.[1]

URACIL AND DIHYDROURACIL PLASMA LEVELS

Endogenous uracil and dihydrouracil levels were quantified in a baseline (pretreatment) plasma sample by using a validated ultra-performance liquid chromatography – tandem mass spectrometry (UPLC-MS/MS) method.[3] Analytes were extracted by protein precipitation; chromatographic separation was performed on an Acquity UPLC HSS T3 column (Acquity Waters, Milford, MA) and were analyzed with MS/MS with an electrospray ionization source.[3] All samples were measured at one institute (The Netherlands Cancer Institute).

PHARMACOKINETIC MEASUREMENTS

Peripheral blood samples for patient 1 were obtained for pharmacokinetic analysis on cycle 1, day 1 (C1D1); cycle 2, day 1 (C2D1); and cycle 2, day 16 (C2D16). Samples on those 3 days were collected at 10 time points up to 10 hours after capecitabine intake (at predose, and at 15 minutes, 30 minutes, 1 hour, 2 hours, 3 hours, 4 hours, 6 hours, 8 hours, and 10 hours after capecitabine intake). For patients 2 and 3, samples were collected only on C1D1, on the same time points as for patient one, except for the latest time point (the sample 10 hours after capecitabine intake was not collected for patient 2 and the last sample was collected at 12 hours instead of 10 hours for patient 3). For patient 1, pharmacokinetic results of the cycle one were known before the start of cycle two. For patients 2 and 3, pharmacokinetic results were not known during treatment, because samples were analyzed after treatment had finished.

Capecitabine and its metabolites 5'-deoxy-5-fluorocytidine (5'-dFCR), 5'-deoxy-5-fluorouridine (5'-dFUR), 5-fluorouracil (5-FU), dihydro-5-fluorouracil (FUH_2), α-fluoro-ureidopropionic acid (FUPA) and fluoro-β-alanine (FBAL), were quantified by using HPLC coupled to electrospray MS/MS. Two individual validated assays were used, one for the simultaneous quantification of capecitabine, 5'-dFCR and 5'-dFUR, and another for 5-FU, FUH_2, FUPA and FBAL.[4] In addition, for patient 1, urine samples were collected for additional analysis on C2D1 and C2D16. Urine was collected per portion from predose up to 10 hours after capecitabine intake on both days. The same HPLC-MS/MS method as for plasma samples was used. Urine samples were diluted 20 times in blank plasma before additional sample pretreatment. All pharmacokinetic samples were measured at the same institute (The Netherlands Cancer Institute).

Supplementary Table 1. *DPYD* genotyping assays.

DPYD SNP	Rs-number	PCR-RFLP forward primer	PCR-RFLP reverse primer	Enzyme	Taqman DME assay
*2A (IVS14+1G>A)	rs3918290	5'-CTTGTTTTAGATGTTAAAT-CACACATA - 3'	5'- CTTGTTTTAGATGT-TAAATCACACATA - 3'	NdeI	C__30633851_20
c.1679T>G	rs55886062	5'-CCAGCTTCAAAAGCTCTTC- 3'	5'- CTTCCGTTTCTGC-CAAGC -3'	TFiI	C_11985548
c.1236G>A	rs56038477	5'- CACTGTACCTTTAGGAT-CAC - 3'	5'- ATGCAGTTTGTTC-GGACTGA -3'	DdeI	C_25596099
c.2846 A>T	rs67376798	5'- CATAGCATTCTAATTC-CAGC - 3'	5'- CAAGTTGTGGC-TATGATCG -3'	Taqa1	C_27530948

Abbreviations: DME: drug metabolizing enzyme; PCR-RFLP: polymerase chain reaction - restriction fragment length polymorphism; Rs: reference SNP number; SNP: single nucleotide polymorphism.

SUPPLEMENTARY RESULTS

DIHYDROURACIL-URACIL LEVELS

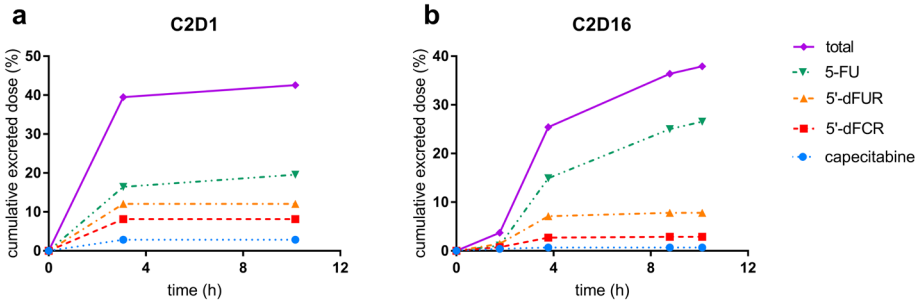
Together with the sample for DPD activity in PBMCs, a pretreatment plasma sample was taken to measure uracil and dihydrouracil levels (**Table 1**). For patient 1, the baseline uracil plasma level was extremely high (1,920 ng/ml) compared with reference levels (median 8.32 ng/ml; range 3.2 to 38.2 ng/ml, N = 550),[5] and no dihydrouracil could be detected. For patients 2 and 3, uracil levels increased compared with reference levels (a value of 28.7 ng/ml for patient 2 and of 35.6 ng/ml for patient 3), and both values were within the top 1% of reference values. Dihydrouracil levels were in the normal range for patients 2 and 3, compared to reference levels.

OTHER HOMOZYGOUS DPYD VARIANT ALLELE CARRIERS

Three additional patients with a homozygous *DPYD* variant genotype were identified during routine *DPYD*-screening (**Table 1**). In these patients, DPD activity in PBMCs was measured pretreatment also and was used to determine the level of dose reduction.
Two out of three patients (patients 5 and 6) were homozygous for the c.1236G>A variant. DPD activity varied between these two patients, from approximately 42% to 79% residual activity. The two patients both received reduced fluoropyrimidine doses without occurrence of severe toxicity. It is unclear why there is a relatively high variation of the effect of this genotype on DPD phenotype in patients, and more research on this variant is advised.

In addition, one patient with a homozygous c.2846A>T genotype (patient 4) was identified. The residual DPD activity in this patient was approximately 10%. This patient was disease free after surgery, so it was decided not to treat this patient with adjuvant

chemotherapy. The remaining activity of patient 4 was lower than of patient 2, who had the same *DPYD* genotype and who had a residual activity of 29%. According to the calculated DPD activity score, as described by Henricks et al.,[6] it could be concluded that a 50% dose reduction would be appropriate for homozygous c.2846A>T carriers, because a 25% dose reduction is recommended for heterozygous carriers of this variant. However, on the basis of the DPD activity results in these two patients, this amount of dose reduction seems insufficient for homozygous c.2846A>T carriers. Pharmacokinetic results in patient 2 showed that the normalized area under the plasma concentration-time curve (AUC) of 5-FU was 3,299 ng*h/ml, which is nine-fold higher than the mean control value of Deenen et al.,[7] which showed that 5-FU clearance might be impaired more than expected on the basis of the value of 29% remaining DPD activity in PBMCs.



Supplementary Figure 1. Pharmacokinetic results in urine of patient 1

Patient 1 was a homozygous *DPYD**2A carrier. Results of urine excretion on cycle 2 day 1 (C2D1, A) and cycle 2 day 16 (C2D16, B) of capecitabine and the metabolites 5'-deoxy-5-fluorocytidine (5'-dFCR), 5'-deoxy-5-fluorouridine (5'-dFUR), 5-fluorouracil (5-FU) and of the total excretion, after intake of 150 mg capecitabine. Excretion was calculated as a percentage of the administered dose of capecitabine.

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PART II

IRINOTECAN

7

INDIVIDUALIZATION OF IRINOTECAN TREATMENT: A REVIEW OF PHARMACOKINETICS, PHARMACODYNAMICS AND PHARMACOGENETICS

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ABSTRACT

Since the clinical introduction in 1998, the topoisomerase I inhibitor irinotecan is widely used in the treatment of solid tumors including colorectal, pancreatic, and lung cancer. Irinotecan therapy is characterized by several dose-limiting toxicities and by large inter-individual pharmacokinetic variability. Irinotecan has a highly complex metabolism, including hydrolyzation by carboxylesterases to its active metabolite SN-38, which is 100-1,000 fold more active compared to irinotecan itself. Several phase I and phase II enzymes, including CYP3A4 and UGT1A, are involved in the formation of inactive metabolites, making its metabolism prone to environmental and genetic influences. Genetic variants in the DNA of these enzymes and transporters could predict a part of the drug related toxicity and efficacy of treatment. This has been shown in retrospective and prospective trials and meta-analyses. Patient characteristics, life-style and co-medication also influence irinotecan pharmacokinetics. Other factors, including dietary restriction, are currently studied. Meanwhile, a more tailored approach to prevent excessive toxicity and optimize efficacy is warranted. This review provides an updated overview on today's literature on irinotecan pharmacokinetics, pharmacodynamics, and pharmacogenetics.

INTRODUCTION

Irinotecan (CPT-11) is a camptothecin derivative that demonstrates anti-cancer activity in many solid tumors. Currently, it is widely used in the treatment of colorectal, pancreatic, and lung cancer. Irinotecan is the prodrug for SN-38, which inhibits topoisomerase-I, an enzyme involved in DNA replication.[1, 2] SN-38 is 100-1,000 fold more cytotoxic than irinotecan, and its exposure is highly variable.[3] SN-38 is inactivated by further enzymatic conversion into SN-38 glucuronide (SN-38G).

PHARMACOKINETICS

DISTRIBUTION

Irinotecan is a hydrophilic compound with a large volume of distribution estimated at almost 400 L/m^2 at steady state.[4] At physiological pH, the lactone-ring of irinotecan and SN-38 can be hydrolyzed to a carboxylate isoform (**Figure 1**). Consequently, a pH-dependent equilibrium between these forms exists.[5] As only the lactone form has antitumor activity, a small change in pH could alter the pharmacokinetics and efficacy of irinotecan.[6] In plasma, however, the carboxylate form of irinotecan and the lactone form of SN-38 dominate.[7, 8] This could be explained by a higher tissue distribution of irinotecan lactone and the preferential binding of SN-38 lactone to plasma proteins.[4, 9] Conversion of irinotecan lactone to carboxylate within the circulation is rapid with an initial half-life between 9-14 minutes, which results in a 50% reduction in irinotecan lactone concentration after 2.5 hours, compared to end of infusion (66% versus 35%).[4, 7, 8]

After end of drug infusion, a rapid decrease in irinotecan plasma concentrations is seen. Peak concentrations of SN-38 are reached within two hours after infusion.[8] Irinotecan is assumed to exhibit linear pharmacokinetics because of the correlation between dose and systemic exposure, which is highly variable between patients.[8] In plasma, the majority of irinotecan and SN-38 is bound to albumin, which has a stronger binding capacity for the more hydrophobic active metabolite, and albumin also stabilizes the lactone forms of irinotecan and SN-38.[10] In blood, SN-38 is almost completely bound and for two-third located in platelets and in – predominantly – red blood cells.[11] The binding constant of SN-38 with erythrocytes is almost fifteen times higher than that of irinotecan.[11]

Thus far, several population pharmacokinetic models of irinotecan have been developed. All models confirmed the large inter-individual variability in pharmacokinetic parameters

of around 30%. In general, a three-compartmental model for irinotecan and a two-compartmental model for SN-38 is assumed.[4, 12-16] A mean SN-38 distribution half-life was estimated to be very short (approximately 8 minutes).[13] Several models showed a second peak in the SN-38 plasma AUC, which was explained by an enterohepatic re-circulation of SN-38. SN-38 is reabsorbed after intestinal deconjugation of SN-38G by (bacterial) β-glucuronidases.[15] Alternatively, release of SN-38 from erythrocytes has also been proposed to cause this second plasma peak.[17]

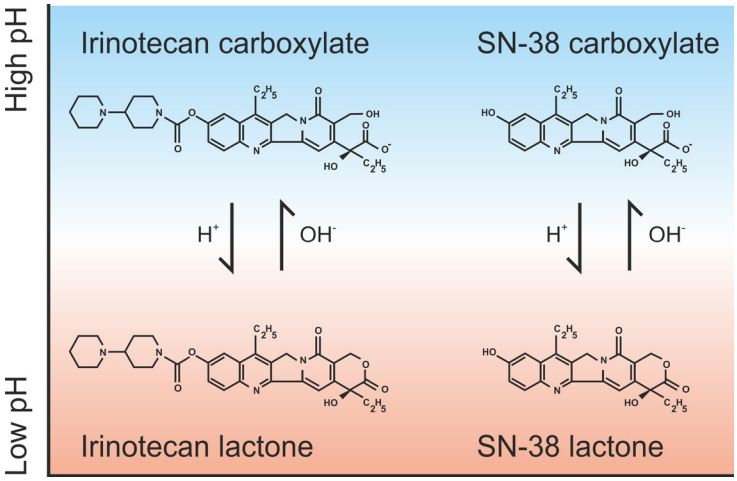

Figure 1. pH-dependent equilibrium of irinotecan and SN-38 isoforms

METABOLISM

Metabolism by carboxylesterases and butyrylcholinesterase

The prodrug irinotecan is hydrolyzed into the active metabolite SN-38 by two isoforms of carboxylesterases (CES1 and 2) and butyrylcholinesterase in the human body (**Figure 2**).[18, 19] CES1 and CES2 are localized in liver, colon, kidney and blood cells. Butyrylcholinesterase is mainly found in plasma.[20] Conversion by these esterases mainly occurs intra-hepatically and is a relatively slow and inefficient process, as only 2-5% of irinotecan is converted into SN-38.[12, 18] CES2 has a 12.5-fold higher affinity for irinotecan than CES1 and is therefore the predominant enzyme in this conversion.[21-23] In addition, this process also occurs in blood, where butyrylcholinesterase has a six-fold higher activity than CES.[20] After conversion, SN-38 is actively transported into the liver by the OATP1B1 transporter (**Figure 2**).[24]

Many studies have investigated intratumoral CES activity, by which irinotecan can be activated at the site of action. Indeed, the amount of CES activity could be related

to irinotecan efficacy, although preclinical work showed conflicting results.[25-30] Many preclinical studies have been performed to selectively increase the intratumoral CES activity with a virus or engineered stem cells, thereby aiming to increase irinotecan efficacy.[31-38] Although a few studies could indeed reverse irinotecan resistance in vitro and in mice, this mechanism has not been investigated in a clinical setting (yet).

To our knowledge, no clinically relevant drug-drug interactions (DDIs) involving CES have been reported for irinotecan, although both inhibitors and inducers of CES have been described, which could potentially influence the rate of irinotecan conversion to SN-38.[39]

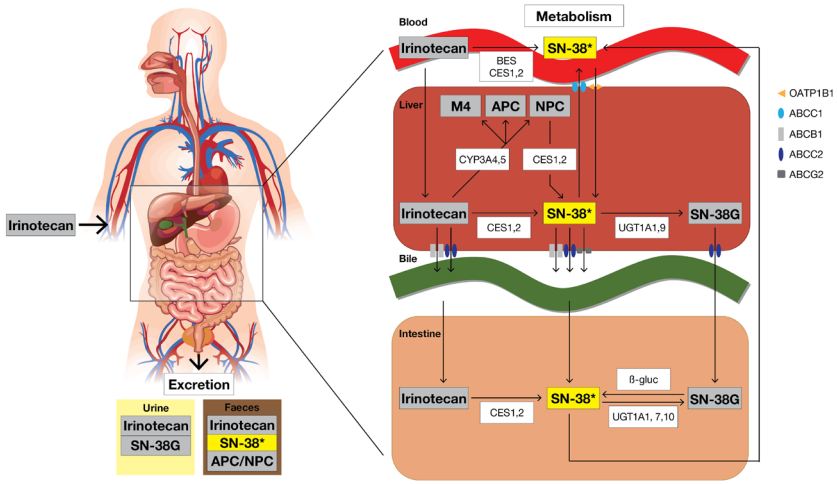

Figure 2 Irinotecan metabolism and excretion

* = active metabolite. Abbreviations: CES = carboxylesterase; BES = butyrylcholinesterase; CYP = cytochrome P450 enzymes; UGT = uridine diphosphate glucronosyltransferase; β-gluc = beta-glucuronidase

Metabolism by uridine diphosphate glucuronosyltransferases

SN-38 is inactivated via glucuronidation to SN-38G by uridine diphosphate glucuronosyl-transferase (UGT) and excreted into the bile.[40, 41] Several UGT subtypes are involved in the hepatic (UGT1A1, UGT1A9) and extrahepatic (UGT1A1, UGT1A7, UGT1A10) conversion of SN-38, of which UGT1A1, UGT1A7 and UGT1A9 are the major iso-enzymes.[42-46] SN-38G is formed almost directly after SN-38 formation, explaining the short half-life of SN-38.[47] Plasma concentrations of SN-38G are the highest amongst all irinotecan metabolites, suggesting a highly efficient glucuronidation rate of SN-38 into SN-38G.[4] UGT1A1 also conjugates bilirubin, and a significant correlation between SN-38 and bilirubin glucuronidation has been observed.[42] In addition, patients

genetically predisposed with decreased UGT1 activity, e.g. in Gilbert's syndrome, are at higher risk for severe toxicity when treated with irinotecan.[48] Also, many other UGT polymorphisms have been described and their influence on irinotecan pharmacokinetics and pharmacodynamics is summarized in section 4.

Metabolism by cytochrome P450 enzymes

Irinotecan is also metabolized by intrahepatic cytochrome P450 (CYP) enzymes, i.e. CYP3A4 and CYP3A5, into inactive metabolites: APC and NPC.[49] In contrast to APC, NPC can be converted to SN-38 by CES1 and CES2 in the liver but to a lesser amount than irinotecan.[50] The importance of CYP3A4 and CYP3A5 in irinotecan metabolism is underlined by the strong correlation between irinotecan clearance and midazolam clearance.[51] Midazolam is an important CYP3A probe drug, and we previously conducted a randomized clinical trial aiming to individualize irinotecan dosing by use of an CYP3A4 phenotype-based algorithm. By dosing on this algorithm the inter-individual variability in irinotecan and SN-38 exposure dramatically reduced compared to conventional dosing.[52] In addition, smoking, some herbal supplements, and co-medication are known to induce or inhibit CYP3A enzymes, resulting in interactions with irinotecan, which are summarized in more detail in section 2.5.

Metabolism by β-glucuronidases

As mentioned before, SN-38G can be deconjugated into SN-38 by β-glucuronidases produced by intestinal bacteria, which could result in an enterohepatic circulation of SN-38.[15, 53-55] In addition, β-glucuronidase activity has been correlated to intestinal damage and diarrhea in rats/mice, which could (potentially) be reduced by inhibiting β-glucuronidase with antibiotics (penicillin and streptomycin) or with amopaxine.[56, 57] Nonetheless, attempts to reduce β-glucuronidase activity by neomycin did not alter irinotecan pharmacokinetic profile in patients significantly.[58]

ELIMINATION

Clearance of irinotecan is mainly biliary (66%) and independent of dose, estimated at 12-21 L/h/m^2.[59, 60] Irinotecan is transported into the bile by several ATP-binding cassette transporters (i.e. ABCB1, ABCC2, and ABCG2, see **Figure 2**).[61-63] In addition, active efflux by ABCB1 has been shown to lead to low intracerebral irinotecan concentrations in mice.[64] All metabolites except for SN-38G, are predominately excreted in feces, although they are also detectable in urine.[4, 59] Terminal elimination half-lives ($t_{1/2}$) between 5-18 hours for irinotecan and between 6-32 hours for SN-38 were reported.[4, 12-14, 59, 65-71] However, later it was shown that the $t_{1/2}$ was initially underestimated, as up to 500 hours after infusion SN-38 concentrations can be detected.[72, 73]

The wide inter-individual variability in irinotecan clearance is still not completely understood. Primarily, a decreased clearance in patients with altered hepatic function has been described.[12, 13] Additionally, increasing age may negatively influence irinotecan clearance, although this could not be confirmed in another analysis.[13, 74] Conflicting effects of gender on irinotecan pharmacokinetics have also been proposed. Several studies reported higher irinotecan exposure in women, which – in part – could be explained by decreased SN-38 (metabolic) clearance [13, 59, 75], while others found no gender effect.[4, 74, 76] Several factors such as dose, timing of administration, enzyme-activity, and hematocrit levels might be responsible for these differences. In addition, firm conclusions can neither be drawn for weight.[13, 77] Worse clinical performance has been demonstrated to decrease irinotecan clearance.[13] However, inter-individual variability does not seem to be related to body size measures as body-surface area (BSA). Although irinotecan dose is generally based on BSA, it has been shown that BSA and other body size measures do not predict irinotecan pharmacokinetics, and that flat-fixed dosing could be a safe alternative.[74, 78]

OTHER FORMULATIONS AND ADMINISTRATIONS

Other formulations

Also, several other irinotecan formulations have been evaluated. Firstly, oral administration of several different formulations has been investigated and deemed feasible in phase-I trials [79-81], but its poor and highly variable bioavailability have limited its current clinical usability.[82]

Secondly, irinotecan drug-eluting beads (DEBIRI) have been developed to control drug release and is mostly used as regional administration. DEBIRI administered into the hepatic artery resulted in higher and prolonged intratumoral irinotecan and SN-38 exposure in liver metastases, whereas systemic exposure was lower than after intravenous administration.[83-85] Hepatic arterial infusion of DEBIRI has been demonstrated to be an effective treatment for unresectable liver metastases.[86]

Thirdly, liposomal irinotecan has been developed and is clinically used. Encapsulated into liposomes, irinotecan is stable for a longer period of time, resulting in increased accumulation in tumor tissue and thereby increasing its effect, as described further in section 3.2.[87]

Other variations in administration

Irinotecan administration based on circadian timing improved clinical outcome in several clinical trials [88-90], probably due to circadian rhythm of enzymes and transporters involved in irinotecan pharmacokinetics and pharmacodynamics.[91-93] However, pharmacokinetic consequences have only been investigated in a small randomized

study, in which an increased metabolic ratio (SN-38 / irinotecan AUC) and smaller interindividual variability were found after circadian-timed dosing.[94]

Trials on two different – more regional infusion methods – have been conducted. Firstly, locoregional therapy with irinotecan infusion into the hepatic artery has been evaluated for the treatment of unresectable liver metastases: different irinotecan formulations have been demonstrated to be safe and effective.[95, 96] This approach resulted in lower systemic exposure to irinotecan and an increased conversion of irinotecan into SN-38 compared to intravenously administered irinotecan.[97] Secondly, use of irinotecan as hyperthermic intraperitoneal chemotherapy (HIPEC) has been investigated as treatment option for colorectal peritoneal metastases.[98-103] A small fraction of irinotecan is rapidly converted intraperitoneally into SN-38: systemic C_{max} of SN-38 has been observed 30 minutes after intraperitoneal administration.[98, 100] Although these different administration methods are investigated for several years, there is yet insufficient evidence that it could be beneficial to implement these strategies in daily care.

DRUG-DRUG INTERACTIONS (DDIs)

DDIs with anti-cancer drugs

Many anticancer agents have been investigated in combination with irinotecan, of which no significant pharmacokinetic interactions with irinotecan have been reported for oxaliplatin, 5-fluorouracil (5-FU) / leucovorin, capecitabine and monoclonal antibodies.[66, 70, 104-123] In contrast, paclitaxel combined with irinotecan in a 3-weekly regimen caused a significant increase of irinotecan, SN-38 and SN-38G exposure, which was assumed to be caused by competitive inhibition of ABCB1 (**Table 1**).[124] Sequencing the administration of paclitaxel after irinotecan seems to improve their synergistic anticancer effects[125], but irinotecan pharmacokinetics is not significantly altered in either sequence. [125, 126] Systemic SN-38 exposure was found to be reduced in patients concomitantly treated with tegafur (S-1) or carboplatin[127-129], of which the latter also reduced irinotecan exposure. Patients seemed to tolerate irinotecan better when thalidomide was co-administered in two phase II studies, in which SN-38G exposure was increased at the expense of SN-38 exposure.[130] However, the pharmacokinetic differences could not be replicated[131, 132] and might be caused by confounding, as half of the patients also used antiepileptic drugs (AEDs).[130]

Tyrosine kinase inhibitors (TKIs) have become very popular in cancer treatment, but are also known for their modulating effects on drug metabolizing enzymes.[133] Several TKIs, i.e. imatinib, pazopanib, sunitinib, lapatinib and gefitinib, have been investigated in combination with irinotecan containing regimens.[134-141] All of these combinations,

except for pazopanib and lapatinib, led to excessive toxicity and have therefore not been evaluated further for clinical use. Increased exposure to irinotecan or SN-38, due to inhibition of CYP3A4, ABCB1 or ABCG2, has been suggested as a cause of the intolerance of irinotecan combined with TKIs, but a pharmacodynamic interaction cannot be ruled out.

DDIs with non-anticancer drugs

Concomitant treatment with non-anticancer drugs such as anti-epileptic agents, certain antidepressants, antiretroviral drugs, and NSAIDs have been shown to affect irinotecan pharmacokinetics or pharmacodynamics. The combination with the potent CYP3A4 inhibitor ketoconazole was one of the first significant DDIs described for irinotecan (**Table 1**).[142] Anecdotally, severe rhabdomyolysis syndrome has been described in a patient using irinotecan and citalopram.[143] Although pharmacokinetic data were not available, competitive metabolism by CYP3A4 was suspected as the underlying mechanism. Hypothetically, other strong CYP3A4 inhibiting antidepressants such as nefazodone could be suspected for an interaction with irinotecan.[144]

AEDs are also known for inducing CYP3A, UGTs and CES.[145] The influence of phenytoin, phenobarbital, and carbamazepine on irinotecan pharmacokinetics was evaluated in a population pharmacokinetic model, which suggested that patients using these AEDs should receive a 1.7 times higher irinotecan dose to reach the same exposure as in patients without AEDs.[75] Individual patients may require an even higher dose, as indicated by a four-fold higher irinotecan clearance and ten-fold lower systemic SN-38 exposure in a patient receiving phenytoin.[146] Therefore, the combination of phenytoin and irinotecan must be avoided (if possible) or dosing must be guided on irinotecan pharmacokinetics to ensure a sufficient exposure. In addition, Innocenti et al. found a decreased exposure to SN-38 when irinotecan was combined with cyclosporine and the AED phenobarbital (**Table 1**).[147] In addition, an important DDI between irinotecan and the combination treatment with ritonavir and lopinavir, caused by CYP3A4, UGT1A1 and ABC-transporter inhibition, resulted in a more than two-fold increase in SN-38 AUC and a 36% decrease in the SN-38G / SN-38 AUC ratio (**Table 1**).[148] A similar effect could be expected of atazanavir, which also is a strong inhibitor of CYP3A4 and UGT1A1.[149] In contrast, by UGT1A induction by methimazole an increase in SN-38 and SN-38G concentrations, as well as an almost 50% increased ratio of SN-38G / SN-38 was found by within patient comparison (**Table 1**).[150] Concerning frequently used drugs such as NSAIDs and proton pump inhibitors, only a possible DDI with celecoxib and omeprazole have been evaluated so far. One of three studies investigating co-administration of irinotecan and celecoxib, described an increased clearance of irinotecan and a decreased AUC of SN-38, although the mechanism is

not clear (**Table 1**).[151, 152, 153]. Although omeprazole influences UGT, CYP3A, ABCB1, and ABCG2, a clinically relevant pharmacokinetic interaction with irinotecan was ruled out in a small cross-over study.[154]

DDIs with herbal and dietary supplements, and lifestyle

In general, herbal and dietary supplements are frequently used by cancer patients.[155, 156] Unfortunately, the potential for herb-drug interactions in oncology is not frequently investigated in clinical studies.[157] Thus far, the effects of St. John's wort (SJW), milk thistle, cigarette smoking, and cannabis tea on irinotecan pharmacokinetics have been investigated. Concomitant use of SJW resulted in a 42% reduction of SN-38 AUC, primarily caused by CYP3A4 induction (**Table 1**).[158] Flavonoids are components of many herbs, such as milk thistle (Silybum marianum), and are able to inhibit CYP3A4, UGT1A1 and ABC-transporters[159-161], but an interaction has not been demonstrated in clinical trials (yet).[161] Cigarette smoking resulted in a decrease in irinotecan and SN-38 exposure, possibly caused by CYP3A induction (**Table 1**).[162] In addition, (medicinal) cannabis can induce CYP3A4 and inhibit ABCB1 and its use is becoming more popular in cancer patients. Although no interaction was demonstrated between irinotecan and medicinal cannabis tea[163], other cannabis formulations contain different concentrations of the enzyme-modulating compounds (e.g. cannabidiol and delta-9-tetrahydrocannabinol (THC)). Therefore, it remains unclear if cannabinoid oils – the most popular formulation nowadays – is safe in combination with irinotecan.

Table 1. Drug-drug interactions with irinotecan

All PK alterations mentioned are significant with p<0.05.

Drug/OTC/Lifestyle	N	Enzyme/ Transporter	Irinotecan Dose	PK alterations		Ref.
Anticancer drugs						
Paclitaxel 135-200 mg/m^2 D8	31	ABCB1	40-60 mg/m^2 D1 + 8, Q3W	Irinotecan SN-38 SN-38G	$AUC_{24.5h}$ 32.7%↑ $AUC_{24.5h}$ 40.4%↑ $AUC_{24.5h}$ 46.2%↑	124
Thalidomide 400mg OD (for 14D)	16		350 mg/m^2, Q3W	SN-38 SN-38G	AUC_{48h} 74%↓ AUC_{48h} 28%↑	130*
S-1 (tegafur) 100/120 mg/m^2, 4-7D	4	ABCG2	100-200 mg/m^2 Q2W	SN-38	AUC_{24h} 50%↓	128
Imatinib 300-600mg OD Cisplatin 30 mg/m^2 D1 + 8	6	CYP3A4,5 CYP2C9	65 mg/m^2 D1 + 8, Q3W	Irinotecan	AUC_{8h} 67%↑, CL 36%↓	134
Lapatinib 1250mg/day Leucovorin 200 mg/m^2 5-FU 600 mg/m^2	12	CYP3A4 OATP1B1 ABCB1 ABCG2	108 mg/m^2 Q2W	SN-38	AUC_{24h} 41%↑, C_{max} 32%↑	137

Table 1 continued.

Drug/OTC/Lifestyle	N	Enzyme/ Transporter	Irinotecan Dose	PK alterations		Ref.
Non-anticancer drugs						
Ketoconazole 200mg OD for 2D	7	CYP3A4	100 mg/m^2 (with ketoconazole) 350 mg/m^2 (alone) Q3W	SN-38 APC	AUC_{500h} 109% ↑ AUC_{500h} 87% ↓	142
Lopinavir 400mg/ Ritonavir 100mg combination drug (Kaletra) BID	8	CYP3A4 UGT1A1 ABCB1	150 mg/m^2 D1 + 10, Q3W	Irinotecan SN-38 SN-38G APC	AUC_{inf} 89% ↑, CL 47% ↓ AUC_{inf} 204% ↑ AUC_{inf} 94% ↑ AUC_{inf} 81% ↓	148
Cyclosporine 5-10mg/kg	43	ABCB1 ABCC2	25-75 mg/m^2 Q1W	Irinotecan SN-38	CL 39-64% ↓ AUC_{24h} 23-630% ↑	147
Cyclosporine + Phenobarbital 90mg for 14D	39	ABCB1 ABCC2 UGT1A1	72-144 mg/m^2 Q1W	Irinotecan SN-38 SN-38G	AUC_{24h} 27% ↓, CL 43% ↑ AUC_{24h} 75% ↓ AUC_{24h} 50% ↓	
Celecoxib 400mg BID	11		50-60 mg/m^2 D1 + 8, Q3W	Irinotecan SN-38	CL 18% ↑ $AUC_{12.5h}$ 21,8% ↓	151*
Methimazole	14	UGT1A	660mg Q3W	SN-38 SN-38G	AUC_{56h} 14% ↑ AUC_{56h} 67% ↑	150
Herbal and dietary supplements, and lifestyle						
Cigarette smoking	190	CYP3A UGT1A1	350 mg/m^2 or 600mg fixed dose Q3W	Irinotecan SN-38	AUC_{100h} 15% ↓, CL 18% ↑ AUC_{100h} 38% ↓	162
St. John's wort 300mg TID	5	CYP3A4	350 mg/m^2 Q3W	SN-38	AUC_{24h} 42% ↓	158

*For thalidomide and celecoxib conflicting data have been published between pharmacokinetic drug interactions with irinotecan. Studies that did not show a significant drug-drug interaction are illustrated in more detail in the text. [131, 132, 152, 153]

Abbreviations: N = sample size; D = day; OD = once daily; BID = twice daily; TID: three times daily; AUC = area under the curve; Inf = infinity; CL = clearance; Q1W = weekly cycle; Q2W = 2-weekly cycle; Q3W = 3-weekly cycle; Ref = reference

PHARMACODYNAMICS

TOXICITY

Irinotecan is known for its dose-limiting adverse events; primarily diarrhea, neutropenia, and asthenia. Of patients with irinotecan monotherapy, 16-31% experience severe diarrhea and a comparable percentage of patients suffer from severe neutropenia and severe asthenia, classified as Common Terminology Criteria for Adverse Events (CTCAE) grade 3 or worse.[164-168] Patients treated with a 5-FU, leucovorin and irinotecan (FOLFIRI) regimen experience severe diarrhea (9-44%) and severe neutropenia (18-54%) to the same extent.[168-173] In addition, neutropenia appears to occur more frequently in

females.[174] Although irinotecan dose is lower in this regimen, also 5-FU could cause these adverse events.

Two types of diarrhea caused by irinotecan can be distinguished: early- and late-onset diarrhea. Early-onset diarrhea starts during, or immediately after drug infusion and is caused by increased cholinergic activity, which stimulates intestinal contractility and reduces the absorptive capacity of the mucosa.[175] In addition, early-onset diarrhea is often part of an acute cholinergic syndrome with diaphoresis and abdominal pain. Overall incidence of this syndrome is approximately 70% without premedication and is reduced to 9% by administration of anticholinergic agents (i.e. atropine or hyoscyamine) before irinotecan infusion.[176, 177] Late-onset diarrhea occurs approximately 8-10 days after irinotecan infusion and is characterized by a more severe course, which is probably caused by damage of the intestinal mucosa due to increased oxidative-stress by biliary secreted or intestinally deconjugated SN-38.[76, 178-180] Several guidelines recommend to treat late-onset diarrhea with loperamide or alternatively octreotide.[181, 182] Antibiotics have been used in clinical practice as well, despite sufficient evidence supporting this strategy.[182] However, these interventions are not always sufficient, which could lead to dose reductions, treatment interruptions and hospitalization.

Conflicting results have been reported regarding the relationship between irinotecan and SN-38 exposure and toxicity (**Table 2**).[60] An initial study suggested the biliary index (i.e. the ratio of SN-38 to SN-38G AUCs multiplied by the AUC of irinotecan) as a better predictor for gastro-intestinal toxicity.[178] Following studies on this subject have been contradictory: a higher biliary index was significantly correlated with higher incidence of severe diarrhea in several studies[76, 178, 183], whereas no significant association was found by others (**Table 2**).[16, 66, 68, 184] The duration of neutropenia has been found to be significantly correlated to a prolonged systemic SN-38 exposure.[73]

Several interventions to prevent diarrhea have been investigated, such as reducing the intestinal exposure of SN-38. In a phase I study, firstly SN-38 excretion in the bile was inhibited by combining irinotecan with cyclosporine (due to ABCC2 and ABCB1 inhibition). Subsequently, phenobarbital (as a UGT1A1 inductor) was added and the combination of cyclosporine/phenobarbital/irinotecan resulted in a 75% reduction of SN-38 AUC.[147] However, when studied in a large randomized phase-III trial, the combination of cyclosporine, irinotecan and panitumumab did not significantly reduce the incidence of severe diarrhea.[185] In another randomized trial, prophylactic use of racecadotril, an anti-secretory drug, also failed to reduce this adverse event.[186]. Alternatively, SN-38 can be bound to activated charcoal or calcium aluminosilicate clay in the intestine. Up till now, only the activated charcoal has been found to reduce

the incidence of diarrhea.[187, 188] However, evidence from a phase-III study and additional pharmacokinetic analysis is warranted to understand the real effect of activated charcoal, which also exhibits a general anti-diarrhoeic effect, and therefore the use of charcoal is not common practice.

Another attempt to reduce toxicity was by inhibition of β-glucuronidase production by antibiotics (i.e. streptomycin, penicillin, and neomycin), amopaxine, and herbal medicines, all without a relevant reduction in diarrhea incidence.[56-58, 189] When combined with cholestyramine to reduce reabsorption, however, β-glucuronidase inhibition by levofloxacine was found to reduce irinotecan-induced diarrhea.[190] In addition, a randomized double blind placebo controlled trial showed a 20% reduction in diarrhea incidence when irinotecan was combined with probiotics. Unfortunately, this did not result in a significant difference between groups, probably due to a lack of statistical power.[191] Lastly, altering the intestinal environment by alkalinization or by reduction of inflammation (by the use of budesonide) did not reduce intestinal toxicity either.[192-197] Currently, fasting before chemotherapy is investigated to reduce toxicity, which has been shown to be effective in mice without affecting the anti-cancer effects. Systemic and hepatic exposure to SN-38 was reduced in these mice, but intratumoral concentrations were unaltered.[198, 199] A prospective trial is currently ongoing in order to assess the effects of fasting in irinotecan-treated patients and to elucidate the underlying biological mechanisms (www.trialregister.nl/; Trial ID: NTR5731).

EFFICACY

Irinotecan is effective in a wide range of malignancies. In metastatic colorectal cancer (mCRC), irinotecan has its most prominent role as monotherapy or within combination therapy. As first line mCRC treatment, the FOLFIRI regimen proved to be superior to 5-FU with leucovorin and to irinotecan monotherapy: a response rate (RR) of 39% and median overall survival (OS) of 14.8-17.4 months has been reported.[168, 169] However, addition of oxaliplatin to this regimen (i.e. FOLFOXIRI) substantially increased treatment efficacy as shown by a RR of 60% and median OS of around 23 months.[204, 205] As second line treatment after 5-FU containing regimens, irinotecan leads to a significantly longer OS than 5-FU with leucovorin or best supportive care (BSC).[166, 167] For patients with a KRAS wild-type tumor, efficacy of palliative treatment could be increased by combining irinotecan monotherapy, FOLFIRI or FOLFOXIRI with monoclonal antibodies (e.g. bevacizumab, cetuximab, panitumumab, ramucirumab).[165, 170-172, 206, 207] In the adjuvant setting, the addition of irinotecan to 5-FU and leucovorin did not result in a survival benefit.[208, 209] Patients with tumors characterized by high microsatellite instability (MSI) have been suggested to respond better to irinotecan based chemotherapy[210, 211], but, a recent meta-analysis failed to show any predictive value of MSI-status in relation to treatment response.[212]

Table 2. Irinotecan toxicity in relation to pharmacokinetics and biliary index

Study	N	Irinotecan Dose	Irinotecan	SN-38	SN-38G	Biliary index
Diarrhea						
Ohe, 1992 [200]	36	5-40 mg/m^2,5D, cont.	**YES**[1]	No	ND	ND
de Forni, 1994 [201]	59	50-145 mg/m^2, Q1W	**YES**[1]	**YES**[1]	ND	ND
Rowinsky, 1994 [65]	32	100-345 mg/m^2, Q3W	No	No	ND	ND
Gupta, 1994 [178]	21	100-175 mg/m^2, Q1W	No	No	No	**YES**[2]
Abigerges, 1995 [202]	64	100-750 mg/m^2, Q3W	**YES**[3]	**YES**[3]	ND	ND
Catimel, 1995 [67]	46	33-115 mg/m^2, D1-D3, Q3W	**YES**[1]	No	ND	ND
Gupta, 1997 [76]	40	145 mg/m^2, Q1W	No	No	No	**YES**[2]
Canal, 1996 [68]	47	350 mg/m^2, Q3W	No	No	No	No
Mick, 1996 [183]	36	145 mg/m^2, Q1W	ND	ND	ND	**YES**[1]
Rothenberg, 1996 [203]	48	125-150 mg/m^2, Q1W	No	**YES**[1]	ND	ND
Herben, 1999 [184]	29	10-12.5 mg/m^2, D14-21, cont.	No	No	No	No
de Jong, 2000 [66]	52	175-300 mg/m^2, Q3W	No	No	ND	No
Xie, 2002 [16]	109	100-350 mg/m^2, Q3W	**YES**[1]	No	**YES**[1]	No
Neutropenia						
Ohe, 1992 [200]	36	5-40 mg/m^2, 5D, cont.	No	**YES**[4]	ND	ND
de Forni, 1994 [201]	59	50-145 mg/m^2, Q1W	**YES**[5]	**YES**[5]	ND	ND
Rowinsky, 1994 [65]	32	100-345 mg/m^2, Q3W	No	**YES**[5]	ND	ND
Abigerges, 1995 [202]	64	100-750 mg/m^2, Q3W	**YES**[4]	**YES**[4]	ND	ND
Catimel, 1995 [67]	46	33-115 mg/m^2, D1-D3, Q3W	No	No	ND	ND
Canal, 1996 [68]	47	350 mg/m^2, Q3W	**YES**[5]	**YES**[5]	No	No
Rothenberg, 1996 [203]	48	125-150 mg/m^2, Q1W	No	No	ND	ND
Herben, 1999 [184]	29	10-12.5 mg/m^2 D14-21, cont.	No	No	No	No
de Jong, 2000 [66]	52	175-300 mg/m^2, Q3W	No	No	ND	ND
Mathijssen, 2002 [73]	26	350 mg/m2, Q3W	ND	**YES**[6]	ND	ND

All assumed relationships mentioned are significant with $p<0.05$. 1 = diarrhea frequency, all grades. 2 = diarrhea grade $\geq$3. 3 = diarrhea $\geq$2. 4 = absolute decrease in neutrophil count, all grades. 5 = % decrease in neutrophil count, all grades. 6 = entire time course of absolute neutrophil count decrease.

Abbreviations: N = sample size; ND = not determined; D = day; Q1W = weekly cycle; Q3W = 3-weekly cycle; cont. = continuously

For advanced esophageal or junction tumors, irinotecan has proven to be effective as monotherapy and when combined with respectively cisplatin, mitomycin, capecitabine and oxaliplatin, 5-FU and leucovorin and docetaxel.[213-219] Of these regimens, however, only irinotecan combined with 5-FU was evaluated in a phase-III trial, in which this combination was inferior to cisplatin/5-FU.[220] In advanced HER2 negative gastric cancer, addition of irinotecan to different combination therapies gave an OS benefit in a pooled analysis of ten studies: median OS was 11.3 months and RR was approximately 38%.[221]

Irinotecan is also used in the treatment of small cell lung cancer (SCLC) and non-small cell lung cancer (NSCLC). For advanced NSCLC, irinotecan combined with respectively

taxanes, platinum, ifosfamide or gemcitabine demonstrated efficacy as first-line treatment in several trials.[222] For advanced SCLC, irinotecan combined with cisplatin or carboplatin had similar RR and median OS as platinum compounds with etoposide (RR 39-84% and median OS 9-13 months) and is therefore used as first-line treatment in Japan, whereas the etoposide containing regimen is preferred elsewhere.[223] Furthermore, irinotecan has demonstrated anti-cancer activity in phase-II trials in a wide range of other solid tumors (i.e. mesothelioma, glioblastoma, gynecological cancers, head and neck cancer), although no phase-III data are available.[224-231]

Finally, in pancreatic cancer, the combination of 5-FU, leucovorin, irinotecan and oxaliplatin (FOLFIRINOX) is used for both first-line adjuvant and palliative treatment, in which it was shown to be superior to gemcitabine monotherapy (median OS 11.1 months, RR 31.6%).[232] Recently, liposomal irinotecan, has been approved as second-line treatment for metastatic pancreatic cancer for patients with progression on gemcitabine-based therapies.[87] Efficacy of this liposomal formulation needs to be explored further in other tumor types.

PHARMACOGENETICS

Expression and functionality of enzymes and drug transporters involved in the metabolism and elimination of irinotecan can be affected by genetic polymorphisms which could influence both irinotecan pharmacokinetics and pharmacodynamics. This section provides an overview of clinical correlations between polymorphisms and irinotecan pharmacokinetics and pharmacodynamics.

ASSOCIATIONS BETWEEN UGT1A1 POLYMORPHISMS AND IRINOTECAN PHARMACODYNAMICS

With more than one hundred reported genetic variants, UGT1A1 is a highly polymorphic enzyme.[233] The most frequently studied UGT1A1 polymorphisms in relation to irinotecan pharmacokinetics and pharmacodynamics are *UGT1A1*6* and *UGT1A1*28*. The majority of the genetic association studies have focused on neutropenia and diarrhea as clinical endpoints.[169]

*Wild-type UGT1A1 is characterized by six thymine-adenine (TA) repeats in the promotor region, whereas UGT1A1*28* (rs8175347) carriers have an extra TA repeat which impairs UGT1A1 transcription and thereby reduces expression by approximately 70%.[234] The incidence of this genetic variant is relatively high among Caucasians

(minor allele frequency, MAF: 26 – 39%) and Africans/African Americans (MAF: 30 – 56%).[235, 236] Among Asians, *UGT1A1*28* is far less common as indicated by an MAF of 9 – 20%.[235, 236] *With an reported MAF of up to 47%, another polymorphism – UGT1A1*6 (rs4148323, 211G>A) – is more common in Asian populations and may therefore be a better predictor for irinotecan-related toxicities in that area of the world.*[237] *UGT1A1*6* also results in a ~70% reduction of UGT1A1 activity in individuals carrying the *UGT1A1*6/*6* genotype.[238]

Both *UGT1A1*28* and **6* polymorphisms result in an increased systemic exposure to irinotecan and SN-38 in patients homozygous for these variants, thereby increasing the risk of irinotecan-associated adverse events.[239, 240] This is also accompanied by increased financial costs of toxicity management.[241] Due to the high number of genetic association studies on the clinical effects of *UGT1A1*6* and **28* on irinotecan pharmacokinetics / pharmacodynamics and large differences between studies in terms of tumor type, dosing regimen, and genetic models, this review will mainly focus on meta-analyses for *UGT1A1*28* and **6* to extract the most relevant information with the highest level of evidence (**Table 3**).

Initially, significant associations between *UGT1A1*28* and hematologic toxicities were only reported for irinotecan doses higher than 180 mg/m^2.[242] More recent meta-analyses, however, did not show a dose-dependent effect of *UGT1A1*28*: also **28* carriers receiving lower irinotecan doses were at risk of neutropenia.[243, 244] These meta-analyses were carried out in a predominantly Caucasian population, thus regardless of scheduled starting dose, genotyping for *UGT1A1*28* and dose reductions in all Caucasian patients homozygous for *UGT1A1*28* may be considered to reduce the risk of severe neutropenia.

Presumably due to the lower incidence of *UGT1A1*28* in Asians, the effects of *UGT1A1*28* on toxicity endpoints are less straightforward in this population. Several meta-analyses in Asian patients with different tumor types and treatment schedules did not show any significant association between *UGT1A1*28* and irinotecan-induced neutropenia.[245, 246] In contrast, *UGT1A1*6* seems to be a more accurate predictor of irinotecan-induced toxicity: Asian patients with gastro-intestinal tumors or with NSCLC were more likely to suffer from neutropenia if they were carrying at least one *UGT1A1*6* allele (**Table 3**).[245, 247] This association does not seem to be dose-dependent.[248]

Both Caucasian and Asian patients homozygous or heterozygous for *UGT1A1*28* have a greater risk of suffering from severe diarrhea compared to wild type patients after receiving irinotecan doses greater than 125 mg/m^2.[249] In another meta-analysis among

Caucasian **28/*28* carriers, this dose-dependent effect was also observed.[244] In Asian patients, *UGT1A1*6* not only correlates well with the risk for irinotecan-induced neutropenia, but is also significantly associated with severe diarrhea.[245, 248] Whether this association is dose-dependent is currently unknown, since no dose subgroup analysis has been carried out.[250]

It seems that response or survival endpoints are not significantly affected by *UGT1A1*6* or **28*. Both *UGT1A1*6* and *UGT1A1*28* genotypes did not have any significant association with tumor response in Asian NSCLC or SCLC patients receiving irinotecan as first- or second-line chemotherapy.[245] Also in Caucasian patients with colorectal cancer, the presence of one or more *UGT1A1*28* alleles did not significantly affect overall and progression-free survival (PFS).[251]

Besides *UGT1A1*6* and *UGT1A1*28*, other common UGT1A1 polymorphisms could theoretically also

affect irinotecan-related toxicity (**Table 3**). For instance, *UGT1A1*60* (rs4124874; 3279T>G) is in linkage with *UGT1A1*28* and is associated with a decrease in transcriptional activity.[238] This genetic variant is common among Caucasians (MAF: 47%) and African Americans (MAF: 85%).[252] Two clinical studies did not report any significant associations between *UGT1A1*60* status and irinotecan-related toxicities[253, 254], irinotecan pharmacokinetics, or tumor response.[253] The only significant association including *UGT1A1*60* was found in a haplotype analysis in which a haplotype consisting of *UGT1A1*28*, **93* and **60* variant alleles was significantly associated with grade 4 neutropenia.[255]

Similar to *UGT1A1*60*, also *UGT1A1*93* (rs10929302; -3156G>A) is in linkage disequilibrium with *UGT1A1*28*.[252] *UGT1A1*93* results in reduced UGT1A1 expression and is associated with elevated bilirubin concentrations in patients homozygous for *UGT1A1*93* [255]. With an MAF of approximately 30% this genetic variant is commonly detected in Caucasians and African Americans.[252] Clinically, *UGT1A1*93* is associated with increased SN-38 AUC, lower neutrophil count, increased incidence hematologic toxicities (including neutropenia), diarrhea, and grade 3 vomiting.[256-261] Moreover, *UGT1A1*93* was also part of a haplotype including variant alleles of *UGT1A1*28*, **60*, **93*, and *UGT1A7*3*, that was associated with increased response rate.[262] A prospective trial on genotype-guided irinotecan dosing based on *UGT1A1*28* and *UGT1A1*93* genotype status is currently ongoing (Trial ID: NTR6612).

ASSOCIATIONS BETWEEN OTHER UGT1A POLYMORPHISMS AND IRINOTECAN PHARMACODYNAMICS: UGT1A7 AND UGT1A9

Compared to patients with *UGT1A9*1/*1*, individuals carrying the *UGT1A9*22* genotype (T9>T10; MAF: 45%) show higher enzyme expression, higher SN-38 glucuronidation and are therefore more at risk for diarrhea.[263, 264] Other *UGT1A9* variants, *UGT1A9*3* (98T>C; MAF: 3%) and *UGT1A9*5* (766G>A; MAF: 1%), are rare in Caucasians and are therefore not likely to significantly affect irinotecan pharmacokinetics and pharmacodynamics in this population.[265] Lower enzyme activity and SN-38 conjugation is observed in *UGT1A7*3* and *UGT1A7*4* polymorphisms.[263, 266]. In line with these findings, *UGT1A7*3/*3* carriers are at greater risk of adverse events while receiving irinotecan chemotherapy.[262, 263] A haplotype consisting of *UGT1A7*3*, *UGT1A9*1*, *UGT1A1*28*, *UGT1A1*60* and *UGT1A1*93* alleles was associated with severe neutropenia in a cohort of 167 colorectal cancer patients treated with FOLFIRI.[259] In the same cohort *UGT1A7*3* was also part of two other haplotypes (including *UGT1A9*, *UGT1A7*, and *UGT1A6* variants) associated with increased risk of grade 3-4 neutropenia (**Table 3)**.

ASSOCIATIONS BETWEEN DRUG TRANSPORTER POLYMORPHISMS AND IRINOTECAN PHARMACOKINETICS AND PHARMACODYNAMICS

Since both irinotecan and SN-38 are substrates of ABC transporters (**Figure 2**), ABC polymorphisms may also affect irinotecan pharmacokinetics [267], as well as irinotecan-related toxicities.[174] In a multivariate analysis including *UGT1A1*93*, *ABCC1* SNPs rs6498588 and rs17501331, these variants were associated with increased SN-38 plasma concentrations and/or decreased absolute neutrophil counts.[256] Opposite effects were reported for the *ABCB1* variant rs12720066 which was associated with decreased SN-38 exposure and increased neutrophils. Carriers of another *ABCB1* SNP (rs1045642) had an increased risk for early toxicity and lower treatment response.[268] In patients with liver metastases treated with hepatic artery infusion of irinotecan, oxaliplatin and 5-fluorouracil and intravenous cetuximab, this SNP was also associated with toxicity (grade 3-4 neutropenia), increased systemic concentrations of oxaliplatin and cetuximab, and prolonged PFS.[269] Furthermore, carriers of the *ABCB1* haplotype (including *rs*1045642, rs1128503, rs2032582) responded less frequently and had shorter survival.[268] In addition to *ABCB1* and *ABCC1*, polymorphisms of *ABCC2* (rs3740066) and *ABCG2* (rs2231137) were reported to be independently predictive for toxicity (i.e. grade 3 diarrhea).[264] In contrast, the *ABCG2 421C>A* SNP seems to have a limited impact on irinotecan exposure.[270] Polymorphisms in the gene for the hepatic efflux transporter ABCC2 may have a protective effect on diarrhea, which is presumably caused by decreased hepatobiliary transport of irinotecan and therefore reduced irinotecan exposure to the gut.[271] This protective effect was observed in Caucasian patients with the *ABCC2*2* haplotype (including six *ABCC2* variants without any *UGT1A1*28* alleles).

Although their role in irinotecan efflux has not yet been established, *ABCC5* and *ABCG1* could also be involved in this process, since several SNPs in these transporters are correlated with severe diarrhea.[272]

OATP1B1, encoded by the *SLCO1B1* gene, is involved in the hepatic uptake of SN-38 (**Figure 2)**. In Caucasian patients carrying at least one *SLCO1B1*1b* variant allele (rs2306283; MAF: 38%), median neutrophil count was approximately two-fold increased, compared with wild-types, presumably by increased hepatic uptake of SN-38 thereby reducing SN-38 plasma concentrations (**Table 3**).[273] This result confirms an earlier genetic association study on the effects of drug transporters on irinotecan neutropenia and pharmacokinetics.[174] In addition, *SLCO1B1*1b* was also associated with increased PFS in patients with colorectal and pancreatic cancer.[274] Thus, potentially *SLCO1B1*1b* could be a protective biomarker for neutropenia and may improve efficacy. In contrast, *SLCO1B1*5* (rs4149056) leads to reduced transporter activity and was associated with increased SN-38 plasma concentrations and an increased risk of neutropenia (in combination with *UGT1A1*28* variant alleles).[274]

IMPLEMENTATION OF GENOTYPE-ADJUSTED IRINOTECAN DOSING GUIDELINES

Both the U.S. Food and Drug Administration (FDA) and Health Canada/Santé Canada (HCSC) recommend a reduction of the irinotecan starting dose in patients who are homozygous for *UGT1A1*28* without specifying the extent of reduction (**Table 4**).[275, 276] In contrast, the Evaluation of Genomic Applications in Practice and Prevention (EGAPP) Working Group did not find sufficient evidence that *UGT1A1* genotyping should be used.[277] Subsequent guidelines, however, do underline the importance of *UGT1A1* genotyping, especially for *UGT1A1*28* variant alleles in Western countries. For example, in France and The Netherlands a reduction of the starting dose of 25-30% is recommended in patients homozygous for *UGT1A1*28* receiving higher doses of irinotecan ($\geq$ 180 mg/m^2).[278, 279] Regarding liposomal irinotecan, the European Medicines Agency (EMA) recommends an initial dose reduction from 80 to 60 mg/m^2 in patients homozygous for *UGT1A1*28*.[280]

In line with the significant associations between *UGT1A1*6* genotype and irinotecan-induced toxicities in Asian populations, the Japanese Pharmaceuticals and Medical Devices Agency (PMDA) recommends screening patients for *UGT1A1*6* and **28* polymorphisms.[281]

Despite the establishment of these guidelines, *UGT1A1* genotyping is currently not routinely performed.[282] This could be explained by the fact that prospective studies evaluating the clinical effects of genotype-directed dosing are scarce. Most likely,

reduction of the irinotecan dose to prevent toxicity in carriers of *UGT1A1*1/*28* and *UGT1A1*28*/*28 is indeed useful, since the maximum-tolerated dose of irinotecan was lower in these patients relative to wild-type patients.[283] Whether a dose reduction of irinotecan affects tumor response in *UGT1A1*28* carriers is yet unknown. On the other hand, patients with the *UGT1A1*1/*1* or *UGT1A1*1/*28* genotype may tolerate higher irinotecan doses than the currently recommended doses and are therefore at risk of suboptimal treatment. Indeed, a phase I dose-finding study showed convincingly that, compared with the recommended irinotecan dose of 180 mg/m^2 in the FOLFIRI regimen, substantial higher doses of irinotecan (up to 420 mg/m^2) were tolerated in patients wild-type or heterozygous for *UGT1A1*28*.[284] More recently, similar findings were observed in patients receiving FOLFIRI in combination with bevacizumab [282], implying that the therapeutic window of irinotecan may be increased for the *UGT1A1*1/*1* and *UGT1A1*1/*28* genotypes.

In summary, particularly for Caucasians *UGT1A1*28* seems to be a good predictor for neutropenia (all irinotecan doses) and diarrhea (doses > 125 mg/m^2). *UGT1A1*28* is also significantly associated with an increased risk for diarrhea in Asian patients at irinotecan doses > 125 mg/m^2. In Asian populations, however, the *UGT1A1*6* variant is more common and appears to be a more accurate predictor for neutropenia (all irinotecan doses) and diarrhea. In addition to *UGT1A1*6* and *UGT1A1*28*, *UGT1A1*93* is also significantly associated with irinotecan-induced toxicity. Less extensively studied polymorphisms such as *UGT1A7*3*, *UGT1A9*1*, and drug transporter polymorphisms (*ABCB1*, *ABCC5*, *ABCC2*, *ABCG1*, *SLCO1B1*) may also be useful predictors for toxicity. Interestingly, *CYP3A4*22* has not been studied thus far, while this SNP has shown relevance for many other CYP3A substrates [285-287]. In order to determine the true value of genotype-driven dosing of irinotecan, the efficacy of this dosing strategy should be evaluated prospectively. Inclusion of additional predictive genetic variants (e.g. *UGT1A1*6*, **93*) in genotype-directed dosing schedules may improve its predictive value.

Table 3. Overview of pharmacogenetic studies on irinotecan toxicity and survival

Polymorphism	Ethnicity	Endpoint	Dose range (mg/m²)	Main findings	Patients (N)	Studies (N)	Ref.
Meta-analyses							
*UGT1A1*28/*28* (rs8175347) vs **1/*28* or **1/*1*	Not reported	Hematologic toxicities	80 – 125	OR = 1.80, 95% CI 0.37 – 8.84, *P* = 0.41	229	3	242
			180	**OR = 3.22, 95% CI 1.52 – 6.81, *P* = 0.008**	410	4	
			200 – 350	**OR = 27.8, 95% CI 4.0 – 195, *P* = 0.005**	184	3	
**28/*28* vs **1/*1*	Mainly Caucasian	Neutropenia	< 150	**OR = 2.43, 95% CI 1.34 – 4.39, *P* = 0.003**	300	4	243
			150 – 250	**OR = 2.00, 95% CI 1.62 – 2.47, *P* < 0.001**	1481	9	
			≥ 250	**OR = 7.22, 95% CI 3.10 – 16.78, *P* < 0.001**	217	3	
**28/*28* vs **1/*28* or **1/*1*	Caucasian	Neutropenia	80 - 350	**OR = 3.44, 95% CI 2.45 – 4.82, *P* < 0.00001**	2015	14	244
**28/*28* vs **1/*28* or **1/*1*		Diarrhea	> 150	**OR = 2.04, 95% CI 1.23 – 3.38, *P* = 0.006**	1317	8	
			< 150	OR = 1.41, 95% CI 0.82 – 2.43, *P* = 0.21	663	6	
**1/*28* or **28/*28* vs **1/*1*	Asian	Neutropenia	50 – 100	OR = 1.47, 95% CI 0.90 – 2.42, *P* = 0.13	515	8	245
**6/*6* (*rs4148323*) vs **1/*6* or **1/*1*		Diarrhea		**OR = 4.90, 95% CI 2.02 – 11.88, *P* = 0.0004**	225	4	
**6/*6* vs **1/*6* or **1/*1*		Tumor response		OR = 1.51, 95% CI 0.78 – 2.92, *P* = 0.22.	225	4	
**28/*28* or **1/*28* vs **1/*1*				OR = 1.03, 95% CI 0.59 – 1.82, *P* = 0.91.	390	7	
**28/*28* vs	Asian	Neutropenia	60 – 200	OR = 1.67, 95% CI 0.94 – 2.97	658	6	246
**6/*28*			30-350	**OR = 2.55, 95% CI 1.82 – 3.58**	886	13	
**6/*6* or **28/*28* or **6/*28* vs **1/*6* or **1/*28* or **1/*1*	Asian	Neutropenia	60 - 350	**OR = 3.275, 95% CI 2.152 – 4.983, *P* = 0.000**	923	11	247
**1/*6* or **6/*6* vs **1/*1*				**OR = 1.542, 95% CI 1.180 – 2.041, *P* = 0.001**	994	9	
**6/*6* vs **1/*1*	Asian	Neutropenia	30 -375	**OR = 4.44, 95% CI 2.42 – 8.14, *P* < 0.001**	833	7	248
**28/*28* or **1/*28* vs **1/*1*	Asian	Diarrhea	> 125	**OR = 3.02, 95% CI 1.42 – 6.44, *P* = 0.004**	309	4	249
**28/*28* or **1/*28* vs **1/*1*	Caucasian			**OR = 1.93, 95% CI 1.38 – 2.70, *P* < 0.001**	1096	11	
**28/*28* vs **1/*1* **1/*28* vs **1/*1*	Caucasian	OS and PFS	60 – 350	All comparisons not significant for both OS and PFS (*P* > 0.05).	1524 (OS) 1494 (PFS)	10	251
Clinical studies							
*UGT1A1*60*	Asian	PK, tumor response, gr. 4 neutropenia, gr. 3 diarrhea, delivered dose	80	*P* > 0.05 for all endpoints.	81	1	253
*UGT1A1*60*	Not specified (probably Korean)	Neutropenia, anorexia, vomiting, diarrhea, abdominal pain	150	*P* > 0.05 for all endpoints.	42	1	254

Table 3 continued.

Polymorphism	Ethnicity	Endpoint	Dose range (mg/m²)	Main findings	Patients (N)	Studies (N)	Ref.
Clinical studies							
*UGT1A1*28, *60, *93* (rs10929302)	Caucasian (50 pts), Black (10 pts), Hispanic (4 pts), Pacific Islander (1 pt), Asian (1 pt)	Neutropenia	350	**- UGT1A1 haplotype (**28/*60/*93*) associated with gr. 4 neutropenia, *P* < 0.001.**	66	1	255
*UGT1A1*28*, **93*, *ABCB1* (rs1045642)	Not specified, presumably Caucasian (France).	Hematologic toxicities	180	**- *UGT1A1*28/*28* and **93/*93*: increased risk of hematologic toxicity (*P* = 0.01).**	184	1	258
*UGT1A1*93*, *ABCB1* (rs12720066), *ABCC1* (rs6498588, rs17501331)	Caucasian (67 pts), African American (11 pts)	ANC nadir, SN-38 AUC	300 or 350	**- Increased SN-38 AUC: UGT1A1*93, ABCC1 (rs6498588).** **- Decreased SN-38 AUC: *ABCB1* (rs12720066).** **- Increased ANC nadir: *ABCB1* (rs12720066.** **- Decreased ANC nadir: *UGT1A1*93*, *ABCC1* (rs17501331).**	78	1	256
*UGT1A1*28* and **93*	Caucasian (94 pts), Asian (2 pts)	Diarrhea	40 - 80, 180, 350	**- *UGT1A1*28/*28* and **93/*93* associated with gr. 3/4 diarrhea (*P* < 0.05).**	96	1	260
		Neutropenia		- No sign. effect on neutropenia.			
*UGT1A1:*28,*93* *UGT1A6*: rs2070959, *UGT1A9*: **22* (rs45625337), *-688A/C* variant *UGT1A7*3* *3'UTR*: *440C>G* variant	Not specified, presumably Caucasian (Canada).	Neutropenia	180	**- *UGT1A1*93* associated with neutropenia.** **- Haplotype ("II" and "III" (variants in *UGT1A9*, *1A7*, *1A6*, and *3'UTR* wild type) associated with gr. 3-4 neutropenia: OR 2.15 and 5.28, respectively.**	167	1	259
Amongst other genes: *UGT1A1*28*, **93*	Caucasian (450 pts), African American (36 pts), Hispanic (16 pts), Asian (9 pts), other (9 pts)	(Febrile) neutropenia, vomiting	125 or 200	**- *UGT1A1*93* associated with gr. 3 febrile neutropenia, gr. 4 neutropenia (*P* < 0.001), and gr. 3 vomiting (*P* = 0.004).**	520	1	261

Table 3 continued.

*UGT1A1:*6, *28* *UGT1A7*3* *UGT1A9*1*	Asian	Adverse events, therapeutic intervention	60, 70, 100 or 180	**- *UGT1A1*6/*28*, *UGT1A7*3/*3* or *UGT1A9*1/*1*: greater risk of adverse events and therapeutic intervention: OR = 11.00, 95% CI 1.633-74.083); *P* = 0.014.**	45	1	263
*UGT1A1*28*, *UGT1A1*60*, *UGT1A1*93*, *UGT1A7*3*, and *UGT1A9*22*	Caucasian	Hematologic toxicity, response rate	180	**- Haplotype II (all variants except *UGT1A1*22*) associated with increased response rate: OR = 8.61; 95% CI 1.75 – 42.38; *P* = 0.01**	250	1	262
Amongst other genes: *SLCO1B1 (rs4149056)* *UGT1A1*6* *UGT1A9*22* *ABCC2 (rs3740066)* *ABCG2 (rs2231137)*	Asian	Neutropenia Diarrhea	65 or 80	**- *SLCO1B1* and *UGT1A1*6*: increased risk for gr. 4 neutropenia** **- *UGT1A9*1/*1, ABCC2* (rs3740066), *ABCG2* (rs2231137): increased risk for gr. 3 diarrhea**	107	1	264
Amongst other genes: *UGT1A1*93*, *ABCC1 (rs*3765129*)*, *SLCO1B1*1b (rs2306283)*	African American (11 pts), Caucasian (67 pts), other (7 pts)	ANC nadir	300, 350	**- *UGT1A1*93, ABCC1 (rs*3765129*):* decreased ANC nadir.** **- *SLCO1B1*1b (rs*2306283**): increased ANC nadir (*P* < 0.05).**	85	1	174
*Amongst other genes: ABCB1 (rs*1045642, rs1128503, rs2032582)	Caucasian	Toxicity Response rate	180	**- *ABCB1* (rs1045642) associated with early toxicity: OR = 3.79 (95% CI 1.09–13.2** **- *ABCB1* haplotype (*rs*1045642, rs1128503, rs2032582): shorter OS, OR = 1.56, 95% CI = 1.01–2.45**	140	1	268
ABCC2: rs1885301, rs2804402, rs717620, rs2273697, rs17216177, rs3740066	Caucasian	Diarrhea	260 - 875 mg	**- Decreased incidence of diarrhea for *ABCC2*2* haplotype (rs1885301, rs2804402, rs717620, rs2273697, rs17216177, rs3740066) without *UGT1A1*28* allele: OR = 0.15 (95% CI = 0.04–0.61), *P* = 0.005.**	167	1	271

Table 3 continued.

Polymorphism	Ethnicity	Endpoint	Dose range (mg/m²)	Main findings	Patients (N)	Studies (N)	Ref.
Clinical studies							
Amongst other genes: *ABCC5* (rs10937158, rs3749438, rs2292997) *ABCG1* (rs225440)	Caucasian	Diarrhea Neutropenia	180	**- Reduced risk of diarrhea for *ABCC5* haplotype (rs10937158 and rs3749438): OR = 0.39 (95% CI = 0.23–0.67, *P* = 0.0006** **- Increased risk of neutropenia for co-occurrence of *ABCG1* and *ABCC5* (rs2292997):OR = 5.93 (95% CI = 2.25–15.59, *P* = 0.0002**	167	1	272
*SLCO1B1*1b*	Caucasian	Neutropenia	300, 350, 380 – 600 (mg)	**- *SLCO1B1*1b*: increased ANC nadir (*P* < 0.05)**	67 (discovery)108 (replication cohort)	1	273
Amongst other genes: *SLCO1B1*1b*, *SLCO1B1*5* (rs4149056)	Caucasian	SN-38 PK, toxicity, PFS	180	- ***SLCO1B1*1b*: increased PFS (*P* < 0.05).** **- *SLCO1B1*5*: increased SN-38 plasma concentration and increased risk of neutropenia (combined with *UGT1A1*28*) (*P* < 0.05).**	127	1	274

Significant findings are shown in bold.

Abbreviations: 95% CI = 95% confidence interval; ANC = absolute neutrophil count; AUC = area under the plasma concentration-time curve; DLT = dose-limiting toxicity; Gr. = grade; OR = odds ratio; OS = overall survival; PFS = progression-free survival; PK = pharmacokinetic. Pt(s) = patient(s); Ref. = reference

Table 4. Overview of guidelines on pharmacogenetic testing for irinotecan

Organization	Country	Year of last update	Genotype recommended for testing	Dose reduction explicitly recommended?	Recommendation	Ref.
U.S. Food and Drug Administration (FDA)	United States of America	2014	*UGT1A1*28*	Yes	*UGT1A1*28/*28*: starting dose reduction by at least one dose level	275
Health Canada/ Santé Canada (HCSC)	Canada	2014	*UGT1A1*28*	Yes	*UGT1A1*28/*28*: reduced starting dose	276
National Pharmacogenetics Network (RNPGx) and the Group of Clinical Onco-pharmacology (GPCO-Unicancer)	France	2015	*UGT1A1*28*	Yes	*UGT1A1*28/*28* and dose 180 – 230 mg/m^2: 25-30% reduction of starting dose. *UGT1A1*28/*28* and dose $\geq$ 240 mg/m^2: irinotecan contra-indicated	278
Royal Dutch Association for the Advancement of Pharmacy (KNMP)	The Netherlands	2011	*UGT1A1*28*	Yes	*UGT1A1*28/*28* and dose > 250 mg/m^2: 30% reduction of starting dose.	279
European Medicines Agency (EMA)	Europe	2017	*UGT1A1*28*	Yes	*UGT1A1*28/*28*: reduce starting dose of liposomal irinotecan from 80 to 60 mg/m^2.	280
Pharmaceuticals and Medical Devices Agency (PMDA)	Japan	2014	*UGT1A1*6* and **28*	No	Use irinotecan with caution in patients with the following genotypes: *UGT1A1*6/*6*, *UGT1A1*28/*28* and *UGT1A1*6/*28*.	281

Abbreviations: Ref. = reference

CONCLUSIONS AND FUTURE PERSPECTIVES

Irinotecan is a crucial anti-cancer drug in treatment regimens for several solid tumors. Many factors which contribute to the large inter-individual pharmacokinetic variability have been elucidated. The last decade, much progress has been made in unraveling the pharmacogenetics influence on systemic exposure, toxicity, and survival. However, this knowledge has not sufficiently been translated into general clinical practice yet.

Based on the pharmacokinetic and pharmacogenetic data discussed in this review article, we recommend dosing adjustments in the following situations:

- Concomitant use of potent CYP3A4 inducers (e.g. rifampicin, phenytoin, phenobarbital, carbamazepine, St. John's wort): avoid combination.
- Concomitant use of potent CYP3A4 inhibitors (e.g. ketoconazole, itraconazole): avoid combination.
- Caucasians: perform genotyping for *UGT1A1*28*. Consider at least a 25% reduction of starting dose in patients homozygous for *UGT1A1*28*.
- Asians: perform genotyping for *UGT1A1*6*. Consider dose reduction of starting dose in patients homozygous for *UGT1A1*6*. Exact dosing adjustments are yet unknown.

Future research should prospectively investigate the added value of individualized irinotecan treatment based on patient characteristics, pharmacogenetics and co-medication. Furthermore, novel drug formulations such as liposomal forms of irinotecan could help to pharmacologically optimize irinotecan treatment.

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PART III

REGORAFENIB

9

INFLUENCE OF THE PROTON PUMP INHIBITOR ESOMEPRAZOLE ON THE BIOAVAILABILITY OF REGORAFENIB: A RANDOMIZED CROSSOVER PHARMACOKINETIC STUDY

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ABSTRACT

BACKGROUND

Regorafenib exposure could potentially be influenced by an interaction with acid reducing drugs.

METHODS

In this cross-over trial, patients were randomized into 2 sequence groups consisting of 3 phases: regorafenib intake alone, regorafenib with concomitant esomeprazole, and regorafenib with esomeprazole 3 hours prior. Primary endpoint was the relative difference (RD) in geometric means for regorafenib $AUC_{0\text{-}24h}$, and was analyzed by a linear mixed model in 14 patients.

RESULTS

$AUC_{0\text{-}24h}$ for regorafenib alone was 55.9 µg*h/mL (CV: 40%), and for regorafenib with concomitant esomeprazole or with esomeprazole 3 hours prior $AUC_{0\text{-}24h}$ was 53.7 µg*h/mL (CV: 34%) and 53.6 µg*h/mL (CV: 43%), respectively. No significant differences were identified when regorafenib alone was compared to regorafenib with concomitant esomeprazole (RD: -3.9%, 95% CI: -20.5-16.1%, P=1.0) or regorafenib with esomeprazole 3 hours prior (RD: -4.1%, 95% CI: -22.8-19.2%, P=1.0).

CONCLUSION

These findings indicate that regorafenib and esomeprazole can be safely combined in clinical practice.

INTRODUCTION

Regorafenib is an oral multi-kinase inhibitor that targets angiogenic, stromal and oncogenic receptor tyrosine kinases (e.g. VEGFR, KIT, BRAF, PDGFR and FGFR).[1] It is currently registered for metastatic colorectal cancer (mCRC), gastro-intestinal stromal tumor (GIST), and hepatocellular carcinoma (HCC).[2-4] Regorafenib is the first and currently only tyrosine kinase inhibitor (TKI) registered for mCRC, although the median overall survival increase for an unselected group in the 3rd or 4th line of treatment is only 1.4 months compared to placebo.[2] For HCC and GIST, regorafenib provides a stronger survival benefit as second and third line TKI-based therapy.[3,4] For several TKIs, systemic exposure has been demonstrated to influence toxicity and efficacy.[5,6]

After oral administration, regorafenib is rapidly absorbed, with a time of maximum concentration (T_{max}) reached at 3-4 hours.[6,7] Most TKIs exhibit pH-dependent solubility.[8] For regorafenib a low basic predicted pK_a of around 2 suggests influence of the gastro-intestinal pH on the absorption, however this is not clearly demonstrated.[9,10] Although the physiochemical properties of regorafenib may not predict significant pH dependent solubility, regorafenib absorption is multifactorial and may be affected by the concomitant use of acid-reducing drugs.[11] For many TKIs, a pharmacokinetic interaction with an acid-suppressive agent has already been demonstrated, for example, erlotinib combined with omeprazole resulted in 46% decrease in systemic exposure.[8] However, for some TKIs this interaction could be ruled out. To our knowledge, for regorafenib there is no study available yet on a possible drug-drug interaction with acid-reducing drugs.

When the exposure is decreased, the efficacy of TKI treatment could potentially also decrease, as was demonstrated for sorafenib and pazopanib among other TKIs.[6] As regorafenib resembles the structure and mechanism of action of sorafenib, an exposure-response relationship could be suspected for regorafenib as well. In a secondary analysis of the phase-3, RESORCE trial in HCC patients, median overall survival and time-to-progression tended to be longer in patients with higher regorafenib exposure during the first treatment cycle, however after correction for several covariates it did not reach statistical significance.[12] To our knowledge, this trial is the only available evidence on a possible exposure-response relationship for regorafenib; therefore, more research is necessary on this point.

Acid-suppressive therapy is frequently used by cancer patients, both as prophylaxis for gastro-intestinal bleeding due to drug-drug interactions (DDI) and as treatment for gastresophageal reflux disease (GERD).[13] In 2013, Smelick et al reported that up

to 33% of all anticancer patients used any form of acid-suppressive therapy, most notably a proton pump inhibitor (PPI).[14] TKIs often cause stomach complaints or GERD, which confronts clinicians with a challenge, as the general consensus is to avoid the combination of TKIs and acid-suppressive agents. Therefore, registration authorities nowadays recommend investigating this DDI before registration of a new TKI. However, for regorafenib, this potential DDI has not been investigated.

In this study we assessed the potential pharmacokinetic interaction between esomeprazole and regorafenib . Furthermore, we also assessed the potential influence of timing of esomeprazole intake relative to that of regorafenib (three hours before regorafenib ingestion or concomitantly).[15]

METHODS

This study was a randomized, two-armed, three-phase, cross-over clinical trial in patients using regorafenib. Between May 2016 and February 2018, the study was performed at the Erasmus Medical Center, Rotterdam, The Netherlands. Approval of the Medical Ethics Committee and the board of directors from the Erasmus University Medical Center, and the competent authorities was obtained. The study was registered at the European Clinical Trials Database (EudraCT 2015-005784-17), and clinicaltrials.gov (NCT02800330).

PATIENTS

Patients were included if they were 18 years or older, had a pathological confirmed diagnosis of mCRC or GIST, Eastern Cooperative Oncology Group (ECOG) performance status ≤ 1, with adequate kidney and liver function. Patients were excluded if they could not abstain from dietary supplements or medication which could interact with regorafenib or esomeprazole, if they could not interrupt acid-suppressive therapy, or if they had a known impaired drug absorption or serious illness that could interfere with study conduct (e.g. infection, bleeding diathesis or hemorrhage, arterial or venous thrombotic or embolic events, uncontrolled hypertension despite optimal medical management, HIV, hepatitis, organ transplants, or kidney, cardiac and respiratory diseases). All patients provided written informed consent before any study related procedure was pursued.

STUDY DESIGN

The main objectives of this study were to compare the area under the curve (AUC)

of regorafenib alone to regorafenib concomitantly used with esomeprazole, and to regorafenib used with esomeprazole three hours prior in patients with mCRC or GIST. Patients started with regorafenib on 120 or 160 mg once daily during a loading phase of 14 consecutive days (**Figure 1**). Regorafenib dose adjustments were only allowed during these first two weeks of the trial. However, due to (reversible) toxicity, the study was allowed to be temporarily interrupted for a maximum of one full regorafenib dosing cycle (i.e. 28 days). After reaching steady-state, patients either used regorafenib alone (phase A), or with esomeprazole (40mg once daily) for five consecutive days (phase B and C). During phase B of the study regorafenib was administered concomitantly with esomeprazole, while during phase C regorafenib was administered three hours after esomeprazole intake, presuming a maximally elevated intragastric pH at the time of regorafenib ingesture.[16] Subjects were randomized into two sequence groups (i.e. A-B-C or C-B-A) to rule out sequence and time effects (**Figure 1**).

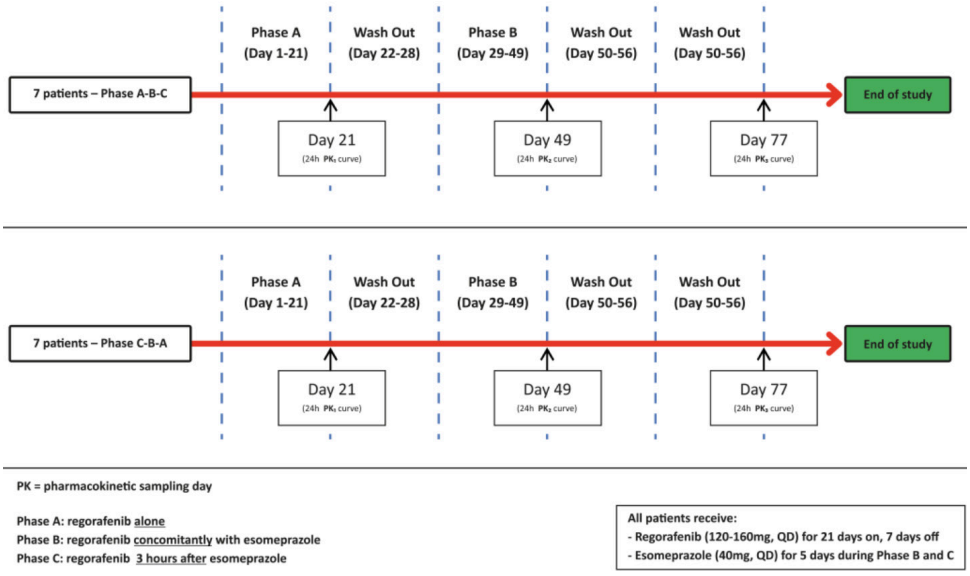

Figure 1. Study procedures

PHARMACOKINETICS

Patients were admitted to the hospital on the 21st, the 49th and the 77th day of the trial for pharmacokinetic blood sampling. Blood samples were collected before regorafenib administration, and at the 0.5h; 1h; 1.5h; 2h, 2.5h; 3h; 3.5h; 4h; 6h; 8h; 12h and 24h time-point after regorafenib administration (at 10:00 AM). Blood samples were collected in

4 mL lithium heparin (Li-He) blood collection tubes, and processed into plasma within 10 minutes by centrifugation for 10 minutes at 2,500*g (at 4°C) and stored at T<-70°C until analysis. Regorafenib, M-2, and M-5 plasma concentrations were measured using a validated liquid chromatography tandem mass spectrometry (UPLC-MS/MS) method (detailed description in **Supplementary Methods**). Pharmacokinetic parameters were calculated by using Phoenix WinNonlin version 7.0, and included exposure expressed as dose corrected area under the curve from pre-intake time point until 24 hours (AUC_{0-24h}), maximum observed concentration (C_{max}), and time until maximum observed concentration (T_{max}).

STATISTICAL ANALYSIS

A difference in systemic exposure to regorafenib of 30% was determined to be clinically relevant. Since two primary comparisons were to be made, i.e. regorafenib with esomeprazole concomitant or three hours prior compared to regorafenib alone, a Bonferroni correction was applied. The Bonferroni correction was implemented by multiplying the obtained p-values by two and calculation of 97.5% confidence intervals (CI) which correspond to the alpha of 0.025 with the interpretation of Bonferroni corrected 95% CIs. It was assumed that the within patient standard deviation in regorafenib pharmacokinetics was 30%. Given a power of 80%, the sample size calculation resulted in a required number of 14 evaluable patients [17]. Patients were considered evaluable when they completed all three phases, including all required blood samples.

Analyses of the AUC_{0-24h} and C_{max} were performed on log-transformed observations since they were assumed to follow a lognormal distribution [18]. Estimates for the mean differences in (log) AUCs and C_{max} of regorafenib, M-2 and M-5 were obtained for the two comparisons separately using a linear mixed effect model with treatment, sequence, and phase as fixed effects and subject within sequence as a random effect [19]. Variance components were estimated based on restricted maximum likelihood (REML) methods and the Kenward-Roger method of computing the denominator degrees of freedom was used. The mean differences and CIs for the differences were exponentiated to provide point estimates of the ratio of geometric means and CIs for these ratios, which can be interpreted as relative differences in percentages. T_{max} was analyzed by means of the Wilcoxon signed rank test and described with medians and interquartile ranges.

Toxicity was described as the incidence of toxicity per phase and was corrected for baseline toxicity by describing only new or worsened toxicity compared to baseline. This study was not powered to detect a difference in toxicity between treatment phases, therefore these results only have a descriptive character.

RESULTS

PATIENT CHARACTERISTICS

A total of 31 patients were included, of which 14 patients were evaluable for the primary endpoint analysis. The evaluable patients were equally distributed over the two treatment sequence groups. Patients were not evaluable due to various reasons: screen failures (n=4); rapid disease progression during treatment (n=8); and premature treatment interruption (n=5). Patients who developed progressive disease during the study period were also equally distributed over the two treatment sequences.

Patient characteristics are detailed in **Table 1**. All patients suffered from mCRC, were of Caucasian origin and predominantly male (71%). Median age was 69 years and most patients had an ECOG performance status of 1 (86%). All patients used regorafenib 120 mg at steady-state on recommendation of the treating physician or due to dose-reductions in the first two weeks of the trial.

Table 1. Patient characteristics

Characteristic	Total
Gender	
Male	10 (71%)
Female	4 (29%)
Age (years)	
Median [IQR]	69 [61-73]
ECOG Performance Status	
0	2 (14%)
1	12 (86%)
Ethnic origin	
Caucasian	14 (100%)
BMI (kg/m^2)	
Median [IQR]	28.6 [24.1-29.9]
eGFR (mL/min)[a]	
Median [IQR]	82 [77-91]
Liver function *(median [IQR])*	
AST	39 [27-68]
ALT	33 [17-39]
Bilirubin	8 [6-13]
Prior therapy	
Surgery	12 (86%)
Radiotherapy	4 (29%)
Chemotherapy	14 (100%)
Monoclonal antibodies[b]	9 (64%)

[a] eGFR was calculated according to the CKD-EPI

[b] Treatment with monoclonal antibodies included bevacizumab, panitumumab, and cetuximab

Abbreviations: AST = aspartate aminotransferase; ALT = alanine aminotransferase; BMI = Body Mass Index; eGFR = estimated glomerular filtration rate; IQR = interquartile range

PHARMACOKINETICS

All obtained pharmacokinetic results are depicted in **Table 2**. No statistical difference in geometric means for regorafenib $AUC_{0\text{-}24h}$ was found when regorafenib alone was compared to regorafenib and esomeprazole concomitantly (relative difference [RD]: -3.9%, 95%CI: -20.5-16.1%, P = 1.0) or when compared to regorafenib and esomeprazole three hours before regorafenib intake (RD: -4.1%, 95%CI: -22.8-19.2%, P = 1.0) (**Figure 2**). Furthermore, no differences could be identified in C_{max} or T_{max} for regorafenib. For M-2 and M-5 no differences could be identified either, although the interindividual variability (expressed as coefficient of variation; CV) was much higher for all these pharmacokinetic parameters compared to regorafenib (**Table 2, Supplementary Figure 1)**. No sequence nor period effects were seen for any of the comparisons of the $AUC_{0\text{-}24h}$ and C_{max} (results not shown).

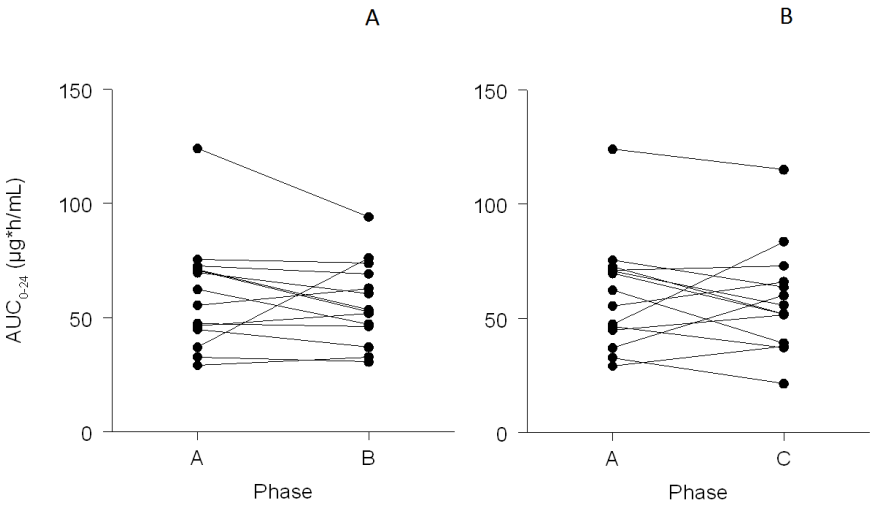

Figure 2. Regorafenib AUC

Regorafenib exposure compared between phase A (regorafenib alone) and phase B (regorafenib concomitantly with esomeprazole) (figure 2A), and between phase A and C (regorafenib with esomeprazole 3 hours prior) (figure 2B)

Abbreviations: $AUC_{0\text{-}24}$ = Area under the curve, timepoint 0h to 24h

Table 2. Regorafenib pharmacokinetics

PK parameters	Regorafenib *(phase A)*	Regorafenib + Esomeprazole concomitant *(phase B)*	Regorafenib + Esomeprazole 3h prior *(phase C)*	Relative difference B vs A (95%CI)	*P-value*	Relative difference C vs A (95%CI)	*P-value*
Regorafenib							
AUC_{0-24h} *(µg*h/mL (CV))*	55.9 (40.3)	53.7 (33.5)	53.6 (42.6)	-3.9% (-20.5-16.1%)	*1.00*	-4.1% (-22.8-19.2%)	*1.00*
C_{max} *(µg/mL (CV))*	5.3 (28.6)	4.4 (24.2)	4.7 (25.5)	-16.5% (-34.9-7.0%)	*0.18*	-12.1% (-32.0-13.8%)	*0.45*
T_{max} *(median hours (IQR))*	2.5 (2.0-3.0)	2.5 (2.0-3.0)	3.0 (2.5-3.1)		*1.00*		*0.83*
M-2							
AUC_{0-24h} *(µg*h/mL (CV))*	36.6 (71.4)	35.1 (66.2)	35.0 (64.5)	-4.0% (-28.6-29.2%)	*1.00*	-4.3% (-30.1-31.0%)	*1.00*
C_{max} *(µg/mL (CV))*	2.9 (72.0)	2.6 (60.9)	2.6 (44.2)	-11.0% (-38.7-29.1%)	*0.88*	-9.3% (-38.1-32.9%)	*1.00*
T_{max} *(median hours (IQR))*	3.3 (2.0-6.0)	2.6 (2.1-3.5)	3.5 (2.5-6.0)		*1.00*		*1.00*
M-5							
AUC_{0-24h} *(µg*h/mL (CV))*	21.9 (103.4)	21.6 (125.7)	20.0 (128.9)	-1.4% (-22.5-25.4%)	*1.00*	-8.9% (-40.4-39.1%)	*1.00*
C_{max} *(µg/mL (CV))*	1.6 (118.8)	1.4 (132.4)	1.4 (107.6)	-10.4% (-34.6-22.8%)	*0.78*	-9.1% (-43.2-45.5%)	*1.00*
T_{max} *(median hours (IQR))*	2.6 (1.5-4.0)	2.3 (1.5-8.0)	3.5 (2.5-6.0)		*1.00*		*0.76*

Abbreviations: AUC_{0-24h} = Area under the curve, timepoint 0h to 24h (expressed as geomean µg*h/mL (CV)); CI = Confidence Interval; C_{max}= maximum concentration (expressed as geomean µg/mL (CV)); CV = coefficient of variation expressed in %; h = hours; IQR = interquartile range; PK = pharmacokinetic; T_{max} = time until maximum concentration (expressed as median hours (IQR))

TOXICITY

Most common adverse events during the whole study period were hoarseness (79%), anorexia (71%), hypertension (71%), hand foot skin reaction (64%), fatigue (71%), stomatitis (57%), and nausea (50%). Also, most common blood value disorders included transaminase increase (79%), bilirubin increase (50%) and hypophosphatemia (29%). The majority of adverse events was of low grade, the incidence of toxicity ≥ grade 3 occurred mainly as hypertension (64%), anorexia (14%) and hand foot skin reaction (14%). The incidence of adverse events seems comparable between different phases. Two patients developed major cardiac events, possibly related to regorafenib treatment: myocardial infarction and atrial fibrillation. One patient developed hypertrichosis, although this rare side effect is seen more often with other TKIs such as erlotinib [20], to our knowledge it has not been described for regorafenib. All observed adverse events are described in **Supplementary Table 1**.

DISCUSSION

This randomized, three-phase, cross-over clinical trial did not reveal a significant pharmacokinetic interaction between esomeprazole and regorafenib at the two time-points studied. Therefore we can conclude that esomeprazole can be combined with regorafenib safely, in contrast to other TKIs.

In this study, esomeprazole was used because it exhibits the strongest pH-reducing effect of all acid-reducing drugs currently available.[8,16] Also, esomeprazole does not influence other enzymes or transporters, such as P-glycoprotein (ABCB1), that could potentially influence the pharmacokinetics of regorafenib's active metabolites M-2 and M-5.[21] Therefore, our findings cannot be extrapolated to other PPIs -- such as pantoprazole -- which is known to influence P-glycoprotein. We examined two time-points regarding the intake time of esomeprazole (i.e. concomitantly or three hours prior regorafenib intake), because PPIs are assumed to have their maximum acid-reducing effect three hours after intake and a possible interaction would be the strongest at this time-point.[15] However, even at this time-point we did not demonstrate an influence of esomeprazole on the pharmacokinetics of regorafenib, M-2 and M-5.

Regorafenib exhibits low solubility, which is mainly caused by its chemical structure as no strong basic or acidic group is attached (regorafenib: 4-[4-({[4-chloro-3-(trifluoromethyl)phenyl]carbamoyl} amino)-3-fluorophenoxy]-N-methylpyridine-2-carboxamide).[22] Furthermore, to improve the solubility, regorafenib is formulated as a

solid dispersion consisting of small powder particles in which the drug and excipient are integrated.[23] Despite this formulation, regorafenib exhibits low solubility compared to other TKIs. As a result regorafenib absorption is, in theory, less affected by intragastric pH-alterations and the results of this study were not totally unexpected. However, since TKI absorption is multifactorial a drug-drug interaction with PPIs cannot always be fully ruled out based on modeling and physiochemical properties alone.[11] Therefore, a drug interaction should always be verified in an *in vivo* setting as was done in this study for regorafenib.

In order to reach the required sample size of 14 evaluable patients a total of 31 patients had to be included in the study, due to the fact that many patients were not able to complete three cycles of regorafenib at 160 or 120 mg due to treatment-related adverse events or progression of disease. In addition, we aimed to include both mCRC and GIST patients, but mainly mCRC patients were included, which resulted in a possible selection bias. In general, mCRC patients are in a worse condition and more heavily pre-treated compared to GIST patients, which could have resulted in more adverse events and a higher drop-out rate. However, we do not think it influenced the pharmacokinetic end points. In addition, the CORRECT trial demonstrated a median overall survival increase of 1.4 months compared to placebo in mCRC patients.[2] Therefore, it was not completely surprising that quite some patients developed early disease progression during study treatment hampering prolonged study participation. In addition, all patients eventually used 120 mg at steady-state instead of 160 mg, due to known severe treatment-related adverse events (e.g. hypertension), which also occurred in up to 50% of patients in the registration studies.[2-4] Furthermore, because this study was designed as a pharmacokinetic cross-over study, we could not compare toxicity between different cycles. However, because we found no differences in regorafenib pharmacokinetics, a difference in exposure-related toxicity seems unlikely. This study was designed to demonstrate a difference based on two primary comparisons on regorafenib exposure depending on esomeprazole intake time (concomitantly or three hours prior). Because of the assumption of a difference between those cycles, we did not include a bioequivalence analysis. However, the boundaries of the adjusted 90%-confidence interval of the relative differences of the regorafenib AUC found in this study almost fit the limits for bioequivalence (B vs A, RD: -3.9%, 90% CI: -18.2-12.9%, and C vs A, RD: -4.1%, 90%CI: -20.3-15.4%)[18], which supports the interpretation of our results.

In conclusion, we have shown that esomeprazole did not influence regorafenib exposure on two different intake time-points, and that these drugs can be combined in clinical practice, without the appearance of a significant pharmacokinetic interaction.

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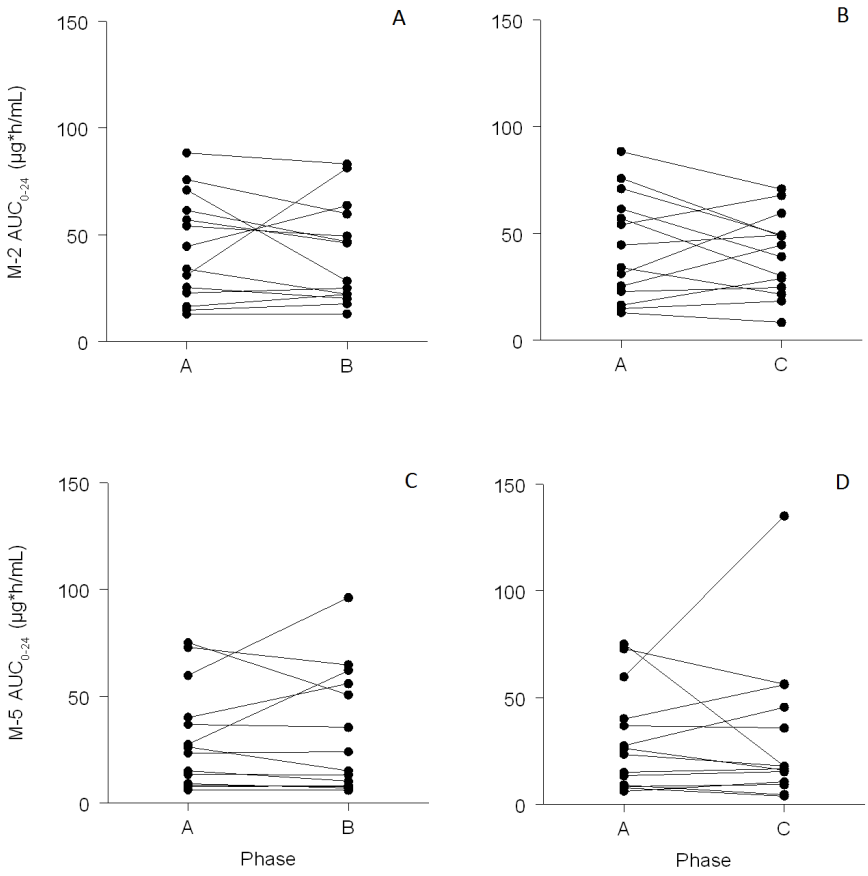
SUPPLEMENTARY INFORMATION
SUPPLEMENTARY METHODS

DETAILED DESCRIPTION ASSAY REGORAFENIB, M-2 AND M-5

Regorafenib and the metabolites M-2- and M-5 were simultaneously quantitated by a validated liquid chromatography tandem triple quadrupole mass spectrometry (UPLC-MS/MS) assay. Aliquots of 25 µL of human lithium heparinized plasma samples for the quantitation of regorafenib and its metabolites were deproteinized, after the addition of 100 µL of Internal Standard (sunitinib-d10). After vigorously mixing for 5 seconds and centrifugation for 10 min at 18,000**g*, aliquots of 1 µL were injected into the UPLC-MS/MS-system. Peak area ratios of analytes versus the Internal Standard were a function of the concentration from 20.0 to 5,000 ng/mL. For regorafenib, the within and between-run precisions at five tested concentrations, including the LLQ, were ≤ 5.94 and ≤ 9.99%, respectively, while the average accuracy ranged from 101.4 to 112.5%. For regorafenib-M2, the within and between-run precisions at five tested concentrations, including the LLQ, were ≤ 5.18 and ≤ 11.4%, respectively, while the average accuracy ranged from 91.0 to 96.7% and for regorafenib-M5, the within and between-run precisions at five tested concentrations, including the LLQ, were ≤ 6.47 and ≤ 11.2%, respectively, while the average accuracy ranged from 92.8 to 99.4%.

SUPPLEMENTARY RESULTS



Supplementary Figure 1. M-2 and M-5 AUC

M-2 and M-5 exposure compared between phase A (regorafenib alone) and phase B (regorafenib concomitantly with esomeprazole) (figure 1A, 1C), and between phase A and C (regorafenib with esomeprazole 3 hours prior) (figure 1B, 1D). Abbreviations: AUC_{0-24} = Area under the curve, timepoint 0h to 24h

Supplementary Table 1. Toxicity

Toxicity[a]	Regorafenib N (%)	Regorafenib + Esomeprazole Concomitant N (%)	Regorafenib + Esomeprazole 3h prior N (%)	Overall[b] N (%)
Gastrointestinal				
Anorexia				
All grades	6 (43)	5 (36)	5 (36)	10 (71)
Grade ≥3	1 (7)	1 (7)	1 (7)	2 (14)
Constipation				
All grades	2 (14)	2 (14)	1 (7)	4 (29)
Grade ≥3	0	0	0	0
Diarrhea				
All grades	0	1 (7)	2 (14)	2 (14)
Grade ≥3		0	0	0
Nausea				
All grades	2 (14)	5 (36)	3 (21)	7 (50)
Grade ≥3	0	0	0	0
Reflux				
All grades	1 (7)	0	1 (7)	2 (14)
Grade ≥3	0	0	0	0
Stomatitis				
All grades	5 (36)	6 (43)	5 (36)	8 (57)
Grade ≥3	0	0	0	0
Vomiting				
All grades	2 (14)	2 (14)	3 (21)	5 (36)
Grade ≥3	0	0	0	0
Respiratory				
Cough				
All grades	0	2 (14)	1 (7)	2 (14)
Grade ≥3		0	0	0
Dry mouth				
All grades	0	1 (7)	3 (21)	3 (21)
Grade ≥3		0	0	0
Dyspnea				
All grades	3 (21)	3 (21)	4 (29)	6 (43)
Grade ≥3	0	0	1 (7)	1 (7)
Ear pain				
All grades	1 (7)	2 (14)	1 (7)	3 (21)
Grade ≥3	0	0	0	0
Hoarseness				
All grades	8 (57)	11 (79)	9 (64)	11 (79)
Grade ≥3	0	0	0	0
Vascular				
Cardiac events[c]				
All grades	0	2 (14)	0	2 (14)
Grade ≥3		0		0
Hypertension				
All grades	4 (29)	4 (29)	5 (36)	10 (71)
Grade ≥3	3 (21)	3 (21)	5 (36)	9 (64)
Skin & Hair				
Erythema				
All grades	1 (7)	2 (14)	1 (7)	4 (29)
Grade ≥3	0	0	0	0

Supplementary Table 1 continued.

Toxicity[a]	Regorafenib N (%)	Regorafenib + Esomeprazole Concomitant N (%)	Regorafenib + Esomeprazole 3h prior N (%)	Overall[b] N (%)
Skin & Hair				
Hand foot skin reaction				
All grades	6 (43)	9 (64)	7 (50)	9 (64)
Grade ≥3	0	1 (7)	1 (7)	2 (14)
Hypertrichosis				
All grades	0	1 (7)	1 (7)	1 (7)
Grade ≥3		0	0	0
General disorders				
Fatigue				
All grades	6 (43)	7 (50)	7 (50)	10 (71)
Grade ≥3	1 (7)	0	1 (7)	1 (7)
Blood value disorders				
AST/ALT increase				
All grades	7 (50)	6 (43)	5 (36)	11 (79)
Grade ≥3	0	1 (7)	0	1 (7)
Bilirubin increase				
All grades	4 (29)	3 (21)	2 (14)	7 (50)
Grade ≥3	0	0	0	0
Hypophosphatemia				
All grades	2 (14)	1 (7)	1 (7)	4 (29)
Grade ≥3	0	0	1 (7)	1 (7)
Platelet count decreased	0	1 (7)	0	1 (7)
All grades		0		0
Grade ≥3				

Number of patients is scored as individual patients per phase.

[a] Toxicity was graded according to the NCI CTC-AE classification (version 4.03)

[b] Overall toxicity was defined as the number of patients during the whole study period (i.e. all three phases)

[c] Cardiac events included atrial fibrillation and myocardial infarction

Abbreviations: AST = aspartate aminotransferase; ALT = alanine aminotransferase; N = number of patients

PART IV

CARBOPLATIN / PACLITAXEL

11

EFFICACY AND TOXICITY OF WEEKLY CARBOPLATIN AND PACLITAXEL AS INDUCTION OR PALLIATIVE TREATMENT IN ADVANCED ESOPHAGEAL CANCER PATIENTS

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ABSTRACT

BACKGROUND

Unfortunately, many patients with esophageal cancer have advanced disease at diagnosis, hence many treatment regimens with induction or palliative intent have been described. However, the most optimal treatment has not been identified. Therefore, we evaluated a weekly regimen of carboplatin (targeted at an area under the curve (AUC) of 4) and paclitaxel 100mg/m^2 as induction or palliative treatment.

METHODS

All patients with advanced (gastro)esophageal cancer treated with this regimen at Erasmus MC between 2002-2018 were included. Exclusion criteria were previous or concurrent radiotherapy on the esophagus, limited data, or treatment elsewhere. Data on toxicity, treatment response and survival were collected. Analyses were performed in two separate groups: induction (iCT) or palliative chemotherapy (pCT). Progression free survival (PFS) and overall survival (OS) were estimated with the Kaplan-Meier method.

RESULTS

A total of 291 patients was included; iCT: 122 and pCT: 169. Most patients had a T3 carcinoma (iCT:54%; pCT:66%), and stage IV disease (iCT:42%; pCT:91%). Toxicity grade ≥3 occurred mainly as hematological toxicity (iCT:71%; pCT:73%), and sometimes as gastrointestinal toxicity (iCT:3%; pCT:5%). Response rates after six cycles were 48% (iCT) and 44% (pCT). For 42% of iCT patients esophagectomy or definitive chemoradiotherapy followed, resulting in a median PFS of 22.1 months (interquartile range (IQR):12.4-114.2) and median OS of 26.8 months (IQR:15.4-91.7). For pCT, median PFS was 8.2 months (IQR:5.1-14.5) and median OS 10.9 months (IQR:6.5-18.3).

CONCLUSION

Weekly carboplatin (AUC4) and paclitaxel (100mg/m^2) is a well-tolerable and effective induction or palliative treatment regimen, and therefore an option for patients with locally advanced or metastatic disease.

INTRODUCTION

Esophageal cancer is currently the 8th most common cancer type worldwide, the incidence is still rising, and its mortality is high.[1-3] Squamous cell carcinoma (SCC) is the most common histology for esophageal cancer worldwide, though adenocarcinomas (AC) are more dominant in the Western world due to typical welfare risk factors including obesity, smoking, and chronic gastresophageal reflex resulting in Barrett's esophagus.[1, 3, 4] Esophageal cancer occurs three to four times more often in male than in female patients in both histological subtypes.[1, 3, 4] However, the reason for the lower incidence in women is not completely understood; it has been suggested that female hormones or the different body fat distribution may protect women against this type of tumor.[5-7] Furthermore, Bohanes et al. demonstrated that male sex is an independent adverse prognostic factor for esophageal cancer-specific survival with a shorter survival for men compared to women in both locally advanced and metastatic disease.[8] Other adverse prognostic factors are performance status of 2 or higher, significant weight loss before diagnosis (i.e. $\geq$ 10%), adenocarcinoma as histological subtype, liver or peritoneal metastases, extensively disseminated disease, and an elevated alkaline phosphatase or lactate dehydrogenase.[9-12] Anatomic origin (i.e. esophageal, esophageal-gastric junction or gastric) was not identified as a significant prognostic factor in two studies[9, 13], while another study demonstrated that tumors arising in the lower one-third of the esophagus did have a worse survival compared to tumors located in the cervical and upper esophagus.[12]

Although the prognosis of esophageal cancer has improved over the last decades, the outcome still remains poor with an overall 5-year survival of 20%.[2, 4] Improvement in prognosis might be caused by recent advances in the treatment of patients with resectable (gastro)esophageal cancer by introduction of neoadjuvant chemoradiotherapy such as the CROSS-regimen.[14, 15] However, almost half of all patients already have non-resectable (gastro)esophageal cancer at diagnosis (i.e. locally advanced tumors or distant metastasis).[16] For patients with locally advanced disease, systemic treatment can be considered in an attempt to downstage the tumor (i.e. induction treatment), which can be followed by surgery or chemoradiotherapy in case of good response. For induction chemotherapy several treatment regimens are described; most of them platinum- or fluoropyrimidine-based.[17-19]

For patients with distant metastases palliative chemotherapy can be considered.[20, 21] Palliative systemic treatment improves survival compared to best supportive care, yet survival benefit is limited and toxicity should be taken into account.[4, 20-22] Many different palliative treatment regimens are described which are often fluoropyrimidine- or

platinum-based doublet or triplet combination regimens. [4, 20, 21] Triplet regimens might be more effective than doublet regimens, however the incidence of severe toxicity increases significantly in triplet regimens compared to doublets.[21, 23] International guidelines often recommend the combination of a fluoropyrimidine and platinum compound as first-line treatment in metastatic (gastro)esophageal cancer but it should be noted that these guideline are sometimes consensus based. The Dutch Esophagus Cancer Guideline describes that chemotherapy can be considered in metastatic esophageal cancer, but does not recommend one specific treatment regimen.[24] A study of the Netherlands Cancer Registry demonstrated that in the Netherlands only, up to 69 different palliative treatment regimens are administered in metastatic (gastro) esophageal cancer patients.[25] This clearly demonstrates that the most optimal palliative treatment in esophageal cancer is not well defined.

Fifteen years ago, our research group performed a phase-1 study of weekly paclitaxel and carboplatin as palliative treatment for patients with metastatic esophageal cancer.[26] This regimen uses the same chemotherapeutic backbone as the CROSS-regimen but is not combined with radiotherapy. As a result, higher dosages are possible with a recommended dose for paclitaxel of 100 mg/m^2 and carboplatin targeted at an area under the curve (AUC) of 4 mg x min/mL.[26] This weekly regimen appeared to be very tolerable and effective with an overall response rate of 54%. Therefore, this regimen was utilized for most patients with advanced or metastatic (gastro)esophageal cancer at the Erasmus University Medical Center, Rotterdam, The Netherlands. The current analysis describes the efficacy and toxicity of this weekly carboplatin and paclitaxel regimen as induction or palliative treatment option in a real-world treatment setting. Furthermore, predictive factors for treatment outcome and prognostic factors for survival will be analyzed.

METHODS

The patient cohort for this analysis was obtained from the Erasmus University Medical Center, Rotterdam, The Netherlands. The primary end point was treatment response in patients with (gastro)esophageal cancer treated with a weekly regimen of carboplatin (AUC 4) and paclitaxel (100 mg/m^2) with induction or palliative treatment intent, respectively. Secondary end points included progression free survival (PFS), OS, the identification of predictive or prognostic factors, and the evaluation of toxicity. As this treatment regimen was considered routine clinical care at the Erasmus University Medical Center, no specific ethics approval or informed consent was required to

retrospectively collect and analyze these data for research purposes.

PATIENTS

All patients with (gastro)esophageal cancer treated with a weekly regimen of carboplatin (AUC 4) and paclitaxel (100 mg/m^2) between October 2002 and May 2018 at the Erasmus University Medical Center, Rotterdam, The Netherlands, were identified by the hospital pharmacy based on drug-dispensing data and evaluated for inclusion. Patients were excluded if radiotherapy on the esophagus was given concurrent or prior to start of treatment with weekly carboplatin and paclitaxel, if one or more cycles were given outside the Erasmus University Medical Center, or if there was limited data on investigated cycles was recorded in the electronic patient file (e.g. due to missing paper files uploaded in the electronic patient file). A multidisciplinary team consisting of a medical oncologist, upper gastro-intestinal surgeon, gastroenterologist, radiologist, and a radiotherapist, decided upon the treatment intent (induction or palliative treatment). Patients with non-resectable disease due to advanced locoregional bulky disease and/or suspected lymph nodes outside the field of possible radiation therapy (i.e. around the common hepatic artery, splenic hilum, or caudal to the celiac artery) were considered for induction treatment. All patients with distant metastasis were considered for palliative treatment. The treatment intent as decided by the multidisciplinary team was used for all further analyses and not retrospectively altered.

TREATMENT

Treatment consisted of a weekly regimen of carboplatin (AUC 4) and paclitaxel (100 mg/m^2) for three weeks, then one week rest, followed by another three weekly cycles. After these six cycles, response-evaluation with CT-scan followed. For patients with regression of the primary tumor or disappearance of distant metastases while not developing new distant metastases after these first six weekly cycles another treatment option could follow (i.e. esophagectomy or definitive chemoradiotherapy [27]). Selection criteria for esophagectomy were: a radical and curative resection was deemed possible, no distant metastases, sufficient clinical condition for surgery, and patient's consent for surgery. Definitive chemoradiotherapy was proposed to all other patients with a good response after the first six weekly cycles.

For patients without other treatment options (e.g. due to distant metastasis) with a good response on these first six weekly cycles, treatment was continued with three 3-weekly cycles of carboplatin (AUC 6) and paclitaxel (175 mg/m^2). All patients and treatment intent were discussed in a multidisciplinary team both before start and after end of treatment.

Paclitaxel and carboplatin were diluted in 500 mL of sodium chloride solution (0.9%), and both administered in a 1h-infusion. All patients received intravenous premedication consisting of dexamethasone 10 mg, ranitidine 50 mg, clemastine 2 mg within 30 minutes before paclitaxel infusion, and granisetron 1 mg administered within 30 minutes before carboplatin infusion [26]. Before start of a new cycle, hematological laboratory values had to fulfill the following requirements; for the first and fourth cycle (day 0 and 28): leukocytes > 3.0 x 10^9/L, thrombocytes > 100 x 10^9/L, and for the second, third, fifth and sixth cycle (day 7, 14, 35 and 42): leukocytes > 1.0 x 10^9/L, thrombocytes > 50 x 10^9/L. There were no restrictions on the absolute neutrophil count. Furthermore, for a full paclitaxel dose, adequate transaminases and bilirubin were required (bilirubin ≤ 1.5 x upper limit of normal (ULN), and AST / ALT ≤ 2.5 x ULN (in case of liver metastases: AST / ALT ≤ 5 x ULN)).

DATA

Data related to patient demographics, tumor characteristics, laboratory results, adverse events, treatment, response and survival were collected. Tumors were (re)staged according to the 7th edition of UICC-AJCC TNM staging manual [28]. Laboratory results and adverse events were scored according to the Common Terminology Criteria for Adverse Events (CTC-AE) version 4.03 [29]. All adverse events occurring during the six weekly cycles and up to one week after treatment were collected and the highest grade per item was used in the analysis. Severe toxicity was defined as adverse events with a CTC-AE grade 3 or higher. Gastro-intestinal toxicity was defined as the occurrence of anorexia, nausea, vomiting, diarrhea, constipation and mucositis. Hematological toxicity was defined as the occurrence of anemia, thrombocytopenia, leukocytopenia and neutropenia.

Response was determined after six cycles on radiological evaluation by CT-scan according to the radiologist (and if possible) by the RECIST criteria [30]. If there was no radiological evaluation possible due to clinical deterioration or death, this was counted as progressive disease (PD) on date of whatever came first. PFS and OS were defined as time from start of chemotherapy till date of radiological or clinical progression or death, respectively.

STATISTICAL ANALYSIS

For all analyses, the patient cohort was divided in patients treated with induction or palliative treatment intent. These groups were analysed separately to prevent bias induced by baseline patient and tumor characteristics. Demographic characteristics and toxicity were described per group. Survival time (i.e. PFS and OS) was estimated according to the Kaplan-Meier method. Predictive factors for treatment response were analysed with logistic regression analysis, where good response was defined

as complete or partial response (CR or PR). Good response was compared to stable disease (SD) and progressive disease (PD) together. Prognostic factors for survival (PFS and OS) were identified by univariate and multivariate Cox regression analyses. All factors with a P-value <0.1 detected in univariate analyses were included in multivariate analyses. A backward selection method was used for the multivariate model where a threshold of P < 0.05 was applied. In general, P-values <0.05 were considered statistically significant. All statistical analyses were performed using Stata version 15.1 (StataCorp. 2017. Statistical Software, College Station, TX: StataCorp LP).

RESULTS

PATIENT, TUMOR AND TREATMENT CHARACTERISTICS

A total of 420 patients with (gastro)esophageal cancer who were treated with carboplatin (AUC 4) and paclitaxel (100 mg/m^2) were screened for inclusion of whom 129 patients were excluded, mainly because of concurrent radiotherapy or referral for chemotherapy in another hospital (**Figure 1**). This resulted in a total of 291 patients evaluable for the analysis of whom 122 patients were included in the induction chemotherapy (iCT) group and 169 patients in the palliative chemotherapy (pCT) group. For 8 patients (iCT: 3 and pCT: 5), date of death was unknown and they were excluded from the overall survival analysis.

The majority of patients in both groups were male (76% and 82%, respectively) and had a good WHO performance status (i.e WHO 0 or 1). Median age was 64 years (IQR: 58-69) in the iCT group and 61 years (IQR: 55-68) in the pCT group. Most patients still smoked tobacco and used alcohol before diagnosis or currently on a regular base. All patient and treatment characteristics are described in **Table 1**. In the iCT group the incidence of the adenocarcinoma was almost equal to squamous cell carcinoma (50% versus 48%) in contrast to the pCT group where the incidence of adenocarcinoma was higher (70% versus 28%). In the iCT group, tumors were most often located in the distal esophagus (47%), but also in the proximal (19%), middle esophagus (24%) and gastro-esophageal junction (11%). In the pCT group, the majority of all tumors were located at the distal esophagus (73%). TNM-stage was also different between the iCT and pCT patients, which resembles the intent of treatment per group, with more M1-disease and higher variety of metastases locations in the pCT group (**Table 1**). The administered mean carboplatin and paclitaxel dose were comparable in both groups, and median number of treatment cycles was 7 in the iCT group and 8 in the pCT group (i.e. six weekly cycles, followed by 3-weekly cycles).

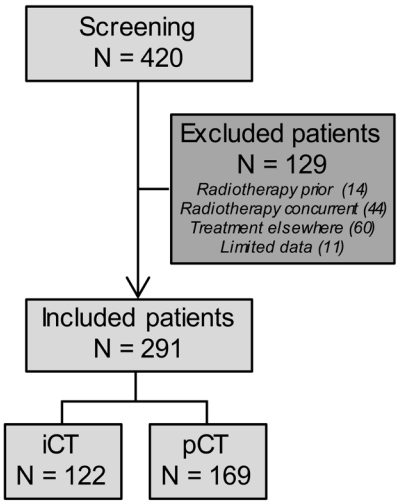

Figure 1. STROBE diagram of included patients

Treatment elsewhere included patients who were referred for one or more cycles of chemotherapy in another hospital.

Abbreviations: iCT = induction chemotherapy; N = number; pCT = palliative chemotherapy

Table 1. Patient, tumor and treatment characteristics

Characteristic	iCT (N=122)	pCT (N=169)
Sex		
Male	93 (76%)	138 (82%)
Female	29 (24%)	31 (18%)
Age (years)		
Median [IQR]	64 [58-69]	61 [55-68]
Performance Status		
WHO 0	28 (23%)	49 (29%)
WHO 1	73 (60%)	90 (53%)
WHO 2	7 (6%)	5 (3%)
Unknown	14 (12%)	25 (13%)
Ethnic Origin		
Caucasian	93 (76%)	120 (71%)
African	1 (0,8%)	3 (2%)
Asian	0	4 (2%)
Unknown	28 (23%)	42 (25%)
BSA (m^2) [a]		
Mean [SD]	1.91 [0.22]	1.89 [0.21]
eGFR (mL/min)[b]		
Median [IQR]	93 [84-99]	92 [78-100]
Unknown	21 (17%)	45 (27%)
Smoking		
Never	15 (12%)	31 (18%)
Before diagnosis	22 (18%)	36 (21%)
Current	80 (66%)	91 (54%)
Unknown	5 (4%)	11 (7%)

Table 1 continued.

Characteristic	iCT (N=122)	pCT (N=169)
Alcohol		
Never	24 (20%)	29 (17%)
Before diagnosis	54 (44%)	81 (48%)
Current	37 (30%)	48 (28%)
Unknown	7 (6%)	11 (7%)
Tumor Location		
Proximal	23 (19%)	8 (5%)
Middle	29 (24%)	26 (15%)
Distal	57 (47%)	123 (73%)
GE-junction	13 (11%)	10 (6%)
Multiple locations	0	2 (1%)
Tumor Type		
Adenocarcinoma	61 (50%)	117 (70%)
Squamous cell carcinoma	59 (48%)	48 (28%)
Other [c]	2 (2%)	3 (2%)
Unknown	0	1 (1%)
Tumor Differentiation		
Good	7 (6%)	3 (2%)
Moderate	38 (31%)	50 (30%)
Poor	48 (39%)	74 (44%)
Unknown	29 (24%)	42 (25%)
T-stage		
T1b	0	3 (2%)
T2	5 (4%)	12 (7%)
T3	66 (54%)	112 (66%)
T4a	27 (22%)	23 (14%)
T4b	24 (20%)	13 (8%)
N-stage		
N0	12 (10%)	21 (12%)
N1	49 (40%)	69 (41%)
N2	48 (39%)	56 (33%)
N3	13 (11%)	23 (14%)
M-stage		
M0	71 (58%)	15 (9%)
M1	51 (42%)	155 (91%)
Metastases Location		
Lymph nodes	50 (41%)	72 (43%)
Liver	0	19 (11%)
Lungs	0	3 (2%)
Other	0	18 (11%)
Multiple locations	1 (1%)*	42 (25%)
Not applicable	71 (58%)	15 (9%)
Disease Stage		
IB	1 (1%)	0
IIA	5 (4%)	2 (1%)
IIB	1 (1%)	2 (1%)
IIIA	17 (14%)	4 (2%)
IIIB	13 (11%)	2 (1%)
IIIC	34 (28%)	5 (3%)
IV	51 (42%)	154 (91%)
Carboplatin dose (mg)		
Mean [SD]	477 [90]	486 [95]

Table 1 continued.

Characteristic	iCT (N=122)	pCT (N=169)
Paclitaxel dose (mg)		
Mean [SD]	191 [22]	187 [22]
Number of treatment cycles [d]		
Median [IQR]	7 [6-9]	8 [6-9]

[a] BSA was calculated according to the Mosteller formula [31]

[b] eGFR was calculated according to the CKD-EPI formula [32]

[c] Tumor Type Other included undifferentiated large cell carcinomas and neuroendocrine carcinomas

[d] Six weekly cycles, followed by 3-weekly cycles

Abbreviations: BSA = body surface area; GE = gastro-esophageal; iCT = induction chemotherapy; IQR = inter-quartile range; mg = milligram; N = number; pCT = palliative chemotherapy; SD = standard deviation; WHO = World Health Organization

TOXICITY

Overall, this treatment regimen was relatively well tolerated. However, the incidence of overall grade ≥ 3 toxicity was 71% in the iCT group and 78% in the pCT group, respectively. These grade 3 or 4 toxicities occurred mainly as hematological toxicity; especially neutropenia with grade 3 in 43% and grade 4 in 25% of patients in both groups. However, febrile neutropenia occurred in ten patients only, resulting in a low incidence of 3% complicated neutropenia (**Table 2**). Gastro-intestinal toxicity was mostly low-graded, gastro-intestinal toxicity grade ≤ 2 occurred mainly as nausea (iCT: 39%, pCT: 46%), constipation (iCT: 34%, pCT: 42%) and anorexia (iCT: 21%, pCT: 26%). Severe gastro-intestinal toxicity (i.e. grade ≥ 3) occurred in 3% of the iCT and 5% of the pCT patients (**Table 2**). Fatigue grade ≤ 2 occurred in 70% of patients of both groups, but severe fatigue in only six patients. Neuropathy was mostly seen as low-graded sensory neuropathy in a quarter of patients in both groups, and motoric neuropathy grade 2 in six patients. Adverse events rarely resulted in a dose reduction of carboplatin or paclitaxel as dose reductions were applied in only 2 patients due to severe nausea in one patient and febrile neutropenia in the other. Transfusion related reactions were more frequent and occurred mainly as reaction to paclitaxel (instead of carboplatin) in around one fifth of the patients.

During the six weekly cycles, cycle delay due to toxicity occurred in 42% and 43% of the patients treated with iCT and pCT, respectively. This delay was mainly caused by thrombocytopenia in 46% of these patients, leukocytopenia (20%) or a combination of both (11%), other reasons included non-neutropenic fever (6%) and gastro-intestinal toxicity (5%). Cycle delay consisted of one week in 70% of patients with delay, and two weeks in 19% of patients, and occurred mostly at cycle 4 (70%). For patients with a cycle delay due to toxicity, this was often one cycle (85%), however, in 18 patients (15%) two

separate cycles were delayed due to hematological toxicity. Premature termination of the planned six weekly cycles due to toxicity occurred in 14 iCT patients (12%) and 28 pCT patients (17%) mostly due to general malaise (43%), or thrombocytopenia (14%) and leukocytopenia (10%) or both (7%). Hospitalization due to toxicity occurred in 18 iCT patients (15%) and 17 pCT patients (10%), which was caused by gastro-intestinal toxicity in 11 patients, febrile neutropenia in 10 patients, non-neutropenic fever in 13 patients, and malaise in one patient. There were no toxic deaths in our cohort.

EFFICACY

After six weekly cycles, overall response rate was 48% for iCT and 44% pCT, with a complete response in 1% of both groups (**Table 3**). For many iCT patients additional treatment was given: definitive chemoradiotherapy (7%), esophagectomy (35%), exploratory laparotomy (4%), or second line chemotherapy (17%) (**Table 3**). In the pCT group some patients received another treatment also: definitive chemoradiotherapy (1%), esophagectomy (4%), exploratory laparotomy (5%), or second line chemotherapy (19%).

Median follow-up for the 27 iCT patients still alive was 43.7 months (range 2-117 months), and 18.4 months in 21 patients for pCT (range 6-124 months). For the whole group of iCT patients, median PFS was 12.4 months (IQR: 7.1-45.3 months) and median OS was 15.6 months (IQR: 9.7-36.3). However, in the 42% of patients who underwent subsequent resection or radiotherapy median PFS was 22.1 months (IQR: 12.4-114.2) and median OS was 26.8 months (IQR: 15.4-91.7). For patients treated with pCT, median PFS was 8.2 months (IQR: 5.1-14.5) and median OS 10.9 months (IQR: 6.5-18.3). The median PFS and OS for patients treated with pCT are comparable to iCT patients who did not receive an esophagectomy or definitive chemoradiotherapy afterwards (**Table 3**).

Table 2. Toxicity and clinical consequences

ADVERSE EVENTS	iCT N=122			pCT N=169		
	Grade 1	Grade 2	Grade ≥3	Grade 1	Grade 2	Grade ≥3
Overall Toxicity	114 (93%)	111 (91%)	86 (71%)	167 (99%)	154 (91%)	131 (78%)
Gastrointestinal Toxicity						
Anorexia	21 (17%)	5 (4%)	1 (1%)	35 (21%)	9 (5%)	3 (2%)
Nausea	39 (32%)	8 (7%)	1 (1%)	68 (40%)	10 (6%)	3 (2%)
Vomiting	19 (16%)	4 (3%)	0	27 (16%)	5 (3%)	3 (2%)
Diarrhea	20 (16%)	1 (1%)	2 (2%)	21 (12%)	10 (6%)	2 (1%)
Constipation	31 (25%)	10 (8%)	0	57 (34%)	15 (9%)	0
Mucositis	4 (3%)	2 (2%)	0	17 (10%)	0	1 (1%)
Other Toxicity						
Alopecia	32 (26%)	40 (33%)	NA	50 (30%)	65 (39%)	NA
Dermatitis	9 (7%)	2 (2%)	0	10 (6%)	4 (2%)	0
Fatigue	59 (48%)	25 (21%)	1 (1%)	82 (49%)	36 (21%)	5 (3%)
Sensory Neuropathy	27 (22%)	3 (3%)	0	40 (24%)	4 (2%)	0
Motoric Neuropathy	1 (2%)	0	0	3 (2%)	6 (4%)	0
Hematological Toxicity						
Anemia	57 (47%)	58 (48%)	6 (5%)	86 (51%)	64 (38%)	17 (10%)
Thrombocytopenia	67 (55%)	22 (18%)	13 (11%)	83 (49%)	27 (16%)	22 (13%)
Leukocytopenia	7 (6%)	56 (46%)	43 (35%)	18 (11%)	74 (44%)	52 (31%)
Neutropenia	0	21 (17%)	82 (67%)	0	24 (14%)	113 (67%)
Other blood value alterations						
Creatinine Increase	6 (5%)	0	0	6 (4%)	0	0
AST Increase	21 (17%)	0	1 (1%)	29 (17%)	2 (1%)	4 (2%)
ALT Increase	20 (16%)	3 (3%)	0	20 (12%)	3 (2%)	2 (1%)
GGT Increase	20 (16%)	4 (3%)	1 (1%)	25 (15%)	14 (8%)	12 (7%)
AF Increase	19 (16%)	0	0	25 (15%)	1 (1%)	6 (4%)
Bilirubin Increase	12 (10%)	5 (4%)	0	7 (4%)	7 (4%)	3 (2%)
CLINICAL CONSEQUENCES	**iCT N=122**			**pCT N=169**		
	No	**Yes: toxicity**	**Yes: other**	**No**	**Yes: toxicity**	**Yes: other**
Febrile Neutropenia	118 (97%)	4 (3%)	NA	163 (96%)	6 (4%)	NA
Dose reduction Carboplatin	117 (96%)	1 (1%)	3 (3%) [a]	126 (75%)	1 (1%)	4 (2%) [a]
Dose reduction Paclitaxel	97 (79%)	1 (1%)	24 (20%) [a]	126 (75%)	1 (1%)	40 (24%) [a]
Treatment delay	64 (53%)	51 (42%)	7 (6%)	84 (50%)	72 (43%)	11 (7%)
Premature end of treatment*	95 (78%)	14 (12%)	13 (11%) [b]	109 (64%)	29 (17%)	31 (19%) [b]
Hospitalization	96 (79%)	18 (15%)	8 (7%)	140 (83%)	17 (10%)	12 (7%)

* Premature end of treatment was defined as end of treatment before the planned six weekly cycles.

[a] Dose reduction other was defined as a transfusion related reaction to paclitaxel or carboplatin, in these cases dose was not reduced but infusion time was prolonged.

[b] Premature end of treatment other included disease progression in 7 (iCT) and 16 (pCT) patients.

Abbreviations: ALT = alanine aminotransferase; AP = alkaline phosphatase; AST = aspartate aminotransferase; GGT = gamma-glutamyltransferase; iCT = induction chemotherapy; N = number; NA = not applicable, pCT = palliative chemotherapy

Table 3. Treatment response

	iCT N=122	pCT N=169
Response after 6 cycles		
Complete Response	1 (1%)	2 (1%)
Partial Response	57 (47%)	72 (43%)
Stable Disease	46 (38%)	56 (33%)
Progressive Disease	11 (9%)	23 (14%)
Unknown	7 (6%)	16 (10%)
Treatment afterwards [a]		
Carboplatin-Paclitaxel [b]	13 (11%)	14 (8%)
Chemotherapy Other [c]	8 (7%)	18 (11%)
Definitive Chemoradiotherapy [d]	9 (7%)	1 (1%)
Esophagectomy	43 (35%)	7 (4%)
PFS (months; median [IQR])		
All patients	12.4 [7.1-45.3]	8.2 [5.1-14.5]
No CRT or esophagectomy afterwards	9.0 [4.3-13.4]	8.0 [5.0-13.2]
CRT or esophagectomy afterwards	22.1 [12.4-114.2]	18.1 [14.8-122.2]
OS (months; median [IQR])		
All patients	15.6 [9.7-36.3]	10.9 [6.5-18.3]
No CRT or esophagectomy afterwards	11.8 [7.3-18.6]	10.6 [6.4-17.2]
CRT or esophagectomy afterwards	26.8 [15.4-91.7]	23.1 [14.8-28.0]

[a] Intervention after last administration of carboplatin (AUC 4) and paclitaxel (100 mg/m^2)

[b] Second period of treatment with carboplatin (AUC 4) and paclitaxel (100 mg/m^2)

[c] Chemotherapy other than carboplatin and paclitaxel; including EOX (epirubicin, oxaliplatin and capecitabine), 5-fluorouracil combined with cisplatin, or phase-1 trial medication combined with docetaxel, irinotecan, or capecitabine

[d] Definitive chemoradiotherapy included six weekly cycles of carboplatin (targeted at AUC 2) and paclitaxel (50 mg/m^2) combined with radiotherapy on the esophagus [27]

Abbreviations: AUC = area under the curve; CRT = definitive chemoradiotherapy; iCT = induction chemotherapy; IQR = inter-quartile range; N = number; pCT = palliative chemotherapy

PREDICTIVE AND PROGNOSTIC FACTORS

In the iCT group, smoking was identified as a predictive factor for poor response (SD/PD) with an odds ratio of 2.30 (95% confidence interval (CI): 1.02-2.21, P = 0.045) for current smokers compared to former and non-smokers. Unfortunately, no other predictive factors for treatment response could be identified in the iCT group, nor in the pCT group, for these results see **Supplementary Table 1**.

For iCT patients smoking was also an adverse prognostic factor for PFS and OS univariately, but only remained significant in the multivariate analysis of PFS with an hazard ratio (HR) of 2.61 (95% CI: 1.17-5.85, P = 0.020) for current smokers versus non-smokers. In addition, elevated thrombocyte number and alkaline phosphatase levels were also adverse prognostic factors for PFS in the multivariate model (HR: 1.00, 95% CI: 1.00-1.01, P = 0.001 and HR: 1.02, 95% CI: 1.00-1.03, P=0.023, respectively). For

OS, higher WHO performance status (HR: 1.87, 95% CI: 1.06-3.29, P = 0.031), higher T-stage (T4B; HR: 1.82, 95% CI: 1.02-3.25, P = 0.044) and thrombocyte number (HR: 1.00, 95% CI: 1.00-1.00, P = 0.025) were adverse prognostic factors in the multivariate model, see **Table 4**.

For pCT patients tumor location and year of diagnosis remained prognostic factors in the multivariate analysis for PFS with patients with a mid-esophageal tumor having a better PFS compared to proximal tumors (HR: 0.29, 95% CI: 0.10-0.80, P = 0.017). For OS, mid-esophageal tumor location was also found to be significant with a comparable HR as for PFS. Other variables included in the multivariate model for OS were body surface area (BSA; HR: 0.34, 95% CI: 0.12-0.91, P = 0.032) and WHO 1/2 versus 0 (HR: 1.69, 95% CI: 1.13-2.52, P = 0.011). For all results, see **Table 4**.

Table 4. Prognostic factors for PFS and OS in patients treated with induction or palliative chemotherapy

BASELINE FACTOR	INDUCTION CHEMOTHERAPY (iCT)											
	Progression Free Survival						Overall Survival					
	Univariate Analysis			Multivariate Analysis			Univariate Analysis			Multivariate Analysis		
	HR	95% CI	*P-value*	HR	95% CI	*P-value*	HR	95% CI	*P-value*	HR	95% CI	*P-value*
SEX(M vs F)	0.77	0.45-1.29	*0.319*				0.83	0.51-1.34	*0.443*			
AGE	1.01	0.98-1.03	*0.600*				1.01	0.99-1.03	*0.496*			
BSA	0.89	0.30-2.62	*0.835*				0.63	0.24-1.64	*0.341*			
WHO (1 vs 0)	1.31	0.77-2.23	*0.322*				2.20	1.30-3.72	***0.003***	1.87	1.06-3.29	***0.031***
ALCOHOL (vs never)			*0.311*						*0.114*			
History	1.03	0.44-2.43					1.49	0.64-3.49				
Current	1.50	0.76-2.96					1.98	0.95-4.14				
SMOKING (vs never)			***0.032***						***0.015***			
History	1.17	0.63-2.18	*0.619*	1.28	0.60-2.75	*0.522*	1.40	0.76-2.60	*0.281*			
Current	2.22	1.14-4.33	***0.019***	2.61	1.17-5.85	***0.020***	2.42	1.26-4.63	***0.008***			
TUMOR LOCATION (vs			*0.937*						*0.429*			
proximal)	1.17	0.57-2.43					0.79	0.43-1.45				
Middle	0.99	0.52-1.91					0.67	0.39-1.16				
Distal	0.94	0.37-2.41					0.55	0.24-1.26				
Junction/Cardia	NA	NA										
Multiple locations												
YEAR OF DIAGNOSIS	1.03	0.96-1.10	*0.381*				1.01	0.95-1.08	*0.689*			
HISTOLOGY (SCC vs AC)	1.06	0.66-1.68	*0.816*				1.22	0.80-1.86	*0.354*			
DIFFERENTIATION												
(poor vs good/moderate)	1.40	0.83-2.37	*0.206*				1.15	0.71-1.86	*0.572*			
T-STAGE												
(iCT: vs T2/T3, pCT: vsT1b/			*0.305*						***0.014***			
T2)	NA	NA					NA	NA	*NA*	NA	NA	NA
T3	1.28	0.74-2.22					0.98	0.57-1.68	*0.948*	1.01	0.56-1.81	*0.984*
T4A	1.61	0.86-3.00					2.20	1.32-3.66	***0.003***	1.82	1.02-3.25	***0.044***
T4B												
N-STAGE (vs N0)			*0.888*						*0.935*			
N1	0.72	0.29-1.75					0.93	0.47-1.83				
N2	0.83	0.35-2.00					0.94	0.48-1.86				
N3	0.79	0.28-2.25					0.77	0.33-1.82				
M-STAGE (vs M0)	0.97	0.61-1.53	*0.880*				0.90	0.59-1.38	*0.634*			
METASTASES LOCATION	NA	NA	NA				NA	NA	NA			
(vs no metastases)												
Nodal												
Liver												
Other												
Multiple locations												
LIVER METASTASES (Y vs No)	NA	NA	NA				NA	NA	NA			
HEMOGLOBIN (mmol/L)	0.99	0.79-1.26	*0.961*				0.83	0.67-1.03	*0.087*			
THROMBOCYTES (10^9/L)	1.00	1.00-1.00	***0.010***	1.00	1.00-1.01	***0.001***	1.00	1.00-1.00	***0.020***	1.00	1.00-1.00	***0.025***
LEUKOCYTES (10^9/L)	1.05	0.97-1.13	*0.206*				1.04	0.97-1.11	*0.270*			
NEUTROPHILS (10^9/L)	1.05	0.96-1.14	*0.310*				1.04	0.96-1.12	*0.312*			
AST (U/L)	1.00	0.97-1.03	*0.958*				0.99	0.97-1.02	*0.599*			
ALT (U/L)	1.00	0.99-1.01	*0.880*				1.00	0.99-1.01	*0.633*			
LD (U/L)	1.00	1.00-1.00	*0.396*				1.00	1.00-1.00	*0.716*			
GGT (U/L)	1.00	1.00-1.01	*0.301*				1.00	1.00-1.01	*0.193*			
AP (U/L)	1.01	1.00-1.01	*0.056*	1.02	1.00-1.03	***0.023***	1.00	1.00-1.01	*0.183*			
BILIRUBIN (µmol/L)	1.01	0.95-1.07	*0.836*				1.02	0.96-1.08	*0.596*			
KREATININ (µmol/L)	0.99	0.97-1.00	*0.114*				1.00	0.98-1.01	*0.665*			

P-values < 0.05 are considered statistically significant and are depicted in bold.

PALLIATIVE CHEMOTHERAPY (pCT)											
Progression Free Survival						Overall Survival					
Univariate Analysis			Multivariate Analysis			Univariate Analysis			Multivariate Analysis		
HR	95% CI	*P-value*	HR	95% CI	*P-value*	HR	95% CI	*P-value*	HR	95% CI	*P-value*
1.00	0.63-1.59	*0.989*				0.90	0.58-1.39	*0.623*			
1.00	0.98-1.03	*0.738*				1.00	0.98-1.02	*0.892*			
0.80	0.33-1.94	*0.618*				0.30	0.13-0.69	***0.005***	0.34	0.12-0.91	***0.032***
1.34	0.90-2.01	*0.154*				1.70	1.16-2.48	***0.006***	1.69	1.13-2.52	***0.011***
		0.498						*0.767*			
0.71	0.40-1.29					0.83	0.50-1.40				
0.90	0.57-1.44					0.87	0.56-1.34				
		0.883						*0.915*			
0.92	0.55-1.53					0.92	0.57-1.47				
1.02	0.58-1.78					0.98	0.59-1.63				
		0.100						***0.045***			
0.32	0.12-0.89	***0.029***	0.29	0.10-0.80	***0.017***	0.27	0.12-0.63	***0.002***	0.27	0.11-0.65	***0.004***
0.59	0.24-1.45	*0.246*	0.55	0.22-1.36	*0.193*	0.45	0.22-0.94	***0.033***	0.54	0.26-1.15	*0.110*
0.69	0.22-2.11	*0.513*	0.68	0.22-2.08	*0.498*	0.46	0.18-1.17	*0.103*	0.49	0.18-1.33	*0.160*
0.85	0.16-4.42	*0.849*	0.73	0.14-3.80	*0.705*	0.87	0.18-4.13	*0.859*			
1.06	1.01-1.12	***0.016***	1.06	1.01-1.12	***0.028***	1.01	0.96-1.05	*0.833*			
0.56	0.36-0.86	***0.008***				0.76	0.52-1.10	*0.146*			
1.06	0.70-1.63	*0.776*				1.06	0.72-1.56	*0.761*			
								0.076			
		0.204				2.03	1.11-3.71				
1.85	0.98-3.48					2.13	1.03-4.41				
1.68	0.76-3.71					1.59	0.71-3.55				
1.36	0.56-3.28										
		0.704						*0.867*			
0.92	0.51-1.65					1.03	0.60-1.76				
1.17	0.65-2.12					1.14	0.65-1.98				
1.15	0.59-2.24					1.23	0.65-2.33				
2.72	1.00-7.38	***0.050***				1.05	0.58-1.90	*0.877*			
		0.048						*0.916*			
1.78	0.71-4.48	*0.219*				1.00	0.54-1.86				
3.43	1.26-9.33	***0.016***				1.26	0.57-2.77				
2.36	0.85-6.57	*0.099*				1.14	0.54-2.42				
2.51	0.98-6.44	*0.055*				1.16	0.60-2.23				
1.47	1.02-2.13	***0.040***				1.12	0.78-1.60	*0.534*			
0.95	0.80-1.12	*0.520*				0.86	0.74-1.00	*0.052*			
1.00	1.00-1.00	*0.695*				1.00	1.00-1.00	*0.684*			
1.01	0.99-1.03	*0.478*				1.00	0.99-1.02	*0.735*			
1.01	0.97-1.05	*0.696*				1.01	0.95-1.08	*0.660*			
1.00	1.00-1.01	*0.145*				1.00	1.00-1.01	*0.883*			
1.00	1.00-1.00	*0.767*				1.00	0.99-1.00	*0.475*			
1.00	1.00-1.00	*0.908*				1.00	1.00-1.00	*0.414*			
1.00	1.00-1.00	*0.608*				1.00	1.00-1.00	*0.973*			
1.00	1.00-1.00	*0.948*				1.00	1.00-1.00	*0.943*			
1.00	0.96-1.03	*0.953*				1.01	0.97-1.04	*0.722*			
1.00	0.99-1.02	*0.552*				0.99	0.98-1.01	*0.356*			

DISCUSSION

This retrospective analysis demonstrated that a weekly regimen of six cycles carboplatin (AUC 4) and paclitaxel (100 mg/m^2) can be used as induction or palliative treatment for patients with advanced or metastatic (gastro-)esophageal cancer. The treatment regimen investigated was well-tolerated as demonstrated by the low incidence of toxicity grade ≥ 3 (except for grade ≥ 3 hematological toxicity).

Almost two third of patients experienced severe neutropenia, which is higher than described for other frequently used regimens.[22] However, the incidence of severe leukocytopenia (i.e. leukocytes lower than 2.0 x 10^9/L) was much lower than of neutropenia observed, and comparable with other frequently used regimens.[22] According to our treatment protocol, this regimen could be safely administered on an outpatient basis at any grade of neutropenia as long as leukocyte number was sufficient (i.e. day 0 and 28: > 3.0 x 10^9/L; day 7, 14, 35 and 42: > 1.0 x 10^9/L), which was also reflected by the low incidence of febrile neutropenia we observed.

Furthermore, the incidence of severe nausea or diarrhea of only one to two percent of patients was much lower than in most other regimens described using combinations with 5-fluorouracil or capecitabine.[22] In patients treated with fluorupyrimidine- and/or platinum-based triplets, severe nausea has been described in 7-21% and severe diarrhea in 3-19% of patients.[22, 33] Gastro-intestinal toxicity is less when doublet treatment is used compared to triplets, but still seems higher than in our regimen, although no direct comparison could be made. In doublets where a fluoropyrimidine is combined with platinum, severe nausea occurred in 7-27% and severe diarrhea in 4-8%, and with irinotecan severe nausea occurred in 7% and severe diarrhea in 22%, respectively.[22] Polyneuropathy was mostly low graded, and occurred in 4% of patients as grade 2 polyneuropathy, while grade 3 was not observed. For example, capecitabine combined with oxaliplatin resulted in 8% grade 3 polyneuropathy.[37] However, the incidence of severe hematological toxicity was only 4% with that regimen, while the incidence of complicated neutropenia was not mentioned.[37] Lastly, 22% of all patients developed an infusion related reaction to paclitaxel, which is most likely caused by its formulation vehicle Cremophor.[34] But this could usually easily managed by prolonging the infusion time of paclitaxel. Infusion related reactions to paclitaxel are not limited to this treatment regimen as much higher incidences up to 44% of patients treated with paclitaxel have been described.[34] The good clinical tolerance of this treatment regimen was also demonstrated by the low incidence of toxicity-related dose-reductions and hospitalization. Premature end of treatment due to toxicity occurred more often and mostly caused by general malaise, which is often multifactorial and therefore probably

not in all patients (fully) caused by our regimen. Furthermore, in palliative treatment quality of life should be considered as well, and a major advantage of the current regimen is that this treatment can be given as outpatient treatment for which no hospitalization or central line is required, although this advantage is not limited to our treatment regimen.

With this regimen an overall response rate of 48% in patients treated with induction intent and 44% in patients treated with palliative intent was achieved. These percentages are slightly lower than the response rate of 54% found in our previous phase-1 study, which can be explained by the low sample size of only 37 patients in that former study and the difference in WHO performance of a selected study population compared to our cohort of non-selected patients.[26] The response rate of 48% in patients with induction intent, is comparable to other regimens with response-rates varying between 20-48% (**Table 5**).[17, 33, 35-44] However, most studies included a mixed population of patients with locally advanced and metastatic disease. Two studies did include only patients with locally irresectable disease without distant metastases and found a response rate of 32% for treatment with cisplatin and fluorouracil, and 45% for a treatment with docetaxel, cisplatin and fluouracil.[17, 18] However, these studies were conducted in patients with SCC, and our induction cohort consisted also for 50% of patients with an adenocarcinoma which makes it difficult to compare these results. Furthermore, for 42% of iCT patients esophagectomy or definitive chemoradiotherapy followed, resulting in a median PFS of 22.1 months and median OS of 26.8 months. For iCT patients, who did not have an esophagectomy or definitive chemoradiation, the median PFS of 9.0 months and OS of 11.8 months was lower, but comparable to the patients treated with palliative intent.

In metastatic (gastro-)esophageal cancer we found a response rate of 44% with our treatment regimen, which is also comparable with the most frequent used other doublet regimens (20-45%) and triplet regimens (31-48%) (**Table 5**).[33, 35-40, 42-44] When comparing survival rates, we found a median PFS of 8.2 months and OS of 10.9 months for palliative treated patients, which is longer than other doublet regimens in this patient group (PFS: 3.7-5.9 months; OS: 8.6-10.7) and is comparable to triplet regimens (PFS: 5.6-7.0; OS: 9.2-11.2) (**Table 5**).[33, 35-40, 42-44] Although we have to interpret these comparisons with caution as no direct comparison can be made, weekly carboplatin and paclitaxel seems at least equally effective, and possibly even more effective compared to other frequently used treatment regimens and was better tolerated than other regimens.

As a secondary aim of the analysis, we tried to identify predictive and prognostic factors. We could only identify smoking as predictive factor for patients with induction

treatment, while unfortunately no predictive factors for treatment outcome in palliative treated patients were found. Nonetheless, we identified several prognostic factors for progression free survival and for overall survival. For induction chemotherapy, current smoking behavior, elevated thrombocyte number and alkaline phosphatase levels, WHO status and T-stage, were identified as adverse prognostic factors. For palliative chemotherapy, tumor location, BSA and WHO status were identified as prognostic factors. We could not confirm other known prognostic factors for survival such as sex or location of metastases.[8, 45, 46] Interestingly, smoking behavior was identified as a negative predictive and prognostic factor. Several reasons for this can be hypothesized, including sarcopenia and factors related to this unhealthy lifestyle. However, the underlying mechanism is not yet unraveled.

Our study has some limitations which need to be mentioned. The retrospective nature of our study could have influenced the quality of the data and the selection of patients. Nevertheless, our patient population included all patients who were treated in a certain time period, hence can be considered as a real-world patient cohort and therefore also representative for daily clinical practice. The retrospective data collection will be mainly of influence on the incidence of low grade adverse events as they are not always recorded, but has no effect on the higher graded adverse events as they have more clinical consequences and were described in detail. Furthermore, we included patients who were considered non-resectable by the multidisciplinary team based on general criteria, but we could not retrospectively retrieve if this decision was possibly (in part) based on certain co-morbidities. Also, we could not retrieve data on HER2 expression of the tumor, as this was not determined in individual patients, which potentially could have influenced the results. Lastly, it was impossible to include a quality of life analysis, which is especially important in treatments with palliative intent.

We demonstrated that weekly paclitaxel and carboplatin is an effective and well-tolerated treatment regimen and could therefore be a valid induction or palliative treatment option in advanced (gastro)esophageal cancer. Despite the fact that chemotherapy in general has limited efficacy in esophageal cancer with only minor differences between different schedules, it will remain the backbone of treatment in metastatic (gastro)esophageal cancer until new treatments are developed. Future research should therefore focus on predictive factors and biomarkers to identify patients on beforehand who will benefit from a certain treatment. Furthermore, the tumor biology should be included in patient selection. Several molecular subtypes of (gastro)esophageal cancer have been identified and provides a rationale to develop tailored treatment for the different subtypes instead of treating all (gastro)esophageal cancers in the same manner.[47] Currently investigated targeted therapies focusses on

targeting the human epidermal growth factor receptor type 2 (HER2) and the vascular endothelial growth factor receptor (VEGF) with limited effect, nevertheless several combination therapies are being evaluated.[48] Lastly, the immune micro-environment of the tumor might be a possible treatment target as demonstrated by the promising results of nivolumab and/or ipilimumab in recent phase-II studies.[49, 50] Furthermore, the phase-III ATTRACTION trial demonstrated that nivolumab significantly increased OS compared to placebo in heavily pretreated Asian patients with metastatic (gastro) esophageal cancer independent of the PD-L1 status.[51] The phase-III KEYNOTE-181 study demonstrated that pembrolizumab as second-line therapy significantly improved OS compared to chemotherapy in patients with metastatic esophageal cancer with a high PD-L1 combined score.[52] Currently, the results of multiple clinical trials evaluating the combination of immunotherapy and chemotherapy are awaited.[48]

In conclusion, we demonstrated that weekly paclitaxel and carboplatin is an effective and well-tolerated induction or palliative treatment regimen in a real-life patient cohort. Future research should directly compare this treatment regimen with other first-line treatment options to determine its true value for clinical practice.

Table 5. Summary of induction and palliative treatment regimens mentioned

Treatment Regimen	Patients (N)	Age (median)	Esophageal Tumor (%)	GEJ Tumor* (%)	Gastric Tumor (%)	Adenocarcinoma (%)	Locally Advanced (%)	Metastatic Disease (%)	Overall Response (%)	Median PFS (months)	Median OS (months)
Fluorouracil, Cisplatin [35]	224	55	0	25	75	90	3	97	25	3.7	8.6
Fluorouracil, Cisplatin [36]	163	59	0	19	81	100	5	95	26	4.2	8.7
Fluorouracil, Cisplatin [17]	163	56[a]	100	0	0	0	100	0	32	NR	11.0
Fluorouracil, Oxaliplatin [37]	51	60	NR	NR	0	88	NR	NR	39	5.3	8.0
Fluorouracil, Oxaliplatin [38]	64	63	100	0	0	0	0	100	41	4.0	10.0
Docetaxel, Cisplatin [44]	76	57	0	26	74	100	5	95	20	5.0	10.5
Paclitaxel, Cisplatin [39]	51	56	100	0	0	61	10	90	43	NR	9.0
Etoposide, Cisplatin [40]	73	60	100	0	0	0	4	96	45	NR	8.5
Epirubicin, Cisplatin, Fluorouracil [33]	263	65	35	29	36	90	21	80	41	6.2	9.9
Epirubicin, Cisplatin, Capecitabine [33]	250	64	30	28	42	90	23	77	46	6.7	9.9
Epirubicin, Oxaliplatin, Fluorouracil [33]	245	61	30	23	37	86	23	77	42	6.5	9.3
Epirubicin, Oxaliplatin, Capecitabine [33]	244	62	34	22	44	87	24	58	48	7.0	11.2
Docetaxel, Cisplatin, Fluorouracil [41]	48	66	100	0	0	0	81	19	31	17.6	NR[b]
Docetaxel, Cisplatin, Fluorouracil [35]	221	55	0	19	81	89	3	96	37	5.6	9.2
Fluorouracil, Folinic Acid, Cisplatin [42]	108	64	0	22	78	100	9	91	25	3.9	8.8
Fluorouracil, Folinic Acid, Oxaliplatin [42]	112	64	0	18	82	100	3	97	35	5.8	10.7
Fluorouracil, Folinic Acid, Irinotecan [36]	170	58	0	20	80	100	4	96	32	5.0	9.0
Fluorouracil, Folinic Acid, Cisplatin, Etoposide [43]	69	55	100	0	0	0	19	81	34	NR	9.5

* If GEJ was not separately mentioned as tumor location; patients were grouped as esophageal tumor

[a] Mean age instead of median age

[b] Median overall survival was not reached, 1-year survival rate was 67.9%

Abbreviations: GEJ = gastro-esophageal junction; N = number; NR = not reported; OS = overall survival; PFS = progression free survival

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SUPPLEMENTARY INFORMATION

Supplementary Table 1. Predictive factors for treatment response in patients treated with induction or palliative chemotherapy

BASELINE FACTORS	Treatment Response (iCT)			Treatment Response (pCT)		
	Univariate Analysis (CR/PR vs SD/PD)			Univariate Analysis (CR/PR vs SD/PD)		
	OR	95% CI	*P-value*	OR	95% CI	*P-value*
SEX (M vs F)	0.89	0.37-2.10	*0.786*	0.84	0.37-1.89	*0.672*
AGE	1.01	0.97-1.05	*0.666*	0.98	0.94-1.01	*0.207*
BSA	0.99	0.19-5.08	*0.993*	0.65	0.15-2.87	*0.566*
WHO (1 vs 0)	1.19	0.50-2.83	*0.702*	0.86	0.42-1.76	*0.678*
ALCOHOL (vs never/history						
Current	1.48	0.66-3.29	*0.341*	0.93	0.47-1.80	*0.820*
SMOKING (vs never/history)						
Current	2.30	1.02-2.21	***0.045***	0.84	0.41-1.73	*0.634*
TUMOR LOCATION (vs proximal)						
Middle	0.86	0.27-2.75	*0.796*	0.13	0.01-1.25	*0.078*
Distal	1.20	0.43-3.31	*0.735*	0.18	0.02-1.51	*0.113*
Junction/Cardia	0.50	0.42-2.40	*0.361*	0.10	0.01-1.29	*0.077*
Multiple locations						
YEAR OF DIAGNOSIS	1.07	0.97-1.18	*0.177*	1.04	0.95-1.14	*0.400*
HISTOLOGY (SCC vs AC)	0.72	0.35-1.52	*0.394*	1.00	0.49-2.01	*0.990*
DIFFERENTIATION (poor vs good/moderate)	1.19	0.52-2.76	*0.680*	0.62	0.29-1.32	*0.215*
T-STAGE (iCT: vs T2/T3, pCT: vs T1b/T2)						
T3				1.53	0.51-4.61	*0.450*
T4A/B	1.38	0.65-2.94	*0.401*	2.13	0.60-7.57	*0.245*
N-STAGE (vs N0)						
N1	1.71	0.43-6.91	*0.449*	0.80	0.30-2.16	*0.657*
N2	1.57	0.40-6.27	*0.527*	0.78	0.28-2.19	*0.639*
N3	0.94	0.17-5.07	*0.940*	0.68	0.19-2.35	*0.537*
M-STAGE (vs M0)	1.03	0.49-2.20	*0.935*	1.55	0.46-5.10	*0.474*
METASTASES LOCATION (vs no metastases)						
Nodal				2.11	0.62-7.16	*0.229*
Liver				0.87	0.20-3.90	*0.858*
Other				4.48	0.99-20.4	*0.052*
Multiple locations				1.24	0.34-4.47	*0.746*
LIVER METASTASES (Y vs No)				0.67	0.34-1.33	*0.254*
HEMOGLOBIN (mmol/L)	0.83	0.57-1.20	*0.313*	1.01	0.76-1.35	*0.921*
THROMBOCYTES (10^9/L)	1.00	1.00-1.00	*0.487*	1.00	0.99-1.00	*0.121*
LEUKOCYTES (10^9/L)	1.05	0.92-1.19	*0.462*	0.99	0.95-1.03	*0.560*
NEUTROPHILS (10^9/L)	1.04	0.89-1.20	*0.641*	0.97	0.91-1.05	*0.487*
AST (U/L)	0.98	0.94-1.03	*0.449*	1.00	0.99-1.01	*0.590*
ALT (U/L)	1.00	0.98-1.02	*0.824*	1.00	0.99-1.01	*0.455*
LD (U/L)	1.00	1.00-1.00	*0.794*	1.00	1.00-1.00	*0.807*
GGT (U/L)	1.00	0.98-1.01	*0.535*	1.00	1.00-1.00	*0.631*
AP (U/L)	1.01	0.99-1.03	*0.220*	1.00	1.00-1.00	*0.413*
BILIRUBIN (µmol/L)	0.99	0.90-1.10	*0.910*	1.00	0.93-1.07	*0.967*

Supplementary Table 1 continued.

BASELINE FACTORS	Treatment Response (iCT)			Treatment Response (pCT)		
	Univariate Analysis (CR/PR vs SD/PD)			Univariate Analysis (CR/PR vs SD/PD)		
	OR	**95% CI**	*P-value*	**OR**	**95% CI**	*P-value*
KREATININ (µmol/L)	1.01	0.99-1.04	*0.163*	1.01	0.99-1.03	*0.276*

P-values < 0.05 are considered statistically significant and are depicted in bold.

Abbreviations: AC = adenocarcinoma; ALT = alanine aminotransferase; AP = alkaline phosphatase; AST = aspartate aminotransferase; F = female; GGT = gamma-glutamyltransferase; iCT = induction chemotherapy; LD = lactate dehydrogenase; M = male; N = number; OR = odds ratio; pCT = palliative chemotherapy; SCC = squamous cell carcinoma; vs = versus (reference category); World Health Organization Performance Status; Y = yes

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ASSOCIATION BETWEEN PACLITAXEL CLEARANCE AND TUMOR RESPONSE IN PATIENTS WITH ESOPHAGEAL CANCER

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ABSTRACT

BACKGROUND

Inter-individual variability in paclitaxel pharmacokinetics may play a role in the response to chemotherapy. Therefore, we studied the association between paclitaxel clearance and treatment response in patients with esophageal cancer.

METHODS

Patients who received paclitaxel (plus carboplatin) treatment for esophageal cancer between 2007 and 2013 were included. Treatment was given as neoadjuvant chemoradiotherapy (nCRT), induction chemotherapy (iCT), or palliative chemotherapy (pCT). Treatment response was assessed by tumour regression grade (TRG) or RECIST1.1, respectively. Unbound paclitaxel clearance (CL) was estimated with NONMEM. Log-transformed clearance was related to response with ANOVA and independent sample t-tests.

RESULTS

A total of 166 patients were included, of whom 113 received nCRT, 23 iCT and 30 pCT. In patients receiving nCRT, paclitaxel clearance was not associated with tumour regression grade (P=0.25), nor with pathologically complete response (geometric mean 561.6 L/h) and residual disease (geometric mean 566.1 L/h, P=0.90). In patients who underwent iCT or pCT, also no association between paclitaxel clearance and RECIST outcome was identified (iCT: P=0.08 and pCT: P=0.81, respectively).

CONCLUSION

Systemic paclitaxel exposure is not associated with response to common paclitaxel based treatment regimens for esophageal cancer. Future studies should focus on tumour exposure in relation to systemic exposure and treatment outcome.

INTRODUCTION

The incidence of esophageal cancer is still rising in the United States and Western Europe and mortality is high.[1, 2] Esophageal cancer is often diagnosed at an advanced stage. Therefore, curative treatment is only attempted in less than fifty percent of patients.[3] Based on the evidence from the Dutch randomised CROSS trial, paclitaxel can be used in combination with carboplatin and radiotherapy as an effective neoadjuvant treatment strategy.[4, 5] In approximately 30% of patients, no vital tumour cells are left in the oesophagectomy specimen following neoadjuvant chemoradiotherapy (nCRT).[4, 6, 7] In another 30% of patients, partial regression of the tumour is observed (1-10% vital tumour cells), while in 25% of patients the resection specimen does not show changes in regression (>50% vital tumour cells). In patients with extensive disease not amendable for surgery, induction or palliative chemotherapy (iCT or pCT respectively) is given, where paclitaxel is also combined with carboplatin.[8-11] In this setting, the dose of paclitaxel is higher (weekly 100 mg/m^2) than in the neoadjuvant setting (weekly 50 mg/m^2).

Paclitaxel is a classic chemotherapeutic agent which stabilises cellular microtubules, thereby blocking chromosomal segregation and mitosis, and eventually inducing apoptosis.[12,13] There is a suggested dose-response relationship for this agent.[14,15] Unfortunately, paclitaxel is also known for its huge inter-individual variability in pharmacokinetics, which is largely explained by (pharmaco-)genetic and environmental differences between patients.[14] As a consequence, differences in (dose-limiting) toxicities may be explained by differences in systemic exposure between patients.[14, 16, 17] However, if differences in outcome could also be explained by variation in systemic paclitaxel exposure is currently unknown.

Therefore, we hypothesized that an increased systemic paclitaxel exposure due to low clearance is associated with a better response to treatment for patients with esophageal cancer. Therefore, in this study, for the first time, the association between systemic exposure to paclitaxel and therapeutic effect in patients with esophageal cancer was studied.

METHODS

PATIENTS

All patients were treated at the Erasmus MC Cancer Institute, Rotterdam, the Netherlands, which is a tertiary referral centre for patients with esophageal cancer. Patients, aged

18 years or older, treated with paclitaxel for histologically proven carcinoma of the intrathoracic esophagus or gastro-esophageal junction between November 2007 and May 2013 were identified from an institutional database (based on a prospective trial registered at www.trialregister.nl as NTR2311, study number MEC 03.264). In this study, all patients who received paclitaxel mono- or combination-therapy, were included. For pharmacokinetic purposes, a limited sampling strategy was used. All patients with esophageal cancer received either paclitaxel in a neoadjuvant chemo-radiotherapy regimen, as induction treatment or in a palliative setting. For each individual patient, a treatment plan was conducted and evaluated during a weekly multidisciplinary team meeting. Ethical approval was given by the ethical committee of the Erasmus MC as an amendment to the prospective trial (NTR2311). All patients provided written informed consent for the mentioned trial.

STAGING

Tumours were (re-)staged according to the 7th UICC-AJCC TNM staging manual.[18] Every patient underwent physical examination and routine biochemical and haematological tests. In every patient, an upper gastro-intestinal endoscopy with biopsies, computed tomography (CT) of the neck, chest and abdomen, and external ultrasonography of the neck with FNA in case of suspected lymph nodes, was performed according to the Dutch esophageal cancer guidelines. Only in T3 tumours PET was proven to be of any additional value at that time, and was not yet standardised.

NEOADJUVANT CHEMO-RADIOTHERAPY

On days 1, 8, 15, 22, and 29, paclitaxel and carboplatin were administered intravenously. A paclitaxel dose of 50 mg/m^2 was administered and the targeted area under the curve (AUC) was 2 mg/mL/min for carboplatin. A total 3D conformal radiation dose of 41.4 Gy was given in 23 fractions of 1.8 Gy each, with 5 fractions administered per week. Radiotherapy started at the first day of the first chemotherapy cycle.[4, 19]

INDUCTION OR PALLIATIVE CHEMOTHERAPY

Weekly 100 mg/m^2 paclitaxel was given together with carboplatin targeting at an AUC of 4 mg/mL/min.[20, 21] In some patients, induction or palliative chemotherapy was continued beyond the planned number of six cycles. This was done to either sustain tumour regression, or in case of partial response, for further downsizing tumour volume. The regimen these patients received consisted of 175 mg/m^2 paclitaxel and carboplatin (targeted at an AUC of 6 mg/mL/min) and administered in three 3-weekly cycles.

SURGERY

If surgery was feasible (after neoadjuvant chemoradiotherapy or after successful

induction chemotherapy), operations were performed or strictly supervised by experienced upper-GI surgeons in four hospitals specialised in esophageal surgery. For tumours of the intrathoracic esophagus and for junctional tumours with positive lymph nodes at or above the carina a transthoracic approach with two-field lymph node dissection was generally performed. In patients with a poor performance status (WHO performance score of 2 or higher) or for tumours substantially involving the gastro-esophageal junction, a transhiatal resection was favoured. [22, 23]

RESPONSE EVALUATION

In patients treated with neoadjuvant chemoradiotherapy, treatment response was based on assessment of the resection specimen. After surgery, the resection specimens were immediately sent to the Department of Pathology and instantly examined by the attending pathologist. Samples of the tumour, lymph nodes and resection margins were obtained before the specimen was fixed in formalin. A radical resection (ypR0, where yp means pathological after neoadjuvant treatment) was defined as no tumour cells within 1 mm of the circumferential, proximal or distal resection margins.[4] Hence, when tumour cells were detected at or within 1 mm of the resection plane it was classified as ypR1. The number of lymph nodes removed and the number of tumour positive lymph nodes removed were assessed. The tumour regression grade (TRG), used to assess the response to neoadjuvant chemoradiotherapy or to induction chemotherapy, was classified into four categories according to a modified Mandard score. TRG 1 means no vital tumour cells in the resection specimen (pathologically complete response of the primary tumour and removed lymph nodes, ypT0N0M0); TRG 2 means less than 10% residual vital tumour cells and/or any residual vital tumour cells in the lymph nodes; TRG 3 means between 10 and 50% residual vital tumour cells; and TRG 4 means more than 50% residual vital tumour cells.[6, 24] For this study, all samples were re-analysed by one pathologist (K.B.).

In patients treated with induction or palliative chemotherapy, treatment response was assessed using CT images after six weekly cycles of chemotherapy and scored according to the response evaluation criteria in solid tumours (RECIST) classification system. A modified RECIST 1.1. score was used, where smaller lesions than required according to definitions for RECIST 1.1 were taken into account as well. All CT images were (re-)evaluated by a single radiologist (N.K.). If no tumour lesions were seen on CT imaging after induction or palliative chemotherapy, patients were classified as having a complete response (CR). When imaging showed regression of the primary tumour and/or lymph nodes or the presence of novel metastatic lesions, patients were classified as having a partial response (PR). If there was no difference in tumour and/or lymph node size and metastatic lesions, patients were classified as having stable disease (SD).

In case of progression in size of the primary tumour and/or lymph nodes or metastatic lesions or development of new lesions, patients were classified as having progressive disease (PD).[25]

PACLITAXEL PHARMACOKINETIC ANALYSES

The analyses for paclitaxel pharmacokinetics were performed according to previous studies.[14, 16, 17] In brief, from each patient blood was taken during one of the five or six (dependent on type of treatment) weekly chemotherapy cycles, using a formerly endorsed limited sampling strategy with 4 to 5 samples within approximately 24 hours after start of paclitaxel infusion.[14, 26] To prevent coagulation, lithium heparin was used in all samples. Subsequent to sample collection, paclitaxel concentrations were determined using a validated method.[16] Next to individual total paclitaxel plasma concentrations, a well-established population pharmacokinetic model and NONMEM software (Icon Development Solutions, Leopardstown Dublin, Ireland) were used to determine the paclitaxel clearance (CL, L/h) in each individual patient.[14]

STATISTICAL ANALYSIS

The primary outcome of this study was the association between paclitaxel clearance and response to systemic treatment in patients with esophageal cancer. Analyses of the unbound paclitaxel clearance were performed on log-transformed clearance values since they were assumed to follow lognormal distribution. Hence, clearance was described by means of geometric means and corresponding coefficients of variation (CV). Differences in clearance between TRG groups were tested by means of ANOVA. Post-hoc tests were only performed if the overall (omnibus) test was significant at the 5% level without correction for multiple testing. The difference between patients with a complete response (TRG1) and patients with residual disease (TRG2-4) was tested by means of the independent samples t-test. In order to interpret the difference found on the log-scale, the difference and corresponding 95% confidence interval (CI) boundaries were exponentiated to represent the geometric mean ratio and its CI on the original scale. Statistical analyses were performed with the use of SPSS software, version 22.0 (SPSS, IBM, New York, NY, USA).

RESULTS

A total of 166 patients with esophageal cancer was included from a prospectively collected database, of whom 113 patients underwent neoadjuvant chemoradiotherapy followed by surgery. Another 23 patients received induction chemotherapy (of whom

11 proceeded to oesophagectomy) and 30 patients underwent palliative treatment. Patient and tumour characteristics of all enrolled patients are listed in **Table 1**. The majority of the patients was male and had an oesophageal adenocarcinoma. In patients receiving neoadjuvant chemoradiotherapy, as well as induction and palliative chemotherapy; cT3 status, cN1 status, a moderately differentiated tumour, and located at the distal oesophagus was seen most. Not all patients received the initially planned courses due to toxicity or the patient's condition (**Table 1**).

Results for individual paclitaxel clearance as measure for paclitaxel exposure is listed per treatment and response group in **Table 2**. Paclitaxel clearance is expressed as geometric mean (GM) with coefficient of variation (CV).

Table 1: Patient and tumour characteristics

Sex			
Male	91 (80.5%)	16 (69.6%)	29 (96.7%)
Age			
Median years (Range)	63 (39-82)	64 (52-77)	64 (47-76)
Tumour type			
Adenocarcinoma	90 (79.6%)	13 (56.5%)	24 (80.0%)
Squamous cell carcinoma	22 (19.5%)	9 (39.1%)	6 (20.0%)
Other+	1 (0.9%)	1 (4.3%)	
Histopathological grading			
G1	3 (2.5%)	0	2 (6.7%)
G2	51 (45.1%)	7 (30.4%)	5 (16.7%)
G3	32 (28.3%)	10 (43.5%)	13 (43.3%)
G4	1 (0.9%)	0	0
Gx or Missing	26 (23.0%)	6 (26.1%)	10 (33.3%)
Tumour localization			
Proximal	0	2 (8.7%)	0
Middle	18 (15.9%)	5 (21.7%)	4 (13.3%)
Distal	80 (70.8%)	10 (43.5%)	19 (63.3%)
Gastro-esophageal junction	15 (13.3%)	6 (26.1%)	7 (23.3%)
Clinical T stage			
cT1	4 (3.5%)*	0	0
cT2	26 (23.0%)*	0	2 (6.7%)
cT3	80 (70.8%)*	17 (73.9%)	16 (53.3%)
cT4	3 (2.7%)*	5 (21.7%)	3 (10.0%)
Missing	0	1 (4.3%)	9 (30.0%)
Clinical N stage			
N0	35 (31.0%)#	3 (13.0%)	3 (10.0%)
N1	41 (36.3%)#	5 (21.7%)	6 (20.0%)
N2	34 (30.1%)#	11 (47.8%)	10 (33.3%)
N3	3 (2.7%)#	4 (17.4%)	5 (16.7%)
Missing	0	0	6 (20.0%)
Clinical M stage			
M0	113 (100%)	21 (91.3%)	2 (6.7%)
M1	0	2 (8.7%)^	28 (93.3%)

Table 1 continued.

TREATMENT REGIMEN			
Neoadjuvant chemoradiotherapy	113 (100%)	X	X
4 courses of Paclitaxel	3 (2.7%)	X	X
5 courses of Paclitaxel	109 (96.5%)	X	X
6 courses of Paclitaxel	1 (0.9%)	X	X
Induction or palliative chemotherapy	X	23 (100%)	30 (100%)
6 courses of Paclitaxel	X	8 (34.8%)	13 (43.3%)
6 + 3 courses of Paclitaxel	X	15 (65.2%)	17 (56.7%)
Resection	113 (100%)	11 (47.8%)	X
Other treatment	X	X	1 (3.3%)£

Abbreviations: nCRT: neoadjuvant chemoradiotherapy, iCT: induction chemotherapy, pCT: palliative chemotherapy, + Other: neuroendocrine tumour.

* uTstage (endosonography) in patients treated with neoadjuvant chemoradiotherapy.

uNstage (endosonography) in patients treated with neoadjuvant chemoradiotherapy.

$ no location possible due to only radiological diagnostics.

^ Submucosal metastasis and suspicion of lung metastasis.

£ brachytherapy

Table 2: Paclitaxel clearance per treatment and response group

Response	Clearance (L/h) Geometric mean (CV, %)	*P-value*
nCRT (n=113)		*0.25*
TRG1 (n=36)	561.6 (34)	
TRG2 (n=28)	591.4 (20)	
TRG3 (n=37)	578.5 (29)	
TRG4 (n=12)	478.5 (56)	
iCT (n=23)		*0.08*
CR (n=2)	358.1 (37)	
PR (n=12)	409.9 (29)	
SD (n=9)	500.7 (8)	
PD (n=0)	X	
		0.81
pCT (n=30)		
CR (n=2)	488.0 (16)	
PR (n=11)	447.1 (35)	
SD (n=9)	440.5 (33)	
PD (n=8)	500.2 (23)	

Abbreviations: nCRT = neoadjuvant chemoradiotherapy; iCT = induction chemotherapy; pCT = palliative chemotherapy; CV = coefficient of variation; TRG = tumour regression grade; CR = complete response, PR = partial response, SD = stable disease, PD = progressive disease.

Thirty-six patients who underwent neoadjuvant CRT had a pathologically complete response (32%) and 77 patients (68%) had a partial or no response based on their oesophagectomy specimen. The tumour regression grade was not significantly associated with paclitaxel clearance (P-value=0.25, Table 2). Post-hoc tests were not

performed because of the non-significant overall effect. Also, when comparing the clearance of patients with a pathologically complete response (TRG1) to the clearance of patients with residual disease (TRG 2-4) no difference was seen (geometric mean ratio = 0.99, 95% CI [0.87-1.13], P-value=0.90, **Table 3**).

The radiological classification of patients --treated either by induction or by palliative chemotherapy-- is also listed in **Table 2.** In none of the 23 patients who underwent induction chemotherapy, progression of disease was seen. A complete response was seen in 2 patients, partial response in 12 patients and stable disease in 9 patients. The response grade according to modified RECIST1.1 was not statistically significantly associated with response (P-value=0.08, **Table 2**). However, a possible trend was seen towards a better response in patients with an increasing paclitaxel exposure, although the number of patients with a clinical complete response was only two. Some 30 patients treated with palliative intent were evaluated in the current analysis of whom 8 patients (27%) had progression of disease at moment of response evaluation after 6 cycles of chemotherapy. Also in this group we could not identify an association between paclitaxel clearance and tumour response (P-value 0.81, **Table 2**).

Table 3: Paclitaxel clearance of patients with pathologically complete response versus patients with residual disease after neoadjuvant chemoradiotherapy followed by surgery

Response	Clearance (L/h) Geometric mean (CV, %)	P-value	Geometric mean ratio
nCRT (n=113)		0.90	0.99 (0.87-1.13)
TRG 1 (n=36)	561.6 (34)		
TRG 2-4 (n=77)	566.1 (32)		

Abbreviations: nCRT = neoadjuvant chemoradiotherapy; CV = coefficient of variation; TRG = tumour regression grade.

DISCUSSION

To our knowledge, this is the first study that investigated the association between systemic exposure to paclitaxel and tumour response in patients with esophageal cancer. Response to paclitaxel in patients receiving neoadjuvant chemoradiotherapy (nCRT), induction chemotherapy or palliative chemotherapy was analysed. In contrast to what was hypothesized, systemic concentrations of paclitaxel were not associated with pathological response or radiological tumour regression.

In patients receiving induction chemotherapy, a possible trend was seen towards patients with a clinically complete response having a lower paclitaxel clearance than patients with a partial response or stable disease. However, as only two patients had a clinically complete response in this subgroup, no hard conclusions can be drawn on this point.

One of the potential reasons why a relation between pharmacokinetics and response was not seen could be that in patients receiving neoadjuvant chemoradiotherapy, the chemotherapy mainly acts as a radiosensitizer. [27-29] Thus, the effects of paclitaxel exposure on treatment outcome can be overshadowed by the combination with radiotherapy. Also the combination with carboplatin chemotherapy (of which no drug concentrations were measured) could have influenced the outcomes of the analyses. Furthermore, the type of tumour (adenocarcinoma *versus* squamous cell carcinoma) affects the response to chemoradiotherapy. Squamous cell carcinoma reacts more effectively to chemoradiotherapy, as indicated by the fact that a pathological response occurs more often in patients with squamous cell carcinomas. However, the CROSS regimen does not distinguish between the two tumour types in clinical practice.[4, 5, 19] In the present study the majority of patients were diagnosed with adenocarcinoma of the esophagus, in line with the incidence in Western world.[30]

Another important reason for the lack of correlation between paclitaxel plasma pharmacokinetics and tumour response is a potential weak correlation between paclitaxel plasma exposure and paclitaxel tumour exposure. As one of its potential resistance mechanisms a tumour may use efflux transporters (*i.e.* ATP-binding cassette (ABC) transporters) to limit intra-tumoural chemotherapy concentrations. Taxanes, including paclitaxel, are known substrates for these transporters.[31,32] Although we did not measure intra-tumoural drug concentrations in this study, due to its retrospective nature, we speculate that intra-tumoural paclitaxel concentrations differ substantially from plasma chemotherapy concentrations. To further explore this, we recently set up a new prospective clinical trial (*i.e.* the PAREO study; registered at www.trialregister.nl as NTR6356, study number MEC 16.696) in which plasma paclitaxel exposure is compared with intra-tumoural concentrations by serial tumour biopsies and simultaneous blood sampling in patients treated for esophageal cancer.

Our study has several limitations. The limited sample size of the induction and palliative treatment group could have influenced our results. However, we do think that a strong relationship between paclitaxel clearance and response still could have been detected. Nevertheless, the results of these two treatment groups should be interpreted with caution. Not all blood samples were collected during the first treatment cycle resulting

in different paclitaxel dosages, especially in the induction and palliative treatment group. However, we used clearance as measure for systemic exposure, which will not be strongly influenced by drug dosage. Next to this, most patients receiving palliative chemotherapy were treated with 6 cycles, while others received more. Response evaluation was performed after six weekly cycles (for the first time) in every patient, but the obtained blood samples for clearance were not strictly regulated to these first six weeks. This feature can be of clinical influence on response, but numbers were too small to characterize.

In summary, in this study, the association between systemic exposure to paclitaxel and pathological response/clinical outcome in patients with oesophageal cancer was studied. The current analysis demonstrates that systemic paclitaxel exposure is not related to response to common paclitaxel based treatment regimens for oesophageal cancer. Future studies should therefore focus on intra-tumoural exposure in relation to systemic exposure and treatment outcome.

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PART V

13

SUMMARY & GENERAL DISCUSSION

This thesis describes several pharmacokinetic and pharmacogenetic studies on four important anti-cancer drugs that are commonly used in the systemic treatment of malignancies in the gastrointestinal tract. In this chapter the results of studies in this thesis will be summarized and discussed per drug. Furthermore, in each part future perspectives will be given on how to translate these results into clinical practice to eventually personalize the oncologic treatment for every patient to reduce toxicity and improve efficacy.

PART I: FLUOROPYRIMIDINES

Fluoropyrimidines are a group of chemotherapeutic agents, which are widely used in the treatment of solid tumors and include intravenously administered 5-fluorouracil (5-FU) and orally administered 5-FU prodrugs capecitabine and tegafur. Depending on the different types of treatment regimens, capecitabine is given either as monotherapy, in combination with other chemotherapeutic agents, or it is combined with radiotherapy. Like other chemotherapeutic agents, capecitabine is in general dosed on body-surface area (BSA) to reduce inter-individual variability in its pharmacokinetics. Fixed-dosing is an alternative dosing strategy, which means that the dose is not adjusted for body size measures. In **Chapter 2** toxicity and effectiveness of fixed-dosed capecitabine in four commonly used treatment regimens is described in a cohort of 1126 patients, and compared to BSA-dosed patients in another cohort of 1193 comparable patients. We found no difference in toxicity between fixed-dosed and BSA-dosed patients, while the prescribed capecitabine dose was 7.1% higher when dosed on BSA compared to fixed-dosed. Interestingly, both cohorts showed only a higher incidence of toxicity, especially diarrhea, in patients of the lowest BSA quartile when capecitabine was combined with radiotherapy. This finding was unexpected and is likely to be caused by the interaction of the two treatment modalities instead of the type of dosing strategy. Several hypotheses for the fact that patients with a lower BSA experience more toxicity from capecitabine combined with radiotherapy can be given; namely: local effects due to the radiosensitizing effect of capecitabine[1], a higher amount of radiated small bowel volume[2, 3] or local rectal irritation by the tumor itself.[4] Future research should therefore be conducted on the potential effects of radiotherapy and BSA on toxicity of this combination treatment in patients with a low BSA.

Besides toxicity, we also investigated the effectiveness of given treatments. No survival difference was identified between the patients with a low BSA and patients with a high BSA per treatment and indication within the fixed-dosed cohort. In addition, the

observed progression free survival (PFS) for capecitabine-oxaliplatin for metastatic colorectal cancer and capecitabine triplet therapy for gastric cancer was comparable to literature data (8.6 and 24.6 months versus 8.0 and 19.2 months, respectively).[5, 6] Several other small studies did not find a survival difference either [7-10], which is in line with our results. Our results indicate that fixed dosing of capecitabine is a reasonable and practical alternative for BSA-based dosing. Therefore, we would recommend implementing fixed dosing in future clinical studies and we have found no arguments why it could not be used in daily clinical care. Unfortunately, we could not compare our results concerning survival with the BSA-dosed cohort as in that second cohort no survival data were collected. Future research should therefore directly compare both dosing strategies with survival as an endpoint. It would be even better if this would be done in a randomized controlled trial setting, however it seems unlikely that this will ever happen. Furthermore, it would be interesting to investigate whether a fixed dosing strategy for capecitabine would lead to fewer prescribing mistakes and possibly reduced costs, as has been demonstrated for some other drugs.[11-13]

Fluoropyrimidines are mainly metabolized by the enzyme dihydopyrimidine dehydrogenase (DPD) which converts more than 80% of 5-FU into the inactive metabolite dihydrofluorouracil. Around 10-30% of patients experience severe or even fatal fluoropyrimidine-related toxicity, which is in almost 60% of these patients caused by reduced DPD activity.[14-19] A reduced DPD activity can be the result of polymorphisms in the *DPYD* gene, which encodes for the DPD enzyme. *DPYD* is a large gene with many genetic variants described, which do not all have functional consequences. Based on a meta-analysis by Meulendijks et al. in 2015, currently, four DPYD variants are considered clinically relevant (*DPYD**2A, c.1679T>G, c.2846A>T, and c.1236G>A) in Caucasian patients, which result in an estimated 50% or 25% reduced DPD-activity.[20] It has already been demonstrated that prospective genotyping for the *DPYD**2A variant and dose-reductions in heterozygote *DPYD**2A carriers improves treatment safety and that it is cost-effective.[21] In **Chapter 3** the results of a large prospective trial, performed in 17 Dutch centers, on personalized fluoropyrimidine dosing based on these four *DPYD* variants are described. All patients were genotyped for these four *DPYD* allele variants before start of fluoropyrimidine treatment. If a variant was detected, patients received a 50% dose reduction for *DPYD**2A and c.1679T>G, and a 25% dose reduction for both c.2846A>T and c.1236G>A. If a homozygote mutation or compound heterozygote mutations were detected, patients were excluded from the trial. All patients were followed during the whole treatment period. Toxicity was compared between *DPYD* variant allele carriers and wild-type patients from this study and with a historical cohort of *DPYD* variant allele carriers treated with the full dose (derived from the previously mentioned meta-analysis [20]). Furthermore, pharmacokinetic analysis of *DPYD* variant

allele carriers treated with a reduced dose was performed.

In total, 1103 evaluable patients were enrolled, of whom 85 *DPYD* variant carriers (7.7%). Overall, severe toxicity was still higher in *DPYD* variant carriers (39%) than in wild-type patients (23%). However, when comparing to the historical cohort, upfront DPYD genotyping markedly reduced the relative risk for severe toxicity for *DPYD**2A (from 4.30 to 1.31) and c.1679T>G carrier (from 4.30 to no toxicity), moderately reduced the relative risk for c.2846A>T (from 3.11 to 2.00), and not reduced the relative risk for c.1236G>A (from 1.72 to 1.69). Furthermore, pharmacokinetic analysis in *DPYD* variant allele carriers treated with a reduced dose demonstrated that fluoropyrimidine exposure was comparable to wild-type patients treated with a full dose. Lastly, this upfront genotyping strategy was likely to be cost-saving or cost-neutral with a moderate cost-saving of €51 per patient as described in **Chapter 4**. Our study demonstrated that upfront *DPYD* genotyping was feasible in routine clinical practice, that it improved patient safety of fluoropyrimidine treatment, and that it is cost-saving. For *DPYD**2A and c.1679T>G carriers, a 50% initial dose reduction seems adequate. For c.1236G>A and c.2846A>T carriers, a larger dose reduction of 50% (instead of 25%) needs further investigation.

Although the results of our pharmacokinetic analysis demonstrated that the *DPYD* variant allele carriers were not underdosed as they had an equal exposure as wildtype patients treated with a full dose, treatment efficacy will remain a concern of critics. A retrospective, matched-pair analysis of *DPYD**2A treated with reduced dose, found no negative effect on overall survival (OS) and PFS.[22] However, this study was underpowered and therefore no firm conclusions can be made based on these data. As no additional studies on survival effects of a reduced dose in *DPYD* variant allele carriers are available, future studies should address this topic. Ideally, a randomized controlled trial on safety and efficacy of *DPYD* genotyping should be performed, but this requires a large sample size and is considered unethical concerning the large amount of evidence currently available, and is therefore not likely to happen. It is therefore necessary that in future prospective studies efficacy of the genotype based reduced dose is included as an endpoint and that efficacy data of different trials by meta-analysis of individual data can be combined.

Based on our study we recommend to endorse worldwide implementation of *DPYD* screening before start of fluoropyrimidine treatment as standard of care to improve safety of fluoropyrimidine treatment.

Henricks et al. already recommended a drug label update to include preemptive *DPYD* genotyping and dose adjustments in all fluoropyrimidine drug labels.[23] The European

Medicine Agency (EMA) supported this request and has now asked the different pharmaceutical companies to adjust the drug labels. Furthermore, partly based on our study and at the request of the French Medicines Agency, EMA is currently reviewing all screening methods for DPD deficiency including *DPYD* genotyping, and evaluating if a screening method should be done in every patient treated with fluoropyrimdines.[24] These latest developments might help to implement *DPYD* genotyping in clinical care. However, clinicians need to be supported with respect to the interpretation of these genotyping results, and therefore integration of pharmacogenetic results and warning systems in the electronic patient file could help to prevent prescription mistakes. Furthermore, in 2017, the Clinical Pharmacogenetic Implementation Consortium (CPIC) updated their guideline on DPD genotyping with dose recommendations on different variants.[25] However, the results of our study were not included in that guideline as our data became available in 2018, and a future update will benefit from more evidence on the most optimal dose for c.1236G>A and c.2846A>T carriers.

Future research can possibly further elucidate the genetic background of fluoropyrimidine induced toxicity. In our study we included four *DPYD* variants currently considered clinically relevant, however these variants are less common in non-Western populations and several other (new) *DPYD* variants are described in these populations.[26-28] Further research is necessary though to determine which *DPYD* variants are clinically relevant in non-Western ethnicities. Therefore, a new prospective observational multicenter trial is currently initiated in the Netherlands. Secondly, DPD activity is not only regulated by *DPYD* variants but also from a post-transcriptional level, for instance by microRNA 27a (miR-27a).[29-31] Lastly, besides *DPYD*, several other genetic variants in enzymes and transporters involved in the fluoropyrimidine metabolism have been related to fluoropyrimidine induced toxicity (including carboxylesterase, thymidylate synthase, cytidine deaminase, thymidine phosphorylase, and the SLC22A7 transporter).[32-40] To identify new variants related to fluoropyrimidine induced toxicity, an additional genome-wide association study (GWAS) will be performed on patient's DNA of our study cohort. All these attempts will hopefully result in a gene-screenings panel that will detect all clinically relevant variants for every patient.

In the previous paragraph, the current status of screening for DPD-deficiency by means of (*DPYD)* genotyping was discussed. However, DPD deficiency can also be identified using different phenotyping tests that measure the DPD activity (in)directly. Several phenotyping methods are currently described, of which DPD activity measurement in peripheral blood monocytes (PBMCs) is the most direct one.[41] More indirect phenotyping tests are related to the measurement of the endogenous DPD substrate uracil or its product dihydrouracil (DHU) such as the ratio between those (DHU/U).[41]

Several studies demonstrated a correlation between uracil levels or the DHU/U ratio and fluorouracil pharmacokinetics, and with the onset of fluoropyrimidine-associated toxicity.[42-49] In **Chapter 5**, in a subset of patients who participated in the prospective trial (described in **Chapter 3 and 4**) several different phenotyping tests were evaluated for their additional value to identify DPD deficiency and to identify patients at risk for severe fluoropyrimidine-induced toxicity. In this exploratory cohort, patients underwent two to four tests before start of fluoropyrimidine-based therapy: endogenous dihydrouracil/uracil (DHU/U) ratio and endogenous uracil in 1037 patients, uracil loading dose in 92 patients [50, 51], and 2-^{13}C-uracil breath test in 82 patients [52]. Phenotyping results were associated with the onset of severe (grade ≥3) fluoropyrimidine-induced toxicity and DPD deficiency (defined as DPD activity ≤ 5.9 nmol/(mg*h) in PBMCs). Clinical validity parameters were calculated per test.

Unfortunately, in our cohort, none of these four tests could predict DPD-deficiency or fluoropyrimidine-induced toxicity very well. Several reasons can be given for these results. Firstly, the sample size for the uracil loading dose and breath test was limited due to difficult patient accrual for these tests. Secondly, a large variation in test results was detected between different study centers, which represents methodological problems with standardization of these tests. Although we did exclude too divergent results per phenotyping test, this will probably have influenced our results. Furthermore, we performed these tests in an unselected population, in contrast to most other studies that evaluated these tests in populations selected or enriched for DPD deficiency or severe treatment-related toxicity. Lastly, the sampling time of the blood withdrawal for the uracil and DHU/U ratio determination was performed on a random time point in our study, although DPD activity exhibits a significant circadian variability.[53] Furthermore, our samples were withdrawn at room temperature and not immediately frozen, while uracil levels are not stable at room temperature.[54] Despite the disappointing results, our study is the first head-to-head comparison of these four tests and highlights several factors that need to be rigidly standardized in future research. Furthermore, the combination of genotyping and phenotyping still remains a promising strategy to improve the amount of patients detected with relevant DPD deficiency. Therefore, a new prospective clinical trial (Alpe2U trial), which recently started, will investigate if a personalized fluoropyrimidine dose based on the combination of pre-treatment *DPYD* genotyping and uracil levels will improve treatment safety. These results have to be awaited before using uracil levels to adjust the fluoropyrimidine dose.

In **Chapter 6**, several patients with multiple *DPYD* variants (i.e. homozygotes or compound heterozygotes), who were treated with personalized fluoropyrimidine treatment were described. In current clinical practice, fluoropyrimidine treatment is

avoided in these patients. However, this could have a large impact on disease outcome for individual patients, as fluoropyrimidines are considered effective anticancer agents and are included in many treatment regimens. As demonstrated in **Chapter 6**, personalized fluoropyrimidine treatment in these patients is an option and treatment should not be withheld. However, it is difficult to predict DPD activity in patients with multiple *DPYD* variants on the genotyping result solely. Therefore, it is strongly recommended to determine DPD-activity in PBMCs in these patients, and adjust the (starting) dose accordingly. Furthermore, these patients should be closely monitored for toxicity and dose-titration based on clinical tolerance is needed to reach a true personalized dose.

PART II: IRINOTECAN

The topoisomerase-I inhibitor irinotecan is widely used in the treatment of solid tumors including colorectal and pancreatic cancer.[55] Irinotecan treatment is characterized by several dose-limiting toxicities such as severe neutropenia and diarrhea in up to a quarter of patients.[56, 57] In **Chapter 7** an overview of current evidence on irinotecan pharmacokinetics, pharmacodynamics, and pharmacogenetics is given. The prodrug irinotecan is hydrolyzed into the active metabolite SN-38 by two carboxylesterase isoforms (CES1 and 2) and butyrylcholinesterase in the human body.[58, 59] SN-38 is 100-1000 fold more active compared to irinotecan itself.[60] SN-38 is inactivated via glucuronidation into SN-38G by uridine diphosphate glucuronosyl-transferase (UGT) and excreted into bile.[61, 62] SN-38G can be deconjugated into SN-38 by β-glucuronidases produced by intestinal bacteria, which could result in an enterohepatic recirculation of SN-38.[63-66] Furthermore, irinotecan is also metabolized by intrahepatic cytochrome P450 (CYP) enzymes, i.e. CYP3A4 and CYP3A5, into the inactive metabolites: APC and NPC.[67] In contrast to APC, NPC can be converted to SN-38 by CES1 and CES2 in the liver but to a lesser amount than irinotecan.[68] Clearance of irinotecan is mainly biliary (66%) and irinotecan is transported into the bile by several ATP-binding cassette (ABC) transporters (i.e. ABCB1, ABCC2, and ABCG2). [55, 69 70-72] This highly complex metabolism makes irinotecan prone to individual, environmental, and genetic influences which will partly explain the large inter-individual variability in irinotecan pharmacokinetics. Many drug-drug interactions have been described with potentially serious consequences. The most famous example is St. John's wort, which resulted in a 42% reduction of SN-38 systemic exposure, primarily caused by CYP3A4 induction.[73] Therefore, prior to start with irinotecan chemotherapy, all patients should be evaluated for possible interactions with co-medication. Furthermore, concomitant use of potent CYP3A4

inducers or inhibitors should be avoided.

In the last decade, much progress has been made in determining the influence of pharmacogenetics on systemic irinotecan exposure, toxicity, and survival. The most frequently studied gene is *UGT1A1*, with currently more than one hundred reported genetic variants; UGT1A1 is a highly polymorphic enzyme.[74] for Caucasians *UGT1A1*28* seems to be a good predictor for neutropenia (all irinotecan doses) and diarrhea (doses >125 mg/m^2). [75-77] *UGT1A1*28* is also significantly associated with an increased risk for diarrhea in Asian patients at irinotecan doses >125 mg/m^2.[77] In Asian populations, however, the *UGT1A1*6* variant is more common and appears to be a more accurate predictor for neutropenia (all irinotecan doses) and diarrhea. [78, 79 80] In addition to *UGT1A1*6* and *UGT1A1*28*, *UGT1A1*93* is also significantly associated with irinotecan-induced toxicity.[81-86] Less extensively studied polymorphisms such as *UGT1A7*3*, *UGT1A9*1*, and drug transporter polymorphisms (*ABCB1*, *ABCC5*, *ABCC2*, *ABCG1*, *SLCO1B1*) may also be useful predictors for toxicity.[81, 87-95] Interestingly, *CYP3A4*22* has not been studied thus far in relation to irinotecan pharmacokinetics or toxicity, while this SNP has shown relevance for many other CYP3A substrates.[96-98]

Several guidelines, including the U.S. Food and Drug Administration (FDA) and Health Canada/Santé Canada (HCSC), recommend a reduction of the irinotecan starting dose in patients who are homozygous for *UGT1A1*28*.[99-102] Furthermore, the Japanese Pharmaceuticals and Medical Devices Agency (PMDA) recommends screening patients for *UGT1A1*6* and **28* polymorphisms.[103] Despite the establishment of these guidelines, *UGT1A1* genotyping is currently not routinely performed.[104] This could be explained by the fact that prospective studies evaluating the clinical effects of genotype-directed dosing are scarce. Therefore, in collaboration with the Catharina Hospital, Eindhoven, and the Leiden University Medical Center, we designed a prospective trial on genotype-guided irinotecan dosing based on *UGT1A1*28* and *UGT1A1*93* genotype status which is currently ongoing (The IRI-28 trial: Trial ID: NTR6612). Hopefully, the results of this trial will endorse implementation of prospective *UGT1A1* screening to personalize the irinotecan treatment in daily clinical care. Future research on irinotecan treatment should include *UGT1A1* screening also, to increase the therapeutic window as there are some studies suggesting that *UGT1A1*1/*1* or *UGT1A1*1/*28* carriers may tolerate higher irinotecan doses than the currently recommended doses and are therefore at risk of suboptimal treatment.[104, 105]

Besides genotyping, several other interventions to reduce treatment related toxicities have been investigated including dietary adjustments. Preclinical studies in animals have demonstrated that by fasting before irinotecan treatment, toxicity can be reduced

while preserving the anti-tumor effects.[106, 107] After 72 hours of fasting, mice experienced significantly less side effects of irinotecan chemotherapy, intra-tumoral SN-38 concentrations tended to be higher, and concentrations in both plasma and healthy liver were significantly lower.[107] **Chapter 8** describes a prospective pharmacokinetic crossover trial investigating a short-term combined caloric and protein restriction regimen (CCPR) in 19 cancer patients with liver metastases treated with irinotecan. Patients were randomized to receive irinotecan preceded by five days of CCPR (~30% calorie and ~70% protein restriction) during the first or second cycle, while the other cycle patients followed their normal diet. During both cycles, 24-hours blood sampling was performed and biopsies of healthy liver and liver metastasis 24-26 hours after irinotecan administration were taken. Interpatient variability in tissue irinotecan and SN-38 concentrations was high. No significant differences in irinotecan and SN-38 concentrations with CCPR in biopsy tissue of healthy liver or liver metastasis were identified, although a trend towards higher concentrations in healthy liver compared to liver metastasis was observed. CCPR significantly increased irinotecan plasma $AUC_{0\text{-}24h}$ with 7.1% (95% CI: 0.3-14.5%, P=0.04) compared to the normal diet, while the SN-38 plasma $AUC_{0\text{-}24h}$ increased with 50.3% (95% CI: 34.6-67.9%, P<0.001). CCPR was well tolerated and no difference in treatment-related grade ≥3 toxicity was identified during CCPR compared to the normal diet (53% vs 42%, P=0.69). Furthermore, no difference was seen with CCPR compared to the normal diet when concerning severe neutropenia (47% vs 32% P=0.38), severe diarrhea (5% vs 21% P=0.25), febrile neutropenia (5% vs 16% P=0.50) and hospitalization (11% vs 21% P=0.634). However, this trial was not designed, nor powered, to detect a difference in toxicity, and therefore these results need to be interpreted with caution. Despite the low sample size, it is still surprising that with the large increase in SN-38 exposure no increase in toxicity was detected, which might have been expected as a concentration-effect relationship has been described for severe diarrhea and neutropenia.[108]

Several mechanisms for the increased SN-38 exposure observed with CCPR can be proposed. Firstly, a decreased elimination of SN-38 caused by reduced UGT-activity or reduced activity of ABC transporters involved in the intestinal elimination of SN-38. In healthy subjects, indeed a reduced UGT metabolism of midazolam was demonstrated after 36 hours of fasting.[109] Furthermore, in patients with morbus Gilbert fasting can induce an increase in bilirubin levels due to reduced UGT activity, which supports this hypothesis too.[110] To further evaluate this possible mechanism, SN-38G concentrations will be determined in retrospect. Preclinical evidence demonstrated an increased ABCC2 activity in mice after 24 hours of fasting. However, to our knowledge this has not been demonstrated for humans, nor was it demonstrated for the other efflux transporters.[111] Secondly, the increased SN-38 concentrations could have been

caused by increased formation of SN-38 by carboxylesterases or intestinal beta-glucuronidases, although there is no evidence supporting this theory. Furthermore, the increased SN-38 concentrations could result from a decreased irinotecan conversion into inactive metabolites by CYP3A4 resulting in increased irinotecan conversion to SN-38. The influence of fasting on CYP3A4 is not fully elucidated as one study found no effect and another an increased CYP3A4 activity, but no decreased activity has been described. [109, 112]

Our findings were unexpected as our preclinical studies in mice demonstrated lower irinotecan and SN-38 concentrations in plasma and healthy tissue and a trend towards increased intra-tumoral concentrations after dietary restriction.[107] Possible explanations for these differences are that in the preclinical study whole tumors and livers were used for pharmacokinetic measurements instead of a single biopsy and the metabolism in mice is not exactly equal to humans.[113-117]

The mechanisms behind the protective effects of dietary restriction are not completely understood, but are currently actively studied. Recently, several small observational trials investigating the beneficial effects of dietary restriction in cancer patients have been conducted. Two small studies demonstrated that short-term fasting resulted in a trend towards less hematological toxicity after chemotherapy.[118, 119] Furthermore, Bauerfeld et al. demonstrated in 34 patients that short term fasting (i.e. 36 hours before till 24 hours after chemotherapy) improved quality of life and fatigue without inducing toxicity during different types of chemotherapeutic regimens.[120] However, none of these studies included pharmacokinetic analyses, while our study demonstrates that the importance of such analyses, especially in view of the narrow therapeutic index of most cytostatic agents. Future studies should therefore include larger sample sizes to evaluate toxicity, including pharmacokinetic analyses of the different cytostatic agents in plasma and preferably repetitively in tumor as well, and perform quality of life analysis. Furthermore, additional research is necessary to determine if dietary restriction does not reduce treatment efficacy as intra-tumoral concentrations were highly variable in our study and other studies did not include efficacy end points also.

PART III: REGORAFENIB

Regorafenib is an oral multi-kinase inhibitor that targets angiogenic and stromal receptor tyrosine kinases both in healthy tissue and in tumors.[121] Regorafenib is currently registered worldwide for the treatment of colorectal cancer (except for the

Netherlands), gastro-intestinal stromal tumors, and hepatocellular carcinoma.[122-124] After oral administration, regorafenib is rapidly absorbed, with a maximum concentration reached at 3-4 hours.[125, 126] Most tyrosine kinase inhibitors (TKIs) exhibit pH-dependent solubility, which makes them prone for drug-drug interactions with acid suppressive agents like proton pump inhibitors.[127] However, for regorafenib it is unknown if these drugs can be combined safely. In **Chapter 9** we describe a prospective randomized three-phase cross-over study in 14 metastatic colorectal cancer patients, on the potential pharmacokinetic interaction between the proton pump inhibitor (PPI) esomeprazole and regorafenib, with special interest in the influence of timing of esomeprazole intake relative to that of taking regorafenib (i.e. concomitantly or three hours prior to the TKI). This clinical trial did not reveal a significant pharmacokinetic interaction between esomeprazole and regorafenib at the two time-points studied. Therefore, we concluded that esomeprazole can be safely combined with regorafenib in clinical practice, in contrast to some other TKIs. These results were not completely unexpected as regorafenib exhibits a low solubility, which is mainly caused by its chemical structure as no strong basic or acidic group is attached.[128] Furthermore, to improve the solubility, regorafenib is formulated as a solid dispersion consisting of small powder particles in which the drug and excipient are integrated [129]. As a result regorafenib absorption is, in theory, less affected by intragastric pH-alterations, which is in line with the results of our study.

In this study, esomeprazole was used because it exhibits the strongest pH-reducing effect of all acid-reducing drugs currently available.[127, 130] Also, esomeprazole does not influence other enzymes or transporters, such as ABCB1, that could potentially influence the pharmacokinetics of regorafenib's active metabolites M-2 and M-5 [131]. Therefore, our findings cannot simply be extrapolated to other PPIs such as pantoprazole which is known to influence ABCB1, but this could be interesting to investigate also. We examined two time-points regarding the intake time of esomeprazole (i.e. concomitantly or three hours prior regorafenib intake), because PPIs are assumed to have their maximum acid-reducing effect three hours after intake and a possible interaction would be the strongest at this time-point.[132] This unique study design can serve as a template for future studies evaluating the influence of PPIs on exposure of oral (anticancer) drugs.

Furthermore, in more than half of all patients, treatment with regorafenib is associated with severe and dose-limiting toxicities such as hypertension and hand foot skin reactions which may not always outweigh treatment benefit.[133] Therefore, there is an urgent need for biomarkers predictive for response to identify specific patients who will, and who will not, benefit from regorafenib treatment. Multiple studies demonstrated that the detection of circulating cell free DNA (cfDNA) and circulating

tumor DNA (ctDNA) could be a powerful tool to monitor and understand the response to anti-cancer agents.[134, 135] However, most of these studies only measured cfDNA and ctDNA at baseline and not after initiation of treatment. In **Chapter 10** we describe an explorative analysis on early cfDNA/ctDNA dynamic changes and correlation with regorafenib pharmacodynamics in 20 metastatic colorectal patients. cfDNA concentrations significantly increased in almost all patients at days 8 and 15, which correlated with ctDNA increase and plasma liver enzyme increases, suggesting that treatment related damage of healthy cells may have confounded these measurements. This finding was somewhat surprising, as we expected cfDNA levels to drop during treatment. The increase in cfDNA could be the result of tumor cell death given the strong correlation with ctDNA, but normal liver tissue damage inflicted by regorafenib may also influence cfDNA levels. The concept that liver damage may increase cfDNA concentrations is supported by a study in 14 mCRC patients treated with chemotherapy and hepatic trans-arterial chemo-embolization that demonstrated that 93% of patients had an increase in cfDNA 24-hours after the first treatment, possibly as a result from local liver cell damage.[136] In two additional studies in non-cancer patients cfDNA increases correlated with several causes of liver cell damage (e.g. non-alcoholic fatty liver disease, liver transplantation, sepsis).[137, 138] Thus, liver cell decay independent of its mechanism seems to increase cfDNA concentrations. These findings implicate that treatment related liver toxicity should be taken into account in any research on cfDNA as potential biomarker for treatment response.

Furthermore, ctDNA dynamics were highly variable between patients, but the majority of patients developed also an absolute increase of mutated molecules per mL at day 8 or 15. An earlier study reported that an increase of ctDNA at day 14 in patients treated with regorafenib was associated with decreased PFS and OS.[139] Our study failed to confirm these findings which may be due to the low sample size of both studies and the dynamic pattern of ctDNA alterations. These conflicting data make it impossible to draw a firm conclusion about the value of ctDNA quantification for response prediction. In our study, the early increase in ctDNA levels tend towards a correlation with increased lactic-dehydrogenase concentration, which may be related to tissue decay, or hypothetically the development of hypoxia in the tumor micro-environment as the anti-angiogenic effects of regorafenib can occur within two weeks after initiation of treatment.[140] In contrast to our finding, Tie et al. measured ctDNA levels in mCRC patients 3 days after treatment with several types of chemotherapy, and found no significant difference in ctDNA levels, which could be explained by the difference in administered anti-cancer agent.[141] Furthermore, in our ctDNA analysis, we could not demonstrate the emergence of new resistance associated mutations or loss of mutations that mark specific therapy sensitive subclones. More specific inhibitors that are used in mCRC such as anti-EGFR

antibodies have been shown to induce the emergence of several resistance associated mutations [135, 142-144]. Together these data indicate that patterns of ctDNA alterations during treatment depend on the type of treatment given. Our study highlights the importance to perform sufficient longitudinal sampling experiments for each type of treatment and correct the outcome for the amount of liver damage before implementing circulating DNA based biomarkers for response prediction.

PART IV: CARBOPLATIN / PACLITAXEL

The combination of carboplatin and paclitaxel is widely used in the treatment of esophageal cancer. Most patients with resectable (gastro)esophageal cancer are treated with carboplatin and paclitaxel based neoadjuvant chemoradiotherapy according to the CROSS-regimen.[145, 146] However, almost half of all patients have already non-resectable (gastro)esophageal cancer at diagnosis due to locally advanced tumors or distant metastasis.[147] For patients with locally advanced disease, systemic treatment can be considered in an attempt to downstage the tumor (i.e. induction treatment), which can be followed by surgery or chemoradiotherapy in case of a good response. For patients with distant metastases, palliative chemotherapy can be considered. Many different induction or palliative treatment regimens are described, which are often fluoropyrimidine- or platinum-based doublet or triplet combination regimens.[148]

At the Erasmus University Medical Center, Rotterdam, a weekly regimen consisting of carboplatin targeted at an area under the curve (AUC) of 4 mg x min/mL and paclitaxel 100 mg/m^2 was developed and implemented as standard of care for all patients with advanced or metastatic esophageal cancer.[149] **Chapter 11** describes the toxicity and efficacy of this weekly carboplatin and paclitaxel regimen as induction or palliative treatment in 291 advanced (gastro)esophageal cancer patients. In general, this treatment regimen was well-tolerated with a low incidence of severe toxicity (i.e. grade ≥3), except for hematological toxicity such as neutropenia. Almost two third of patients experienced severe neutropenia, which is higher than described for other frequently used regimens.[150] However, severe leukopenia occurred in one third of patients and was therefore comparable with other frequently used regimens.[150] According to our treatment protocol, this regimen could be safely administered on an outpatient basis at any grade of neutropenia as long as leukocyte number was sufficient (i.e. day 0 and 28: > 3.0 x 10^9/L; day 7, 14, 35 and 42: > 1.0 x 10^9/L), which was also reflected by the low incidence of febrile neutropenia in only three percent of patients. Furthermore, the incidence of severe nausea or diarrhea of only one to two percent of patients was much lower than

in most other fluoropyrimidine-based doublet regimens with an incidence up to 8% for severe diarrhea and up to 20% for severe nausea, and for triplet-based regimens are these incidences even higher.[150, 151] The good clinical tolerance of this treatment regimen was also demonstrated by the low incidence of toxicity-related dose-reductions in one percent and drug-related hospitalization in twelve percent of patients.

With this regimen an overall response rate of 48% in patients treated with induction intent and 44% in patients treated with palliative intent was achieved. The response rate of 48% in patients with induction intent, is comparable to other regimens with response-rates varying between 20-48% .[151-163] Furthermore, for 42% of patients treated with induction intent, an esophagectomy or definitive chemoradiotherapy followed, which resulted in a median PFS of 22.1 months and median OS of 26.8 months. For the other patients, who did not have an esophagectomy or definitive chemoradiation, the median PFS of 9.0 months and OS of 11.8 months was lower, but comparable to the patients treated with palliative intent. In patients with metastatic (gastro-)esophageal cancer we found a response rate of 44%, which is also comparable with the most frequently used other doublet regimens (20-45%) and triplet regimens (31-48%) .[151-153, 155-158, 160-162] When comparing survival rates, we found a median PFS of 8.2 months and OS of 10.9 months for palliative treated patients, which is longer than other doublet regimens in this patient group (PFS: 3.7-5.9 months; OS: 8.6-10.7) and is comparable to triplet regimens (PFS: 5.6-7.0; OS: 9.2-11.2) .[151-153, 155-158, 160-162] Although we have to interpret these comparisons with caution as we did not make a direct head to head comparison, the weekly combination of carboplatin and paclitaxel seems to be at least equally effective, and possibly even more effective compared to other frequently used treatment regimens and was better tolerated than other regimens.

Furthermore, we tried to identify predictive factors for treatment outcome and prognostic factors for PFS and OS. We could only identify smoking as predictive factor for patients with induction treatment, while unfortunately no predictive factors for treatment outcome in palliative treated patients were found. Nonetheless, we identified several prognostic factors for PFS and OS. For induction chemotherapy, current smoking behavior, elevated thrombocyte number and alkaline phosphatase levels, WHO status and T-stage, were identified as adverse prognostic factors. For palliative chemotherapy, tumor location, BSA and WHO status were identified as prognostic factors. In our population studied, we could not confirm other known prognostic factors for survival such as sex or location of metastases.[164-166]

Paclitaxel is also characterized by a large inter-individual variability in exposure and a dose-response relation has been suggested.[167, 168] Therefore, we hypothesized that an

increased systemic paclitaxel exposure (lower clearance) is associated with a better response to treatment for patients with esophageal cancer. Unfortunately, in **Chapter 12** we demonstrate the absence of any association between paclitaxel clearance and treatment outcome in 166 patients with esophageal cancer treated with common paclitaxel-based treatment regimens (e.g. neo-adjuvant chemoradiotherapy, induction and palliative chemotherapy). Several reasons can be hypothesized for the absence of this correlation. Firstly, in neoadjuvant chemoradiotherapy, the chemotherapy is administered in a lower dose and mainly acts as a radiosensitizer.[169, 170] Also, the combination with carboplatin chemotherapy (of which no drug concentrations were measured) could have influenced the outcomes of the analyses. Furthermore, the type of tumor (adenocarcinoma versus squamous cell carcinoma) affects the response to chemoradiotherapy indicated by the fact that a histological response occurs more often in patients with squamous cell carcinomas. However, the CROSS regimen does not distinguish between the two tumor types in clinical practice.[145, 146] In the present study, the majority of patients were diagnosed with an adenocarcinoma of the esophagus, in line with the incidence in Western world.[171] Another important reason for the lack of correlation between paclitaxel plasma pharmacokinetics and tumor response is a potential weak correlation between paclitaxel plasma exposure and paclitaxel tumor exposure. As one of its potential resistance mechanisms a tumor may use efflux transporters (i.e. ABC-transporters) to limit intra-tumoral chemotherapy concentrations. Taxanes, including paclitaxel, are known substrates for these transporters.[172, 173] Although we did not measure intra-tumoral drug concentrations in this study, due to its retrospective nature, we speculate that intra-tumoral paclitaxel concentrations differ substantially from plasma chemotherapy concentrations. To further explore this, we have set up a new prospective clinical trial (i.e. the PAREO study; registered as NTR6356) in which plasma paclitaxel exposure is compared with intra-tumoral concentrations by serial tumor biopsies and simultaneous blood sampling in patients treated for esophageal cancer.

Although the prognosis of esophageal cancer has improved over the last decades, unfortunately the outcome still remains poor with an overall 5-year survival of 20%.[174, 175] Improvement in prognosis might be caused by recent advances in the treatment of patients with resectable (gastro)esophageal cancer by introduction of neoadjuvant chemoradiotherapy such as the CROSS-regimen.[145, 146] However, as stated before, almost half of all patients already have non-resectable (gastro)esophageal cancer at diagnosis.[147] Especially for these patients, improvement of current treatments is highly warranted. In general, chemotherapy has limited efficacy in advanced esophageal cancer with only minor differences between different schedules, but it will remain the backbone of treatment in metastatic (gastro)esophageal cancer until new treatments

are developed. Future research should therefore focus on predictive factors and biomarkers to identify patients beforehand who will benefit from a certain treatment. Furthermore, the tumor biology should be included in patient selection. Several molecular subtypes of (gastro)esophageal cancer have been identified and provides a rationale to develop tailored treatment for the different subtypes instead of treating all (gastro)esophageal cancers in the same manner.[176] Currently investigated targeted therapies focusses on targeting the human epidermal growth factor receptor type 2 (HER2) and the vascular endothelial growth factor receptor (VEGF) with limited effect, nevertheless several combination therapies are being evaluated.[177]

Since the publication of the impressive results of immunotherapy in other tumor types such as melanoma, the immune micro-environment of esophageal tumors is evaluated as possible treatment target as well. Recent phase-II studies demonstrate promising results of nivolumab and/or ipilimumab in esophageal cancer.[178, 179] Furthermore, the phase-III ATTRACTION trial demonstrated that nivolumab significantly increased OS compared to placebo in heavily pretreated Asian patients with metastatic (gastro) esophageal cancer independent of the PD-L1 status.[180] The phase-III KEYNOTE-181 study demonstrated that pembrolizumab as second-line therapy significantly improved OS compared to chemotherapy in patients with metastatic esophageal cancer with a high PD-L1 combined score.[181] Currently, the results of multiple clinical trials evaluating immunotherapy and the combination of immunotherapy and chemotherapy are awaited.[177] Therefore, the true value of immunotherapy in (metastatic) esophageal cancer is not clear yet.

CONCLUSION

This thesis describes several pharmacokinetic, -dynamic and pharmacogenetic studies on four frequently used anti-cancer drugs in cancers of the gastro-intestinal tract. We used many different research methods, of which intra-tumoral pharmacokinetics and circulating DNA are relatively new in the field and the true potential of these methods needs to be further explored. Pharmacogenetic-based dosing is currently not yet standard of clinical care and hopefully this will change soon for fluoropyrimidine treatment and *DPYD* genotyping. The preliminary data suggest that this may also be the case for irintotecan treatment and *UGT1A1* genotyping in the future. When the data from pharmacokinetic and pharmacogenetic research are combined in medical oncology, this will give us the tools that will eventually lead to a true personalized dosing regimen for every patient.

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PART VI

A

APPENDICES

APPENDIX 1.
NEDERLANDSE SAMENVATTING

Dit proefschrift beschrijft verschillende onderzoeken gericht op met name de 'farmacokinetiek' en 'farmacogenetica' van vier verschillende anti-kanker medicijnen die veelvuldig worden gebruikt in de behandeling van gastro-intestinale kankersoorten. Farmacokinetiek beschrijft alles wat het lichaam doet met het geneesmiddel, terwijl farmacogenetica zich meer richt op de erfelijke factoren die hiervoor van belang zijn. Het overkoepelende doel van al deze onderzoeken is om factoren te identificeren die kunnen helpen om de anti-kanker behandeling voor iedere patiënt op maat te maken met als voornaamste doelen de bijwerkingen (toxiciteit) te minimaliseren en (de kans op en de duur van) het aanslaan van de behandeling (effectiviteit) te vergroten.

DEEL I: FLUOROPYRIMIDINES

Fluoropyrimidines waaronder de geneesmiddelen 5-fluouracil en capecitabine vallen, behoren tot de meest gebruikte anti-kanker middelen. Afhankelijk van het type behandeling wordt capecitabine gegeven als monotherapie, dan wel in combinatie met andere anti-kanker middelen, of in combinatie met radiotherapie. Net als vele andere anti-kanker middelen wordt capecitabine in het algemeen gedoseerd op basis van het lichaamsoppervlakte (berekend uit de lengte en het gewicht) van een patiënt. Een alternatief hiervoor is een 'vaste' of 'gefixeerde' dosering per indicatie, waarbij de dosering dus niet is aangepast aan deze lichaamsmaat. In **Hoofdstuk 2** wordt de toxiciteit en effectiviteit van het vast doseren van capecitabine in vier verschillende behandelregimes in een groep van 1126 patiënten vergeleken met 1193 vergelijkbare patiënten die op lichaamsoppervlakte zijn gedoseerd. We hebben geen verschil in toxiciteit gevonden tussen deze twee verschillende manieren om capecitabine te doseren. Daarnaast werd geen verschil in effectiviteit van de behandeling gevonden. Onze resultaten laten dus zien dat deze vaste dosering van capecitabine een goed en praktisch alternatief voor het doseren op lichaamsoppervlakte is.

Fluoropyrimidines worden voor meer dan 80% omgezet in niet werkzame (inactieve) stoffen door het enzym dihydopyrimidine dehydrogenase (DPD). Ongeveer 10-30% van alle patiënten die behandeld worden met fluoropyrimidines ervaart helaas ernstige of zelfs fatale toxiciteit. Deze ernstige toxiciteit wordt in bijna 60% van deze patiënten veroorzaakt door een verlaagde DPD activiteit, die vaak wordt veroorzaakt door afwijkingen in het gen *DPYD* dat codeert voor het enzym DPD. Er zijn momenteel vier klinisch relevante genetische varianten bekend: *DPYD**2A, c.1679T>G, c.2846A>T, en c.1236G>A, die zorgen voor een geschatte 25-50% afname van de DPD activiteit. In **Hoofdstuk 3** wordt een groot onderzoek in 17 Nederlandse ziekenhuizen

beschreven, waarbij er gescreend werd op deze vier genetische varianten en daarop de fluoropyrimidine dosering werd aangepast (met een 25% of 50% dosisreductie afhankelijk van de erfelijke variant). In totaal werden er 1103 patiënten geïncludeerd, waarvan 85 patiënten met een *DPYD* variant (7.7%). De incidentie van ernstige toxiciteit was ondanks de dosisreducties nog steeds hoger in mensen met *DPYD* varianten (39%) vergeleken met patiënten zonder een mutatie (23%). Maar wanneer we onze resultaten vergelijken met een historisch cohort met *DPYD* variant dragers die met een volledige dosering werden behandeld, nam het (relatieve) risico op ernstige bijwerkingen duidelijk af voor de varianten *DPYD**2A en c.1679T>G, verminderde het risico gedeeltelijk voor c.2846A>T, maar werd het risico voor mensen met de c.1236G>A variant niet verminderd. Patiënten met deze laatste twee varianten, kregen een dosisreductie van (slechts) 25% en mogelijk is dit niet voldoende om de toxiciteit te verlagen. Daarnaast hebben we in **Hoofdstuk 4** laten zien dat deze doseermethode kosteneffectief is.

Behalve *DPYD* genotyperen, kan er ook (in)direct naar de werking van het DPD-enzym gekeken worden door middel van zogenaamde 'fenotyperings'-testen. Onder directe fenotyperingstesten verstaan we het direct meten van DPD activiteit in bijvoorbeeld bloedcellen. Indirecte fenotyperingstesten richten zich op de meting van het endogene substraat van DPD (namelijk uracil) of op de stoffen die ontstaan na verwerking door DPD (zoals dihydrouracil). In **Hoofdstuk 5** beschrijven we vier verschillende DPD fenotyperingstesten die verricht zijn bij een gedeelte van de patiënten van het eerdere onderzoek. Helaas kon bij geen van deze vier testen DPD-deficiëntie of fluoropyrimidine gerelateerde bijwerkingen goed voorspellen. Er is daarom verder onderzoek naar deze fenotyperingstesten nodig voor we ze in de klinische praktijk kunnen gaan gebruiken.
In **Hoofdstuk 6** worden patiënten met meerdere *DPYD* varianten per patiënt beschreven die we hebben behandeld met een gepersonaliseerde fluoropyrimidine dosis. Dit hoofdstuk laat zien dat ondanks de genetische variatie in deze patienten een individuele behandeling goed mogelijk gebleken.

DEEL II: IRINOTECAN

Al twee decennia is irinotecan een anti-kanker middel wat veelvuldig gebruikt wordt in de behandeling van onder andere darm- en alvleesklierkanker. De behandeling met irinotecan wordt gekenmerkt door verschillende dosis-limiterende bijwerkingen zoals een daling in de witte bloedcellen en ernstige diarree. Irinotecan heeft een complex metabolisme waarbij irinotecan eerst moeten worden omgezet in de werkzame stof SN-38. Hierdoor kunnen er vele indiviuele, omgevings- en genetische factoren van invloed

zijn op de irinotecan en SN-38 blootstelling. In **Hoofdstuk 7** geven we een overzicht van de kennis op het gebied van irinotecan farmacokinetiek, 'farmacodynamiek' (wat het geneesmiddel met het lichaam doet) en farmacogenetica. De laatste jaren is er meer aandacht voor de farmacogenetica van irinotecan, waarbij varianten van het *UGT1A1* gen gerelateerd zijn aan een verhoogde kans op bijwerkingen. Dosisaanpassing op basis van het *UGT1A1* zou daarmee ook de toxiciteit van de behandeling kunnen verlagen.

Er zijn vele andere interventies onderzocht die het risico op toxiciteit zouden kunnen verlagen, waaronder dieet aanpassingen. Onderzoeken in proefdieren hebben aangetoond dat door te vasten voor de behandeling met irinotecan, de toxiciteit werd verlaagd terwijl het anti-kanker effect bleef behouden. Gezien deze positieve bevindingen werd in **Hoofdstuk 8** een onderzoek beschreven waarbij we een vijf daags eiwit- en caloriebeperkt dieet onderzoeken in 19 patiënten met darmkanker voor behandeling met irinotecan. In dit onderzoek werd op de dag van irinotecan toediening verschillende bloedafnames gedaan en de dag erna een biopt van zowel gezond leverweefsel als een biopt uit een leveruitzaaiing verricht. Er was een hoge variatie in de irinotecan en SN-38 concentraties in de biopten van deze patiënten en daarbij werd geen duidelijk verschil gevonden met of zonder dieet. Echter, het dieet zorgde voor een toename van 7% in de irinotecan blootstelling en maar liefst 50% toename in de SN-38 blootstelling in het bloed. Het dieet werd goed verdragen en we zagen geen verschil in bijwerkingen. Bij deze sterke toename van de SN-38 blootstelling had een toename in de toxiciteit verwacht kunnen worden, maar dit werd dus niet gezien, wat veelbelovend is. Verder onderzoek moet verricht worden om te bepalen of de effectiviteit en toxiciteit van irinotecan inderdaad verbeterd kan worden met dieetrestrictie.

DEEL III: REGORAFENIB

Regorafenib is een oraal anti-kanker middel, dat wereldwijd gebruikt wordt in de behandeling van darmkanker (behalve in Nederland), gastro-intestinale stromale tumoren en leverkanker. Na inname wordt regorafenib snel geabsorbeerd in het maagdarmstelsel en kan daarna worden opgenomen in het bloed. Voor vergelijkbare middelen is aangetoond dat voor deze absorptie de zuurgraad van de maag sterk van belang is. Bij het gebruik van maagzuurremmers zou daarmee de opname van het medicijn verlaagd kunnen worden en daarmee leiden tot een verminderde blootstelling. In **Hoofdstuk 9** beschrijven we een onderzoek dat laat zien dat als de maagzuurremmer

esomeprazol gelijktijdig met of drie uur eerder dan de regorafenib wordt ingenomen, de blootstelling van regorafenib niet wordt beinvloed. Deze maagzuurremmer kan dus veilig worden gecombineerd met regorafenib.

In meer dan de helft van alle patienten geeft regorafenib ernstige bijwerkingen en de effectiviteit van de behandeling weegt soms niet op tegen deze toxiciteit. Daarom is het belangrijk om te voorspellen wie voordeel heeft van deze behandeling en wie niet. Circulerend DNA (erfelijk materiaal), zowel van de tumor (ctDNA) als de combinatie van tumor en gezonde weefsels (cfDNA), is een relatief nieuwe methode om te kijken naar het effect van de behandeling. In **Hoofdstuk 10** beschrijven we de ctDNA en cfDNA dynamiek in 20 patiënten behandeld met regorafenib. Deze analyse laat zien dat ctDNA en cfDNA concentraties beide toenemen na 1 tot 2 weken behandeling met regorafenib en dat dit waarschijnlijk deels wordt veroorzaakt door schade van de regorafenib op de gezonde weefsels in het lichaam.

DEEL IV: CARBOPLATIN / PACLITAXEL

De combinatie van de twee (per infuus toegediende) anti-kanker middelen carboplatin en paclitaxel wordt veel gebruikt in de behandeling van slokdarmkanker. Bij patiënten met resectabele slokdarmkanker wordt dit in combinatie met radiotherapie (bestraling) gegeven voorafgaand aan de operatie (we noemen dit ook wel een neo-adjuvante behandeling). Echter, meer dan de helft van alle patiënten heeft helaas niet-resectabele ziekte bij diagnose en zij kunnen dan soms een behandeling krijgen om de tumor kleiner te maken om alsnog een eventuele operatie mogelijk te maken (we noemen dit inductie therapie). Wanneer er al uitzaaiingen zijn, dan is de behandeling gericht op verlenging van het leven (dit is een palliatieve behandeling). In **Hoofdstuk 11** beschrijven we de toxiciteit en effectiviteit van een wekelijks behandelschema met carboplatin en paclitaxel als inductie of palliatieve behandeling. De effectiviteit van deze behandeling bleek vergelijkbaar te zijn met andere behandelingen met deze indicatie, terwijl de toxiciteit milder lijkt te zijn. Dit is dus een goede behandeloptie voor patiënten met slokdarmcarcinoom.

Desalniettemin is er voor paclitaxel een grote variatie in farmacokinetiek tussen patiënten beschreven. Daarom hebben we in **Hoofdstuk 12** gekeken of er een relatie was tussen paclitaxel blootstelling en uitkomst van de behandeling in 166 patiënten met slokdarmkanker (zowel neoadjuvant, inductie als palliatief). We hebben helaas geen relatie tussen paclitaxel blootstelling en effect van de behandeling kunnen aan

tonen. Verder onderzoek (zoals farmacokinetiek van paclitaxel in de tumor) is nodig om factoren te identificeren die het effect van de behandeling kunnen voorspellen.

APPENDIX 2.
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APPENDIX 3. CURRICULUM VITAE

Femke Marloes de Man was born on February 2th 1990 in Rotterdam, The Netherlands. During her last two years of high school education at the Erasmiaans Gymnasium at Rotterdam, she was selected for and participated in the Junior Med School program of the Erasmus University Medical Center, Rotterdam. In 2007 she successfully finished this program with a preclinical research project on the hormonal treatment of breast cancer under supervision of Prof. dr. P.M.J.J. Berns at the department of Medical Oncology in the Josephine Nefkens Institute at the Erasmus Medical Center. After graduation, she studied medicine at the Erasmus University Medical Center, Rotterdam. During her study she performed extra-curricular research on pre-transplant tacrolimus exposure as prediction for post-transplant dose requirements in kidney transplant patients under supervision of dr. N. Shuker and Prof. dr. T. van Gelder at the department of Pharmacy in collaboration with the department of Nephrology at the Erasmus University Medical Center. For her master thesis, she participated in a prospective study on drug-drug interactions in patients treated for cancer under supervision of dr. R.W.F. van Leeuwen. During her study she actively participated in the student rowing club A.R.S.R. Skadi, and participated in several committees and the board of the Vertical VUUR. She went for her internships of choice to the department of Internal Medicine at the Albert Schweitzer Hospital, Dordrecht and to department of Medical Oncology, at the Daniel den Hoed Hospital, Rotterdam. Next, in November 2015 she obtained her MD degree and started in December 2015 with her PhD program at the group of Translational Pharmacology at the department of Medical Oncology under supervision of Prof. dr. A.H.J. Mathijssen, Prof. dr. T. van Gelder and dr. M.P.J.K. Lolkema. During her PhD she supervised five medical students in extracurricular research and their master thesis. She was also actively involved in education, and obtained the first part of the Basic Education Qualification in 2018. Also, she organized several monthly research meetings and participated in the board of the AAV representing all medical residents within the Erasmus University Medical Center. Furthermore, she was selected for the Female Talent Class of the Erasmus Medical Center. After nearly 3.5 years of PhD-period, in May 2019, she started working as a medical resident at the department of Internal Medicine at Reinier de Graaf Gasthuis, Delft, The Netherlands, under supervision of dr. H. Boom (internist-nephrologist).

APPENDIX 4.
LIST OF PUBLICATIONS

de Man FM, van Eerden RAG, Oomen-de Hoop E, Veraart JN, van Doorn N, van Doorn L, van der Gaast A, Mathijssen RHJ
Efficacy and toxicity of weekly paclitaxel and carboplatin as induction or palliative treatment in advanced esophageal cancer patients
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Nederlands Tijdschrift voor Oncologie 2014 Juni;11:133-40

APPENDIX 5.
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Research School:	Molecular Medicine
Promotors:	Prof. dr. A.H.J. Mathijssen
	Prof. T. van Gelder
Co-promoter:	Dr. M.P.J.K. Lolkema

1. PhD traininig	Year	Workload (ECTS)
General courses		
BROK (GCP) course	2015, 2019	1.0
Research Integrity	2017	0.3
Biomedical English Writing and Communication	2018	3.0
Specific courses		
OpenClinica database building	2015	0.4
MS Excel: Basic workshop	2016	0.3
MS Excel: Advanced workshop	2016	0.3
CPO minicourse in methodology	2016	0.3
The Molmed annual course on molecular medicine	2016	0.3
Basic introduction course on SPSS	2016	1.0
Workshop Omgaan met groepen	2016	0.5
Genetics for Dummies	2016	0.5
Biostatistical Methods I: Basic Principles (CCO2)	2016	5.7
Survival Analysis Course	2017	0.6
Workshop: Hoorcollege geven	2017	0.3
Workshop: Onderhandelen	2018	0.3
Teach the Teacher I	2018	0.7
Oral presentations		
NVKF&B Scientific Meeting	2015	0.2
Annual Clinical Pharmacology Meeting	2016	0.2
Translational Pharmacology Meeting	2015-2018	0.8
Medical Oncology Research Meeting	2016-2018	0.6
Scientific meeting, Medical Oncology	2017	0.2
Poster presentations		
ESMO congress	2017	0.2
ESMO congress	2018	0.2
ICPAD	2018	0.2
NVKF&B Scientific Meeting (2x)	2019	0.4

(Inter)national conferences		
NVKFB Scientific meeting	2015-2019	2.5
Young oncologist evening, Erasmus MC	2016, 2017, 2019	1.5
Regional GI-symposium	2016, 2018	1.0
Daniel den Hoed day	2016	0.5
Therapeutic sequence mCRPC and beyond	2016	0.5
Annual clinical pharmacology meeting	2016	0.5
CPCT symposium	2016	0.5
ESMO congress	2017, 2018	4.0
ICPAD	2018	0.5
Other		
Scientific meeting Medical Oncology	2016-2018	0.6
Translational Pharmacology meetings	2015-2019	2.0
Clinical Pharmacology meetings	2015-2019	2.0
2. Teaching		
Lecturing		
Department of dietetics, Erasmus MC	2016	0.2
Young oncologist evening, Erasmus MC	2017, 2019	0.4
CPO course	2018	0.2
Internal Medicine Clinical Demonstration, Erasmus MC	2018	0.2
Tutoring		
Tutorial class first-year medical students	2016-2018	4.5
Supervising students in extracurricular research		
Marijn Veerman	2015-2016	2.0
Ruben van Eerden	2016	1.0
Supervising Master's thesis		
Mirjam de With	2016	1.0
Marijn Veerman	2017	1.0
Ruben van Eerden	2017	1.0
Ivo Bijl	2018	1.0
Joris Veraart	2018	1.0
3. Other		
Peer review of manuscripts	2016-2017	0.4
Organisation meetings (Research Meeting Translational Pharmacology 2017, MORM 2017-2018)	2017-2018	2.0
Board of medical residents (AAV), Erasmus MC	2017-2019	5.0

APPENDIX 6. DANKWOORD

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