DRUG INTERACTIONS WITH ANTI-CANCER AGENTS

A PHARMACOKINETIC AND PHARMACODYNAMIC APPROACH

KOEN HUSSAARTS

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een farmacokinetische en farmacodynamische benadering

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It always seems impossible until it is done

Nelson Mandela

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CHAPTER 1

GENERAL INTRODUCTION

In the last century, cancer has become one of the most important health issues worldwide.[1] With increasing incidence and total number of deaths, it is currently one of the leading causes of death before the age of seventy in the Western World.[2] Simultaneously, there has been a significant rise in the number of treatment options with an exponential growth in the last twenty to thirty years, starting with radiotherapy and surgery as treatment options in the early 1900's. Nowadays there are multiple treatment options, among which intravenously administered chemotherapy and orally administered targeted drugs (e.g. tyrosine kinase inhibitors).[1, 3] Despite the still growing arsenal of therapeutic options there remains an urgent need for optimalisation of the already registered drugs to guarantee the most optimal treatment for each individual patient.

In case of treatment with anti-cancer drugs, one of the most important parameters to ensure optimal treatment efficacy is the systemic exposure to that particular anti-cancer drug. Optimal systemic drug exposure, or bioavailability, is determined by several individual factors (e.g. organ function, body size-measures), disease (tumor burden, etc.), (pharmaco-) genetic factors and environmental factors (e.g. co-medication).[4] Most of these factors may influence systemic drug exposure by interacting with either drug 'pharmacokinetics' and/or 'pharmacodynamics'. Pharmacokinetics describe the process of how the body affects a drug. This process globally consists of four major components: absorption, distribution, metabolism and excretion, which together illustrates the journey of a drug throughout the body.[5-7] Pharmacodynamics describe the biochemical and physiologic effects (e.g. effect and toxicity) of drugs on both the body and the disease.[6] Alterations in pharmacodynamics or pharmacokinetics, due to the earlier mentioned patient and environmental factors, may have a significant impact on treatment efficacy in cancer patients, and patient may be deprived from optimal anticancer therapy [8, 9]

This thesis describes pharmacokinetic and pharmacodynamic drug-interaction studies for several (commonly used) anti-cancer agents. It is important to investigate these drug-interactions to either gain knowledge about possible interaction mechanisms in general and to find ways to avoid or deal with these drug-interactions, therefore assuring an optimal treatment for every individual patient.

PART I: DRUG-DRUG INTERACTIONS

Together with the increase in treatment modalities, there has also been an significant increase in the overall quality of life and life expectancy of cancer patients.[10, 11] Despite this prolonged survival time, many patients suffer from comorbidities and side-effects caused by both the disease and the treatment, often forcing them to use multiple co-medications. Therefore, cancer patients are at major risk for polypharmacy, i.e. the use of multiple drugs concomitantly with the anti-cancer drugs. Polypharmacy is associated with an increased risk of drug-drug interactions, which may lead to treatment failure and/or increased toxicity.[12, 13] Pharmacokinetic drug interactions influence the pharmacokinetics and therefore exposure to certain drugs at several levels (**figure 1**).[14] Absorption is the uptake of a drug from the gastro-intestinal tract into the blood stream. This is a complex process, which is mainly driven by several 'pumps' or drug-transporters. After the absorption phase, the drug is distributed to the liver where a complex enzyme driven process (mainly by enzymes of the cytochrome P450 enzyme system) breaks down the drug into several metabolites; these metabolites can be both active and inactive. This breakdown process is called drug metabolism. After the metabolism phase, a drug is distributed further through the body by the blood stream and eventually excreted through the bile or urine. Medication may have an influence on every of these four steps of drug pharmacokinetics. Nonetheless, most (important) drug-drug interactions appear in the absorption and metabolism phase.[9]

Chapter 2 gives a detailed overview of several clinically relevant drug-drug interactions on both the absorption and metabolism level involved with orally administered small molecule kinase inhibitors (SMKIs). SMKIs are a relatively new class of drugs used for the treatment of various malignancies. SMKIs includes the group of tyrosine kinase inhibitors (TKIs). SMKIs and TKIs target specific so-called tyrosine kinases, that activate several cellular pathways involved in (cancer)cell growth, differentiation, death and a series of biochemical and physiological processes among other things.[15] These drugs inhibit the phosphorylation (activation) of these tyrosine kinases, leading to blockage of these cellular pathways, thereby preventing tumor growth and stimulating tumor death. SMKIs are administered orally, and as a consequence, are therefore highly prone to drug-drug interactions, since they also have to undergo the absorption step of the pharmacokinetic process in contrast to intravenously administered drugs, which bypass this step.[16]

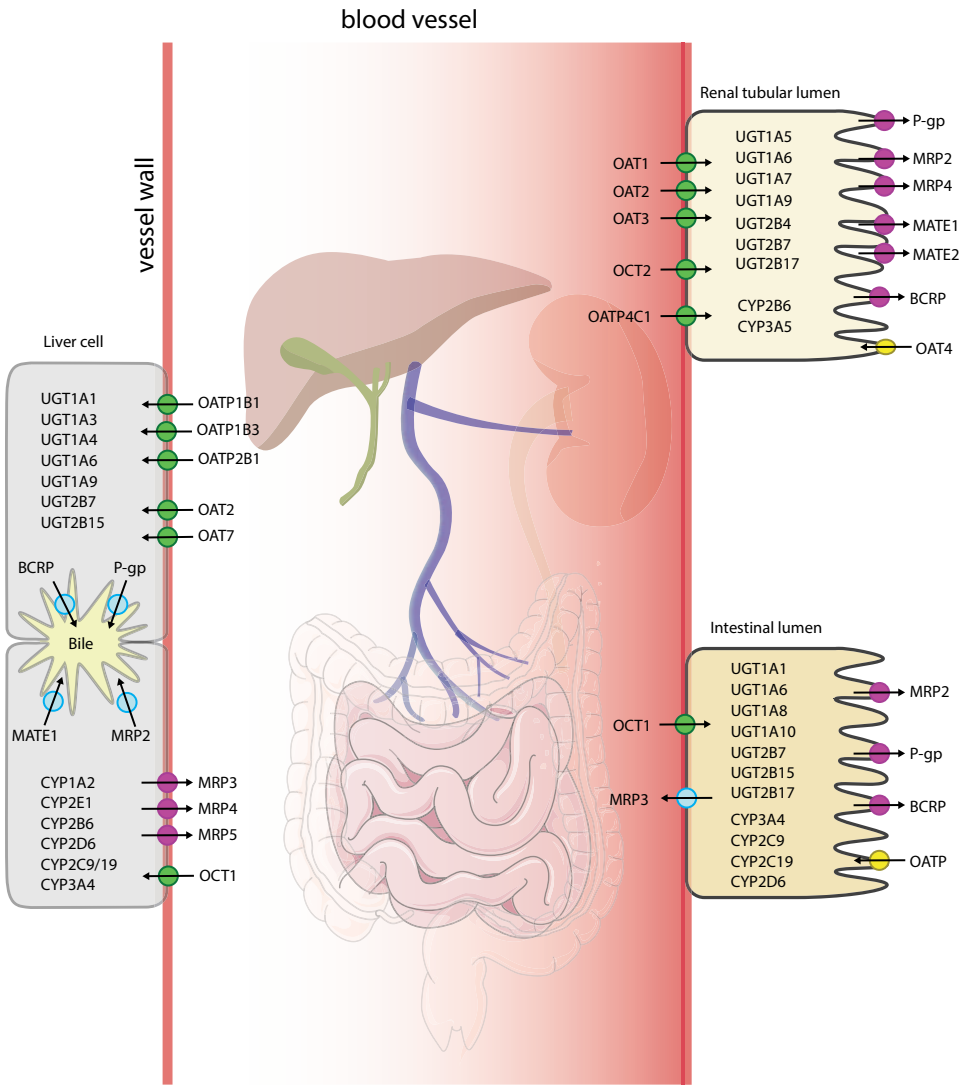

FIGURE 1: Mechanism of pharmacokinetic drug-interactions [14]

Another example of a drug-drug interaction --involving the absorption phase-- is the concomitant use of proton pump inhibitors (PPIs). PPIs are used extensively by cancer patients for the treatment of, for example gastroesophageal reflux disease, counting up to 33% of all cancer patients.[17] Co-administration of PPIs can cause a significant decrease in drug-exposure (area under the curve; AUC) of several SMKIs, even up to 61% for dasatinib.[18] Since many anti-cancer drugs among which the SMKIs dissolve

better in an acidic environment, the decrease in drug-exposure can be explained by the increase in stomach pH following PPI administration, which has a significant impact on drug absorption and thus drug exposure.[9, 16] Since drug exposure decreases significantly with PPIs this may have an impact on overall survival, as was proven for several drugs like pazopanib.[19] Furthermore, Olivier et al. and Chu et al. have shown that the use of PPIs in general next to the regular anticancer treatment leads to a significant decrease in survival for patients using sunitinib or capecitabine, respectively, which may be explained by the drug-drug interaction with PPIs leading to a decrease in anti-cancer drug exposure.[20, 21] In **chapter 3** a comment to the research from Chu et al. is described about the pitfalls in their work. Despite the interesting results of this study, the lack of data on type of PPI used, PPI dose, and period of time the combination was used, makes the interpretation of the data difficult.

An example of a more optimal research strategy to study drug-drug interactions with PPIs in cancer patients is presented in **chapter 4**. Here, the possible drug-drug interaction between regorafenib, a new multi-kinase inhibitor that targets angiogenic, stromal and oncogenic receptor tyrosine kinases (e.g. VEGFR, KIT, BRAF, PDGFR and FGFR), and the PPI esomeprazole is described. Regorafenib is used in the treatment of metastatic colorectal cancer, hepatocellular carcinoma and gastrointestinal stromal tumor. We concluded (chapter 2) that regorafenib is unlikely to result in a clinically relevant drug-interaction with PPIs. However this interaction has not been studied in a clinical setting. Therefore this study investigated whether there is a significant interaction between the PPI esomeprazole and regorafenib, or not.

Drug-drug interactions involving drug metabolism are the most important and most prevalent drug-drug interactions in clinical practice.[22] Most drugs are extensively metabolized in the liver in active (e.g. tamoxifen, sorafenib) and inactive metabolites (e.g. afatinib) by cytochromes of the P450 (CYP) system. Drug interactions involve either induction or inhibition of a certain enzyme resulting in decreased or increased drug concentrations respectively.[9] In **chapter 5** a study is described in which a metabolic drug-drug interaction (docetaxel with prednisone) is investigated. Docetaxel, a chemotherapeutic agent in the class of taxanes, is used in the regular treatment of several cancer types such as breast cancer, but also showed an important survival benefit in the first line treatment of metastatic hormone sensitive prostate cancer (mHSPC).[23] In this TAX327 study, docetaxel was combined with prednisone to equally compare it to mitoxantrone therapy, which is also combined with prednisone. Two large clinical trials (CHAARTED and STAMPEDE) assessed the survival benefit of docetaxel compared to standard of care resulting in a comparable survival benefit of 10.4 and 16.0 months in the CHAARTED and STAMPEDE respectively. [24, 25] However,

one of the main differences between these trials was the absence of prednisone in the CHAARTED trial whereas the STAMPEDE trial added prednisone to the treatment. There was no significant difference in toxicity between the CHAARTED and STAMPEDE trial, which raised the question whether prednisone could be removed from the treatment regimen to prevent (long-term) toxicity in these patients. Furthermore prednisone is a mild CYP3A4 inducer and may theoretically alter docetaxel pharmacokinetics, since docetaxel is primarily metabolized by CYP3A4.[26] To clarify this, this interaction is investigated and described in chapter 5 of this thesis.

Anticancer drugs, and especially some SMKIs, are known for their narrow therapeutic window, which is the balance between side-effects on one hand and underdosing and ineffective dosing on the other.[8] In this case a drug-interaction may have a significant impact on both patient wellbeing and therapy efficacy. When two agents with a small therapeutic window have to be combined for clinical reasons, and metabolic pathways overlap, the risk for a significant drug-interaction increases significantly since only a small alteration may have a major impact on toxicity or efficacy of such a drug. For instance patients who receive immunosuppressants after undergoing a liver transplantation for HCC may develop a recurrent tumor in the transplant liver in approximately 20% of the cases.[27, 28] The first line of treatment in this case is the SMKI sorafenib, which also has a narrow therapeutic window and is associated with many side-effects. Furthermore, most patients who underwent a liver transplantation receive immunosuppressants, which are usually strong inhibitors of several metabolizing enzymes such as CYP3A4 and may therefore alter sorafenib pharmacokinetics and theoretically also efficacy and toxicity.[29] *Vice versa*, sorafenib may also alter immunosuppressant pharmacokinetics as well by inhibiting CYP3A4, which is the main metabolic enzyme for many immunosuppressants. In **chapter 6** a case-series is presented in patients using this combination of agents.

Sorafenib is associated with many side-effects of which hand-foot skin reaction (HFSR) is one of the most common and debiliating.[30, 31] HFSR is a particularly painful complication seen most frequently during the early weeks of treatment with SMKIs, such as sorafenib, sunitinib, and pazopanib, in which hyperkeratotic plaques develop predominantly over sites of pressure or friction. Unfortunately, there is currently no effective treatment option for HFSR besides dose-reduction or discontinuation. However, the finding by Zimmerman et al. that a drug transporter (OAT6) in keratinocytes is responsible for the uptake of sorafenib in the skin might potentially offer a possibility to prevent this side-effect.[32] The preclinical study showed that by selectively inhibiting OAT6 with probenecid, a drug used in the treatment of gout, HFSR was prevented in

mice. However, probenecid may also alter sorafenib metabolism and/or excretion.[33] Therefore, a clinical study investigating the possible interaction between sorafenib and probenecid and the influence of this combination on HFSR was performed (**chapter 7**).

Another example of an important pharmacodynamic drug-drug interaction is the prolongation of the QTc-interval, which gives a significant risk of cardiac arrhythmias and sudden cardiac death.[34, 35] The QTc-interval is determined as the time on an electrocardiogram (ECG) from the start of the QRS complex, to the end of T wave, as it returns to baseline.[36] A group of agents that is known to prolong the QTc-interval includes the serotonin reuptake inhibitors (SRIs), used in the treatment of depression and anxiety disorders.[37] These drugs are often prescribed to patients with cancer, because of their high prevalence of depressive complaints.[38] Among these, breast cancer patients experience the highest prevalence of depression.[39] Many of these patients use antihormonal therapy, most often the selective estrogen receptor inhibitor tamoxifen, which may also prolong the QTc-interval by itself.[40] Therefore, **Chapter 8** describes the effect on the QTc-interval of the combination of these two classes of agents compared to tamoxifen monotherapy.

PART II FOOD-DRUG INTERACTIONS

Besides the use of multiple drugs, and the risk for drug-drug interactions, there is also an increasing trend in the use of complementary and alternative medication.[41] Nowadays, the use of food and herbs is becoming more and more popular among (cancer) patients as an alternative strategy for the treatment of cancer and the treatment of cancer and treatment related symptoms (e.g. pain and nausea).[41] About 48-88% of all cancer patients use alternative medication including food supplements.[41] Besides the potentially favorable effects, food and supplements may also have an impact on the pharmacokinetics of several drugs and may therefore deprive patients from an optimal therapy.[42] For example, the intake with a fat meal increases the exposure of abiraterone; a drug used in the treatment of castrate resistant prostate cancer by 10-fold, compared to intake in a fasted state.[43] Since food or supplements may also have a significant impact on the pharmacokinetics of drugs like SMKIs, an extensive review is presented in **chapter 9** about important currently known food-drug interactions with SMKIs.

As mentioned in part I of this introduction, significant drug-interactions between PPIs and SMKIs exist, resulting in a significant and clinically relevant reduction in drug exposure, and therefore potential reduction in therapy efficacy. Because of its low acid dissociation constant (pKa-value; resulting in a rapidly decreasing solubility at

higher pH), erlotinib, a SMKI used in the treatment of non-small cell lung cancer, is also highly prone for a drug-drug interaction with PPIs. Concomitant use of erlotinib with esomeprazole results in a decrease of AUC of almost 50%.[44, 45] Cancer patients often have a hard indication for the use of a PPI and therefore cannot stop or decrease their PPI therapy. Therefore, a practical way to bypass this interaction with the popular beverage cola is presented in **chapter 10.** Coca-Cola is a very acidic beverage (pH=2.5). By taking erlotinib simultaneously with cola --instead of water-- the negative effects on erlotinib absorption may theoretically be bypassed, caused by a temporarily decrease in stomach pH and therefore an increase in erlotinib solubility.

Although intake of erlotinib with cola possibly offers a clear advantage over intake with water, when erlotinib is used concomitantly with a PPI, cola also knows many disadvantages. Erlotinib is often administered in the morning at an empty stomach, which makes ingestion with cola difficult for many patients. Many SMKIs, like erlotinib, have the ability to dissolve better in a fatty environment compared to water. Therefore, intake with (fat) food alters the exposure of several SMKIs as described in chapter 9 of this thesis. Consequently, intake of a SMKI with a fatty drink, such as full cow's milk, may potentially be another and healthier alternative, compared to cola to increase the systemic exposure to erlotinib (**chapter 11**). This chapter studies the effects of coadministration of a PPI on erlotinib plasma exposure. Furthermore the influence of full cow's milk, as a fatty beverage, on erlotinib exposure in patients using erlotinib with or without a PPI is described.

Since the use of food and herbs (and supplements) is becoming increasingly popular among cancer patients, the risk of a relevant food-drug interaction is also increasing. One of the most popular herbs among cancer patients, especially breast cancer patients, is curcumin, which is derived from the root of the *curcuma longa* and is used in traditional Asian cuisine and medicine. Curcumin is believed to induce several health benefits and to also possibly have an additional anti-tumor effect.[46, 47] However, curcumin itself may also have an effect on drug pharmacokinetics in rats as was demonstrated by Cho et al.[48] They showed a 33%-64% increase in the area under the curve (AUC) or exposure of tamoxifen. However, since these data were preclinical, the translation to a clinical setting --especially in cancer patients-- remains difficult. Tamoxifen is a prodrug and has to be metabolized first, mainly by the cytochrome P450 enzymes CYP2D6 and CYP3A4, into active metabolites of which endoxifen is the most potent. Furthermore, tamoxifen shows a large interindividual variability in drug exposure.[49] These characteristics make tamoxifen prone for drug interactions, which is of major relevance, as there is a suggested threshold for endoxifen efficacy, and a drug interaction might potentially lead to subtherapeutic endoxifen concentrations.[50]

Since many breast cancer patients use both tamoxifen and curcumin in daily practice, a clinical study in breast cancer patients is described in **chapter 12**. Here, this possible pharmacokinetic interaction with regards to curcumin and tamoxifen with or without the bio enhancer piperine, is studied.

Next to curcumin, one of the other supplements that is becoming more and more popular among (breast) cancer patients is green tea, which contains high amounts of epigallocatechin gallate (EGCG). EGCG is a flavonoid compound (i.e. an organic nitrogen-free organic structure often found in plants) with a proposed anti-cancer effect.[51] However, flavonoids may also cause a drug-interaction as was shown by Misaka et al. for nadolol.[53, 53] They found a 85% decrease in nadolol plasma levels, when nadolol was administered with green tea. EGCG may potentially also influence tamoxifen metabolism. **Chapter 13** describes the interaction between green tea extract capsules and tamoxifen in breast cancer patients.

In conclusion, this thesis gives an overview of different pharmacokinetic and pharmacodynamic aspects in the field of drug-drug and drug-food interactions. However, this is just a tip of the iceberg and more research is needed to fully understand the mechanisms, the complexity and impact of drug-interactions in daily clinical oncology practice. Recognition of drug-drug and drug-food interactions may improve therapy efficacy, reduce side-effects and therefore increase the quality of life of (cancer) patients, and should have a more prominent place in both clinical research and practice.

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PART I

DRUG-DRUG INTERACTIONS

CHAPTER 2

CLINICALLY RELEVANT DRUG INTERACTIONS WITH MULTIKINASE INHIBITORS: A REVIEW

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ABSTRACT

Multi-kinase inhibitors (MKIs), including the tyrosine kinase inhibitors (TKIs), have rapidly become an established factor in daily (hemato-) oncology practice. Although the oral route of administration offers improved flexibility and convenience for the patient, challenges arise in the use of MKIs. As MKIs are prescribed extensively, patients are at increased risk for (severe) drug-drug interactions (DDIs). As a result of these DDIs, plasma pharmacokinetics of MKIs may vary significantly, thereby leading to high interpatient variability and subsequent risk for increased toxicity or diminished therapeutic outcome.

Most clinically relevant DDIs with MKIs concern altered absorption and metabolism. The absorption of MKIs may be decreased by concomitant use of gastric acid suppressive agents (e.g. proton pump inhibitors) as many kinase inhibitors show pH-dependent solubility. In addition, DDIs concerning drug (uptake and efflux) transporters may be of significant clinical relevance during MKI therapy. Furthermore, since many MKIs are substrates for cytochrome P450 isoenzymes (CYPs), induction or inhibition with strong CYP inhibitors or inducers may lead to significant alterations in MKI exposure.

In conclusion, DDIs are of major concern during MKI therapy and need to be monitored closely in clinical practice. Based on the current knowledge and available literature, practical recommendations for management of these DDIs in clinical practice are presented in this review.

INTRODUCTION

Although cancer is still the leading cause of death among men and women worldwide, novel treatment options are rapidly evolving. In order to improve treatment efficacy and minimize toxicity more specific targets have been identified. One of the most promising classes of targeted anticancer agents are the multi-kinase inhibitors (MKIs), including the tyrosine kinase inhibitors (TKIs). MKIs target specific tyrosine kinases within the tumor cell, where they play a key role in the signal transduction, gene transcription, and DNA synthesis.[1] MKIs like osimertinib (for lung cancer) and cabozantinib (for kidney cancer) rapidly gained a place in standard of care treatment for multiple or new indications [e.g. regorafenib in primary liver cancer, after earlier approvals for gastrointestinal stromal tumor (GIST) and colorectal cancer.

MKIs include both small molecule MKIs and large molecule MKIs. In this review we will solely focus on the small molecule MKIs. Small molecule MKIs are administered orally, which gives them a clear advantage over conventional chemotherapy in terms of flexibility and patient convenience. Many MKIs show a narrow therapeutic window, whereas intra- and interpatient exposure is highly variable and multifactorial.[2-4] Also factors like food, beverages, lifestyle, and pharmacogenetic polymorphisms may alter MKI bioavailability significantly.[5] For example, as MKIs are predominately metabolized through phase I (e.g. CYP enzymes) or phase II enzymes (e.g. UDP-glucuronosyltransferase) or almost exclusively by phase II enzymes (e.g. in the case of afatinib), this makes them highly prone for drug-drug interactions (DDIs) involving drug metabolism.[6] Moreover, since cancer patients often use multiple drugs concomitantly with their anticancer therapy, they are even more at risk for DDIs, compared to other patient groups.[7]

DDIs can be classified as pharmacodynamic or pharmacokinetic.[8] Pharmacokinetic DDIs are defined as drug interactions regarding drug absorption, metabolism, distribution and elimination leading to altered plasma concentrations of a drug and possible unfavorable outcomes (e.g. increased toxicity and reduced treatment efficacy). A pharmacodynamic interaction is the altered response in terms of toxicity and efficacy when two or more drugs affect similar molecular targets (e.g. membrane receptors). Pharmacodynamic DDIs can be additive, antagonistic or synergistic. For instance, epidermal growth factor receptor (EGFR) kinase inhibitors often show synergistic antitumor effects when combined with chemotherapy.[9]

Both the United States Food and Drug Administration (US FDA) and the European Medicines Agency (EMA) present guidelines for the interpretation of DDIs. However,

because of discrepancies between recommendations, currently no clear general consensus for the management of DDIs is available. Therefore, the management of DDIs is challenging for clinicians and the need for a general consensus is urgent.

This review-article presents an overview of known pharmacokinetic DDIs regarding orally taken MKIs currently approved by the US FDA and EMA. Moreover, if possible, practical recommendations are given for the management of DDIs during MKI therapy in clinical practice.

METHODS

We conducted a search in PubMed and the Embase databases for English language studies published until 2 July 2018 for randomized clinical trials, observational studies, and reviews about US FDA and EMA-approved MKIs. We used the following search MESH terms: '(Drug interactions) OR (Drug combination) AND (Drug name)'. In Embase, we used 'clinical studies', 'humans' and 'only in English' as additional search limits. The search results were manually screened for relevance. In addition, all MKI (US FDA and EMA) assessment reports were screened on the latest updates regarding DDIs in the scientific updates available at the EMA and US FDA website until 2 July 2018. We included clinical drug–drug interaction studies in human and preclinical pharmacokinetic studies investigating possible interactions. We excluded studies which did not focus on pharmacokinetics or drug interactions. Clinical relevance of the interaction was scored on the basis of the US FDA-classification of the effect of drug interactions and the level of available evidence as a 'major', 'moderate' or 'minor' interaction. If there was no clinical pharmacokinetic study performed, the interaction potential was estimated on the basis of the inhibitory concentration or pKa and the advice in the assessment reports.[10]

ABSORPTION

Intragastric pH

The absorption of MKIs can be significantly affected by altered intragastric pH. When intragastric pH is elevated (e.g. due to proton pump inhibitors; PPIs), the MKI solubility, bioavailability, and eventually treatment efficacy may be significantly influenced (**Figure 1**).[8,11–13] The impact of this 'pH effect' is highly variable per MKI and the clinical relevance of the DDI between MKIs and acid-suppressive agents (e.g. PPIs, H2-antagonists and antacids) must be assessed on an individual basis. A complete overview can be found in **Table 1**.[14–35]

Indecisive guidelines and the fact that 20–30% of all cancer patients have an indication for the use of acid-suppressive agents (ASAs) complicate the management of this DDI.[36] The general consensus is, if possible, to avoid the combination between MKIs and ASAs.[37] However, if there is a distinct indication for an ASA (e.g. Barrett's esophagus), a clear and practical advice to manage the DDI between MKIs and ASAs is essential to safeguard optimal MKI therapy. Based on the pharmacokinetics and pharmacodynamics of both MKIs and ASAs, practical advice can be given for the management of the DDI between MKIs and PPIs, H2-antagonists (H2As) and antacids (see Figure 1 and Table 1).[13] This advice may be extrapolated to newly introduced MKIs with a known or suspected drug interaction with gastric suppressive agents and thus with a great impact of the 'pH effect' as mentioned in Figure 1 and **Table 1**.

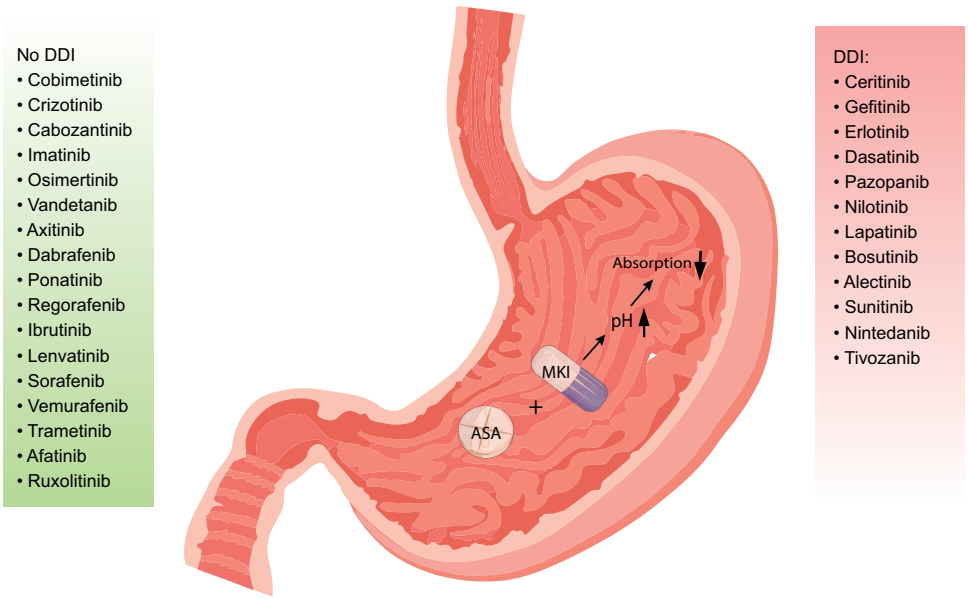

FIGURE 1. Working mechanism of the drug–drug interaction with an ASA: MKIs are arranged according to the clinical relevance and magnitude of the interaction in a descending order, with the most relevant interactions on top of the list. A PPI increases stomach pH after intake and thereby decreases absorption of MKIs and therefore bioavailability of MKIs. Abbrevations: ASA, acid-suppressive agent; DDI, drug–drug interaction; MKI, multikinase.

MKIs and PPIs. Since PPIs do not elevate intragastric pH over the full 24 h-range, a window of relatively low intragastric pH may be used to manage the DDI.[38] If there is a hard indication for PPI use, MKIs should be taken at least 2h before the PPI in the morning in a once-daily regimen, since MKIs can be absorbed completely within this window.[13,38]

TABLE 1. DDIs regarding gastric acid suppression

MKI (year of marketing approval)	Acid-suppressive compound	Decrease in C_{max}	Decrease in AUC	Clinical Relevance	Recommendations	References
Afatinib (2013)	Not reported yet (a clinical trial is currently ongoing (NTR: 6652))	NA	NA	Minor	Based on pKa a non clinically relevant interaction is expected.	14, 15
Alectinib (2017)	Esomeprazole at least one hour before a regular breakfast for 5 days. Alectinib was administered 30 minutes after breakfast	16%	22%	Minor	Although the effects are minimal preferably avoid the use of acid-suppressive agents. Otherwise apply separate administration times or consider short-acting antacids.	14-16
Axitinib (2012)	Rabeprazole 20 mg for 5 consecutive days 3h prior to axitinib intake	42%	5%	Minor	No interventions needed. Concomitant acid-suppression can be used safely.	14, 15, 17
Bosutinib (2013)	Lansoprazole 60mg/day for 2 consecutive days	46%	26%	Minor	Avoid the use of acid-suppressive agents. Otherwise apply separate administration times or consider short-acting antacids.	14, 15, 18
Cabozantinib (2016)	Esomeprazole 40mg delayed release capsule for 6 days 1 hour before cabozantinib intake	10%	9%	Minor	No interventions needed. Concomitant acid-suppression can be used safely.	14, 15, 19
Ceritinib (2015)	Esomeprazole 40mg for 6 consecutive days 1 hour before ceritinib intake	79% (healthy subjects) 25% (patients)	76% (healthy subjects 30% (patients)	Moderate	Avoid the use of acid-suppressive agents. Otherwise separate administration times. Antacids might be used 4h before or 2h after ceritinib intake or H_2-antagonists can be used 10h before or 2h after ceritinib intake.	14, 15, 20
Cobimetinib (2015)	Rabeprazole 20mg for 5 days prior to cobimetinib administration in a fasted and non-fasted state. In the fasted state concomitantly with cobimetinib and 1 h before cobimetinib in the non-fasted state.	14% in the non-fasted state	<11%	Minor	No interventions needed. Concomitant acid-suppression can be used safely.	14, 15, 21
Crizotinib (2012)	Esomeprazole 40mg for 5 days concomitant with crizotinib	0%	10%	Minor	No interventions needed. Concomitant acid-suppression can be used safely.	14, 15
Dabrafenib (2013)	Rabeprazole 40 mg for 4 consecutive days concomitant with dabrafenib	12%	3%	Minor	No interventions needed. Concomitant acid-suppression is considered safe.	14, 15
Dasatinib (2006)	Omeprazole 40mg for 4 consecutive days with dasatinib Maalox 30ml concomitantly with dasatinib Maalox 30ml 2h before dasatinib Famotidine 40mg 10h before dasatinib	42% 58% 26% 63%	43% 55% NA 61%	Moderate Moderate Minor Moderate	Avoid the use of acid-suppressive agents. Otherwise apply separate administration times. H_2-antagonist can be used 2h after dasatinib intake. Antacids can be used 2 hours before or after dasatinib intake.	14, 15, 22

TABLE 1. Continued

MKI (year of marketing approval)	Acid-suppressive compound	Decrease in C_{max}	Decrease in AUC	Clinical Relevance	Recommendations	References
Erlotinib (2005)	Omeprazole 40mg for 7 consecutive days with erlotinib Ranitidine 300 mg once daily concomitantly with erlotinib Ranitidine 150mg twice daily concomitantly with erlotinib	61% 54% 17%	46% 33% 15%	Moderate Minor Minor	Avoid the use of acid-suppressive agents. Otherwise apply separate administration times. Or H_2-antagonist should be used 2h after erlotinib intake. Antacids can be used 4 hours before or 2 hours after erlotinib intake. Furthermore cola may increase erlotinib absorption.	14, 15, 23, 24
Gefitinib (2009)	Ranitidine 450 mg BID 1 day before gefitinib intake	71%	47%	Moderate	Avoid the use of acid-suppressive agents. Otherwise apply separate administration times. Antacids may be used 2h before or after gefitinib intake.	14, 15, 25
Ibrutinib (2014)	Omeprazole 40mg for 5 days in a fasted condition 2h before ibrutinib intake	63%	non significant difference	Minor	No interventions needed. Concomitant acid-suppression can be used safely.	14, 15, 26
Imatinib (2001)	Omeprazole 40mg for 5 consecutive days 15 min before imatinib intake	3%	7%	Minor	No interventions needed. Concomitant acid-suppression can be used safely	14, 15, 27, 28
Lapatinib (2008)	Esomeprazole 40mg for 7 consecutive days in the evening (12h before lapatinib intake)	NA	26%	Minor	Avoid the use of acid-suppressive agents. Otherwise apply separate administration times. Antacids might be used 2h before or after lapatinib intake.	14, 15
Lenvatinib (2015)	H2-blockers, antacids, PPIs not further specified in a PBPK analysis	non significant difference	non significant difference	Minor	No clinical studies, but concomitant use with acid-suppressive therapy is considered safe due to a PBPK analysis.	14, 15
Nilotinib (2007)	Esomeprazole 40mg for 5 consecutive days 1h before nilotinib intake	27%	34%	Minor	Avoid the use of acid-suppressive agents. Otherwise apply separate administration times. Antacids might be used 2h before or after nilotinib intake or H_2-antagonists can be used 10h before or 2h after nilotinib intake.	14, 15, 29-31
Nintedanib (2015)	No clinical study	NA	NA	Moderate	No clinical studies available, however nintedanib bioavailability decreases rapidly with increasing pH so a gastric acid suppressive drug is likely to give a DDI.	14, 15
Osimertinib (2016)	Omeprazole 40 mg in a fasted state for 5 consecutive days	2%	7%	Minor	No interventions needed. Concomitant acid-suppression can be used safely.	14, 15

TABLE 1. Continued

MKI (year of marketing approval)	Acid-suppressive compound	Decrease in C_{max}	Decrease in AUC	Clinical Relevance	Recommendations	References
Pazopanib (2010)	Esomeprazole 40mg for 5 consecutive days	42%	40%	Minor	Pazopanib should be taken at least 2 h before or 10 h after a dose of an H2-antagonist. Antacids can be used 4 h before or 2 h after pazopanib intake. PPIs should be administered concomitantly with pazopanib in the evening.	14, 15, 32
Ponatinib (2013)	Lansoprazole 60 mg for 2 consecutive days concomitantly with ponatinib	25%	1%	Minor	No interventions needed. Concomitant acid-suppressive therapy is considered safe.	14, 15, 33
Regorafenib (2013)	Esomeprazole 40 mg for 5 consecutive days 3h before and concomitantly with regorafenib. A clinical study is currently ongoing (NCT02800330)	NA	NA	Minor	No clinical studies available. However regorafenib is considered to be safe since regorafenib pKa is high.	14, 15
Ruxolitinib (2012)	No clinical study	NA	NA	Minor	No clinical studies available. Concomitant acid-suppressive therapy is considered safe, since pKa of ruxolitinib is high.	14, 15
Sorafenib (2006)	Omeprazole 40 mg for 5 consecutive days	no significant difference	no significant difference	Minor	No interventions needed. Concomitant acid-suppressive therapy is considered safe.	14, 15
Sunitinib (2006)	No clinical study	NA	NA	Minor	Sunitinib shows high solubility and therefore concomitant acid-suppressive therapy is considered safe. However survival seems to be lower in patients using ASA.	14, 15, 34
Tivozanib (2017)	No clinical study	NA	NA	Moderate	No clinical studies available. However adverse event rate was higher in PPI users, which suggests higher tivozanib plasma levels due to a DDI.	14, 15
Trametinib (2014)	No clinical study	NA	NA	Minor	Trametinib shows consistent solubility over all pH values. Therefore concomitant acid-suppressive therapy is considered safe.	14, 15
Vandetanib (2012)	Omeprazole 40mg for 5 days concomitantly 150mg ranitidine for 5 days concomitantly with vandetanib	15% 8%	6% 1%	Minor Minor	No interventions needed. Concomitant acid-suppressive therapy is considered safe.	14, 15, 35
Vemurafenib (2012)	No clinical study	NA	NA	Minor	No interventions needed. Concomitant acid-suppressive therapy is considered safe.	14, 15

Legend: *Clinical relevance is scored by means of the FDA Clinical Drug Interaction Studies — Study Design, Data Analysis, and Clinical Implications Guidance for Industry as a guideline as Major (AUC increase ≥80%), Moderate (AUC increase ≥ 50% to < 80%), Minor (AUC increase ≥20% to <50%) and by taking into account the performed study and the available evidence regarding pKa and the available assessment report. NA is not applicable/unknown.* [10,14,15]

Another possibility is to administer a MKI with an acidic beverage such as cola (pH = 2.5) to manage the DDI, since the acidic beverage temporarily decreases stomach pH resulting in better MKI solubility and absorption.[23] Furthermore, the influence of other acidic beverages [e.g. sprite (pH = 3.4) or orange juice (pH = 3.3)] on the absorption of MKIs has not been studied yet.

MKIs and H2-antagonists. Since most H2-antagonists show a short plasma half-life and are administered in a twice daily regimen (e.g. ranitidine), MKIs should be taken at least 2h before or 10h after the H2-antagonist intake according to US FDA and EMA guidelines.[14,15]

MKIs and antacids. Antacids are relatively short-acting agents (e.g. magnesium hydroxide). MKIs should be administered at least 2h before, or 4h after antacid intake, to manage this DDI.[14,15]

Drug transporters and intestinal enzymes

As mentioned previously, MKI absorption is a multifactorial process mediated and affected by passive diffusion, active transport through multiple drug transporters, and intestinal metabolism.[7] The activity of these drug transporters and intestinal enzymes may significantly influence MKI bioavailability.

Drug transporters are located throughout the body, especially in the gut, bile ducts, kidneys and the blood–brain barrier (**Figure 2**).[39] The US FDA states: 'membrane transporters can have clinically relevant effects on the pharmacokinetics and pharmacodynamics of a drug in various organs and tissues by controlling its absorption, distribution, and elimination. In contrast to drug metabolizingenzymes that are largely expressed in the liver and small intestines'.[10] Therefore, the effect of a DDI considering drug transporters may be of greater clinical relevance then is assumed so far.

Furthermore, efflux drug transporters like P-glycoprotein, or P-gp (ATP-binding cassette subfamily B member 1, ABCB1) and also breast cancer resistance protein (BCRP; ATP-binding cassette subfamily G member 2, ABCG2) may play a crucial role in drug absorption and enterohepatic recirculation. Enterohepatic recirculation is the process in which foreign chemicals are absorbed into the portal blood stream and metabolized by hepatocytes, secreted into the bile and eventually are reabsorbed after secretion of bile in the gut lumen.[40] In this multi-step process drug transporters like P-gp and BCRP play a significant role. Other drug efflux transporters that may influence MKI bioavailability are the multidrug resistance protein subfamily (ATP-binding cassette subfamily C member 1 to 12, ABCC1 to 12, like MRP1) and the multi-antimicrobial

extrusion protein (MATE), while several uptake transporters may be involved as well [e.g. organic anion transporting peptides (OATPs), organic anion transporters (OATs), and organic cation transporters (OCTs), see **Figure 2**].

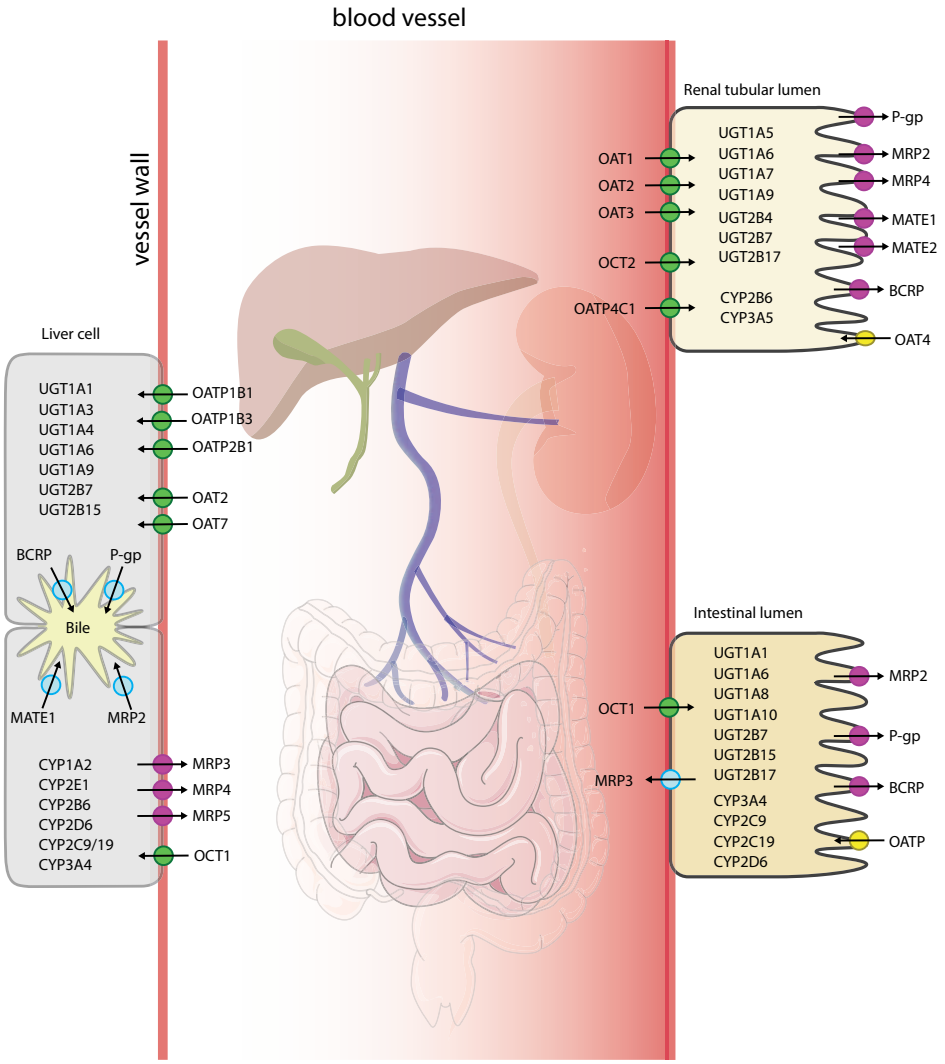

FIGURE 2. Distribution of drug transporters and metabolizing enzymes: A complete overview of all the drug transporters and metabolizing phase I and phase II enzymes are presented in this figure for the main organs involved in the pharmacokinetics of drugs. BCRP, breast cancer resistance protein (ABCG2); CYP, cytochrome P450 iso-enzyme, MATE, multi-antimicrobial extrusion protein; MRP, multidrug resistance associated protein; OAT, organic anion transporters; OATP, organic anion ransporting peptides; OCT, organic cation transporters; P-gp, P-glycoprotein (ABCB1); UGT, UDP-glucuronosyltransferase.

Many drugs are known P-gp inhibitors (e.g. verapamil) or act as a strong P-gp-inducer (e.g. rifampicin). Drugs like cyclosporine, an inhibitor of several OATPs (e.g. OATP1B1 and BCRP) and cimetidine (OCT2 inhibitor) may influence other drug transporters as well.[41] For example, nintedanib showed a decrease in both area under the curve (AUC) and maximum concentration (C_{max}) when co-administered with rifampicin. Since nintedanib is almost exclusively metabolized by phase II enzymes, this effect on AUC and C_{max} is most likely due to P-gp induction.[42] In general the use of strong P-gp or BCRP inhibitors or inducers is discouraged when MKIs are substrates for these transporters. Furthermore, many MKIs show inhibition of several drug transporters by themselves (**Table 2**).[14,15,18,21,35,41,43–59] When a MKI acts like an inhibitor of these transporters and is co-administered with drug transporter substrates with a narrow therapeutic window (e.g. digoxin), close monitoring of side effects (e.g. severe arrhythmia for digoxin) is warranted. For some MKIs the clinical relevance of DDIs regarding drug transporters is negligible and the combination with inhibitory or inducing compounds is considered to be well tolerated (e.g. bosutinib).[14,15]

In contrast with the above mentioned unwanted adverse effects, mostly found in preclinical studies, DDIs concerning drug transporters and MKIs may also be used in a beneficial way. For example, MKIs may potentially increase chemotherapy concentrations through P-gp or BCRP inhibition (e.g. increased paclitaxel plasma concentration resulting from P-gp inhibition by nilotinib or increased nilotinib concentrations as a result of P-gp inhibition by imatinib).[60,61]

In conclusion, we found only a limited number of clinical studies, which investigated the effects of inhibition or induction of drug transporters by MKIs, since this is a relatively novel field of DDI research. Combinations between strong drug transporter inhibitory or inducing compounds should be avoided for most MKIs as mentioned in **Table 2**.

Intestinal metabolism

Another important factor in drug absorption is intestinal metabolism. Many MKIs are metabolized in the gut wall through intestinal CYP3A4, which is often in close proximity of drug transporters, such as P-gp. When a MKI is given concomitantly with an intestinal CYP3A4 inducer (e.g. rifampicin) or inhibitor (e.g. grapefruit juice) this may significantly change MKI bioavailability.[62] However, in contrast, Van Erp and colleagues failed to show a significant increase in sunitinib exposure, when co-administered with grapefruit juice.[63] Moreover, since many MKIs undergo extensive first-pass metabolism and are thus dependent of both intestinal and hepatic metabolism, it is difficult to determine whether intestinal metabolism or hepatic metabolism is the main contributor to an altered drug bioavailability.

TABLE 2. DDIs with drug-transporters

MKI	Substrate	Inhibits	C_{max}	AUC	Clinical implications	Interaction potential	References
Afatinib	P-gp, BCRP	*In vitro*: P-gp, BCRP	Ritonavir: 38% increase Rifampicin: 22% decrease	Ritonavir: 48% increase Rifampicin: 34% decrease	For strong P-gp and BCRP inhibitors (e.g. ritonavir, cyclosporine); use staggered dosing, preferably 6 hours or 12 hours apart from afatinib. When afatinib is administered with a strong P-gp inducer (e.g. rifampicin) increase the afatinib dose with 10mg with close monitoring of side-effects. For substrates of P-gp and BCRP close monitoring of side-effects is recommended.	Moderate	14, 15, 43
Alectinib	M4 is a P-gp substrate	*in vitro*: P-gp, BCRP	NA	NA	When alectinib is co-administered with P-gp or BCRP substrates appropriate monitoring of side-effects of these substrates is recommended.	Minor	14, 15, 44
Axitinib	P-gp, BCRP	*in vitro*: P-gp, BCRP	NA	NA	appropriate monitoring of side-effects is recommended when axitinib used with P-gp and BCRP substrates or inhibitors and inducers.	Minor, since there is only *in vitro* evidence and axitinib is only a weak P-gp and BCRP substrate	14, 15
Bosutinib	P-gp	*In vitro*: P-gp, BCRP, OCT1 dabigatran (P-gp substrate): no effect on dabigatran pharmacokinetics	NA	NA	Clinical relevant interactions with drug transporters are not likely to appear.	Minor	14, 15, 18, 45
Cabozantinib	MRP2	*in vitro*: P-gp, BCRP, MATE1, MATE2	NA	NA	Appropriate monitoring is recommended when using substrates of P-gp of BCRP. Interactions with MATE1-2 in clinically relevant concentrations are unlikely. If necessary a 20mg dose alteration may be applied. Close monitoring of side-effects is warranted when administered with strong MRP2 inhibitors (e.g. cyclosporine).	Moderate	14, 15

TABLE 2. Continued

MKI	Substrate	Inhibits	C_{max}	AUC	Clinical implications	Interaction potential	References
Ceritinib	P-gp	P-gp, BCRP	NA	NA	Concomitant administration with strong inducers or inhibitors of P-gp must be avoided since plasma concentration of ceritinib might be altered. Close monitoring of side-effects is warranted when administered with strong P-gp or BCRP substrates. However CYP DDIs are of greater influence.	Minor, since interactions regarding CYP enzymes are of greater clinical importance	14, 15
Cobimetinib	P-gp	*in vitro*: BCRP, OATP1B1, OATP1B3, OCT1	NA	NA	Concomitant administration with strong P-gp inducers or inhibitors must be avoided. Appropriate monitoring is recommended when using BCRP, OATP1B1, OATP1B3, OCT1 substrates.	Moderate	14, 15, 21
Crizotinib	P-gp	*in vitro*: P-gp, OCT1, OCT2	NA	NA	Appropriate monitoring of side-effects is recommended when using concomitant P-gp substrates, inhibitors and inducers. Furthermore close monitoring is recommended when using P-gp, OCT1, OCT2 substrates.	Minor, since CYP interactions are of greater clinical importance	14, 15
Dabrafenib	P-gp, BCRP	*in vitro*: OATP1B1, OATP1B3, BCRP	Rosuvastatin: 160% increase	Rosuvastatin: 7% increase	Dabrafenib is not likely to have a clinically relevant interaction with OATP1B1, OATP1B3 and BCRP. Concomitant use with substrates of these transporters is considered safe. The influence of P-gp and BCRP inhibitors or inducers is considered to be small since the bioavailability of dabrafenib is high (95%), only limited pharmacokinetic effects can be expected	Minor	14, 15
Dasatinib	P-gp, BCRP	*NA*	NA	NA	Concomitant administration with strong inducers or inhibitors of P-gp and BCRP must be avoided or side-effects must be monitored closely when administered with strong inhibitors.	Minor	14, 15, 46

TABLE 2. Continued

MKI	Substrate	Inhibits	C_{max}	AUC	Clinical implications	Interaction potential	References
Erlotinib	P-gp, BCRP	*In vitro:* OCT2, OAT3	NA	NA	Concomitant administration with strong inducers or inhibitors of P-gp or BCRP must be avoided since an altered plasma concentrations possible. Administration with OCT2 and OAT3 substrates should be avoided.	Moderate	14, 15, 47-49
Gefitinib	P-gp, BCRP	*in vitro*: BCRP, P-gp	NA	*In vitro* Irinotecan: AUC irinotecan 63% increase	Concomitant administration with P-gp and BCRP substrates should be avoided. BCRP inhibition is 10-fold stronger than P-gp inhibition. So especially be careful when gefitinib is combined with BCRP substrates. Avoid the use of strong BCRP or P-gp inhibitors or inducers since gefitinib plasma concentration may be altered.	Moderate	14, 15, 50
Ibrutinib	NA	*in vitro*: P-gp, BCRP	NA	NA	When P-gp or BCRP substrates are used, they should be taken at least 6 hours before or after ibrutinib intake. Inhibitors or inducers of transporters are not likely to result in clinically meaningful changes in ibrutinib pharmacokinetics and can be used concomitantly.	Minor	14, 15, 51
Imatinib	P-gp, BCRP	*In vitro:* BCRP	NA	NA	A clinical relevant interaction with P-gp or BCRP inhibitors or inducers may be possible. Close monitoring of substrate specific side-effects is advised when used concomitantly with BCRP substrates. Although the interaction potential is considered to be low.	Minor	14, 15, 52
Lapatinib	P-gp, BCRP	*in vitro*: P-gp, BCRP, OATP1B1	Digoxin (P-gp substrate): 100% increase (digoxin)	Digoxin (P-gp substrate): 60-80% increase (digoxin)	Lapatinib is highly susceptible for interactions regarding drug transporters. When using P-gp, BCRP, OATP1B1 substrates close monitoring of side-effects is recommended. The use of strong P-gp and BCRP inhibitors or inducers should be avoided.	**Major**	14, 15, 53

TABLE 2. Continued

MKI	Substrate	Inhibits	C_{max}	AUC	Clinical implications	Interaction potential	References
Lenvatinib	P-gp, BCRP, MDR1	*in vitro*: P-gp, BCRP, OATP1B3	Ketoconazole: 19% increase single-dose rifampicin: 33% increase	Ketoconazole: 15% increase single-dose rifampicin: 31% increase	Clinical relevant interactions with strong inhibitors or inducers of P-gp, BCRP are not likely to appear, but close monitoring for lenvatinib specific side-effects is recommended.. Concomitant administration with P-gp, BCRP and OATP1B3 substrates should be avoided.	Minor	14, 15, 54, 55
Nilotinib	P-gp, BCRP	*in vitro*: P-gp, BCRP	NA	Imatinib (CYP3A4/P-gp inhibitor): nilotinib AUC increased with 18-40%	Concomitant administration with strong P-gp or BCRP inducers or inhibitors must be avoided since an altered plasma concentration is possible otherwise side-effects should be monitored closely.	Minor	14, 15, 56
Nintedanib	P-gp	*in vitro*: P-gp, OCT1, BCRP	Ketoconazole: 83% increase Rifampicin: 60% decrease	Ketoconazole: 61% increase Rifampicin: 50% decrease	when administered with strong P-gp inhibitors a 100mg step-wise dose reduction must be considered. The duration of therapy with strong inducers must be minimized since inadequate plasma levels of nintedanib might occur. Concomitant administration with P-gp, BCRP and OCT1 substrates should be avoided.	**Major**	14, 15
Osimertinib	P-gp, BCRP	*in vitro*: P-gp, BCRP	Rosuvastatin (BCRP substrate): 72% increase	Rosuvastatin (BCRP substrate): 35% increase	Concomitant administration with strong P-gp and BCRP inducers or inhibitors must be avoided since an altered plasma concentration is likely. When co-administered with BCRP or P-gp substrates close monitoring of side-effects is recommended.	Minor	14, 15
Pazopanib	P-gp, BCRP	*in vitro*: OATP1B1, P-gp, BCRP	Lapatinib (P-gp and BCRP inhibitor) 60% Increase	Lapatinib(P-gp and BCRP inhibitor): 50% increase	Co-administration with strong P-gp or BCRP inhibitors must be avoided. Close monitoring of side-effects is advised when used concomitantly with P-gp or BCRP substrates.	Moderate	14, 15

TABLE 2. Continued

MKI	Substrate	Inhibits	C_{max}	AUC	Clinical implications	Interaction potential	References
Ponatinib	P-gp, BCRP	*in vitro*: P-gp, BCRP	NA	NA	Appropriate monitoring is recommended when co-administered with P-gp or BCRP substrates. Also the use of strong inhibitors or inducers of P-gp, BCRP must be avoided, although DDI potential is considered to be low since ponatinib is only a weak substrate for P-gp and BCRP.	Minor	14, 15
Regorafenib	P-gp, BCRP	*In vitro*: BCRP Regorafenib has no effect on digoxin AUC	Rosuvastatin (BCRP substrate): 360% increase	Rosuvastatin (BCRP substrate): 280% increase	BCRP substrates should be used with caution. When administered with strong inhibitors or inducers of P-gp and BCRP close observation of side-effects is warranted.	**Major**	14, 15
Ruxolitinib	NA	*in vitro*: P-gp, BCRP	NA	NA	When ruxolitinib is administered with P-gp or BCRP substrates close monitoring is advised for these substrates. DDI potential can be minimized if time between administration is kept apart as long as possible.	Minor	14, 15
Sorafenib	P-gp, OATP1B1, OATP1B3, MRP2-3	P-gp	NA	NA	Concomitant administration with strong inhibitors or inducers of P-gp, OATP1B1, OATP1B3(rifampicin) and MRP2-3 should be avoided. Administration with P-gp substrates should be done with caution.	Moderate	14, 15, 57
Sunitinib	P-gp	*in vitro*: P-gp, BCRP coadministration with gefitinib (BCRP inhibitor) did not result in significant AUC changes of sunitinib	NA	NA	Appropriate monitoring is recommended when co-administered with P-gp or BCRP substrates. Also the use of strong inhibitors or inducers of P-gp must be avoided.	Minor	14, 15
Tivozanib	NA	*In vitro*: BCRP	NA	NA	Co-administration with BCRP substrates must be avoided or side-effects must be monitored closely.	Minor	14, 15

TABLE 2. Continued

MKI	Substrate	Inhibits	C_{max}	AUC	Clinical implications	Interaction potential	References
Trametinib	P-gp	*In vitro*: P-gp, BCRP, OAT1, OAT3, OATP1B1, OATP1B3, OATP2B1, OCT2, and MATE1	NA	NA	Co-administration of strong inhibitors or inducers of P-gp must be avoided. When P-gp, BCRP, OAT1, OAT3, OATP1B1, OATP1B3, OCT2 and MATE1 substrates are used, staggered dosing must be applied (at least 2 hours apart) to minimize DDI risk. However, based on the low dose and low clinical systemic exposure relative to the in vitro inhibition or induction potential this is not expected to be of in vivo significance.	Minor	14, 15
Vandetanib	NA	*in vitro*: P-gp, BCRP, OCT2	Metformin (OCT-2 substrate) increased with 50% Digoxin (P-gp substrate) increased with 29%	Metformin (OCT-2 substrate) increased with 74% Digoxin (P-gp substrate) increased with 23%	Co-administration with P-gp, BCRP, OCT2 substrates must be avoided and side-effects must be monitored closely. Concomitant intake with strong inhibitors or inducers of drug transporters is safe.	Moderate	14, 15, 35
Vemurafenib	P-gp, BCRP	*In vitro*: P-gp, BCRP	Digoxin (P-gp substrate) increased 50%	Digoxin (P-gp substrate) increased 80%	Concomitant administration with strong inhibitors or inducers of P-gp and BCRP should be avoided. Appropriate monitoring is recommended when co-administered with P-gp or BCRP substrates.	**Major**	14, 15, 59

Legend: *Clinical relevance is scored by means of the FDA Clinical Drug Interaction Studies — Study Design, Data Analysis, and Clinical Implications Guidance for Industry as a guideline as Major (AUC increase ≥80%), Moderate (AUC increase ≥ 50% to < 80%), Minor (AUC increase ≥20% to <50%) taken into account the available evidence for both change in AUC of MKI and change in AUC for transporter substrates, since there is no separate scoring system for drug-transporter interactions. If there was no clinical evidence, clinical relevance was estimated on the basis of available evidence regarding inhibitory concentrations and the assessment report. Interaction potential was then scored as Minor or at most Moderate. NA is not applicable or only preclinical data available. Abbreviations: P-gp = P-glycoprotein (ABCB1), BCRP = Breast Cancer Resistance Protein (ABCG2), MRP = Multidrug resistance associated protein, MATE = Multi-Antimicrobial Extrusion Protein, OATP = organic anion transporting peptides (OATP), OAT = organic anion transporters and OCT = organic cation transporters Strong drug transporter inhibitors: P-gp: amiodarone, carvedilol, clarithromycin, dronedarone, itraconazole, lapatinib, lopinavir, propafenone, quinidine, ranolazine, ritonavir, saquinavir, telaprevir, tipranavir and ritonavir, verapamil. BCRP: curcumin, cyclosporine, eltrombopag OATP1B1/OATP1B3: atazanavir, ritonavir, clarithromycin, cyclosporine, erythromycin, gemfibrozil, lopinavir, rifampin (single dose), simeprevir OAT1/OAT3: p-aminohippuric acid (PAH), probenecid, teriflunomide, MATE1/MATE2-K: cimetidine, dolutegravir, isavuconazole, ranolazine, trimethoprim, vandetanib strong drug transporter inducers: P-gp: rifampicin, carbamazepine, phenytoin, St. John's wort, ritonavir* [1, 58]

Metabolism

In the liver, MKIs are predominately metabolized by CYP enzymes into either active or inactive metabolites. For some MKIs, like nintedanib, phase II metabolism through UDP-glycosyltransferases (UGTs), glutathione S-transferases and sulfotransferases (SULTs) is dominant in their metabolism.[6,64,65] Inhibition or induction of these phase I and II enzymes by coadministered medication may lead to either (severe) toxicity or loss of effective MKI therapy, respectively.

As DDIs with strong CYP3A4 inhibitors and inducers (e.g. ketoconazole and rifampicin, respectively) play a significant role in MKI therapy, they are usually well described and clear recommendations for the management of these DDIs are presented in the assessment report. There are many (strong) inducers or inhibitors of CYP enzymes for which a complete overview can be found at the FDA and EMA websites.[41,66] Moreover, some MKIs (e.g. imatinib, pazopanib) also displayed inhibitory or inducing activity by themselves.[67–70] The general advice is to avoid concomitant administration with strong inhibitors or inducers of CYP enzymes. If this is not possible, a MKI dose adjustment, based on the advice given in the assessment report is recommended. For strong inducers a gradual dose escalation of the prescribed dose is advised with close monitoring of MKIspecific side effects. For an overview of clinically relevant DDIs and for practical recommendations see **Table 3**.[14,15,41,43,44,67-69,71–93]

Interactions with novel MKIs

In the last decade there has been a significant increase in the development of and treatment with MKIs resulting in more than a doubling of registered MKIs in the past 5 years. Earlier, we described the DDIs with MKIs which were approved before 1 August 2013.[6] Here, we give an extensive overview of the DDI potential and management of the novel MKIs, which have been approved after August 2013. A complete overview including all (new and older) MKIs is presented in **Tables 1–3**.

Afatinib.

Afatinib is used in the treatment of nonsmall cell lung cancer (NSCLC). It is a substrate of P-gp and BCRP and is mainly metabolized through enzyme-catalyzed Michael adduct formation (phase II) and only in a minor extent to phase I enzymes like CYP3A4 and FMO (2%).[14,15] Concomitant administration with ritonavir (a P-gp inhibitor) showed a 48% increase in AUC and 39% increase in Cmax.[43] Treatment with a potent P-gp inducer (rifampicin) prior to singledose afatinib showed a moderate effect on both afatinib AUC and C_{max} (34% and 22% decrease respectively).[43] When afatinib is administered with strong P-gp and BCRP inhibitors, staggered dosing may be used, preferably 6h

or 12h apart from afatinib intake. When afatinib is administered with strong P-gp inducers the dose may be increased with 10mg with close monitoring of side effects. Administration with strong CYP inducers or inhibitors is considered safe, since no CYP enzymes are involved in afatinib metabolism. Furthermore in vitro studies showed afatinib itself to be an inhibitor of P-gp and BCRP, so close monitoring of side effects when administered with substrates for these transporters with a narrow therapeutic window is recommended.[14,15]

Alectinib.

The anaplastic lymphoma kinase (ALK) inhibitor alectinib is used in the treatment of metastatic lung cancer. Alectinib as well as its M4 metabolite are considered equally active. Alectinib is primary metabolized by CYP3A4.[14,15] Co-administration with the strong CYP3A4 inhibitor posaconazole resulted in a 75% increase of AUC, while co-administration with rifampicin led to a 73% decrease in alectinib AUC.[44] Since alectinib and M4 are equally active, a dose modification is not necessary (unless patients experience a significant increase in toxicity) when alectinib is administered with strong inhibitors or inducers of CYP3A4. Since alectinib is a P-gp and BCRP inhibitor, close monitoring of side effects of these substrates is recommended, especially for drugs with a narrow therapeutic window (e.g. digoxin).

Bosutinib.

Bosutinib is used in the treatment of chronic myeloid leukemia (CML). Although bosutinib is a P-gp substrate and inhibitor, DDIs are not likely to appear, since clinical studies demonstrated no significant effect on dabigatran (P-gp substrate) or bosutinib (when administered with the P-gp inhibitor lansoprazole) pharmacokinetics.[18,45] Therefore no bosutinib dose reductions are necessary, when administered with strong P-gp inducers or inhibitors. Bosutinib is mainly metabolized through CYP3A4 and coadministration with the strong inhibitor ketoconazole resulted in 420% increase in C_{max} and 760% increase in AUC.[74] Administration with rifampicin showed a significant 86% reduction in C_{max} and a 92% decrease in AUC of bosutinib. Administration with the moderate inhibitor aprepitant also showed an increase in AUC and C_{max}.[73] In conclusion; strong inhibitors or inducers of CYP3A4 must be avoided or a gradual 20% dose reduction should be applied, when co-administered with strong inhibitors of CYP3A4. Increasing the bosutinib dose is not useful, when co-administered with strong CYP3A4 inducers, since a maximal tolerated bosutinib dose of 600mg is often not sufficient to compensate for the relatively large loss of exposure.[14,15]

TABLE 3. DDIs regarding drug metabolism

MKI	Major CYP	Minor CYPs and others	Inhibitory activity	Inducing activity	Inhibitory compound	Inducing compound	Change in C_{max}	Change in AUC	Clinical recommendations	Clinical relevance	Reference
Afatinib	mainly due to non enzyme-catalysed Michael adduct formation	FMO3, CYP3A4	NA	NA	ritonavir		38 % increase	48 % increase	No DDIs is expected, combination with CYP inducers or inhibitors is considered safe. The effect is most likely through P-gp induction and inhibition.	Minor	14, 15, 43
						rifampicin	22 % decrease	34% decrease			
Alectinib	CYP3A4	CYP2C8, CYP3A5	There was no influence on midazolam (CYP3A4) pharmacokinetics	CYP1A2, CYP2B6, CYP3A4 (*in vitro*)	Posaconazole		18% increase	75% increase	Since alectinib metabolites are equally effective as alectinib strong inhibitors or inducers of CYP3A4 can be safely combined with close monitoring of side-effects from alectinib.	Minor (since alectinib metabolites are equally active)	14, 15, 44
						rifampicin	51% decrease	73% decrease			
Axitinib	CYP3A4	CYP3A5, CYP1A2, CYP2C19, UGT1A1	UGT1A4, UGT1A7, UGT1A9, CYP1A2	NA	ketoconazole		50% increase	106% increase	50% dose reduction of axitinib is recommended when concomitantly used with strong inhibitors of CYP3A4 and slow dose escalation is advised for strong inducers of CYP3A4. Smoking is not allowed since it might alter CYP1A2 metabolism.	Moderate	14, 15, 71, 72
						rifampicin	71% decrease	79% decrease			
Bosutinib	CYP3A4	Mono-oxygenase enzymes (FMO)	NA	NA	ketoconazole aprepitant (moderate CYP3A4 inhibitor)		420% increase 50% increase	760% increase 100% increase	Avoid strong and moderate CYP3A4 inhibitors or inducers. Otherwise stop bosutinib treatment or reduce bosutinib dose by 20%. Dose escalation is often not useful since adequate plasma levels aren't reached with a maximum dose of 600mg qd.	**Major**	14, 15, 73, 74
						rifampicin	86 % decrease	92% decrease			
Cabozantinib	CYP3A4	CYP2C9	CYP2C9, CYP3A, CYP2C19 (*in vitro*) No significant effect on Rosiglitazone AUC (CYP2C8 substrate)	NA	ketoconazole		no significant difference	38% increase	(Chronic) co-administration of strong inhibitors and inducers of CYP3A4 must be avoided. If necessary a 20mg dose alteration may be applied. For CYP2C9, CYP2C19 or CYP3A4 substrates with a narrow therapeutic window close monitoring of side-effects is recommended, however the inhibitory and inducing potential of cabozantinib is likely to be low.	Moderate	14, 15, 75
						rifampicin	no significant difference	77% decrease			
Ceritinib	CYP3A4	NA	CYP3A4, CYP2C9, CYP2A6, CYP2E1 (*in vitro*)	CYP3A4	ketoconazole		20% increase	190% increase	A 30% dose reduction may be applied when ceritinib is administered with strong inhibitors of CYP3A4. Concomitant use of strong inducers should be avoided. When administered with CYP2C9, CYP2A6, CYP2E1 or CYP3A4 substrates close monitoring of side-effects is recommended.	Moderate	14, 15
						rifampicin	44 % decrease	70% decrease			

TABLE 3. Continued

MKI	Major CYP	Minor CYPs and others	Inhibitory activity	Inducing activity	Inhibitory compound	Inducing compound	Change in C_{max}	Change in AUC	Clinical recommendations	Clinical relevance	Reference
Cobimetinib	CYP3A4	CYP2C19, CYP2D6, UGT2B7	Dextromethorphan (CYP2D6 substrate) and midazolam exposure was not altered by cobimetinib.	CYP1A2 (*in vitro*)	itraconazole		220% increase	570% increase	Avoid the (chronic) use of strong CYP3A4 inhibitors or inducers (especially treatment with strong inhibitors). If treatment is necessary monitoring of side-effects must be applied and the use must be limited. Also a 20 mg dose adjustment may be made. Concomitant administration with CYP1A2 substrates must be avoided or side-effects must be monitored closely.	**Major**	14, 15, 76
						rifampicin (PBPK-model)	63% decrease	83% decrease			
Crizotinib	CYP3A4	CYP3A5, CYP2C8, CYP2C19, CYP2D6	CYP3A4, CYP2B6, UGT1A1, UGT2B7 Midazolam AUC increased with 270%	UGT1A1, CYP2B6, CYP2C8, CYP2C9	ketoconazole		40% increase	220% increase	Avoid the (chronic) use of strong CYP3A4 inhibitors or inducers. If treatment is necessary monitoring of side-effects is recommended. When administered with CYP3A4, UGT1A1, UGT2B7, CYP2C8, CYP2C9 or CYP2B6 substrates close monitoring is recommended.	**Major**	14, 15, 77
						rifampicin	79% decrease	84% decrease			
Dabrafenib	CYP2C8	CYP3A4	CYP1A2, CYP2D6 R-warfarin (CYP2C19 substrate) AUC decreased with 33% and C_{max} increased with 19% S-warfarin (CYP2C9 substrate) AUC decreased with 37% and C_{max} increased with 17%	CYP3A4, CYP2B6 midazolam (a CYP3A4 substrate) AUC and C_{max} decreased with 47% and 65% respectively	ketoconazole gemfibrozil		33% increase no significant difference	71% increase 47% increase	Avoid the (chronic) use of strong CYP3A4 and CYP2C8 inhibitors or inducers. If there is a hard indication for the use of strong inhibitors or inducers, the duration of use must be limited. When used with CYP3A4, CYP1A2, CYP2B6, CYP2C9 and CYP2C19 substrates side-effects must be monitored closely, especially in the first 3 days of use.	Minor	14, 15, 78
						rifampicin	27% decrease	34% decrease			
Dasatinib	CYP3A4	FMO, UGT	CYP2C8, CYP3A4 simvastatin (CYP3A4 substrate) AUC and C_{max} increased with 20% and 37% respectively.	NA	Ketoconazole		384% increase	256% increase	Avoid strong CYP3A4 inducers or inhibitors. When administered with strong inhibitors dasatinib dose must be reduced with 20-40 mg. When administered with strong inducers a dose escalation must be applied with close monitoring of side-effects. When administered with CYP2C8 or CYP3A4 substrates close monitoring of side-effect is recommended.	**Major**	14, 15, 79
						rifampicin	81% decrease	82% decrease			

TABLE 3. Continued

MKI	Major CYP	Minor CYPs and others	Inhibitory activity	Inducing activity	Inhibitory compound	Inducing compound	Change in C_{max}	Change in AUC	Clinical recommendations	Clinical relevance	Reference
Erlotinib	CYP3A4	CYP1A2, CYP1A1, CYP1B1, CYP3A5	CYP1A1, CYP3A4, CYP2C8 and UGT1A1 Midazolam AUC decreased with 24% Paclitaxel (CYP2C8) AUC was unchanged	NA	Ketoconazole Ciprofloxacin (CYP1A2 inhibitor)		69% increase No significant difference	86% increase 39% increase	When strong CYP3A4, CYP1A2 inducers are used dose increase up to 300mg is advised with monitoring of side-effects. For strong inhibitors a 50 mg dose reduction is recommended. Use of CYP1A2 inducers or inhibitors (e.g. smoking) is encouraged. When administered with CYP3A4, CYP1A1, CYP2C8 and UGT1A1 substrates close monitoring of side-effects is recommended.	Moderate	14, 15, 80
						rifampicin	29% decrease	69% decrease			
Gefitinib	CYP3A4	CYP3A5, CYP2C19 CYP2D6	CYP2D6 and CYP2C19 Metoprolol (a CYP2D6 substrate) AUC increased with 35%	NA	itraconazole		61% increase	78% increase	Dose reduction is not necessary, when combined with strong CYP3A4 inhibitors, since gefitinib has a good tolerability profile. The use of strong CYP3A4 inducers needs to be avoided. When combined with CYP2D6 or CYP2C19 substrates close monitoring of side-effects is recommended.	**Major**	14, 15, 81
						rifampicin	65% decrease	83% decrease			
Ibrutinib	CYP3A4	CYP2D6	CYP3A4	CYP2B6	ketoconazole grapefruit juice erythromycin voriconazole		2800% increase 250% increase 240% increase 570% increase	2300% increase 120% increase 200% increase 470% increase	If the use of strong CYP3A4 inhibitors is necessary reduce ibrutinib dose to 140mg or temporarily (<7 days) stop ibrutinib therapy. For moderate inhibitors reduce ibrutinib dose to 280mg. Minimize the time of use for strong inducers of CYP3A4. Strong inhibitors or inducers of CYP2D6 must be used with caution.	**Major**	14, 15, 82
						Rifampicin	92% decrease	90% decrease			
Imatinib	CYP3A4	CYP3A5, CYP1A2, CYP2D6, CYP2C9, CYP2C19	CYP2C9 Cyclosporin (a CYP3A4/CYP2C8 substrate) concentration raised with 26% during imatinib therapy metoprolol (CYP2D6 substrate) AUC increased with 23% simvastatin (CYP3A4 substrate) AUC increased with 250%	NA	Ketoconazole		26% increase	40% increase	No intervention is needed for strong CYP3A4 inhibitors but monitoring for toxic effects is recommended and duration of strong CYP3A4 inhibitor compounds needs to be minimized. For CYP3A4 inducers a 50% imatinib dose increase may be applied. Also close monitoring is recommended for concomitant use of CYP3A4, CYP2C9 and CYP2B6 substrates with narrow therapeutic windows.	Moderate	14, 15, 67-69
						rifampicin	54% decrease	74% decrease			
Lapatinib	CYP3A4	CYP3A5, CYP1A2, CYP2D6, CYP2C8, CYP2C9, CYP2C19	CYP3A4, CYP2C8 Midazolam (CYP3A4 substrate) AUC increased with 45% Paclitaxel (CYP2C8 substrate) AUC increased with 37% concomitant with pazopanib	NA	ketoconazole		114% increase	257% increase	For strong inhibitors lapatinib dose must be lowered to 500mg. For strong inducers a gradual increase of lapatinib dose must be administered with close monitoring of side-effects. When administered with CYP3A4 or CYP2C8 substrates close monitoring of side-effects is recommended.	Moderate	14, 15, 83, 84
						carbamazepine	59% decrease	72% decrease			

TABLE 3. Continued

MKI	Major CYP	Minor CYPs and others	Inhibitory activity	Inducing activity	Inhibitory compound	Inducing compound	Change in C_{max}	Change in AUC	Clinical recommendations	Clinical relevance	Reference
Lenvatinib	Oxidase by aldehyde oxydase and conjugation by glutathione	CYP3A4	NA	NA	ketoconazole		19% increase	15% increase	Lenvatinib administration with CYP3A4 inducers or inhibitors is considered safe.	Minor	14, 15
						rifampicin	no significant difference	18% decrease			
Nilotinib	CYP3A4	CYP2C8, CYP1A1, CYP1A2, CYP1B1	CYP2D6, CYP2C9, CYP3A4, CYP2C8, UGT1A1 (*in vitro*) Midazolam AUC increased 160% and C_{max} 100% Warfarin (CYP2C9 substrate) AUC did nog change	CYP2B6, CYP2C8, CYP2C9 (*in vitro*)	ketoconazole		84% increase	201% increase	For strong CYP3A4 inhibitors nilotinib dose must be lowered to 400mg once daily. For strong inducers nilotinib dose must be gradually increased depending on toxic side-effects. When administered with CYP2D6, CYP2C8 or CYP3A4, CYP2C9, UGT1A1 substrates close monitoring of side-effects is recommended.	**Major**	14, 15, 85
						rifampicin	64% decrease	80% decrease			
Nintedanib	Hydrolysis due to esterases	UGT1A1, UGT1A7, UGT1A8, UGT1A10, CYP's (5%)	NA	NA	ketoconazole		83% increase	61% increase	Nintedanib co-administration with strong CYP inducers or inhibitors is considered safe since only a small part is metabolized by CYP enzymes and the interaction is more likely through P-gp inhibition or induction.	Minor	14, 15
						rifampicin	60% decrease	50% decrease			
Osimertinib	CYP3A4	CYP3A5, CYP1A2, CYP2A6, CYP2C9, CYP2E1	CYP1A2, CYP2C8, UGT1A1(*in vitro)* CYP3A4, CYP3A5 Simvastatin AUC and C_{max} decreased with 9% and 23% respectively	CYP3A4, CYP1A2	itraconazole		20% decrease	24% increase	Administration with strong inhibitors of CYP3A4 is considered safe. Strong inducers of CYP3A4 must be used with caution with close monitoring of side-effects. When administered with CYP3A4/3A5, CYP1A2, CYP2C8 and UGT1A1 substrates close monitoring of side-effects is recommended.	Moderate	14, 15 ,86, 87
						Rifampicin	73% decrease	78% decrease			
Pazopanib	CYP3A4	CYP1A2, CYP2C8	*In vitro:* CYP3A4, CYP2B6, CYP2C8, CYP2D6, CYP2E1, UGT1A1 midazolam AUC and C_{max} increased both with 30% respectively dextromethorphan (CYP2D6 substrate) AUC and C_{max} increased with 33% an 64% respectively paclitaxel (a CYP2C8 substrate) AUC and C_{max} increased with 26% and 31% respectively Caffeine (CYP1A2 substrate), Warfarin (CYP2C9 substrate) and omeprazole (CYP2C19 substrate) AUC did not change	NA	ketoconazole		45% increase	66% increase	When a strong CYP3A4 inhibitor is administered a 50% pazopanib dose reduction may be applied. For strong inducers close monitoring of side-effects must be applied and therapy with inducers must be limited. Close observations for CYP2C8, CYP2D6, CYP2E1, UGT1A1 and CYP3A4 with narrow therapeutic windows must be applied when co-administered with pazopanib.	Minor	14, 15, 83
						Phenytoin or carbamazepine	50% decrease	30% decrease			

TABLE 3. Continued

MKI	Major CYP	Minor CYPs and others	Inhibitory activity	Inducing activity	Inhibitory compound	Inducing compound	Change in C_{max}	Change in AUC	Clinical recommendations	Clinical relevance	Reference
Ponatinib	CYP3A4	CYP2D6, CYP2C8, CYP3A5	NA	NA	ketoconazole		47% increase	78% increase	When administered with strong CYP3A4 inhibitors a dose-reduction to 30mg may be administered. The co-administration of strong inducers should be avoided or therapy duration should be minimized.	Moderate	14, 15, 88, 89
						Rifampicin	42% decrease	62% decrease			
Regorafenib	CYP3A4	UGT1A9	*In vitro:* UGT1A1, UGT1A9, CYP2C8, CYP2B6, CYP2C9, CYP2C19, CYP3A4 Irinotecan metabolite (SN-38) (substrate of UGT1A1) AUC increased with 44%	NA	ketoconazole		40% increase	33% increase	Co-administration with strong inhibitors or inducers of CYP3A4 and UGT1A9 should be avoided. Influence on regorafenib plasma levels is relatively small. Regorafenib dose must be gradually increased when administered with strong CYP3A4 inhibitors and close monitoring of side-effect is recommended when administered with strong CYP3A4 inducers. Toxicity must be monitored for UGT1A1, UGT1A9, CYP2C8, CYP2C9, CYP2C19 or CYP3A4 substrates, however pharmacokinetic data did not result in clinically meaningfull interactions.	Moderate	14, 15
						Rifampicin	20% decrease	50% decrease			
Ruxolitinib	CYP3A4	CYP2C9	Intestinal CYP3A4	NA	ketoconazole erythromycin		33% increase 8% increase	91% increase 27% increase	When administered with strong inhibitors of CYP3A4 and CYP2C9 a 50% dose reduction may be applied if there is relevant toxicity. For moderate inhibitors a dose reduction is not necessary. For strong CYP3A4 and CYP2C9 inducers side-effects should be closely monitored.	Moderate	14, 15, 90
						Rifampicin	52% decrease	71% decrease			
Sorafenib	CYP3A4	UGT1A9	UGT1A9, UGT1A1 Administration with cyclophosphamide (a CYP2B6 substrate), warfarin, midazolam, dextromethorphan, omeprazoel or paclitaxel did not result in any significant changes in AUC of these substrates.	NA	ketoconazole		26% increase	11% increase	Sorafenib administration with strong inhibitors or inducers of CYP3A4 is considered safe. For UGT1A1 and UGT1A9 substrate specific side-effects should be closely monitored. The use of strong UGT1A9 inhibitors or inducers should be avoided.	Minor	14, 15
						Rifampicin	no significant difference	37% reduction			
Sunitinib	CYP3A4	CYP1A2	NA	NA	ketoconazole		49% increase	51% increase	Dose reduction is advised when co-administered with strong CYP3A4 inhibitors to a minimum of 37.5mg for GIST and metastatic renal cell carcinoma or 25mg for neuro-endocrine tumors based on monitoring of tolerability. For strong CYP3A4 inducers an increase in 12.5 mg increments may be applied with monitoring of tolerability.	Minor	14, 15
						Rifampicin	23% decrease	46% decrease			

TABLE 3. Continued

MKI	Major CYP	Minor CYPs and others	Inhibitory activity	Inducing activity	Inhibitory compound	Inducing compound	Change in C_{max}	Change in AUC	Clinical recommendations	Clinical relevance	Reference
Tivozanib	CYP3A4	UGT1A, CYP1A1	CYP2B6, CYP2C8	NA	Ketoconazole		3% decrease	5% increase	Administration with strong inhibitors of CYP3A4 Is considered safe. The use of strong CYP3A4 inducers must be minimized and close monitoring of side-effect is recommended. Also close monitoring of side-effects is recommended when administered with CYP2B6 or CYP2C8 substrates.	Moderate	14, 15, 91
						Rifampicin	9% increase	52% decrease			
Trametinib	Deacetylation and glucuronidation	CYP3A4	CYP2C8, CYP2C9, CYP2C19 (*in vitro*)	CYP3A4 (*in vitro*)	No studies available		NA	NA	Administration with strong inhibitors or inducers of CYP enzymes is considered safe since primary metabolism is not due to metabolism. DDI potential is likely to be low.	Minor	14, 15
						no studies available	NA	NA			
Vandetanib	CYP3A4	FMO1, FMO3	CYP2D6	CYP1A2, CYP2C9, CYP3A4 Midazolam AUC did not change	Itraconazole		4% decrease	9% increase	Administration with strong inhibitors and inducers of CYP3A4 is considered safe. Concomitant administration with strong inducers must be avoided or dose may be gradually increased. When administered with substrates for CYP2D6, CYP1A2, CYP2C9 and CYP3A4 close monitoring of side-effects is recommended.	Minor	14, 15, 92
						rifampicin	3% increase	40% decrease			
Vemurafenib	CYP3A4	UGT	*In vitro:* CYP1A2, CYP2C8, CYP2C9 150% increase in caffeine (CYP1A2 substrate) exposurewas seen Warfarin (CYP2C9 substrate) exposure increased with 18%	CYP3A4, CYP2B6 Midazolam AUC decreased with 32%	no completed clinical study		NA	NA	The influence of CYP3A4 or UGT inhibitors or inducers is considered minimal. When administered with CYP1A2, CYP2C8, CYP2C9, CYP3A4 or CYP2B6 substrates close monitoring of side-effects is recommended.	Minor	14, 15
						rifampicin	unknown	40% decrease			

Legend: Clinical relevance is scored by means of the FDA Clinical Drug Interaction Studies — Study Design, Data Analysis, and Clinical Implications Guidance for Industry, for inducers as Major (AUC decrease ≥80%), Moderate (AUC decrease ≥ 50% to < 80%), Minor (AUC decrease ≥20% to <50%) or unknown and for inhibitors as Major (AUC increase ≥ 400%), Moderate (AUC increase ≥ 100% to < 400%), Minor (AUC increase ≥25% to <100%) or unknown as on the basis of the available evidence regarding inhibitory concentrations and the assessment report. Clinical relevance was scored on the basis of the highest score. NA is not applicable/not available. Abbreviations: CYP = Cytochrome P450 iso-enzyme, UGT = UDP-glucuronosyltransferase FMO = Flavin-containing monooxygenase Major CYP inhibitors: CYP1A2: Ciprofloxacin, enoxacin, fluvoxamine, zafirlukast CYP2C8: clopidogrel, gemfibrozil CYP2C9: fluconazole CYP2C19: fluconazole, fluoxetine, fluvoxamine, ticlopidine CYP2D6: bupropion, fluoxetine, paroxetine, quinidine, terbinafine, cinacalcet CYP3A4: boceprevir, cobicistat, conivaptan, danoprevir, elvitegravir, ritonavir, grapefruit juice, indinavir, itraconazole, ketoconazole, lopinavir, paritaprevir, posaconazole, ritonavir, saquinavir, telaprevir, tipranavir, troleandomycin, voriconazole, clarithromycin, diltiazem, idelalisib, nefazodone, nelfinavir, itraconazole, ketoconazole Major CYP inducers: CYP2B6: carbamazepine CYP2C9: carbamazepine, enzalutamide CYP2C19: enzalutamide, rifampicin, ritonavir CYP3A4: carbamazepine, enzalutamide, mitotane, phenytoin, rifampin, St. John's wort (Administration, 2018; Konig, Muller, & Fromm, 2013)1, 58, 93

Cabozantinib.

Cabozantinib is used in the treatment of medullary thyroid carcinoma and renal cell carcinoma (RCC). Since cabozantinib is a P-gp and BCRP inhibitor, close monitoring of side effects of substrates with a narrow therapeutic window is recommended when co-administered with cabozantinib.[14,15] A study with ketoconazole and rifampicin showed a significant change in AUC (38% increase and 77% decrease, respectively).[75] There was no significant effect of cabozantinib on rosiglitazone (a CYP2C8 substrate) plasma pharmacokinetics, indicating no inhibitory effect on CYP2C8 in contrast to the in vitro data.[75] The product label recommends minimizing the risk of a DDI by avoiding co-administration with strong inducers or inhibitors of CYP3A4. If necessary, a dose adjustment (decrease or increase) of 20mg following a step-by-step approach may be warranted.

Ceritinib.

Ceritinib is used in the treatment of ALK-positive NSCLC. Ceritinib is a substrate and inhibitor for P-gp. Furthermore, ceritinib is mainly metabolized by CYP3A4. Treatment with ketoconazole resulted in 190% and 20% increase in ceritinib AUC and C_{max}, respectively.[14,15] Coadministration with rifampicin showed a 70% and 44% decrease in AUC and C_{max}, respectively.[14,15] If concomitant administration with strong inhibitors of CYP3A4 is unavoidable a dose reduction by one third of the initial dose is necessary (rounded to units of 150mg). For strong CYP3A4 inducers gradual dose escalation is possible with close monitoring of MKI-specific side effects.

Cobimetinib.

Cobimetinib is a BRAF inhibitor used in the treatment of melanoma. It is a substrate for P-gp and inhibits BCRP, OATP1B1, OATP1B3, and OCT1.[14,15] Therefore, close monitoring of side effects is warranted when cobimetinib is administered with BCRP (e.g. rosuvastatin), OATP1B1, OATP1B3 (e.g. atorvastatin) or OCT1 substrates (metformin) with a narrow therapeutic window. Cobimetinib is primarily metabolized by CYP3A4 and UGT2B7. When co-administered with itraconazole 570% and 220% increase in AUC and C_{max} was seen, respectively.[14,15] A physiologically based pharmacokinetic (PBPK) model demonstrated rifampicin to decrease cobimetinib AUC by 83% and C_{max} by 63%.[76] So, the co-administration with strong inhibitors or inducers of CYP3A4 and P-gp must be avoided. However, rabeprazole (a P-gp inhibitor) showed no effects on the pharmacokinetics of cobimetinib.[21] If concomitant use of cobimetinib and strong CYP3A4 inhibitors is unavoidable, the cobimetinib dose should be decreased with

20mg (33%) following a step-by-step approach. Furthermore, since cobimetinib is a CYP1A2 inhibitor, concomitant use with CYP1A2 substrates (e.g. haloperidol) may lead to altered plasma concentrations of these substrates.[14,15]

Dabrafenib.

Dabrafenib is a BRAF inhibitor used in the treatment of advanced melanoma and NSCLC. Dabrafenib was shown to be a substrate for P-gp and BCRP. Since the bioavailability of dabrafenib is high (95%), only limited pharmacokinetic effects can be expected with inhibitors and inducers of these drug transporters. Dabrafenib is metabolized by both CYP3A4 (24%) and CYP2C8 (67%). Administration of dabrafenib with ketoconazole, gemfibrozil (a CYP2C8 inhibitor), and rifampicin showed significant changes in AUC, however these effects were mostly relatively small.[14,15] Furthermore, dabrafenib is known to auto-induce CYP3A4 mediated metabolism.[14,15] In conclusion, concomitant administration with strong CYP3A4 and CYP2C8 inhibitors or inducers must be avoided. Furthermore, a study with warfarin showed a 37% and 33% decrease in AUC and an 18% and 19% decrease in Cmax for S-warfarin (a CYP2C9 substrate) and R-warfarin (a CYP3A4/CYP1A2 substrate), respectively.[78] Therefore, dabrafenib is characterized as a moderate CYP3A4 inducer and a weak CYP2C9 inducer and as a result concomitant use of substrates for these enzymes must be avoided.[78]

Ibrutinib.

Ibrutinib is used as treatment for chronic lymphatic leukemia (CLL) and mantle cell lymphoma. Ibrutinib is an inhibitor of P-gp and BCRP.[14,15] Ibrutinib is mainly metabolized by CYP3A4. Ketoconazole gave 2800% and 2300% increase in C_{max} and AUC respectively.[14,15,51] Furthermore concomitant administration with rifampicin showed 92% and 90% decrease in C_{max} and AUC respectively.[14,15] Administration with a moderate inhibitor of CYP3A4 (e.g. erythromycin) led to 240% and 200% increase in C_{max} and AUC respectively.[14,15,82] Overall concomitant administration with strong CYP3A4 inhibitors or inducers must be avoided. If ibrutinib is administered with moderate and strong CYP3A4 inhibitors the ibrutinib dose should be reduced to 280mg and 140mg respectively. When ibrutinib is administered with substrates of P-gp and BCRP monitoring of side effects of these substrates is warranted. When toxicity appears the dose of these substrates may be decreased.

Lenvatinib.

Lenvatinib is used in the treatment of RCC and advanced thyroid carcinoma. It was shown to be a MDR1 substrate, a P-gp and BCRP substrate and inhibitor and an OATP1B3 inhibitor in vitro.[14,15] When lenvatinib is administered with ketoconazole or

rifampicin, only marginal changes in AUC and C_{max} were observed.[54,55] Since lenvatinib is mainly metabolized through several phase II mechanisms (e.g. aldehyde oxidase and glutathione conjugation) into less active metabolites and only for a small part by CYP3A4, these changes were most likely due to an interaction with P-gp.[14,15] Lenvatinib has an overall low DDI potential and dose modifications are currently not considered necessary.

Nintedanib.

Nintedanib is used in the treatment of NSCLC. It is a substrate and weak inhibitor of P-gp.[14,15,94] When nintedanib is administered with a strong P-gp inhibitor, a 100mg (25%) step-wise daily dose reduction must be considered with close monitoring of side effects. Use of strong P-gp inducers must be avoided, since nintedanib plasma concentrations may decrease. Nintedanib is mainly metabolized due to hydrolysis by esterases and glucuronidated by UGT with only a minor involvement of CYP enzymes (CYP3A4; 5%).[14,15] Administration with ketoconazole resulted in 61% and 83% increase in AUC and C_{max} respectively and administration with rifampicin demonstrated a decrease in AUC of 50% and 60% of C_{max} respectively.[42] These differences were probably due to a DDI with P-gp. Therefore, concomitant administration with strong inhibitors or inducers of CYP3A4 is considered safe.

Osimertinib.

Osimertinib is used in the treatment of NSCLC.[14,15] Osimertinib is a substrate and inhibitor for P-gp and BCRP.[14,15] A study with rosuvastatin (a sensitive BCRP substrate) showed an increase in AUC and C_{max} of 35% and 72% of rosuvastatin respectively.[87] Osimertinib is mainly metabolized by CYP3A4 and CYP3A5, but only rifampicin resulted in a significant change in both AUC and C_{max} in contrast to itraconazole.[86] A study with simvastatin (a CYP3A4 substrate) resulted in a slight decrease in AUC and C_{max} of simvastatin of 9% and 23%, but these changes are not considered to be of clinical significance.[87] In conclusion only strong CYP3A4 inducers must be used with caution and close monitoring of side effects of osimertinib is warranted.

Ponatinib.

Ponatinib is used in the treatment of CML and Acute lymphatic leukemia (ALL). Ponatinib is a substrate and inhibitor of P-gp and BCRP.[14,15] Therefore, concomitant use of ponatinib with strong inhibitors or inducers of these transporters should be avoided. Ponatinib is mainly metabolized into nonactive metabolites by CYP3A4 and to a lesser extent by CYP2D6, CYP2C8 and CYP3A5.[14,15] A study with concomitant ketoconazole administration showed an increase in C_{max} of 47% and 78% in AUC of

ponatinib.[88] Multiple dosing of rifampicin demonstrated a decrease in AUC and C_{max} of 42% and 62% respectively.[89] As a consequence, concomitant administration with inhibitors of CYP3A4 and P-gp should be avoided or a dose reduction to 30mg should be applied when administered concomitantly. Moreover, the use of strong CYP3A4 or P-gp inducers must be avoided or duration must be minimized, since ponatinib exposure may change.

Tivozanib.

Tivozanib is used in the treatment of RCC. Tivozanib is an inhibitor of BCRP and is metabolized by multiple liver enzymes, including CYP3A4, CYP1A1 and several UGT1A enzymes (e.g. UGT1A1, UGT1A3 and UGT1A7).[14,15] A study with rifampicin showed a 52% decrease in tivozanib AUC. Therefore, the administration with strong CYP3A4 inducers should be avoided. A dose escalation is not necessary since the effect on tivozanib exposure is relatively small. Ketoconazole did not result in significant changes in tivozanib exposure.[14,15,91] Administration with strong CYP3A4 inhibitors is therefore considered safe. Furthermore, the concomitant administration with strong UGT inhibitors or inducers (e.g. probenecid or ibuprofen) should be avoided since tivozanib plasma concentrations potentially may change.

Trametinib.

Trametinib is used in the treatment of melanoma and NSCLC. It is a known inhibitor of P-gp, BCRP, OAT1, OAT3, OATP1B1, OATP1B3, OAT2B1, OCT2 and MATE1 and a substrate for P-gp.[14,15] As a result, the use of strong inhibitors or inducers of P-gp (e.g. ketoconazole) must be avoided. Trametinib is metabolized through deacetylation, oxidation and glucuronidation pathways.[14,15] No drug interaction studies are available to date, however since trametinib is not dependent on CYP isoenzymes, no DDIs with CYPs are to be expected.

DDI studies with longer available MKIs

In recent years several new studies have been published that investigated DDIs with longer available MKIs. Most of these studies are listed in **Tables 1–3**. There are only a few clinical DDI studies concerning drug transporters, since most studies mainly focus on CYP interactions. A phase I study investigated the combination of gefitinib and irinotecan and found an increase in SN-38 (the active irinotecan metabolite) and irinotecan plasma exposure, attributed to an enhanced BCRP activity in the gut.[50] Moreover, in patients using sorafenib with rifampicin, the concentration of the metabolite sorafenib-glucuronide increased, suggesting inhibition of OATP1B1 by rifampicin and confirms sorafenib as an OATP1B1 substrate.[57]

Several new studies investigated possible DDIs regarding drug metabolism. For a complete overview see **Table 3**. For example: imatinib coadministration caused a 26% increase in cyclosporine (CYP3A4 and CYP2C8 substrate) plasma levels, explained by CYP3A4 inhibition by imatinib.[69] In addition, lapatinib and pazopanib demonstrated an increase of 23% and 26% in paclitaxel AUC respectively, suggesting inhibition of CYP2C8 by these MKIs.[83,95] Furthermore, regorafenib significantly increased the exposure to irinotecan and its active metabolite SN-38 due to UGT1A1 inhibition.[96,97]

Although most MKIs are metabolized through CYP enzymes it becomes more apparent that MKI metabolism is multifactorial and the inhibition and induction of other pathways (such as drug transporters) may also significantly influence MKI exposure. More research is needed to fully assess the DDI potential of these new pathways and their clinical relevance.

DISCUSSION

Many MKIs have a narrow therapeutic window, with a clear relation between exposure and response on one hand and toxicity on the other.[98] For example, sunitinib and pazopanib show increasing severe toxicity with raising plasma concentration, leading to dose reductions and discontinuation of treatment in many patients.[99,100] Meanwhile, a threshold for efficacy for these drugs is seen.[98–100] Therefore, it is important to provide the right dose for the individual patient, in order to optimize treatment efficacy and minimize toxicity. To accomplish this, there is a shifting paradigm towards personalized dosing in oncology practice.[5] Along with other factors, DDIs are key factors influencing MKI exposure and subsequent clinical outcome. In addition, cancer patients are at greater risk for DDIs.[7] Therefore, a structured medication review for clinically relevant DDIs should take place on a regular basis.

To create a solid base for medication review, more DDI studies are strongly needed and results should be weighed on their clinical relevance. Specific and practical guidelines must be developed to guide clinicians and pharmacists in the management of DDIs in clinical practice. A practical way to reach this goal is by establishing clinical expert groups for consensus-based evaluation of clinical significance and management of the DDIs.[101]

ASAs may strongly decrease MKI bioavailability. Since there is no clear general consensus on the management of this DDI we presented a practical advice for all ASAs. However, another problem is that there is no standard design for clinical DDI research with ASAs. Ideally, drug exposure should be compared in a crossover design

between MKI monotherapy and during co-administration of the strongest ASA [e.g. the PPI esomeprazole (40mg)] 3 h prior to MKI administration, since maximum intragastric pH elevating effect of this PPI is reached after this time period.[38] In that case, when no effects are seen, a DDI between MKIs and PPIs can be ruled out. When a significant DDI with H2-antagonists and antacids is expected, a corresponding treatment arm may be added. A more standardized study design of these ASA-DDI studies may provide a solid basis for practical management of this DDI, since study results could more easily be interpreted and compared between different MKIs.

Drug transporters are located throughout the body and thus potentially influence pharmacokinetics on multiple levels.[39] To date, insufficient attention has been given to the clinical relevance of these DDIs concerning drug transporters. Unfortunately, there is a lack of clinical studies investigating this type of DDI. Furthermore, many registration studies use ketoconazole or rifampicin as an inhibitor or inducer of CYP3A4, but these drugs are also strong inhibitors or inducers of P-gp. As a result, the P-gp effect may be underestimated or overestimated in the assessment reports. More research is needed to fully assess the DDI potential concerning drug transporters.

In contrast, DDIs with drug transporters may also be used for beneficial purposes. For instance, inhibition of certain drug transporters (e.g. P-gp) in the blood–brain barrier might theoretically lead to altered blood–brain barrier penetration, which may result in better brain (metastasis) penetration of a MKI, for example, osimertinib.[102] In addition, Zimmerman and colleagues demonstrated a protective effect on hand-foot skin reaction in mice, a frequently seen side effect of sorafenib, when sorafenib was concomitantly taken with the OAT6 inhibitor probenecid.[103]

Furthermore erlotinib may reduce cisplatin toxicity (e.g. nephrotoxicity and ototoxicity) through OCT2 inhibition.[48] Such potentially useful applications of DDIs between MKIs and drug transporters need to be further explored, and may in the future result in more effective MKI therapy.

In current DDI research there is a trend towards a model-based DDI prediction, like the PBPKmodels.[104,105] PBPK-models are multi-compartmental (often represented as single organs or tissues) models which use (in vitro) pharmacokinetic data and human physiologically-dependent system parameters to predict DDIs with a mathematical model.[106] A disadvantage of PBPK modeling is the lack of sufficient in vivo data that adds to the uncertainty in the predictions of the PBPK model. Also, the lack of knowledge regarding multifactorial physiologic changes in, for instance, enzyme and transporter

expression and activity might be a possible confounding factor. Despite the evident benefits of PBPK modeling in current DDI research, confirmatory evidence from clinical trials in humans is needed to assess a good predicting model.[105]

Another novel approach in oncology in managing DDIs is therapeutic drug monitoring (TDM). For many MKIs there is a clear relationship between exposure, toxicity and treatment efficacy (e.g. imatinib, pazopanib and sunitinib).[98,100,107] For some MKIs TDM could be an alternative way to manage DDIs in MKI therapy, where dose adjustments can be made if plasma levels are outside the therapeutic range. Furthermore, TDM has the advantage of monitoring MKI treatment, continuously over a longer time period which may result in better therapy efficacy. However, further research is needed to confirm the clinical relevance of TDM as a tool in DDI management.

In conclusion, most MKIs are highly prone to cause DDIs. Drugs that elevate intragastric pH, strong inhibitors or inducers of CYP enzymes and drug transporters can result in clinically relevant changes in MKI exposure. For many DDIs the only evidence for a potential DDI comes from in vitro data or is predicted based on PBPK modeling. Without clinical data it is difficult to determine the exact clinical relevance of these possible DDIs. In this review, we present practical recommendations for management of MKI interactions in clinical practice. Acknowledging these DDIs by clinicians may eventually result in a more personalized and effective treatment with MKIs.

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CHAPTER 3

FACTORS AFFECTING THE ASSOCIATION OF PROTON PUMP INHIBITORS AND CAPECITABINE EFFICACY IN ADVANCED GASTROESOPHAGEAL CANCER

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With interest, we read the report by Chu and colleagues, investigating the influence of proton pump inhibitors (PPIs) on capecitabine efficacy in advanced gastroesophageal cancer.[1] This retrospective analysis revealed a significant difference in treatment efficacy in patients treated with capecitabine and oxaliplatin (CapOx) with and without concomitant proton pump inhibitor (PPI) use, probably due to an increased intragastric pH --and subsequent-- impaired capecitabine absorption.

Although this is a highly clinically relevant finding, the conclusions are preliminary in our opinion. One of our main concerns is that the time of PPI intake (in particular in relation to the time of capecitabine intake) was not studied. We believe this is of importance, since PPIs show a delayed onset of action and reach their maximum elevation in intragastric pH only 3-4 hours after administration.[2] Furthermore, the elevation of intragastric pH by PPIs is only present for approximately 12 hours, which theoretically makes it possible to combine these agents with less (or even without) negative effects on the exposure of capecitabine.[2] Meanwhile, if the PPI is taken 4-16 hours before capecitabine, the effect on capecitabine exposure (due to the maximal elevation of intragastric pH by the PPI) may be larger compared to a simultaneous intake. In this particular subgroup, also the effects on outcome may be worse than published by Chu et al.

Two other relevant aspects, i.e. PPI dose and type of PPI, have not been taken into account in this study either. There is a significant difference in acid suppression potential between the various PPI variants.[3] In addition, the dose of the PPI used has a positive correlation with the magnitude and duration of gastric acid suppression.[4] Therefore, these issues need to be addressed before a general conclusion on the (negative) effects of PPI use on capecitabine efficacy can be drawn.

A final concern that needs to be addressed is the lack of sufficient medication verification during the study. Along with other factors, drug interactions can significantly alter anticancer drug exposure and efficacy. Drug interactions between CapOx and other concomitantly taken medications could not be excluded, as was already mentioned by Chu et al. However, it cannot be emphasized enough, that drug interactions are frequently seen in cancer patients.[5]

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CHAPTER 4

INFLUENCE OF THE PROTON PUMP INHIBITOR ESOMEPRAZOLE ON THE BIOAVAILABILITY OF REGORAFENIB:

A RANDOMIZED CROSSOVER PHARMACOKINETIC STUDY

CLINICAL PHARMACOLOGY & THERAPEUTICS; 2019 JUN;105(6):1456-1461

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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**).

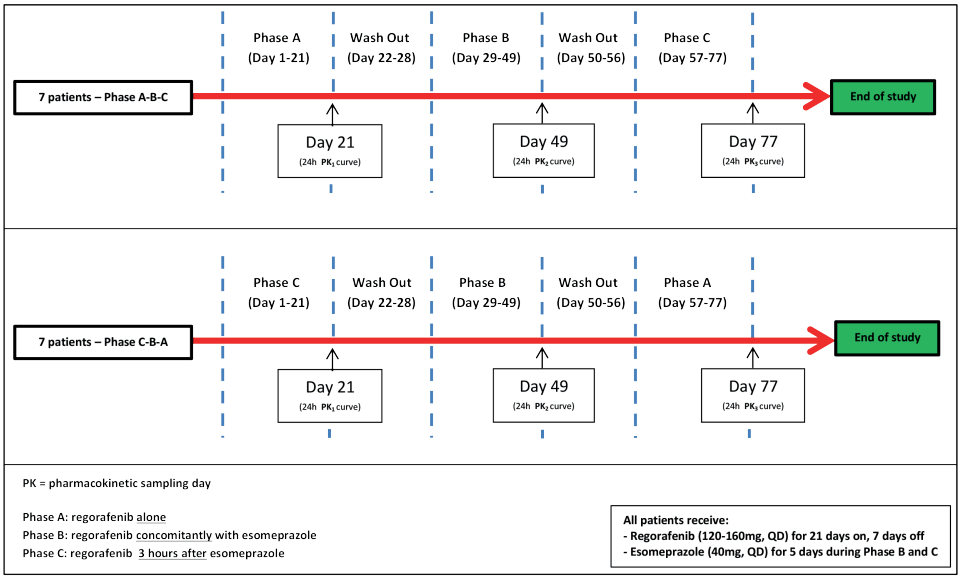

FIGURE 1. Study procedures

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.

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

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.

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).

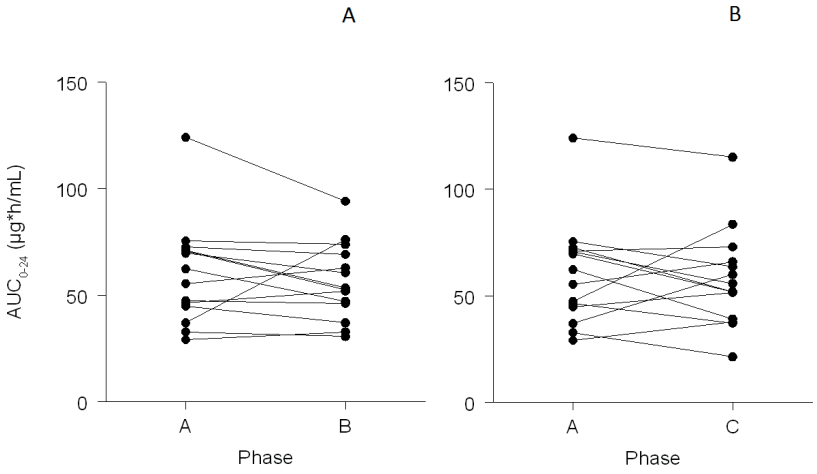

FIGURE 2. Regorafenib AUC**.** Regorafenib exposure compared between phase A (regorafenib alone) and phase B (regorafenib concomitantly with esomeprazole) (figure 2A), and between phase A and C (regorafenib with esomeprazole 3 hours prior) (figure 2B)
Abbreviations: $AUC_{0\text{-}24}$ = Area under the 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							
$AUC_{0\text{-}24h}$ (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							
$AUC_{0\text{-}24h}$ (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							
$AUC_{0\text{-}24h}$ (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: $AUC_{0\text{-}24h}$ = 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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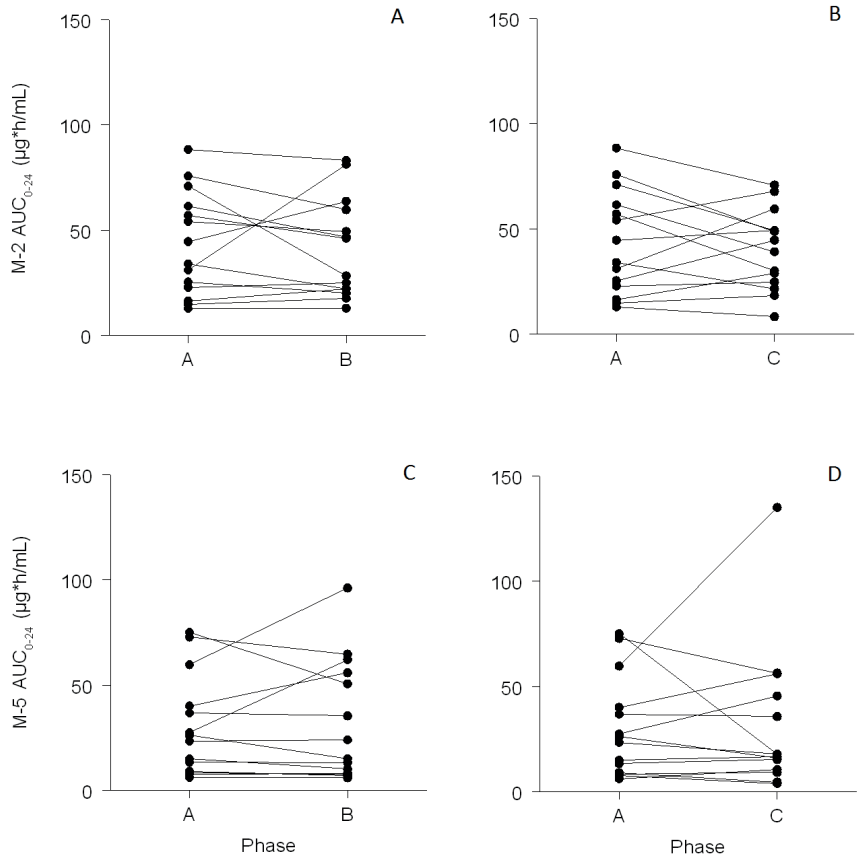
SUPPLEMENTARY INFORMATION

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

SUPPLEMENTARY TABLE 1. Continued

Toxicity[a]	Regorafenib N (%)	Regorafenib + Esomeprazole Concomitant N (%)	Regorafenib + Esomeprazole 3h prior N (%)	Overall[b] N (%)
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
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

CHAPTER 5

EFFECTS OF PREDNISONE ON DOCETAXEL PHARMACOKINETICS IN MEN WITH METASTATIC PROSTATE CANCER:

A RANDOMIZED DRUG-DRUG INTERACTION STUDY

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ABSTRACT

Aim. Docetaxel has been approved for the treatment of metastatic prostate cancer in combination with prednisone. Since prednisone is known to induce the cytochrome P450 iso-enzyme CYP3A4, which is the main metabolizing enzyme of docetaxel in the liver, a potential drug-drug interaction (DDI) may occur. In this prospective randomized pharmacokinetic cross-over study we investigated docetaxel exposure with concomitant prednisone, compared to docetaxel monotherapy in men with metastatic prostate cancer.

Methods. Patients scheduled to receive at least 6 cycles of docetaxel (75 mg/m2) and who lent written informed consent, were randomized to receive either the first 3 cycles, or the last 3 consecutive cycles with prednisone (BID 5mg). Pharmacokinetic blood sampling was performed during cycle 3 and cycle 6. Primary endpoint was difference in docetaxel exposure, calculated as area under the curve ($AUC_{0\text{-}inf}$) and analyzed by means of a linear mixed model. Given the cross-over design the study was powered on eighteen patients to answer the primary, pharmacokinetic, endpoint.

Results. Eighteen evaluable patients were included in the trial. Docetaxel concentration with concomitant prednisone ($AUC_{0\text{-}inf}$ 2784 ng*h/mL, 95% CI 2436-3183 ng*h/mL) was similar to the concentration of docetaxel monotherapy ($AUC_{0\text{-}inf}$ 2647 ng*h/mL, 95%CI 2377-2949 ng*h/mL). Exploratory analysis showed no toxicity differences between docetaxel monotherapy and docetaxel cycles with prednisone.

Conclusion. No significant difference in docetaxel concentrations was observed. In addition, we found similar toxicity profiles in absence and presence of prednisone. Therefore, from a pharmacokinetic point of view, docetaxel may be administrated with or without prednisone.

INTRODUCTION

Docetaxel, a taxane chemotherapeutic agent, was approved by the Food and Drug Administration (FDA) and European Medicines Agency (EMA) in 2004 as first-line chemotherapy for metastatic castration-resistant prostate cancer (mCRPC) as a result of survival benefit obtained in TAX327.[1,2] In that study, mitoxantrone plus prednisone treatment was compared to a 3-weekly docetaxel (75 mg/m^2) regimen in mCRPC patients. Prednisone (5 mg BID) was added to docetaxel to equally compare both treatment arms, although the preceding phase 2 trials with docetaxel (36 mg/m^2, weekly) in mCRPC had been conducted without prednisone.[3,4] In the final analysis, treatment with docetaxel plus prednisone improved overall survival (OS) with 2.9 months compared to the mitoxantrone group. Subsequently, docetaxel and prednisone became first-line chemotherapy for mCRPC .

After the registration of docetaxel plus prednisone, the role of corticosteroids in the treatment of mCRPC remained controversial. In patients with symptomatic bone metastases corticosteroids may have a favorable palliative effect, and a reduction in docetaxel-induced toxicity has been suggested.[5-7] However, the effect of prednisone on OS in mCRPC patients remains unclear.[6,8] Of note, prolonged use of corticosteroids may lead to the development of multiple severe toxicities including osteoporosis, adrenal insufficiency, immune suppression, and may exacerbate comorbidities like diabetes.[9] These side-effects of long-term corticosteroid are a justifiable reason to reconsider the addition of prednisone to the docetaxel regimen.

Recently, two large clinical trials, CHAARTED and STAMPEDE, assessed the survival benefit of docetaxel combined with androgen-deprivation therapy (ADT) in metastatic hormone-sensitive prostate cancer (mHSPC).[10,11] In order to avoid long term exposure to steroids, the investigators of the CHAARTED trial decided to administer docetaxel without prednisone, whereas docetaxel was administered with prednisone in the STAMPEDE study. At the time of the initiation of our study, only the results of CHAARTED were available, showing a robust survival benefit of 13.6 months compared to androgen deprivation therapy alone. Toxicity rates were similar to previously published work on docetaxel plus prednisone in mCRPC patients , except for a higher febrile neutropenia rate in CHAARTED without prednisone, as compared to TAX327 where docetaxel was administered with prednisone.[1,12] Likewise, a retrospective trial by Kongsted *et al.* showed that the toxicity rates of febrile neutropenia and edema were significantly higher in the docetaxel monotherapy group compared to the docetaxel plus prednisone-group (for an overview of toxicity rates previously reported on docetaxel with or without prednisone, see **Table 1**).[5]

TABLE 1. Literature review of docetaxel toxicities with and without prednisone

Trials	Prednisone	Neutropenia (Gr3-4)	Febrile neutropenia
TAX-327	Yes	32%	3%
Venice	Yes	7%	<1%
Mainsail	Yes	16%	5%
GETUG-AFU15	No	32%	8%
CHAARTED	No	12%	6%
STAMPEDE	Yes	12%	15%
Kongsted et al.	No	-	25%
	Yes	-	10%

As a underlying mechanism, prednisone could influence docetaxel pharmacokinetics via the CYP3A4 iso-enzyme. Glucocorticoids are known as CYP3A inducers, and docetaxel is mainly metabolized in the liver by the cytochrome P450 iso-enzymes CYP3A4 and CYP3A5.[13] Consequently, this potential drug-drug interaction could lead to higher clearance of docetaxel and therefore diminished docetaxel exposure. In this study, we therefore investigated the effects of prednisone on docetaxel pharmacokinetics in patients with metastatic prostate cancer.

METHODS

This prospective, randomized, cross-over pharmacokinetic trial was carried out between September 2016 and February 2018 at the Erasmus MC Cancer Institute in Rotterdam, the Netherlands. The study protocol was approved by the Ethical board of the Erasmus MC, and the study was conducted according to the ethical guidelines of the Declaration of Helsinki. All participants signed informed consent before start of the study. The study was registered at the European Clinical Trials Database (EudraCT 2016-001269-10) and the Dutch Trial Register ('www.trailregister.nl' by NTR-number NTR6037 or acronym Doc-Pred).

Patients

We included patients with histologically confirmed metastatic prostate cancer, both hormone-sensitive or castration-resistant, who were scheduled to receive a minimum of 6 cycles of docetaxel chemotherapy. Eligible patients were 18 years and older, with an Eastern Cooperative Oncology Group (ECOG) performance status of 0 or 1. Adequate organ function was required, defined by creatinine clearance > 60mL/min, bilirubin levels <1x ULN, ALAT/ASAT <2.5x ULN, alkaline phosphatase (AF) < 5x ULN,

absolute neutrophil count > 1,5x10^9/L and platelets > 100x10^9/L. Patients had to be castrated either by continued androgen deprivation therapy (ADT) with gonadotropin releasing hormone (GnRH) analogues or by surgical orchiectomy. It was preferred ADT started four weeks prior to chemotherapy, to reach castration-levels of testosterone before treatment start. Prior hormonal treatment, like enzalutamide and abiraterone, was allowed. However, these therapies, including prednisone, had to be stopped at least 6 weeks before the start of this study. Medication or herbal supplements known to induce or inhibit CYP3A pathway were prohibited.

Study Design

Patients received 6 consecutive cycles of 3-weekly docetaxel (75 mg/m^2) and were digitally randomized to receive either the first 3 docetaxel cycles or the last 3 cycles with prednisone (cross-over). Prednisone 5 mg BID was administered during three consecutive cycles. Prednisone started at day 1 of cycle 1 or cycle 4 and was stopped after the last day of cycle 3 or cycle 6 (depending on randomization arm, A or B respectively). Prednisone dose-modifications were not allowed during the last week before pharmacokinetic sampling (cycle 3 day 1 and cycle 6 day 1) and patient compliance was assessed through a patient diary. Docetaxel dose-modifications because of hematological or non-hematological toxicities were allowed, and schedule modifications were allowed up to one week. Dexamethasone is a strong CYP3A4 inducer, its use, as premedication, was restricted to only 12 and 3 hours before docetaxel-infusion to reduce its influence on docetaxel pharmacokinetics.

Pharmacokinetic sampling

To have maximum inducible effects of prednisone on the CYP-enzymes and to ensure a sufficient wash-out period after prednisone, we decided to undertake PK-samples during cycle 3 and cycle 6. Hospital admission during the first day of the 3rd and the 6th docetaxel cycle was required to obtain 24-hour pharmacokinetic-blood samples. Blood/plasma samples for determination of docetaxel pharmacokinetics were taken at predefined time points (pre-infusion and at 0.5, 0.92, 1.25, 1.5, 2, 3, 4, 6, 8 and 24 hours after the start of docetaxel). Plasma concentrations of docetaxel were measured using a validated liquid chromatography with tandem mass spectrometry method (UP-LCMS/MS).[14] Pharmacokinetic parameters were docetaxel concentration, expressed as dose-corrected area under the curve from pre-infusion time-point to infinity ($AUC_{0\text{-}inf}$), maximum drug concentration (C_{max}), docetaxel half-life ($t_{1/2}$) and docetaxel clearance. $AUC_{0\text{-}inf}$ was calculated using a linear pharmacokinetic curve to estimate the residual AUC from the latest measurable pharmacokinetic point (24h-time-point).

Toxicity

Secondary endpoint was describing toxicity rates during docetaxel monotherapy cycles and docetaxel with prednisone cycles. Standard laboratory control was performed prior to each docetaxel cycle and when indicated according to the physician. Toxicities were scored using the CTCAE (v.4.0) grading. If relevant differences in toxicity rates between the treatment arms occurred, these were analyzed by means of McNemar test.

Statistical analysis

A difference in systemic exposure to docetaxel of 25% was determined to be clinically relevant and it was assumed that the within-patient standard deviation in docetaxel pharmacokinetics was 25%. Given a power of 80% and a two-sided alpha of 5%, 18 patients were required to detect a difference.[15] Since docetaxel dose-modifications were allowed, a dose-correction was applied for all docetaxel concentrations to the standard dose of 75 mg/m^2. All docetaxel cycles with prednisone were compared to all docetaxel cycles without prednisone, regardless of the randomization arm. Analyses of the $AUC_{0\text{-inf}}$ and C_{max} were performed on log-transformed values, since these parameters were assumed to follow a lognormal distribution.[16] Estimates for the mean differences in (log) $AUC_{0\text{-inf}}$, C_{max} and clearance were obtained using a linear mixed effect model with treatment, sequence, and period as fixed effects and subject within sequence as a random effect.[17] 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 their 95% CIs were exponentiated to provide point estimates of the ratio of geometric means and 95% CIs for these ratios, which can be interpreted as relative differences in percentages. $T_{1/2}$ was analysed by means of the Wilcoxon signed rank test and described with medians and interquartile ranges.

RESULTS

Patients

Twenty-nine patients were screened, of whom four were screen failures which were excluded from study participation (Figure 1). We randomized 25 patients to receive either cycles 1-3 with concomitant prednisone, and cycles 4-6 without prednisone (arm A, N=11), or *vice versa* (arm B, N=7). During treatment one patient withdrew consent in arm A, and six patients stopped treatment in arm B due to radiologic confirmed progression (*N*=3) or withdrawal of consent (*N*=3). Baseline patient and disease characteristics are shown in Table 2. All patients, except three mHSPC patients, received

the first cycle of docetaxel 4 weeks after initiation of ADT, to reach castration levels of testosterone. However, all patients received ADT for more than a month before PK samples during cycle 3 were withdrawn.

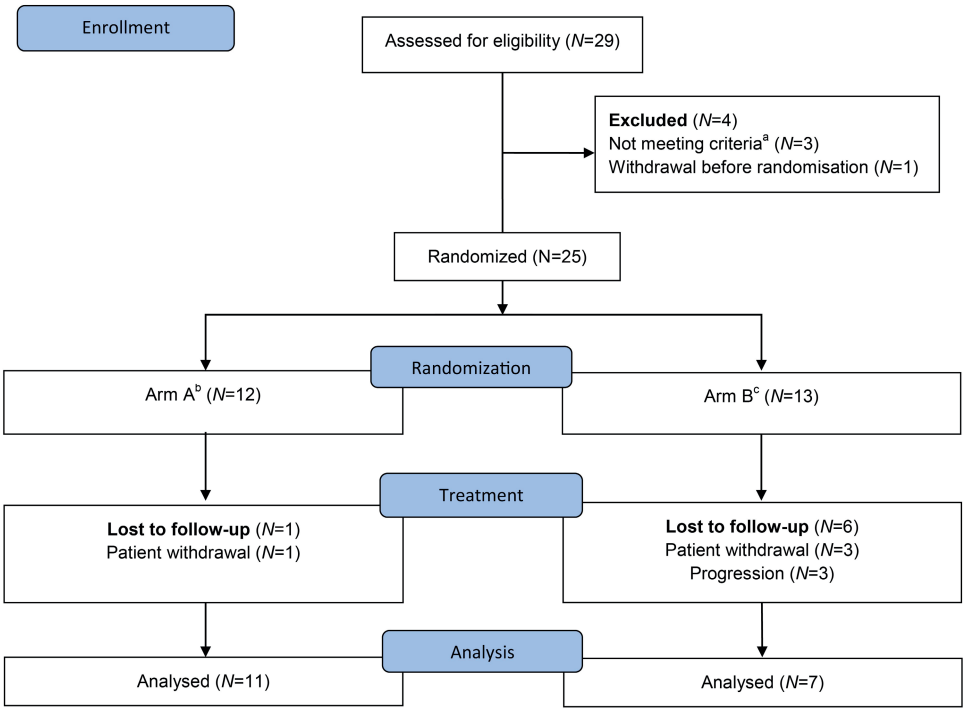

FIGURE 1. Flowchart

a. due to inadequate laboratory values

b. Arm A: Three cycles of docetaxel plusprednisone followed by three cycles of docetaxel alone

c. Arm B: Three cycles of docetaxel alone followed by three cycles of docetaxel plus prednisone

Pharmacokinetic parameters

The geometric mean exposure of docetaxel was not significantly different (1.9%, 95% CI -9.9% till 15.2%, P=0.75) during docetaxel with concomitant prednisone treatment (AUC_{0-inf} of 2784 ng*h/mL, 95% CI 2436-3183 ng*h/mL) compared to docetaxel monotherapy (AUC_{0-inf} of 2647 ng*h/mL, 95% CI 2377-2949 ng*h/mL). The pharmacokinetic variation, as expressed by coefficient of variation, was slightly higher in the docetaxel with prednisone arm as compared to docetaxel monotherapy (27% and 22% respectively). All pharmacokinetic parameters are shown in **Table 3** and were not significantly different for docetaxel with or without prednisone. Additionally, we graphically showed

TABLE 2. Patient and disease characteristics

Characteristic	***N* (%)**
Patients	18 (100)
Age (Median, IQR)	70 (62-73)
BMI (Median, IQR)	25.8(24.6-28.7)
WHO Performance Status	
0	8 (44)
1	10 (56)
Hormone Status	
Hormone sensitive	11 (61)
Castration resistant	7 (39)
Metastatic stage at screening	
M0	5 (28)
M1a	4 (22)
M1b	8 (44)
M1c	1 (6)
Gleason score at diagnosis	
≤ 7	4 (22)
> 7	14 (77)
Type of castration	
Bilateral orchidectomy	1 (6)
LHRH analogues	17 (94)
Previous therapy	
Radicale prostatectomy	1 (6)
RTx prostate	3 (16)
Hormone therapy	
Bicalutamide	6 (33)
Enzalutamide	2 (11)
Radium-223	1 (6)
Experimental therapy	1 (6)
Lab results at baseline	Median (IQR)
PSA, µg/L	20 (3-87)
Hb, mmol/L	8 (7-10)
LDH, U/L	196 (178-216)
AP, U/L	103 (70-160)
Albumin, g/L	44 (43-46)

Abbreviations: IQR = Inter Quartile Range, BMI = Body Mass Index, WHO = World Health Organizations, LHRH = luteinizing hormone releasing hormone, RTx = radiotherapy, PSA = prostate specific antigen, Hb = hemoglobin, LDH = lactate dehydrogenase, AP = alkaline phosphatase

differences in exposure of docetaxel in mCRPC patients (blue line) and mHSPC patients (red line), separately in arm A and arm B; see **Figure 2**. We performed a t-test on the complete patient group (arm A and arm B combined) and found no significant (P=0.2) difference between the exposure in mCRPC patients and mHSPC patients. Of note, we found a 13.4% (95% CI 2.1%-23.4%, P=0.025) lower exposure of docetaxel over time, independent from randomization or disease setting. This, so called, period-effect shows lower measured concentrations of docetaxel in cycle 6 compared to the concentrations in cycle 3, regardless of the addition of prednisone (**Figure 2**).

TABLE 3. Docetaxel pharmacokinetics

Docetaxel PK parameters	Docetaxel (*N*=18)	Docetaxel+Prednisone (*N*=18)	Relative difference (95% CI)	P-value
$AUC_{0\text{-}inf}$ [a] geomean ng*h/mL (CV%)	2647 (22)	2784 (27)	1.9% (-9.9 till 15.2)	0.75
C_{max} [a] geomean ng/mL (CV%)	2454 (26)	2505 (25)	-1.4% (-15.3 till 14.8)	0.85
CL[a] geomean, L/h (CV%)	55 (26)	53 (26)	-2.3% (-9.5 till 5-6)	0.53
$T_{1/2}$ [b] median, h (IQR)	12.6 (10.6-14.5)	13.7 (11.3-16.3)		0.31

Abbreviations: $AUC_{0\text{-}inf}$ = Area under curve timepoint zero until infinity, C_{max} = maximum concentration, CL = clearance, $T_{1/2}$ = half-life, geomean = geometric mean, CV% = coefficient of variation, CI = confidence interval, [a]= analyzed by means of a linear effect model, [b]= analyzed by means of Wilcoxon signed rank test.

Toxicity

Toxicity rates were similar between the cycles with and without prednisone, see **Table 4**, except for neutropenia. A non-significant trend towards a higher rate of all grade (gr 1-4) neutropenia (N=12) was observed in patients treated without prednisone as compared to with prednisone (44 vs 22%, P=0.22). Seven patients (39%) experienced an episode of grade 3 – 4 neutropenia. Three febrile neutropenia hospitalizations were observed, two of which happened during co-administration of prednisone. There was no difference in the disease setting; toxicity was equally distributed in castration-resistant and hormone-sensitive setting (data not shown).

TABLE 4. Toxicity with or without prednisone

	All grades	
Toxicity	**With prednisone *N* (%)**	**Without prednisone *N* (%)**
Nausea	3 (17)	5 (28)
Mucositis	9 (50)	8 (44)
Diarrhea	5 (28)	2 (11)
Sens PNP	6 (33)	6 (33)
Fatigue	12 (67)	13 (72)
Neutropenia	4 (22)	8 (44)
Febrile neutropenia	1 (6)	2 (11)
Nail toxicity	5 (28)	6 (33)
Edema	0 (0)	1 (6)
Dysgeusia	1 (6)	1 (6)

Abbreviations: Sens PNP = sensory polyneuropathy

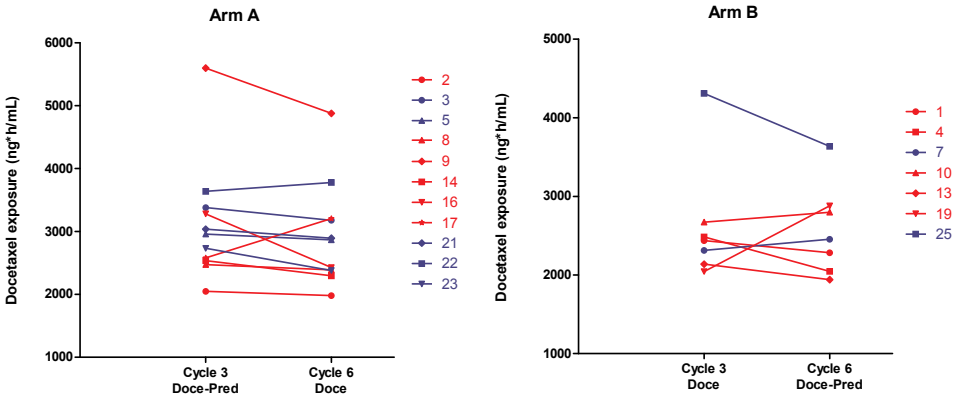

FIGURE 2. Docetaxel concentration by disease setting. Each line represents a patient for whom the measured docetaxel concentration (geomean $AUC_{0\text{-inf}}$) at cycle 3 and cycle 6 were connected with a line to visualize the concentration differences between the cycles. In the majority of the patients the measured concentration in cycle 6 is lower than in cycle 3, reflecting the period-effect observed in this study. The red lines represent patients in the hormone-sensitive disease setting and the blue lines represent the castration-resistant patients, subdivided by randomization arm.

DISCUSSION

In this randomized study, the effects of prednisone on the pharmacokinetics of docetaxel were evaluated. No significant difference in docetaxel exposure with or without the administration of prednisone was observed. This is the first *randomized* pharmacokinetic study investigating the effects of prednisone on the pharmacokinetics of docetaxel. From a pharmacological perspective, we conclude that prednisone did not affect the exposure of the docetaxel regimen.

Glucocorticoids are classified as inducers of the CYP3A enzyme,[18] and docetaxel is metabolized primarily by this iso-enzyme. Previously, an interaction study of docetaxel and prednisone has been published in the Clinical Study Report (CSR) of docetaxel and no relevant drug-drug interaction was reported.[19] However, that PK-study was not randomized and included only two docetaxel cycles; one

with prednisone and one without, possibly not providing enough time for optimal CYP-induction by prednisone. Moreover, that study was limited by sparse PK-sampling (only 6 samples during each cycle) and by limited pharmacokinetic endpoints of docetaxel (clearance only). Therefore, in our study, we used a randomized cross-over design including 6 cycles of docetaxel (3 cycles in absence and 3 cycles in presence of prednisone), an enriched sampling scheme with more relevant pharmacokinetic endpoints.

Although we corrected for dose-reductions due to toxicity over time, we unexpectedly did find a significant period-effect in this study. This means that a decrease in docetaxel exposure occurred in the consecutive cycles independent of randomization or treatment. This might be an explanation for the trend towards an overall higher incidence of (febrile) neutropenia seen at the start of chemotherapy cycles. There are a few potential explanations for this phenomenon. First, a time-dependent induction of CYP3A4 by upregulation of Pregnane X receptor (PXR) due to repetitive docetaxel exposure could occur.[20-23] This phenomenon is called 'auto-induction' and is previously described with several other agents, e.g. dabrafenib.[24] A second possible explanation is an upregulation of ABCB1 (P-glycoprotein) by docetaxel. P-glycoprotein is an active drug-efflux transporter at the cell membrane of hepatocytes, kidney cells and intestine cells. Its upregulation leads to an increased efflux of docetaxel out of the circulation, resulting in decreased plasma concentrations.[25] This phenomenon could even lead to pharmacokinetic resistance to the drug.[26,27] This period effect is unlikely to be caused by

castration-levels of the patients, since the maximum induction effect of ADT is reached after approximately 4 weeks , whereas in our study patients had received at least nine weeks of ADT at the time of PK sampling.

Interestingly, we observed no difference in docetaxel-induced toxicities in the absence or presence of prednisone, except for a non-significant difference in neutropenia. Because our study was not powered or designed for toxicity related questions, we can only conclude from a pharmacokinetic point of view that prednisone could be safely omitted from the docetaxel regimen.

The major benefit of administering docetaxel without prednisone could be a reduced treatment-period of prednisone for patients with metastatic prostate cancer. Long-term corticosteroid use, albeit in low dosage, may contribute to the development of severe toxicities, as mentioned before.[9] By excluding prednisone from the initial docetaxel chemotherapy regimen patients will no longer be unnecessarily exposed to these side-effects. Especially for those patients in the hormone-sensitive phase, who usually have a long life expectancy, excluding prednisone will be of relevance to avoid long-term toxicity with unclear antitumor activity. In this light, Ghatalia *et al.* found no positive effect on survival nor on cabazitaxel-induced toxicity in patients with mCRPC.[28]

Limitations of our study include the administration of the standard pre-medication dexamethasone, which is another CYP3A inducer. We aimed to minimize the pharmacokinetic effect of dexamethasone on docetaxel by excluding the latest gift of dexamethasone before docetaxel infusion. Strengths of our study are the randomized design with extensive PK sampling at multiple time points.

In conclusion, we found no influence of prednisone on docetaxel pharmacokinetics. Docetaxel is registered with concomitant prednisone in the mCRPC setting. In metastatic hormone-sensitive disease, the use of prednisone should be supported by other arguments balancing the benefit of prednisone versus the potential long-term side effects of corticosteroid use.

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CHAPTER 6

COMBINING SORAFENIB AND IMMUNOSUPPRESSION IN LIVER TRANSPLANT RECIPIENTS WITH HEPATOCELLULAR CARCINOMA

SUBMITTED

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ABSTRACT

Hepatocellular carcinoma (HCC) recurrence after liver transplantation occurs in approximately 20% of patients. Most of these patients use immunosuppressant drugs. Meanwhile, patients with HCC recurrence are frequently treated with the small molecule kinase inhibitor (SMKI) sorafenib. However, sorafenib and many immunosuppressants are substrates of the same enzymatic pathways (*e.g.* CYP3A4), which may potentially result in altered SMKI or immunosuppressant plasma levels. Therefore, we investigated changes in drug exposure of both sorafenib and immunosuppressants over time in four patients with systemic immunosuppressant and sorafenib treatment after HCC recurrence. In this study, sorafenib exposure declined over time during combined treatment with immunosuppressants, while two patients also experienced declining tacrolimus plasma levels. Importantly, patients were unable to increase the sorafenib dose higher than 200 mg b.i.d. without experiencing significant toxicity. We recommend to treat patients using both sorafenib and immunosuppressants with a sorafenib starting dose of 200 mg b.i.d.

INTRODUCTION

Hepatocellular carcinoma (HCC) is the sixth most frequently diagnosed cancer type worldwide and the fourth leading cause of cancer death in the world.[1] Liver transplantation [2] is indicated in patients with localized HCC, with a 5-year survival rate of approximately 70%.[3] Still, HCC recurrence in the transplanted liver occurs in about 20% of patients.[3]

After HCC recurrence, one of the most applied therapies is sorafenib, an orally active multi-kinase inhibitor approved for the treatment of HCC, resulting in a median overall survival benefit of 7.4 months.[4-7] Usually sorafenib is started in a 200mg b.i.d. dose in this patient group due to expected sorafenib side-effects in patients after liver transplantation and is gradually increased based on toxicity. Patients with HCC recurrence after liver transplantation seem to be more susceptible to sorafenib related side effects. Sorafenib side effects include --among others—gastro-intestinal related side effects (e.g. diarrhea) and cutaneous side effects (e.g. hand-foot skin reaction). These side effects lead to dose reduction or even cessation of sorafenib therapy in 15%-77% of the treated patients after liver transplantation.[8] The higher incidence of side effects in patients with a liver transplantation may be due to a pharmacokinetic and/or pharmacodynamic drug-drug interaction with immunosuppressants.[9-11] Sorafenib and immunosuppressants have overlapping metabolic pathways, which increases the risk of a drug-drug interaction. Sorafenib is metabolized by CYP3A4 and by UGT1A9, while CYP3A4 is also the most important enzyme in the metabolism of several immunosuppressive drugs (e.g. tacrolimus, MTOR inhibitors).[11,12]

Here, we present a case series of four patients with HCC recurrence after liver transplant using tacrolimus concomitantly with sorafenib which allowed to study a possible drug-drug interaction. In all four patients serial blood samples for the determination of both sorafenib and tacrolimus have been taken as part of usual clinical care, for patient safety reasons. Blood samples were taken at day 7 and 14 after the start of sorafenib for the determination of sorafenib area under the curve ($AUC_{0\text{-}7.5}$) and C_{max}, at time point t=0h (before intake of sorafenib) as well as 2, 4, and 7.5 hour after intake of sorafenib. At timepoint t=0h, blood was also taken for the determination of tacrolimus $C_{trough.}$. Next, both tacrolimus and sorafenib C_{trough} were determined on a regular basis at the outpatient clinic. All patients gave written consent for the use of these samples and clinical data for scientific purposes, including this publication.

CASE 1

A 62-year old male patient was referred to the department of Medical Oncology for systemic treatment with sorafenib. He had been diagnosed with chronic hepatitis C before and underwent a liver transplantation for HCC in 2015, followed by tacrolimus monotherapy without previous systemic or local therapy. In June 2017, sorafenib 200 mg b.i.d. was started after HCC recurrence with pulmonary metastases, at which time tacrolimus was dosed at 3 mg once daily providing a tacrolimus trough concentration (C_{trough}) of 5.9 µg/L (reference: 4-8 ug/L).

The $AUC_{0\text{-}7.5h}$ of sorafenib was 2.1% higher at day 14 compared to day 7, while the sorafenib C_{max} was 24% lower (**Table 1**). In general, both sorafenib and tacrolimus trough levels showed a relevant decrease in the first months of treatment, up to a 90% decrease for sorafenib plasma trough levels compared to the baseline trough level and up to 64% for tacrolimus (**Figure 1**).

The tacrolimus dose was increased to 4 mg once daily (q.d.) in August 2017, in an attempt to maintain adequate tacrolimus concentrations. As a result, tacrolimus levels increased, while sorafenib levels further decreased. Therefore, also the sorafenib dose was increased with 50% to 200 mg in the morning and 400 mg in the evening in December 2017, after which also the sorafenib C_{trough} increased. Due to CTCAE grade 3 liver toxicity, the sorafenib dose had to be reduced again to 200 mg b.i.d. at first and to 300 mg q.d. (400mg one day and 200mg the other) in February 2018. Subsequently, sorafenib concentrations decreased and tacrolimus concentrations further increased. Sorafenib was stopped in May 2018 after progressive disease was noticed at the CT scan.

TABLE 1. $AUC_{0\text{-}7.5h}$ and C_{max} of each individual case.

	Day 7		Day 14			
Case	**$AUC_{0\text{-}7.5h}$ sorafenib (mg*h/L)**	**C_{max} sorafenib (mg/L)**	**$AUC_{0\text{-}7.5h}$ sorafenib (mg*h/L)**	**C_{max} sorafenib (mg/L)**	**RD $AUC_{0\text{-}7.5h}$ (%)**	**RD (%) C_{max}**
1	33.4	8.5	34.1	6.4	+2.1	-24.4
2	47.8	8.7	48.2	10.7	+0.9	+22.1
3	37.6	6.3	22.6	4.0	-37.3	-40.0
4	24.9	6.0	13.9	2.3	-62.1	-44.0

All patients used sorafenib 200 mg b.i.d. Abbreviations: AUC= area under the plasma curve, RD= relative difference; C_{max} = maximum concentration; RD = relative difference

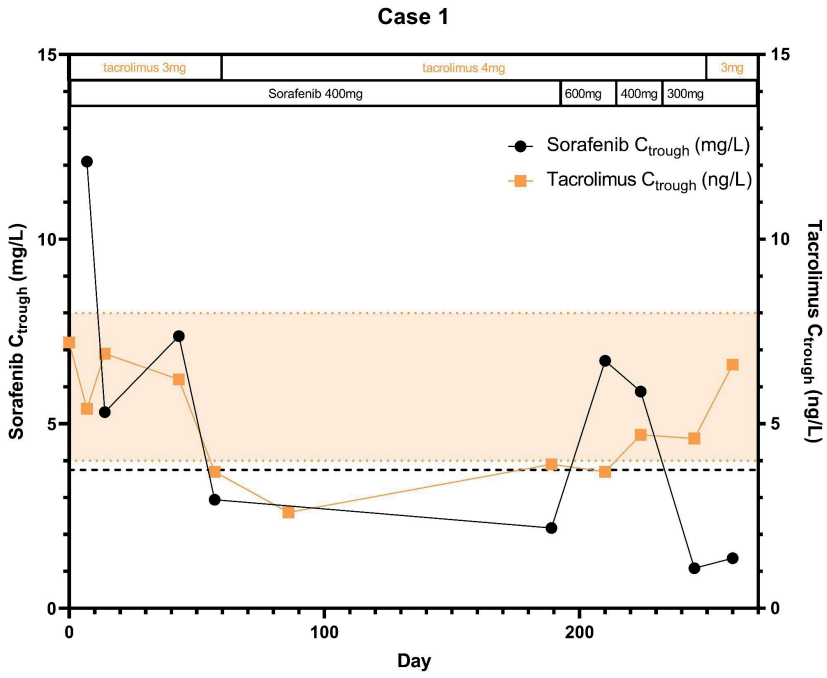

FIGURE 1. Sorafenib and tacrolimus C_{trough} concentrations over time for Subject 1: The C_{trough} levels are displayed over time after the start of sorafenib treatment. Furthermore the optimal C_{trough} levels of both sorafenib and tacrolimus are provided.

CASE 2

A 70-year old female with alcohol induced liver cirrhosis was diagnosed with HCC in 2009, which was at first successfully treated with trans-arterial chemo-embolization (TACE). She underwent a liver transplantation in January 2011. She developed disease recurrence with pulmonary metastases in 2018, after which she was referred to the department of Medical Oncology for systemic treatment with sorafenib, which was started at a 200 mg b.i.d. dose in July 2018. Patient had no signs of liver fibrosis and had a normal liver function when sorafenib was started. Before start of sorafenib, the tacrolimus dose was 4 mg daily and tacrolimus C_{trough} was 5.2 µg/L. On day 14, $AUC_{0\text{-}7.5h}$ and C_{max} of sorafenib were respectively 0.9% and 22.1% higher than at day 7 (**Table 1**). Sorafenib C_{trough} remained stable during the first 2 weeks of concomitant treatment with tacrolimus but generally declined over time (**Figure 2**). Hereafter, in August 2018,

immunosuppressant therapy was stopped by the treating gastroenterologist and sorafenib concentrations further decreased over time. In August 2019, this patient had proven progressive disease and sorafenib was stopped after 19 months of treatment.

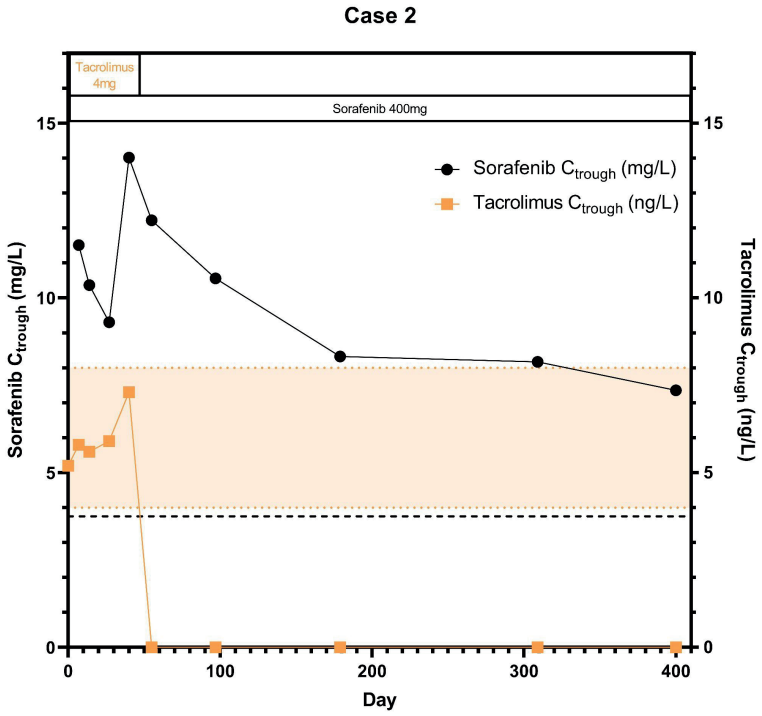

FIGURE 2. Sorafenib and tacrolimus C_{trough} concentrations over time for Subject 2: The C_{trough} levels over time after the start of sorafenib treatment. Furthermore the optimal C_{trough} levels of both sorafenib and tacrolimus are provided.

CASE 3

A 65-year old male patient with chronic hepatitis C was diagnosed with HCC for which he received a liver transplantation in 2018. Immunosuppressive treatment consisted of mycophenolate mofetil (MMF) 1000 mg b.i.d. and tacrolimus (4 mg b.i.d., which was later reduced to 4 mg q.d.). Later, the patient switched from MMF to sirolimus (2 mg q.d.) due to livertoxicity. In April 2019, the patient had a recurrence of disease after which sorafenib was started in a dose of 200 mg b.i.d. Both tacrolimus and sirolimus concentrations were adequate at baseline (C_{trough} = 4.7 µg/L and C_{trough} = 8.0

µg/L, respectively). At day eight of sorafenib treatment, tacrolimus was stopped by the gastroenterologist according to physician's choice and the patient continued with sirolimus monotherapy. After cessation of tacrolimus, the sorafenib concentration initially decreased and remained relatively stable until disease progression, which was also the case for sirolimus concentration (**Figure 3**). $AUC_{0\text{-}7.5h}$ and C_{max} of sorafenib decreased with 40.0% and 37.3% respectively at day 14 compared to day 7 (**Table 1**). After just 2 months of treatment, this patient had disease progression after which sorafenib treatment was stopped and best supportive care was started. After stopping sorafenib therapy, the sirolimus plasma levels further decreased with 42.6% compared to the latest C_{trough} with the combination therapy.

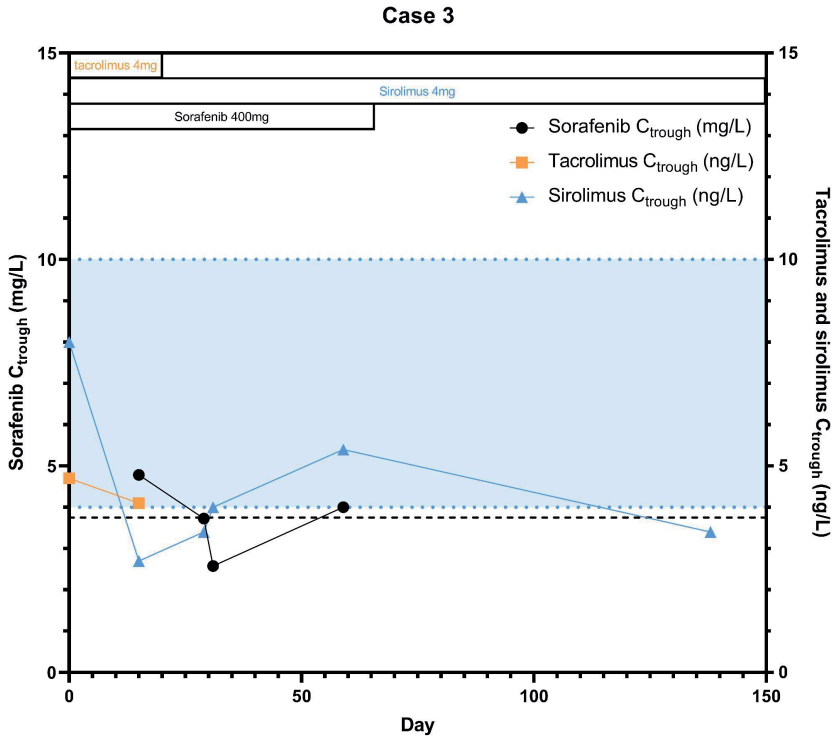

FIGURE 3. Sorafenib and tacrolimus C_{trough} concentrations over time for Subject 3: The C_{trough} levels are displayed over time after the start of sorafenib treatment. Furthermore the optimal C_{trough} levels of both sorafenib and sirolimus are provided. Case 3 was initially treated with both sirolimus and tacrolimus but stopped tacrolimus short after start of sorafenib as was shown in this figure.

CASE 4

A 69-year old male with alcohol induced liver cirrhosis was diagnosed with HCC and underwent a liver transplantation in March 2019. Due to rapid disease recurrence, this patient started with sorafenib in June 2019. His dose of tacrolimus was 10 mg q.d., with a baseline tacrolimus C_{trough} of 3.9 µg/L. Sorafenib exposure was remarkably lower at day 14 than at day 7, as the AUC_{last} decreased with 44% and C_{max} with 62% respectively (**Table 1)**. During the further treatment, sorafenib showed a decrease in plasma trough levels over time despite a dose increase to 200 mg once daily and 400 mg once daily (**Figure 4**). On the other hand, the tacrolimus plasma concentration remained relatively stable over time. In October 2019 sorafenib was stopped due to progression of disease.

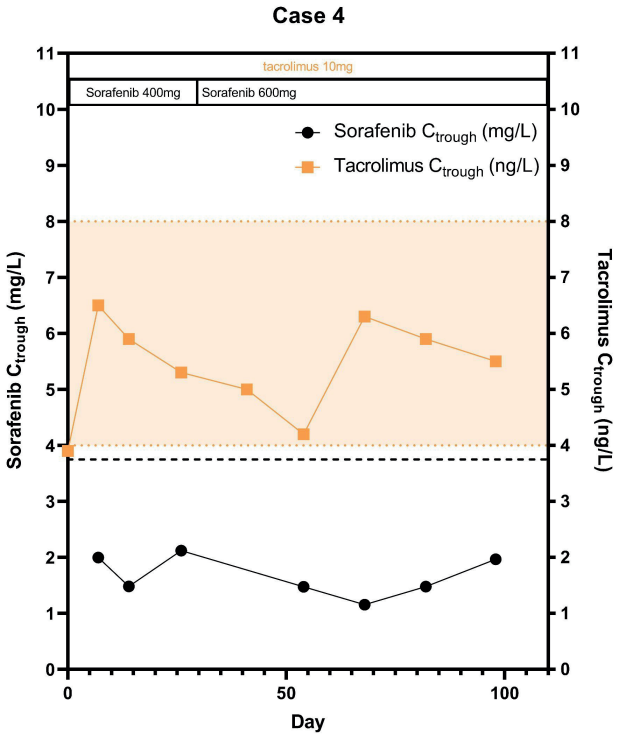

FIGURE 4. Sorafenib and tacrolimus C_{trough} concentrations over time for Subject 4: The C_{trough} levels are displayed over time after the start of sorafenib treatment. Furthermore the optimal C_{trough} levels of both sorafenib and tacrolimus are provided.

DISCUSSION

In this study we present the first case series of patients treated with sorafenib for HCC recurrence after liver transplantation investigating both sorafenib and immunosuppressant plasma concentration over time. In all four patients the plasma pharmacokinetics of both immunosuppressants and sorafenib were longitudinally monitored until sorafenib discontinuation. Sorafenib plasma concentrations (C_{trough}) decreased over time in every case, even after discontinuation of tacrolimus in two of four cases. Long term decrease in TKI exposure is a recognized phenomenon and we cannot distill a consequent pharmacokinetic influence of immunosuppression on the gradually decreased sorafenib exposure from our results. This decline in sorafenib exposure may be induced by autoinduction of CYP3A4, which results in declining plasma levels over time as was demonstrated for imatinib before.[13,14] However, variation in immunosuppression concentrations was not structural (two patients showed a decline in immunosuppression plasma exposure, while the other two patients showed opposite effects), which makes structural CYP3A4 induction less likely.[15] Potentially sorafenib non-adherence may have contributed to the decline in sorafenib concentrations over time: about 50% of patients on long term oral anticancer drug therapy tend to be non-adherent to their treatment resulting in a diminished therapy efficacy and (unexplained) decline in plasma levels.[16]

Although a clear pharmacokinetic interaction of tacrolimus and sorafenib was not found, a sorafenib dose increment to 600 mg daily led to severe hepatotoxicity in case 1. Although sorafenib concentrations increased prior to occurrence of the adverse events, the absolute concentrations of sorafenib did not exceed those measured at start of therapy, which contradicts a sole pharmacokinetic explanation. Both laboratory and imaging findings did not show other causes of hepatotoxicity (e.g. viral hepatitis) and other side -effects in our patients. Therefore, it is likely that an additional pharmacodynamic mechanism is causing the high incidence of sorafenib-induced toxicity after liver transplantation. As mentioned before, sorafenib toxicity rates are higher in patients treated with immunosuppression. In several studies, a high incidence of sorafenib dose reduction or discontinuation (15%-77%) has been reported in patients with HCC after liver transplantation when starting with a 400 mg b.i.d. dose.[17-19] However, the proportion of patients in need of dose reduction or discontinuation seemed to be lower in Asian population studies, suggesting a possible genetic difference.[4] Based on these observations, starting with a lower than regular sorafenib dose seems to be justified in most patients, since the majority of patients required a dose reduction and most patients did not experience significant toxicity at lower dosing levels.[19]

Unfortunately none of these studies investigated sorafenib or immunosuppressant pharmacokinetics. Because sorafenib plasma trough concentrations showed a decrease in our patients, the underlying mechanism of this increase in side effects most likely is of pharmacodynamic origin. Moreover, the immunocompromised status of these patients may be related to an increased incidence of side effects in post liver transplantation patients. However, the exact mechanism remains unknown.

Moreover, an important aspect in the immunosuppressant treatment of patients with HCC recurrence after liver transplantation is the class of immunosuppressants used. Latest evidence suggest survival benefit of treatment with mammalian target of rapamycin (mTOR) inhibitors compared to calcineurin inhibitors like tacrolimus especially when used with sorafenib.[6] . However, general consensus on this topic is not yet reached and alternative therapies, such as lowering immunosuppressant dosing as much as possible, are used in clinical practice. All the patients in this study are treated according to the national treatment guidelines in the Netheralnds. From a pharmacokinetic point of view most CNIs have similar pharmacokinetic properties compared to mTOR inhibitors the effects seen in this case-series may also be applied for these class of immunosuppressants.

Several lessons can be learned from this case series. First of all, there is currently a lack of knowledge in the management of the combination of sorafenib and tacrolimus. Oncologists often determine the sorafenib starting dose on the basis of personal experience with this treatment combination. Overall, there is a decrease in sorafenib plasma levels over time, even when it is not combined with tacrolimus. Due to an increased risk of side effects in patients with a liver transplantation,[9] and based on the high incidence of side effects with higher sorafenib doses we would recommend to start treatment with a reduced daily dose of 200 mg b.i.d.[4] Based on tolerability, the dose can then gradually be escalated. Moreover, a daily sorafenib dose of 200mg b.i.d. has demonstrated to be an effective dosing strategy, which indicates a possible overdosing in most patients treated with sorafenib.[20]

In conclusion, the interaction between sorafenib and immunosuppressive drugs is clinically relevant in view of the high toxicity rates compared to patients without a liver transplantation. More research is needed to investigate the pharmacokinetic aspects of this drug-drug interaction.

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CHAPTER 7

INFLUENCE OF PROBENECID ON THE PHARMACOKINETICS AND PHARMACODYNAMICS OF SORAFENIB

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ABSTRACT

Prior studies have demonstrated an organic anion transporter 6 (OAT6)-mediated accumulation of sorafenib in keratinocytes. The OAT6 inhibitor probenecid decreases sorafenib uptake in skin and might, therefore, decrease sorafenib-induced cutaneous adverse events. Here, the influence of probenecid on sorafenib pharmacokinetics and toxicity was investigated. Pharmacokinetic sampling was performed in 16 patients on steady-state sorafenib treatment at days 1 and 15 of the study. Patients received sorafenib (200–800 mg daily) in combination with probenecid (500 mg two times daily (b.i.d.)) on days 2–15. This study was designed to determine bioequivalence with geometric mean Area under the curve from zero to twelve hours ($AUC_{0-12\,h}$) as primary endpoint. During concomitant probenecid, sorafenib plasma $AUC_{0-12\ h}$ decreased by 27% (90% CI: −38% to −14%; $P < 0.01$). Furthermore, peak and trough levels of sorafenib, as well as sorafenib concentrations in skin, decreased to a similar extent in the presence of probenecid. The metabolic ratio of sorafenib-glucuronide to parent drug increased (+29%) in the presence of probenecid. A decrease in systemic sorafenib concentrations during probenecid administration seems to have influenced cutaneous concentrations. Since sorafenib-glucuronide concentrations increased compared with sorafenib and sorafenib-*N*-oxide, probenecid may have interrupted enterohepatic circulation of sorafenib by inhibition of the organic anion transporting polypeptides 1B1 (OATP1B1). Sorafenib treatment with probenecid is, therefore, not bioequivalent to sorafenib monotherapy. A clear effect of probenecid on sorafenib toxicity could not be identified in this study.

INTRODUCTION

Over the last two decades, systemic anti-cancer treatment options have been expanded from traditional cytotoxic chemotherapy to targeted agents, including tyrosine kinase inhibitors (TKIs). TKIs offer a number of important advantages over conventional cytotoxic chemotherapy like the oral administration of the drugs, but they are still afflicted by some major problems, including large interindividual pharmacokinetic variability, a narrow therapeutic window, and debilitating adverse events. [1]

Cutaneous adverse events are among the most frequently observed toxicities with many TKIs, and their intensity can significantly affect both quality of life and health care economics.[2] A particularly painful complication seen most frequently during the early weeks of use with TKIs, such as sorafenib, sunitinib, and regorafenib, is called hand-foot skin reaction (HFSR), in which painful hyperkeratotic plaques develop predominantly over sites of pressure or friction [3,4] The clinical incidence of HFSR varies among TKIs with a particularly high incidence (20% ≥ grade 3) being observed with sorafenib [5], an orally administered multikinase inhibitor, registered for treatment of advanced hepatocellular carcinoma and advanced renal cell carcinoma as well as iodine-refractory advanced thyroid cancer [3,4,6,7] Furthermore, it is investigated as a treatment option for acute myeloid leukemia.[8]

The pathogenesis of TKI-induced HFSR remains currently unknown, and the only effective treatment options involve either dose reduction or discontinuation of therapy, which theoretically may have negative effects on disease management.[9,10] However, previous *in vitro* and in vivo research showed that sorafenib can accumulate in human epidermal keratinocytes mediated by the organic anion transporter 6 (OAT6) [11], and that sorafenib-induced skin toxicity can be prevented by cotreatment with the OAT6 inhibitor probenecid without negatively influencing the antitumor properties of sorafenib.[11]

Probenecid is an uricosuric agent indicated for the maintenance treatment of hyperuricemia associated with gout and gouty arthritis. It was also used as an adjuvant for therapy with certain antibiotics, such as penicillin, ampicillin, or methicillin, because it elevates and prolongs their plasma levels by inhibition of renal excretion.[12] Probenecid is usually well tolerated at a dose of 500 mg two times daily and is usually taken for (many) months. Probenecid is also known as a pan- uridine diphosphate glucuronosyltransferase (UGT) inhibitor, used in drug registration studies and, therefore, could potentially influence pharmacokinetics of several drugs, including sorafenib that undergoes cytochrome P450 3A4 (CYP3A4)-mediated oxidation into

its active metabolite (pyridine-*N*-oxide) and UGT1A9-mediated glucuronidation into sorafenib glucuronide.[13-15] Furthermore, probenecid is known to alter the activity of several drug transporters like OAT and the organic anion transporting polypeptides (OATP), which play a main role in renal and hepatic excretion.[16] However, the extent of this possible effect is not yet determined in clinical studies and the safety of the combination of these drugs is currently unknown. As part of an ongoing project to develop translationally useful prevention strategies for sorafenib-induced HFSR, in the current study, we evaluated the pharmacokinetics (PK) and safety of sorafenib when concomitantly used with probenecid.

PATIENTS AND METHODS

This non-randomized, cross-over study was performed between November 2017 and November 2019 at the Erasmus University MC Cancer Institute. The study was approved by the local ethics committee of the Erasmus University MC (METC-17-490, date of approval 16-11-2017) and competent authority and was registered at the European Clinical Trials Database (EudraCT 2017-002470-40) and the Dutch trial registry (www.trialregister.nl; number NL6783).

Patients

Patients who had a confirmed diagnosis of advanced hepatocellular carcinoma (HCC) or differentiated thyroid carcinoma with an indication for sorafenib treatment, and who were at least 18 years of age, were included in this study. Furthermore, patients had to have an Eastern Cooperative Oncology Group (ECOG) performance status of ≤2 and an adequate hematological, renal, and liver function defined as a Common Terminology Criteria for Adverse Events (CTCAE) grade of ≤2 at baseline. Besides, patients with known contraindications for probenecid use (e.g., history of uric acid kidney stones, an acute gouty attack, or blood dyscrasias) and/or the use of drugs that are strong CYP3A4 or UGT1A9 inducers or inhibitors were excluded. All included patients gave written informed consent.

Study procedures

Patients received sorafenib for at least two weeks to ensure steady-state pharmacokinetics of sorafenib. Since sorafenib has linear pharmacokinetics,[17] dose reductions were allowed after the start of the study. Sorafenib was administered at a 200–800-mg daily dose during the 15-day study period and was given concomitantly with probenecid (500 mg b.i.d.) from day 2 to day 15 of the study. Both sorafenib and probenecid were ingested at predefined timepoints at 10:00 a.m. and 10:00 p.m.

Pharmacokinetic sampling

Patients were admitted to the hospital on days 1 and 15 of the study for pharmacokinetic blood sampling. A total of nine blood samples for the determination of sorafenib, sorafenib *N*-oxide, and sorafenib glucuronide were obtained at predefined time points (T = pre, T = 0.5 h, T = 1 h, T = 2 h, T = 4 h, T = 6 h, T = 8 h, T = 10 h, and T = 12 h). Blood samples were processed into plasma within 30 min, by vortex mixing and centrifugation for 10 min at 2500 g at 4 °C. Plasma concentrations were determined using a validated liquid chromatography tandem mass spectrometry (LC-MS/MS) method [18], at both the laboratory of Translational Pharmacology in the Erasmus MC, Rotterdam, and the laboratory of Pharmaceutics and Pharmaceutical Chemistry, Ohio State University, OH. Predefined pharmacokinetic endpoints were the dose-corrected area under the curve from preadministration time point until 12 h after sorafenib intake ($AUC_{0-12\ h}$), maximum concentration (C_{max}), time until maximum concentration (T_{max}), and lowest plasma concentration (C_{trough}) and were determined using WinNonlin v. 7.0 (Phoenix, Certara, 5349 AB, Oss, the Netherlands) for sorafenib, sorafenib-*N*-oxide, and sorafenib glucuronide.

Skin biopsies

A 3-mm skin biopsy was obtained at days 1 and 15 of the study during PK sampling days for pharmacokinetic analysis. Skin biopsies were taken from either the forearm or the shoulder region, but always from the same region at the same timepoint in an individual patient during the two consecutive PK sampling days. If patients had HFSR lesions at the hand at the first PK day, an additional skin biopsy was performed from the thenar eminence region of the hand for pathologic analysis on days 1 and 15. The biopsies were graded according to the scoring for interface dermatitis as used for graft-versus-host disease by an experienced pathologist (J.D.). There is no other pathologic grading scale for HFSR and our grading scale shows the most overlapping features from a pathologic perspective [19]. Furthermore, concentrations of sorafenib were determined from the skin biopsies after dilution in human plasma and homogenization using the validated liquid chromatography tandem mass spectrometry (LC-MS/MS) method described earlier [18].

In vitro transport assay

Transport assays assessing probenecid's inhibition of OATP1B1 were conducted as previously described [20]. The [3H]estradiol-17b-d-glucuronide, a positive control substrate for OATP1B1 [21], was obtained from American Radiolabeled Chemicals. Water-soluble probenecid was obtained from Invitrogen (Molecular Probes). The generation and characterization of Flp-In T-Rex293 cells expressing inducible OATP1B1 have been

reported previously [22, 23]. Cells expressing OATP1B1 or vector control (VC) were cultured in Dulbecco's Modified Eagle's Medium (DMEM; Invitrogen) supplemented with 10% Fetal Bovine Serum (FBS), hygromycin B (25 mg/mL; Invitrogen), and blasticidin (37.5 mg/mL; Biovision, California, United States of America).

Cells were seeded in 24-well plates in phenol red-free DMEM containing 10% FBS, hygromycin (25 mg/mL), blasticidin (37.5 mg/mL), and doxycycline (1 µg/mL) and were incubated at 37 °C for 24 h. Cells were then washed with warm PBS and pre-incubated with the indicated concentration of probenecid in phenol red-free DMEM (without FBS and supplements) at 37 °C for 15 min. Cells were then incubated with phenol red-free DMEM containing the indicated concentration of probenecid and 0.2 µM [3H]estradiol-17b-D-glucuronide for an additional 15 min. The experiment was terminated by washing three times with ice-cold PBS. Cells were lysed in 1 N NaOH at 4 °C overnight, and then the solution was neutralized with 2 mol/L HCl. Total protein was measured using a Pierce BCA Protein Assay Kit (Thermo Scientific), and total protein content was quantified using a microplate spectrophotometer. Drug concentrations were determined in the remaining cell lysate by liquid scintillation counting using a scintillation counter. OATP1B1-mediated uptake was calculated by dividing the disintegrations per minute (dpm) from each replicate by the amount of protein (mg) and subtracting the dpm/mg protein in VC cell line from the dpm/mg protein in OATP1B1 overexpressing cells at each concentration of probenecid. OATP1B1-mediated uptake at each concentration of probenecid was then compared with OATP1B1-mediated uptake when only an equal volume of vehicle was added without probenecid (i.e., % control). The half maximal inhibitory concentration (IC_{50}) was calculated using a nonlinear fit comparing concentration of probenecid versus response.

Toxicity

Toxicity rates were determined at baseline, days 1 and day 15 of the study using Common Terminology Criteria for Adverse Events (CTCAE version 4.0, National Cancer Institute, Bethesda, Maryland, United States), and by evaluating the patient diaries during the sorafenib monotherapy phase and sorafenib concomitant with probenecid phase.

Statistical analysis

The primary objective of this study was to determine bioequivalence between sorafenib monotherapy and sorafenib concomitantly with probenecid to determine whether it is a safe option in clinical practice. Bioequivalence can be concluded when the 90% confidence interval (CI) of the ratio of geometric means is within 80% and 125%. Assuming a standard deviation of the difference of 0.25 for log($AUC_{sorafenib}$), using a 90%

power and two-sided alpha of 5%, the required number of evaluable patients was 16. Analyses of $AUC_{0\text{-}12h}$, $C_{trough,}$ and C_{max} were performed on log-transformed observations by means of the paired t-test. The point estimates and CIs were transformed back to the original scale in order to give the point estimates for the ratio of the geometric means and the CIs. 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. This was corrected for baseline toxicity and was only taken into account in case of an increase in CTCAE grade per PK sampling day. Since the design of this study was not appropriate to detect a significant difference in toxicity, these results had a descriptive character.

RESULTS

Patient Characteristics

Seventeen patients were included, of whom 16 patients were evaluable due to withdrawal of one patient. Most patients (n = 14) were male and had an HCC (n = 12). Eight patients with HCC had underlying liver cirrhosis due to alcohol abuse (n = 3) or chronic viral hepatitis (n = 5). Other patient characteristics can be found in **Table 1.**

Pharmacokinetics

When sorafenib was administered with concomitant probenecid, the geometric mean sorafenib $AUC_{0-12\,h}$ was 26.8% (90% CI: −37.7% to −14.1%) lower than when sorafenib was administered alone. Similarly, sorafenib plasma C_{max} and C_{trough} decreased significantly by 25.1% (90% CI: −44.3% to −19.7%) and 26.0% (90% CI: −43.4% to −3.4%), respectively. (**Table 2 and Figure 1**).

Sorafenib metabolites showed a similar decrease in plasma concentration, although there was a substantial interpatient variability. The sorafenib-*N*-Oxide $AUC_{0-12\,h}$ decreased by 36.3% (90% CI: −52.8% to −14.1%) and C_{max} showed a similar significant decrease of 39.2% (90% CI: −54.6% to −18.7%). Interestingly, cotreatment with probenecid did not decrease the sorafenib-glucuronide $AUC_{0-12\,h}$ to a similar extent (5.5%; 90% CI: −18.0% to 8.9%), did not significantly influence C_{max} (6.1%; 90% CI: −21.7% to 12.7%), and, thus, shows bioequivalence (**Table 2 and Figure 1**). The ratio of sorafenib-glucuronide to sorafenib increased by 29% when sorafenib was co-administered with probenecid, whereas other metabolic ratios did not change significantly. Sorafenib concomitant with probenecid is not bioequivalent to sorafenib monotherapy.

TABLE 1. Patient characteristics.

Characteristic	Evaluable patients (n=16)
Sex	
Male	14 (88%)
Female	2 (12%)
Age (years) median [IQR]	66.5 [58-75]
Performance	
ECOG 0	1 (6%)
ECOG 1	13 (82%)
ECOG 2	2 (12%)
Tumor type	
HCC	12 (72%)
liver cirrhosis	*8 (66%)*
Pre-existent hepatitis	*5 (42%)*
Thyroid carcinoma	4 (28%)
BMI (kg/m²) median [IQR]	25.2 [22-30]
Race	
Caucasian	11 (70%)
African	1 (6%)
Arabic	3 (18%)
Asian	1 (6%)
Sorafenib daily dose at start of study	
200 milligrams	1 (6%)
400 milligrams	10 (63%)
600 milligrams	2 (12%)
800 milligrams	3 (19%)

Abbreviations: BMI= body mass index, ECOG = Eastern Cooperative Oncology Group; HCC = hepatocellular carcinoma, n = number of patients; IQR = interquartile range.

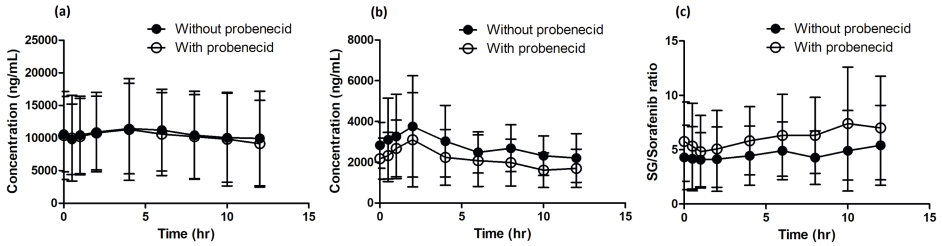

FIGURE 1. Pharmacokinetic results are displayed for a) sorafenib-glucuronide concentration, b) sorafenib concentration, c) Sorafenib-glucuronide (SG) to sorafenib ratio

TABLE 2. Pharmacokinetic results.

Pharmacokinetic parameters	Sorafenib mono-therapy	Metabolic ratio (metabolite/ sorafenib)	Sorafenib with probenecid	Metabolic ratio (metabolite/ sorafenib)	RD sorafenib monotherapy vs sorafenib + probenecid (90%CI)	P-value
Sorafenib						
$AUC_{0\text{-}12h}$ (CV %) geomean µg*h/mL	33457.8 (42)		24476.8 (57)		-26.8% (-37.7% to -14.1%)	<0.01
C_{max} (CV %) geomean µg/mL	4457.8 (52)		3337.2 (63)		-25.1% (-44.3% to -19.7%)	<0.01
C_{trough} (CV %) geomean µg/mL	2125.5 (60)		1571.9 (61)		-26.0% (-43.4% to -3.4%)	0.07
T_{max} (IQR) median hours	3.7 (1.5-4.15)		2.2 (1.0-2.01)		NA	0.53
Sorafenib concentration in keratinocytes Geomean ng/mL (CV %)	50.0 (61)	$1.49 * 10^{-3}$	36.0 (63)	$1.47* 10^{-3}$	-28.1% (-46.3% to -3.7%)	0.07
Sorafenib-N-oxide						
$AUC_{0\text{-}12h}$ (CV %) geomean µg*h/mL	3442.8 (78)	0.10	2192.3 (77)	0.09	-36.3% (-52.8% to -14.1%)	0.02
C_{max} (CV %) geomean µg/mL	467.3 (77)		283.9 (74)		-39.2% (-54.6% to 18.7%)	<0.01
C_{trough} (CV %) geomean µg/mL	271.1 (282)		(71)		-35.2% (-59.7% to 4.3%)	0.13
Sorafenib-glucuronide						
$AUC_{0\text{-}12h}$ (CV %) geomean µg*h/mL	120660 (55)	3.61	113995 (59)	4.66	-5.5% (-18.0% to 8.9%)	0.49
C_{max} (CV %) geomean µg/mL	12704 (51)		11931 (64)		-6.1% (-21.7% to 12.7%)	0.56
C_{trough} (CV %) geomean µg/mL	9159 (65)		8400 (67)		-8.3% (-21.3% to 6.9%)	0.34

Abbreviations: $AUC_{0\text{-}12h}$ = area under the curve, time point 0h to 12h; CI = Confidence Interval; RD = relative difference; C_{max} = maximum concentration; C_{trough} = minimum concentration; CV = coefficient of variation; h = hours; n = number of patients; T_{max} = time until maximum concentration, IQR = interquartile range; NA = not applicable.

Sorafenib concentration in skin decreased in the presence of probenecid by 28.1% (90% CI: −46.3% to −3.7%), with a similar plasma sorafenib/sorafenib in skin ratio (**Table 2**). Furthermore, there was no difference between patients with or without liver cirrhosis in sorafenib plasma AUC (−6.3%; 90% CI: −32.9% to 30.7%; P = 0.73) and C_{trough} (−7.4%; 90% CI: −46.8% to 61.3%; P = 0.81).

In vitro transport assay

Subsequently, we hypothesized that probenecid interferes with enterohepatic sorafenib circulation via OATP1B1 inhibition and, therefore, measured the impact of probenecid on the cellular uptake of a probe OATP1B1 substrate, [3H]estradiol-17b-d-glucuronide, in a cell line overexpressing OATP1B1. Probenecid inhibited OATP1B1 function with an IC_{50} of 182 μM (**Figure 2**).). Given that probenecid achieves plasma concentrations higher than 200 μM at clinically relevant doses,[24] the results of this experiment support our hypothesis that OATP1B1 contributes to the observed drug–drug interaction between probenecid and sorafenib.

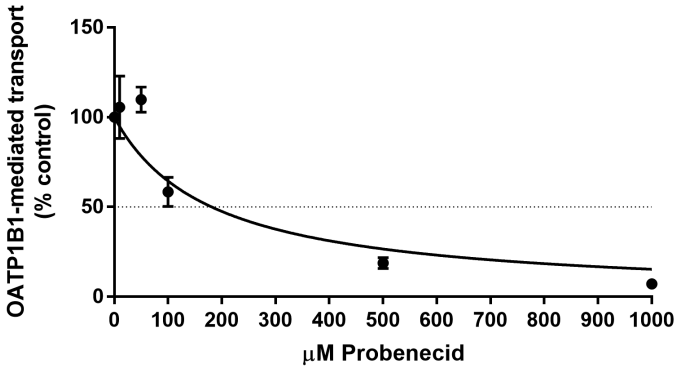

FIGURE 2. Inhibition of OATP1B1 function by probenecid in vitro. HEK293 cells expressing OATP1B1 or VC were pre-incubated with probenecid at the indicated concentrations for 15 minutes before incubation with probenecid and [3H]estradiol-17b-D-glucuronide for 15 minutes. Data represent uptake of OATP1B1-expressing cells at each concentration compared against vehicle after subtracting uptake by VC cells (mean ± SEM). Each concentration consists of 3-9 technical replicates across 1-3 biological replicates.

Toxicity

There were three serious adverse events, which were assumed to be not related to any of the study drugs (gastroenteritis with dehydration and dyspnea grade 3 during the probenecid part and atrial fibrillation *de novo* in the monotherapy part, all complicated with unplanned hospitalization). HFSR, rash, anorexia, and fatigue occurred more

frequently during probenecid administration (65%) than during sorafenib monotherapy (43%) (**Table 3, Supplementary Table 1**). HFSR occurred in 10 of 16 patients with five patients experiencing HFSR at the first day of PK sampling. Three of these patients experienced progression of HFSR symptoms during the study. Most toxicity occurred after 3–6 weeks of treatment. Most grades 2 and 3 adverse events were seen when sorafenib was administered with probenecid. A total of five patients experienced HFSR at PK sampling day 1 and a biopsy of the thenar eminence region was taken in these patients on days 1 and 15 of the study. There was no difference in the grading of the HFSR between the first and second PK sampling day in these patients.

TABLE 3. Patient reported adverse events during study period.

	Sorafenib mono (N = 16)		**Sorafenib concomitantly with probenecid (N = 16)**	
Adverse event	Grade 1-2	Grade 3	Grade 1-2	Grade 3
Rash	1	-	3	1
Nausea	1	-	2	-
Vomiting	0	-	1	-
Oral mucositis	1	-	1	-
Diarrhea	1	-	2	-
Constipation	2	-	3	-
Anorexia	4	-	7	-
Dyspnea	-	-	-	1
Edema	-	-	1	-
Fatigue	2	-	6	1
fever	1	-	-	-
Pain	1	1	2	1
Serious adverse events (SAE)	1		2	

There were three serious adverse events (Atrial fibrillation de novo, dyspnea grade 3 and severe gastroenteritis with dehydration) during sorafenib therapy for which hospitalization was necessary

DISCUSSION

In this study, we investigated the influence of the OAT6 and OATP1B1 inhibitor probenecid on sorafenib pharmacokinetics and toxicity in patients, and found a significant decrease in the geometric mean of sorafenib plasma exposure and a nearly significant decrease in intracutaneous sorafenib exposure during concomitant probenecid administration

making sorafenib concomitantly with probenecid not bioequivalent to sorafenib monotherapy. Of the metabolites, systemic sorafenib-*N*-oxide concentrations decreased proportionally with the parent drug, but the sorafenib-glucuronide to sorafenib ratio increased after probenecid administration, which does not support the hypothesis of UGT inhibition. This is in line with our previous findings on enterohepatic circulation of sorafenib-glucuronide, which demonstrated that OATP1B inhibition leads to an increase in plasma sorafenib glucuronide levels.[13,25] Next to the relative increase in systemic sorafenib-glucuronide exposure, its reduced hepatocellular secretion would also explain the decrease in systemic sorafenib concentrations after probenecid administration, as these concentrations are less maintained via enterohepatic circulation of deconjugated sorafenib glucuronide. Moreover, as we found probenecid to inhibit OATP1B1-mediated transport in vitro at clinically relevant concentrations and as we previously showed that OATP1B1 contributes to enterohepatic sorafenib cycling,[13] it is plausible that reduced enterohepatic circulation of sorafenib led to its significant decrease in systemic exposure after probenecid. Alternatively, the relative systemic accumulation of sorafenib-glucuronide compared with sorafenib and sorafenib-*N*-oxide might be caused by decreased tubular secretion in the kidney, where probenecid is known to inhibit prominent drug transporters as OAT1 and OAT3.[16] However, data regarding this potential interaction are lacking. This study was not designed to quantify these mechanisms and it should be noted that all patients followed the same sequence of treatment, i.e., sorafenib monotherapy followed by concomitant probenecid, which complicates the differentiation between effects of probenecid and time. Regardless of its etiology, the decrease in systemic sorafenib exposure rather than inhibited OAT6-mediated transport seemed to determine cutaneous sorafenib concentrations, as systemic and cutaneous sorafenib concentrations decreased proportionally and protective effect of probenecid on cutaneous exposure could not be demonstrated. This follows a recent population PK analysis in which systemic sorafenib and sorafenib-*N*-oxide were associated with earlier occurrence of HFSR.[26]

Despite lower sorafenib exposure, adverse events occurred more frequently during probenecid cotreatment. It is known that the prevalence of adverse events increases during the first weeks of sorafenib treatment.[27] The difference in adverse events is, therefore, unlikely a result of the drug interaction observed in this study. Patients participated in the study at a relatively early stage of the TKI treatment (i.e., maximal six weeks after start of sorafenib treatment). Usually sorafenib adverse events such as hypertension occur early during TKI treatment [27] and HFSR usually develops 2–4 weeks after initiation of sorafenib. Hence, it is not likely that we missed this adverse event in our study population.[19,27] In the five patients who experienced HFSR at the first day of study, pathologic characteristics of skin biopsies from the thenar eminence region did

not change during the study, potentially due to the non-specificity for HFSR of the used grading scale, i.e., the interface dermatitis score, or due to the absence of high-grade HFSR in our study population. Therefore, subtle HFSR specific changes could have been missed.

In conclusion, both systemic and cutaneous sorafenib exposure decreased proportionally during concomitant probenecid administration, which may have been caused by interruption of enterohepatic cycling via OATP1B1 inhibition.

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SUPPLEMENTARY TABLE 1. Toxicity per patient

Patient	Duration of sorafenib at baseline (days)	Sorafenib daily dose (mg)	Study phase	HFSR	Rash	Nausea	Diarrhea	Constipation	Anorexia	Fatigue	Pain	Hoarseness
			Baseline					1	1	1	1	
1	14	800	PK day 1		2			1		1	1	
		800	PK day 2					1	1	1	1	
			Baseline			1			1		1	
2	28	400	PK day 1							1	1	
		400	PK day 2			1			1	1	1	
			Baseline						1	1	1	
3	9	400	PK day 1		1				2	2	1	
		400	PK day 2						1	1	1	
			Baseline							1	1	
4	27	600	PK day 1				1		1	1	1	
		600	PK day 2	1					2	2	1	
			Baseline					1	1	1		
5	23	800	PK day 1	1				1	1	1	1	1
		800	PK day 2					2	1	1	1	
			Baseline									
6	35	800	PK day 1			1				1	1	
		800	PK day 2	3	2					1	2	
			Baseline	1				1		1	1	1
7	22	400	PK day 1	1				1		1	1	
		400	PK day 2	2				1		1	1	
			Baseline							1	1	1
8	8	400	PK day 1							1	1	
		600	PK day 2	1					1	1	1	
			Baseline						1	1		
9	11	400	PK day 1					1	1	1		
		400	PK day 2	1	1			1	1	1		
			Baseline						1	1	1	1
10	23	200	PK day 1	1					1	1	1	
		200	PK day 2			1			1	2	1	1
			Baseline		1						1	1
11	42	400	PK day 1							2	1	
		400	PK day 2				1			2		

SUPPLEMENTARY TABLE 1. Continued

Patient	Duration of sorafenib at baseline (days)	Sorafenib daily dose (mg)	Study phase	HFSR	Rash	Nausea	Diarrhea	Constipation	Anorexia	Fatigue	Pain	Hoarseness
			Baseline		1							1
12	12	400	PK day 1									
		400	PK day 2	2	1					1		
			Baseline	1						1	1	1
13	41	400	PK day 1	1					1	1	3	
		400	PK day 2	2				1	1	1	3	1
			Baseline	1						2		1
14	29	400	PK day 1	1					1	2		1
		400	PK day 2		3				2	3	1	1
			Baseline		1		1		1			
15	24	400	PK day 1				1		1	1		
		400	PK day 2				1		1	1		
			Baseline						1	2		
16	23	400	PK day 1						1	2		
		400	PK day 2				2		2	2		

Legend: Toxicity per phase per patient according to the CTCAE grading. In general there is an increase in adverse events during the study. Fields are left blank if toxicity is scored 0.

CHAPTER 8

THE RISK OF QTC-INTERVAL PROLONGATION IN BREAST CANCER PATIENTS TREATED WITH TAMOXIFEN IN COMBINATION WITH SEROTONIN REUPTAKE INHIBITORS

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ABSTRACT

Purpose. Antidepressants like the serotonin reuptake inhibitors (SRIs) are often used concomitantly with tamoxifen (e.g. for treatment of depression). This may lead to an additional prolongation of the QTc-interval, with an increased risk of cardiac side effects. Therefore we investigated whether there is a drug-drug interaction between tamoxifen and SRIs resulting in a prolonged QTc-interval.

Methods. Electrocardiograms (ECGs) of 100 patients were collected at steady state tamoxifen treatment, with or without concomitant SRI co-medication. QTc-interval was manually measured and calculated using the Fridericia formula. Primary outcome was difference in QTc-interval between tamoxifen monotherapy and tamoxifen concomitantly with an SRI.

Results. The mean QTc-interval was 12.4 ms longer when tamoxifen was given concomitantly with an SRI (95% CI:1.8–23.1 ms; P = 0.023). Prolongation of the QTc-interval was particularly pronounced for paroxetine (17.2 ms; 95%CI:1.4–33.0 ms; P = 0.04), escitalopram (12.5 ms; 95%CI:4.4–20.6 ms; P < 0.01) and citalopram (20.7 ms; 95%CI:0.7–40.7 ms; P = 0.047), where other agents like venlafaxine did not seem to prolong the QTc-interval. None of the patients had a QTc-interval of >500 ms.

Conclusions. Concomitant use of tamoxifen and SRIs resulted in a significantly higher mean QTc-interval, which was especially the case for paroxetine, escitalopram and citalopram. When concomitant administration with an SRI is warranted venlafaxine is preferred.

INTRODUCTION

One of the most common causes of cessation of therapeutic use of drugs which have already been marketed is prolongation of the QT-interval, which is defined as a QT interval > 470 ms in females and > 450 ms in males according to European Society of Cardiology (ESC) guidelines[1,2]. QT-interval or the heart-rate corrected QT (QTc) interval prolongation is associated with higher risk of polymorphic ventricular tachycardia or Torsade des Pointes (TdP), which may ultimately lead to sudden cardiac death (SCD)[1,3]. The QTc-interval represents the duration between the onset of ventricular depolarization and the completion of repolarization of the myocardium. Several risk factors are associated with an increased risk for QTc-interval prolongation (e.g hypopotassemia, renal impairment, use of diuretics and other QTc-prolonging drugs and unmodifiable risk factors such as age > 65 years and female gender)[1,4–6]. Furthermore, it has become evident that several classes of anti-cancer drugs are associated with QT prolongation and, therefore, this offers a great challenge in the treatment of cancer patients[7–9].

The suggested mechanism of drug-induced QTc-interval prolongation is inhibition or reduced expression of the Human ether-a-go-go related (hERG) gene that encodes a potassium channel that regulates repolarizing currents (Ikr) in the cardiomyocytes or inhibition of late sodium currents [1,10]. Inhibition of these Ikr results in a delay in the ventricular repolarization causing prolongation of the QT-interval (Fig. 1). Some drugs are known Ikr inhibitors, but failed to demonstrate a clinical significant QTc-interval prolongation at dosages used in routine clinical practice (e.g. fexofenadine), although some of these drugs still give an increased risk of experiencing TdP [1]. Therefore, the risk of experiencing TdP is not fully linear with the extent of QTc-interval prolongation. Combining QTc-prolonging drugs (drug-drug interaction) may also increase the risk of SCD [1,8]. The combination of two known QTc-prolonging drugs may result in a cumulative or synergistic prolongation of the QTc-interval and thus increased risk for TdP [11,12].

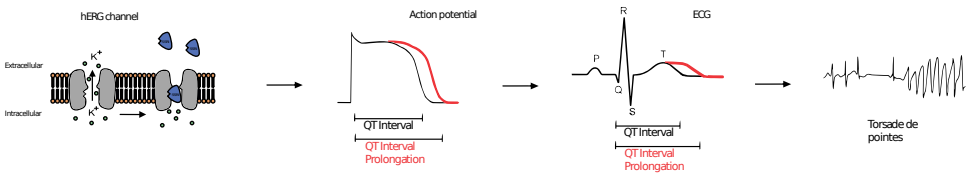

FIGURE 1. Mechanism of QTc-interval prolongation. Serotonin reuptake inhibitors (SRIs) inhibit the hERG channel and therefore the Ikr (repolarizing potassium (K+) current) in the cardiomyocyte. This results in a delay of the ventricular repolarization time and therefore in a prolongation of the QTc-interval. Prolongation of the QTc-interval may result in cardiac arrhythmias such as TdP.

The risk of drug-induced QTc-interval prolongation is determined according to Adverse Drug Event Causality Analysis into QTc-prolonging drugs with a 'known', 'possible' or 'conditional' risk for TdP [13,14]. A drug is categorized as a drug 'with a known risk of TdP' if there is substantial positive evidence of prolongation of the QTc-interval and an association with TdP. The risk is scored as 'possible' if there is substantial evidence which supports the conclusion that drugs can prolong the QTc interval, but there is insufficient evidence that these drugs are associated with TdP. Finally, the risk is scored as 'conditional' if there is substantial evidence of QT-interval prolongation with an association with TdP development but only under certain conditions (e.g. overdosing) or because the drug has shown ability to create one or more conditions that facilitate induction of TdP (e.g. by inhibiting metabolism of QTc-proloning drugs). Drugs with a 'known' risk for QTc-interval prolongation are escitalopram and citalopram. Venlafaxine, imipramine, nortriptyline and tamoxifen are classified as 'possible' and paroxetine, amitriptyline, sertraline, fluoxetine and fluvoxamine are classified as conditional according to Crediblemeds®.

One of the anti-cancer drugs, which is a known Ikr inhibitor, is the selective ER modulator (SERM) tamoxifen [8,15,16]. Since decades, tamoxifen is used in the treatment of breast cancer, where it provides suppression of ER-dependent proliferation of breast cancer cells and therefore reduces the risk of disease recurrence and mortality. However, tamoxifen may also lead to Ikr inhibition in cardiac tissue and ultimately to prolongation of the QTc-interval [15]. After absorption tamoxifen is converted into several pharmacologically active metabolites of which endoxifen is the most potent. The cytochrome P450 enzymes CYP2D6 and CYP3A4 play a dominant role in the biotransformation of tamoxifen [17]. It has been shown that the use of CYP2D6 or CYP3A4 inhibitors or inducers may lead to a significant alteration in tamoxifen and endoxifen exposure [18–20]. One of the classes of drugs that is known for its ability to inhibit CYP2D6 are the serotonin reuptake inhibitors (SRIs) like the selective serotonin reuptake inhibitors (SSRI) and the serotonin norepinephrine reuptake inhibitor (SNRI) venlafaxine [21]. These drugs are frequently used (by breast cancer patients) for the treatment of depression, anxiety disorders or (tamoxifen-related) hot flashes [22]. The most potent CYP2D6-inhibiting SRIs are paroxetine and fluoxetine [21]. When coadministration of an SRI is necessary with tamoxifen therapy, patients are often treated with weak CYP2D6-inhibiting SRIs like citalopram or escitalopram to minimize the risk of changes in endoxifen plasma concentrations [19,20]. However, SRIs such as citalopram and escitalopram are also known to cause prolongation of the QTc-interval [23].

Since both tamoxifen and SRIs may prolong the QTc-interval, the combined use of these drugs may result in an enhanced risk of prolongation of the QTc-interval and therefore ventricular arrhythmias, especially in breast cancer patients since they have often more additional risk factors (e.g. female gender, often older age). At present it is unknown if the effect of combined treatment on the QTc-interval is additive or synergistic. Hence, the objective of this study was to determine whether there is a clinically relevant drug-drug interaction between tamoxifen and SRIs resulting in a prolonged QTc-interval.

MATERIALS AND METHODS

Study Design

This observational study was performed between February 2012 and October 2018. Electrocardiogams (ECGs) were collected in the Erasmus University Medical Center in Rotterdam, the Franciscus Vlietland & Gasthuis in Schiedam and the Elisabeth-Tweesteden hospital in Tilburg, the Netherlands. This study has focused on the QTc-interval during treatment with tamoxifen monotherapy compared to treatment with tamoxifen and SRIs (i.e. SSRIs, SNRIs and tricyclic antidepressants). The study was approved by the local ethics committee of the Erasmus Medical Center in Rotterdam (MEC12–109).

Study Population

We included a total of 100 adult patients with breast cancer for whom treatment with tamoxifen was indicated. Fifty patients also used an SSRI, venlafaxine or a tricyclic antidepressant, which also inhibits serotonin reuptake and may increase the risk for QTc-interval prolongation (e.g. amitriptyline). ECGs were taken at any time interval following drug intake. Patients were on tamoxifen treatment for at least 4 weeks. Patients were included either retrospectively or prospectively. Patients should not have received chemotherapy or radiotherapy within 4 weeks prior to the ECG-recording. If patients were included prospectively, written informed consent was obtained. If the ECG of patients showed a left or right bundle branch block (LBBB/RBBB), atrial fibrillation or other ECG abnormalities due to cardiac pathology, ischaemia or bigeminy, they were excluded from further analysis owing to interference of these factors with the QTc-interval. ECGs showing a QRS complex of >120 ms, RR intervals >1800 ms (defined as the time between two consecutive R waves) or < 500 ms or ECGs with a QTc interval > 700 ms or < 300 ms were also excluded, since the QTc-interval could not be reliably measured. In addition, patients who used other strong inhibitors/inducers of CYP2D6 and/or CYP3A4 (according to the Flockhart table) were excluded from the

analysis [21]. Medication with a 'known' risk of TdP according to the CredibleMeds list of QTc-prolonging drugs, except for tamoxifen and SRIs, was prohibited and considered as exclusion criterion [13].

Outcome Measures and Data Collection

The primary outcome measure of this study was the difference in QTc-interval duration between tamoxifen monotherapy and tamoxifen therapy with concomitant use of SRIs. Secondary outcomes were the difference in the prevalence of QTc-interval prolongation between the two groups and the identification of risk factors for QTc-interval prolongation. QTc-interval prolongation was defined as a QTc-time of >470 ms in females and > 450 ms in males, based on the ESC guidelines [2]. Twelve-lead ECGs were recorded and QT-intervals were measured manually by the same researcher for all patients, preferably from lead II, from the onset of the QRS complex to the end of the T-wave, according to the tangent method, and were corrected for heart rate using the Fridericia formula (QTcF) [24]. The Fridericia formula is formulated as the QT-interval divided by the RR-interval to the power 0.33 (QTcB = QT/(RR0.33)) [25]. For each patient data on characteristics such as age, sex, medical history, tumor localization, previous anti-cancer treatment, laboratory analysis (i.e. liver function [AST, ALT, bilirubin], renal function [creatinin, glomerular filtration rate(eGFR)], electrolytes [sodium, potassium, calcium, magnesium]) and medication was obtained from electronic patient records (HIX, Chipsoft b.v., Amsterdam, the Netherlands). ECGs were obtained during tamoxifen or tamoxifen concomitant with an SRI therapy, when steady state therapy for both therapies was reached (determined as at least four weeks of use for tamoxifen and one week for SRIs). A baseline ECG was determined as an ECG before start of tamoxifen or SRI therapy.

Statistical Analysis

QTc-intervals were compared between patients receiving tamoxifen monotherapy and patients receiving tamoxifen with concomitant SRI therapy. To detect a difference of 15 ms, assuming a standard deviation for QTc-interval time of 26 ms, in mean QTc-interval between both groups with 80% power, a total of one hundred patients was required. Therefore, a total of fifty evaluable patients using tamoxifen monotherapy and fifty evaluable patients using tamoxifen concomitant with an SRI were included in the study. A p value ≤0.05 was considered statistically significant. Data was analyzed using IBM SPSS Statistics version 24 (IBM Corporation, Armonk, NY). A t-test for independent samples was used to compare the mean QTc-interval between the treatment groups. Furthermore, difference between treatment groups in mean age was also determined using a t-test. For the other patient characteristics the chi-square test was used.

Moreover for age, renal function, sodium, potassium, calcium and magnesium a Pearson correlation coefficient was estimated to determine the correlation with the QTc-interval. Correlation for other parameters as tumor localization and previous therapy (e.g. anthracyclines, trastuzumab and radiotherapy) was estimated using a Spearman correlation coefficient. For the secondary outcome the QTc-interval was dichotomized as either prolonged if >470 ms for females or not prolonged if otherwise, according to the ESC guidelines [2]. Difference in proportion of QT-interval prolongation between groups was determined using the Fisher's exact test. Univariate logistic regression analysis was performed to determine associated risk factors. If there were any significant risk factors they were put into a multivariate analysis.

RESULTS

Participants

A total of 111 breast cancer patients were initially included in this study. Eleven patients were excluded due to a variety of ECG abnormalities at baseline resulting in a total of 100 evaluable patients. Fifty patients were treated with tamoxifen in combination with an SRI (further referred to as index group) and 50 patients were treated with tamoxifen without an SRI (further referred to as control group). All patients were female. The median age of patients in the control group (60; interquartile range (IQR) = 50–66 years) was significantly higher than the median age of patients in the index group (50; IQR = 45–59 years; P = 0.01). There were no other statistically significant differences between the two groups and none of the patients experienced cardiac arrhythmias. The most frequently used SRIs in the index group were venlafaxine (30%) and paroxetine (20%). A more detailed overview of the patient characteristics is presented in **Table 1.**

Primary Outcome Measures

Mean QTc interval was 407.5 ± 22.1 ms in the control group and 419.9 ± 24.1 ms in the index group. This resulted in a significant difference in mean QTc interval of 12.4 ms (95%CI 1.8–23.1 ms; P = 0.023) (**Table 2**). Heart rate was not significantly different between the control group and the index group.

TABLE 1. Patient characteristics

Characteristic	Index group (N=50) (%)	Control group (N=50) (%)	P-value of the difference
Age (Median, IQR)*	50 (45-59)	60 (50-66)	0.01*
<65 years	41 (82)	37 (74)	
≥65 years	9 (18)	13 (26)	
Sex			NA
Female	50	50	
Race			0.28
Caucasian	45 (90)	45 (90)	
Arabic	4 (8)	1 (2)	
African	1 (2)	1 (2)	
Latino	-	3 (6)	
Breast cancer localization#			0.54
Left	25 (50)	22 (44)	
Right	23 (46)	28 (56)	
Trastuzumab pretreatment			0.20
Yes	8 (16)	3 (6)	
No	42 (84)	47 (94)	
Anthracycline pretreatment			1.0
Doxorubicin	21 (42)	17 (34)	
Epirubicin	19 (38)	22 (44)	
No	10 (20)	11 (22)	
Radiotherapy			0.69
Yes	26 (52)	29 (58)	
no	24 (48)	21 (42)	
Number of drugs	2 (0-6)	2 (0-4)	0.93
Tamoxifen dose			0.20
20 mg	45 (90)	49 (98)	
40 mg	5 (10)	1 (2)	
Type of antidepressant			NA
Venlafaxine	15 (30)		
Paroxetine	10 (20)		
Escitalopram	5 (10)		
Citalopram	5 (10)		
Amitriptyline	5 (10)		
Sertraline	4 (8)		
Fluoxetine	3 (6)		
Other	3 (6)		
Renal dysfunction	1	3	0.30

TABLE 1. Continued

Characteristic	Index group (N=50) (%)	Control group (N=50) (%)	P-value of the difference
Electrolyte disturbances			
Hyponatremia	2	0	0.50
Hypopotassemia	0	0	-
Hypocalcemia	3	2	1.0
Hypomagnesemia	1	2	1.0
Hepatic dysfunction	1	1	1.0
Antidiabetic use	4 (8)	3 (6)	1.0
Loopdiuretic use	0 (0)	1 (2)	1.0

*Abbrevations: IQR = interquartile range, NA=not applicable, Other type of antidepressant were fluvoxamine (n=1), imipramine (n=1) and nortriptyline (n=1). Renal dysfunction was defined as estimated Glomerular Filtration Rate (eGFR) <60m/min/1,73m^2, Hyponatremia was defined as a sodium value <136 mmol/l, Hypopotassemia was defined as a potassium value <3.5 mmol/l, hypocalcemia was defined as a calcium value < 2.2mmol/l, hypomagnesemia was defined as a magnesium value <0.7mmol/l and hepatic dysfunction was defined as increased bilirubin (>16umol/l), increased alanine aminotransferase (ALAT) (>40 U/l) or increased aspartate transaminase (ASAT) (>35 U/l). Missing values: Hepatic function (N=33), Sodium (N=31), potassium (N=27), calcium (N=60), magnesium (N=66) and renal function (N=28); * = P-value < 0.05. # For breast cancer localization the equation was made for left or right. There was 1 patient with breast cancer on both sides at primary diagnosis and 1 patient for which data regarding tumor localization was unknown due to lack of information from the referring center. These patients were both excluded from this analysis.*

TABLE 2. QTc interval

	Mean QTc (Fridericia) time (ms) ± SD	Patients with QTc prolongation	Mean Difference (ms) (95%CI)	P-value	Mean Heart rate (beats/ min) ± SD
Tamoxifen monotherapy	407.5 ± 22.1	1 (2%)	+12.4 (1.8 to 23.1)	P=0.023*	70 ± 13.6
Tamoxifen with SSRI	419.9 ± 24.1	0 (0%)#			69 ± 10.9
Venlafaxine	408.8 ± 21.5		+1.3 (-11.4 to 14.0)	0.84	
Paroxetine	424.7 ± 29.2		+17.2 (1.4 to 33.0)	0.04*	
Escitalopram	420.0 ± 6.0		+12.5 (4.4 to 20.6)	0.007*	
Citalopram	428.2 ± 16.6		+20.7 (0.7 to 40.7)	0.047*	
Amitriptyline	428.8 ± 32.5		+21.3 (-0.1 to 42.5)	0.054	
Sertraline	424.3 ± 24.1		+17.0 (-5.6 to 39.6)	0.147	
Fluoxetine	414.7 ± 25.6		+7.2 (-18.7 to 33.1)	0.59	

*Legend: This table shows the QTc times and difference in QTc-interval between treatment groups. * P-value <0.05. For the analysis of the differences an independent samples t-test was used. #Difference in number of patients with QTc prolongation was not significant (P=1.0).*

TABLE 3. Risk factors for QTc-interval prolongation

Patients	QTc-interval prolongation (N=1)	Correlation coefficient (P-value)
Age		0.24 (0.02)*
Age >65	1	0.18 (0.07)
Race		0.07 (0.47)
Caucasian	0	
Arab	0	
African	0	
Latino	1	
Breast cancer localization*		-0.16 (0.11)
Left	1	
right	0	
Trastuzumab	0	-0.03 (0.81)
Anthracyclines		0.14 (0.15)
Doxorubicin	0	
epirubicin	0	
No	1	
Radiotherapy	1	0.10 (0.34)
Use of >1 concomitant drug	1	0.23 (0.02)*
SRI use	0	0.25 (0.01)*
Type of SRI		0.27 (0.06)
Venlafaxine	0	
Paroxetine	0	
Escitalopram	0	
Citalopram	0	
Amitriptyline	0	
sertraline	0	
Fluoxetine	0	
Other	1	
Renal dysfunction	1	-0.24 (0.04)*
Electrolyte disturbances		
Hyponatremia	0	-0.19 (0.12)
Hypopotassemia	0	-0.28 (0.02)*
Hypocalcemia	0	-0.14 (0.39)
Hypomagnesemia	0	0.29 (0.09)
Hepatic dysfunction	0	0.20 (0.10)
Antidiabetics	1	-0.12 (0.24)
Loop diuretics	0	-0.10 (0.32)

*Legend: Number of patients which show QTc-interval prolongation (QTc>470ms), when using the Fridericia formula. Furthermore the correlation coefficient was calculated and displayed. For breast cancer localization the equation was made for left or right. There was 1 patient with breast cancer on both sides at primary diagnosis and 1 patient for which data regarding tumor localization was unknown due to lack of information from the referring center. These patients were both excluded from this analysis. * P-value<0.05*

Secondary Outcome Measures

Analysis with the Fridericia formula resulted in 1 patient with a prolonged QTc-interval, which was in the control group. This resulted in a prevalence of 2% in the control group and a prevalence of 0% in the index group, which was a non-significant difference (P = 1.0). None of the patients had a QTc-interval of >500 ms. SRI subgroup analysis showed a significant difference in mean QTc-interval time for paroxetine (17.2 ms; 95%CI 1.4–33.0 ms; P = 0.04), escitalopram (12.5 ms; 95%CI 4.4–20.6 ms; P = <0.01) and citalopram (20.7 ms; 95%CI 0.7–40.7 ms; P = 0.047) compared to the control group in contrast to the other SRIs, which did not show a significant difference in QTc-interval (**Table 3**).

For the known risk factors for QTc-interval prolongation, only SSRI use (Spearman r = 0.25; P = 0.01), age (Pearson r = 0.24; P = 0.02), plasma potassium levels (Pearson r = −0.28, P = 0.02), renal dysfunction (Pearson r = −0.24; P = 0.04) and the use of >1 concomitant drugs used (Spearman r = 0.23, P = 0.02) showed significant correlation with QTc-interval duration. There were no other factors which showed a significant correlation with QTc-interval in general (**Table 3**). Furthermore possible risk factors as tumor localization (left vs. right) and (pre)treatment with anthracyclines, radiotherapy or trastuzumab did not show statistically significant correlation with QTc-interval in general. Univariate analysis did not reveal any significant risk factors and therefore a multivariate analysis was not performed. The odds ratios for the individual risk factors could not be measured reliably, since the prevalence of QTc prolongation was low.

DISCUSSION

To our knowledge, this is the first study that investigated the additional risk of developing QTc- prolongation in patients using tamoxifen in combination with an SRI. This study showed a significant difference in the mean QTc-interval between patients treated with tamoxifen monotherapy and patients treated with tamoxifen therapy concomitantly with an SRI, suggesting an additional QTc-prolonging effect if tamoxifen is combined with an SRI. Furthermore, in this study 1% of the patients had a prolonged QTc-interval (>470 ms). This prevalence is in line with other clinical findings and a recent investigation in cancer patients treated with conventional or targeted anti-cancer therapy [26,27].

In this study, ECGs were retrospectively or prospectively collected during tamoxifen steady-state monotherapy or tamoxifen therapy combined with an SRI. One of the main limitations of this study was the absence of a baseline measurement in most of the patients. Therefore, a 'change from baseline' analysis could not be performed.

There was a significant difference in mean QTc-interval time between the tamoxifen monotherapy and tamoxifen with SRI treated patients, which is most likely related to the additive effect of the SRI. As mentioned earlier tamoxifen is an assumed QTc-interval prolonging agent, especially in higher doses [8,16]. Furthermore there is substantial evidence regarding QTc-interval prolongation by SRIs, showing an average increase in QTc-interval of 10-20 ms. QTc-interval prolonging effects seem most prominent in nortriptyline and citalopram with increases of more than 30 ms [28,29]. Therefore an additive effect of SRIs seems possible on top of the QTc-interval prolonging effects of tamoxifen. However, to determine whether the use of an SRI in combination with tamoxifen is a significant/clinically relevant factor influencing the QTc-interval, more research is needed in patients having both a baseline ECG during tamoxifen use and at least a second ECG where tamoxifen is used in combination with an SRI.

Interestingly, a subgroup analysis of the different SRIs showed a significant increase of the QTc-interval for citalopram, escitalopram and paroxetine, which is in line with the classification on the CredibleMeds list. In this list, citalopram and also escitalopram has been clearly associated with QTc-interval prolongation. On this list paroxetine is classified as a drug which gives a 'conditional' risk of TdP. Several additional factors like antidiabetic drug use, renal dysfunction and multiple drug use may have contributed to QTc-interval prolongation in some of these patients. Furthermore, patients in the control group were significantly older than in the index group. The QTc-interval increases with age, and therefore in elderly patients, the criteria for QTc-interval prolongation will be met more frequently in the index group. We do acknowledge that due to limited sample size in the subgroup our study was underpowered to make definitive conclusions regarding individual drugs.

Although QTc-interval prolongation is carefully investigated during early drug development, its actual influence on overall survival remains unclear. It is clear that QTc-interval prolongation can lead to ventricular tachyarrhythmias (e.g. TdP) and SCD [1,3]. A recent systematic review from Arunachalam et al. showed that ventricular tachyarrhythmias were observed in 2.6% of patients using QTc-interval prolonging drugs, however TdP (0.33%) and SCD (0.03%) were relatively rare [27]. Since the absolute risk of cardiac events is small, physicians always need to weigh the benefits of cessation of a QTc-interval prolonging drug to the disadvantage of discontinuation of a potentially useful drug. If a QTc-interval prolonging drug can be replaced by a non QTc-interval prolonging agent, this should always be considered.

The interaction investigated in this study may be explained at a pharmacodynamic or pharmacokinetic level. Both tamoxifen and SRIs inhibit the Ikr and therefore, both

may prolong the QTc-interval. Inhibition of CYP2D6 by SRIs results in lower endoxifen plasma levels (especially for strong CYP2D6 inhibitors like paroxetine) and possible more Ikr inhibition, because of the higher tamoxifen plasma levels. However preclinical evidence suggests similar Ikr-inhibition by both tamoxifen and its metabolites, making this a less likely explanation [20,30–32]. SRIs like fluoxetine and paroxetine are well known strong CYP2D6 inhibitors, which could alter endoxifen concentrations and deprive patients from optimal oncologic therapy. Escitalopram, citalopram and venlafaxine are weak CYP2D6 inhibitors and therefore are considered safe when administered concomitantly with tamoxifen [20]. However, since escitalopram and citalopram are also 'known' QTc-interval prolonging drugs, the combination with tamoxifen is not desirable and venlafaxine may be a better alternative since it seems to prolong the QTc-interval in only a minor extent, as was shown in this study (**Table 2**). However more research is needed to verify this point.

In conclusion, this study is the first clinical study that investigated the additional risk of QTc-interval prolongation in patients using an SRI concomitantly with tamoxifen. There was a significantly longer mean QTc-interval in the patients who used an SRI, which tended to be most prominent in patients receiving citalopram, escitalopram or paroxetine. The other SRIs, like venlafaxine and fluvoxamine, were not clearly associated with QTc-interval prolonging effects. Based on our data we recommend avoiding citalopram, escitalopram and paroxetine in tamoxifen treated women, and use the others SRIs that do not have this QTc-prolonging effect (e.g. venlafaxine and fluvoxamine) to minimize the possible risk of TdP and cardiac arrhythmias. As the degree of QTc-interval prolongation was limited, and none of the patients in this study reached a QTc-interval of >500 ms, routinely checking ECGs in patients on combined tamoxifen+SRI treatment does not seem necessary. For patients who have multiple other risk factors for QTc-interval prolongation and are using paroxetine, escitalopram and citalopram checking the QTc-interval duration may increase patient safety.

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PART II

FOOD-DRUG INTERACTIONS

CHAPTER 9

CLINICAL IMPLICATIONS OF FOOD–DRUG INTERACTIONS WITH SMALL MOLECULE KINASE INHIBITORS

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ABSTRACT

During the past two decades, small-molecule kinase inhibitors have proven to be valuable in the treatment of solid and haematological tumours. However, because of their oral administration, the intrapatient and interpatient exposure to small-molecule kinase inhibitors (SMKIs) is highly variable and is affected by many factors, such as concomitant use of food and herbs. Food–drug interactions are capable of altering the systemic bioavailability and pharmacokinetics of these drugs. The most important mechanisms underlying food–drug interactions are gastrointestinal drug absorption and hepatic metabolism through cytochrome P450 isoenzymes. As food–drug interactions can lead to therapy failure or severe toxicity, knowledge of these interactions is essential. This Review provides a comprehensive overview of published studies involving food–drug interactions and herb–drug interactions for all registered SMKIs up to Oct 1, 2019. We critically discuss US Food and Drug Administration (FDA) and European Medicines Agency (EMA) guidelines concerning food-drug interactions and offer clear recommendations for their management in clinical practice.

INTRODUCTION

Since the start of this millennium, a new class of anticancer drugs has gained an important role in the treatment of solid and haematological tumours: the small-molecule kinase inhibitors (SMKIs). SMKIs cause cell-cycle arrest, induce apoptosis, inhibit angiogenesis, and modulate tumour immunity by specifically inhibiting cellular signal transduction through blocking dysregulated protein kinases.[1] Some SMKIs are registered for specific oncogenic driver mutations, which need to be determined using molecular diagnostics. As a result, this tailored treatment approach often results in better efficacy with a favourable risk-benefit balance when compared with chemotherapy.[2,3]

With the introduction of SMKIs, new challenges have emerged. Different from most chemotherapeutic drugs, which are administered intravenously, SMKIs are administered orally. Although oral intake improves patient comfort and flexibility of treatment (eg, place and timing of intake), the variability in intrapatient and interpatient exposure to SMKIs is high[4] and is affected by many factors, such as drug-drug interactions, concomitant use of food and medicinal herbs, genetic variance, and lifestyle.[5] The effect of food on drug exposure could be clinically significant. For example, administration of lapatinib, combined with a high-fat meal, increases its plasma concentrations more than three times.[6] Besides a concomitant meal, other specific foods and beverages might cause food–drug interactions (FDIs). Additionally, some herbal products that are frequently used by patients with cancer have substantial potency to cause herb–drug interactions (HDIs).[7] FDIs and HDIs can affect plasma drug concentration, which is a result of the absorption, distribution, metabolism, and elimination of a drug (ie, pharmacokinetic interactions). Most patients and clinicians are insufficiently aware of possible FDIs and HDIs and their potential risk for treatment inefficacy or toxicity.[8] Hence, it is crucial to have thorough knowledge of these FDIs and HDIs for safe and optimal treatment of patients with cancer.

The US Food and Drug Administration (FDA) and the European Medicines Agency (EMA) provide recommendations for assessing possible FDIs, to ensure optimal dose finding and drug labelling.[2,3] To define whether or not food intake clinically relevantly affects the plasma concentration of a drug, the FDA applies the bioequivalence range of 80–125% for the 90% CI of total exposure—known as the area under the curve (AUC)—or maximal plasma concentration (C_{max}). SMKI administration during the fasting state serves as the reference.[3] Regarding herbs, only EMA states that efforts should be made to investigate a possible HDI when reports suggest a clinically relevant interaction.[2]

This Review presents a comprehensive overview of published studies regarding FDIs and HDIs for all registered SMKIs (until Oct 1, 2019). It discusses the most important mechanisms underlying FDIs and HDIs and aims to provide clear recommendations to manage clinically relevant interactions in daily practice.

ABSORPTION

Gastrointestinal absorption has a key role in the plasma concentrations of SMKIs. Before entering the portal bloodstream, drugs must first dissolve and pass enterocyte cell membranes. The solubility of weakly basic drugs, such as SMKIs, is largely dependent on the intragastric pH. The intragastric pH is increased by food, acid-suppressing drugs, or both.[9] Postprandial rise in intragastric pH shifts the drug's ionised/non-ionised equilibrium to the non-ionised form, and reduces SMKI solubility and absorption. Since most SMKIs are also lipophilic drugs,[10] they probably dissolve better when administered concomitantly with a (fat) meal. Additionally, food enhances splanchnic blood flow and bile secretion and it increases intragastric and intestinal retention and transit time,[11] thus increasing drug absorption potential.

Besides passive diffusion, multiple drug transporters are important for drug permeability. Organic anion and cation (uptake) peptides actively transport the SMKI into the enterocyte from which the drug is transported (or can diffuse) to the portal vein. Contemporaneous counter (efflux) transport to the intestinal lumen can occur by P-glycoprotein (ATP-binding cassette B1; ABCB1) and breast cancer resistance protein (BCRP; ABCG2).[5] In case of high-passive diffusion, food interactions with transporters are not likely to result in clinically significant altered exposures. Various food constituents (eg, curcumin, flavonoids, bitter melon) and beverages (eg, tea catechins) are known to inhibit P-glycoprotein, whereas St John's wort is a potent P-glycoprotein inducer, which could decrease drug exposure.[12]

FOOD–DRUG INTERACTIONS

High-fat meal

High-fat test meals consist of 800–1000 kcal of which approximately 500–600 kcal is derived from fat and 250 kcal from carbohydrates (eg, a full English breakfast).[2,3] Concomitant SMKI administration with a high-fat meal resulted in a clinically significant increase of C_{max} and AUC for twelve SMKIs, and in a 29–51% decrease of C_{max} and AUC for three SMKIs (ie, afatinib, dabrafenib, and sorafenib). We noted the relative changes in the C_{max} and AUC when an SMKI at its therapeutic dose is administered with a high-fat,

moderate-fat, or low-fat meal, compared with the fasted state (**Table 1**).[2, 3, 6, 10, 13-45] On the contrary, for seventeen SMKIs, concomitant food intake showed no relevant effect on its AUC. However, brigatinib, encorafenib, ruxolitinib, tivozanib, and trametinib had decreases in C_{max} of 20% or more with a high-fat meal, but had no effect on their AUC.

Moderate-fat and low-fat meals

Moderate-fat test meals contain half the caloric content of a high-fat meal, with fat contributing to approximately 150 kcal.[2] Low-fat test meals are less consistent between studies because they are neither defined by FDA nor EMA,[2, 3] but these meals roughly consist of less than 100 kcal derived from fat (eg, a continental breakfast). Similar to high-fat meals, concomitant SMKI administration with moderate-fat meals did not result in clinically significant FDIs for axitinib and sorafenib. The absence of clinically relevant FDIs also applies to the low-fat meals that were studied for eight SMKIs. On one hand, only when FDIs with a high-fat meal are known to occur, further investigation of drug intake with other types of meals (eg, moderate-fat or low-fat meals) is indicated to improve patient comfort, or to reduce drug dosage and costs. On the other hand, when FDIs do not occur with high-fat diets, doing additional FDI studies with lower fat meals is not indicated, because a high-fat meal functions for lipophilic drugs as proof-of-principle with maximal interacting potential.

General recommendations

Alteration of C_{max} or AUC caused by an FDI could potentially alter the drugs' toxicity and effectiveness. Recommendations on food intake should be based on combining optimal effectiveness with the lowest toxicity possible. In general, these recommendations are straightforward: when food greatly decreases an SMKI's exposure, patients should be instructed to take the SMKI without food, because it could decrease the effectiveness of the drug. When food does not affect an SMKI's AUC, patients should be given free choice whether to use the SMKI with or without food. However, when food substantially increases a SMKI's exposure without affecting its tolerability, more balanced recommendations should be given. Only when safety has been confirmed, SMKIs with FDIs that increase the exposure are allowed to be administered with food. For several SMKIs, viable or promising correlations have been reported between pharmacokinetic parameters (eg, AUC or plasma trough concentration) and survival or response.[46] In such a case, the optimal method to individualise SMKI treatment is the frequent monitoring of SMKI plasma concentrations, also known as therapeutic drug monitoring.[47] When plasma concentrations decrease to less than the therapeutic threshold, despite a good adherence, patients might be advised to take the SMKI concomitantly with a meal.

TABLE 1. Overview of the relative changes in the C_{max} and AUC when an SMKI at its therapeutic dose is administered with a high-fat, moderate-fat, or low-fat meals, compared with the fasted state

	Change in C_{max} (%)	Change in AUC (%)	Importance	FDA or EMA recommendation	Author recommendation
Afatinib[2, 3, 13]					
High-fat meal	−50%	−39%	Moderate	Take without food	Take without food
Alectinib[2, 14]					
High-fat meal	170%	192% to 210%	Major	Take with food	Take with food*
Axitinib[2, 3, 15]					
High-fat meal	11%	19%	Minor	Take with or without food	Take with or without food
Moderate-fat meal	−16%	−10%	Minor	Take with or without food	Take with or without food
Binimetinib[2, 3]					
High-fat meal	−17%	−1%	Minor	Take with or without food	Take with or without food
Low-fat meal	−29%	No effect	Minor	Take with or without food	Take with or without food
Bosutinib[2, 3, 16]					
High-fat meal	42% to 80%	54% to 70%	Major	Take with food	Take with food
Brigatinib[2, 3, 45]					
High-fat meal	−24% to −13%	−2%	Minor	Take with or without food	Take with or without food
Cabozantinib[2, 3, 17]					
High-fat meal	41%	57%	Moderate	Take without food	Take without food
Ceritinib[2, 3, 18, 19]					
High-fat meal	41%	73%	Major	Take 450 mg with food or 750 mg without food	Take preferably 450 mg with food, or 750 mg without food
Low-fat meal	43% to 45%	54% to 58%	Moderate	Take 450 mg with food or 750 mg without food	Take preferably 450 mg with food, or 750 mg without food
Low-fat meal (450 mg dose) versus fasted (750 mg dose)	3%	4%	Minor	Take 450 mg with food or 750 mg without food	Take preferably 450 mg with food, or 750 mg without food
Low-fat meal (600 mg dose) versus fasted (750 mg dose)	25%	24%	Moderate	Take 450 mg with food or 750 mg without food	Take preferably 450 mg with food, or 750 mg without food
Cobimetinib[2, 3, 20]					
High-fat meal	0% to 7%	0% to 10%	Minor	Take with or without food	Take with or without food
Crizotinib[2, 3, 21, 22]					
High-fat meal	−14% to 0%	−14% to 0%	Minor	Take with or without food	Take with or without food
Dabrafenib[2, 3, 23]					
High-fat	−51%	−31%	Moderate	Take without food	Take without food
Dasatinib[2, 3]					

TABLE 1. Continued

	Change in C_{max} (%)	Change in AUC (%)	Importance	FDA or EMA recommendation	Author recommendation
High-fat meal	NA	14%	Minor	Take with or without food	Take with or without food
Low-fat meal	NA	21%	Minor	Take with or without food	Take with or without food
Encorafenib[2, 3]					
High-fat meal	−36%	−4% to 0%	Minor	Take with or without food	Take with or without food
Erlotinib[24, 25]					
High-fat meal	33% to 56%	33% to 66%	Moderate	Take without food	Take without food
Gefitinib[2, 26]					
High-fat meal	32%	37%	Moderate	Take with or without food	Take with or without food
Ibrutinib[2, 3, 27, 28]					
High-fat meal	163% to 400%	62% to 200%	Major	Take with or without food	Take with food
Imatinib[2, 29]					
High-fat meal	−15% to −11%	−7%	Minor	Take with food	Take with or without food
Lapatinib[2, 3, 6, 30]					
High-fat meal	166% to 203%	100% to 325%	Major	Take without food	Take with a low-fat meal
Low-fat meal	90% to 150%	80% to 200%	Major	Take without food	Take with a low-fat meal
Lenvatinib[2, 3, 31, 32]					
High-fat meal	−4% to 0%	0% to +6%	Minor	Take with or without food	Take with or without food
Nilotinib[2, 3, 33]					
High-fat meal	48% to 112%	43% to 82%	Major	Take without food	Take without food
Low-fat meal	33% to 55%	15% to 29%	Moderate	Take without food	Take without food
Nintedanib[2, 3]					
High-fat meal	19%	21%	Minor	Take with food	Take with or without food
Osimertinib[2, 3, 34, 35]					
High-fat meal	−7% to 14%	6% to 19%	Minor	Take with or without food	Take with or without food
Pazopanib[2, 3, 36, 44]					
High-fat meal	108%	134%	Major	Take without food	Take preferably 600 mg with food, or 800 mg without food
Low-fat meal	110%	92%	Major	Take without food	Take preferably 600 mg with food, or 800 mg without food

TABLE 1. Continued

	Change in C_{max} (%)	Change in AUC (%)	Importance	FDA or EMA recommendation	Author recommendation
Low-fat meal (600 mg dose) versus fasted (800 mg dose)	12%	9%	Minor	Take without food	Take preferably 600 mg with food, or 800 mg without food
Ponatinib[2, 3, 37]					
High-fat meal	−6% to 0%	0% to 10%	Minor	Take with or without food	Take with or without food
Low-fat meal	−6 % to 0%	−2% to 0%	Minor	Take with or without food	Take with or without food
Regorafenib[2, 3]					
High-fat meal	73%	48%	Moderate	Take with food or low-fat meal	Take without food
Low-fat meal	54%	36%	Moderate	Take with food or low-fat meal	Take without food
Ruxolitinib[2, 3, 38]					
High-fat meal	−24%	5%	Minor	Take with or without food	Take with or without food
Sorafenib[2, 3]					
High-fat meal	NA	−30% to −29%	Moderate	Take without food	Take without food
Moderate-fat meal	NA	no effect	Minor	Take without food	Take without food
Sunitinib[2, 3, 39]					
High-fat meal	0% to 4%	0% to 12%	Minor	Take with or without food	Take with or without food
Tivozanib[2, 3, 40]					
High-fat meal	−23%	7%	Minor	Take with or without food	Take with or without food
Trametinib[2, 3, 41]					
High-fat meal	−70%	−10%	Minor	Take without food	Take with or without food
Vandetanib[3, 42]					
High-fat meal	−11% to 17%	0% to 10%	Minor	Take with or without food	Take with or without food
Vemurafenib[2, 3, 43]					
High-fat meal	114% to 150%	150% to 400%	Major	Take with or without food	Take with food

*The recommendation for all SMKIs is to reduce dose if intolerable toxic effects occur. Importance of the food-drug interaction is considered minor (not clinically relevant) when AUC is <20% decreased or <25% increased, moderate when AUC is ≥20% and <50% decreased or ≥25% and <67% increased, and major when AUC is ≥67% increased or ≥50% decreased. C_{max}=maximal plasma concentration. AUC=area under the curve. SMKI=small-molecule kinase inhibitors.*As alternative for dose reduction, consider administration without food if intolerable toxic effects occur.*

Specific recommendations

We noted the relative changes in C_{max} and AUC of all SMKIs when taken with a high-fat meal (**Figure 1**). FDA and EMA recommendations are expected to be strict: SMKIs shown in the grey area can be taken with or without food, whereas SMKIs outside the grey area can only be taken without food (ie, fasted). However, for some SMKIs, FDA and EMA recommendations are not in accordance with these principles.

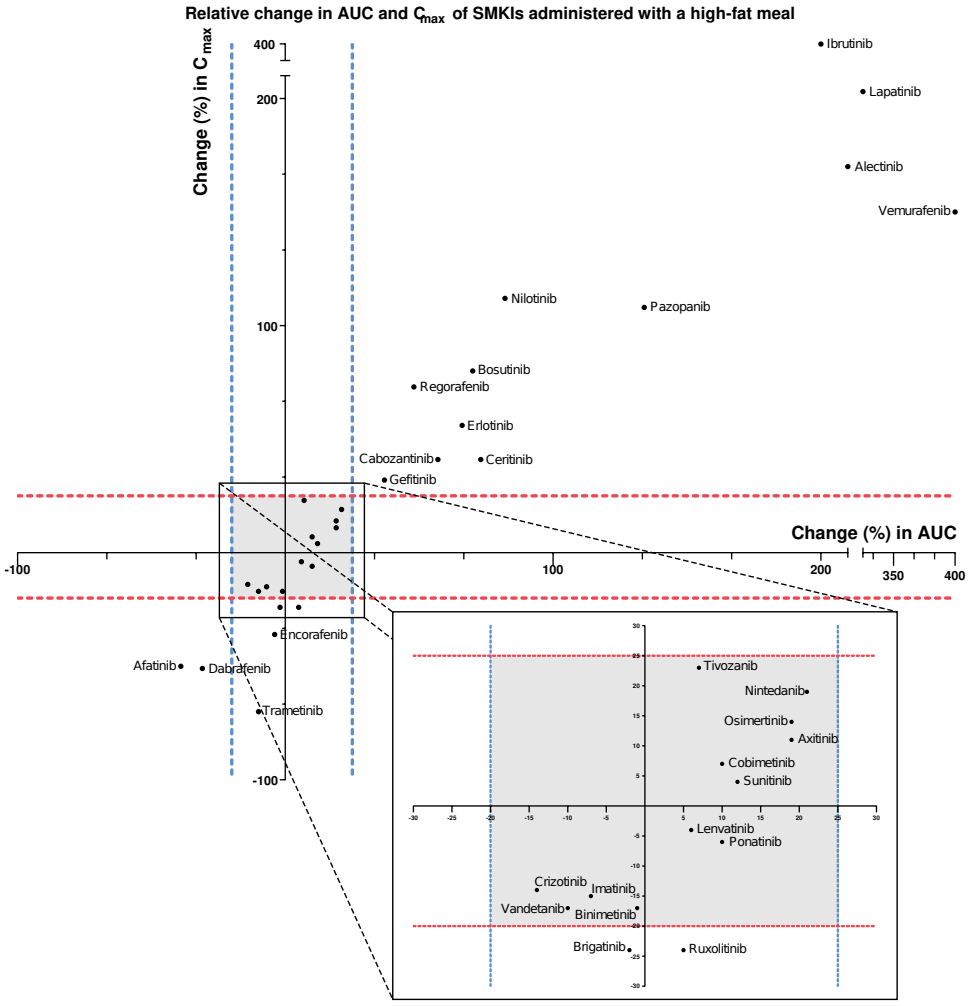

FIGURE 1. Relative change in AUC and C_{max} of SMKIs administered with a high-fat meal
Data derived from **Table 1**. *The central grey area emphasises the range of 80% to 125% in which no clinically relevant FDI occurs. Dabrafenib and sorafenib are not displayed because of unavailable C_{max} data. AUC=area under the curve. C_{max}=maximal concentration. SMKIs=small-molecule kinase inhibitors. FDI=food–drug interaction.*

Gefitinib, ibrutinib, and vemurafenib

Gefitinib, ibrutinib, and vemurafenib are known for having a clinically significant food effect, but nonetheless they are recommended by FDA and EMA to be administered with or without food **(Table 1**). Especially for vemurafenib, in which a high-fat meal increases its C_{max} by 150% and AUC by 400%,[43] this recommendation is remarkable. Considering vemurafenib's plasma concentration to be associated with overall survival and developing common terminology criteria for adverse events (CTCAE) grade of 2 or higher skin rash,[48] it is important to reach an effective exposure with minimal toxicity. With the current recommendation, 14% of treated patients do not reach plasma trough concentrations.[48] As vemurafenib has a substantial FDI, and interpatient variability is decreased with food by 49%,[43] we recommend vemurafenib to be taken with food. Awareness and counselling for possible skin rash (CTCAE grade ≥2) are also important. Furthermore, therapeutic drug monitoring could be used to establish drug concentrations at therapeutic levels in the fasted state. For ibrutinib, there is no conclusive evidence for an exposure–toxicity relationship,[3, 27, 28] albeit complete target receptor occupation (and possibly response) is exposure dependent.[49] Therefore, we advise ibrutinib to be taken with food. Gefitinib has the most moderate FDI (32% increase in C_{max} and 37% increase in AUC),[26] which might be the reason for its liberal food recommendation (ie, administration irrespective of food intake).

Alectinib, bosutinib, and regorafenib

Although alectinib, bosutinib, and regorafenib are affected by FDIs, they are specifically recommended to be administered with food. The registration study of alectinib was done with concomitant food administration[50] and no differences in side-effects with fasted conditions were found,[14] therefore patients should be instructed to take alectinib with food. Furthermore, bosutinib was shown to be better tolerated with food at therapeutic doses because the incidence of gastrointestinal adverse events decreased when bosutinib was taken with food.[16] EMA's rationale to recommend administration concomitant with a low-fat meal is based on a better exposure to regorafenib's active metabolites.[2] However, no toxicity data of these studies are reported.[2, 3] Hence, we cannot endorse the recommendation of both FDA and EMA to take regorafenib with a (light) meal.[2, 3] Theoretically, when drug absorption and systemic exposure is increased, the residual gastrointestinal drug fraction with accompanied gastrointestinal toxicity is reduced. For SMKIs, tolerability depends more on local than systemic adverse events, therefore an FDI could optimise efficacy and decrease toxicity simultaneously.[16]

Imatinib, nintedanib, and trametinib

In line with this assumption, imatinib and nintedanib are recommended to be taken concomitantly with food, even though absorption is not clinically affected by food consumption. For both SMKIs toxicity data are lacking. We suggest a recommendation based on patient's preference—ie, intake with or without food. However, even though food does not affect trametinib's AUC, FDA and EMA recommend taking trametinib without food. This recommendation is based on extrapolated calculations by Cox and colleagues,[41] who studied the single-dose pharmacokinetics of trametinib. In our opinion, both single-dose pharmacokinetic studies and pharmacokinetic modelling studies do not adequately show the in-vivo impact of an FDI. Consistently, because trametinib does not have an FDI, trametinib can be administered irrespective of food consumption.

Lapatinib

A multiple-dose FDI study with lapatinib showed a major FDI with no unexpected toxicity when it was taken 1 h after high-fat food consumption.[30] This effect is similar to administration concomitant with a low-fat meal.[6] Extrapolating these results, we recommend lapatinib intake with a low-fat meal. That would additionally allow lapatinib to be co-administered with capecitabine, when given as combination treatment for HER2-positive breast cancer.[2, 3]

Ceritinib and pazopanib

The exposure to ceritinib and pazopanib is greatly affected by FDIs. However, multiple-dose FDI studies compared exposure of standard SMKI dose taken without food with reduced dose taken with food[2, 19, 44, 51] To maintain equivalent exposure to 750 mg ceritinib taken fasted, 450 mg and 600 mg doses of ceritinib were administered with a low-fat meal. Administration of 600 mg ceritinib led to a substantially higher exposure compared with 750 mg in the fasted state, but this is however not clinically relevant. Although 450 mg resulted in an equal exposure (4% AUC increase) in comparison to 750 mg taken fasted, less dose reductions occurred (24% *vs* 65%) due to less gastrointestinal toxicity.[19] Conclusively, treatment efficacy in terms of overall response rate, disease control rate, and time to response was shown to be consistent as well.[51] A 2019 study[44] showed that continental breakfast (ie, low-fat meal) consumption with 600 mg pazopanib had similar exposure and toxicity to 800 mg pazopanib administered without food (9% AUC increase). Additionally, in this study, 68% of patients preferred concomitant food intake over fasting. However, the FDA recommendation is intake of both SMKIs without food,[3] whereas EMA's advice is to swallow 450 mg ceritinib with

food, or 750 mg pazopanib without food.[2] Considering the better tolerability and economic benefits of a lower dose of ceritinib and pazopanib, both SMKIs (450 mg ceritinib and 600 mg pazopanib) could be administered with a low-fat meal.

Clinical implications

Despite these practical recommendations, not all patients will be able to meet them. For example, if patients cannot eat food or if they are on a special diet, SMKI administration with a meal might be complicated. Fasted intake is possible for SMKIs that are recommended to be taken without food or irrespective of food intake. However, for SMKIs we advise to take with food (to maximise exposure), dose escalation to initial registered doses is an option for ceritinib and pazopanib (**Table 1**). Efficacy data of alectinib and bosutinib, when administered without food, are lacking. Alternative administration routes or even alternative therapies should, therefore, be considered. Because current labels of ibrutinib, lapatinib, and vemurafenib do not oblige food intake, it is still safe to take them without food. Also, for some SMKIs, therapeutic drug monitoring should be considered to monitor steady-state exposure and optimise dosage.

Ultimately, clinical application of FDIs can be regarded as food-dependent dose individualisation—ie, dosing based on a patient's food consumption. In another 2019 study,[52] by use of therapeutic drug monitoring to determine exposure, pazopanib was administered with a low-fat meal and the dose was escalated or reduced after evaluation of toxicity. With 64% of the initial registered dose of pazopanib, therapeutic target plasma concentrations were reached for multiple cycles.[52] Preferably, pazopanib dose should have been based on its trough concentration. Food-based dose individualisation could then increase SMKI efficacy and lower its drug costs simultaneously. However, high-fat meals should be advised with caution, because fatty acids showed harmful molecular effects, including increased tumour progression and metastasis.[53]

Nowadays, several combination treatments of SMKIs with immunotherapy are under clinical investigation or already used in clinical practice, for instance the combination of axitinib and pembrolizumab in renal cell carinoma.[54] Since immunotherapy is administered parenterally, an FDI with immunotherapy is not expected to occur. However, when food alters the exposure to the co-administered SMKI, total efficacy or toxicity of the combined SMKI-immunotherapy could be affected. Therefore, it is important to be aware of these FDIs when patients have toxic effects from SMKI-immunotherapy combinations, but also when new combinations are investigated.

Most FDI studies use high-fat meals to find the maximal food effect. This effect could, however, be far from the average daily practice, considering that not all patients with cancer are capable of eating high-fat meals. Furthermore, one study found that 21% of patients did not always follow strict fasting recommendations.[55] The effects of this lack of compliance can be considerable. Illustrative for erlotinib, occasional food intake increased its C_{max} by 35% and AUC by 33%, whereas missing a concomitant meal led to a 14% decrease in C_{max} and 15% decrease in AUC.[24] Moreover, other factors, such as therapy compliance, will seriously affect exposure.[55] Hence, FDIs are an important link in the chain to obtain and maintain an adequate systemic drug exposure.

Other pharmacokinetic food effects

The effects of food on the variability and time to reach maximum concentrations of SMKIs are presented in **Table 2**.[2, 3, 6, 10, 13-45] Additionally,the absolute bioavailability and biopharmaceutical classification system (BCS) classes are reported in **Table 3**.[2, 3, 10]

Bioavailability

The absolute bioavailability is the amount of unchanged drug that has been absorbed by the gastrointestinal tract and has entered systemic circulation after hepatic first-pass metabolism. On one hand, when absolute bioavailability is low, a (high-fat) meal could increase absorption and, therefore, could also increase systemic exposure. On the other hand, FDIs could cause a decrease in exposure for the three SMKIs (dabrafenib, imatinib, and ruxolitinib) with a bioavailability of almost 100%. Food does not affect imatinib or ruxolitinib exposure, but decreases the exposure to dabrafenib with 31%.

Biopharmaceutical classification system

The BCS is based on the aqueous solubility of a drug and its intestinal permeability, which are the most important elements affecting drug absorption. BCS classes are divided in four categories: class I drugs have both high solubility and permeability, class II drugs have low solubility and high permeability, class III drugs have high solubility and low permeability, and class IV drugs have both low solubility and permeability.[3] Taking into consideration their solubility limited absorption, BCS class II and IV drugs could have more FDIs, because high-fat meals can increase drug solubility. The relative changes in AUC with a high-fat meal are categorised by BCS class in **Figure 2**.[10] Most SMKIs are class II drugs, yet some encounter FDIs, which are both increasing and decreasing exposure. FDI prevalence is balanced in the second largest BCS class (IV), all giving an increased exposure. Albeit only seven SMKIs are BCS classes I or III, only afatinib is negatively affected by food. Hence, high solubility could be associated with lower prevalence of FDIs.

TABLE 2. Effect of food on the variability and time to reach maximum concentrations of small-molecule kinase inhibitors

	Change in C_{max} variability (%)	Change in AUC variability (%)	Change in T_{max} (%; fed T_{max})
Afatinib[2, 3, 13]			
High-fat meal	+63%	+2%	+130% (6·9 h)
Alectinib[2, 14]			
High-fat meal	−20%	−6%	+100% (4 h)
Axitinib[2, 3, 15]			
High-fat meal	−45%	−9%	+50% (3 h)
Moderate-fat meal	−34%	+9%	+40% (2·8 h)
Binimetinib[2, 3]			
High-fat meal	−56%	−33%	+132% (2 h)
Low-fat meal	−50%	−8%	+43% (1·3 h)
Bosutinib[2, 3, 16]			
High-fat meal	−61%	−70%	+100% (6 h)
Brigatinib[2, 3, 45]			
High-fat meal	−31%	−11%	+250% (5 h)
Cabozantinib[2, 3, 17]			
High-fat meal	−5%	+4%	+50% (6 h)
Ceritinib[2, 3, 18, 19]			
High-fat meal	−32%	−24%	+25% (10 h)
Low-fat meal	−55% to −44%	−49% to −46%	−12% to +33% (7 to 8 h)
Low-fat meal (450 mg dose) versus fasted (750 mg dose)	NA	NA	+2% (6 h)
Low-fat 600 mg versus fasted 750 mg	NA	NA	+2% (6 h)
Cobimetinib[2, 3, 20]			
High-fat meal	+29%	+22%	+300% (6 h)
Crizotinib[2, 3, 21, 22]			
High-fat meal	+12%	+8%	No effect (5 h)
Dabrafenib[2, 3, 23]			
High-fat meal	−11%	−12%	+200% (6 h)
Dasatinib[2, 3]			
High-fat meal	NA	NA	NA
Low-fat meal	NA	NA	NA
Encorafenib[2, 3]			
High-fat meal	+37%	−9%	+130% (3·5 h)
Erlotinib[24, 25]			
High-fat	−51% to +9%	−38% to +25%	+39% to 74% (3·9 to 4·2 h)

TABLE 2. Continued

	Change in C_{max} variability (%)	Change in AUC variability (%)	Change in T_{max} (%; fed T_{max})
Gefitinib[2, 26]			
High-fat meal	−21%	−3%	No effect (5 h)
Ibrutinib[2, 3, 27, 28]			
High-fat meal	−63% to +2%	−18% to +2%	−53% to +167% (1·5 to 4 h)
Imatinib[2, 29]			
High-fat meal	−20%	−37%	+37% (3·7 h)
Lapatinib[2, 3, 6, 30]			
High-fat meal	No effect	−20%	+50% to 67% (5 to 6 h)
Low-fat meal	−16%	−13%	0% to 30% (3·9 to 4 h)
Lenvatinib[2, 3, 31, 32]			
High-fat meal	−52%	−24%	+100% to 150% (4 to 5 h)
Nilotinib[2, 3, 33]			
High-fat meal	−16% to −13%	−14% to +25%	+20% to 25% (3 to 5 h)
Low-fat meal	−5% to +16%	+7% to 43%	No effect (4 h)
Nintedanib[2, 3]			
High-fat meal	+2%	+63%	+99% (4 h)
Osimertinib[2, 3, 34, 35]			
High-fat meal	+16%	+9%	+33% (8 h)
Pazopanib[2, 3, 36, 44]			
High-fat meal	+23%	+32%	+50% (6 h)
Low-fat meal	−6%	−5%	+50% (6 h)
Low-fat meal (600 mg dose) versus fasted (800 mg dose)	+12%	+6%	+33% (4h)
Ponatinib[2, 3, 37]			
High-fat meal	−1%	+3%	No effect (6 h)
Low-fat meal	+4%	+5%	−17% (5 h)
Regorafenib[2, 3]			
High-fat meal	NA	NA	NA
Low-fat meal	NA	NA	NA
Ruxolitinib[2, 3, 38]			
High-fat meal	+45%	+9%	+150% (2·5 h)
Sorafenib[2, 3]			
High-fat meal	NA	NA	NA
Moderate-fat	NA	NA	NA
Sunitinib[2, 3, 39]			

TABLE 2. Continued

	Change in C_{max} variability (%)	Change in AUC variability (%)	Change in T_{max} (%; fed T_{max})
High-fat meal	−13%	+3%	+2% (8 h)
Tivozanib[2, 4, 40]			
High-fat meal	−23%	−1%	+683% (23·5 h)
Trametinib[2, 3, 41]			
High-fat meal	−12%	+3%	+169% (4 h)
Vandetanib[3, 42]			
High-fat meal	No effect	No effect	+33% (8 h)
Vemurafenib[2, 3, 43]			
High-fat meal	−56%	−49%	+100% (8 h)

C_{max}=maximal plasma concentration. AUC=area under the curve. T_{max}=time to reach C_{max}. NA=not available.

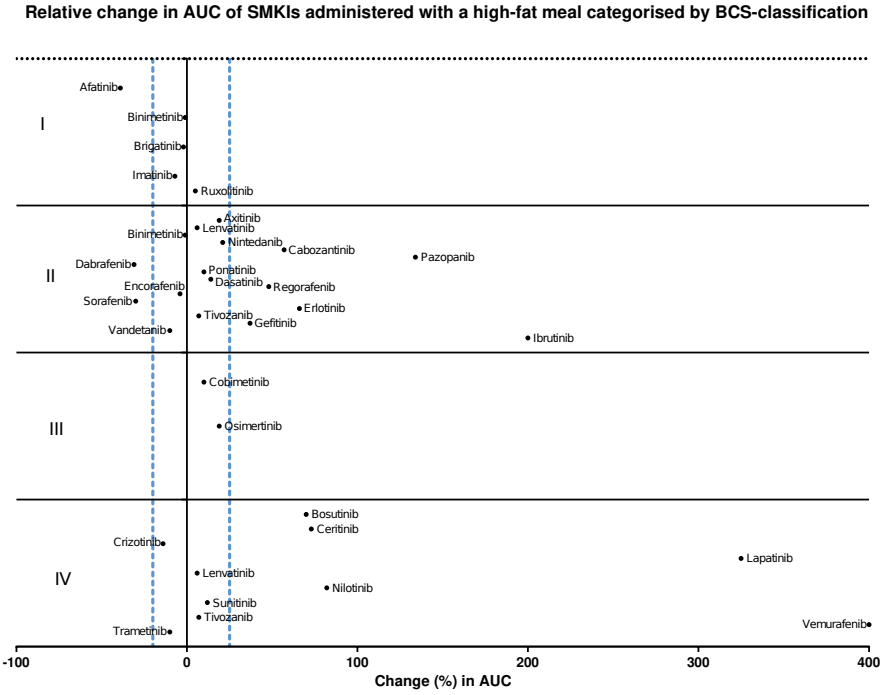

FIGURE 2. Relative change in AUC of SMKIs administered with a high-fat meal categorised by BCS class. Class I drugs have both high solubility and permeability, class II drugs have low solubility and high permeability, class III drugs have high solubility and low permeability, and class IV drugs have both low solubility and permeability. Data derived from **Table 1** and **Table 3.** The dashed lines represent the minus 20% and plus 25% in AUC wherein no FDI is present. AUC=area under the curve. SMKIs=small-molecule kinase inhibitors. BCS=biopharmaceutical classification system. FDI=food–drug interaction.

TABLE 3. Absolute bioavailability and BCS classes of small-molecule kinase inhibitors

	BCS class[2, 10]	**Bioavailability (%)**[2, 3, 10]
Afatinib	I	NA
Alectinib	IV	37%
Axitinib	II	58%
Binimetinib	I or II*	50%
Bosutinib	IV	34%
Brigatinib	I	NA
Cabozantinib	II	NA
Ceritinib	IV	NA
Cobimetinib	III	46%
Crizotinib	IV	43%
Dabrafenib	II	95%
Dasatinib	II	NA
Encorafenib	II	86%
Erlotinib	II	59%
Gefitinib	II	57–60%
Ibrutinib	II	2·9%
Imatinib	I	98%
Lapatinib	IV	NA
Lenvatinib	II or IV†	85%
Nilotinib	IV	30%
Nintedanib	II	4·7%
Osimertinib	III	70%
Pazopanib	II	21%
Ponatinib	II	NA
Regorafenib	II	NA
Ruxolitinib	I	>95%
Sorafenib	II	NA
Sunitinib	IV	NA
Tivozanib	II or IV†	NA
Trametinib	IV	72%
Vandetanib	II	NA
Vemurafenib	IV	64%

BCS=biopharmaceutical classification system. NA=not available. Binimetinib shows low solubility (Class II) at physiological pH but higher (Class I) at acidic pH. † Permeability unknown.*

For SMKIs in which absorption is dissolution-limited, a change of formulation to a solid dispersion (ie, small one-phase powder drug particles) could optimise absorption. Therewith, the effect of food and intragastric pH on solubility is reduced.[56]

Time to reach C_{max} (T_{max})

T_{max} is the time when the balance between drug absorption and distribution results in the C_{max}. High-fat meals increased or had no effect on the average T_{max}—eg, for 25 SMKIs a high-fat meal increased T_{max} by 25% or more (**Table 2**). In contrast, other meal types had the potential to decrease T_{max} (eg, for lapatinib and ponatinib). A longer T_{max} could potentially reduce toxicity, because absorption is spread over a longer period. Also, this could prolong gastrointestinal food–drug and drug–drug interaction time. We, therefore, advise specific counselling when patients use interacting drugs or herbs, and administer their SMKI with food.

Variability in exposure

A high-fat meal reduced interpatient variability in AUC by 20% or more for seven SMKIs and increased this variability by 25% or more for two SMKIs (ie, nintedanib and pazopanib). Interpatient variability was similar for pazopanib when administered with a low-fat meal and was regardless of its dose. Furthermore, the majority of SMKIs showed no noteworthy change with food consumption. However, this was measured in different clinical trials in which timing and caloric intake were monitored closely. In real life, the variation in food intake will probably be higher than reported in these clinical trials. Minimal interpatient variability might have favourable clinical consequences, because efficacy and tolerability could be optimal when exposure is within the therapeutic window. As earlier described, food recommendation negligence occurs frequently in daily life and might lead to substantial alterations in exposure.[24,55] Considering the major differences between a study and normal daily life, results of interpatient variability should be interpreted with caution.

METABOLISM

After uptake by enterocytes, some SMKIs undergo intestinal metabolism by cytochrome P450 (CYP) iso-enzymes. Because most drugs are (largely) metabolised by CYP3A4,[5] most interaction studies are focused on this iso-enzyme. St John's wort strongly induces CYP3A4, therefore, reduces drug bioavailability and exposure. Common foods, such as garlic, red wine, and grapefruit, inhibit CYP3A4,[57] potentially increasing drug exposure.

After reaching the portal vein, SMKIs are metabolised in the liver. Hepatic (phase I) CYP enzymes are responsible for this oxidative metabolism of the majority of SMKIs. As an exception, nintedanib largely undergoes (other phase I) hydrolysis by esterases. Afatinib and binimetinib are mainly metabolised by conjugating phase II enzymes. Lenvatinib and trametinib show predominantly CYP independent phase I and II metabolism (ie, deacetylation, oxidation, and glucuronidation).[2, 3]

SMKI metabolism by CYP enzymes mainly results in inactive metabolites. On one hand, CYP induction hence leads to decreased exposure with potentially reduced efficacy and toxicity. On the other hand, CYP inhibition increases exposure, which could result in accumulation of potentially life-threatening side-effects. Grapefruit is a widely known comestible inhibitor of hepatic CYP3A4 and St John's is a known inducer of hepatic CYP3A4.

SPECIFIC FOODS

Grapefruit (juice)

Grapefruit is considered to be a strong inhibitor of intestinal and hepatic CYP3A4 and it induces drug efflux by P-glycoprotein transporters.[58] Furthermore, grapefruit's flavonoids (naringin) inhibit the uptake transporter OATP1A2, therefore decreasing drug bioavailability.[59] The high interspecies variability in concentrations of grapefruit's interacting compounds create inconvenient diversity in interaction studies.[60] Results should be carefully interpreted. In general, one single grapefruit or 200 mL or more of grapefruit juice can cause relevant escalation of drug concentrations.[61]

There is, however, a difference between concomitant grapefruit juice intake, which predominantly affects absorption through intestinal CYP inhibition, and chronic (non-concomitant) grapefruit consumption that inhibits hepatic CYP metabolism.[62] The known effects of CYP inducing and inhibiting compounds on SMKI bioavailability are presented in **Table 4**.[2, 3, 7, 63-70] Both the study of sunitinib in humans[70] or the study of sorafenib in rats[68] showed no noteworthy FDIs with chronic grapefruit usage.

TABLE 4. The effects of CYP inducing and inhibiting compounds on the bioavailability of small-molecule kinase inhibitors

	Major CYP	Minor CYP and others	Inhibiting compound	Inducing compound	Recommendations
Afatinib[2, 3]	Mainly due to non-enzyme catalysed Michael adduct formation	..	..	..	..
Alectinib[2]	CYP3A4	CYP2C8, CYP3A5	Grapefruit (juice)	St John's wort	When either grapefruit (juice) or St John's wort are co-administered, monitoring is recommended
Axitinib[2, 3]	CYP3A4	CYP3A5, CYP1A2, CYP2C19, UGT1A1	Grapefruit (juice)	St John's wort	Avoid use of grapefruit (juice; when co-administered, decrease dose by approximately 50%); avoid use of St John's wort (when co-administered, a gradual dose increase is recommended)
Binimetinib[2]	UGT1A1	CYP1A2, CYP2C19	..	St John's wort	Avoid use
Bosutinib[2, 3]	CYP3A4	Mono-oxygenase enzymes	Grapefruit (juice)	St John's wort	Avoid use of grapefruit (juice) and of St John's wort
Brigatinib[2, 3]	CYP2C8, CYP3A4	CYP3A5	Grapefruit (juice)	St John's wort	Avoid use of grapefruit (juice; when co-administered, reduce dose by approximately 50%); avoid use of St John's wort
Cabozantinib[2, 3]	CYP3A4	CYP2C9	Grapefruit (juice)	St John's wort	Avoid use of grapefruit (juice; when co-administered, a dose decrease with 33% is recommended); avoid use of St John's wort (when co-administered, a dose decrease with 33% is recommended)
Ceritinib[2, 3]	CYP3A4	..	Grapefruit (juice)	St John's wort	Avoid use of grapefruit (juice) and of St John's wort
Cobimetinib[2, 3]	CYP3A4	UGT2B7	Grapefruit (juice)	St John's wort	Avoid use of grapefruit (juice; when co-administered, monitoring is recommended; interruption when St John's wort is used for less than 8 days should be considered); avoid use of St John's wort
Crizotinib[2, 3]	CYP3A4	CYP3A5, CYP2C8, CYP2C19, CYP2D6	Grapefruit (juice)	St John's wort	Avoid use of grapefruit (juice) and of St John's wort
Dabrafenib[3]	CYP2C8	CYP3A4	..	St John's wort	Avoid use (when co-administered, monitoring is recommended)

TABLE 4. Continued

	Major CYP	Minor CYP and others	Inhibiting compound	Inducing compound	Recommendations
Dasatinib[2, 3]	CYP3A4	FMO3, UGT	Grapefruit (juice)	St John's wort	Avoid use of grapefruit (juice; when co-administered, monitoring is recommended; reducing dasatinib dose by 20 mg or 40 mg when total dose is 120 mg or 140 mg daily, respectively, should be considered); avoid use of St John's wort (when co-administered, monitoring is recommended; increasing dasatinib dose shoud be considered)
Encorafenib[2, 3]	CYP3A4	CYP2C19, CYP 2D6	Grapefruit (juice)	St John's wort	Avoid use of grapefruit (juice; when co-administered, reduce dose to 33% of the encorafenib dose); avoid use of St John's wort
Erlotinib[3, 63]	CYP3A4	CYP1A2, CYP1A1, CYP1B1, CYP3A5	Grapefruit (juice)	St John's wort; green tea extract (C_{max} 16% and AUC 21%)*	Take caution when grapefruit (juice) is co-administered (dose reduction should be considered when side-effects occur); avoid use of St John's wort; avoid use of green tea extract
Gefitinib[1, 2, 3, 64, 65]	CYP3A4, CYP2D6	CYP3A5, CYP2C19	..	St. John's wort; bawu decoction (C_{max} −79% and AUC −61%);* guipi decoction (C_{max} −23% and AUC no effect);* ginseng, mushrooms, and selenium†	Avoid use of St John's wort (dose increase to 500 mg daily should be considered when coadministered); avoid use of bawu decoction; safe to use guipi decoction; avoid use of ginseng, mushrooms, and selenium
Ibrutinib[2, 3]	CYP3A4	CYP2D6	Grapefruit (juice)	St John's wort	Avoid use of grapefruit (juice) and of St John's wort
Imatinib[1, 3, 7, 66, 67]	CYP3A4	CYP2C8, CYP3A5, CYP1A2, CYP2D6, CYP2C9, CYP2C19	Grapefruit (juice)	St John's wort (C_{max} −29% to −15% and AUC −32% to −30%); ginseng‡	Avoid use of grapefruit (juice); avoid use of St John's wort; avoid use of ginseng (when co-administered, dose should be increased by at least 50% and clinical response should be carefully monitored)
Lapatinib[2, 3, 63]	CYP3A4	CYP3A5, CYP1A2, CYP2D6, CYP2C8, CYP2C9, CYP2C19	Grapefruit (juice)	St John's wort; green tea extract (C_{max} −14% and AUC −22%)*	Avoid use of grapefruit (juice); avoid use of St John's wort (when co-administered, dose should be gradually increased from 1250 to 4500 mg per day and from 1500 to 5500 mg daily); avoid use of green tea extract
Lenvatinib[2, 3]	Aldehyde oxidase & gluthatione conjugation	CYP3A4	..	..	..

TABLE 4. Continued

	Major CYP	Minor CYP and others	Inhibiting compound	Inducing compound	Recommendations
Nilotinib[2, 3]	CYP3A4	CYP2C8, CYP1A1, CYP1A2, CYP1B1	Grapefruit (juice)	St John's wort	Avoid use of grapefruit (juice) and of St John's wort
Nintedanib[2, 3]	Hydrolysis due to esterases	UGT1A1, UGT 1A7, UGT1A8, UGT1A10, CYP3A4	..	St John's wort	Avoid use of St John's wort
Osimertinib[2, 3]	CYP3A4	CYP3A5, CYP1A2, CYP2A6, CYP2C9, CYP2E1	..	St John's wort	Avoid use of St John's wort
Pazopanib[2, 3]	CYP3A4	CYP1A2, CYP2C8	Grapefruit (juice)	..	Avoid use of grapefruit (juice)
Ponatinib[2, 3]	CYP3A4	CYP2D6, CYP2C8, CYP3A5	Grapefruit (juice)	St John's wort	When grapefruit (juice) is co-administered, reduce to 30 mg daily; avoid use of St John's wort
Regorafenib[2, 3]	CYP3A4	UGT1A9	Grapefruit (juice)	St John's wort	Avoid use of grapefruit (juice) and of St John's wort
Ruxolitinib[2, 3]	CYP3A4	CYP2C9	Grapefruit (juice)	St John's wort	Avoid use of grapefruit (juice; when co-administered, reduce to 10 mg twice daily); concurrent administration should be avoided in patients with <100 × 10^9 platelets per L; when using St John's wort monitor closely and titrate dose
Sorafenib[3, 68, 69]	CYP3A4	UGT1A9	Grapefruit (juice) (C_{max} +10% and AUC −16%)*; triptolide (Cmax +44% to +63% and AUC +73% to +83%)*	Long-Dan-Xie-Gan-Tang (C_{max} −4% and AUC −12%);* St John's wort	Avoid use of grapefruit (juice), triptolide, Long-Dan-Xie-Gan-Rang, and St John's wort (when co-administered, consider dose increase)
Sunitinib[2, 3, 70]	CYP3A4	CYP1A2	Grapefruit (juice) (C_{max}+11% and AUC +11%)	St John's wort	Avoid use of grapefruit (juice; when co-administered, dose decrease should be considered to a minimum of 37·5 mg daily for GIST and mRCC or 25 mg daily for pNET); avoid use of St John's wort (when co-administered, consider dose increase in 12·5 mg increments up to 87·5 mg daily for GIST and mRCC or 62·5 mg daily for pNET)

TABLE 4. Continued

	Major CYP	Minor CYP and others	Inhibiting compound	Inducing compound	Recommendations
Tivozanib[2‡]	CYP3A4	UGT1A, CYP1A1	..	St John's wort	Avoid use of St John's wort
Trametinib[2, 3]	Deacetylation, oxidation and glucoronidation	CYP3A4	..	..	..
Vandetanib[2]	CYP3A4	FMO1, FMO3	..	St John's wort	Avoid use of St John's wort
Vemurafenib[2]	CYP3A4	UGT	..	St John's wort	Avoid use of St John's wort

C_{max}=maximal plasma concentration. AUC=area under the curve. CYP=cytochrome P450. UGT=UDP-glucuronosyltransferase. FMO=flavine mono-oxygenase. GIST=gastro-intestinal stromal tumour. mRCC=metastatic renal cell carcinomas. pNET=pancreatic neuroendocrine tumours. In vivo rat study results. † Case report. ‡ Only EMA approved.*

Other foods and beverages can have an effect on SMKI bioavailability **(Table 5**).[2, 9, 27, 63, 64, 71, 72, 73, 74] Concomitant grapefruit juice intake was studied for ibrutinib (115% AUC increase),[27] imatinib (2% increase in minimal plasma concentration [C_{min}]),[71] and nilotinib (29% AUC increase).[73] However, since the three study designs are very different, it is difficult to extrapolate their results to other CYP3A4-metabolised SMKIs. FDA and EMA recommendations for all CYP3A4-metabolised SMKIs are to avoid grapefruit. In case of concomitant use, dose reductions are advised for axitinib, brigatinib, cabozantinib, dasatinib, encorafenib, ponatinib, ruxolitinib, and sunitinib, thus minimising potentially dangerous increases of their blood concentration.[2, 3] Even in those cases when evidence of FDIs with SMKIs is scarce, we concur with FDA and EMA in the advice to avoid grapefruit completely during SMKI treatment of CYP3A4 substrates, because the composition of grapefruit and subsequent effect on CYP3A4 is variable and unpredictable.

Beverages

Most beverages are known for their low pH and high-sugar content.[75] Because of its phosphoric acid ingredient, cola was found to be acidic enough to overcome the drug–drug interaction with erlotinib and a proton-pump inhibitor.[9] Most soda, fruit, and energy drinks have a mean pH less than 4, making them suitable for researching similar purposes.[75] Likewise, hypothetically exploring erlotinib's lipophilicity, a potential FDI with fatty milk was studied. (ESMO 2019, #1540P)[76] Since no FDI was found, erlotinib administration with milk is safe. Furthermore, green tea extract caused major FDIs with erlotinib, lapatinib, and sunitinib in rats, decreasing their AUCs by 51–74%.[63, 74] We thus recommend avoiding green tea extract during SMKI therapy until proven safe in humans. Nonetheless, some patients with cancer are not capable of taking their SMKI with water. In that specific situation, albeit only proven for nilotinib, administration with a teaspoon of non-fat plain yoghurt or applesauce is considered safe,[2, 72] and we would extrapolate those outcomes to all SMKIs.

TABLE 5. The effects of other foods and beverages on the bioavailability of small-molecule kinase inhibitors

	Study intervention	Change in C_{max}	Change in C_{max} variability (%)	Change in AUC	Change in AUC relative variability (%)	Change in T_{max} % (fed T_{max})	Importance*	Recommendations
Erlotinib								
Green tea (extract)[63†]	Single-dose erlotinib immediately after green tea extract	−68%	214%	−70%	−12%	No effect (1 h)	Major	Avoid use of green tea (extract)
Coca-Cola[9]	Multiple doses of erlotinib with Coca-Cola	No effect	−6%	+9%	−9%	+16% (NA)	Minor	Safe to use
Coca-Cola[9]	Multiple doses of erlotinib with esomeprazol and Coca-Cola	+42%	−26%	+39%	−25%	No effect (NA)	Major	Consider taking with Coca-Cola when using PPI or take PPI >3 h after erlotinib
Gefitinib								
Bawu decoction[64†]	Single-dose gefitinib 5 min and 1 h after herb	−88% to −67%	+35% to 92%	−75% to −60%	−57% to −17%	+271% to 393% (5·2 to 7·4 h)	Major	Avoid use
Guipi decoction[64†]	Single-dose gefitinib 5 min and 1 h after herb	−36% to −22%	+28% to 78%	−21% to −19%	−6% to +17%	−7% to +60% (1·3 to 2·4 h)	Moderate	Avoid concomitant administration
Ibrutinib								
Grapefruit juice[27]	Single-dose ibrutinib the evening before and concomitant grapefruit juice	+260%	+52%	+115%	+57%	−15% (1·5 h)	Major	Avoid use of grapefruit (juice)
Imatinib								
Grapefruit juice[71]	Multiple doses of imatinib concomitant grapefruit juice	−2%	NA	NA; C_{min}+2%	NA	NA	Minor	Avoid grapefruit (juice), since its composition is variable and unpredictable
Lapatinib								
Green tea (extract)[63†]	Single-dose lapatinib immediately after green tea extract	−70%	+235%	−74%	−47%	No effect (1 h)	Major	Avoid use of green tea (extract)
Nilotinib								
Grapefruit juice[73]	Single-dose nilotinib with grapefruit juice	+60%	−26%	+29%	−14%	No effect (4 h)	Moderate	Avoid use of grapefruit (juice)

TABLE 5. Continued

	Study intervention	Change in C_{max}	Change in C_{max} variability (%)	Change in AUC	Change in AUC relative variability (%)	Change in T_{max} % (fed T_{max})	Importance*	Recommendations
Non-fat plain yoghurt[2, 72]	Single-dose nilotinib with non-fat plain yoghurt	+31%	−6%	+8%	+2%	No effect (4 h)	Minor	Safe to use
Applesauce[2, 72]	Single-dose nilotinib with applesauce	−5%	+3%	−3%	−8%	−25% (3 h)	Minor	Safe to use
Sunitinib								
Green tea (extract)[741]	Single-dose sunitinib concomitant with green tea polyphenol epigallocatechin-3-gallate	−48%	−46%	−51%	−24%	+37% (4·9 h)	Major	Avoid use of green tea (extract)

*Only small-molecule kinase inhibitors with known interactions are shown in this table. C_{max}=maximal plasma concentration. C_{min}=minimal plasma concentration. AUC=area under the curve. NA=not available. PPI=proton-pump inhibitor. * Importance of the food-drug interaction is considered minor (not clinically relevant) when AUC is <20% decreased or <25% increased, moderate when AUC is ≥20% and <50% decreased or ≥25% and <67% increased and major when AUC is ≥67% increased or ≥50% decreased. † In vivo rat study results.*

HERB–DRUG INTERACTIONS

St John's wort

St John's wort (*Hypericum perforatum*) is frequently used as an antidepressive compound. Its active substance, hyperforin, induces hepatic CYP3A4 and inhibits P-glycoprotein mediated drug efflux.[12, 77] Clinical and pharmacokinetic effects have been proven to be positively associated with hyperforin concentrations in different studies.[77] St John's wort's HDI was investigated in two clinical trials that showed a 30–32% decrease in drug exposure following consumption of St John's wort (**Table 4**).[7, 67] Because concentrations of active substances fluctuate by 5–8 times between brands or abstracts,[77] standardisation of study methods to investigate the HDI for all SMKIs is very difficult. Therefore, we recommend avoiding consumption of St John's wort during SMKI treatment, which is in accordance with FDA and EMA recommendations.[2, 3]

Oriental herbs

Herbal products are used by 13–63% of patients with cancer. Up to 72% of these patients do not inform their oncologist about their supplemental herb intake, therefore interaction potential with conventional anticancer treatment may be substantial.[8]

Much is unknown about HDIs with SMKIs in clinical practice. Numerous oriental herbs can inhibit multiple CYP-enzymes,[8] though no standardised HDI studies in humans with SMKIs were found (**Table 4, Table 5**). Two case reports describe reversible severe toxicity and therapy failure, probably due to ginseng (potential CYP-inducer) and other alternative preparations.[65, 66] The decoctions bawu and guipi are traditional oriental medicines. Bawu is a mixture of eight herbs and guipi is a mixture of 12 herbs, both include ginseng. These traditional medicines are considered to be purifying and are used to treat various diseases. Bawu decreased gefitinib's AUC in rats by 61–75%, without regard to administration time.[64] Guipi was found not to have an HDI when co-administration with gefitinib was avoided, because it caused a 21% decrease in gefitinib exposure otherwise.[64] Triptolide (derived from *Tripterygium wilfordii*) escalated sorafenib's AUC with 83% in rats, possibly through CYP3A4 inhibition.[69]

EMA and FDA recommendations for SMKIs do not specifically mention safe or dangerous herbal preparations, but patients are instructed to communicate herb use to their health-care provider.[2, 3] Because conclusive data are missing, clinicians are faced with questions that are practically unanswerable. Current advice is to avoid products with possible interacting compounds, to minimise the risk of HDIs. To provide clinicians and patients clear recommendations concerning the dangers or safety of herbal preparations, more research to HDIs and SMKIs is warranted.

FOOD–DRUG INTERACTION STUDIES

Registered therapeutic doses of SMKI are generally based on the maximum tolerated dose that is found in phase I trials. Once a decision is made for SMKI administration in fed or fasted state, all consecutive registration studies maintain this food recommendation. To change these recommendations, for instance to reduce drug costs by allowing food consumption with a reduced dose, solid evidence that FDIs affect drug tolerability or anticancer activity must be shown. Since toxicity develops generally after a loading phase of several weeks, FDI studies should ideally include multiple drug doses over a long period (ie, multiple weeks). Phase I studies can be used for this purpose. In studies in which an FDI is present, repeating the drug's registration studies from phase I to phase III can guarantee safety and efficacy. Single-dose FDI studies, which miss a reliable safety and efficacy assessment of the loading phase, are thus limited for extrapolation of established FDIs to clinical recommendations. Their strength solely lies in the exclusion of an FDI, when no clinically significant change in exposure is found. The FDA, however, recommends only a single-dose study design to research FDIs.[3]

Currently, popularity of calorie-restricted dietary interventions, such as cyclic fasting or fasting-mimicking diets, is increasing. Their safety and effectivity are being investigated in various clinical trials (NCT03340935, NL5624, and NCT03595540), because much is unknown about their efficacy and potential pharmacokinetic effect on anticancer drugs.

Animal models are not suited as replacement for human in-vivo studies, because interspecies differences can bias FDIs. For example, no FDI for gefitinib (in dogs) and pazopanib (in monkeys) was found,[2] whereas in humans there is a food effect (**Table 1**). The relevance of in-vitro data for HDIs is limited, although some prediction models that mimic HDIs (eg, midazolam as model substrate for CYP3A4) show promising results and could be feasible.[78] In-vitro research could be used as an indicator to identify herbs that should be studied in vivo, as requested by EMA.[2] We recommend studying in-vivo FDIs and HDIs at steady state, with a multiple-dose instead of a single-dose study, with enough patients or healthy volunteers to examine exposure with subsequent efficacy and tolerability. Results of such studies would be conclusive for providing useful advice for clinical practice.

CONCLUSIONS

FDIs and HDIs can alter the systemic bioavailability and pharmacokinetics of many clinically approved SMKIs. The major mechanisms underlying FDIs and HDIs concern gastrointestinal drug absorption and metabolism through cytochrome P450 isoenzymes. FDIs and HDIs might lead to therapy failure or (acute) severe toxicity, therefore knowledge of these interactions is essential.

SEARCH STRATEGY AND SELECTION CRITERIA

A literature search for European Medicines Agency-approved small-molecule kinase inhibitors (SMKIs) used in haemato-oncology, with the exception of mTOR- and CDK4/6-inhibitors, was done in Embase and Pubmed from database inception until Oct 1, 2019, using the MESH terms: "(food-drug interactions) OR (herb-drug combination) OR ((complementary therapies OR combination OR interaction OR supplement) AND (diet OR food OR herb OR drink)) AND (drug name)". In Embase, we applicated "clinical studies", "humans", and "only in English" as quick search limits. Prior to full-text screening, abstracts and titles were screened. Also, articles concerning pharmacokinetic effects of possible in-vivo FDIs or HDIs were included. FDA and EMA assessment reports (including

updates) and “Summary of Product Characteristics“ were additionally examined for FDIs and HDIs for each SMKI. Practical recommendations were formulated based on available evidence and the FDA’s definition of an FDI.

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CHAPTER 10

INFLUENCE OF THE ACIDIC BEVERAGE COLA ON THE ABSORPTION OF ERLOTINIB IN PATIENTS WITH NON-SMALL-CELL LUNG CANCER

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ABSTRACT

Purpose. Erlotinib depends on stomach pH for its bioavailability. When erlotinib is taken concurrently with a proton pump inhibitor (PPI), stomach pH increases, which results in a clinically relevant decrease of erlotinib bioavailability. We hypothesized that this drug-drug interaction is reversed by taking erlotinib with the acidic beverage cola. The effects of cola on erlotinib bioavailability in patients not treated with a PPI were also studied.

Patients and Methods. In this randomized, cross-over, pharmacokinetic study in patients with non–small-cell lung cancer, we studied intrapatient differences in absorption (area under the plasma concentration time curve [$AUC_{0\text{-}12h}$]) after a 7-day period of concomitant treatment with erlotinib, with or without esomeprazole, with either cola or water. At the 7th and 14th day, patients were hospitalized for 1 day for pharmacokinetic sampling.

Results. Twenty-eight evaluable patients were included in the analysis. In patients treated with erlotinib and esomeprazole with cola, the mean $AUC_{0\text{-}12h}$ increased 39% (range, 212% to 136%; P = .004), whereas in patients not treated with the PPI, the mean $AUC_{0\text{-}12h}$ was only slightly higher (9%; range, 210% to +30%; P = .03) after erlotinib intake with cola.

Conclusion. Cola intake led to a clinically relevant and statistically significant increase in the bioavailability of erlotinib during esomeprazole treatment. In patients not treated with the PPI, the effects of cola were marginal. These findings can be used to optimize the management of drug-drug interactions between PPIs and erlotinib.

INTRODUCTION

Erlotinib is an oral reversible tyrosine kinase inhibitor (TKI) of the epidermal growth factor receptor effective in non–small-cell lung cancer (NSCLC). The advantage of the oral administration route of erlotinib causes a highly relevant new problem. The GI absorption of erlotinib is a complex multifactorial process characterized by a poor and variable bioavailability that results in significant intrasubject and intersubject variability in exposure.[1] One of the most important factors that influences erlotinib absorption is intragastric pH.[2,3] Because of its weakly basic properties, erlotinib can be present in either the ionized or the nonionized form, which depends on the intragastric pH. In the case of elevated intragastric pH, equilibrium shifts toward the less-soluble nonionized erlotinib form, and drug absorption decreases. The concomitant use of acid-reducing agents, such as proton pump inhibitors (PPIs), therefore, leads to a clinically significant drug-drug interaction (DDI) with erlotinib.[2-5] In a study of healthy volunteers, the concurrent use of the PPI omeprazole significantly reduced the area under the curve (AUC) and maximum serum concentration (C_{max}) of erlotinib with 46% and 61%, respectively.[6] As a result, the product label of erlotinib states that PPIs should not be taken concurrently with erlotinib. Recently, the concomitant use of erlotinib and acid-suppressive agents was shown to be associated with decreased erlotinib efficacy in patients with NSCLC.[5] Because a PPI often is indicated during erlotinib therapy, pharmacists and medical oncologists are confronted with challenges.[2,7] A solution for managing this DDI is not yet available. A practical way to bypass the DDI between erlotinib and PPIs could be to temporarily lower the stomach pH by taking erlotinib with an acidic beverage, such as cola. The classic form of this beverage has a pH of 2.5, which leads to a temporary decrease of the stomach pH after intake. Other studies have shown that the absorption of weakly basic drugs, such as ketoconazole and itraconazole, is enhanced when taken concomitantly with Coca-Cola (The CocaCola Company, Atlanta, GA).[8,9] We hypothesize that due to similar physicochemical basic properties, this positive effect also is the case with erlotinib. We evaluated the impact of cola on the absorption of erlotinib (with and without esomeprazole) in patients with lung cancer.

MATERIAL AND METHODS

Study Design and Procedures

This study was an open-label, two-way, randomized, cross-over design in patients treated with erlotinib for NSCLC. The study was performed at the Erasmus MC Cancer Institute in Rotterdam, the Netherlands, between March 2014 and June 2015. The

medical ethics committee of the Erasmus Medical Center (MEC14-046) approved the study, which was registered through the Dutch Trial Registry. Twenty-eight patients on steady-state erlotinib therapy were assigned to one of two study groups of 14 patients each (**Figure 1**). Study group 1 received erlotinib (Tarceva [Genentech, South San Francisco, CA] at any dose, days 1 to 14, 10 AM) taken with 250 mL Coca-Cola Classic or 250 mL water. Study group 2 received erlotinib (Tarceva at any dose, days 1 to 14, 10 AM) and esomeprazole 40 mg (Nexium [AstraZeneca, London, UK], days 5 to 7 and 12 to 14, 7 AM) taken with 250 mL Coca-Cola Classic or 250 mL water. After allocation to one of the study groups, patients were randomly assigned to two sequence arms of seven patients each. Sequence arm A first took erlotinib with water for 7 days followed by Coca-Cola Classic for 7 days. Sequence arm B took erlotinib with Coca-Cola Classic and water in the reversed order. On days 7 and 14, patients were admitted for 24 h to the hospital for pharmacokinetics (PK) sampling (Fig 1). Before signing informed consent, the use of interacting comedications (including over-the-counter drugs, herbal/food supplements) was collected in a structured anamnesis with the patient. Patient medication use was assessed for DDIs by using the DDI software program Micromedex (Truven Health Analytics, Greenwood Village, CO). To ensure steady-state concentrations, patients had to use erlotinib for a minimum of 14 days before entering the study. In study group 2, esomeprazole 40 mg once daily was given for at least 3 days before both PK sampling days (3 h before erlotinib intake) to achieve maximum elevation in intragastric pH.[10,11] Furthermore, patients who used PPIs long term before entering the study were allowed to participate as long as they were willing to use esomeprazole 40 mg (Nexium) for 3 consecutive days before both PK sampling days according to the protocol. Patients fasted overnight before both PK sampling days. On the PK sampling days, erlotinib was taken in the hospital. Because the study population comprised regular patients with lung cancer with an indication for an epidermal growth factor receptor TKI, erlotinib dose reductions due to toxicity were allowed. Compliance was assessed through patient diaries.

Eligibility

Eligibility criteria were age 18 years and older, histology or cytology confirmed diagnosis of lung cancer for which treatment with erlotinib monotherapy was indicated, use of erlotinib monotherapy at any dose for at least 2 weeks before study participation, World Health Organization performance status of 0 or 1, and no concurrent use of over-the-counter medications or medications known to interact with either erlotinib or esomeprazole. Exclusion criteria were pregnancy or lactation and a clear language barrier.

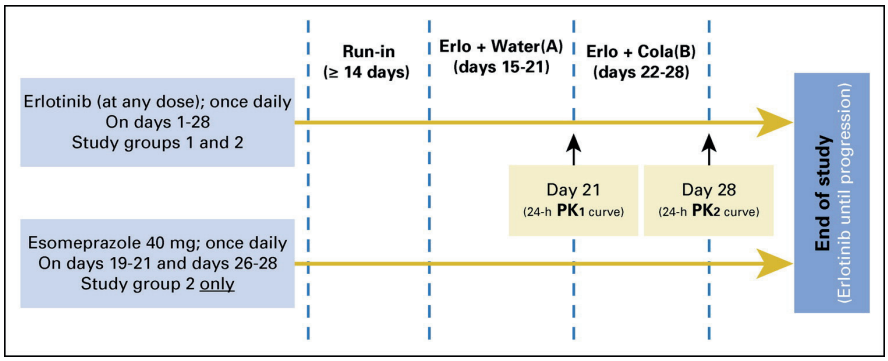

FIGURE 1: Study design. A= sequence arm A; B= sequence arm B; Erlo = erlotinib; PK= pharmacokinetic.

Pharmacokinetics

Blood samples for the analysis of erlotinib were collected before erlotinib dosing and at 0.5, 1, 1.5, 2, 2.5, 3, 3.5, 4, 6, 8, 12, and 24 h (13 samples per hospitalization for both sequence arms) after erlotinib administration. Esomeprazole levels were not measured. At each time point, blood samples were collected in 6-mL lithium heparin blood collection tubes. After collection, blood samples were processed to plasma within 10 min by centrifugation (2,000 3 *g*; 10 min; 4°C). Plasma was transferred into polypropylene tubes (1.8-mL Nunc CryoTube [Roskilde, Denmark] vials), which were subsequently stored at less than 270°C until the time of analysis at the Laboratory of Translational Pharmacology (Josephine Nefkens Institute, Erasmus MC Cancer Institute, Rotterdam, the Netherlands). PK parameters of erlotinib were calculated by using weighted non-compartmental analyses with Phoenix WinNonlin 6.3 software (Pharsight, a Certara Company, Princeton, NJ) and included the area under the plasma concentration time curve ($AUC_{0\text{-}12h}$), maximum serum concentration (C_{max}), and time to Cmax (T_{max}).

Statistics

The primary objective was to determine the intrapatient differences in absorption (expressed by $AUC_{0\text{-}12h}$ and C_{max}) after a 7-day period of concomitant treatment of erlotinib (with or without esomeprazole for 3 days) taken with cola and 7 days of erlotinib taken with water and vice versa. Each patient acted as his or her own control subject. In this exploratory study, the primary end point was the relative difference (RD) between erlotinib AUC_{cola} and erlotinib AUC_{water} calculated for each patient as follows: RD = (AUC_{cola} – AUC_{water}) / AUC_{water}. Cola was considered to have an impact on the erlotinib AUC when the absolute value of RD was at least 25% (ie, ≤25% or at least

+25%).[12] By assuming an intraindividual standard deviation of the difference between AUC_{cola} and AUC_{water} of 30%, 14 evaluable patients per study group (without or with esomeprazole) had to be included to obtain 80% power (two-sided significance level a = 0.05) to detect this difference of ≥ 25%.[13] To evaluate the impact of cola on the AUC (ie, compare AUC_{cola} and AUC_{water}), we used the STATA command pkcross, which analyzes cross-over experiments (STATA 13; StataCorp, College Station, TX). This command uses analysis of variance models to analyze the data.[14] In this way, possible period effects (first v second PK sampling period) and sequence effects (A → B v B → A) were taken into account by assuming that no carryover effect existed. In case of a dose reduction (due to toxicity), PK data were normalized to a dose of 150 mg erlotinib. The P value to indicate whether the mean AUC and mean C_{max} were significantly different after water versus after cola was assigned to the treatment effect by using the pkcross command. This was evaluated separately for patients who used esomeprazole and for those who did not.

RESULTS

Patient Characteristics

Thirty-five patients were enrolled of whom 28 were evaluable (14 in each study group). Seven patients were excluded from the study for various reasons (ie, use of Diet Coke [n = 1; The Coca-Cola Company], use of a generic brand of esomeprazole instead of Nexium [n = 1], progression of disease before both PK sampling periods [n = 2], impossibility of venipuncture [n = 1]) and on patients' own initiative (ie, withdrawal of consent [n = 2]). Baseline characteristics are shown in **Table 1**. The majority of patients were male (61%), and the median age was 63 years.

PK, Safety, and Tolerability

In patients treated with erlotinib and esomeprazole (study group 2; **Table 2**), the mean AUC0-12h was 39% higher (range, 212% to +136%; P = .004) and mean Cmax 42% higher (range, 24% to +199%; P = .019) after cola compared with water intake (Fig 2). In patients treated with erlotinib without esomeprazole (study group 1; **Table 3**), the mean $AUC_{0\text{-}12h}$ was 9% higher (range, 210% to +30%; P = .03) and mean C_{max} comparable (0%; range, 219% to +18%; P = .62) after cola intake (Fig 2). Time to C_{max} was not significantly altered in study group 1 (18%; range, 260% to +194%; P = .75) and study group 2 (0%; range, 220% to +52%; P = .99).

TABLE 1. Subject characteristics at baseline

Characteristics	**No**	**%**
No of patients	28	100
Age (years)		
Median (range)	63 (39-77)	
Sex		
Female	11	39
Male	17	61
Race		
Caucasian	24	86
Asian	4	14
BMI (kg/m^2)		
Mean (range)	24,2 (19-31)	
Tobacco use		
Current (< 1 month)	2	7
None	26	93
ECOG-performance status		
0	15	54
1	13	46
Pre-treatment chemotherapy		
Yes	8	29
No	20	71
EGFR mutation		
Yes	14	50
No	10	36
Unknown	4	14
Dosage erlotinib		
50mg	1	4
100mg	4	14
150mg	23	82

Abbreviations: ECOG, Eastern Cooperative Oncology Group; EGFR, epidermal growth factor receptor

Adverse events were generally mild and resolved without medical intervention. Erlotinib was well tolerated when administered with either cola or water (also in patients with known gastroesophageal reflux disease). Grade 3 skin toxicity developed in one patient in study group 1, and hospital admission was required. After standard-of-care treatment, the patient was discharged without sequelae but showed progression during erlotinib therapy In this patient, erlotinib was stopped, and the patient was

excluded from the study. Erlotinib treatment-related adverse effects primarily affected the skin (eg, grade 1 rash) and the GI system (eg, nausea, diarrhea). Details are shown in **Table 4**. There were no known deviations in the patient diaries with regard to study adherence.

TABLE 2. Summary of pharmacokinetic parameters study group 2 (Erlotinib+Esomeprazole + water vs. Cola)

Parameter	Erlo+Esom+Water (A)	Erlo+Esom+Cola (B)	Difference % (range)
Erlotinib dose*	150 (100-150)	150 (50-150)	
Erlotinib			
$AUC_{0\text{-}12h}$ (µg×h/ml), geometric mean (geometric mean CV%)	9.0 (19.9%)	11.8 (14.9%)	39% (-12% to +136%), *P*=.004
C_{max} (µg/ml), geometric mean (geometric mean CV%)	1.08 (152%)	1.43 (112%)	42% (-4% to +199%), *P*=.019

Abbreviations: A, sequence arm A; $AUC_{0\text{-}12H}$, area under the plasma concentration time curve; B, sequence arm B; C_{max}, maximum plasma concentration; CV%, percentage of coefficient of variation defined by (standard deviation / mean) 3 100; Erlo, erlotinib; Esom, esomeprazole 40 mg once daily.
**Median (range).*
†In case of a dose reduction (due to toxicity), pharmacokinetic data were normalized to a dose of 150 mg erlotinib.
‡Based on individual patient data.

TABLE 3. Summary of pharmacokinetic parameters for study group 1 (Erlotinib+water vs. Cola)

Parameter	Erlo + Water (A)	Erlo + Cola (B)	Difference % (range)
Erlotinib dose*	150 (50-150)	150 (50-150)	
Erlotinib**			
$AUC_{0\text{-}12h}$ (µg×h/ml), geometric mean (geometric mean CV%)	17.3 (8.5%)	18.6 (7.7%)	9% (-10% to 30%), *P*=.03
C_{max} (µg/ml), geometric mean (geometric mean CV%)	2.10 (68%)	2.09 (64%)	0% (-19% to +18%), *P*=.62

Abbreviations: A, sequence arm A; $AUC_{0\text{-}12H}$, area under the plasma concentration time curve; B, sequence arm B; C_{max}, maximum plasma concentration; CV%, percentage of coefficient of variation defined by (standar deviation / mean) x 100; Erlo, erlotinib; Esom, esomeprazole 40mg once daily
** Median (range)*
†In case of a dose reduction (due to toxicity), pharmacokinetic data were normalized to a dose of 150 mg erlotinib.
‡Based on individual patient data.

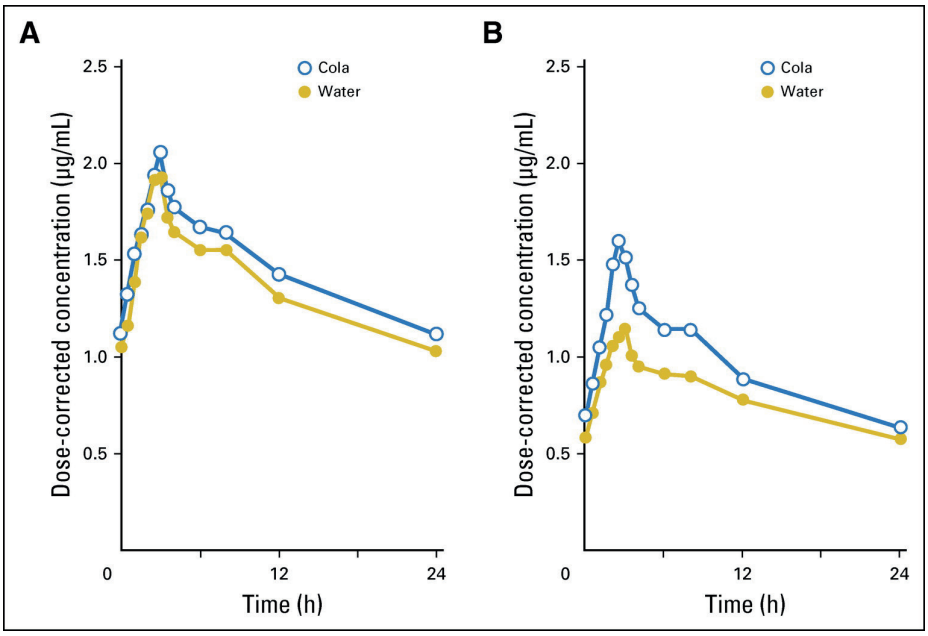

FIGURE 2: Pharmacokinetic profile. Mean dose-corrected concentration v time profiles are shown for erlotinib alone administered with water or cola (A; n = 14) and erlotinib + esomeprazole with water or cola (B; n = 14).

TABLE 4. Treatment related adverse events during study period

	PPI (n=14), No. (%)		Non-PPI (n=14), No. (%)	
	Water	**Cola**	**Water**	**Cola**
Diarrhea*	2 (14%)	2 (14%)	3 (21%)	6 (43%)
Nausea*	1 (7%)	2 (14%)	1 (7%)	1 (7%)
Vomiting*	0	0	1 (7%)	0
Rash†‡	9 (64%)	11 (79%)	9 (64%)	11 (79%)
Fatigue†	8 (57%)	8 (57%)	7 (50%)	7 (50%)

Abbreviation: PPI, proton pump inhibitor.
**All grade 1 according to Common Terminology Criteria for Adverse Events (CTCAE) version 4.03.*
†Grade 1 or 2 according to CTCAE version 4.03
‡One patient with grade 3 skin toxicity according to CTCAE version 4.03.

DISCUSSION

We show that the use of cola significantly increases the mean exposure of erlotinib in patients treated with esomeprazole probably because of increased solubility and absorption. Furthermore, in patients who took the medications with cola instead of water, the mean exposure to erlotinib also significantly increased, although this effect was clinically irrelevant. The observed PK parameters were comparable to previous reports.[6] The current study confirms that pH-dependent solubility plays a key role in erlotinib absorption and that a 250 mL of cola can enhance erlotinib absorption by temporarily lowering intragastric pH. Although H2 antagonists (eg, ranitidine) and antacids can substantially affect erlotinib bioavailability, we used esomeprazole (Nexium at the regular dosage of 40 mg once daily) in this study because it is currently the most effective acid-reducing agent on the market.[2,10,11] Furthermore, when using esomeprazole instead of other PPIs (eg, pantoprazole), other factors such as inhibition of relevant drug transporters that may also alter erlotinib PK (eg, P-glycoprotein) can be ruled out.[15] To our knowledge, no other interactions (eg, those based on altered metabolism or clearance) exist between erlotinib and esomeprazole, besides those based on altered intragastric pH, that may alter erlotinib PK. A 3-day period before PK sampling days was assumed to maximize acid-reducing effects and to minimize the period a patient was exposed to the unwanted DDI between esomeprazole and erlotinib.[6,10,11] This assumption was supported by the observation of no significant differences in $AUC_{0\text{-}12h}$ and C_{max} between patients treated with esomeprazole for 3 days and those treated longer term. In this study, a large interpatient variability in either AUC or other PK parameters was observed. Several factors could explain this variability. Most probably, the absorption from the gut itself varies highly between patients. Adherence to the protocol during the study period (eg, by drinking other [volumes of] acidic beverages or by not taking erlotinib on an empty stomach) is unlikely to be the cause of variability because the study protocol was explained thoroughly, and patient diaries were heavily protocoled and checked by the investigators. Other probable reasons are interpatient differences in gastric emptying and GI motility. Cola may not enhance absorption in all patients because gastric pH may also physiologically vary; thus, the effects may be lower if the gastric pH is lower in one patient compared with another.[1]

A limitation of this study is that we did not measure intragastric pH. Because some patients might experience altered gastric acid secretion (eg, achlorhydria or Zollinger-Ellison syndrome), large interpatient variations in intragastric pH can be expected. Because of the weak basic properties and an acid dissociation constant (ie, the pH

at which equilibrium is reached between the ionized and the nonionized form) near the stomach pH range of 1 to 4 (erlotinib pKa = 5.4), intragastric pH shifts lead to a more significant shift toward the nonionized (less-soluble) form and subsequent lower bioavailability compared with TKIs with a higher pKa value (eg, sunitinib, afatinib[6]). This may partly explain the large variation in erlotinib absorption seen in the current study. The measurement of the intragastric pH per patient might give additional insights into the effect of cola intake on intragastric pH and subsequent absorption. Another limitation of this study is that it was not designed to explore the effects of long-term cola coadministration on the outcome of anticancer treatment with erlotinib. Because of the study design being purely based on PK and chemical parameters (ie, pH effect and subsequent erlotinib solubility and absorption) and the relatively short time (ie, 7 days) that patients were treated with erlotinib and cola (instead of water), the study did not allow us to evaluate the impact of cola on erlotinib efficacy. Therefore, the clinical impact of cola on erlotinib efficacy should be unraveled in future research.

In theory, in patients with elevated intragastric pH and subsequent impaired absorption (eg, achlorhydria, gastrectomy), the use of cola may increase bioavailability of erlotinib or other TKIs with a relatively low pKa value. Because of the nocturnal duodenogastric reflux peak during sleep, the intragastric pH at night is, on average, higher than that in the morning.[11] Many patients take a TKI ante noctem.[16] In theory, when a patient decides to take erlotinib ante noctem, cola could help to increase bioavailability by temporarily lowering intragastric pH. The effect of cola on these subgroups should be explored further in future studies.

In clinical practice, a hard indication for the use of PPIs during erlotinib therapy (eg, patients treated with corticosteroids and nonsteroidal anti-inflammatory drugs or with [recurrent] gastroesophageal reflux disease) often exists. On the other hand, physicians are faced with product label guidelines that advise to avoid the combination or to switch to less-effective H2 antagonists or antacids (taken 2 h after erlotinib).[6] When erlotinib and a PPI are given concomitantly, the AUC of erlotinib steeply decreases,[6] which suggests that lower bioavailability due to PPI use (up to 46% for erlotinib[6]) may deprive patients from optimal therapy.[5,17] Thus, in the case that the combination of a PPI and erlotinib is inevitable, the pH-lowering effects of cola may help physicians to optimize erlotinib therapy.

Although ingredients of cola, such as caffeine, may potentially interact with erlotinib PK, pH-dependent solubility more likely is the predominant factor in erlotinib absorption.[3,18] Erlotinib is a Biopharmaceutics Classification System[19] class II drug characterized by

poor solubility but high intestinal permeability, which means that in vivo erlotinib bioavailability is predominantly limited by its solubility.[3,19] When dissolved, erlotinib is rapidly and extensively (> 90%) absorbed across the intestinal membrane.[3]

Although cola can be associated with several disadvantages, such as dental corrosion and gastroesophageal irritation, it is (for most people) a palatable drink readily available worldwide. Furthermore, Coca-Cola Classic has the clear advantage of a substantially lower pH (approximately 2.5) compared with other acidic beverages, such as orange juice (pH approximately 4), 7-Up (pH approximately 3.5; Dr Pepper/Seven Up, Plano, TX), and diet (cola) products (pH approximately 3 to 4). In theory, drinks with higher pH might not be as effective at enhancing erlotinib absorption as Coca-Cola Classic. Furthermore, although not studied, higher volumes of cola might acidify the stomach even more, and erlotinib absorption could be further enhanced. However, in the current study, 250 mL of cola was well tolerated, and higher volumes might be less convenient for the patient (especially in the morning).

In conclusion, the use of cola provides a potential and easyto-implement way to significantly improve erlotinib bioavailability, especially during concomitant use of esomeprazole. These findings can be used to optimize the management of the existing DDI between erlotinib and PPIs. Potentially, the effects of cola on erlotinib exposure may be extrapolated to other TKIs with a pH-dependent solubility (eg, dasatinib, gefitinib, nilotinib), but this remains to be evaluated in future studies. Furthermore, other acidic beverages (ie, orange juice, other carbonated drinks) may have similar effects as cola and should be explored in future trials.

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CHAPTER 11

INFLUENCE OF COW'S MILK AND ESOMEPRAZOLE ON THE ABSORPTION OF ERLOTINIB:

A RANDOMIZED, CROSSOVER PHARMACOKINETIC STUDY IN LUNG CANCER PATIENTS

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ABSTRACT:

Introduction. Erlotinib's gastrointestinal solubility and absorption are decreased by proton pump inhibitors (PPIs). Since erlotinib is a lipophilic drug, we hypothesized that concomitant intake with the fatty beverage milk may be a feasible way to increase erlotinib uptake. We performed a two-period, randomized, crossover study to investigate the influence of cow's milk with 3.9% fat on the exposure of erlotinib with and without the PPI esomeprazole in patients with non-small cell lung cancer (NSCLC). The effect of esomeprazole was studied in an additional intrapatient comparison.

Method. Pharmacokinetic sampling was performed on days 7 and 14 during 24 consecutive hours. During the 7 days prior to pharmacokinetic sampling, erlotinib was taken daily with 250 mL of either water or milk. In the PPI arm, esomeprazole (40 mg once daily 3 h prior to erlotinib) was taken for 3 days.

Results. Erlotinib area under the curve from time zero to 24 h (AUC_{24}) did not significantly change when administered with milk, compared with water, in both non-PPI users (n = 14; − 3%; 95% conidence interval [CI] − 12 to 8%; p = 0.57) and patients who used esomeprazole (n = 15; 0%; 95% CI − 15 to 17%; p = 0.95). Esomeprazole decreased erlotinib AUC_{24} by 47% (n = 9; 95% CI − 57 to − 34%; p < 0.001) and C_{max} by 56% (95% CI − 64 to − 46%; p < 0.001). No differences in toxicities were observed between milk and water.

Conclusion. Milk with 3.9% fat has no effect on the exposure to erlotinib in NSCLC patients, independent of PPI use. The combination with milk is safe and well tolerated. Concomitant esomeprazole treatment strongly decreased both erlotinib AUC_{24} and C_{max} and should be avoided if possible.

INTRODUCTION

Erlotinib is a tyrosine kinase inhibitor (TKI) registered for the treatment of epidermal growth factor receptor (EGFR) mutated metastatic non-small cell lung cancer (NSCLC) [1, 2]. It is indicated in combination with gemcitabine as firstline therapy for unresectable or metastatic pancreatic cancer [1]. Erlotinib is orally administered on a daily basis at a dose of 150 and 100 mg once daily for NSCLC and pancreatic cancer, respectively. Intra- and interpatient variability differs significantly due to interactions with food [3], concomitant medication [4], and lifestyle factors (*i.e*. smoking) [5, 6].

The bioavailability of erlotinib largely depends on its solubility in the stomach and passive diffusion and probable active cellular transport in the gastrointestinal tract [7]. Optimal drug absorption is reached at a physiologically low intragastric pH (*i.e*. pH value of 1), since erlotinib is then protonized and is thus better soluble [8]. However, various acid-reducing drugs, including histamine-2 receptor antagonists (*e.g.* ranitidine) and proton pump inhibitors (PPIs; e.g. omeprazole) may lead to a 40–50% decrease in erlotinib absorption due to an increase in intragastric pH [9]. It has been previously demonstrated that this impaired systemic exposure to erlotinib can be corrected when administered in combination with the acidic beverage cola [10]. However, daily intake of acidic and highly caloric beverages such as cola or orange juice has disadvantages, such as dental problems, disrupted bone mineral composition, and weight gain [11]. We hypothesized that a healthier way to enhance erlotinib bioavailability could be by making use of the effects of other food components. The exposure of erlotinib is increased 33–66% when administered concomitantly with a high-fat meal [3]. We explored this potentially positive food effect as a *proof-of-principle* by optimizing erlotinib absorption in the presence of a beverage containing fat. In the past, milk-based drug formulations have shown to be equally effective compared with standard formulations in terms of solubility and dispersion [12]. Milk is consumed worldwide by billions of people. It is a healthy beverage that contains essential proteins, vitamins and minerals (*e.g.* calcium and phosphorus). Cow's milk accounts for more than 80% of the global milk production [13].

This is the first study that investigates the efects of erlotinib administered concomitantly with high-fat whole cow's milk compared with water. In addition, a direct intrapatient comparison to study the effects of esomeprazole on the systemic exposure of erlotinib has never been made. Therefore, we also explored the potential drug–drug interaction of esomeprazole use on the absorption of erlotinib.

METHODS

Patient eligibility

Adult NSCLC patients were eligible for inclusion in this study if they had an Eastern Cooperative Oncology Group (ECOG) performance status [14] of ≤ 1, were treated with a stable dose of erlotinib for at least 2 weeks (to guarantee steady-state plasma concentrations) and did not use any other (complementary or alternative) medicine or compounds that may have the potential to interact with either erlotinib or esomeprazole. Patients who concomitantly used any prescribed PPI could only participate in the PPI arm of this study when willing to switch to esomeprazole. It was possible for patients to participate in both study arms if PPI use was discontinued or if they were willing to take esomeprazole as required for this study. All participating patients were asked to sign a written informed consent form. The study was approved by the local Ethics Committee (Erasmus University Medical Center Rotterdam; MEC 16–590) and was registered in the Dutch Trial Registry (number NL5984; NTR6148) [15].

Study design

This was a single center, randomized, two-period, crossover pharmacokinetic study with two study arms. **Figure 1** shows the study low chart. After signing informed consent and after screening, patients were allocated to the non-PPI (arm A) or PPI (arm B) study arms. Hereafter, they were randomized to start with erlotinib with 250 mL of water (period 1) or cow's milk containing 3.9% fat (period 2) for 7 consecutive days (days 1–7 or 8–14). The 7-day period was chosen to ensure that erlotinib concentrations reached steady state. At days 7 and 14, patients were electively admitted for 24-h pharmacokinetic blood sampling. During each admission, 13 blood samples were collected; < 5 min before erlotinib intake (t = 0 h) and at time points (0.5, 1, 1.5, 2, 2.5, 3, 3.5, 4, 6, 8, 12 and 24 h) after erlotinib intake. Patients had to take erlotinib according to its label, *i.e.* fasted for at least 2 h prior to and 1 h after administration. Additionally, on the day of hospital admission, food intake was prohibited between 4 h prior to and 1 h after erlotinib administration. Consumption of beverages was restricted for 1 h before and after erlotinib intake. In the PPI arm, patients were required to take esomeprazole (40 mg once daily) 3 h prior to erlotinib intake on days 5, 6 and 7 and days 12, 13 and 14 after the start of the study. The timing of esomeprazole intake was chosen to ensure maximal inhibition of gastric acid secretion at the time of erlotinib intake [16]. All samples were analyzed by a validated liquid chromatography-tandem mass spectrometric assay for precise quantiication of erlotinib plasma concentrations [17].

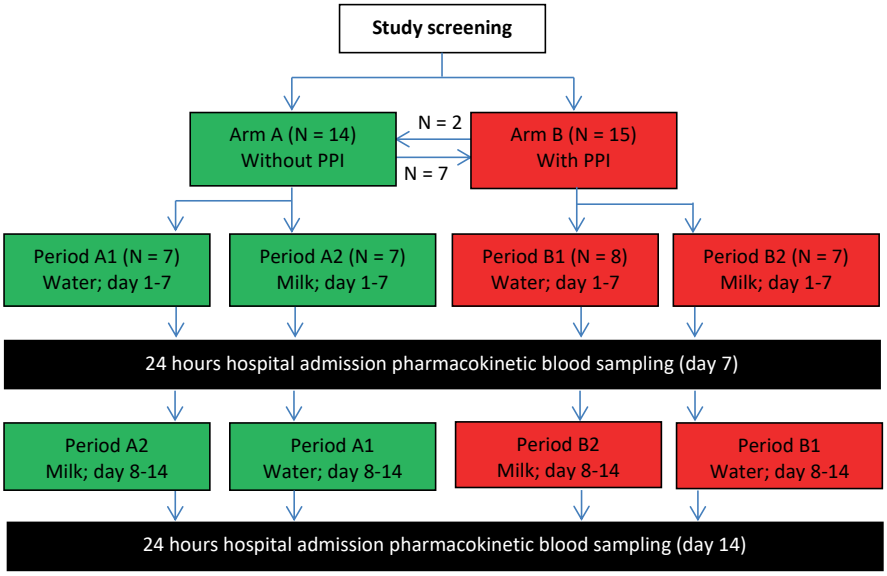

FIGURE 1: Study flowchart. After screening, patients were allocated to the non-PPI (arm A) or PPI (arm B) arms. Hereafter, they were randomized to start with administration of either concomitant water (period 1) or cow's milk (period 2). Subsequent participation in both arms was allowed and is illustrated with the arrows between arms A and B. Hospital admissions for pharmacokinetic blood sampling took place at days 7 and 14. Esomeprazole 40 mg once daily was administered in arm B at days 5, 6 and 7, and days 12, 13 and 14. PPI proton pump inhibitor

Study objectives

The primary objective was the diference in geometric mean of the area under the curve from time zero to 24 h (AUC_{24}) between periods with concomitant cow's milk compared with water, both with and without esomeprazole. Secondary objectives were the effects of esomeprazole intake in patients who were included in both arms, other pharmacokinetic outcomes (*i.e.* clearance, maximum concentration [C_{max}] and time to C_{max} [T_{max}]), and comparison of (the incidence and severity of) the adverse effects of treatment with erlotinib between periods and study arms.

Adverse event monitoring

Toxicity was scored by the investigator at baseline and during hospital admission in accordance with the US National Cancer Institute Common Terminology Criteria for Adverse Events (CTCAE) grades, version 4.03 [18]. Patients were provided with a diary to report any (ongoing) adverse events during the study.

Statistical analyses

Given a clinically relevant difference of 30% in AUC, a within-patient standard deviation of 25%, 80% power and a two-sided significance level of 5%, 14 evaluable patients were required per study group (*i.e.* with or without esomeprazole) [19]; hence, a total of 28 patients had to be included.

Analyses of AUC_{24} and C_{max} were performed on logtransformed values, since these parameters were assumed to follow a log-normal distribution [20]. Estimates for the mean differences in (log) AUC_{24} and C_{max} between milk and water were obtained for both study arms separately (with or without esomeprazole) using a linear mixed-effect model with treatment (water or milk), sequence and period as fixed effects, and subject-within-sequence as a random effect [21]. 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 their 95% conidence intervals (CIs) were exponentiated to provide point estimates of the ratio of geometric means and 95% CIs for these ratios, which can be interpreted as relative differences in percentages. T_{max} was analyzed using the nonparametric Wilcoxon signed-rank test. Analyses to study the effect of esomeprazole were performed in a similar way, although they also included the effect of water versus milk as a mixed effect and only included patients who participated in both study arms. Toxicity was described as the incidence of toxicity per period. This was taken into account in case of an increase in CTCAE grade per cycle. Since the design of this study was not appropriate to detect a significant difference in toxicity, these results had a descriptive character. All statistical analyses were performed using Stata (StataCorp. 2017. Stata: Release 15.1. Statistical Software. College Station, TX, USA: StataCorp LP).

RESULTS

Patients

A total of 21 unique patients were included between February 2017 and November 2019. The patient demographics are presented in **Table 1**. For personal reasons, one patient withdrew informed consent after completion of the first period. Nine patients were included in both the non-PPI and PPI arms; hence, 29 pairs of study periods were completed—14 in the non-PPI arm and 15 in the PPI arm (**Figure 1**).

TABLE 1. Patients' characteristics.

Characteristic	Total included (n=20)
Sex	
Male	7 (35%)
Female	13 (65%)
Age (years) median [IQR]	67.5 [55-73.5]
Performance	
ECOG 0	10 (50%)
ECOG 1	10 (50%)
Race	
Caucasian	16 (80%)
Asian	3 (15%)
African	1 (5%)
Current smoker	0 (0%)
Erlotinib dose	
150 milligrams	17 (85%)
100 milligrams	2 (10%)
50 milligrams	1 (5%)

Abbreviations: ECOG = Eastern Cooperative Oncology Group; n = number of patients; IQR = interquartile range.

Pharmacokinetic effects of milk

The pharmacokinetics of erlotinib when taken with milk or water are presented in **Table 2**. Erlotinib AUC_{24} decreased non-significantly by 3% (95% CI − 12 to 8%; p = 0.567) when administered with milk, compared with water, in the non-PPI patients. In addition, in those patients who used esomeprazole, erlotinib exposure did not signiicantly differ as a result of intake with either water or milk (0%; 95% CI − 15 to 17%; p = 0.953). **Figures 2a and b** show the absence of an efect of milk in both study arms. C_{max} did not difer in non-PPI or PPI users, with relative diferences of a 6% and 1% increase, respectively (95% CI − 21 to 11%, p = 0.409; and 95% CI − 12 to 17%, p = 0.831, respectively). In both study arms, T_{max} increased non-significantly at 0.5 h; in the non-PPI arm from 2.0 to 2.5 h (p = 0.729) and in the PPI arm from 2.5 to 3.0 h (p = 0.306). Interpatient variability, measured by the coeicient of variation (CV), was lower with milk compared with water in both study periods and for both AUC_{24} and C_{max}. This lower variability in AUC_{24} with milk intake was most pronounced in the PPI arm (CV 38% vs. 61%) (**Table 2**).

TABLE 2. Pharmacokinetic results per period.

Pharmacokinetic parameters	No-PPI with water (n=14)	No-PPI with milk (n=14)	PPI with water (n=15)	PPI with milk (n=15)	RD no-PPI with milk vs no-PPI with water (95% CI)	P-value	RD PPI with milk vs PPI with water (95% CI)	P-value
Erlotinib								
$AUC_{0\text{-}24h}$ (CV %) geomean µg*h/mL	23.0 (37)	22.4 (35)	11.7 (61)	11.6 (38)	-2.7% (-12 to 8%)	0.567	-0.5% (-15 to 17%)	0.953
C_{max} (CV %) geomean µg/mL	1.85 (38)	1.73 (21)	0.81 (55)	0.82 (40)	-6.4% (-21 to 11%)	0.409	1.5% (-12 to 17%)	0.831
T_{max} (IQR) median hours	2.00 (1.52-2.50)	2.50 (2.00-3.00)	2.52 (2.05-3.50)	3.00 (2.50-3.52)	NA	0.729	NA	0.306

Abbreviations: AUC_{24} area under the curve from time zero to 24 h, CI conidence interval, RD relative diference, C_{max} maximum concentration, CV coefficient of variation, T_{max} time until maximum concentration, IQR interquartile range, NA not applicable, PPI proton pump inhibitor

TABLE 3. Pharmacokinetic results per period

Pharmacokinetic parameters	No-PPI (n=9)	PPI (n=9)	RD PPI vs no-PPI (95% CI)	P-value
Erlotinib				
$AUC_{0\text{-}24h}$ (CV %) geomean µg*h/mL	20.1 (30)	10.6 (51)	-47% (-58 to -34%)	<0.001
C_{max} (CV %) geomean µg/mL	1.72 (32)	0.75 (46)	-56% (-64 to -46%)	<0.001

AUC_{24} area under the curve from time zero to 24 h, CI conidence interval, RD relative diference, C_{max} maximum concentration, CV coefficient of variation, T_{max} time until maximum concentration, IQR interquartile range, NA not applicable, PPI proton pump inhibitor

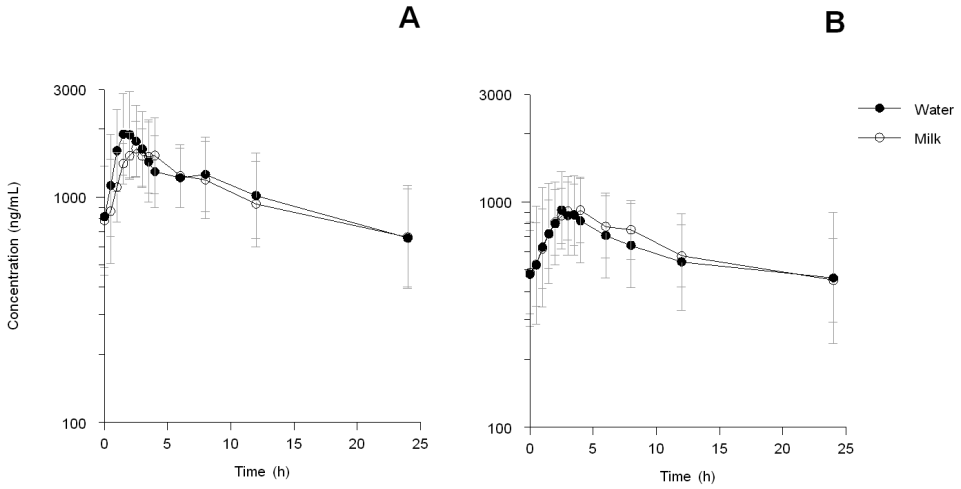

FIGURE 2. Efect of cow's milk on erlotinib concentrations. Erlotinib taken with 250 mL of cow's milk or water, a without and b with concomitant esomeprazole administration

Effects of esomeprazole on erlotinib pharmacokinetics

Based on data from the nine patients who participated in both study arms, esomeprazole decreased erlotinib AUC_{24} by an average of 47% (95% CI − 58 to − 34%; $p < 0.001$) and C_{max} by 56% (95% CI − 64 to − 46%; $p < 0.001$) compared with the period in which esomeprazole was not used. These results are displayed in **Figure 3** and **Table 3**. T_{max} seemed longer for both the milk and water periods, especially in the PPI arm (**Table 2**). In the setting of administration with water, the interpatient variability in AUC_{24} increased from 37 to 61% due to esomeprazole co-treatment. When erlotinib was taken with milk, the interpatient variability in AUC_{24} was not afected by esomeprazole co-treatment (CV 38% vs. 35%) (**Table 2**).

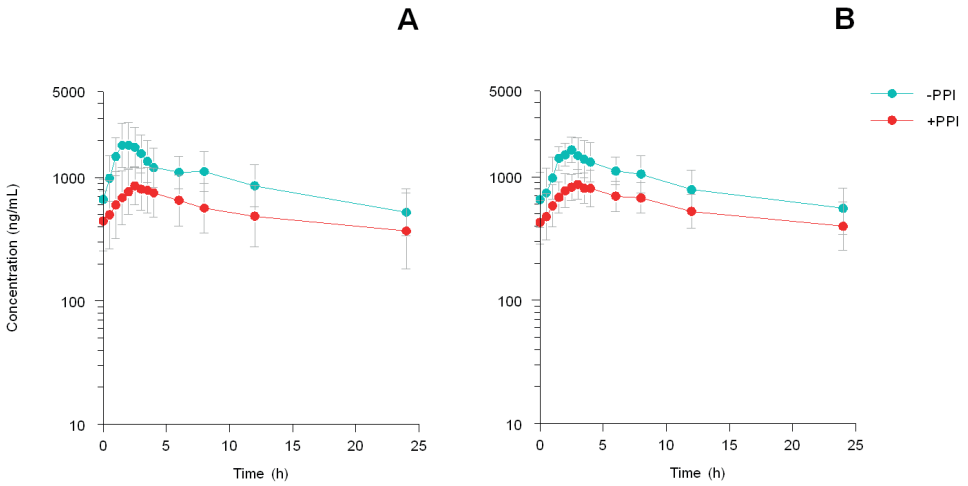

FIGURE 3. Efect of esomeprazole on erlotinib concentrations. Erlotinib taken with a 250 mL water or b cow's milk. In the PPI arm, esomeprazole was administered 3 h prior to erlotinib intake. PPI proton pump inhibitor

Toxicity

Table 4 presents all adverse events experienced. Overall, patient-reported adverse events during this study did not increase compared with baseline. Independent of study arm, no diferences in toxicities were observed between study periods. Furthermore, patients reported almost equal adverse event grades in both the non-PPI and PPI arms (data not shown). Two grade 3 adverse events occurred—one period of nausea that fluctuated for several weeks, and one increase in skin rash during concomitant nadroparine treatment. Both patients used erlotinib for more than 3 months prior to this increase in toxicity. For the first patient, erlotinib was temporarily discontinued several weeks after study completion and restarted at a reduced dosage. For the second patient, erlotinib was temporarily discontinued and its dosage reduced. These dose reductions were effective in reducing toxicity in both cases. There was one serious adverse event (SAE) in this study, namely a CTCAE grade 3 malignant spinal fracture, which occurred after randomization and before the first study period. This SAE required hospital admission and was considered to be not related to study procedures, therefore erlotinib treatment was continued. No eminent study intervention-related toxicity occurred.

TABLE 4. Patient-reported adverse events during study period.

	Baseline (N = 30)		**Water (N = 29)**		**Milk (N = 30)**	
Adverse event	Grade 1-2	Grade 3	Grade 1-2	Grade 3	Grade 1-2	Grade 3
All events	29 (97%)	-	28 (97%)	-	28 (93%)	2 (6%)
Reported in ≥10% of patients						
Nausea	1 (3%)	-	3 (10%)	-	3 (10%)	1 (3%)
Diarrhea	6 (20%)	-	3 (10%)	-	3 (10%)	-
Constipation	5 (17%)	-	1 (3%)	-	3 (10%)	-
Fatigue	10 (33%)	-	10 (34%)	-	6 (20%)	-
Pain	5 (17%)	-	7 (24%)	-	9 (30%)	-
Rash	23 (77%)	-	18 (62%)	-	20 (67%)	1 (3%)
Alopecia	12 (40%)	-	11 (38%)	-	11 (37%)	-
Serious adverse event	-	1 (3%)*	-	-	-	-

*Data are expressed as n (%) Water = both periods wherein patients used water to take erlotinib, both without and with a PPI Milk = both periods wherein patients used cow's milk to take erlotinib, both without and with a PPI. PPI proton pump inhibitor * Serious adverse event was a spinal fracture that needed hospital admission during which erlotinib was continued*

DISCUSSION

This study reports the absence of a pharmacokinetic efect of cow's milk with 3.9% fat on exposure to erlotinib in NSCLC patients, independent of PPI use. Additionally, this study showed a decrease in erlotinib AUC_{24} of almost 50% and a decrease in C_{max} of more than 50% when erlotinib was administered 3 h after esomeprazole intake.

A possible explanation for the lack of effect of milk on erlotinib exposure is that the 3.9% fat content of cow's milk is not high enough to affect absorption. In absolute values, patients were administered 9.75 g (250 mL × 3.9%) of fat from milk. This is relatively low in comparison with a high-fat meal, which consists of 500–600 kilocalories of pure fat [3] (*c.q*. 56–67 g). The efect of a high-fat meal on erlotinib disposition ranges from a 33% AUC increase when taken 2 h after erlotinib administration [22], to a 66% increase in AUC of erlotinib when food and drug are taken concomitantly [23]. In theory, the negative efect of esomeprazole of almost 50% decrease in AUC_{24} could be overcome by coadministration of a high-fat meal.

An additional reported efect of increasing the bioavailability of erlotinib with coadministration of a high-fat meal was a decrease in interpatient variability [3]. The benefits of less interpatient variability are a more predictable effectivity and toxicity on a large scale, since more patients will be administered within the therapeutic window.

Our data show that milk also reduced interpatient variability, especially in the PPI arm (**Table 2**). Although, on average, bioavailability did not change, the lower interpatient variability would be an argument in favor of erlotinib administration with milk instead of water.

Another reason why erlotinib absorption was not affected by milk could be that the strong pH buffering capacity of milk [12] prevents the intragastric pH from decreasing. Hence, the beneficial efect of the milk's fat is counteracted by switching erlotinib to its less soluble, non-ionized form, which is not an optimal condition for transluminal transportation across gastrointestinal cells. Furthermore, there is no evidence of milk interacting with drug transporters or hepatic cytochrome P450 isoenzymes.

Average milk consists of 3–4% fat [13]. Since we used cow's milk with the highest fat content (3.9%) commercially available, it is unlikely that lighter variants of cow's milk would have a higher efect on the bioavailability of erlotinib. Nevertheless, cow's milk may be of interest for increasing systemic exposure of TKIs with vaster food efects*, i.e.* lapatinib (up to 325% and 200% AUC increase with a high- and low-fat meal, respectively) [3]. In line with milk, yoghurt (0.4% fat [24]) is not expected to interact with erlotinib absorption and could also be considered safe. Coadministration with yoghurt was previously studied and was considered safe for the TKI nilotinib [3, 25].

Moreover, for the first time, we conducted an intrapatient comparison on the effects of esomeprazole on the AUC_{24} and C_{max} of erlotinib, which is in line with previous research with erlotinib and omeprazole [9]. We hence warn patients and prescribers of this possible harmful interaction, which could lead to therapy ineffectiveness. Potential solutions for patients who are dependent on PPI use may be a delayed PPI intake until erlotinib is fully absorbed or by taking erlotinib concomitantly with cola [10]. Albeit practical, the most feasible solution is a critical reconsideration of the need to prescribe a PPI and discontinuation of the PPI where possible.

Another way to increase the aqueous solubility, and therewith bioavailability, of erlotinib could be to improve its formulation [26]. A phospholipid formulation showed an improved pharmacokinetic proile in rats [27]. Before this new formulation could be considered to be implemented in clinical practice, further research should first be conducted to determine its possible benefits and deficits.

Furthermore, the absence of a milk efect on erlotinib exposure is probably also the reason why this study found no differences in patient-reported toxicity. This is not surprising as, for erlotinib, the plasma concentration is correlated with the occurrence

of the most prevalent adverse efects of skin rash and diarrhea [28]. Erlotinib intake with milk is just as safe as intake with water, and could thus be advised to patients as an alternative for administration with water, for example to mitigate mild gastrointestinal reflux complaints or as the patient's preference.

Interestingly, although esomeprazole reduced erlotinib exposure by half, patients did not report less toxicity; however, the 3-day period during which patients had to take esomeprazole was most likely too short to have a noticeable effect on toxicity. When esomeprazole is taken for a longer period of time, the chronic decrease in erlotinib exposure could have a more distinctive effect of less toxicity.

CONCLUSION

Whole cow's milk with 3.9% fat has no clinically relevant efects on the exposure of erlotinib in NSCLC patients, independent of PPI use. The combination with milk instead of water is safe and well tolerated, and may be a good alternative for some patients. Meanwhile, the use of esomeprazole 3 h prior to erlotinib intake strongly decreased both erlotinib AUC_{24} and C_{max}, and should be avoided if possible.

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CHAPTER 12

IMPACT OF CURCUMIN WITH OR WITHOUT PIPERINE ON THE PHARMACOKINETICS OF TAMOXIFEN

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ABSTRACT

Tamoxifen is a prodrug that is primarily metabolized into the pharmacologically active metabolite endoxifen and eventually into inactive metabolites. The herb curcumin may increase endoxifen exposure by affecting phase II metabolism. We compared endoxifen and tamoxifen exposure in breast cancer patients with or without curcumin, and with addition of the bio-enhancer piperine. Tamoxifen (20-30mg q.d.) was either given alone, or combined with curcumin (1,200mg t.i.d.) +/- piperine (10mg t.i.d.). The primary endpoint of this study was difference in geometric means for the area under the curve (AUC) of endoxifen. Genotyping was performed to determine CYP2D6 and CYP3A4 phenotypes. The endoxifen $AUC_{0\text{-}24h}$ decreased with 7.7% (95%CI: -15.4 to 0.7%; P=0.07) with curcumin and 12.4% (95%CI: -21.9 to -1.9%; P=0.02) with curcumin and piperine, compared to tamoxifen alone. Tamoxifen $AUC_{0\text{-}24h}$ showed similar results. For patients with an extensive CYP2D6 metabolism phenotype (EM), effects were more pronounced than for intermediate CYP2D6 metabolizers (IM). In conclusion the exposure to tamoxifen and endoxifen was significantly decreased by concomitant use of curcumin (+/-piperine). Therefore co-treatment with curcumin could lower endoxifen concentrations below the threshold for efficacy (potentially 20-40% of the patients), especially in EM patients.

INTRODUCTION

Breast cancer is one of the most commonly diagnosed malignancies worldwide and one of the leading causes of cancer related deaths in women.[1] Since decades, patients with estrogen receptor positive breast cancer are extensively treated with endocrine therapy such as tamoxifen. Tamoxifen acts as a selective estrogen receptor modulator in breast cancer tissue, thereby reducing the risk of disease recurrence and breast cancer specific mortality.[2]

Currently, there is a trend towards the use of natural herbs and dietary products among cancer patients. Nearly 20-30% of all cancer patients, especially breast cancer patients, use herbal medicine besides their conventional therapy.[3] Curcumin, also called *´turmeric´*; a spice recovered from the roots of the *curcuma longa* plant, is becoming increasingly popular among cancer patients because of its supposed anti-cancer effects.[4] Curcumin is characterized by a poor bioavailability due to poor absorption and rapid metabolism.[5] Therefore, curcumin is often used in combination with piperine (a component of black pepper). Piperine increases curcumin bioavailability 20-fold by increasing curcumin absorption and inhibition of curcumin glucuronidation.[6]

Tamoxifen shows a complex and multi-pathway metabolism, which mainly occurs in the liver. Tamoxifen is metabolized into several (active) metabolites, through several phase I and phase II metabolizing enzymes; mainly by the cytochrome P450 (CYP) enzymes CYP2D6 and CYP3A4 (Figure 1).[7] Based on its relatively high plasma concentrations and potency, endoxifen is believed to be one of the most important metabolites in the efficacy of tamoxifen therapy.[7, 8] Moreover, endoxifen is excreted (mainly in the feces) after phase II metabolism through UDP-glucuronyltransferases (UGTs) and sulfotransferase (SULT).[8]

A study in rats demonstrated an increase in tamoxifen plasma concentration of 33-64%, suggesting an inhibitory effect of curcumin on tamoxifen metabolism.[9] Several studies (both *in vitro* and *in vivo*) demonstrated that curcumin has an inhibitory effect on several CYP enzymes among which CYP3A4 and CYP2D6.[10] Another important effect of curcumin is inhibition of phase II drug metabolism by inhibition of UGT. Furthermore, curcumin could potentially inhibit or induce several drug-efflux transporters (e.g. P-glycoprotein (P-gp)).[10, 11]

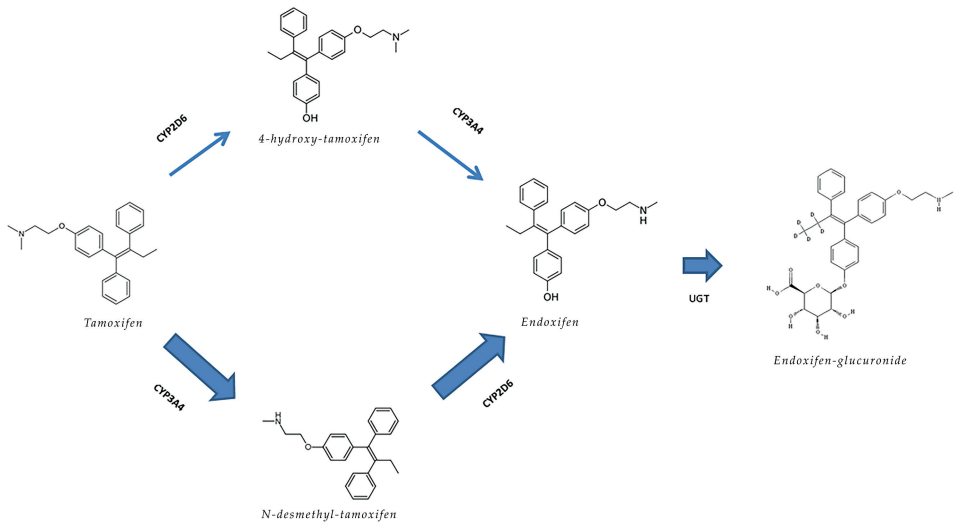

FIGURE 1: The major primary metabolite N-desmethyl-tamoxifen and the minor primary metabolite 4-hydroxytamoxifen are formed by N-demethylation and 4-hydroxylation of tamoxifen, through CYP3A4 and CYP2D6 metabolism respectively. Further CYP-mediated metabolism of these metabolites results in the formation of 4-hydroxy-N-desmetyltamoxifen (endoxifen). Endoxifen is ultimately metabolized through phase II metabolism into a.o. endoxifen-glucoronide through UGT and also through SULT enzymes.

In this study, it was hypothesized that endoxifen plasma concentrations may increase when tamoxifen is administered with curcumin, mainly through UGT enzyme inhibition. In addition, concomitant administration with the bio-enhancer piperine may potentiate effects on tamoxifen and endoxifen plasma pharmacokinetics. In a pharmacokinetic cross-over study we therefore explored the impact of curcumin --with and without piperine-- on tamoxifen and endoxifen pharmacokinetics.

RESULTS

Patient Characteristics

Seventeen patients were included in the study of whom one patient was excluded due to voluntary withdrawal, resulting in 16 evaluable patients. Patient characteristics can be found in Table 1. DNA analysis showed no variants for CYP3A4*22. As this polymorphism is considered most relevant for CYP3A4, a predicted normal CYP3A4 phenotype for all study patients based on genotype was assumed.[12, 13] Based on CYP2D6 genotyping, seven patients (44%) showed an extensive CYP2D6 metabolism

TABLE 1. Patient characteristics

Characteristic	N (%)
Patients	16 (100)
Randomization sequence	
ABC	9 (56)
CBA	7 (44)
Age (Median, IQR)	45 (42-58)
Sex	
Female	15 (94)
Male	1 (6)
Race	
Caucasian	15 (94)
Arabic	1 (6)
Height (Median, IQR)	171 (167-176)
Weight (Median, IQR)	73 (65-91)
BMI (Median, IQR)	25 (23-29)
WHO Performance Status	
0	13 (81)
1	3 (19)
Previous chemotherapy	
Yes	12 (75)
TAC	2 (13)
AC - paclitaxel	4 (25)
FEC - docetaxel	6 (37)
No	4 (25)
Previous RTx	
Yes	10 (63)
No	6 (37)
Tamoxifen dose	
20 mg	15 (94)
30 mg	1 (6)
Genotype	
CYP3A4*22	
EM	16 (100)
CYP2D6	
EM	7 (44)
IM	7 (44)
PM	1 (6)
UM	1 (6)

Abbrevations: IQR = interquartile range, TAC = Docetaxel, doxorubicin and cyclofosfamide, AC = doxorubicin, cyclofosfamide, FEC = 5FU, epirubicine and cyclofosfamide, RTx = radiotherapy, EM = extensive metabolism phenotype, IM = intermediate metabolism phenotype, PM = poor metabolism phenotype, UM = Ultra-rapid metabolism phenotype.

phenotype (EM), while seven other patients (44%) exhibited intermediate CYP2D6 metabolism (IM). The other two patients (12%) demonstrated ultra-rapid metabolism (UM) and poor CYP2D6 metabolism (PM), respectively.

Pharmacokinetics

In patients treated with tamoxifen and curcumin, the geometric mean $AUC_{0\text{-}24h}$ and C_{trough} of tamoxifen decreased with 8.0% (95%CI: -14.1% to –1.4%, P=0.02) and 7.1% (95%CI: -17.1% to 4.0%, P=0.25), respectively, compared to tamoxifen monotherapy (Table 2). Furthermore, $AUC_{0\text{-}24h}$ and C_{trough} of endoxifen decreased with 7.7% (95%CI: – 15.4% to 0.7%, P=0.07), and 5.6% (95%CI: -15.6% to 5.5%, P=0.43), respectively, with concomitant curcumin treatment.

When tamoxifen was administered with curcumin and piperine, the effects were more pronounced; tamoxifen $AUC_{0\text{-}24h}$ and C_{trough} decreased with 12.8% (95%CI: -19.2% to -5.9%, P<0.01) and 12.2% (95%CI: -21.5% to -1.8%, P=0.02), respectively, compared to tamoxifen monotherapy. The endoxifen $AUC_{0\text{-}24h}$ decreased with 12.4% (95%CI: -21.9% to -1.9%, P=0.02), while the C_{trough} decreased with 12.4% (95%CI: -20.9% to -3.0%, P=0.01). Further pharmacokinetic results are shown in Table 2**.** Pharmacokinetic parameters for 4-hydroxy-tamoxifen and N-desmethyl tamoxifen showed a decrease in almost every pharmacokinetic parameter when administered with curcumin --with and without piperine-- although only $AUC_{0\text{-}24h}$ of N-desmethyl tamoxifen and 4-hydroxytamoxifen with curcumin and $AUC_{0\text{-}24h}$ of N-desmethyl tamoxifen with curcumin and piperine demonstrated a significant difference.

When analyzing the CYP2D6 predicted phenotypes separately, both endoxifen and tamoxifen showed a more pronounced decrease for both $AUC_{0\text{-}24h}$ and C_{trough} (especially during treatment with curcumin and piperine) in patients with an extensive metabolism, compared to those with an intermediate metabolism **(**Table 3**)**. In patients with an IM treated with curcumin and piperine the $AUC_{0\text{-}24h}$ decreased with 5.3% (95%CI: -13.1% to +3.1% , P=0.16) and 10.3% (95%CI: -23.5% to 5.3%, P=0.14) for tamoxifen and endoxifen, respectively. In patients with an EM treated with curcumin and piperine the tamoxifen and endoxifen $AUC_{0\text{-}24h}$ decreased with 22.0% (95%CI: -29.0% to -14.2%, P<0.01) and 18.4% (95%CI: -36.1% to 4.3%, P=0.09), respectively. C_{trough} showed similar results **(**Table 3**)**. Although the interaction term was only significant for tamoxifen $AUC_{0\text{-}24h}$ and C_{trough} with curcumin and piperine. There was no period effect, which implicates no decline in tamoxifen nor endoxifen plasma concentrations based on altered tamoxifen metabolism over time. Individual tamoxifen and endoxifen $AUC_{0\text{-}24h}$ can be found in Figure 2.

TABLE 2. Tamoxifen pharmacokinetics

PK parameters	Tamoxifen monotherapy	Tamoxifen + curcumin	Tamoxifen + curcumin + piperine	Relative difference A vs B (95%CI)	*P*-value	Relative difference (A vs C) (95%CI)	*P*-value
Tamoxifen							
$AUC_{0\text{-}24h}$	5951 (20)	5460 (24)	5171 (23)	-8.0% (-14.1 to -1.4)	0.02	-12.8% (-19.2 to -5.9)	<0.01
C_{trough}	213 (27)	198 (28)	187 (24)	-7.1% (-17.1 to +4.0)	0.25	-12.2% (-21.5 to -1.8)	0.02
C_{max}	356 (16)	324 (21)	313 (22)	-8.4% (-16.4 to +0.5)	0.07	-11.1% (-18.1 to -3.6)	<0.01
T_{max}	2.4 (1.9 to 3.1)	2.4 (1.9 to 3.0)	2.7 (1.9 to 3.8)		0.74		0.34
Endoxifen							
$AUC_{0\text{-}24h}$	597 (59)	556 (52)	518 (54)	-7.7 % (-15.4 to +0.7)	0.07	-12.4% (-21.9 to -1.9)	0.02
C_{trough}	25 (60)	23 (53)	21 (55)	-5.6 % (-15.6 to +5.5)	0.43	-12.4% (-20.9 to - 3.0)	0.01
C_{max}	31 (56)	28 (50)	27 (51)	-7.1% (-16.3 to +3.2)	0.20	-9.8% (-20.1 to +1.8)	0.10
T_{max}	2.0 (1.3 to 3.0)	1.7 (1.2 to 2.6)	1.8 (1.1 to 3.1)		0.88		0.62
4-hydroxy-tamoxifen							
$AUC_{0\text{-}24h}$	113 (31)	106 (24)	103 (28)	-6.3 % (-11.6 to -0.73)	0.03	-8.2% (-17.0 to +1.6)	0.11
C_{trough}	4.4 (34)	4.2 (26)	4.1 (28)	-4.3 % (-12.3 to +4.4)	0.45	-7.3 (-17.6 to +4.3)	0.26
C_{max}	6.0 (32)	5.4 (26)	5.4 (31)	-10.0% (-16.8 to -2.6)	<0.01	-8.8% (-20.0 to +4.0)	0.20
T_{max}	2.7 (1.9 to 3.9)	2.4 (1.8 to 3.3)	2.8 (2.0 to 3.8)		0.42		0.37
N-desmethyl-tamoxifen							
$AUC_{0\text{-}24h}$	11596 (21)	10766 (24)	10084 (31)	-7.0% (-13.1 to -0.6)	0.03	-12.4% (-22.3 to -1.3)	0.03
C_{trough}	463 (28)	430 (29)	411 (32)	-7.2% (-15.0 to +1.2)	0.10	-10.9% (-21.6 to +1.3)	0.08
C_{max}	602 (21)	556 (24)	540 (32)	-7.2% (-14.9 to +1.1)	0.09	-9.7% (-20.2 to +2.3)	0.12
T_{max}	2.6 (1.8 to 3.7)	1.7 (1.2 to 2.4)	2.1 (1.4 to 3.2)		0.24		0.88

*Abbreviations: PK = pharmacokinetics; CI = Confidence Interval; $AUC_{0\text{-}24h}$= Area under the curve, timepoint 0h to 24h (expressed as geomean nM*h/mL (CV%)); C_{trough}= minimum concentration (expressed as geomean nM/mL (CV%)); C_{max}= maximum concentration (expressed as geomean nM/mL (CV%)); T_{max}= time until maximum concentration (expressed as median h (IQR)); CV% = coefficient of variation, IQR = interquartile range.*

TABLE 3. Tamoxifen pharmacokinetics, based on CYP2D6 phenotype

PK parameters	Tamoxifen monotherapy (A)	Tamoxifen + curcumin (B)	Tamoxifen + curcumin + piperine (C)	Relative difference A vs B (95%CI)	P- value	Relative difference A vs C (95%CI)	P- value
Intermediate Metabolizers (IM)							
Tamoxifen $AUC_{0\text{-}24h}$	5795 (4895 – 6859)	5427 (4313 – 6830)	5518 (4679 – 6508)	-7.2% (-18.2 to +5.4)	0.19	-5.3% (-13.1 to +3.1)	0.16*
Tamoxifen C_{trough}	200 (160 – 251)	191 (146 – 249)	199 (167 – 237)	-5.9% (-20.9to +11.9)	0.41	-1.3% (-15.3 to 15.1)	0.84*
Endoxifen $AUC_{0\text{-}24h}$	523 (362 –755)	472 (339 – 656)	477 (340 – 669)	-9.4% (-21.7 to +4.8)	0.14	-10.3% (-23.5 to 5.3)	0.14
Endoxifen C_{trough}	21 (14 – 32)	19 (13 – 27)	19 (14 – 27)	-10.7% (-28.2 to 11.2)	0.24	-8.3% (-27.2 to 15.4)	0.38
Extensive Metabolizers (EM)							
Tamoxifen $AUC_{0\text{-}24h}$	6077 (4882 – 7565)	5471 (4247 – 7047)	4836 (3720 – 6288)	-10.3% (-19.7 to +0.3)	0.06	-22.0% (-29.0 to - 4.2)	<0.01*
Tamoxifen C_{trough}	218 (163 – 291)	199 (148 –268)	170 (132 – 218)	-9.6% (-26.4 to +11.2)	0.27	-24.6% (-33.9 to - 14.1)	<0.01*
Endoxifen $AUC_{0\text{-}24h}$	745 (576 – 963)	716 (574 – 893)	596 (495 – 717)	-5.7% (-19.6 to +10.7)	0.39	-18.4% (-36.1 to +4.3)	0.09
Endoxifen C_{trough}	30 (23 – 39)	31 (25 – 38)	25 (20 – 30)	-0.3% (-12.8 to +13.9)	0.96	-17.2% (-26.1 to - 7.3)	<0.01*

*Abbreviations: PK = pharmacokinetics; CI = Confidence Interval; $AUC_{0\text{-}24h}$= Area under the curve, timepoint 0h to 24h (expressed as geomean nM*h/mL (95% CI)); C_{trough}= minimum concentration (expressed as geomean nM/mL (95%CI)); *Interaction term reached statistical significance (P<0.05).*

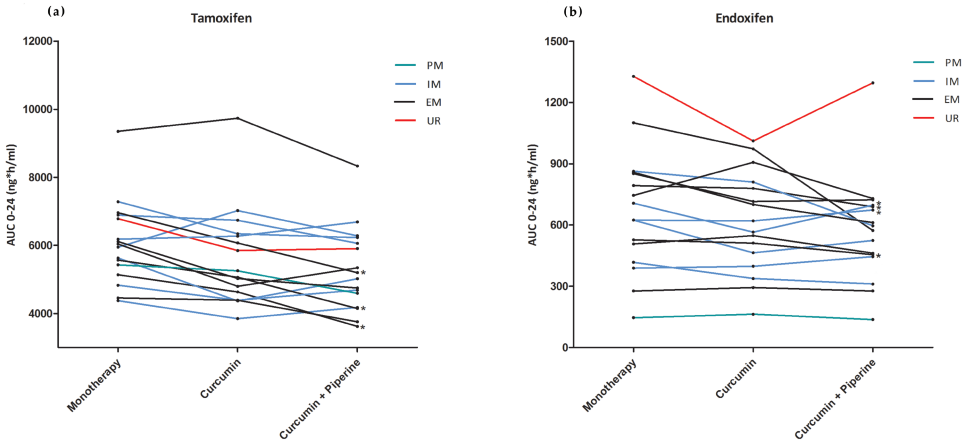

FIGURE 2. Endoxifen and tamoxifen $AUC_{0\text{-}24h}$ per individual patient per treatment phase: (a) Tamoxifen $AUC_{0\text{-}24h}$ per individual patients per treatment phase. (b) endoxifen $AUC_{0\text{-}24h}$ per individual patients per treatment phase. Patients with an Intermediate CYP2D6 metabolism (IM) were colored blue. Patients with an extensive CYP2D6 metabolism (EM) were colored black. Poor CYP2D6 metabolizers (PM) and Ultra-rapid CYP2D6 metabolizers (UR) were colored green and red respectively; *:decrease in $AUC_{0\text{-}24h}$>25% ; a total of 4 patients showed a >25% decrease in endoxifen $AUC_{0\text{-}24h}$ and 3 patients in tamoxifen $AUC_{0\text{-}24h}$ when tamoxifen was administered with curcumin and piperine compared to tamoxifen monotherapy

Toxicities

There were no unexpected serious adverse events (SAE) during combined treatment with curcumin or curcumin plus piperine related to the study procedures. There was one serious adverse event, which was assumed to be not related to any of the study drugs (collaps with unknown origin). Toxicity profiles were similar between the different treatment phases, although more hot flashes and fatigue were observed in patients treated with curcumin +/- piperine compared to tamoxifen monotherapy (Table 4). Interestingly, 3 patients suffered from grade 2-3 diarrhea during treatment with curcumin and piperine, whereas none of the patients experienced diarrhea when treated with tamoxifen monotherapy.

TABLE 4. Toxicity

Toxicity	Tamoxifen monotherapy *N* (%)	Tamoxifen with curcumin *N* (%)	Tamoxifen with curcumin and piperine *N* (%)
Nausea	2(13)	1(6)	1(6)
Diarrhea	0	1(6)	3(19)
Constipation	2(13)	4(25)	1(6)
Fatigue	2(13)	3(19)	3(19)
Hot flashes	3(19)	5(31)	4(25)
Reflux	1(6)	1(6)	0
Dyspnea	0	1(6)	0
Anorexia	1(6)	0	1(6)
Pain	4(25)	0	2(13)
Rash	1(6)	0	1(6)
Hypophosphatemia	0	0	1(6)
Hyperlipidemia	1(6)	1(6)	1(6)

Legend: Number of patients with all CTCAE grade toxicity when treated with tamoxifen with or without curcumin and piperine expressed as number of patients (% of total number of patients).

DISCUSSION

In this study, a modest but significant decrease was found in both tamoxifen and endoxifen plasma concentrations during concomitant administration of tamoxifen and curcumin, compared to tamoxifen monotherapy. This effect was even more pronounced when tamoxifen was administered with curcumin and piperine. Furthermore, patients with an extensive CYP2D6 phenotype seem to be at greater risk of experiencing this herb-drug interaction, compared to CYP2D6 intermediate metabolizers.

Since tamoxifen metabolism and excretion is complex and involves multiple enzymes and transporter-proteins, the likelihood of a drug-drug interaction (DDI) with modulators and inhibitors of enzymes and drug-transporters (e.g. CYP3A4 and P-glycoprotein) involved in tamoxifen metabolism is high.[7, 8] Based on preclinical data, curcumin is a compound which could potentially lead to such a DDI.[10] When designing our study, we based our hypothesis on preclinical data, as no studies in cancer patients studying the effects of curcumin on the pharmacokinetics of anti-cancer drugs were available in the literature. Cho *et al.* demonstrated an increase in tamoxifen exposure and a decrease in 4-hydroxy-tamoxifen/tamoxifen AUC ratio, suggesting a decrease in CYP-mediated metabolism or P-glycoprotein mediated efflux of tamoxifen.[9] *In vitro* results indicate an inhibitory effect of curcumin on phase II metabolism, in which enzymes such as

UGT are involved.[10, 14] UGTs are also involved in the metabolism of tamoxifen (Figure 1), in theory resulting in increased endoxifen plasma concentrations.[10] In contrast to the study in rats by Cho *et al*., we found both a decrease in tamoxifen and endoxifen plasma concentrations. We do not have a conclusive explanation for this observation, as several mechanisms might be involved. As endoxifen plasma concentrations did not increase during concomitant treatment with curcumin and piperine, an inhibitory effect on phase II metabolism is unlikely as these results rather indicate phase II induction. A more likely explanation is inhibition of CYP2D6 –which is underlined by the larger effect in EM patients—although this cannot be the only explanation, as tamoxifen concentrations also decreased due to curcumin co-treatment. Another explanation may be found in a potential interaction with P-glycoprotein. This transporter, which is responsible for the efflux of tamoxifen out of the epithelial cells into the gut and bile, was studied before *in vitro* and led to contrasting results (both induction and inhibition).[10, 15, 16] In case curcumin acts as a P-glycoprotein inducer, tamoxifen and metabolite plasma concentrations would all decrease, which is in line with our findings, resulting from a diminished absorption of tamoxifen into the blood stream.

One of the main problems of clinical research with curcumin is to standardize the formulation of curcumin. There are many curcumin formulations available. Potentially these formulations may differ in bio-availability, which makes it difficult to determine the individual impact of these formulations and to give a general advise.[17] Moreover, many of these formulations exist of multiple non-standardized ingredients. In this study standardized formulations of both curcumin and piperine of 1200mg and 10mg capsules respectively were used from a single production batch.

Moreover curcumin knows low bio-availability and the suggested interpatient variability is high.[4, 18] We only measured tamoxifen pharmacokinetics and did not determine plasma levels of curcumin, which gives a possible limitation of this study since the magnitude of a possible interaction may differ between patients depending on curcumin plasma levels.[18] However a curcumin dose of 3.6 g q.d. is considered to reach significant plasma concentrations and is therefore most likely to achieve a significant drug-interaction.[18]

Furthermore the interaction term for CYP2D6 metabolism only reached significance for tamoxifen $AUC_{0\text{-}24h}$ and C_{trough} when coadministered with curcumin and piperine. Since the design of this study was not sufficient to detect a significant difference in other pharmacokinetic endoxifen and tamoxifen comparisons this result must be confirmed in future clinical trials.

Besides curcumin, piperine might also influence pharmacokinetics of tamoxifen and endoxifen by itself.[19] Piperine affects the structure of the intestinal lumen and wall resulting in a higher passive drug influx. Concomitant piperine use might alter gastric emptying time use in a dose- and time dependent manner, and in addition, piperine is known to be a P-glycoprotein inhibitor *in vivo.*[19, 20] Moreover piperine might enhance plasma concentrations of several drugs due to inhibition of CYP enzymes (e.g. CYP3A4 , CYP2D6).[19] Therefore the effect of piperine on tamoxifen and endoxifen pharmacokinetics may not be underestimated and the results in this study could not be solely attributed to curcumin.

Although the relatively small effects on tamoxifen and endoxifen pharmacokinetics and the high inter-patient variability of endoxifen (CV= 50-60%) this study may seem of limited clinical relevance. However, individual patients may be deprived from an optimal therapy, since tamoxifen and endoxifen concentrations may drop below the threshold for efficacy (~16 nM for endoxifen) due to co-treatment with curcumin.[7, 21] Especially, considering the fact that 20-30% of all treated patients have an endoxifen plasma concentration below this threshold and an additional 20% has endoxifen plasma concentrations just above this threshold.[21-23] This scenario is in particular the case in patients with an extensive metabolism CYP2D6 phenotype, as effects of curcumin with and without piperine were most pronounced in this group of patients.

This was the first study, which investigated the influence of curcumin (with and without piperine) on tamoxifen pharmacokinetics. The use of curcumin (with and without piperine significantly decreased tamoxifen and endoxifen pharmacokinetics, especially in EM patients. Patients may be deprived from optimal tamoxifen treatment and endoxifen plasma levels may even drop below the threshold for treatment efficacy. Therefore patients should be advised to stop curcumin use during tamoxifen treatment or treatment efficacy of tamoxifen should be adequately monitored.

MATERIALS AND METHODS

This 2-arm, 3-period, randomized, cross-over study was performed between January 2017 and May 2018 at the Erasmus University Medical Center. The study was approved by the local ethics committee and competent authority in accordance to the declaration of Helsinki and was registered at the European Clinical Trials Database (EudraCT 2016-004008-71) and the Dutch trial registry (www.trialregister.nl; number NTR6149).

Patients

We included patients who had a histological or cytological confirmed diagnosis of breast cancer with an indication for tamoxifen treatment and who were at least 18 years of age. In addition, an Eastern Cooperative Oncology Group (ECOG) performance status of 0 or 1, adequate hematological, renal, and liver function defined as a CTCAE grade of ≤ 1 were required, and patients should be able and willing to abstain from curry, grapefruit (juice), (herbal) dietary supplements, herbals or over-the-counter medication (except for paracetamol and ibuprofen) for the duration of the study. Patients were excluded if they had known impaired drug absorption (e.g. gastrectomy), serious illness or medical unstable conditions requiring treatment (e.g. infection, heart failure) or if they used strong CYP3A4, CYP2D6, UGT or P-glycoprotein inhibitors or inducers. All included patients gave written informed consent.

Study procedures

Patients received tamoxifen at the same dose for at least 28 days before entering the study to ensure steady-state pharmacokinetics. No dose alterations were allowed after inclusion in the study. Patients were digitally randomly assigned into two sequence groups, using block randomization, to rule out sequence effects. Tamoxifen was administered at a constant dose (20-30 mg q.d.) during three consecutive cycles. During cycle 1, patients received tamoxifen monotherapy; in cycle 2, patients swallowed tamoxifen concomitant with curcumin (three times daily 1,200 mg), and in cycle 3, tamoxifen was taken concomitantly with curcumin and piperine (three times daily 1,200 mg and three times daily 10 mg, respectively) in the first sequence group or vice versa in the other group. Curcumin and piperine were taken at predefined time points (10 AM, 4 PM, and 10 PM). Patient compliance was assessed through a patient diary until end of study after three consecutive cycles. Furthermore CYP2D6 and CYP3A4*22 mutational analysis was performed.

Pharmacokinetic sampling

Patients were admitted to the hospital on days 28, 56, and 84 of the study for pharmacokinetic blood sampling. Blood samples for determination of tamoxifen, 4-hydroxytamoxifen, n-desmethyl-tamoxifen, and endoxifen pharmacokinetics were obtained at predefined time points (t=0 (before tamoxifen intake); and 0.5h; 1h; 1.5h; 2h, 2.5h; 3h; 3.5h; 4h; 6h; 8h; 12h, and 24h after tamoxifen intake). Blood samples were processed into plasma within 30 minutes by vortex mixing and centrifugation for 10 min at 2,500-3,000 g at 4°C. Plasma concentrations were measured using a validated liquid chromatography with tandem mass spectrometry method (UP-LCMS/MS).[24] Predefined pharmacokinetic parameters were tamoxifen and endoxifen exposure

(expressed as dose corrected area under the curve from pre-infusion time point until 24h ($AUC_{0\text{-}24h}$)), maximum concentration (C_{max}), time until maximum concentration (T_{max}) and lowest plasma concentration (C_{trough}).

Toxicity

Toxicity rates during tamoxifen monotherapy and tamoxifen concomitantly with curcumin with or without piperine were determined during patient follow-up until the end of the study using Common Terminology Criteria for Adverse Events (CTCAE version 4.0), and by evaluating the patient diaries.

Statistical analysis

A difference in systemic exposure ($AUC_{0\text{-}24h}$) to endoxifen of 25% between treatment cycles was considered to be clinically relevant. It was assumed that the within patient standard deviation in endoxifen pharmacokinetics was 20%.[25] Given a power of 80%, this resulted in a sample size of 16 evaluable patients.[26]

Analyses of $AUC_{0\text{-}24h}$, C_{trough} and C_{max} were performed on log-transformed observations since these were assumed to follow a log-normal distribution.[27] Estimates for the mean differences in (log) $AUC_{0\text{-}24h}$, C_{trough} and C_{max} were obtained for the two comparisons (i.e. curcumin versus tamoxifen monotherapy, and curcumin plus piperine versus tamoxifen monotherapy) separately using a linear mixed effect model with treatment, sequence, and period as fixed effects and subject within sequence as a random effect.[28] 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.[28] Since two primary comparisons were made, a Bonferroni correction was applied to correct for multiple testing (two-sided alpha of 5%/2=2.5%). T_{max} was analyzed by means of the Wilcoxon signed rank test and described with medians and interquartile ranges .

For a comparison between extensive and intermediate CYP2D6 phenotype an interaction term between metabolism and treatment was added to the linear mixed effects models. Only if the interaction term turned out to be significant, subsequent subgroup analyses were performed.

Toxicity was described as the incidence of toxicity per phase. This was corrected for baseline toxicity and was only taken into account in case of an increase in CTCAE grade per cycle. Since the design of this study was not appropriate to detect a significant difference in toxicity, these results had a descriptive character.

CONCLUSION

In conclusion, this is the first study in patients, which investigated the influence of curcumin (with and without piperine) on tamoxifen pharmacokinetics. Curcumin (with and without piperine) significantly decreased tamoxifen and endoxifen pharmacokinetics, especially in EM patients. Therefore patients using curcumin should be adequately monitored or should be advised to stop curcumin use during tamoxifen treatment.

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CHAPTER 13

INFLUENCE OF GREEN TEA CONSUMPTION ON ENDOXIFEN STEADY-STATE CONCENTRATION IN BREAST CANCER PATIENTS TREATED WITH TAMOXIFEN

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ABSTRACT

Background. Many cancer patients use additional herbs or supplements in combination with their anti-cancer therapy. Green tea – active ingredient epigallocatechin-3-gallate (EGCG) – is one of the most commonly used dietary supplements among breast cancer patients. EGCG may alter the metabolism of tamoxifen. Therefore, the aim of this study was to investigate the influence of green tea supplements on the pharmacokinetics of endoxifen; the most relevant active metabolite of tamoxifen.

Methods. In this single center, randomized cross-over trial, effects of green tea capsules on endoxifen levels were evaluated. Patients treated with tamoxifen for at least 3 months were eligible for this study. After inclusion, patients were consecutively treated with tamoxifen monotherapy for 28 days and in combination with green tea supplements (1 g twice daily; containing 300 mg EGCG) for 14 days (or *vice versa*). Blood samples were collected on the last day of monotherapy or combination therapy. Area under the curve ($AUC_{0\text{-}24h}$), maximum concentration (C_{max}) and minimum concentration (C_{trough}) were obtained from individual plasma concentration-time curves.

Results. No difference was found in geometric mean endoxifen $AUC_{0\text{-}24h}$ in the period with green tea versus tamoxifen monotherapy (-0.4%; 95% CI: -8.6 – 8.5%; p=0.92). Furthermore, no differences in C_{max} (-2.8%; -10.6 – 5.6%; p=0.47) nor C_{trough} (1.2%; -7.3 – 10.5%; p=0.77) were found. Moreover, no severe toxicity was reported during the whole study period.

Conclusions. This study demonstrated the absence of a pharmacokinetic interaction between green tea supplements and tamoxifen. Therefore, the use of green tea by patients with tamoxifen does not have to be discouraged.

INTRODUCTION

Breast cancer is the most commonly diagnosed type of cancer among women.[1] In the adjuvant treatment of hormone sensitive breast cancer, tamoxifen is the most frequently used and an effective oral endocrine therapy.[2] Many cancer patients – with estimates up to 80% – use complementary and alternative medicines in combination with their anti-cancer therapy.[3–7] One of the most popular herbal supplements among breast cancer patients are green tea (*camellia sinensis*) supplements.[4,5,8]

Green tea contains a large number of bioactive compounds, such as catechins and flavonoids.[9,10] The active pharmacological ingredient of green tea is epigallocatechin-3-gallate (EGCG).[11] EGCG is believed to contribute to various cancer-preventive effects resulting from its high antioxidant potential.[11–14] *In vitro* and animal studies reported a number of cancer-preventative effects of EGCG including: attenuation of oxidative stress, inhibition of angiogenesis, induction of apoptosis and alterations in expression of cell cycle regulatory proteins.[11,12,14–17] None of these effects have been proven clinically. However, there are also signs that green tea and associated substances can influence other prescribed drugs. For example, it has been reported that EGCG could significantly reduce the systemic exposure of nadolol, folic acid and digoxin in subjects with approximately 85%, 39% and 31%, respectively.[18–20] Moreover, EGCG significantly increased the bioavailability of for example simvastatin and verapamil in rat studies.[21,22] The described interactions with these drugs are the result of altered bioavailability or decreased metabolism, and can mechanistically be explained by inhibition of influx transporter organic anion transporter polypeptide (OATP) or efflux transporter P-glycoprotein and several phase I and II metabolizing enzymes (*e.g.* cytochrome P450 (CYP) 3A and UDP-glucuronosyltransferase (UGT)).[18–27] Simultaneous administration with green tea is therefore not recommended for these drugs. However, the impact of green tea on tamoxifen pharmacokinetics remains unclear.

Tamoxifen pharmacokinetics depend on a multi-pathway biotransformation (**Figure 1**).[28] After hepatic uptake by – among others – OATP1B1, the cytochrome P450 iso-enzymes CYP2D6 and CYP3A4 metabolize tamoxifen into the main metabolite endoxifen.[28–31] Endoxifen is ultimately glucuronidated by UGT into an inactive metabolite and excreted through bile and feces.[30] In view of the involvement of drug transporting proteins and metabolizing enzymes, green tea could potentially interfere with the tamoxifen metabolism. Herb-drug interactions with tamoxifen could negatively impact the pharmacokinetic profile, as was previously shown with the combination of tamoxifen and curcumin.[32] Therefore, the primary objective of this study was to evaluate the

possible pharmacokinetic interaction between green tea supplements and tamoxifen. The secondary objective was to assess the safety profile of green tea in combination with tamoxifen.

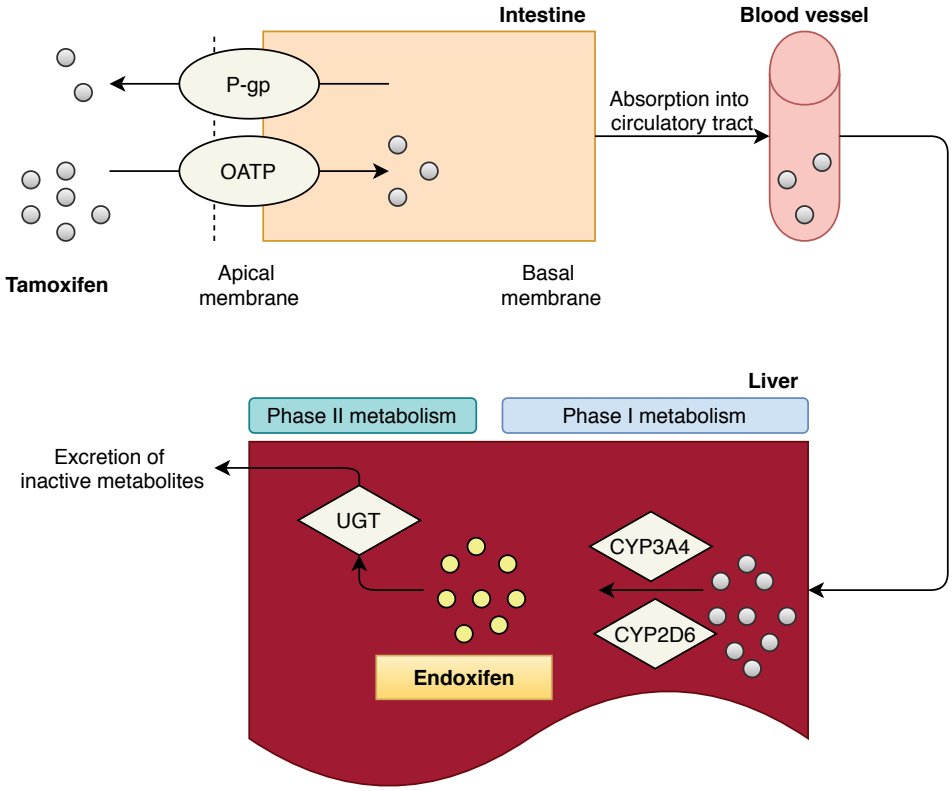

FIGURE 1: Main metabolism pathway of tamoxifen. After absorption tamoxifen is metabolized mainly by CYP2D6 in its active metabolite endoxifen. Tamoxifen relies on phase II metabolism before it can be excreted from the body. Endoxifen is ultimately glucuronidated into endoxifen-glucuronide mainly by UGTs. Several in vitro studies suggest inhibition by green tea of several phase I enzymes (CYP2D6 and CYP3A4) and inhibition of several drug-transporters which the efflux transporter P-gP (ABCB1) and sever influx-transporters like OATP. P-gP, P-glycoprotein; CYP, cytochrome P450; OATP, organic anion transporting polypeptide; UGT, UDP-glucuronosyltransferase.

METHODS

Study design

This single-center, randomized, two-armed, open-label, pharmacokinetic cross-over trial aimed to investigate the endoxifen exposure in breast cancer participants using tamoxifen with or without green tea. The study protocol was written in conformity with the declaration of Helsinki and approved by the local medical ethics committee and registered at the Netherlands Trial Registry (number NL8144). Enrollment took place after written informed consent at the Erasmus University Medical Center, Rotterdam, The Netherlands. Patients with a confirmed histological or cytological diagnosis of primary breast cancer, a World Health Organization (WHO) performance status of ≤ 1 and on tamoxifen treatment at a stable dose of 20 or 40 mg q.d. for at least 3 months (ensuring steady-state concentration) were included. Participant demographics, medical history, CYP2D6 phenotype status and serum biochemistry were assessed before study entry. Participants were excluded if they were CYP2D6 poor or ultra-rapid metabolizers or if they had an impaired drug absorption. Furthermore, all participants were required to abstain from herbal or dietary supplements and strong inhibitors or inducers of CYP3A4, CYP2D6, UGT and P-glycoprotein. Depending on randomization, participants either started with tamoxifen monotherapy (20 or 40 mg q.d.; 10 AM) for 28 consecutive days or tamoxifen and green tea (1000 mg b.i.d.; containing 150 mg of EGCG; 10 AM and 10 PM) concomitantly for 14 consecutive days. This dose of green tea capsules is equivalent to approximately 5-6 cups of regular green tea and is also in line with previous clinical studies. Thereafter, participants received tamoxifen and green tea concomitantly for 14 consecutive days or tamoxifen monotherapy for 28 days, respectively. The green tea capsules were manufactured by a qualified Dutch Pharmacy (NatuurApotheek, Pijnacker, the Netherlands) and the batch was provided with a certificate of analysis for verification of the EGCG content. Participants were hospitalized for 24-hour pharmacokinetic blood sampling on days 14 and 42, after one night of fasting. Blood samples were collected periodically at 13 predefined time points (t=0; 0.5; 1.5; 2; 2.5; 3; 3.5; 4; 6; 8; 12 and 24 h after tamoxifen intake) and after processing to plasma stored at -80 °C until analysis. Plasma samples were analyzed by a validated liquid chromatography tandem mass spectrometry (UPLC-MS/MS) method in accordance with U.S. Food and Drug Administration (FDA) bioanalytical method validation guidelines.[33] Adverse events were graded using the Common Terminology Criteria for Adverse Events version 5.0 (CTCAEv.5, National Cancer Institute, Bethesda, MD, USA).

Pharmacokinetic analysis

A non-compartmental pharmacokinetic analysis of steady-state concentrations was performed using Phoenix WinNonlin version 8.1 (Pharsight, a Certara Company, Princeton, NJ, USA). Main pharmacokinetic parameters including area under the curve ($AUC_{0\text{-}24h}$), maximum observed concentration (C_{max}) and minimum observed concentration (C_{trough}) were constructed by individual plasma concentration-time curves.

Statistical analysis

The main objective of this trial was to compare the concentration of endoxifen with and without green tea supplements by comparing the $AUC_{0–24h}$ between days 14 and 42, where one comparison was made: endoxifen monotherapy versus combined with green tea supplements. A relative difference in $AUC_{0–24h}$ of at least 25% was considered to be clinically relevant and the within-patient deviation was assumed to be 20%. Given a power of 90% and a two-sided alpha of 5%, this resulted in a sample size of 14 evaluable patients (7 in both treatment arms). Analyses of AUC of tamoxifen, and C_{trough} and C_{max} of both endoxifen and tamoxifen were performed on log-transformed observations since these are assumed to follow a log-normal distribution. Estimates for the mean differences in C_{trough} and C_{max} were obtained for one comparison (tamoxifen concomitantly with green tea monotherapy versus tamoxifen monotherapy) separately using a linear mixed effect model treatment with sequence, and period as fixed effects and subject within sequence as a random effect. 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 antilog were taken from the effect estimate and corresponding 95% confidence interval boundaries for the comparisons of tamoxifen concomitantly with green tea versus tamoxifen monotherapy to interpret the results (interpreted as ratios of the geometric means).

RESULTS

Trial participants

Between October 2019 and February 2020, a total of 14 breast cancer patients were enrolled. All participants completed this trial and were evaluable. An overview of baseline characteristics is presented in **Table 1**. Participants were predominantly of Caucasian origin (86%) and were extensive metabolizers of CYP2D6 (79%). All participants were treated with adjuvant tamoxifen in this trial. The vast majority of

patients used tamoxifen in a dose of 20 mg once daily (93%) and one patient used tamoxifen in a dose of 40 mg once daily (7%). In addition, the median duration of tamoxifen use before enrollment in this trial was 11.8 (range 6.0 – 12.9) months.

TABLE 1. Baseline characteristics of evaluable participants (n=14).

Characteristic	**N (%) or median (range)**	
Sex		
Female	14	(100%)
Male	0	(0%)
Age, years	58.5	(50.8 – 68.3)
BMI, $kg \cdot m^{-2}$	27.4	(23.9 – 28.5)
WHO performance status		
0	12	(86%)
1	2	(14%)
Ethnic origin		
Caucasian	12	(86%)
Afro-Caribbean	2	(14%)
CYP2D6 phenotype		
EM	11	(79%)
IM	3	(21%)
Biochemistry		
AST (U/L)	21	(17.8 – 27.0)
ALT (U/L)	15	(11.8 – 21.0)
ALP (U/L)	53.5	(43 – 67)
GGT (U/L)	21	(16.5 – 29.5)
Total bilirubin (µmol/L)	6	(5.3 – 8.5)
Albumin (g/L)	36	(35 – 37)
LD (U/L)	189	(181.5 – 196.5)
Hb (mmol/L)	8.1	(7.7 – 8.3)
Creatinine (µmol/L)	76.5	(71.8 – 87.3)
Previous treatment		
Surgery	14	(100%)
Radiotherapy	9	(64%)
Chemotherapy	3	(21%)
Tamoxifen dose		
20 mg	13	(93%)
40 mg	1	(7%)
Duration of adjuvant tamoxifen use, months	11.8	(6.0 - 12.9)

BMI, body mass index; EM, extensive metabolism; IM, intermediate metabolism; AST, aspartate aminotransferase; ALT, alanine aminotransferase; ALP, alkaline phosphatase; GGT, gamma-glutamyltransferase; LD, lactate dehydrogenase; Hb, hemoglobin.

Pharmacokinetics

Tamoxifen and endoxifen levels were detectable in all collected blood samples. Estimates of main pharmacokinetic parameters for tamoxifen monotherapy versus tamoxifen with green tea supplements are presented in **Table 2**. The individual AUC values for endoxifen and tamoxifen exposure without and with green tea supplements are displayed in **Figure 2 and 3**. The geometric mean of endoxifen $AUC_{0\text{-}24h}$ during concomitant administration of green tea was comparable to tamoxifen monotherapy (746 nmol.h.L^{-1} ; coefficient of variation (CV): 38.6% vs 749 nmol.h.L^{-1}; CV 41.1%). The corresponding relative difference (RD) in endoxifen $AUC_{0\text{-}24h}$ between the cycle with and without green tea was -0.4% (95% CI: -8.6 – 8.5%; p=0.92). Endoxifen geometric means of C_{max} 38.5 nmol/L; CV 37.3% vs 39.6 nmol/L; CV 41.7% and C_{trough} 32.2 nmol/L; CV 34.1% vs 31.9 nmol/L; CV 39.8% also did not significantly differ between with or without green tea.

The plasma pharmacokinetic parameters of tamoxifen showed a clear resemblance in $AUC_{0\text{-}24h}$ with and without green tea (RD 4.1% (95% CI: -6.6 – 16.1%; p=0.44). Likewise, the determined relative difference of tamoxifen C_{max} (RD -2.2% (95% CI: -11.8 – 8.4%; p=0.64) and C_{trough} (RD 6.2% (95% CI: -6.8 – 20.9%; p=0.34) also shared similar results between both treatments. No differences between CYP2D6 phenotype groups and endoxifen exposure was found.

TABLE 2. Main pharmacokinetic parameters of tamoxifen and endoxifen.

PK parameters	Tamoxifen monotherapy[a]	Tamoxifen with green tea[a]	*p*-value	Relative difference (%) (95% CI)
Endoxifen				
$AUC_{0\text{-}24h}$ (nmol·h·L^{-1})	749 (41.1)	746 (38.6)	0.92	-0.4 (-8.6 – 8.5)
C_{max} (nmol/L)	39.6 (41.7)	38.5 (37.3)	0.47	-2.8 (-10.6 – 5.6)
C_{min} (nmol/L)	31.9 (39.8)	32.2 (34.1)	0.77	1.2 (-7.3 – 10.5)
Tamoxifen				
$AUC_{0\text{-}24h}$ (nmol·h·L^{-1})	6867 (26.1)	7150 (22.9)	0.44	4.1 (-6.6 – 16.1)
C_{max} (nmol/L)	401.5 (28.1)	392.6 (25.1)	0.64	-2.2 (-11.8 – 8.4)
C_{min} (nmol/mL)	257.1 (35.6)	273.0 (24.4)	0.34	6.2 (-6.8 – 20.9)

PK, pharmacokinetic; CI, confidence interval; AUC, area under the plasma-concentration time curve; C_{max}, maximum observed concentration; C_{min}, minimum observed concentration.
a = values are geometric mean (% coefficient of variation).

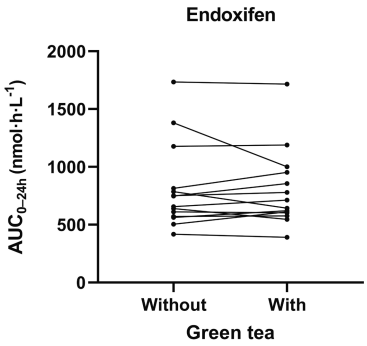

FIGURE 2: Pharmacokinetics of endoxifen without and with concomitant green tea supplements.

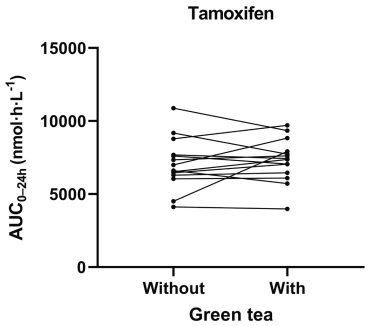

FIGURE 3: Pharmacokinetics of tamoxifen without and with concomitant green tea supplements.

Treatment-related adverse events

An overview of treatment-related adverse events is presented in **Table 3**. Headache, gastro-intestinal side-effects (*e.g.* constipation and dyspepsia) and polyuria were reported more often during the treatment with green tea vs tamoxifen monotherapy. A few changes in liver biochemical parameters (AST, ALT, GGT) occurred during administration with green tea, as well as a creatinine increase and platelet count decrease. Hot flashes were the most reported side-effects, but its occurrence count remained the same independent of green tea consumption. Adverse events were mild and serious adverse events (grade 3 or higher) were not observed during the study period.

TABLE 3. Treatment-related adverse events, graded according to CTCAEv.5

Adverse event	Tamoxifen monotherapy (N)	Tamoxifen with green tea (N)
Grade 1		
General		
Abdominal pain	2	
Headache	2	4
Hot flashes	5	5
Restlessness		1
Gastro-intestinal		
Nausea	1	
Dyspepsia		1
Gastroesophageal reflux		1
Constipation		1
Belching		1
Bloating		1
Urogenital		
Polyuria		3
Irregular menstruation		1
Menorrhagia	1	1
Biochemistry		
ASAT increased		1
ALAT increased		1
GGT increased		1
Creatinine increased		1
Platelet count decreased		2
Grade ≥3	0	0

ASAT, aspartate aminotransferase; ALAT, alanine aminotransferase; GGT, gamma-glutamyltransferase.

DISCUSSION

This randomized, cross-over, pharmacokinetic study clearly demonstrated that green tea supplements did not cause a pharmacokinetic interaction with tamoxifen or endoxifen in breast cancer patients. Therefore, we can conclude that tamoxifen absorption and metabolism were not affected by green tea from a pharmacokinetic point of view. Furthermore, serious or severe green tea related adverse events were not reported during the whole study period.

These results were unexpected as preclinical studies showed that green tea did modify important targets of tamoxifen metabolism (*e.g.* OATP, P-glycoprotein, UGT and CYP enzymes).[23,25–27,34] Several mechanisms for drug interactions resulting in an altered bioavailability or metabolism have been reported, including inhibition of influx- or efflux-transporters and cytochrome P450 enzymes.[18–22] Furthermore, other green tea-drug combinations were previously studied in humans, and significant herb-drug interactions with clinical implications were found.[18,20] Consequently, it was hypothesized that green tea would induce changes in the systemic exposure of tamoxifen and endoxifen, but no differences in endoxifen and tamoxifen exposure between the phase with and without green tea were found in this study.

The non-significant effect is not consistent with the outcomes of a study that reported EGCG (range 3 to 10 mg/kg) significantly altered the pharmacokinetic parameters of tamoxifen in rats.[35] This animal study suggested that EGCG might be effective to obstruct CYP3A4-mediated metabolism and P-glycoprotein mediated efflux pathways in the intestine and liver. However, a lower dose EGCG (0.5 mg/kg) did not significantly alter the metabolite formation of tamoxifen in rats.[35] This phenomenon suggests a dose-dependent effect of EGCG on the pharmacokinetic profile of tamoxifen. In this trial, the EGCG dose used is equivalent to a dose of approximately 4 mg/kg.

In this study a commercially available green tea extract was administered, in what is considered a high, but safe dose for humans (2000 mg green tea per day of which 300 mg is EGCG) and in line with dosages used in previous clinical studies and with what we observe in breast cancer patients in our out-patient clinic. [10,35–39] This EGCG dose is equivalent to approximately about 5-6 cups of green tea. According to the European Food and Safety Association (European agency funded by the European Union) 300 mg EGCG is comparable to the maximum mean daily EGCG intake from the consumption of regular green tea in beverage form.[38] However, it is worth noting that routes of administration other than green tea supplements (*e.g.* green tea beverages) may in theory affect green tea absorption and bioavailability and therefore may affect tamoxifen pharmacokinetics. Therefore, it is possible that green tea beverages show a different bioavailability of EGCG compared with green tea capsules. However a possible interaction with the green tea beverage less likely since similar EGCG levels are likely to be obtained in human plasma. Apparently, administration of green tea capsules influence the phase II metabolism of tamoxifen to a very limited extend.

The main reported adverse events in this trial were headaches, hot flashes, gastro-intestinal toxicity, polyuria and minor abnormalities in liver biochemical parameters. The incidences of headache, polyuria, gastro-intestinal adverse events and minor liver

biochemical disturbances were increased in the green tea phase, whereas abdominal pain was more present without green tea. All reported adverse events during this study were mild (grade 1). Previous studies found similar gastro-intestinal and hepatic adverse events related to the administration of high doses of green tea.[36,37,40] In addition, headache, polyuria and restlessness are well-known side-effects of caffeine, one of the substituents of green tea supplements (140 mg per day, equivalent to approximately 200 mL of filtered coffee). These green tea related adverse events suggest that green tea was sufficiently absorbed, which is important because of its low oral bioavailability.[13,41,42] To ensure adequate green tea absorption, we administered the daily dose in two dosages and patients with known impaired drug absorption were excluded.

In conclusion, this study clearly indicated that tamoxifen and endoxifen pharmacokinetics were not affected by green tea supplements. Concomitant treatment with green tea and tamoxifen was well-tolerated in this real-life breast cancer cohort. Therefore, the use of green tea among breast cancer patients does not have to be actively discouraged by physicians.

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CHAPTER 14

SUMMARY AND GENERAL DISCUSSION

In this thesis pharmacokinetic and pharmacodynamic **drug-drug interactions** (DDIs) and **drug-food interactions** (DFIs) with several commonly prescribed anti-cancer drugs have been addressed. This chapter gives a general overview of this thesis with an in-depth discussion of the individual chapters. Furthermore, recommendations and future perspectives on the management and investigation of these interactions will be presented.

PART I: DRUG-DRUG INTERACTIONS (DDIS)

A drug interaction is defined as the pharmacological or clinical response to the administration or co-exposure of a drug with another substance that modifies the patient's response to the drug.[1] In the case of a drug-drug interaction, the interacting substance is another drug used by the patient. There are several risk factors for experiencing a drug interaction in clinical practice such as age and polypharmacy, but also cancer patients in general are at an increased risk, because they often use multiple drugs as part of their cancer treatment or for the management of comorbidity.[2,3]

Drug interactions intervene with the pharmacokinetic or pharmacodynamic mechanisms of drugs. Pharmacokinetic drug interactions involve the absorption, distribution, metabolism and/or excretion of drugs resulting in altered plasma concentrations, which results in possible wanted (e.g. increased efficacy) or unwanted effects (e.g. increase of side-effects). Pharmacodynamic drug-interactions occur when two drugs have similar molecular targets, which may result in an increase in toxicity or response to these agents.[1,4] In **Chapter 2** the drug-drug interaction potential of 29 small molecule kinase inhibitors (SMKIs), including the tyrosine kinase inhibitors (TKIs) is described. The main interaction categories are pH-dependent drug-interactions, drug-transporter interactions and interactions with drug metabolism. Many SMKIs show pH-dependent solubility resulting in a decreased plasma exposure when co-administered with acid-suppressive medication like proton pump inhibitors (PPIs). For example gefitinib, a TKI used in the treatment of non-small cell lung cancer (NSCLC), shows a decrease in plasma exposure of 47% when co-administered with the PPI lansoprazole or esomeprazole.[5] However, for many TKIs like tivozanib and ruxolitinib, clinical data is missing and the current advice regarding these interactions is only based on chemical (Pka, acid-dissociation constant) data. Also for drug-transporter interactions clinical data is frequently missing. These interactions may have a major impact on drug pharmacokinetics as is the case for the SMKIs lapatinib and vemurafenib (P-gp inhibitors), regorafenib (BCRP-inhibitor) and nintedanib (a P-gp substrate). Finally, SMKIs are prone to drug interactions involving drug metabolism, which gives

either inhibition or induction of phase I (cytochrome P450 enzymes) or phase II drug metabolism (e.g. glucuronidases and sulfatases). Especially bosutinib, cobimetinib, crizotinib, gefitinib, ibrutinib, and nilotinib are highly prone for clinically relevant drug interactions regarding drug metabolism. In conclusion, most SMKIs are prone for DDIs, which may result in an altered systemic exposure. Taken into account that about 34% of all the treated patients is underdosed during treatment,[6] it is important to accomplish optimal individual exposure to SMKIs and to provide the right dose for each individual patient. Therefore, clinicians always need to pay attention to DDIs since they may have a major impact on drug pharmacokinetics and thus efficacy and/or toxicity. One of the possible solutions for the management of these interactions is the use of therapeutic drug monitoring (TDM).[7,8] TDM has proven its additional value for several drugs like tamoxifen and pazopanib, where a significant recurrence benefit was found when drug concentrations were above a certain threshold.[9,10] Overall, there is lack of decent clinical drug interaction studies with SMKIs, especially for drug-transporter interactions and interactions with acid-suppressive medication. Therefore, it is necessary to perform more clinical studies to better understand the underlying mechanisms and advise both clinicians and patients.

There is some evidence suggesting a survival benefit for patients treated with SMKI monotherapy compared to SMKI therapy concomitantly taken with a PPI. For example, Mir *et al.* [11] found a significant difference in median overall survival (OS) in patients treated with pazopanib using acid-suppressive agents compared to patients without an acid-suppressive agent of 8.0 vs 12.6 months (HR, 1.81; 95% CI, 1.31–2.49; P < 0.01), with duration of treatment with acid-suppressive agents as a significant risk factor for a worse outcome. There are other examples of anti-cancer drugs besides the SMKIs, which also show a decrease in survival when treated with acid-suppressive agents. For example, Chu *et al.*[12] demonstrated a significant survival difference for capecitabine, an oral chemotherapeutic agent used in the treatment of colorectal cancer among others, when co-administered with PPIs compared to capecitabine without PPIs. They found that patients treated with a PPI had a worse progression free survival (PFS) (HR: 1.68; 95% CI, 1.42-1.94; P < 0.001) and overall survival (HR: 1.41; 95% CI, 1.11-1.71; P = 0.001) compared to capecitabine without a PPI suggesting a significant DDI between capecitabine and PPIs. However, **Chapter 3** describes many remarks to this study by Chu *et al*. PPI use was defined as 20% or more overlap between PPI prescription and trial treatment duration. However, total PPI duration was not taken into account in the primary analysis. Furthermore, both PPI dose and type of PPI were not taken into account, even though there is a significant difference in acid suppression among various PPI variants and also the PPI dose is positively correlated with both magnitude and duration of gastric acid suppression.[13,14] Moreover, time of intake of

both capecitabine and PPIs was not taken into account, which is of clinical relevance since PPIs reach their maximum pH elevating effects 2-4 hours after administration and their pH-elevating effects change during the day.[15] Because of the complex mechanism involved in this interaction, there is a need for a more standardized research method for the investigation of a possible drug-interaction with PPIs.

An example of a new method to investigate a potential drug-interaction with PPIs is provided in **Chapter 4** of this thesis. Regorafenib, a multi-kinase inhibitor used in the treatment of several tumor types such as colorectal carcinoma and hepatocellular carcinoma, has a predicted pKa of around 2, suggesting a minor influence of the gastrointestinal pH on the absorption of the drug.[16-18] In this pharmacokinetic cross-over study, fourteen patients were consecutively treated with regorafenib monotherapy, regorafenib concomitantly with esomeprazole, and regorafenib three hours after esomeprazole intake (in this order or *vice versa*). In this study, no difference in regorafenib plasma area under the curve ($AUC_{0\text{-}24h}$) was found for regorafenib monotherapy compared with concomitant esomeprazole (relative difference: −3.9%; 95%CI: −20.5 to 16.1%; P = 1.0) or compared with regorafenib with esomeprazole three hours prior to regorafenib intake (relative difference: −4.1%; 95% CI: −22.8 to 19.2%; P = 1.0). These findings indicate that regorafenib can be safely combined with PPIs at all timepoints. This study demonstrates that this study design can serve as a template for future studies investigating the influence of PPIs on the pharmacokinetics of anti-cancer agents. This was not unexpected since regorafenib exhibits a low solubility in general, which is due to its complex chemical structure where no acidic or basic group is attached.[18] As esomeprazole was used, which is the strongest pH-elevating PPI,[15,19] we expect these findings to be generalizable for other PPIs. Nevertheless, results of this study are not by definition applicable to other acid-suppressive compounds. Esomeprazole is a known inhibitor of CYP2C19, but it is not known to induce or inhibit drug-transporters or enzymes involved in the pharmacokinetics of regorafenib, making a potential DDI most likely caused by an alteration in stomach pH. For example H2-receptor antagonists, such as pantoprazole may still cause a DDI with regorafenib, since regorafenib is a substrate for P-gp (ABCB1) and pantoprazole inhibits this drug-transporter.[20] By inhibition of this transporter, a significant alteration in regorafenib plasma exposure may still occur.

Unlike interactions at the level of drug absorption, there is more clinical evidence regarding drug metabolism interactions, i.e. interactions that involve phase I or phase II metabolism. Most drugs are metabolized in the liver by enzymes of the cytochrome P450 system of which CYP3A4 usually is the most important. Many anti-cancer agents, such as the taxanes (e.g. docetaxel and cabazitaxel) display CYP3A4-dependent metabolism

in the liver and are thus prone for DDIs with drugs that induce or inhibit the CYP3A4 enzyme. For example enzalutamide, used in combination with taxanes in the treatment of metastatic castration-resistent prostate cancer (mCRPC), amongst others decreases the $AUC_{0\text{-}24h}$ with 22% for cabazitaxel compared to cabazitaxel monotherapy.[21] Another taxane, which is often prescribed in the treatment of metastatic prostate cancer, is docetaxel.[22] Two large trials investigated the value of docetaxel in the hormone-sensitive setting; one trial used docetaxel in combination with prednisone and one trial used docetaxel without prednisone.[23,24] Both trials showed a similar overall survival benefit of 13.4 and 15.0 months respectively, which raised the question whether prednisone could be removed from the regular docetaxel treatment. Furthermore prednisone, like many corticosteroids, is a mild CYP3A4 inducer and therefore may decrease docetaxel exposure.[25,26] In **Chapter 5** our cross-over study in 18 patients with metastatic hormone-sensitive prostate cancer showed similar docetaxel concentrations with ($AUC_{0\text{-}inf}$ 2784 ng*h/mL, 95%CI: 2436-3183 ng*h/mL) or without ($AUC_{0\text{-}inf}$ 2647 ng*h/mL, 95%CI: 2377-2949 ng*h/mL) concomitant prednisone treatment. Furthermore, no difference in toxicity between the treatment regimens was observed. Therefore it can be stated that, from a pharmacokinetic point of view, prednisone can be safely removed from the treatment with docetaxel in patients with mCRPC. The major benefit of administering docetaxel without prednisone is the prevention of long-term side-effects of prednisone such as osteoporosis and adrenal insufficiency.[27,28] However, this study was only designed to detect a difference in pharmacokinetics and therefore more research is needed to detect a possible difference in toxicity or survival. In the literature there is an ongoing debate about the utility of prednisone, since prednisone may have an anti-tumor effect by itself and decreases the PSA level in about 15% of the patients.[29] Furthermore prednisone is believed to reduce taxane-induced toxicity such as fatigue.[30] However a clear survival benefit from the addition of prednisone is not yet found, when combined with chemotherapy in mCRPC patients.[23,31] Additionally, no difference in toxicity was observed when treating patients with or without prednisone and also long-term prednisone use is thought to promote resistance mechanisms to taxane therapy. [23,32]

A narrow therapeutic window can increase the risk of a significant DDI, especially when this applies to both interacting drugs. SMKIs, for example, are known to have a small therapeutic window and an increase or decrease in plasma exposure may therefore have a significant impact on both therapy efficacy and patient wellbeing.[33] An example of a clinical situation where this balance is very delicate, is the oncological treatment of transplanted patients with SMKIs and immunosuppressant drugs (e.g. tacrolimus). Immunosuppressant drugs often show similar pharmacokinetic pathways as several anti-cancer drugs among which the SMKIs and are highly prone to clinically

relevant drug interactions.[34] For instance, patients with hepatocellular carcinoma (HCC) sometimes undergo liver transplantation as an initial treatment for their HCC, after which immunosuppressant therapy is started.[35] Unfortunately, 20% of these patients develop an HCC recurrence in the transplanted liver and need systemic anti-cancer treatment.[36] In the case of a recurrence, patients are often treated with sorafenib, which may lead to severe toxicity (e.g. rash, hypertension) despite the known survival benefit.[37,38] Sorafenib, similar to most immunosuppressants, is mainly metabolized by CYP3A4 into several metabolites, which increases the risk for a DDI since both drugs are a substrate of the same enzyme. **Chapter 6** describes four patients who used sorafenib in combination with immunosuppressant drugs. We found that the sorafenib concentration decreased over time when combined with immunosuppressant drugs, without major effects on tacrolimus plasma levels. Treatment of patients with HCC recurrence after liver transplantation remains controversial, since there is a clear lack of knowledge in managing this interaction. An explanation for the interaction is auto-induction of CYP3A4 by sorafenib, which results in an increased sorafenib metabolism over time. This principle was shown for imatinib and may apply to sorafenib as well.[39,40] This study offers the first evidence, although it has to be confirmed in a larger clinical study, that combining immunosuppressant drugs with sorafenib decreases sorafenib plasma exposure over time. Interestingly, all patients in this study experienced severe side-effects when administered more than 400 mg of sorafenib daily, probably due to a pharmacodynamic DDI between sorafenib and tacrolimus. Since side-effects are more prone in patients with a liver transplantation, starting with a dose of 200 mg twice a day seems a better and safer option in these patients than the regularly advised dose in non-transplant patients.[41]

As described in Chapter 2 of this thesis, drug transporters are important in the pharmacokinetics of many drugs among which the SMKIs. Drug transporters are divided in solute carrier (SLC) and ATP-binding cassette (ABC) transporters.[42] These transporters are involved in either the uptake (e.g. OCT; SLC22A and OATP; SLCO) or efflux (e.g. P-glycoprotein; ABCB1 and BCRP; ABCG2) of drugs in and out of the cell.[42,43] Many SMKIs are substrate for specific drug transporters and are therefore prone to drug-transporter interactions. For example nintedanib, a TKI which is exclusively metabolized by phase II enzymes, shows a 61% increase in plasma exposure, when treated concomitantly with the strong P-glycoprotein (P-gp) inhibitor ketoconazole. Furthermore, treatment with rifampicin, a strong P-gp inducer, decreased nintedanib plasma exposure by 50%.[44] Similar to nintedanib, sorafenib is also prone for interactions with drug-transporters, such as inhibition of OATP1B by rifampicin, which results in an increase in sorafenib-glucuronide plasma levels in mice.[45] Besides the advantage of sorafenib therapy on survival, it also knows many side-effects of which hand-foot skin

reaction (HFSR) is the most common and debilitating.[37,46] HFSR is a painful complication of the hands and feet in which hyperkeratotic plaques develop predominantly over sites of pressure or friction, which occurs in 20-40% of sorafenib-treated patients.[47,48] There is currently no good treatment option for HFSR except for dose reduction or cessation of sorafenib therapy. Zimmerman *et al.*[49] found that accumulation of sorafenib in keratinocytes is the possible mechanism behind HFSR in mice. The uptake of sorafenib is facilitated by the OAT6 (SLC22A20) transporter and inhibition of this transporter with probenecid, a drug used in the treatment of gouty arthritis, resulted in prevention of the HFSR. However probenecid is a known pan-UGT inhibitor and may therefore alter sorafenib pharmacokinetics, since sorafenib is metabolized by UGT1A9. In **Chapter 7** we investigated the influence of probenecid on sorafenib pharmacokinetics and toxicity in patients treated with sorafenib. Probenecid decreased the sorafenib $AUC_{0\text{-}12h}$ 26.8% (90%CI: -37.7% to -14.1%; $P < 0.01$). Furthermore peak concentrations (-25.1%, 90%CI: -44.3% to -19.7%; $P < 0.01$) and trough levels of sorafenib (-26.0%, 90%CI: -43.4% to -3.4%; $P < 0.01$) decreased. Moreover, sorafenib concentrations in keratinocytes decreased in the presence of probenecid with 28.1% (90%CI: -46.3% to -3.7%, $P = 0.07$). Unfortunately, there was no clear difference in skin toxicity between sorafenib monotherapy and sorafenib concomitantly with probenecid. Nonetheless, this study demonstrates that probenecid is likely to alter sorafenib concentrations, due to OATP1B1 inhibition, which alters the hepatic circulation of sorafenib and therefore changes systemic sorafenib concentration. These results offer probenecid as a new inhibitory agent for clinical interaction studies.[50,51] In short, both systemic and cutaneous sorafenib exposure decreased proportional during concomitant probenecid administration, which may have been caused by interruption of enterohepatic cycling via OATP1B1 inhibition.

Besides pharmacokinetic DDIs there are also pharmacodynamic DDIs. A well-known example of the latter interaction is prolongation of the QTc-interval, which is associated with sudden cardiac death and (fatal) arrhythmias.[52] The suggested mechanism of drug-induced QTc-interval is the inhibition of the potassium channel encoded by the human ether-a-go-go related gene (hERG). Inhibition of this potassium channel leads to a delay in ventricular repolarization and therefore a prolongation of the QTc-interval.[52,53] Prolongation of the QTc-interval is one of the most common causes of cessation of therapeutic use of drugs that have already been marketed.[52] There are many drugs known to prolong the QTc-interval among which the (selective) serotonin reuptake inhibitors, which are frequently used by (cancer) patients for the treatment of depressive symptoms.[54,55] Another drug that is known to prolong the QTc-interval, is tamoxifen.[56,57] Tamoxifen is a selective estrogen receptor modulator (SERM) used in the adjuvant treatment of breast cancer. Tamoxifen inhibits the estrogen-dependent

proliferation of breast cancer cells and therefore reduces the risk of disease recurrence and mortality. Combining drugs that prolong the QTc-interval may result in a synergistic prolongation of the QTc-interval with an increased risk of cardiac side-effects as a consequence.[58] **Chapter 8** presents an observational study in 50 patients using tamoxifen monotherapy and 50 patients using tamoxifen in combination with a serotonin reuptake inhibitor (SRI). In our study, concomitant use of tamoxifen with SRIs significantly prolonged the mean QTc-interval with 12.4 ms (95%CI 1.8 to 23.1 ms; P = 0.023) when analyzed with the Fridericia formula. This prolongation was most prominent for patients using paroxetine, escitalopram or citalopram. Taken into account the pharmacokinetic drug interaction profile of both tamoxifen and SRIs, it should be advised to administer tamoxifen concomitantly with venlafaxine or fluvoxamine, which are not likely to result in QTc-interval prolongation. In contrast to other SRIs, these agents do not influence tamoxifen pharmacokinetics.[59] In the general population there is a large interindividual variability of the QTc-interval making it hard for physicians to determine the clinical relevance of the QTc-interval prolongation on a single ECG.[60] In the case of the combination of tamoxifen and SRIs like venlafaxine and fluvoxamine, monitoring of the ECG is not warranted, since the additional prolongation of the QTc-interval is only minor. However, when using SRIs like paroxetine and escitalopram, monitoring is adviced since the prolongation of the QTc-interval may approach clinical significance. Furthermore, there are many individual risk factors like polypharmacy and age, that increase the risk of clinically relevant QTc-prolongation and explain the large interindividual variability in patients.

In conclusion, multiple DDIs are of major clinical relevance. In daily clinical oncology practice DDIs are common and need to be investigated further since sufficient clinical data is frequently lacking.

PART II: FOOD-DRUG INTERACTIONS (FDIS)

Besides co-medication there are many other factors that can influence the pharmacokinetics of drugs, such as lifestyle and food. Nowadays there is an increasing trend in the use of complementary and alternative medication (CAM) as a treatment strategy. This especially applies to cancer patients who use CAM increasingly more often as an alternative for the treatment of cancer and the complaints of both the disease and therapy-related side-effects.[61] In total 48-88% of all cancer patients use alternative medication or food (supplements) next to their regular anti-cancer therapy.[61,62] Besides the possible anti-cancer effects of food and supplements, these substances can also have a significant impact on the pharmacokinetics of several drugs and may therefore

deprive patients from optimal therapy. This is for instance the case for St. John's wort, a herb used for treatment of depressive symptoms. The intake of this herb decreased the plasma levels of the active metabolite of irinotecan (SN-38) , a chemotherapeutic agent used in several tumor types among which colorectal carcinoma, by 42% (95%CI: 14%-70%) by alteration of irinotecan metabolism.[63] Food and herbs can also alter drug absorption. A well-known example is the influence of a fat meal on the absorption of the chemotherapeutic agent abiratarone, which is used in the treatment of prostate cancer. Administration of abiratarone with a high-fat meal resulted in a 10-fold increase in abiratarone plasma exposure compared to abiratarone intake without food.[64] **Chapter 9** gives an overview of known food interactions with SMKIs. We found that many SMKIs show a significant increase in both maximum concentration (C_{max}) and AUC. For example, lapatinib showed an increase in the AUC of 100%-325% when administered with a high-fat meal compared to intake of lapatinib on an empty stomach. Furthermore, some food substances like grapefruit juice (an inhibitor of intestinal and hepatic CYP3A4) can alter SMKI exposure in a significant extent due to an interaction with TKI metabolism. Coadministration of grapefruit juice with ibrutinib showed an increase in AUC of 115% compared to ibrutinib with water.[65] Additionally, intake with other beverages like green tea can alter SMKI pharmacokinetics as was shown for erlotinib, lapatinib, and sunitinib in rats resulting in a decrease in AUC of these drugs ranging from 51-74%.[66,67] Besides food substances, herbs can also alter metabolism of SMKIs with St. John's wort being the most familiaras was mentioned earlier.[68] Therefore it is advised to abstain from St. John's wort when using SMKIs in general. This review also found that the advices of the European Medicines Agency (EMA) and the Food and Drug Administration (FDA) were not always consistent, making the management of these interactions challenging for clinicians. A more uniform advise is necessary when interpreting a possible FDI with SMKIs and is presented in this review. It is difficult to estimate the impact of a particular diet of an individual patient on the pharmacokinetics of anti-cancer drugs since the interpatient variability is high. When an SMKI is advised to be administered without food, patients have strict dietary instructions. However, about 21% of the patients treated with a SMKI does not always follow these instructions resulting in altered pharmacokinetics of the SMKIs in these patients compared to patients who comply to the dietary instructions.[68] This lack of compliance could potentially lead to a significant alteration in plasma pharmacokinetics as was shown for erlotinib, as missing a concomitant meal led to a decrease in erlotinib AUC of 15%.[69] Moreover, individual SMKI variability is high and many other factors may influence its exposure. Besides the negative effects of intake with food, food can also

be used for food-dependent dose individualization. By taking SMKIs with food, some SMKI plasma levels increase, due to which the dose of these SMKIs can be lowered, which has many other advantages such as a reduction in drug costs simultaneously.

In chapters 2 to 4 of this thesis the DDIs of drugs with PPIs were already described. An SMKI which is known for its interaction with PPIs and other gastric acid suppressive agents, is erlotinib. Co-administration with PPIs results in a significant decrease in erlotinib plasma levels and ultimately reduces therapy efficacy.[-12, 70, 71] However, many patients have an indication for the use of PPIs and cannot stop PPI therapy. A practical way to bypass this interaction is the intake of erlotinib with an acidic beverage to temporarily lower stomach pH and increase erlotinib absorption, as the TKI will dissolve better in this acidic environment. An acidic beverage which is frequently used in the modern society, is cola. The brand Coca-cola has a pH-value of 2.5 and can therefore act as a pH-lowering beverage. In patients using ketoconazole, the intake of ketoconazole with cola resulted in a significant increase in the AUC of ketoconazole.[72] In **Chapter 10** we investigated the concomitant intake of erlotinib with Coca-Cola with and without a PPI in 28 patients. When erlotinib and esomeprazole were administered with Coca-Cola instead of water, this resulted in a significant and clinically relevant increase of 39% (95%CI: −12% to +136%; P = .004) in the $AUC_{0\text{-}12h}$ of erlotinib. This means that it partly corrects the negative effect of PPIs on the erlotinib AUC. This study therefore offers a practical way of using a beverage to improve erlotinib therapy in patients using a PPI. However, the almost 50% decrease in plasma levels of erlotinib with concomitant use of a PPI can also be bypassed using separate intake times of the PPI and erlotinib as was suggested in chapter 2 of this thesis.[73] This suggestion also bypasses the disadvantages such as irritation of the stomach and long term side-effects as dental problems that adding an acidic beverage offers to the treatment with an SMKI.[74,75]

In **Chapter 11** a healthier way of managing the interaction between erlotinib and PPIs is presented. Erlotinib, as most TKIs, knows a high solubility in a fatty environment as was also discussed in Chapter 9 of this thesis. Therefore, a fatty beverage may also increase the erlotinib absorption to bypass the interaction with PPIs. In Chapter 11, erlotinib was administered in 29 patients with either cow's milk or water, both with and without a PPI. High-fat cow's milk has proven to significantly interact with drugs depending on various mechanisms. For example, several antibiotic drugs (e.g. minocycline and tetracyclin) show a significantly decreased absorption, due to calcium binding of the drugs resulting in a decrease in plasma exposure.[76] We found no significant difference in plasma pharmacokinetics of erlotinib when it was combined with high-fat cow's milk (both with and without PPIs). Based on the results of this study, we can conclude that erlotinib intake with milk is safe and well-tolerated, but not sufficient to bypass the

decrease in plasma exposure when combining erlotinib with PPIs. When erlotinib was taken with a fatty meal, the AUC increased with 33-66% indicating a moderate FDI. High-fat cow's milk has a caloric content of 68 kcal/ 100 mL and contained 3.9 g of fat per 100 mL. Patients took erlotinib with a total of 170 kcal in this study, whereas a standardized high-fat meal contains 800-1000 kcal.[77] Since the total amount of calories and fat is (much) lower compared to a standardized high-fat meal, this could explain the absence of a significant interaction.

Besides FDIs at the absorption phase, there are many other substances (e.g. grapefruit juice and St. John's wort) that can alter drug pharmacokinetics due to an interaction with drug metabolism. The use of food (supplements) and herbs is very popular among cancer patients, and curcumin is probably one of the most popular herbs, especially among breast cancer patients.[61,62] This herb is derived from the root of the *curcuma longa* plant and is believed to have anti-tumor effects.[78,79] However, curcumin can also alter pharmacokinetics of drugs as was shown by Cho *et al.*[80] in rats using tamoxifen. Tamoxifen acts as a prodrug and needs to be metabolized into several active metabolites of which endoxifen is the most important one.[81] Metabolic enzymes involved in tamoxifen metabolism are mainly CYP2D6 and CYP3A4. Next, UDP-glucuronosyltransferase (UGT) enzymes inactivate endoxifen into endoxifen-glucuronide.[81,82] This complex metabolic route makes tamoxifen prone for drug interactions as was shown previously with e.g. several SSRIs and rifampicin.[55,83] In **Chapter 12** the FDI between curcumin and tamoxifen was investigated in 16 patients. Compared to tamoxifen alone, we found a significant decrease in endoxifen AUC_{0-24h} of 7.7% (95%CI: −15.4 to 0.7%; P = 0.07) with curcumin and a decrease of 12.4% (95%CI: −21.9 to −1.9%; P = 0.02) with curcumin and piperine (a bio-enhancer used to increase curcumin plasma concentration). These effects were most prominent in patients with an extensive (normal) functioning metabolizing CYP2D6 enzyme. The decrease in AUC seems relatively small, but taking into account that a large part of patients is under or just marginally above the therapeutic threshold of endoxifen, using curcumin may still result in subtherapeutic endoxifen concentrations.[9,84] This study emphasizes that the concurrent use of additional food or herbs is not by definition safe. The effect of curcumin on the exposure of endoxifen may be explained by a decreased absorption in the gut resulting in ultimately lower endoxifen plasma levels, but decent clinical evidence is not yet available.[85] Moreover, a difference was seen in patients with an extensive CYP2D6 metabolism phenotype and an intermediate metabolism phenotype. This difference can only partly be explained by the inhibitory potential of curcumin on CYP2D6[86], since CYP2D6 inhibition only results in a decrease in endoxifen concentrations in patients with a normal functioning CYP2D6 phenotype. More research is needed to determine

the exact mechanisms behind the effects of curcumin on the pharmacokinetics of tamoxifen. Meanwhile, patients should be discouraged to take curcumin supplements next to their tamoxifen therapy.

Another substance used extensively by cancer patients is green tea, which can be administered as either a hot drink, or in high concentrations as a capsule. Green tea is believed to have anti-cancer effects resulting from catechins, a class of flavonoids that exert potent antioxidant activity, of which (-)-epigallocatechin-3-gallate (EGCG) has the highest antioxidant and thus anti-cancer potential.[87,88] However, in contrast, several flavonoids such as green tea may cause significant unwanted FDIs as was shown for the beta blocker nadolol, where co-administration with green tea resulted in a huge decrease in nadolol plasma concentration of 85%.[89,90] In **Chapter 13**, we studied the effects of green tea capsules on tamoxifen and endoxifen concentrations in a cross-over design in 14 patients who were on steady state tamoxifen. We found no significant effect in endoxifen plasma concentration suggesting that administration of tamoxifen with green tea is safe from a pharmacokinetic point of view. We were unable to demonstrate a meaningful effect probably due to the lack of clinically relevant inhibition of phase I and II metabolizing enzymes. The effect on nadolol pharmacokinetics was probably due to OATP1B inhibition, which is a drug-transporter with no effect on tamoxifen or endoxifen pharmacokinetics. In general, the results in literature regarding green tea are in contradiction with these findings.[89] There are several preclinical studies in cell cultures and mice that suggest an inhibitory effect on several pharmacokinetic levels, among which the phase I and II metabolizing enzymes.[89,91] However, these effects could not be demonstrated in human subjects in this study. This underlines the need for clinical studies to investigate the magnitude of a certain interaction.

CONCLUSIONS AND IMPLICATIONS

Over the years, treatment of cancer has developed rapidly with a raise in treatment options and treatment strategies. It is important to ensure an optimal treatment for every individual patient by using personalized treatment strategies, where ideally individual patient characteristics should be taken into account. As mentioned thoroughly in this thesis, there are many factors that may interfere with anti-cancer therapy through alteration of drug pharmacokinetics, making DDIs and FDIs major influencing factors in daily oncology practice since they can influence anti-cancer drug pharmacokinetics and therefore efficacy and patient well-being.[1,2] Drug interactions are responsible for 20-30% of all the adverse reactions to drugs and account for up to 5% of the total hospital admissions.[1,92] These interactions may lead to an altered drug exposure based

on several mechanisms – as discussed in this thesis – usually leading to either a decline in therapy efficacy or an increase in side-effects. Most interactions take place during either the absorption phase, or the metabolism phase of an anti-cancer drug. There is a substantial amount of evidence about the interaction with strong inhibitors or inducers of metabolizing CYP enzymes and absorption interactions with high-fat meals. However, there is a clear lack of decent clinical research in the field of other drugs or substances, like compounds that can inhibit or induce drug transporters. The exact role of drug transporters is not fully determined, but is nevertheless considered to be of major clinical relevance.[42] Drug transporters are located throughout the body and may have an influence in different ways. They can act as a barrier mechanism preventing drugs from entering organs such as the brain and they can even cause drug-resistance, because drug efflux transporters such as P-gp may be upregulated in tumor cells.[93]

The inhibition or induction of drug transporters on the exposure and pharmacokinetics of anti-cancer agents may potentially be an even larger clinical problem than interactions with drug metabolism (in terms of side-effects and efficacy). because of the wide spread of these drug-transporters in the body. Nevertheless, as there is only a limited number of clinical studies investigating these interactions, more research is definitely needed to better understand and predict the impact of inhibition or induction of these transporters on drug pharmacokinetics. Drug interactions can sometimes also be beneficial. For example, co-administration of a P-gp inhibitor can increase the sensitivity of a drug-resistant tumor and therefore improve the response of a patient to the particular anti-cancer agent.[94,95] Furthermore, inhibition of specific transporters involved in the blood-brain barrier can alter the uptake of a drug in the brain, which leads to potentially better treatment of brain metastases and tumors.[96,97]

The most often implemented form of DDI research used for the registration of drugs, is inhibition of several metabolizing enzymes with strong CYP3A4 inhibitors or inducers (i.e. ketoconazole or rifampicin). However, clinical data is lacking for moderate or mild inhibitors and inducers or the interaction potential of several substances like herbs used by patients, even though these still could have clinically relevant effects. Currently, a trial aimed at exploring such an interaction is ongoing regarding the effects of the moderate CYP3A4 inhibitor erythromycin on the pharmacokinetics of palbociclib in cancer patients (Dutch Trial Registry number NL7549). Furthermore, it is important to acknowledge the fact that patients want to treat their cancer by using alternative medication or herbs. Nowadays, there are many (new) interesting substances that are becoming more and more popular under cancer patients such as cannabis. To better advice patients in clinical practice it is important to keep investigating possible interactions with these substances.

In conclusion, drug-drug and food-drug interactions are of major clinical relevance, but more research is needed to better understand the mechanism and impact of these interactions on patients quality of life and therapy.

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PART III

APPENDICES

APPENDIX 1. NEDERLANDSE SAMENVATTING

In dit proefschrift worden verschillende onderzoeken beschreven over de wisselwerking tussen anti-kankermedicatie en (veelgebruikte) andere medicatie en voedingsmiddelen. In deze onderzoeken wordt gekeken naar de manier waarop het lichaam omgaat met het geneesmiddel ('farmacokinetiek'), dan wel naar de manier waarop het geneesmiddel een effect heeft op het lichaam of de tumor ('farmacodynamiek'). Binnen de geneesmiddelenleer, ook wel de farmacologie genoemd, bepalen deze twee onderdelen de uiteindelijke effecten van een geneesmiddel, zowel gewenst (een betere werking van je therapie) als ongewenst (meer bijwerkingen). Het overkoepelende doel van alle deelonderzoeken in dit proefschrift is om eventuele wisselwerkingen te identificeren waarmee de bijwerkingen (toxiciteit) van een medicijn kunnen worden geminimaliseerd of de effectiviteit van de behandeling kan worden verbeterd. Op die manier kan de behandeling van kanker verder worden geoptimaliseerd.

Deel 1: Wisselwerking tussen anti-kankermedicatie en comedicatie

De behandeling van kanker is in de afgelopen decennia drastisch verbeterd, wat heeft geleid tot een langere levensverwachting voor patiënten met kanker. Doordat de overleving toeneemt, neemt echter ook het aantal patiënten met langetermijnklachten van de kanker (of de behandeling van de ziekte) toe. Hierdoor gebruiken kankerpatiënten vaak meerdere soorten medicijnen tegelijkertijd, wat de kans op een wisselwerking (of interactie) enorm vergroot. Deze wisselwerkingen kunnen zowel voordelig als nadelig werken.

Een wisselwerking vaak invloed op de farmacokinetiek van een middel. De farmacokinetiek bestaat uit 4 stappen: 1) de opname of absorptie van een medicijn uit het maagdarmkanaal, 2) de verdeling of distributie van een medicijn via de bloedbaan, 3) het metabolisme; oftewel de verwerking c.q. afbraak van een medicijn (meestal door de lever) en 4) de uitscheiding van een medicijn via de gal en/of de urine. Medicatie heeft vaak een invloed op de absorptie dan wel het metabolisme van een anti-kankermedicijn. In **hoofdstuk 2** is een uitgebreid overzicht beschreven van alle bekende wisselwerkingen van de zogenaamde small-molecule kinase inhibitors (SMKI's) met andere medicatie. SMKI's worden gebruikt in de behandeling van verschillende soorten kanker, zoals long- en darmkanker. Deze middelen grijpen in op verschillende processen in de kankercel, waardoor deze niet meer goed kan functioneren en uiteindelijk doodgaat. Deze anti-kankermiddelen worden via de mond (oraal) ingenomen en moeten dus alle vier de stappen van het farmacokinetische proces doorlopen. Hierdoor lopen SMKI's,

vergeleken met intraveneus toegediende anti-kankermiddelen, dan ook een grotere kans op wisselwerking met andere middelen, zoals ook blijkt uit het uitgebreide overzicht in **hoofdstuk 2.**

Een bekend voorbeeld van een wisselwerking in de absorptiefase heeft betrekking op maagbeschermers, zoals proton pomp remmers (PPI's). Deze middelen worden door ongeveer een derde van alle kankerpatiënten gebruikt voor de behandeling van verschillende klachten zoals maagzuurbranden. PPI's zorgen ervoor dat de maaginhoud minder zuur wordt, waardoor het maagzuur minder irritatie en pijn geeft. Sommige geneesmiddelen zoals de SMKI's hebben echter een zure omgeving van de maag nodig om goed en volledig op te lossen. Als maagbeschermers ervoor zorgen dat de maag minder zuur wordt, kan het zijn dat de SMKI's minder goed oplossen en daardoor uiteindelijk ook minder goed worden opgenomen in het bloed. Voor de SMKI dasatinib kan dit bijvoorbeeld zelfs tot meer dan een halvering van de opname leiden. Wanneer minder van het anti-kankermedicijn in het bloed terechtkomt, leidt dit mogelijk ook tot een mindere werking van het medicijn. Patiënten die het anti-kankermiddel capecitabine, dat o.a. wordt gebruikt voor de behandeling van darmkanker, tegelijkertijd innemen met een maagbeschermer leven bijvoorbeeld minder lang dan patiënten die geen maagbeschermer gebruiken. In **hoofdstuk 3** is onze reactie op dit onderzoek met capecitabine en maagbeschermers beschreven, met daarin een opsomming van enkele tekortkomingen van deze studie. Tevens is een voorstel voor een nieuwe onderzoeksmethode naar deze wisselwerking beschreven. Deze nieuwe onderzoeksmethode is vervolgens toegepast in het onderzoek dat is beschreven in **hoofdstuk 4** van dit proefschrift. Zoals gezegd zijn SMKI's over het algemeen erg gevoelig voor de wisselwerking met maagbeschermers. Een SMKI, waar dit nog niet voor was uitgezocht bij mensen is regorafenib. Dit is een relatief nieuwe SMKI die wordt gebruikt voor de behandeling van onder andere darm- en leverkanker. In het onderzoek hebben we gekeken of de PPI esomeprazol invloed heeft op de blootstelling aan regorafenib. Bij 14 patiënten, die zowel regorafenib zonder esomeprazol als regorafenib met esomeprazol (op 2 verschillende momenten) hebben gekregen, zagen we geen verschil in blootstelling aan regorafenib. Op basis van dit onderzoek hebben we geconcludeerd dat de wisselwerking met maagbeschermers niet opgaat voor regorafenib en dat deze dus veilig samen gebruikt kunnen worden.

Naast de wisselwerkingen op het gebied van absorptie vinden de meeste wisselwerkingen plaats op het gebied van het metabolisme van medicatie, dat meestal in de lever plaatsvindt. De meeste medicijnen worden door bepaalde eiwitten (ook wel (CYP-)enzymen genoemd) in het lichaam afgebroken tot zogenaamde metabolieten. Deze eiwitten kunnen door andere medicatie worden gestimuleerd of afgeremd,

wat kan zorgen voor een lagere of hogere blootstelling aan het medicijn. Een bekend voorbeeld hiervan is het anti-schimmelmiddel ketoconazol. Dit medicijn remt het enzym CYP3A4, waardoor de blootstelling aan medicamenten dramatisch kan toenemen, zoals het geval is bij ibrutinib, een SMKI gebruikt bij chronische leukemie. Wanneer ibrutinib tegelijk wordt ingenomen met ketoconazol, wordt een 23 keer (!) hogere concentratie van ibrutinib in het lichaam bereikt dan zonder ketoconazol. Er zijn nog zeer veel andere medicijnen die in meer of mindere mate invloed hebben op CYP-enzymen, waaronder de corticosteroïden (zoals prednison). Deze middelen worden gebruikt voor bijvoorbeeld het remmen van het immuunsysteem en worden veelvuldig in combinatie met chemotherapie gegeven. Zo worden patiënten met uitgezaaide prostaatkanker vaak behandeld met een chemotherapeuticum, docetaxel genaamd, in combinatie met prednison. Uit grote onderzoeken is echter gebleken dat er geen duidelijk verschil is in effectiviteit en bijwerkingen wanneer docetaxel met of zonder prednison gebruikt wordt. Prednison geeft daarnaast veel bijwerkingen, met name bij langdurig gebruik, waardoor het weglaten van prednison uit het behandelschema wellicht gunstiger is voor de patiënt. In **hoofdstuk 5** is gekeken naar de invloed van prednison op de blootstelling aan docetaxel om te onderzoeken of prednison veilig uit het normale behandelschema kan worden geschrapt. In deze studie onder 18 patiënten met prostaatkanker is er geen verschil in de blootstelling aan docetaxel te zien tussen patiënten die daarnaast worden behandeld met prednison ten opzichte van docetaxel zonder prednison. Hieruit kan worden geconcludeerd dat – op basis van de farmacokinetiek – prednison veilig kan worden weggelaten uit het behandelschema.

Een relevant probleem met anti-kankermedicijnen is het nauwe evenwicht (ook wel 'therapeutisch venster' genoemd) tussen enerzijds de mogelijke bijwerkingen en anderzijds te weinig blootstelling aan het medicijn om effectief te zijn. Dit principe geldt ook voor de SMKI's waarbij deze balans erg smal is. Wanneer in dit geval een wisselwerking plaatsvindt met andere medicatie, zeker als die andere medicatie ook nog eens een smal therapeutisch venster heeft, dan heeft dit potentieel grote klinische consequenties. Dit is bijvoorbeeld te zien bij patiënten met leverkanker na een eerdere levertransplantatie. Deze patiënten worden over het algemeen behandeld met de SMKI sorafenib en hebben vaak meer last van bijwerkingen van dit medicijn dan patiënten zonder een levertransplantatie. Dit kan komen doordat ze vaak middelen gebruiken die het immuunsysteem onderdrukken om afstoting van de lever te voorkomen (zogenaamde immunosuppressiva) zoals tacrolimus. Deze middelen kunnen potentieel het metabolisme van sorafenib en daarmee de concentratie ervan veranderen. In **hoofdstuk 6** is gekeken naar de blootstelling aan zowel sorafenib als de immunosuppressiva in 4 patiënten die deze specifieke combinatie van geneesmiddelen gebruikten. Bij deze patiënten wordt in de loop van de tijd een duidelijke daling van

de blootstelling aan sorafenib waargenomen, maar tegelijkertijd lijden deze patiënten aan veel bijwerkingen van de behandeling met sorafenib waardoor ophogen van het medicijn erg lastig is. Het advies is dan ook om deze patiënten geen hoge dosis sorafenib te geven, omdat dit meestal leidt tot te veel bijwerkingen waardoor patiënten (tijdelijk of definitief) moeten stoppen met de behandeling.

Sorafenib kent veel bijwerkingen, zoals een hoge bloeddruk en allerlei bijwerkingen met betrekking tot de huid. Het hand-voetsyndroom is hiervan misschien wel de belangrijkste bijwerking met ook een grote impact op de kwaliteit van leven van deze patiënten. Het hand-voetsyndroom kenmerkt zich door pijnlijke blaar- en eeltvorming op de handpalmen en voetzolen. Er is geen andere behandeling voor deze bijwerking voorhanden dan het verlagen van de dosering dan wel het (tijdelijk) staken van de toediening van sorafenib. Onderzoek in muizen heeft laten zien dat het ontstaan van het hand-voetsyndroom kan worden voorkomen door het remmen van de functie van een geneesmiddel-transporter die zorgt voor de opname van sorafenib in huidcellen. Een bekende remmer van deze transporter is probenecid; een oud medicijn dat soms wordt gebruikt in de behandeling van jicht. De combinatie van probenocid met sorafenib is echter nog nooit in mensen getest. In **hoofdstuk 7** is het resultaat beschreven van een onderzoek onder 16 patiënten naar de potentiële invloed van probenecid op de farmacokinetiek van sorafenib. Gelijktijdige inname van sorafenib met probenecid geeft een aanzienlijk lagere blootstelling aan sorafenib en de afbraakprodukten (metabolieten) ervan, zonder een duidelijk effect op de bijwerkingen. Deze combinatie is dan ook niet goed toepasbaar in de dagelijkse praktijk, maar toont wel aan dat probenecid een wisselwerking kan geven en dus wellicht kan worden gebruikt in toekomstig onderzoek naar interacties.

Ook op het gebied van farmacodynamiek kunnen er interacties optreden. Twee middelen kunnen bijvoorbeeld eenzelfde soort effect hebben en daardoor additief of zelfs synergistisch werken, maar ze kunnen elkaar ook juist tegenwerken (antagonistisch). Een voorbeeld van zo'n interactie is verlenging van het QTc-interval op het elektrocardiogram (ECG); oftewel de tijd van de ontspanning van de hartkamers, wat een risico geeft op hartritmestoornissen. Bekende medicijnen die dit effect hebben zijn de zogenaamde serotonine reuptake blokkers (SRI's), oftewel antidepressiva. Een groep patiënten die veel last heeft van depressieve klachten, en daarvoor worden behandeld met antidepressiva, zijn borstkankerpatiënten. Deze patiënten worden vaak behandeld met tamoxifen; een (anti-)hormonale therapie die ook een QTc-intervalverlenging kan veroorzaken. In **hoofdstuk 8** is gekeken of gelijktijdige toediening van een SRI met tamoxifen de QTc-intervalverlenging versterkt t.o.v. tamoxifen alleen. In dit onderzoek is een duidelijke toename in verlenging van het QTc-interval gevonden, met name bij

de anti-depressiva paroxetine, escitalopram en citalopram. De verlenging was niet dusdanig groot dat bij iedere patiënt actie is vereist. Echter, op basis van ons onderzoek verdient het wel de voorkeur om een SRI te geven die geen sterke QTc-verlening geeft en ook niet inwerkt op het metabolisme van tamoxifen, zoals venlafaxine.

Deel 2: Wisselwerking tussen anti-kankermedicatie en voeding

Naast het gebruik van comedicatie is er de laatste decennia ook een grote toename te zien in het gebruik van alternatieve medicatie en/of voedingssupplementen. Ongeveer 48-88% van alle kankerpatiënten gebruikt tegenwoordig voedingsmiddelen en kruiden als een alternatieve of aanvullende strategie voor de behandeling van kanker. Naast de veronderstelde gunstige effecten op de ziekte kunnen voeding en kruiden helaas ook een nadelige invloed hebben op de farmacokinetiek van anti-kankermedicatie door een wisselwerking. Een voorbeeld hiervan is het middel abiratarone, gebruikt voor de behandeling van prostaatkanker. Inname met vette voeding geeft maar liefst een 10 keer hogere blootstelling van abiratarone vergeleken met inname zonder voedsel. Zoals eerder genoemd zijn SMKI's erg gevoelig voor een wisselwerking met medicatie en voeding. Een uitgebreid overzicht van voor SMKI's bekende interacties met voeding en kruiden is gepresenteerd in **hoofdstuk 9** van dit proefschrift. Het valt met name op dat er veel verschillen tussen de SMKI's zijn in hoeverre ze goed oplossen in een vet milieu. Daarnaast zijn er ook voedingsmiddelen en kruiden die een wisselwerking op het niveau van het metabolisme veroorzaken. De bekendste voorbeelden hiervan zijn St. Janskruid en grapefruit(sap), die respectievelijk het enzym CYP3A4 stimuleren (inductie) en het enzym CYP3A4 remmen (inhibitie) en daardoor de bloostelling aan SMKI's veranderen.

Zoals reeds genoemd in het eerste deel van dit proefschrift, zijn SMKIs erg gevoelig voor verschillende interacties met name een wisselwerking met PPI's. Een bekend voorbeeld van een SMKI die erg gevoelig is voor deze interactie, is erlotinib. Dit middel wordt gebruikt in de behandeling van longkanker en lost het best op bij een erg lage zuurgraad van de maag. Aangezien PPI's de maag minder zuur maken lost dit middel bij gebruik daarvan veel minder goed op, wat een lagere blootstelling tot gevolg heeft. Wanneer de pH van de maag tijdelijk verlaagd wordt tijdens de absorptiefase, omzeilt dat potentieel het negatieve effect van PPI's. Een veelgebruikte frisdrank met een lage pH is cola (Coca-Cola; pH-waarde = 2.5). In **hoofdstuk 10** is onderzocht wat inname van erlotinib met cola t.o.v. water doet met de opname van erlotinib in patiënten die al dan niet een maagbeschermer gebruiken. We zagen in deze patiënten dat het negatieve effect van de PPI's nagenoeg wordt opgeheven door de tijdelijke verlaging van de pH door cola.

Hoewel cola een duidelijk voordeel geeft ten aanzien van de opname van erlotinib, is inname met cola niet ideaal. Langdurig gebruik van cola kan namelijk ook resulteren in tandproblemen en gewichtstoename. Daarnaast moet erlotinib in de ochtend op een nuchtere maag met cola worden ingenomen wat ook niet voor iedere patiënt is weggelegd. Een alternatieve oplossing ligt mogelijk in gelijktijdige inname met een vette drank, omdat erlotinib tevens goed oplost in een vet milieu (zoals met vette voeding). In **hoofdstuk 11** is een dergelijke strategie met volle koemelk (ongeveer 4% vet) besproken. In deze studie met 28 patiënten werd echter geen duidelijk effect van volle melk op de opname van erlotinib gezien, wat deze strategie geen geschikt alternatief voor het gebruik van cola maakt. Daarnaast is in deze studie gekeken naar het effect van de PPI esomeprazol op de blootstelling van erlotinib. Het blijkt dat esomeprazol een zeer forse daling geeft van de blootstelling aan erlotinib. Dit onderzoek bevestigt dus het grote probleem van gelijktijdige toediening. Patiënten kunnen erlotinib veilig met melk innemen indien dit gewenst is, omdat er geen duidelijke wisselwerking op het gebied van de farmacokinetiek is, maar het gelijktijdig gebruik van PPI's wordt wel afgeraden.

Het gebruik van alternatieve medicatie wordt steeds populairder onder kankerpatiënten. Daarmee neemt ook het risico op een significante wisselwerking met anti-kankermedicatie toe. Een bijzonder populair kruid is kurkuma, dat wordt gewonnen uit de wortel van de *curcuma longa* plant en wordt gebruikt in de traditionele Aziatische keuken en geneeskunde. Onderzoek in muizen heeft laten zien dat kurkuma ook een effect kan hebben op het metabolisme van tamoxifen. Tamoxifen moet door verschillende enzymen (met name CYP2D6 en CYP3A4) in verschillende stappen worden omgezet in de actieve stof endoxifen. **Hoofdstuk 12** beschrijft een studie in 16 patiënten met borstkanker die tamoxifen met en zonder kurkuma (en een periode met kurkuma en zwarte-peperextract, dat er waarschijnlijk voor zorgt dat kurkuma beter in het bloed wordt opgenomen). Gelijktijdige inname van tamoxifen met kurkuma zorgt voor een significante daling van 12% in de blootstelling aan endoxifen. Hoewel dit een relatief klein effect is, kan dit een potentieel gevaarlijke wisselwerking zijn, aangezien voor de werkzaamheid van tamoxifen een bepaalde concentratie nodig is die door deze wisselwerking wellicht niet kan worden bereikt. Dit effect van kurkuma op de blootstelling aan tamoxifen en endoxifen is met name te zien in patiënten waarbij het metabolisme normaal functioneert, wat het merendeel van de patiënten betreft.

Naast kurkuma is er een ander supplement dat veel door borstkankerpatiënten wordt gebruikt, namelijk groene thee (capsules). Groene thee bevat namelijk zogenaamde flavinoïden (een stikstofvrije organische structuur die in planten voorkomt) waarvan epigallocatechine gallaat (EGCG) het belangrijkste is en mogelijk een anti-kankerwerking

heeft. Echter, EGCG kan ook een significante wisselwerking geven met medicatie, zoals eerder is aangetoond bij het geneesmiddel nadolol (een medicijn dat wordt gegeven aan patiënten met een ritmestoornis). Gelijktijdige toediening met groene-theecapsules gaf een afname van 85% van de blootstelling aan nadolol. Deze wisselwerking geldt mogelijk ook voor patiënten die worden behandeld met tamoxifen. In **hoofdstuk 13** is een onderzoek onder 14 patiënten gepresenteerd dat laat zien dat gelijktijdige toediening van tamoxifen met groene thee geen invloed heeft op de blootstelling aan endoxifen t.o.v. toediening zonder groene thee. Gelijktijdige toediening van tamoxifen met groene thee is dus veilig vanuit een farmacologisch gezichtspunt.

Al met al kunnen wisselwerkingen met medicatie en voeding een belangrijke invloed hebben op de werking van anti-kankertherapie. Potentieel kan er veel goed, maar ook veel fout gaan. Het is daarom belangrijk dat er voldoende aandacht en kennis is voor deze interacties met andere geneesmiddelen en voeding in de klinische praktijk en dat er veel onderzoek plaats blijft vinden. Op die manier kan patiënten een optimale gepersonaliseerde behandeling worden geboden, waarbij rekening wordt gehouden met (of advies gegeven kan worden over) alle medicatie en voeding/kruiden die een patiënt gebruikt.

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APPENDIX 3. CURRICULUM VITAE

Gerardus Antonie Maria 'Koen' Hussaarts was born on December 29th 1990 in Roosendaal, the Netherlands. After finishing secondary school (Norbertus College, Roosendaal) in 2009 he studied Biomedical Sciences at the University of Antwerp. After finishing his first year, he started to study medicine at the Erasmus University Rotterdam in 2010. During his study, he participated in a prospective drug-drug interaction study for his master thesis, under supervision of Dr. R.W.F. van Leeuwen and Prof. dr. A.H.J. Mathijssen. He obtained his medical degree in September 2016 after which he started his PhD program at the department of Medical Oncology, section Translational Pharmacology of the Erasmus MC Cancer Institute, under supervision of Prof. dr. A.H.J. Mathijssen, Prof. dr. T. van Gelder and Dr. R.W.F. van Leeuwen. His PhD studies focused on drug-drug interactions with anti-cancer agents. During this period he obtained the first part of the Basic Education Qualification. Furthermore, he developed educational material (i.e. a clinical case for 'klinisch redeneren'), in collaboration with the department of General Practice of the Erasmus Medical Center for medical students of the Erasmus MC. After a PhD-period of nearly 3.5 years, in January 2020, he started working as a medical resident at the Emergency Department of the Bravis Hospital, Roosendaal and Bergen op Zoom, The Netherlands until August 2020. In September 2020 he started as a general practicioner in training at the Erasmus MC.

APPENDIX 4. LIST OF PUBLICATIONS

Hussaarts KGAM, van Doorn L, Eechoute K, Damman J, Fu Q, van Doorn N, Eisenmann ED, Gibson AA, Oomen-de Hoop E, de Bruijn P, Baker SD, Koolen SLW, van Gelder T, van Leeuwen RWF, Mathijssen RHJ, Sparreboom A, Bins S.
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Influence of the Acidic Beverage Cola on the Absorption of Erlotinib in Patients With Non-Small-Cell Lung Cancer.
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APPENDIX 5. PHD PORTFOLIO

PhD Portfolio

Summary of PhD training and teaching

Name PhD student:	G.A.M. Hussaarts
Erasmus MC Department:	Medical Oncology
Research School:	Molecular Medicine
PhD period:	September 2016 – December 2019
Promotor(s):	prof. dr. A.H.J. Mathijssen
	prof. dr. T. van Gelder
Co-promotor:	dr. R.W.F. van Leeuwen

1. PhD training

	Year	Workload ECTS
General courses		
BROK Course and learning, March 2nd – 17th	2017	1.5
Integrity in Research (integriteitscursus), September 25th	2018	0.3
Biostatistical Methods I: Basic Principles (CCO2) (NIHES) September 18th – October 11th	2018	5.7
Specific courses (e.g. Research school, Medical Training)		
OpenClinica Database building	2016	0.3
Basic introduction course on SPSS, April 3rd – 5th, Rotterdam	2017	1.0
Microsoft Excel 2010: Basic, February 6th, Rotterdam	2017	0.3
Microsoft Excel 2010: Advanced, May 30th, Rotterdam	2017	0.4
Deel BKO (TT1): June 25th -26th	2018	1.5
CPO course	2019	0.3
Oral presentations		
Medical Oncology Research Meeting	2016-2019	1.5
Translational Pharmacology Meeting	2016-2019	1.5
Scientific meetings, Medical Oncology, Erasmus MC	2017-2019	1.5
Cardio-oncology symposium	2019	0.3
Research day ACE Pharmacology & Therapeutics	2019	0.3
International Workshop on Clinical Pharmacology of Anticancer Drugs (ICPAD), Amsterdam	2019	0.5
Poster presentations		
ASCO Annual meeting, Chicago, IL, USA	2018	0.5
International Workshop on Clinical Pharmacology of Anticancer Drugs (ICPAD),	2018-2019	0.4
Amsterdam	2019	0.5
ESMO congress, Barcelona, Spain	2019	0.2
NVKF&B Scientific Meeting		

(Inter)national conferences		
NVKFB Scientific meeting	2017-2019	1.5
Symposium Therapeutic Drug Monitoring Oncolytics	2018	0.3
ASCO Annual meeting, Chicago, IL, USA	2018	2.0
ESMO Congress, Barcelona, Spain	2019	2.0
International Workshop on Clinical Pharmacology of Anticancer Drugs (ICPAD), Amsterdam	2018-2019	1.0
Other		
Scientific meetings, Dept. of Medical Oncology	2017-2019	0.6
Translational Pharmacology Meeting	2016-2019	2.0
Clinical Pharmacology Meeting	2016-2019	2.0

2. Teaching

	Year	Workload ECTS
Lecturing		
Scholing “Interacties in de oncologie” voor Surplus Zevenbergen	2019	0.3
Clinical Demonstration Dept. of Internal Medicine	2019	0.3
Supervising Master’s thesis		
Leonie van Harten	2017	1.0
Stan Berghuis	2018	1.0
Marijn Veerman	2018	1.0
Nadia van Doorn	2019	1.0
Niels Heersche	2019	1.0
Total		**35.5**

APPENDIX 6. DANKWOORD

Dit proefschrift is uiteraard niet het werk van mij alleen. Zonder de hulp en steun van velen had dit werk nooit af kunnen komen. Allereerst wil ik alle patiënten en hun naasten bedanken voor alle toewijding en hun belangeloze inzet voor de verschillende onderzoeken in een fase van hun leven waarin ik zelf waarschijnlijk heel andere prioriteiten zou hebben. Zonder deze inzet zou (medisch-)wetenschappelijk onderzoek niet uitgevoerd kunnen worden en zou de geneeskunde niet staan waar zij nu staat. Daarnaast wil ik ook nog een hoop andere mensen in het bijzonder bedanken.

Allereerst wil ik mijn beide promotoren bedanken. Professor dr. Mathijssen, beste Ron, jij hebt ooit het vertrouwen in mij uitgesproken en mij over weten te halen om onder jouw hoede een promotietraject aan te gaan. Gelukkig heb ik hier geen spijt van gekregen en heb ik enorm veel geleerd in deze betrekkelijk korte tijd. Jouw enthousiasme en toewijding werken aanstekelijk en met name de tijd en moeite die je ondanks je drukke agenda telkens weer vrij hebt kunnen maken om stukken te lezen en lastige vragen te beantwoorden, hebben ervoor gezorgd dat dit proefschrift af is gekomen en ook dat jouw onderzoeksgroep zo floreert. Ik weet dat je het privé niet altijd makkelijk hebt gehad, maar als promovendus heb ik dit nooit gemerkt waarvoor ik alleen maar respect kan hebben. Wat mij betreft had je de 'promotor van het jaar' award zeker moeten winnen.

Professor Dr. Van Gelder, beste Teun, we hebben elkaar niet heel veel gezien tijdens onze promotie, maar desondanks heb ik veel aan je gehad. Je stond klaar voor alle (onderzoeksgerelateerde) vragen en dacht ook actief mee over het vormgeven van mijn toekomst. Uiteraard vind ik je transfer naar het LUMC een groot gemis voor het Erasmus MC, maar ik weet zeker dat ze daar erg blij met je zijn. Hopelijk kun je daar nu eindelijk wat mensen vinden die wel voor je favoriete voetbalclub zijn.

Beste Roelof, onze samenwerkingsperiode was een tumultueuze aangezien we beiden veel hebben meegemaakt, met en los van elkaar. Ik hoop dat je je ambities waar kunt gaan maken zonder daarbij jezelf voorbij te lopen, ik gun het je in ieder geval van harte! Daarnaast is het natuurlijk een eer om je eerste echte promovendus te zijn en we gaan het mooi afsluiten straks tijdens (en na) de verdediging.

Prof. Dr. Gelderblom, Prof. Dr. Dingemans, Prof. Dr. Huitema, bedankt dat jullie de moeite en tijd hebben genomen om dit manuscript te beoordelen. Daarnaast wil ik alle overige leden van de commissie, Prof. Dr. Sparreboom, Prof. Dr. Reyners, Prof. Dr. Aerts en Dr. Frank Jansman bedanken voor het plaatsnemen in de oppositie.

Ruben, mijn EE-19 maatje, zonder jou had mijn algemene muziek-, sport- en Rotterdamkennis nooit geweest wat het nu is. Jouw verbluffend mooie(?) muzieksmaak van nog steeds legendarische artiesten als Snelle, Frenna en Josylvio heeft duidelijk zijn sporen nagelaten. Een locomotief zal nooit meer hetzelfde zijn. En uiteraard mis ik onze vrijdagmiddag afterparty, die altijd een mooi begin van het weekend was. Laten we dit, nadat je me hebt bijgestaan bij de promotie, snel nog maar eens overdoen.

Edwin, samen zijn wij gestart met ons promotietraject. Als AE-306 nomaden voelden we ons vaak een beetje geïsoleerd van de groep, maar we hebben ons er maar mooi doorheen geslagen. Nogmaals dank voor alle gezellige momenten en hulp (met name met technische zaken als computers). Leuk dat je nu weer terug bij de groep bent na je coschappen en ik weet zeker dat je je promotie nu ook goed af gaat sluiten. Daarnaast vind ik het erg fijn dat jij me tijdens de promotie een steuntje in de rug wil geven.

Femke, lieve Fem, jouw georganiseerde aanpak en doorzettingsvermogen heeft velen van ons geholpen en geïnspireerd bij onze promotie. Niet voor niks is jouw opbouw van de persoonlijke mappen nog steeds heilig en ben je cum laude gepromoveerd. Onze gesprekken bij een bak koffie waren altijd een fijne start van de dag. Ik weet zeker dat je het nog heel ver gaat schoppen als oncoloog en als kersverse echtgenote. Dat speciaalbiertje in Delft moet er binnenkort toch maar eens van komen!

Lieve Flo, op het eind waren wij toch mooi ineens de ervaren promovendi, maar jij natuurlijk veruit de oudste. Ik heb altijd erg genoten van jouw naïviteit, je chaotische buien en soms toch een beetje onhandige momenten. Zelf heb ik hier graag misbruik van gemaakt en je (samen met Ruben) veelvuldig voor de gek gehouden. Gelukkig hebben we er samen altijd goed om kunnen lachen en heb ik aan jou een geweldige collega gehad. Het meest legendarische moment voor mij blijft toch wel het shoppen bij Macy's in Chicago (ik bedoel, hoe dan!).

Bodine, lieve Bo, ondanks dat je toch heel anders bent dan ik, lijken we toch ook veel op elkaar. Helaas heb ik nooit lang bij je op de kamer mogen zitten, maar ondanks dat hebben we toch veel leuke gesprekken gehad en vooral lekker over sport en flauwekul geluld. Jouw heerlijk fanatieke en eerlijke houding heb ik enorm gewaardeerd en deze gaat je zeker nog ver brengen als mens en als uroloog. Daarnaast vind ik het ergens ook een fijn idee dat ik later met al mijn plasproblemen bij je terecht kan.

Marijn, jouw persoonlijkheid is uniek. Je hebt een eigen mening en komt daar ook gewoon lekker voor uit. Gelukkig heb ik je in de loop van de tijd ook wat beter leren

kennen en ben ik erachter gekomen dat je stiekem toch wel een klein hartje hebt. Uiteraard hebben we samen veel gelachen en heb ik altijd erg fijn met je samengewerkt, met de publicatie in Lancet Oncology als hoogtepunt.

Porrazzo, als niet-promovendus toch lid worden van de promovendigroep is natuurlijk een topprestatie. Sowieso heb ik bewondering voor hoe je bent opgeklommen de afgelopen jaren. Je bent een goede vriend geworden en we hebben samen veel leuke dingen meegemaakt. Bijna overvallen op Rotterdam Zuid, legendarische potjes FIFA, pizza eten en uiteraard je carnaval ontmaagding (als je je dat tenminste nog kunt herinneren). Ik hoop dat er nog veel van dit soort momenten mogen volgen.

Louwrens, ik heb jouw ontwikkeling vanaf dag 1 mee mogen maken. Eerst student in AE-306 en daarna doorgegaan met een promotietraject bij onze groep. Jouw kennis over tamoxifen is ongeëvenaard en je bent intussen de apotheker met het meeste directe patiëntencontact van Nederland. Je gaat je promotie zeker goed afsluiten met een mooi proefschrift waarin de TEA-studie uiteraard het hoogtepunt gaat zijn.

Daan, jij was gelijk een unieke aanwinst voor de club van promovendi met al je levenservaring, er is volgens mij niks wat jij niet hebt meegemaakt. Altijd gezellig aanwezig en ik heb veel met je gelachen (als je dan uiteindelijk op het werk verscheen tenminste). Ondanks dit alles heb je ook tijd gevonden om je promotie in relatief korte tijd af te ronden en dat gaat sowieso leiden tot een erg mooi proefschrift en vast ook een mooie vervolgcarrière.

Nikki, wat hebben wij stiekem al veel meegemaakt samen. We gaan uiteraard terug tot het COLA-studie dreamteam en we zijn (jij weliswaar via een omweg) ook allebei in een promotietraject binnen de klinische farmacologie terecht gekomen. Ik heb altijd erg fijn met je kunnen werken en vooral ook goed kunnen praten. Je bent echt een vriendin voor me geworden in al die jaren. Onze dubbeldate in Haarlem moeten we maar snel plannen, al is het maar voor wat extra triatlonadviezen.

Leni, wat heb ik fijn met jou mogen samenwerken tijdens mijn promotie met name bij onze gezamenlijke sorafenib-projecten. De klinische ervaring die jij meebrengt, is gigantisch en daardoor ook onmisbaar in het functioneren van de onderzoeksgroep en de afdeling oncologie van het Erasmus MC. Naast het werk was er ook altijd tijd voor een informeel (uitgebreid) gesprek of activiteit met de silent disco toch wel als hoogtepunt. Je gaat het zeker fantastisch doen tijdens jouw promotie.

Mirjam, in de korte tijd die we hebben samengewerkt, heb ik een heel fijne collega aan je gehad. We moesten als streekgenoten de Brabantse eer toch altijd hooghouden en

dat is volgens mij prima gelukt. De ESMO van 2019 was ons gezamenlijke hoogtepunt waarbij we naast de gezelligheid onze kamergenoten toch echt hebben moeten opvoeden. Ik ga er uiteraard vanuit dat je dit ook blijft doen met al je huidige mannelijke collega's, dan blijft de onderzoeksgroep toch een beetje in het gareel als Ron er niet is. Heel veel succes met je verdere carrière.

Stefan, als mijn opvolger van de interactie-lijn binnen de onderzoeksgroep weet ik zeker dat je een mooi traject en uiteindelijk een mooie promotie tegemoet gaat. Daarnaast gaan we elkaar zeker in de gaten houden op Strava.

Wesley, jij bent een erg leuke en unieke aanvulling voor de promovendi groep. Helaas snap ik niet heel goed waar je onderzoek over gaat, maar je kunt er in ieder geval altijd goed over vertellen. We hebben daarnaast samen veel kunnen lachen bij alle gezamenlijke besprekingen en borrels. Verder ben ik erg blij dat je nu af en toe even op mijn vriendin past.

Inge, Lindsay, Lisanne, Pauline en Melissa, oftewel de meiden van de overkant. Ik heb jullie altijd heel fijne en gezellige collega's gevonden en met name op de ASCO en ESMO hebben we heel veel leuke momenten samen beleefd. Bedankt voor de gezelligheid en het ga jullie goed! Lieve Inge, wat is jouw leven enorm veranderd sinds je weg bent gegaan uit het Erasmus MC. Een nieuwe baan, getrouwd en natuurlijk een klein menneke erbij! Het geluk lacht je toe en ik kom snel maar eens die kant op om bij te kletsen, zoals we altijd hebben gedaan tijdens onze vele koffieafspraakjes.

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Azi, thanks for all the 'gezellige' coffee moments. We have had a lot of interesting conversations and also helped each other during difficult times. I hope you can finish your PhD-trajectory on short notice and continue your career in Belgium, in the beautiful city of Antwerp. When the COVID-19 pandemic is attenuated, we must certainly catch up.

Peter, Inge en Mei, zonder jullie zou dit hele boekje slechts een kaft met wat tekst zijn. Ik heb altijd fijn met jullie samengewerkt en vind het elke keer weer bijzonder wat er eigenlijk allemaal kan met die ingewikkelde machines. In de loop van de jaren heb ik jullie gelukkig ook op een betere manier leren kennen tijdens de labuitjes en gesprekken bij een bak koffie.

Bimla en Carla, ook jullie werk wordt vaak onderschat maar met een stappenteller zou al snel duidelijk worden hoeveel jullie doen voor het lab. Bimla, jij was mijn tweede moeder de afgelopen jaren en ik heb altijd erg genoten van je Surinaamse kookkunsten en temperament. Carla, het was altijd gezellig om samen op de kamer te zitten tijdens PK-dagen en te carpoolen naar labuitjes en barbecues vanuit het zuiden van Nederland.

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Veel van het werk dat bij klinisch onderzoek komt kijken zou niet mogelijk zijn zonder alle baliemedewerkers, secretaresses en verpleegkundigen die achter de schermen veel voor ons doen en regelen. Jullie zijn met te veel om allemaal afzonderlijk te bedanken, maar ik ben jullie allen ontzettend dankbaar voor de hulp en inzet de afgelopen jaren. In het bijzonder Petra, Chantal, Willy, José en Eline, bedankt voor alles.

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bedanken, jullie hebben zonder dat jullie het misschien zelf beseffen heel veel voor mij betekent in een heel moeilijke periode. Ik kijk ernaar uit om te knallen volgend jaar in de tweede divisie.

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