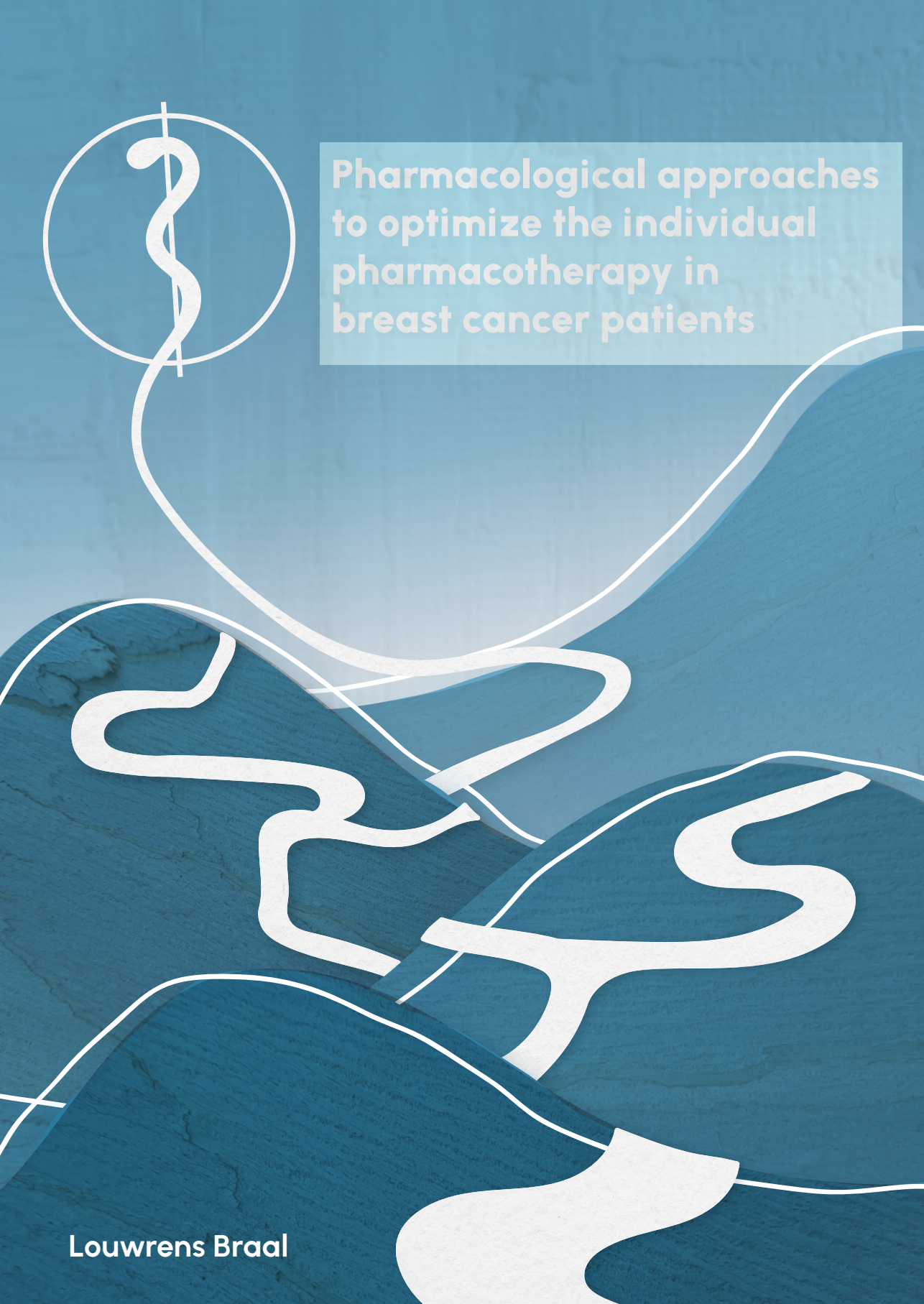

Pharmacological approaches to optimize the individual pharmacotherapy in breast cancer patients

Louwrens Braal

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Pharmacological Approaches to Optimize the Individual Pharmacotherapy in Breast Cancer Patients

Farmacologische benaderingen om de individuele farmacotherapie te optimaliseren in patiënten met borstkanker

Proefschrift

Ter verkrijging van de graad van doctor aan de Erasmus Universiteit Rotterdam

op gezag van de rector magnificus

Prof. dr. A.L. Bredenoord

en volgens het besluit van het College van Promoties.

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Cornelis Louwrens Braal

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	Prof. dr. B.C.P. Koch
	Prof. dr. S.C. Linn

Voor mijn ouders

Contents

Section II: CDK4/6 inhibitors

Section III: Conclusions

CHAPTER 1

General introduction and aim of the investigations

Pharmacotherapy in early and metastatic breast cancer

Worldwide, breast cancer is the most frequently diagnosed cancer among women.[1] In the pharmacotherapeutic arsenal of breast cancer, chemotherapy, monoclonal antibodies, protein kinase inhibitors, and endocrine therapy play an eminent role.[2,3] This thesis focuses on two important oral breast cancer drugs, namely the selective estrogen receptor modulator tamoxifen and the cyclin dependent kinase (CDK) 4/6 inhibitors (*i.e.* palbociclib, ribociclib and abemaciclib).

Tamoxifen specifically targets the estrogen receptor and is therefore considered the first targeted therapy in oncology.[4,5] Tamoxifen is frequently prescribed in the adjuvant setting of early breast cancer and leads to a reduction in disease recurrence.[6,7] In premenopausal women, tamoxifen is indicated, while postmenopausal women are often advised a sequential treatment of tamoxifen followed by an aromatase inhibitor (*i.e.* letrozole, anastrozole and exemestane).[3]

Tamoxifen is a prodrug and has a complex metabolite profile, of which endoxifen is the most important.[8] The prodrug is mainly metabolized by cytochrome P450 (CYP) 2D6 and 3A4 into its main active metabolites 4-hydroxy-tamoxifen and endoxifen (**Figure 1**). The two main active metabolites have a 30-100 higher binding affinity for the estrogen receptor compared with tamoxifen. In addition, endoxifen achieves a 5 to 10 times higher plasma concentration than 4-hydroxy-tamoxifen. Therefore, endoxifen is regarded as the most important metabolite.[8–10]

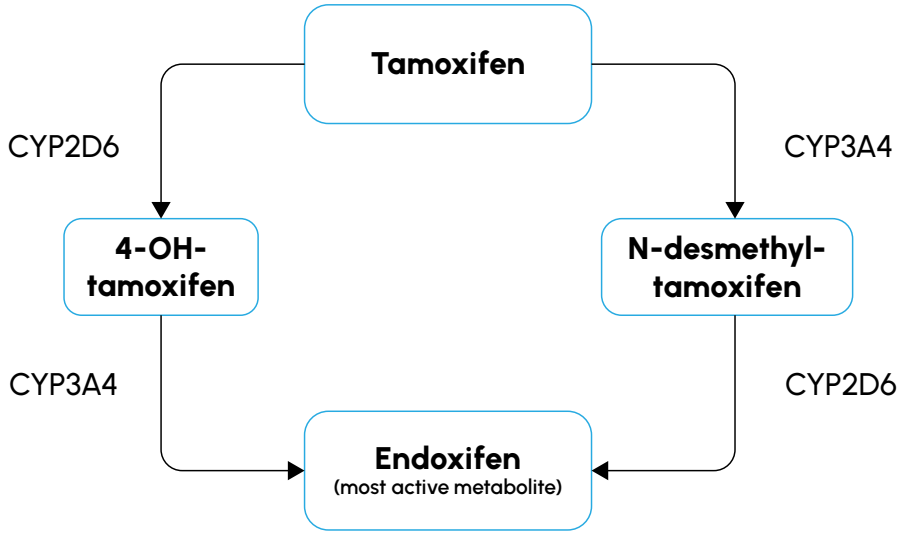
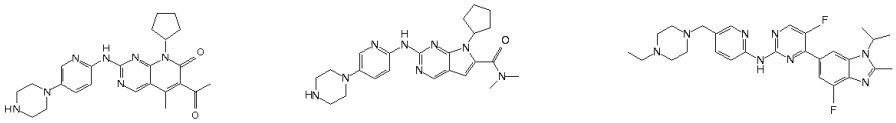


Figure 1 – Simplistic representation of biotransformation of tamoxifen into its most important metabolites.

Recently, CDK4/6 inhibitors have been approved for the treatment of advanced or metastatic breast cancer. The chemical structures of the various CDK4/6 inhibitors show many similarities (**Figure 2**). As advanced or metastatic breast cancer represents an incurable disease, the main purpose of treatment is to delay disease progression, preferably with anticancer drugs that are patient-friendly in their use and toxicity profile. The effectiveness of CDK4/6 inhibitors can be increased by combining them with drugs that prevent the downstream estrogen-dependent stimulation of the cancer cell. Inhibition of the estrogen pathway results in downregulation of cyclin D1 and reduced complexation of CDK4 and CDK6.[11] Therefore, the selective CDK4/6 inhibitors palbociclib, ribociclib, and abemaciclib are given in combination with endocrine therapy (aromatase inhibitors or fulvestrant) in the treatment of hormone receptor positive (HR+) and human epidermal growth factor 2 negative (HER2–) breast cancer. On the basis of their efficacy, all three CDK4/6 inhibitors now play an important role in the treatment of patients with HR+, HER2- breast cancer. Treatment with a CDK4/6 inhibitor leads to a significant improvement in both progression free survival and overall survival.[12,13]



Palbociclib Ribociclib Abemaciclib

Figure 2 – Chemical structures of palbociclib, ribociclib and abemaciclib.

Interindividual variability in response

After administration of the same dose of an oral drug to a group of patients, there are usually large differences in pharmacological responses. For many drugs there is a sigmoidal relationship between the degree of exposure and the pharmacological effect.[14] Variability in response has several causes and depends also on the type of drug. The most important causes of variability are genetic constitution, demographic characteristics, drug interactions and compliance.[15,16] In addition to differences between individual patients with regard to the relationship between dose and effect, variation can also occur within the individual patient. For instance, because a patient's disease activity changes over time, or because drugs are added or discontinued, causing a relevant drug-drug interaction to arise or disappear.[17]

Individualization of pharmacotherapy

The field of pharmacology that studies the changes of drug concentrations in the body in relation to time – in this case the rate and speed of the processes of absorption, distribution, metabolism and excretion – is defined as pharmacokinetics. Pharmacodynamics describes the response that a drug has after interacting with receptors in the body, as well as its intensity and duration of action. The causes of variability in drug response can often be traced to differences between patients in pharmacokinetics and pharmacodynamics. Despite large differences between patients, tamoxifen and the CDK4/6 inhibitors are prescribed at a fixed dose.[12]

Based on current oncological dosing strategies, there are indications that a significant proportion of patients are underdosed or overdosed.[18–24] In clinical practice, the starting dose is empirically reduced after the occurrence of unacceptable toxicity. However, particularly underdosing is difficult to interpret on the basis of observations. The 'one-size-fits-all fixed dosing strategy' therefore appears to be too rough as measure for dosing oral drugs. This thesis focuses on individualization of pharmacotherapy for breast cancer patients. The aim is to provide each individual patient with the best possible treatment based on pharmacological data. There are currently two strategies available to individualize dosing i) genotype-guided dosing and ii) therapeutic drug monitoring guided dosing.

Genotype-guided dosing can especially reduce the proportion of toxicity of a treatment by anticipating with a dose intervention at the start of therapy. This is particularly useful when starting with a toxic drug, such as the pyrimidine antagonists.[25] However, interindividual variability in pharmacokinetics is caused by several factors.[15] If there is sufficient time to adequately select the right dose for an individual patient based on measured plasma concentrations, therapeutic drug monitoring seems a desirable application in clinical practice.[26–28]

Aim of the investigations

In general, limited information in the area of applied individual pharmacotherapy of both tamoxifen and the CDK4/6 inhibitors was available at the start of this thesis. Therefore the main aim of this thesis is to develop a deeper understanding of the interrelationship between dosage, pharmacokinetics, pharmacodynamics and effects of tamoxifen and CDK4/6 inhibitors to further optimize pharmacotherapy in breast cancer patients (**Figure 3**).

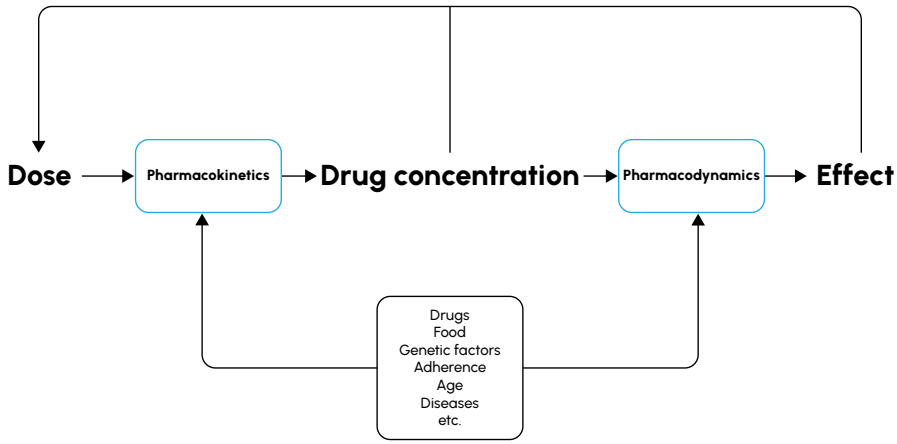


Figure 3 – Schematic interrelationships of dose, pharmacokinetics, pharmacodynamics and drug effects, as well as possible factors and conditions affecting these.

In section I, the individualization of tamoxifen is studied from a multidimensional approach. At the start of this study, a minimum endoxifen concentration of 16 nM (5.97 ng/mL) was suggested for effectiveness.[29] The influence of different interventions (such as TDM-guided dosing or combination with another drug or food supplement) on tamoxifen variability is evaluated from a pharmacokinetic, pharmacodynamic and economic perspective.

In section II, the pharmacological properties of different CDK4/6 inhibitors are investigated. This exposition revealed that the influence of a moderate CYP3A4 inhibitor on the metabolism of a CDK4/6 inhibitor is of limited predictability. Similarly, more research is needed into the relationship between exposure and (pharmacological) response, hence the desirability of developing a more simple analytical method for pharmacokinetic analysis in future studies. In summary, many translational questions regarding optimization of both tamoxifen and CDK4/6 pharmacotherapy need to be clarified in this thesis.

Outline of this thesis

Chapter 2 evaluates the results of a predictive model for endoxifen concentration in steady-state.

Chapter 3 describes the relevance of a well-designed study to investigate the association between endoxifen exposure and clinical outcomes.

Chapter 4 evaluates the feasibility of therapeutic drug monitoring guided tamoxifen dosing in the adjuvant setting.

Chapter 5 evaluates the cost-effectiveness of therapeutic drug monitoring guided tamoxifen dosing in early breast cancer patients.

Chapter 6 describes the health-related quality of life and productivity costs in breast cancer patients treated with tamoxifen.

Chapter 7 reports the results of an observational study of tamoxifen related liver steatosis in patients with early breast cancer by means of transient elastographic evaluation.

Chapter 8 investigates a possible interaction mechanism between tamoxifen and green tea (EGCG) consumption.

Chapter 9 reports the results of the pharmacokinetic interaction between tamoxifen and probenecid in patients with low endoxifen exposure at steady-state concentration.

Chapter 10 summarizes the pharmacological similarities and differences between the different CDK4/6 inhibitors – palbociclib, ribociclib and abemaciclib – in advanced or metastatic breast cancer patients.

Chapter 11 reports the results of the effects of a moderate CYP3A4 inhibitor (erythromycin) on the pharmacokinetics of palbociclib.

Chapter 12 describes a simple and patient-friendly analytical method validation for quantitative determination of ribociclib in a dried blood spot matrix.

Chapter 13 summarizes the key points of this thesis. The results presented in this thesis are briefly reviewed and some possible consequences for further investigations are discussed.

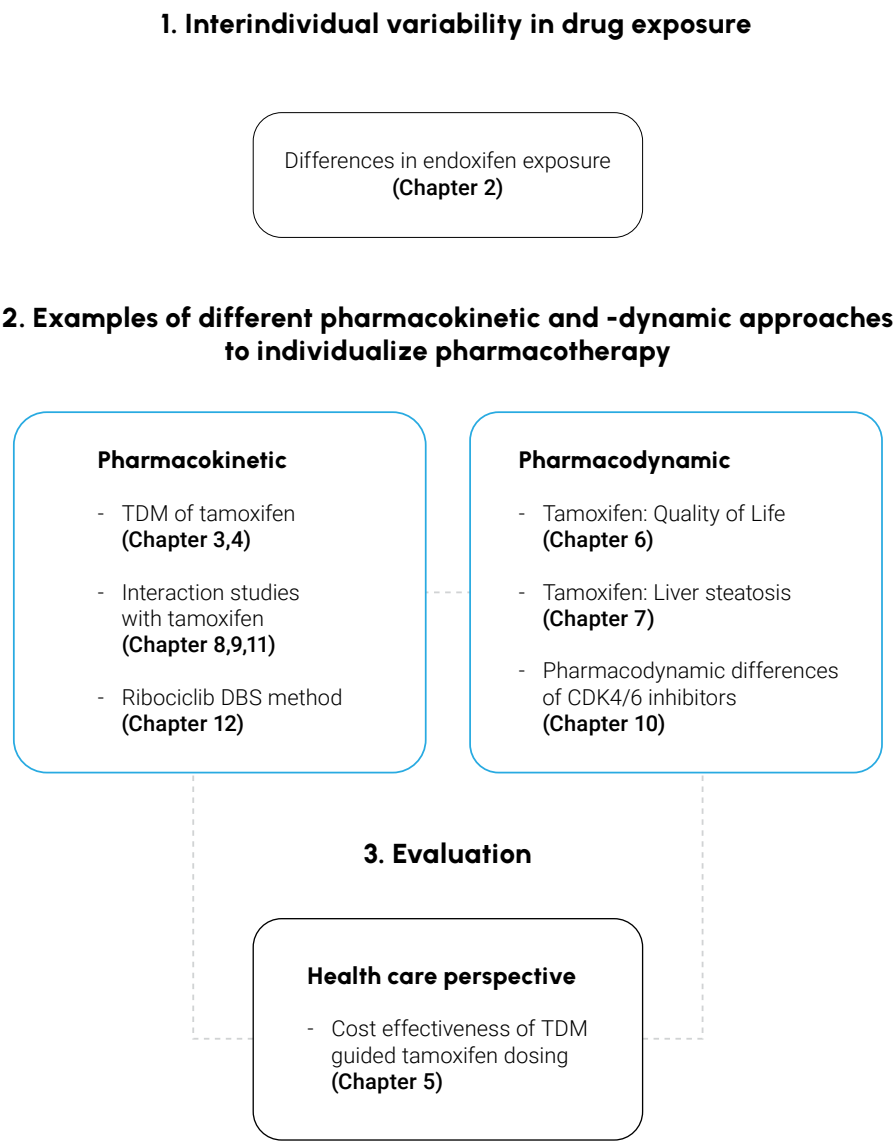


Figure 4 – Outline of this thesis. DBS; dried blood spot, TDM; therapeutic drug monitoring.

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CHAPTER 3

Relevance of endoxifen concentrations: Absence of evidence is not evidence of absence

C. Louwrens Braal , Jos H. Beijnen , Stijn L.W. Koolen , Esther Oomen-de Hoop , Neeltje Steeghs , Agnes Jager , Alwin D.R. Huitema , Ron H.J. Mathijssen

Journal of Clinical Oncology 2019 Aug 1;37(22):1980-1981

With interest we read the publication by Sanchez-Spitman *et al.*, reporting on their 'CYPTAM' study about the association between *CYP2D6* genotype and clinical outcome in the adjuvant treatment of breast cancer with tamoxifen.[1] We acknowledge that a lack of association was shown, and that solely the determination of *CYP2D6* genotype has limited relevance for clinical practice in this setting.

However, we do not agree with Sanchez-Spitman *et al.* on their interpretation of the data on the association between endoxifen concentrations (which is the most relevant tamoxifen metabolite) and recurrence of breast cancer. In our opinion, the authors are jumping to conclusions, as the primary endpoint of this study was not to investigate the relationship between endoxifen concentrations and clinical outcome. Instead, this study was amended in 2017 to explore endoxifen concentrations in relation to recurrence free survival, and was underpowered to draw solid conclusions on this endpoint with a hazard ratio of 2.0 as input to their sample size calculations. This hazard ratio cannot be considered realistic to study the effects of endoxifen concentrations on recurrence free survival.[2]

The CYPTAM study included 662 patients between the years 2008 and 2011. The trial was originally designed to study *CYP2D6* genotype and recurrence free survival. In the protocol, one serum sample for pharmacokinetic purposes was taken (at a random moment during the first year of treatment) and retrospectively the authors correlated the measured endoxifen concentrations with outcome. In 2011, Madlensky *et al.* reported a hazard ratio of 1.4 for patients with endoxifen concentrations below versus above 5.97 ng/mL.[3] Considering this hazard ratio, and the same assumptions as were made in the CYPTAM protocol (2 years of patient inclusion and 2 years of follow-up), a prospectively designed study would have required 276 events in at least 3,150 patients. Importantly, the ratio between patients with endoxifen concentrations below and above the cut-off point (1:4) differs from the ratio between the phenotype groups as assumed in the original sample size calculations of the CYPTAM study (1:1.25). Hence, an even larger sample size would have been required if the correct ratio was taken into account, leading to almost 4,500 patients in case of 2 years of inclusion and 2 years of follow-up. Sanchez-Spitman *et al.* do not mention the observed number of events, nor do they present a Kaplan-Meier curve for relapse free survival stratified for endoxifen concentration that could provide some insight into this number. However, the wide confidence intervals of the hazard ratios for different risks groups suggest a low number of events. In addition, according to the study protocol several patients with an intermediate or poor metabolizer phenotype received a temporarily tamoxifen dose increment. It is unclear from the manuscript if these patients were included in this analysis, but if so, this has confounded the outcome of the study. In addition, the authors do not discuss

their conflicting results about endoxifen concentrations and clinical outcome in comparison with previous studies.[3–5]

Moreover, just one serum sample does not reflect systemic exposure throughout the tamoxifen treatment course. For example, use of co-medication may change over time, and may seriously affect systemic endoxifen concentrations.[6] It is known that the combination of tamoxifen and strong CYP2D6 inhibitors is still popular amongst breast cancer patients.[7] Therefore, we believe that it is a shortcoming that data on co-medication, or other factors (temporarily) influencing endoxifen concentrations (e.g. low and variable compliance[8]) is missing in this analysis.

Sanchez-Spitman *et al.* conclude their manuscript by stating that '*our data do not justify therapeutic drug monitoring based on endoxifen concentrations in patients with breast cancer receiving tamoxifen*'. However, based on the reflections mentioned above, this conclusion cannot be drawn from their study, especially in light of the available literature.[3–5] Instead, we are opting for a large prospective – if possible randomized – clinical trial, to study the value of endoxifen-based therapeutic drug monitoring in tamoxifen treatment.[9,10]

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CHAPTER 4

Therapeutic drug monitoring of endoxifen for tamoxifen precision dosing: feasible in patients with hormone-sensitive breast cancer

C. Louwrens Braal, Agnes Jager, Esther Oomen–de Hoop, Justin D. Westenberg, Koen M.W.T. Lommen, Peter de Bruijn, Mijntje B. Vastbinder, Quirine C. van Rossum–Schornagel, Martine F. Thijs–Visser, Robbert J. van Alphen, Liesbeth E.M. Struik, Hanneke J.M. Zuetenhorst, Ron H.J. Mathijssen, Stijn L.W. Koolen

Clinical Pharmacokinetics 2022 Apr; 61(4):527-537

Abstract

Background

Endoxifen is the most important active metabolite of tamoxifen. Several retrospective studies suggested a minimal, or threshold, endoxifen systemic concentration of 14–16 nM for a lower recurrence rate. The aim of this study was to investigate the feasibility of reaching a predefined endoxifen level (≥16 nM; 5.97 ng/mL) – over time – using therapeutic drug monitoring (TDM).

Methods

In this prospective, open label, intervention study, patients who started treatment with a standard dose of tamoxifen – 20 mg once daily – for early breast cancer were enrolled. An outpatient visit was combined with a TDM sample at 3, 4.5 and 6 months after initiation of the tamoxifen treatment. The tamoxifen dose was escalated to a maximum of 40 mg if patients had an endoxifen concentration below 16 nM. Primary endpoint of the study was the percentage of patients with an endoxifen level ≥16 nM at 6 months after the start of therapy compared with historical data, in other words 80% of patients with endoxifen levels ≥16 nM with standard therapy.

Results

In total, 145 patients were included. After 6 months, 89% of the patients had endoxifen levels ≥16 nM, compared with a literature based 80% of patients with endoxifen levels ≥16 nM at baseline (95% CI, 82 to 94%; P = 0.007). In patients with an affected CYP2D6 allele, it was not always feasible to reach the predefined endoxifen level ≥16 nM. No increase in tamoxifen related adverse events was reported after dose escalation.

Conclusion

This study demonstrates that it is feasible to increase the percentage of patients with endoxifen levels ≥16 nM by means of TDM. TDM is a safe strategy and offers a possibility to nearly halve the number of patients with endoxifen levels <16 nM.

1. Introduction

Tamoxifen significantly reduces the risk of disease recurrence and mortality in patients with hormone receptor positive (HR+) breast cancer.[1–3] In premenopausal women, tamoxifen monotherapy (preferably with ovarian suppression) is indicated for a period of 5 years, while postmenopausal women are often advised a sequential treatment of tamoxifen followed by an aromatase inhibitor.[4,5] Despite adjuvant endocrine treatment, in 11-23% of the patients their disease returns within five years and in about 30% it returns within 15 years.[6,7]

Tamoxifen is a prodrug and is mainly metabolized by cytochrome P450 (CYP) 2D6 and 3A4 into its main active metabolites (**Figure 1**). The two main active metabolites (4-hydroxy-tamoxifen and endoxifen) have a 30-100 greater binding affinity for the estrogen receptor (ER) compared with tamoxifen. In addition, endoxifen achieves a 5 to 10 times higher plasma concentration than 4-hydroxy-tamoxifen. Therefore, endoxifen is regarded as the most important metabolite.[8–11] The complex metabolic profile contributes to a high observed inter-individual variability in endoxifen concentrations[12]

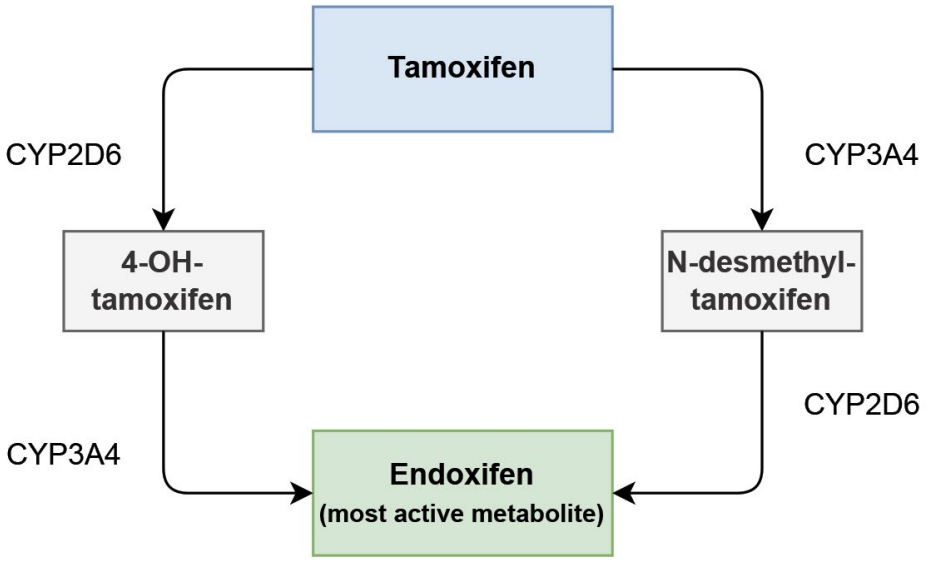

Figure 1 – Main metabolic pathway of tamoxifen into its most active metabolite endoxifen.

In several studies, the endoxifen concentration has been inversely associated with the risk of breast cancer recurrence. In a retrospective analysis, Madlensky and colleagues reported an endoxifen threshold of 16 nM. This study included 1,370 pre- and postmenopausal early breast cancer patients who were treated with tamoxifen in the adjuvant setting for 5 years. Patients with an endoxifen concentration below the lowest quintile (<16 nM) showed a 26% lower disease free survival compared to patients with an endoxifen levels above 16 nM.[13] In a smaller study among 306 premenopausal patients, the endoxifen levels were divided in quartiles. Compared to endoxifen concentrations in the highest quartile (i.e. >35 nM), endoxifen concentrations in the lowest quartile (<14 nM) were associated with an almost two times higher risk of distant recurrence.[14] At the current standard dose of tamoxifen of 20 mg, approximately 20-24% of patients have an endoxifen level below 16 nM.[13,15]

Given this exposure response relationship, dose optimization may reduce the risk for a recurrence of breast cancer. Dose optimization based on CYP2D6 genotype has been attempted since approximately 39% of the variability in endoxifen concentration can be explained by CYP2D6 genotype.[16] However, conflicting results have been reported regarding the association between CYP2D6 genotyping and recurrence rate.[17–22] These conflicting results underline the importance of considering other factors that may influence the variability in endoxifen concentration, including concomitant medication, dietary- or food supplements, adherence, age, body mass index (BMI), hormonal status and circadian rhythm.[23–27] Therefore, therapeutic drug monitoring (TDM)-guided dose individualisation appears to be a valid method to optimize the endoxifen level.[28] Therapeutic drug monitoring is a commonly used tool to select the right dose of a drug for individuals based on plasma concentrations of the drug or active metabolite.[29] The aim of this prospective study was to investigate the feasibility of increasing the proportion of patients reaching a prespecified endoxifen threshold concentration using TDM. Although the exact threshold of the endoxifen level is currently unknown, we opted for the highest threshold value as described in the literature (≥16 nM) in order to minimize the risk of underdosing.[14]

2. Patients and Methods

2.1 Study design and population

The TOTAM (TDM Of TAMoxifen) trial is an open-label, single arm, intervention study performed at the Erasmus University Medical Center in Rotterdam, the Netherlands; approved by the institutional review board and registered in the Netherlands Trial Registry (www.trialregister.nl; NL6918). Patients who started treatment with a standard dose of tamoxifen (20 mg once daily) for early breast cancer and who were able and willing to give written informed consent, were eligible for participation in this trial. Exclusion criteria were: patients taken tamoxifen for a

period longer than 3 months, a starting dose higher than 20 mg once daily, a prior diagnosis of endometrial cancer (≤3 years ago), or a diagnosis with advanced or metastatic breast cancer.

A hospital visit was combined with TDM samples at 3, 4.5 and 6 months after initiation of the tamoxifen treatment. A hospital visit included registration of co-medication or supplements, adverse events using the U.S. National Cancer Institute's Common Terminology Criteria for Adverse Events version 5 (CTCAEv5) and monitoring of drug adherence using the Morisky Medication Adherence Scale; a widely used self-report questionnaire resulting in a high (score 8), medium (score 6-7) or low (score <5) adherence rate.[30,31] Endoxifen trough concentrations at steady-state were sampled and processed to plasma. For quantification of tamoxifen and endoxifen, a validated liquid chromatography tandem mass spectrometry (LC-MS/MS) method was used.[32] After laboratory analysis, both the tamoxifen and endoxifen concentration was evaluated by a pharmacist or clinical pharmacologist. The tamoxifen dose was advised to escalate in patients with a high adherence score and a measured endoxifen level below 16 nM. Before increasing the dose of tamoxifen, the investigator discussed possible individual factors reasoning an endoxifen level below the threshold. Only in patients with non-adherence or potential drug-drug interactions which could be avoided, the tamoxifen dose was initially not escalated. In other cases the tamoxifen dose was escalated to a maximal daily dose of 40 mg, such as described in the drug label of tamoxifen. Patients with an endoxifen level in the range 12 - <16 nM were advised to escalate to 30 mg, while patients with endoxifen levels below 12 nM were escalated to the maximal daily dose of 40 mg once daily. CYP2D6 genotyping was performed using the Infiniti test (Autogenomics, Carlsbad, CA) and the Quantstudio test (ThermoFisher Scientific, Waltham, MA). Variation in the CYP2D6 gene is responsible to alterations in enzyme activity compared with wild type.[33] CYP2D6 phenotype was assayed in the laboratory on the genetic variants *2-10, *12, *14, *17, *29 and *41 and thereafter patients were classified into four phenotypes based on enzyme function, including ultra-rapid metabolizer (UM), extensive metabolizer (EM), intermediate metabolizer (IM) and poor metabolizer (PM). Classification and interpretation were done based on the Clinical Pharmacogenetics Implementation Consortium (CPIC) guideline for CYP2D6 and tamoxifen therapy.[33]

2.2 Primary endpoint and statistical analysis

The primary endpoint was the percentage of patients with an endoxifen level ≥16 nM at six months after initiation of adjuvant tamoxifen treatment. The secondary endpoint was the incidence of tamoxifen-related adverse events (CTCAEv5) after dose escalation. This study was powered to demonstrate that 90% of the patients will have endoxifen levels ≥16 nM at six month after start of tamoxifen by means of TDM-guided dose individualization. For the sample size calculation it was assumed that 80% of the patients had endoxifen levels ≥16 nM without

TDM-guided dose-individualization, as retrieved from literature data.[13,15] To test this hypothesis with a two-sided alpha of 0.05, a power of 80%, and with applying a continuity correction, at least 118 evaluable patients were required. Patients were defined as evaluable if TDM samples were taken at 3, 4.5 and 6 months after start with tamoxifen treatment. Secondary, the influence of the covariate age categorized as ≤45 or ≥55 years at the start of tamoxifen treatment – on endoxifen exposure was tested by a t-test. The association between CYP2D6 phenotype and BMI with baseline levels endoxifen were tested by means of ANOVA. Statistical analysis was performed using SPSS statistics software (version 26, IBM, Chicago, IL).

3. Results

Between January 2018 and June 2019, a total of 145 women with early breast cancer who were treated with adjuvant tamoxifen were enrolled in this trial. To compensate for dropouts in the period to reach the primary endpoint, more than the required 118 patients were included for the primary endpoint analysis. A total of 136 (94%) participants were evaluable for the primary endpoint analysis as 9 of the 145 patients – five due to subtherapeutic endoxifen concentrations and four due to tamoxifen-related toxicity – were switched to an aromatase inhibitor in the meantime. The median age of the patients was 57 years (range 46 – 66). Prior to tamoxifen treatment, most patients underwent surgery in combination with either radiotherapy, (neo)-adjuvant chemotherapy or a combination. *CYP2D6* was successfully genotyped in all patients and were conform Hardy-Weinberg equilibrium distribution (P <0.05). An overview of relevant baseline characteristics are depicted in **Table 1**.

The primary objective of the study was reached by means of TDM-guided dose individualization. After six months, 121 out of 136 (89%) of the patients had an endoxifen concentration above the threshold of 16 nM (95% CI, 82 to 94%, *P* = 0.007). In total, 130 out of 145 (90%) of the participants reached the target concentration or successfully switched to an aromatase inhibitor within the study period of six months (**Figure 2**). The pharmacokinetic profile of subtherapeutic endoxifen levels over time is depicted in **Figure 3A** and the endoxifen profile of all patients stratified on *CYP2D6* phenotype is presented in **Figure 3B** .

Table 1 - Baseline characteristics of 145 TOTAM study participants.

Characteristic	Value	
Age, years	57.0	(46.0 – 66.0)
BMI, $kg \cdot m^{-2}$	25.9	(22.9 – 28.7)
Tumor stage		
T1	67	(46)
T2	67	(46)
T3/4	11	(8)
Nodal stage		
N0	79	(55)
N1	49	(34)
N2	12	(8)
N3	5	(3)
Histologic classification		
Ductal adenocarcinoma	100	(69)
Lobular adenocarcinoma	34	(23)
Mucinous carcinoma	4	(3)
Other[a]	7	(5)
Histologic grade		
1	20	(14)
2	94	(65)
3	31	(21)
PR status		
0%	16	(11)
1-10%	14	(10)
>10%	115	(79)
HER2 status		
Positive	13	(9)
Negative	132	(91)
Prior treatment		
Surgery		
Mastectomy	63	(43)
Lumpectomy	82	(57)
Radiotherapy		
Yes	106	(73)
No	39	(27)
Neo-adjuvant chemotherapy		
Yes	28	(19)
No	117	(81)
Adjuvant chemotherapy		
Yes	32	(22)
no	113	(78)

Table 1 (continued)

Characteristic	Value	
***CYP2D6* predicted phenotype**[b]		
UM	1	(1)
EM	92	(63)
IM	41	(28)
PM	11	(8)
Co-medications		
CYP2D6 inhibitors		
Weak[c]	1	(1)
Moderate[d]	1	(1)
Potent[e]	1	(1)
CYP3A4 inhibitors		
Weak[f]	18	(12)
Moderate[g]	1	(1)
Potent	0	(0)
Morisky Medication Adherence Scale		
High adherence	132	(91)
Medium adherence	7	(5)
Low adherence	6	(4)

Data are presented as N (%) or median (interquartile range). Baseline, visit 1 (T1) 3 months after start with tamoxifen therapy; BMI, body mass index; CYP, cytochrome P450; EM, extensive metabolizer; HER2, Human Epidermal growth factor receptor 2; IM, intermediate metabolizer; PM, poor metabolism; PR, progesterone receptor; UM, ultra-rapid metabolizer.

[a] *Papillary lesions and inflammatory breast cancer.*

[b] *CYP2D6* phenotype was assayed in the laboratory and genetic variants, including *2-10, *12, *14, *17, *29 and *41. Classification and interpretation were done based on the *Clinical Pharmacogenetics Implementation Consortium guideline for CYP2D6 and tamoxifen therapy*

[c] *Citalopram*

[d] *Sertraline*

[e] *Quinidine*

[f] *Esomeprazole, omeprazole, pantoprazole*

[g] *Diltiazem*

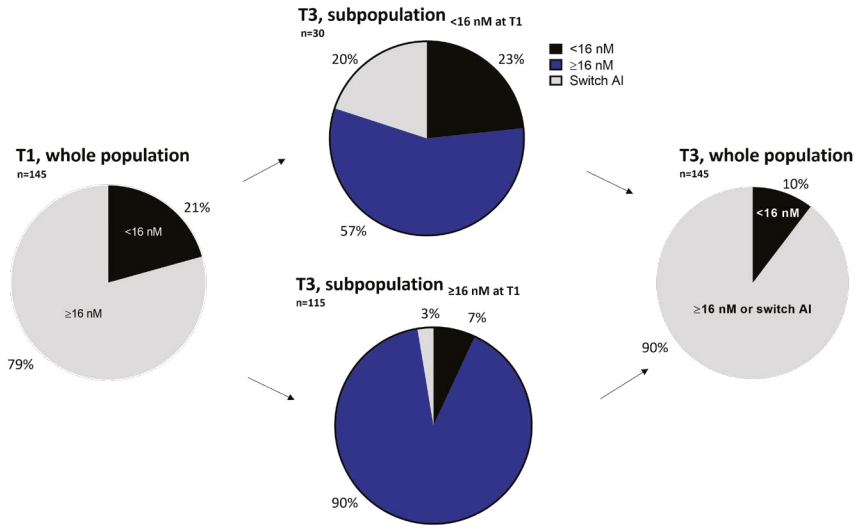

Figure 2 – Therapeutic drug monitoring over time (n=145). Endoxifen concentrations (nM) were stratified based on threshold (1) <16 nM or (2) ≥16 nM. AI; aromatase inhibitor (i.e. letrozole, anastrazole or exemestane); T1, 3 months after start with tamoxifen; T3, 6 months after start with tamoxifen.

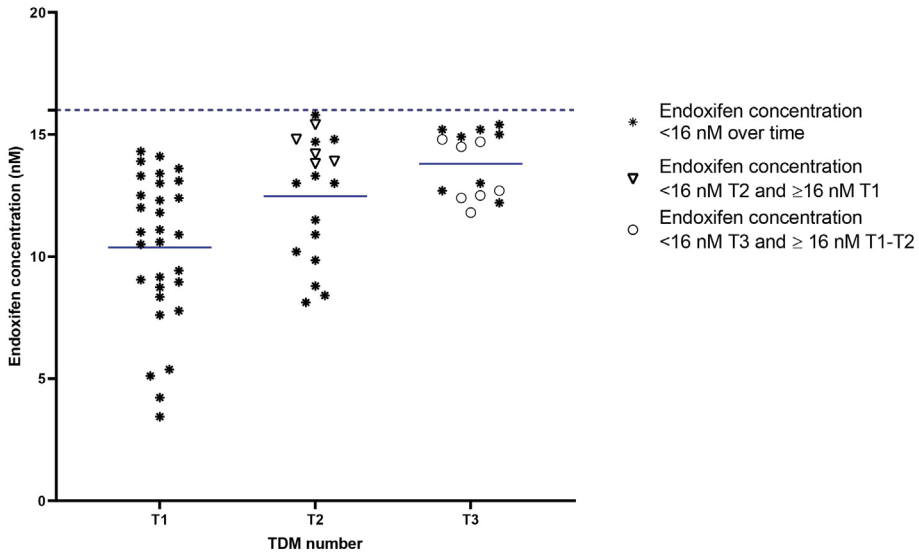

Figure 3A – Pharmacokinetic profile of endoxifen levels below threshold; 3 months (n=30); 4.5 months (n=18) and 6 months (n=15) after start with tamoxifen treatment. The horizontal line represents the mean endoxifen concentration and the horizontal dashed line represents the predefined endoxifen threshold (≥16 nM). T1, 3 months after start with tamoxifen; T2, 4.5 months after start with tamoxifen; T3, 6 months after start with tamoxifen.

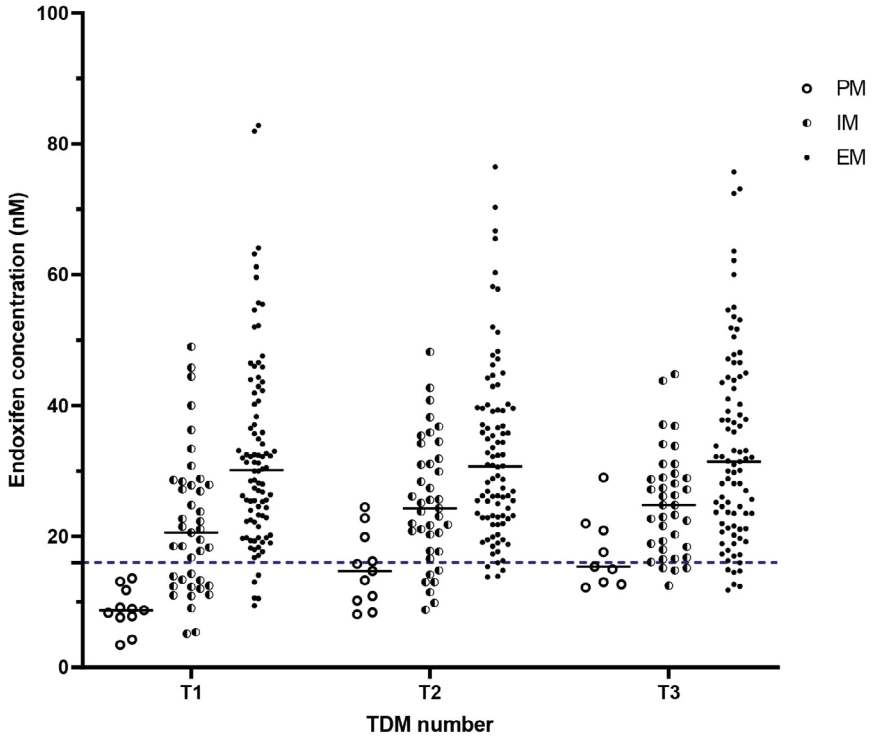

Figure 3B – Pharmacokinetic profile of endoxifen levels stratified based on *CYP2D6* phenotype. The horizontal line represents the mean endoxifen concentration and the horizontal dashed line represents the predefined endoxifen threshold (≥16 nM). T1 (n=145), 3 months after start with tamoxifen; T2 (n=141), 4.5 months after start with tamoxifen; T3 (n=136), 6 months after start with tamoxifen. EM; extensive metabolizer, IM; intermediate metabolizer, PM; poor metabolizer.

At the first TDM-visit (T1) after 3 months of tamoxifen treatment, 30 (21%) patients had an endoxifen level below the threshold of 16 nM. The tamoxifen dose was escalated to 30 or 40 mg in 27 patients and no dose escalation – on discretion of the physician – took place in three patients. The mean concentration of tamoxifen and endoxifen at T1 was 315 ± standard deviation (SD) 99 nM and 27.7 ± SD 14.8 nM, respectively (**Table 2**). At the second TDM-visit (T2) after 4.5 months of tamoxifen treatment, 18 patients (tamoxifen dose 20 mg (n=6); 30 mg (n=3); and 40 mg (n=9)) had an endoxifen level below 16 nM. In 7 of these patients the tamoxifen dose was escalated after T2. The mean endoxifen level at T2 of the 5 patients who had an endoxifen level ≥16 nM at T1 and <16 nM at T2 was 14.4 nM (range 13.8 – 15.4 nM)). Finally, six months after initiation of tamoxifen therapy (T3), 15 out of 136 (11%) patients (tamoxifen dose 20 mg (n=8); and 40 mg (n=7)) showed an endoxifen level <16 nM.

Within the first six months of tamoxifen treatment, a total of 31 patients were escalated to a dosage of 30 or 40 mg tamoxifen; with one or two dose-escalation steps After dose escalation, 21 (68%) of these patients reached the threshold. Of the 10 patients with subtherapeutic endoxifen concentrations 5 (50%) switched to an aromatase inhibitor within 6 months, 3 out of 10 (30%) switched to an aromatase inhibitor after 6 months and 2 out of 10 (20%) continued tamoxifen treatment with 40 mg once daily. Four participants (mean endoxifen level 23 ± SD 4.9 nM) switched to an aromatase inhibitor because of tamoxifen-related adverse events while receiving tamoxifen 20 mg QD (**Table 3**). In our study population, age and BMI had no clear effect on tamoxifen metabolism (P = 0.27 and P >0.60, respectively). CYP2D6 phenotype (poor metabolizer (PM), intermediate metabolizer (IM), and normal or extensive metabolizer (EM)) had a statistically significant effect on endoxifen exposure (P <0.001), with PM patients having the lowest systemic endoxifen concentrations (**Table 4**).

Table 2 – Main pharmacokinetic results of tamoxifen of 145 TOTAM study participants.

Concentration (nM)	3 months (T1)	4.5 months (T2)	6 months (T3)
Tamoxifen			
TOTAM cohort[a]	315 ± 99	345 ± 154	347 ± 165
20 mg cohort[b]	315 ± 99	303 ± 104	306 ± 124
Endoxifen			
TOTAM cohort[a]	27.7 ± 14.8	29.4 ± 13.1	30.2 ± 13.6
20 mg cohort[b]	32.3 ± 12.5	32.3 ± 12.6	31.9 ± 13.6

Data are presented as mean ± standard deviation.
[a]TOTAM cohort, patients with a tamoxifen dose in the range of 20–40 mg once daily (n = 145 [T1], n = 141 [T2], and n = 136 [T3]); [b]20 mg cohort (n=106), patients with a tamoxifen dose of 20 mg once daily during the whole study period (T1–T3). T1; 3 months after starting tamoxifen, T2; 4.5 months after starting tamoxifen, T3; 6 months after starting tamoxifen.

Table 3 – Therapeutic Drug Monitoring over time (n=145).

Variable	T1: 3 months	T2: 4.5 months	T3: 6 months
Tamoxifen users	145 (100)	141 (97)	136 (94)
Endoxifen concentration ≥16 nM	115 (79)	123 (87)	121 (89)[a]
≥16 nM resulting from dose escalation		15/27 (56)	5/7 (71)
Endoxifen concentration <16 nM	30 (21)	18 (13)	15 (11)
<16 nM without dose escalation		1 (5)	6 (40)
<16 nM besides dose escalation		12 (67)	2 (13)
<16 nM T2 but ≥16 nM T1		5 (28)	-
<16 nM T3 but ≥16 nM T2		-	7 (47)
Tamoxifen dose (mg)			
10		2 (1)	2 (2)
20	145 (100)	112 (80)	108 (79)
30		6 (4)	4 (3)
40		21 (15)	22 (16)
Switch aromatase inhibitor[b], reason		4 (3)	9 (6)
Endoxifen concentration <16 nM without dose escalation		2 (1.5)	2 (1)
Endoxifen concentration <16 nM after dose escalation		-	3 (2)
Toxicity on 20 mg tamoxifen		2 (1.5)	4 (3)
Toxicity on 30-40 mg tamoxifen		-	-

Data are presented as n (%) or n/N (%) unless otherwise indicated. T1; 3 months after starting tamoxifen, T2; 4.5 months after starting tamoxifen, T3; 6 months after starting tamoxifen. [a]Evaluable population (n = 136) compared with a literature-based 80% of patients with endoxifen levels ≥16 nM without TDM-guided dosing (95% confidence interval 82–94; P = 0.007). [b]anastrozole, letrozole, or exemestane.

Table 4 – Pharmacokinetic results of tamoxifen after dose escalation and stratified on CYP2D6 phenotype.

Endoxifen concentration (nM)	Before dose escalation (T1 or T2)	After dose escalation (T2 or T3)
Dose escalation		
+10 mg, n=7	12.4 ± 1.3	20.7 ± 9.1
+20 mg, n=24	10.5 ± 3.3	21.8 ± 10.4
CYP2D6 phenotypes		
EM, n=92	32.5 ± 14.5	-
<16 nM	6/92 (6.5)	0/6 (0)
IM, n=41	21.9 ± 10.8	-
<16 nM	14/41 (34.1)	3/14 (21)
PM, n=11	8.8 ± 3.2	-
<16 nM	11/11 (100)	7/11 (64)

Data are presented as mean ± standard deviation or n/N (%). EM; extensive metabolizer, IM; intermediate metabolizer, PM; poor metabolizer, T1; 3 months after starting tamoxifen, T2; 4.5 months after starting tamoxifen, T3; 6 months after starting tamoxifen.

Low endoxifen levels were found in the majority of patients with an IM or PM *CYP2D6* phenotype. At T1, 7% of the patients with a EM phenotype had a endoxifen level <16 nM, whereas 34% of the IMs and 100% of the PMs were below this target. Also 8% of the participants (with a mean endoxifen concentration of 19.1 nM (16.6 – 19.9) declined below the threshold during the relative short study period, while a preliminary measurement was above 16 nM. After dose escalation, the pharmacokinetic target of ≥16 nM was achieved in 100% of the EMs, 79% of the IMs and 36% of the PMs. Also the endoxifen concentrations increased linearly from 10.5 ± SD 3.3 nM to 21.8 ± SD 10.4 nM after dose escalation to 40 mg tamoxifen once daily. In the non-escalated group (n=106) stable endoxifen concentrations over time were observed compared with T1; with mean endoxifen levels of 32.3 ± SD 12.5 nM and 31.9 ± SD 13.6 nM at baseline and six months after initiation of treatment, respectively (**Table 2**). In the non-escalated group a relatively low intra-individual variability of 19% was found.

Hot flashes (61%), arthralgia (19%), fatigue (11%), vaginal dryness (8%) and mood swings (6%) were the most commonly reported tamoxifen-related adverse events (all CTCAE grade 1) during the first six months of this clinical trial. Low grade toxicity, adverse events were often persistent and perceived as limiting and therefore, four patients decided to discontinue their tamoxifen treatment (between T1 and T3). After dose escalation, no increase in tamoxifen related adverse events, severe or serious adverse events or treatment discontinuation were reported. Morisky adherence scores were reported in our population of 91%, 5% and 4% for high, medium and low adherence scores, respectively; these results imply a high adherence rate in our study population (**Table 1**). The use of concomitant CYP2D6 or CYP3A4 inhibitors was limited in the study population. Only two patients used a moderate-potent CYP2D6 inhibitor (i.e. sertraline 50 mg q.d. and quinidine 200 mg b.i.d.) and one patient used a moderate-potent CYP3A4 inhibitor (i.e. diltiazem 120 mg t.i.d.). Also one patients used a weak CYP2D6 inhibitor (i.e. citalopram) and 18 patients used a weak CYP3A4 inhibitor (i.e. esomeprazole, omeprazole or pantoprazole).

4. Discussion

The TOTAM study demonstrates that TDM-guided dose individualisation leads to a statistically significant and clinically relevant increase in the number of patients with endoxifen levels above the predefined threshold of 16 nM after 6 months of treatment. By using TDM guided dose individualization dosing for 3 months, nearly 50% of patients with an initial endoxifen level below the predefined threshold reached endoxifen levels ≥16 nM. Therefore, our study offers tools for applying TDM of tamoxifen in clinical practice.

Dose escalation resulted in a significant increase of both tamoxifen and endoxifen concentrations (independent of CYP2D6 status), which is consistent with previous data.[12,34–38] An Australian dose escalation study showed a higher percentage of patients achieving the threshold – 94% versus 76% at baseline – compared with our data.[15] However, it should be noted that the defined threshold was set differently (>15 nM versus ≥16 nM), the dose of tamoxifen was increased to a maximum of 60 mg (instead of 40 mg), and the tamoxifen dose was increased in those patients with endoxifen levels below 30 nM.

Stratification based on the CYP2D6 genotype showed differences in mean endoxifen levels both before and after dose escalation. After dose escalation, the predefined endoxifen target was achieved in 100% EMs and 79% IMs, but only 36% of the PMs. This result implies that to achieve therapeutic endoxifen concentrations early in treatment, it is advisable to anticipate – if available – on CYP2D6 genotype status of the patient. Our results indicating that PMs might benefit with a start dosing of 40 mg tamoxifen once daily combined with TDM; and IMs might benefit with the standard dose combined with TDM. For NMs 20 mg once daily tamoxifen might be sufficient without TDM for most of the tamoxifen users. Currently, a proof-of-concept study is ongoing in CYP2D6 IM and PM patients to combine tamoxifen with probenecid (an UGT-inhibitor), aiming to reduce the conversion of endoxifen in inactive metabolites (www.trialregister.nl, study number NL8444). In addition, another trial is ongoing to evaluate the effect of suppletion of Z-endoxifen according to CYP2D6 genotype or plasma levels to reach a predefined endoxifen threshold (NCT03931928). However also a switch to an aromatase inhibitor might be a valid option for the subgroup of patients with persistently low endoxifen levels after a tamoxifen dose adjustment to 40 mg.

Also in patients with adequate CYP2D6 function, at least one TDM sample is advisable for every patient treated with tamoxifen.[16,39] Next to CYP2D6 genotype, multiple other factors can contribute to lower endoxifen levels, such as concomitant CYP2D6 inhibitors, adherence, menopausal status and decreased absorption.[23,40] However, due to the low incidence of concomitant use of CYP2D6 inhibitors and median age of 57 years in our analysis, only CYP2D6 phenotype was highly predictive for endoxifen exposure at baseline. Despite the relatively low intra-individual variability of 19% (as found in our study), 8% of the participants fell below the threshold somewhere during the relative short study period, while TDM sample 1 or 2 was above 16 nM (mean endoxifen concentration of 19.1 nM (16.6 – 19.9). This suggests that patients with endoxifen levels in the range of 16 to 20 nM should be monitored more frequently than patients with endoxifen levels above 20 nM at the first measurement at 3 months after the start of treatment. Long term (2 years of follow-up) intra-individual data collection in the TOTAM study for tamoxifen pharmacokinetics is still ongoing. These data will ultimately represent the

pharmacokinetic profile during the first 24 months of treatment with tamoxifen in this cohort of patients.

After increasing the tamoxifen dose, no increase in the degree or severity of toxicity was observed. Although the absolute number of patients with increased doses is relatively low, the toxicity data are in agreement with the literature. Prospective studies have shown that there is no correlation between the dose of tamoxifen and the incidence of side effects.[34,35,41,42] A longer follow-up should reveal whether this also applies to the rare or long-term side effects, such as the risk of developing endometrial carcinoma, deep vein thrombosis and pulmonary embolism.[43–45] Due to the likelihood of these serious side effects, a conscious decision was made to increase the dose up to the maximal registered tamoxifen dose of 40 mg once daily.

In our study, there was a high degree of adherence; 91% of the population were scored with the highest Morisky medication adherence score. Both the adherence questionnaire and tamoxifen levels above 100 nM indicate that the participants of this study were highly motivated for tamoxifen treatment. Another contributor to this high degree of tamoxifen adherence could be the serial therapeutic drug monitoring including active counselling by a pharmacist or medical oncologist. However, in real-life the degree of adherence fluctuates between 41-88% whereby the relevance of TDM can be increased outside the context of a clinical trial or a longer follow-up period.[46,47] In literature, discontinuation of tamoxifen therapy is mostly observed during the first year of treatment.[46] For example, a recent study already exemplified that TDM is a useful tool for detecting non-adherence (tamoxifen level <100 nM) in an early stage of treatment.[46–48] As a result of earlier research almost all concomitant moderate and strong CYP2D6 inhibitors have been included in the medication monitoring system of Dutch pharmacies as a monitoring signal.[49] Therefore, in our population minimal concomitant CYP2D6 or CYP3A4 inhibitors were noticed.

A methodological strength of our study is the design with repeated measures of tamoxifen TDM-samples compared with mostly single sampling as described in literature.[35] However, the intensive monitoring could also, paradoxically, be considered a potential limitation. The intensive monitoring strategy potentially positively contributes to the adherence and motivation of patients for tamoxifen treatment. Another strength of our study was the inclusion of a real life population of tamoxifen users as well as (1) both pre- and postmenopausal patients, (2) evaluation of an approved on-label dosage (maximal 40 mg tamoxifen QD), and (3) evaluation of a switch to an aromatase inhibitor.

The proportion of breast cancer patients with subtherapeutic endoxifen levels are determined based on the threshold first reported by Madlensky and colleagues of 16 nM.[13] If the true value of this threshold is lower, this could imply that a lower proportion of patient's treatment is categorized as subtherapeutic before TDM. Based on this, the uncertainty regarding the effectiveness of tamoxifen below the current threshold of 16 nM is an important limitation underlying the presented outcome.

Still conflicting results are reported for an endoxifen exposure-response relationship for the adjuvant tamoxifen treatment. A preclinical study in mice showed an association between endoxifen levels and tumour growth and a xenograft model found a dose-dependent association between concentration and degree of gene expression in a MCF7 cell line.[50,51] These preclinical data support the findings of the retrospective analyses by Madlensky *et al.* and Saladores *et al.* with an endoxifen threshold in the range of 14-16 nM.[13,14] In contrast to these findings, in the CYPTAM trial – in which 667 women were treated with tamoxifen – no association was found between CYP2D6 genotype and endoxifen concentration in relapse free survival.[52] However, the design of this study has some limitations and power was insufficient to conclude that there is no exposure-response relationship. Therefore, an exposure-response relationship remains disputed.[53–57] A prospective randomized controlled TDM study could provide clarity. However, such a trial is probably impossible, since it would require many thousands of patients to participate and a follow-up period of more than a decade.[54] To break out of this potential dead end, physicians are encouraged to implement TDM in the meantime in clinical practice, pending further prospective data. In our opinion, TDM of endoxifen is the most suitable approach for tamoxifen precision dosing due to several factors could be affecting the endoxifen concentrations in patients. TDM is mainly recommended in polypharmacy (many concomitant drugs or food supplements) patients, premenopausal patients and in patients diagnosed with an affected CYP2D6 allele. Importantly, an infrastructure to quantify endoxifen concentrations in human plasma is easy to implement in clinical practice.

In conclusion, the current TOTAM study clearly demonstrates the feasibility of therapeutic drug monitoring in personalizing tamoxifen treatment in patients with breast cancer. This strategy offers a possibility to safely halve the number of patients with endoxifen levels below 16 nM, without introducing additional toxicity.

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CHAPTER 5

Therapeutic drug monitoring-guided adjuvant tamoxifen dosing in early breast cancer patients: A cost-effectiveness analysis from the prospective TOTAM trial

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Abstract

Background and objectives: Endoxifen is the active metabolite of tamoxifen and a minimal plasma concentration of 16 nM has been suggested as a threshold above which it is effective in reducing the risk of breast cancer recurrence. The aim of the current analysis was to investigate the cost-effectiveness of therapeutic drug monitoring (TDM) guided tamoxifen dosing.

Methods: A cost-effectiveness analysis was performed from a Dutch health care perspective, using a partitioned survival model and a lifetime horizon. The reduction in subtherapeutic treatment following TDM is modelled as improved rates of recurrence free survival (RFS) and overall survival (OS) in comparison to standard tamoxifen treatment. A probabilistic sensitivity analysis (PSA) and a series of scenario analyses were performed to assess the robustness of the results.

Results: Base case results estimated a total increase in life years and quality-adjusted life years (QALYs) for TDM of 0.40 and 0.53, respectively. Total costs for TDM and standard tamoxifen treatment are €32,893 and €39,524, respectively. The TDM intervention results in both more QALYs and less health care costs, indicating a dominating effect for TDM. The PSA results indicate that the probability of TDM being cost-effective is 92% when using a willingness to pay threshold of €20,000.

Conclusions: TDM-guided dose optimization of tamoxifen is estimated to save costs and increase QALYs for early breast cancer patients.

1. Introduction

For more than 40 years, tamoxifen has been the standard adjuvant treatment for estrogen receptor positive (ER+) breast cancer.[1] Treatment regimens often consist of 5 years of tamoxifen for premenopausal women and at least 2.5 years of tamoxifen followed by an aromatase inhibitor (i.e. letrozole, anastrazole or exemestane) in postmenopausal women.[2–4] Tamoxifen treatment reduces recurrence rates by approximately one third compared with control (no tamoxifen treatment).[1] The effectiveness of tamoxifen is exerted through its active metabolite endoxifen.[2]

The rate at which tamoxifen is converted to endoxifen varies greatly between individuals. This means that the same tamoxifen dose does not necessarily translate to the same endoxifen plasma concentrations at the individual level.[5] The results from two retrospective studies investigating the effectiveness of tamoxifen at different plasma concentrations of endoxifen suggested that a minimal endoxifen concentration of 14–16 nM is needed for an optimal risk reduction for recurrence.[6,7,8] In the main retrospective analysis of these two studies, Madlensky et al. reported an endoxifen threshold of 16 nM. This study included 1,370 pre- and postmenopausal early breast cancer patients who were treated with tamoxifen in the adjuvant setting for 5 years. Patients with an endoxifen concentration below the lowest quintile (<16 nM) showed a 26% lower disease free survival compared to patients with endoxifen levels above 16 nM.[6] Findings from subsequent studies indicate that endoxifen concentrations remained below 16 nM in 20–24% of patients treated with tamoxifen.[6,8,9] These findings suggested that the effectiveness of tamoxifen treatment may be enhanced in patients with subtherapeutic endoxifen concentrations.

Therapeutic drug monitoring (TDM)-guided dose individualization of tamoxifen is a strategy to attain endoxifen levels above a predefined threshold and thereby enhancing its therapeutic effectiveness. TDM consists of regular monitoring of endoxifen plasma concentrations and increasing tamoxifen dosage when the endoxifen concentration is below 16 nM. The feasibility of TDM for breast cancer patients treated with adjuvant tamoxifen has recently been established in the TOTAM study, in which the number of patients with subtherapeutic endoxifen levels was reduced by 50% within three months from the first assessment of endoxifen levels.[10] As such, TDM is expected to increase the effectiveness of tamoxifen treatment at the expense of additional health care resource use due to TDM. This raises the question whether TDM is a cost-effective intervention relative to standard tamoxifen treatment.

The cost-effectiveness of TDM of tamoxifen in the Netherlands was investigated previously, in the absence of data from a clinical trial on TDM, from a theoretical perspective.[11] This study

relied on several assumptions, for example regarding the outcomes of TDM, additional health care resource use and patients' quality of life. that were not in line with the current implementation and results of TDM in TOTAM. The results of that study indicated that TDM is cost-effective, but an important question remains regarding whether the same outcome is obtained from a cost-effectiveness analysis (CEA) based on the implementation and results of a prospective TDM clinical trial. Here we reported the results of a CEA of TDM-guided adjuvant tamoxifen therapy in hormone-sensitive breast cancer from a Dutch health care perspective. [12,13]

2. Methods

2.1 Patients, intervention and comparator

The results from the prospective, open label TOTAM study (Dutch Trial Registry; NL6918) that included 145 patients with breast cancer who were treated with TDM-guided adjuvant tamoxifen dosing were used.[10] Patient and disease characteristics are presented in **Appendix I**. This study was designed to investigate the feasibility of establishing a predefined endoxifen concentration (≥16 nM) in a period of 6 months after start with tamoxifen. All patients initially received a tamoxifen dose of 20 mg once daily. The tamoxifen dose was escalated over time to a maximum of 40 mg once daily for patients with an endoxifen concentration below 16 nM. Furthermore, a switch to an aromatase inhibitor (i.e. letrozole, anastrozole or exemestane) can be a valid option for the subgroup of patients with persistently low endoxifen levels after a tamoxifen dose adjustment to 40 mg. Endoxifen plasma concentrations were monitored at 3, 4.5 and 6 months after start of treatment, followed by dose escalations when applicable. The results from TOTAM showed that in 20.7% of the patients the endoxifen concentration was below 16 nM after 3 months of treatment. After 6 months, 11.0% of the patients remained below the threshold (p=0.007). Standard tamoxifen treatment was assumed to consist of 20 mg once daily, as recommended in the treatment guideline for early breast cancer. Given the lack of (dose-related) adverse events that are associated with tamoxifen, these were not included in the analysis.[9,14,15]

2.2 Model structure

A partitioned survival model was constructed in Excel (Microsoft 2016, Redmond, WA, USA) that consisted of three health states: recurrence free survival (RFS), recurrent disease (RD), and Death (**Figure 1**). All patients started the model in RFS, where they could either remain in the next model cycle, transition to RD upon diagnosis with a local recurrence or distant metastases, or die. Patients in RD can either remain in RD in the next model cycle or die. A model cycle length of 3 months is used, which corresponds with the duration of the TOTAM study.

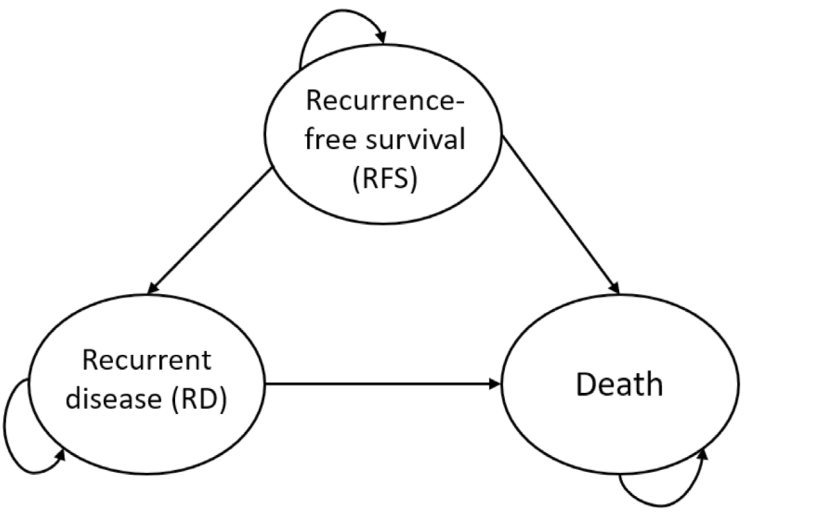

Figure 1 – Health-state structure of the partitioned survival model.

2.3 Perspective, time horizon and discounting

The analysis was performed from a Dutch health care perspective. A scenario analysis was performed that included costs due to lost productivity from paid and unpaid work to approximate a societal perspective. A lifetime time horizon was used to capture all relevant costs and effects, which were discounted – according to the Dutch guidelines for economic evaluations in healthcare – at an annual rate of 4% and 1.5%, respectively.[16,17]

2.4 Model input parameters

Survival estimates

Data on RFS and overall survival (OS) were extracted from a meta-analysis by the early breast cancer trialists' collaborative group (EBCTCG) on 10,238 women who received treatment with adjuvant tamoxifen for about five years or no tamoxifen, with a follow-up time period of 10 – 15 years.[18] Patient-level data were estimated using the methods described by Tierney et al., 2007 and Hoyle and Henley, 2011.[19,20] Extrapolations of RFS and OS were performed using the package 'survival' in R[21], using exponential, Weibull, lognormal and log-logistic parametric functions. For both RFS and OS, the Weibull curves were selected based on statistical fit as indicated by the Akaike Information Criterion (AIC), visual fit, and clinical plausibility. OS extrapolations were adjusted for background mortality using life tables provided by Statistics Netherlands (CBS). To prevent logical inconsistencies, RFS was restricted using OS as its upper bound in the model.

The clinical benefit of TDM was modelled based on the assumption that the RFS and OS curves of patients treated with standard tamoxifen dose (20 mg tamoxifen once daily; without the intervention TDM) included 20% of patients with subtherapeutic endoxifen levels below 16 nM, which was reduced to 10% with TDM in line with results from TOTAM and other studies.[6,9] It was assumed that RFS and OS of patients with subtherapeutic endoxifen levels are equal to RFS and OS of patients who were not treated with tamoxifen in the EBCTCG study. This assumption was supported by clinical and pharmacological expert opinion.[22]

Health-related quality of life

Health-related quality of life data were collected in TOTAM using the EQ-5D-5L.[23] Subsequently, the descriptive health profiles were valued using the Dutch tariff to generate utilities for patients in RFS.[24] The average utility value obtained after 3 months of treatment (i.e. before any dose adjustments) was used to represent patients receiving standard tamoxifen treatment, and the average utility value obtained after 6 months of treatment (i.e. after dose adjustments were performed if needed) was used to represent patients receiving TDM-guided adjuvant tamoxifen dosing. In absence of data from TOTAM on the utilities of patients in RD, a utility value for RD was sourced from the literature. The results from a study in Finland that assessed the utilities of patients in different stages of breast cancer provided a value of 0.74 (standard deviation (SD) ± 0.26)) for metastatic disease.[25] The utilities that were used in the model are presented in **Table 1** alongside other model input parameters. An age-dependent decline in utility was applied using the method by Ara and Brazier.[26]

Resource use and costs

Drug acquisition costs for TDM-guided adjuvant tamoxifen were calculated using the proportions of patients receiving each dose (i.e. 20, 30 or 40 mg once daily) in TOTAM or 20 mg once daily for standard tamoxifen. The costs of tamoxifen and anastrozole were sourced from the Dutch national health care institute Zorginstituut Nederland, Diemen, the Netherlands).[27,28] It was assumed that tamoxifen was provided per three-monthly prescription, for which pharmacy drug dispensing costs were included. Health care resource use in RFS was based on the Dutch (adopted from the ESMO guidelines) treatment guideline for breast cancer,[29–32] and included outpatient hospital visits and visits to the general practitioner (GP). Health state costs for RFS were subdivided to account for differences in resource use during the first year, use experienced between year one and five, and after 5 years. Intervention costs occurred only during the first year and consisted of three additional outpatient oncology visits, a phone consultation and two endoxifen tests (€95 each). It was assumed that after one year, resource use in RFS was the same for both treatments. Unit costs were derived from the Dutch manual for cost research: methodology of cost research and reference prices for economic evaluations in health care[23], and published hospital declaration prices. The health state costs in RD were assumed to be the same for both interventions, and were informed by published

health care cost estimates of Dutch patients with local and distant recurrence during the first year and thereafter.[33] A weighted, per-cycle cost estimate is calculated assuming an average time spent in RD of 3 years and equal proportions of local and distant metastases.[34] The RD health state costs included drug costs, surgical procedures, radiotherapy, diagnostic resources, and in- and outpatient visits. All costs included in the analysis are expressed in 2019 euros, and costs sourced from a prior year were updated using the consumer price indexes provided by Statistics Netherlands (CBS). An overview of the health state costs for RFS and RD is provided in **Table 1**.

Table 1 – Input parameters used in deterministic and probabilistic sensitivity analysis. Parameter adjustments for scenario analyses are indicated per scenario.

Variable	Mean value TDM (SE)	Mean value tamoxifen SC (SE)	Distribution	References
Age, years	57	57	Fixed	[10]
Discount rates				
Costs (%)	4	4	n.a.	[30]
Effects (%)	1.5	1.5	n.a.	
Transition state				
RFS			Weibull	[18]
AIC	11,055.72	11,729.69		
Intercept	3.4116	3.3832		
log(scale)	-0.0449	-0.0039		
OS			Weibull	
AIC	10,885.48	11,344.88		
Intercept	3.1694	3.1392		
log(scale)	-0.3305	-0.3295		
Utility				
RFS state	0.88 (0.22)	0.87 (0.20)	Beta	[10,25]
RD state	0.74 (0.26)	0.74 (0.26)	Beta	
Endoxifen concentration				
<16 nM (%)	10	20	Fixed	[10]
≥16 nM (%)	90	80	Fixed	
Costs per cycle - RFS				
Drug acquisition costs	€ 33	€ 27		[13,27,28]
Resources hospital				
Year 0-1	€ 383	€ 261	Gamma	[10,30]
Year 1-5	€ 90	€ 90	Gamma	
Resources GP	€ 38	€ 38	Gamma	[29,30]
Costs per cycle - RD	€ 10,153	€ 10,153	Gamma	[33]
Productivity loss				
RFS	€ 0	€ 0	Gamma	
RD	€ 0	€ 0	Gamma	
Death	€ 0	€ 0	Gamma	

Table 1 (continued)

Variable	Mean value TDM (SE)	Mean value tamoxifen SC (SE)	Distribution	References
Alternative parameters scenario analysis				
S1a. Endoxifen concentration (100% on threshold)				
<16 nM (%)	0	20	Fixed	
≥16 nM (%)	100	80	Fixed	
RFS			Weibull	[18]
AIC	10,526.35	11,729.69		
Intercept	3.4355	3.3832		
log(scale)	-0.0885	-0.0039		
OS			Weibull	
AIC	11,154.34	11,344.88		
Intercept	3.2161	3.1392		
log(scale)	-0.3234	-0.3295		
S1b. Endoxifen concentration (95% on threshold)				
<16 nM (%)	5	20	Fixed	
≥16 nM (%)	95	80	Fixed	
RFS			Weibull	[18]
AIC	10,783.76	11,729.69		
Intercept	3.42592	3.3832		
log(scale)	-0.0663	-0.0039		
OS			Weibull	
AIC	10,746.67	11,344.88		
Intercept	3.18265	3.1392		
log(scale)	-0.3309	-0.3295		
S1c. Endoxifen concentration (85% on threshold)				
<16 nM (%)	15	20	Fixed	
≥16 nM (%)	85	80	Fixed	
RFS			Weibull	
AIC	11,325.18	11,729.69		
Intercept	3.39738	3.3832		
log(scale)	-0.0245	-0.0039		
OS			Weibull	
AIC	10,651.92	11,344.88		
Intercept	3.18965	3.1392		
log(scale)	-0.3282	-0.3295		
S1d. Correction factor recurrence rate assumption				
<16 nM (%)	-	20	Fixed	
≥16 nM (%)	-	80	Fixed	
RFS			Weibull	[18]
AIC	10,526.35	11,729.69		
Intercept	3.4355	3.3832		
log(scale)	-0.0885	-0.0039		

Table 1 (continued)

Variable	Mean value TDM (SE)	Mean value tamoxifen SC (SE)	Distribution	References
OS			Weibull	
AIC	11,154.34	11,344.88		
Intercept	3.2161	3.1392		
log(scale)	-0.3234	-0.3295		
S2. RD costs				
A. RD -high	€ 11,990	€ 11,990	Gamma	[33]
B. RD- low	€ 3194	€ 3194	Gamma	
S3. Productivity loss				
RFS	€ 397	€ 397	NA	[10,35]
RD	€ 4477	€ 4477	NA	
Death	€ 4477	€ 4477	NA	
S4a. Alternative curve fit: Loglogistic				
RFS			Loglogistic	[18]
AIC	11,060.01	11,732.21		
Intercept	3.1388	3.0864		
log(scale)	-0.1572	-0.1249		
OS			Loglogistic	
AIC	10,895.21	11,354.41		
Intercept	2.9835	2.9484		
log(scale)	-0.4212	-0.4238		
S4b. Alternative curve fit: Lognormal				
RFS			Lognormal	[18]
AIC	11,055.81	11,720.28		
Intercept	3.2897	3.2222		
log(scale)	0.4914	0.5116		
OS			Lognormal	
AIC	10,937.21	11,397.70		
Intercept	3.1116	3.0674		
log(scale)	0.2460	0.2378		
S4c. Alternative curve fit: Exponential				
RFS			Exponential	[18]
AIC	11,056.43	11,727.71		
Intercept	3.4648	3.3875		
log(scale)	0	0		
OS			Exponential	
AIC	11,022.10	11,488.17		
Intercept	3.5119	3.4704		
log(scale)	0	0		

AIC; Akaike Information Criteria, GP; general practitioner, nM; nmol/L, OS; overall survival, RD; recurrent disease, RFS; recurrence-free survival, SC; tamoxifen standard care (without TDM intervention), SE; standard error, TDM; therapeutic drug monitoring-guided tamoxifen dosing.

Productivity costs

Productivity loss data were collected in TOTAM using the iMTA Productivity Costs Questionnaire and included short-term absence from paid work, presenteeism at paid work, and productivity losses at unpaid work as reported in the 28-day recall period.[35] Reported productivity losses as a result of long-term absenteeism starting before tamoxifen treatment, during primary cancer treatment, were excluded from the final estimate. Costs due to productivity losses at paid work were valued using the average hourly wage for women aged 55-60 (€ 27.18/hour) as provided by Statistics Netherlands (CBS). For unpaid work this was valued using the reference cost of informal care (€ 14.00/hour) provided by the Dutch guidelines for health economic evaluations.[30] A maximum loss of productivity was assumed (i.e. based on the average of reported productivity at baseline) for both RD and death. Productivity losses were included for all subjects up to the current Dutch retirement age of 67 years.

2.5 Sensitivity analyses

Probabilistic sensitivity analysis

A probabilistic sensitivity analysis is performed to assess the sensitivity of the results to the uncertainty surrounding all input parameters. Parameter uncertainty is expressed using as a distribution around the mean values with a corresponding standard error (SE). If SEs are not available an arbitrary percentage of the mean value is applied using 10% of the mean for fixed unit costs and 20% for health care resource use parameters, reflecting the expectation that resource use is more variable than unit costs. The proportions of patients receiving a tamoxifen dose escalation (i.e. in TDM-guided adjuvant tamoxifen dosing) are varied using a Dirichlet distribution. The uncertainty surrounding the extrapolated survival curves is estimated using a Cholesky correlation matrix.[36] In each model simulation a random value is drawn for each parameter from its corresponding distribution. Simulations are repeated 1,000 times and cost-effectiveness outcomes are presented in a cost-effectiveness (CE)-plane. The probability of cost-effectiveness is represented by the percentage of simulations below the applied willingness to pay (WTP) threshold. The probability of cost-effectiveness is assessed at a WTP of €20,000, in line with the Dutch standard.[37,38]

2.6 Scenario analyses

In addition to the base case analysis, several scenario analyses were performed to assess the sensitivity of the results to alternative values for model input parameters. In scenario 1 (S1), three different sets of survival curves were used: an absolute increase of 20% (S1a; 100% on target), 15% (S1b; 95% on target) or 5% (S1c; 85% on target) of the patients in the whole population with an endoxifen level ≥16 nM, in contrast to the absolute increase of 10% (90%

on target) who benefits as in the base case. S1a, therefore, describes the maximal attainable effectiveness of TDM for another distribution of patients below the predefined endoxifen threshold of 16 nM. S1b represents of a scenario where the TDM threshold is set at a lower value. S1c describes a scenario in which TDM is less effective at improving endoxifen serum concentrations. Analogous to the base case, it is assumed that that the effect of tamoxifen below this threshold is equal to control. A fourth set of survival curves (S1d) was constructed using the survival curve of S1a where all patients are above the threshold in combination with the assumption that an improved recurrence rate can be found in patients with endoxifen levels above 16 nM (Hazard Ratio (HR) 0.74).[6] Due to uncertainty in duration of tamoxifen treatment in the Madlensky paper, the hazard ratio assumption of 0.74 was not included in the base case scenario. After applying the HR, a curve is obtained representing the RFS below the 16 nM threshold.[6] This survival curve is again combined with the curve of S1a to construct a final curve where 90% is above the 16 nM threshold and 10% is below based on data obtained in the TOTAM trial.

In scenario 2 (S2) two different per-cycle cost estimates for RD are used, being either almost twice as high (S2a) or almost half the original estimate (S2b) per cycle for the base case analysis.[33] Parameter estimates are informed by the literature which describes costs estimates of costs experienced after the first year of metastases (S2a) and costs experienced during the first year of recurrence (S2b). Using these alternative cost estimates, the influence of RD costs on cost-effectiveness is assessed. The costs estimates are also informed by the costs for local and distant recurrence. In this situation an unweighted average was applied.[33] For the low estimate, costs are based on local recurrence during the first year of disease. For the high estimate, experienced costs are based on the metastatic disease after the first year.[33]

In scenario 3 (S3), costs due to productivity losses were included to approximate a societal perspective. Lastly, scenario analyses were performed based on alternative parametric functions for the extrapolations of RFS and OS, using loglogistic (S4a), lognormal (S4b), and exponential (S4c) parametric functions for both RFS and OS.

3. Results

3.1 Base case and probabilistic sensitivity analysis

The base case results are presented in **Table 2** and show a total increase in QALYs and LYs for TDM of 0.53 and 0.40, respectively. Total, lifetime costs were € 32,893 for TDM-guided adjuvant tamoxifen therapy and € 39,524 for standard tamoxifen (20 mg once daily, without TDM). In terms of cost-effectiveness, TDM dominates standard tamoxifen due to both positive

incremental QALYs and negative incremental costs (i.e. TDM is cost saving in comparison to standard tamoxifen). An explanation for this result is the lower risk of recurrent disease – and hence, lower costs associated with this stage of disease – with an adequate endoxifen level. Probabilistic sensitivity analysis results for the base case model are presented in the CE-plane in **Figure 2**. The probability that TDM-guided adjuvant tamoxifen therapy is cost-effective relative to standard tamoxifen is 92% at a WTP threshold of € 20,000 per QALY gained.[38]

3.2 Scenario analyses endoxifen threshold (S1a, S1b and S1c)

S1a assessed the effect of assuming that all patients achieve endoxifen concentrations ≥16 nM. These survival curves aimed to estimate the maximum obtainable effect of TDM for tamoxifen treatment following our modelling approach. This scenario results in a total of 1.03 incremental QALYs, with a dominant ICER per QALY gained for TDM-guided adjuvant tamoxifen therapy. Deterministic results are presented in **Table 2** and a graphic representation is presented in **Figure 3**. In both the base case scenario and most of the scenario analyses the intervention TDM dominates. Scenario 1b aims to provide an estimate of the cost-effectiveness of TDM when using a lower threshold of 14 nM. According to our results TDM-guided adjuvant tamoxifen therapy is dominant given this scenario as a result of an increase in LYs gained. Scenario 1c provides an opposing scenario where the effectiveness of TDM improving the endoxifen concentrations is lower than found by the TOTAM study. This scenario illustrates only 85% of the population achieving endoxifen concentrations ≥16 nM after TDM, rather than the 90% of the population in the base case. This results in an incremental increase in QALYs of 0.71 and an ICER of € 2,177 per QALY gained which means TDM-guided adjuvant tamoxifen therapy would induce higher costs though remaining well under the WTP threshold.

3.3 Scenario analyses costs in progressed disease (S1d)

A third set of survival curves (S1d) is constructed using the survival curve of S1a and the hazard ratio of 0.74 for recurrence rate in patients with endoxifen levels above 16 nM based on the Madlensky data. This results in an increase in incremental QALYs of 0.76 and a dominant ICER for TDM-guided adjuvant tamoxifen therapy (**Table 2**).

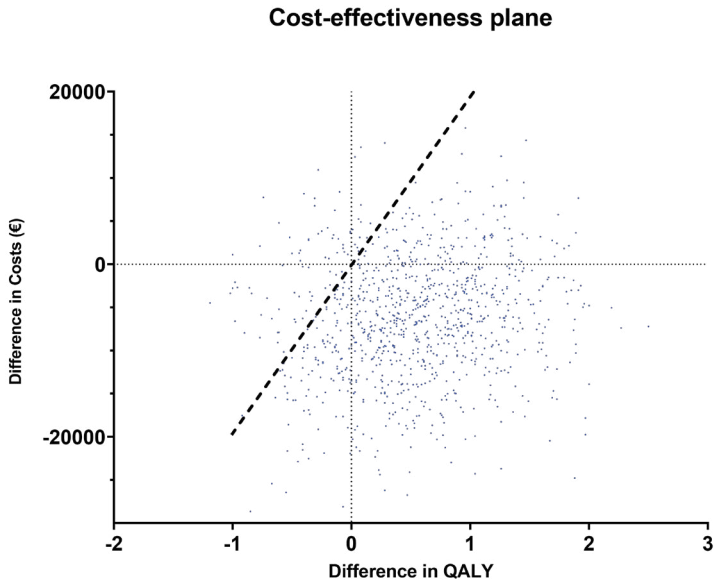

Figure 2 – Cost-effectiveness plane of the probabilistic sensitivity analysis for base case model in the cost-effectiveness analysis of therapeutic drug monitoring of tamoxifen adjuvant therapy versus standard of care (without TDM intervention). Straight line indicates the Dutch conservative willingness to pay threshold of €20,000. All model simulations below this threshold are considered cost-effective from a healthcare perspective.

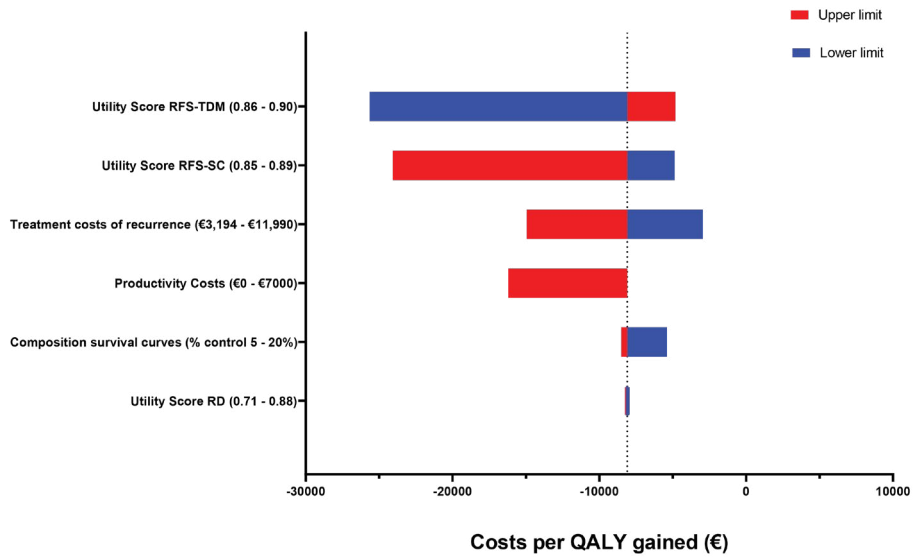

Figure 3 – Tornado diagram illustrating the effect of alternative parameter values in a deterministic sensitivity analysis on the incremental cost-effectiveness ratio for therapeutic drug monitoring of tamoxifen adjuvant therapy versus standard of care (without TDM intervention). RFS; recurrence-free survival, RD; recurrent disease.

3.4 Scenario analyses costs in progressed disease (S2a and S2b)

The second scenario included two alternative estimates, a high and a low estimate, for the per-cycle health care costs in RD. The results of these scenario's indicated that for both the high and the low estimate cost-effectiveness remains TDM-guided adjuvant tamoxifen therapy dominated, despite the large difference in total costs between the alternatives. These results illustrated that the costs associated with recurrence of breast cancer are an important driver of the cost-effectiveness of TDM-guided adjuvant tamoxifen therapy. Given that TDM aims to improve treatment effectiveness and therefore is expected to reduce recurrence, assuming higher costs for RD improves the cost-effectiveness of TDM relative to standard tamoxifen (**Table 2**).

3.5 Scenario health care perspective (S3)

In this scenario total incremental costs are increased to -€ 9549, resulting in an increasingly dominant ICER per QALY gained for TDM-guided adjuvant tamoxifen therapy. In this scenario, 30% of the difference in incremental costs can be attributed to the reduction in lost productivity resulting from fewer transitions to RD over time with TDM-guided dose individualization (**Table 2**).

3.6 Alternative curve fit (S4a-c)

In these scenarios the costs and effects of TDM-guided adjuvant tamoxifen therapy are presented when using alternative curve fits for survival extrapolation. Importantly, in all scenarios TDM-guided adjuvant tamoxifen therapy resulted in higher costs for resource use, and lower costs for treatment costs in RD and RFS. The difference in QALYs gained was very small and the increment in QALYs is mainly caused by the difference in life expectancy, because more people stayed longer in RFS (**Table 2**).

Table 2 - Results for base case and scenario analyses in the cost-effectiveness analysis of TDM-guided tamoxifen dosing versus the standard care (without TDM).

	Total costs	Total LYs	Total QALYs	Incremental costs	Incremental LYs	Incremental QALYs	ICER (costs QALY gained)
Base case							
TDM	€ 32,893	19.84	16.51	-€ 6631	0.40	0.53	TDM dominates
SC	€ 39,524	19.45	15.98				
S1a. 100% on threshold (20% benefits)							
TDM	€ 26,369	20.41	17.01	-€ 13,155	0.96	1.03	TDM dominates
SC	€ 39,524	19.45	15.98				
S1b. 95% on threshold (15% benefits)							
TDM	€ 28,975	20.01	16.67	-€ 10,549	0.57	0.69	TDM dominates
SC	€ 39,524	19.45	15.98				
S1c. 85% on threshold (5% benefits)							
TDM	€ 41,076	20.09	16.69	€ 1552	0.64	0.71	€ 2177
SC	€ 39,524	19.45	15.98				
S1d. Correction factor recurrence rate assumption							
TDM	€ 26,573	20.10	16.76	-€ 12,951	0.65	0.76	TDM dominates
SC	€ 39,524	19.45	16.00				
S2a. RD high							
TDM	€ 37,786	19.84	16.53	-€ 7966	0.40	0.53	TDM dominates
SC	€ 45,752	19.45	16.00				
S2b. RD low							
TDM	€ 14,359	19.84	16.53	-€ 1573	0.40	0.53	TDM dominates
SC	€ 15,932	19.45	16.00				
S3. Productivity loss							
TDM	€ 70,531	19.84	16.53	-€ 9549	0.40	0.53	TDM dominates
SC	€ 80,080	19.45	16.00				
S4a. Alternative curve fit: Loglogistic							
TDM	€ 34,716	17.36	20.83	-€7511	0.34	0.49	TDM dominates
SC	€ 42,227	16.86	20.49				
S4b. Alternative curve fit: Lognormal							
TDM	€ 34,997	17.96	21.56	-€ 7429	0.34	0.50	TDM dominates
SC	€ 42,426	17.47	21.22				
S4c. Alternative curve fit: Exponential							
TDM	€ 21,917	18.14	21.72	-€ 12,397	0.34	0.52	TDM dominates
SC	€ 34,314	17.62	21.38				

ICER; incremental cost-effectiveness ratio, LYs; life years, QALYs; quality-adjusted life years, S; Scenario, SC; tamoxifen standard care (without TDM intervention), TDM; therapeutic drug monitoring-*guided tamoxifen dosing*.

4. Discussion

Our study is the first CEA of TDM-guided dose individualization of tamoxifen in patients with early breast cancer that is based on data obtained from a clinical trial. The results indicated that TDM strategy is cost-effective for patients with early ER+ breast cancer compared to standard tamoxifen. Base case results showed that TDM-guided adjuvant tamoxifen therapy is dominant over standard tamoxifen due to incremental QALYs of 0.53 and cost savings of € 6631.

In a previous CEA, TDM of tamoxifen resulted in a similar outcome regarding the cost-saving potential of TDM when also assuming an endoxifen threshold of 16 nM.[11] In contrast to the current findings, the analysis by van Nuland *et al.* indicated an increment in QALYs of 0.0115 whereas our results showed a much higher estimate, including age-adjustment.[11] However, considerable differences between both studies can be identified in terms of methodology and data used to inform input parameters. In addition to using trial data from TOTAM and different literature sources to inform the current study, the main differences involve the assumptions for OS, and a different approach to the modelling of the clinical benefit of TDM. Regarding assumptions for OS, an important difference is that the current study assumed a difference in OS between standard care and TDM guided tamoxifen dosing, whereas van Nuland *et al.* assumed equal OS between treatments. Regarding the modelling of the clinical benefit of TDM-guided adjuvant tamoxifen therapy, for the current study survival curves were constructed from patients who received about 5 years of tamoxifen or no tamoxifen based on the assumption that patients with subtherapeutic endoxifen levels have the same OS and RFS as patients who were not treated with tamoxifen and represent 20% of the patients who were treated with standard tamoxifen. In contrast, van Nuland *et al.* applied an HR from Madlensky *et al.* to obtain RFS for high and low endoxifen levels. However, this HR did not represent patients who were treated with tamoxifen for a period approximating 5 years (Madlensky, personal communication) and therefore its applicability to the EBCTCG data is questionable.

The strengths of this economic evaluation included the availability of prospectively collected data by TOTAM study, and therefore its representative description of the Dutch breast cancer population. Further, until now there are no published QoL scores or productivity losses for Dutch breast cancer patients treated with tamoxifen in the adjuvant setting.

This analysis had some limitations. Foremost, these results are based on some important assumptions regarding the exposure-response relationship of endoxifen affecting the potential effectiveness of TDM and should, therefore, be interpreted carefully. Firstly, the improvement seen in the proportion of patients considered above the threshold is based on a threshold value

for endoxifen of 16 nM. Also, the expected effect below this threshold is assumed to be equal to control in our model. However, based on the Madlensky data it was not possible to construct overall survival rates, because of uncertainty in duration of treatment with tamoxifen. Scenario 1d, which assumed that women with a endoxifen level ≥16 nM had a 26% lower recurrence rate, showed an enhanced dominant effect for TDM than in the base case scenario. Despite the initial evidence for an exposure-response relationship, two later prospective clinical studies reported no associations between endoxifen plasma concentrations and clinical outcome.[39,40] However, based on the considerable amount of criticism published following these reported results, no affirmative prospective evidence exists on the complete absence of this relationship.[41–43] In addition to this, if a threshold exists, currently no agreement exists on the exact value of this threshold as two other thresholds have been suggested at a lower value of 14 nM and 9 nM.[44–46] Currently, the proportion of breast cancer patients with subtherapeutic endoxifen levels are determined and incorporated in the model based on the threshold first reported by Madlensky and colleagues of 16 nM.[6] Importantly, all issues of uncertainty in assumptions were included in the sensitivity analysis. If the true value of this threshold is lower (S1a-c), this could imply that a lower proportion of patients' treatment is categorized as subtherapeutic before TDM. Because the method of survival extrapolation was based on these proportions, this could have implications for the effect of TDM, as well as lower its cost-effectiveness. The uncertainty regarding the effectiveness of tamoxifen below the current threshold of 16 nM is an important source of uncertainty. Yet, the cost-effectiveness model can be adjusted to re-evaluate the cost-effectiveness of TDM-guided adjuvant tamoxifen therapy in the light of new developments.

A prospective randomized controlled TDM study could provide clarity. However, such a trial is probably not feasible since it would require many thousands of patients to participate and a follow-up period of more than a decade.[41] To break out of this potential dead end, physicians are encouraged to implement TDM – a feasible intervention– in the meantime in clinical practice, pending further prospective data.[22]

Further limitations pertained to data availability and uncertainty in underlying assumptions. First, the lack of prognostics on the influence of TDM-guided adjuvant tamoxifen therapy on RFS and OS based on prospective clinical trials required additional assumptions and computation of the expected effect, introducing additional uncertainty.[41]

A final important consideration was based on the incidence at which women with ER+ early breast cancer are treated with tamoxifen for a total of 5 years. As described in the introduction, post-menopausal women often start treatment with tamoxifen and switch to an aromatase inhibitor (i.e. letrozole, anastrozole or exemestane) after 2 to 3 years of treatment.[47] Considering

a significant proportion of women with breast cancer are post-menopausal, this implicates a smaller role for TDM in this subgroup of patients, as compared to breast cancer patients who are treated with tamoxifen for 5 years.

5. Conclusion

In conclusion, the current economic evaluation aimed to determine the cost-effectiveness of TDM for adjuvant tamoxifen therapy – resulting in dose optimization or a switch in pharmacotherapy to an aromatase inhibitor – compared to the current standard of care in the Netherlands. Our results indicated that TDM-guided adjuvant tamoxifen therapy dominated standard tamoxifen in terms of cost-effectiveness, gaining QALYs (0.53) life years (0.40) and saving costs (€6631). The results of this economic evaluation indicate that TDM provides good value for money, which may support policy makers at both the hospital, insurer and Dutch national level in decisions on the routine implementation of TDM for tamoxifen adjuvant therapy in the clinical setting.

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Supplementary Information

Appendix I - Baseline characteristics of participants TOTAM trial (n=145).

Characteristic	N (%) or Median (interquartile range)	
Age, years	57.0	(46.0 – 66.0)
BMI, $kg \cdot m^{-2}$	25.9	(22.9 – 28.7)
Tumor stage		
T1	67	(46)
T2	67	(46)
T3/4	11	(8)
Nodal stage		
N0	79	(55)
N1	49	(34)
N2	12	(8)
N3	5	(3)
Histologic classification		
Ductal adenocarcinoma	100	(69)
Lobular adenocarcinoma	34	(23)
Mucinous carcinoma	4	(3)
Other	7	(5)
Histologic grade		
1	20	(14)
2	94	(65)
3	31	(21)
ER status		
Positive	145	(100)
PR status		
0%	16	(11)
1-10%	14	(10)
>10%	115	(79)
HER2 status		
Positive	13	(9)
Surgery		
Mastectomy	63	(43)
Lumpectomy	82	(57)
Radiotherapy		
Yes	106	(73)
Neo-adjuvant chemotherapy		
Yes	28	(19)
Adjuvant chemotherapy		
Yes	32	(22)

BMI, body mass index; ER, estrogen receptor; HER2, Human epidermal growth factor receptor 2; PR, progesterone receptor.

CHAPTER 7

Tamoxifen use and potential effects on liver parenchyma: a long-term prospective transient elastographic evaluation

C. Louwrens Braal, Rob J. de Knegt, Agnes Jager, Stijn L.W. Koolen, Ron H.J. Mathijssen, Karel Eechoute

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Abstract

Aim

Tamoxifen is a commonly prescribed drug in both early and metastatic breast cancer. Prospective studies in Asian populations demonstrated that tamoxifen-related liver steatosis occurred in more than 30% of the patients within 2 years after start of treatment. No well-designed prospective studies on potential tamoxifen-related liver steatosis have been conducted in Caucasian patients so far. Therefore, our prospective study aimed to assess the incidence of tamoxifen-related liver steatosis for a period of 2 years in a population of Caucasian breast cancer patients treated with tamoxifen.

Methods

Patients with an indication for adjuvant treatment with tamoxifen were included in this study. Data was collected at 3 months (T1) and at 2 years (T2) after start of tamoxifen treatment. For the quantification of liver steatosis, patients underwent liver stiffness measurement by transient elastography with simultaneous controlled attenuation parameter determination using the FibroScan.

Results

A total of 95 Caucasian breast cancer patients were included in this evaluation. Liver steatosis was observed in 48% and 51% of the patients at T1 and T2, respectively. No clinically relevant increase in liver steatosis was observed during the treatment period of 2 years with tamoxifen (median CAP 243 ± 49 dB/m (T1) and 253 ± 55 dB/m (T2), respectively, p=0.038).

Conclusion

In conclusion, in this prospective longitudinal study in Caucasian breast cancer patients, no clinical relevant alterations in liver steatosis in terms of CAP values and liver/lipid parameters were observed after 2 years of tamoxifen treatment. This study therefore demonstrates an absence of severe tamoxifen-related liver toxicity such as steatosis, fibrosis or cirrhosis. As recent data advocate longer adjuvant treatment periods, these data give no reason to doubt about long-term safety.

Tamoxifen is a commonly prescribed drug in both early stage and metastatic breast cancer. (1) Although the toxicity profile is relatively mild, tamoxifen use is associated with development of fatty liver disease. Prospective studies in Asian populations demonstrated that tamoxifen-related liver steatosis occurred in more than 30% of the patients within 2 years after start of treatment. (2,3) The concept of primary liver steatosis (related to metabolic risk factors) and secondary (e.g. drug use) can intermingle in clinical practice. Earlier, we described a Caucasian patient who developed a severe stage of liver steatosis, six months after starting with daily tamoxifen treatment. (4) Despite of these data, no well-designed prospective studies on potential tamoxifen-related liver steatosis have been conducted in Caucasian patients so far.

Considering that the majority of patients with early stage breast cancer have a good prognosis, preventing severe long-term side effects, such as fatty liver disease, is highly relevant. More so, as recent data suggest a clinical benefit of extending tamoxifen therapy to 10 years especially in premenopausal, young patients. (5,6) Our prospective, observational study aimed to assess the incidence of tamoxifen-related liver steatosis for a period of 2 years in a population of Caucasian breast cancer patients treated with tamoxifen.

Caucasian patients with an indication for adjuvant treatment with tamoxifen were included in this study. Patients who had longer than 3 months tamoxifen treatment or started with a dose higher than 20 mg once daily and patients with a non-Caucasian ethnicity, were non-eligible for inclusion. The study was approved as a secondary endpoint by the Local Ethics Committee (Erasmus MC, Rotterdam) and was registered in the Dutch Trial Registry (www.trialregister.nl; NL6918). (7) Written informed consent was obtained from all patients participating in this study. All patients were evaluated for a period of 2 years after start of tamoxifen therapy. Data was collected at 3 months (T1) and at 2 years (T2) after start of tamoxifen treatment, during 2 outpatient visits, including blood sampling for liver function (*e.g.* ALT, AST, GGT, ALP and TB) and lipid spectrum.

For the quantification of liver steatosis, patients underwent liver stiffness measurement (LSM) by transient elastography with simultaneous controlled attenuation parameter (CAP) determination using the FibroScan Touch 502 software version C 3.2 (Echosens, Paris, France). Experienced operators performed all FibroScan examinations as per the manufacturer's recommendations. Primary endpoint in this observational study was the alteration in liver steatosis two years after start with tamoxifen treatment compared with baseline measurements (T1). Statistical differences between groups or paired data points were calculated by appropriate parametric or non-parametric tests. All tests were 2-sided and $p<0.05$ was considered statistically significant.

5 percent of our patients were excluded from analysis due to loss to follow-up (not for a medical reason) and therefore, a total of 95 Caucasian breast cancer patients (age 55.9 ± 12.0 years and BMI 25.5 ± 3.8 $kg.m^{-2}$) were included in this evaluation and all 190 FibroScan assessments were performed and eligible for analyses. Liver steatosis (defined by a CAP >248 dB/m; according to validation report EchoSens) was observed in 48% and 51% of the patients at T1 and T2, respectively. No clinically relevant increase in liver steatosis was observed during the treatment period of 2 years with tamoxifen (median CAP 243 ± 49 dB/m (T1) and 253 ± 55 dB/m (T2), respectively, p=0.038). Also, no alterations were observed in fibrosis scores between 3 months and 2 years of treatment with tamoxifen (4.6 ± 1.4 kPa (T1) and 4.4 ± 1.4 kPa (T2), respectively, p>0.05). Results of the FibroScan assessments are presented in Table 1.

Liver fibrosis, defined by LSM >7.0 kPa, was diagnosed in 9 patients (9%) at T1 and in 6 patients (6%) at T2, respectively. In case of a suspicion of severe liver fibrosis (>9.5 kPa), patients were referred to a hepatologist for a second opinion. In all cases, no diagnosis of hepatitis was made by the hepatologist. Lifestyle advices (limited alcohol intake, exercise, diet etcetera) were given and follow-up for liver fibrosis was advised. These consultations did not lead to dose alterations, interruptions or discontinuations. Furthermore, the liver parameters were stable over time in these patients. A statistically significant difference was found between biochemistry parameters at 3 months compared with 2 years of tamoxifen treatment, including an increase in mean AST, triglycerides, Apo-B and glucose; and a decrease in mean total bilirubin, ALP, LDL. No differences were observed between T1 and T2 for weight and body mass index (BMI). In our population, 13/95 (14%) patients used drugs for diabetes mellitus, hypertension or hypercholesterolemia. No association between those drugs and liver steatosis at T1 or T2 was found. Also, liver fibrosis stiffness score was stable over time in patients with steatosis compared with patients without steatosis. In general, patients with a CAP >248 dB/m were characterized by a higher: BMI (26.9 ± 3.7), age (58.9 ± 11.6) or triglycerides levels (1.8 ± 0.8) compared with the population below 248 dB/m. These findings clearly indicate "lifestyle factors" as major risk factor for the development of liver steatosis. Main parameters of the population tamoxifen users are depicted in **Table 1**.

Previously, a prospective observational study in 175 Chinese patients demonstrated a cumulative incidence of liver steatosis of 38% after 2 years of tamoxifen use. (3) However, to the best of our knowledge, this is the first prospective, observational study to investigate the potential effect of tamoxifen on liver steatosis in a Caucasian population. Both studies show no clinically relevant alterations of liver enzymes after extensive tamoxifen use during 2 years. (3) In contrast to an Asian population, no increase in liver steatosis was observed in our Caucasian population.

Table 1 - Main parameters of evaluable patients after 3 months (T1) and 24 months (T2) after start with tamoxifen treatment (*n*=95).

	T1 (*n*=95) **N (%) or Mean ± SD**	**T2 (*n*=95)** **N (%) or Mean ± SD**	**P-value**
Age, years	55.9 ± 12.0	-	-
Weight, kg	72.1 ± 10.8	72.5 ± 10.5	0.28
BMI, $kg.m^{-2}$	25.5 ± 3.8	25.7 ± 3.8	0.22
Medication			
DM, hypertension, hypercholesterolemia	13 (14)	n.a.	
Liver steatosis			
CAP (dB/m)	243 ± 49	253 ± 55	0.038*
Steatosis (CAP >248[a] dB/m), %	46 (48)	48 (51)	-
Liver fibrosis			
LSM (kPa)	4.6 ± 1.4	4.4 ± 1.4	0.9
Fibrosis (>7.0 kPa), %	9 (10)	6 (6)	-
Biochemistry			
ALT, U/L	20.7 ± 7.6	20.7 ± 9.7	0.9
AST, U/L	22.6 ± 5.3	24.7 ± 6.2	<0.001***
GGT, U/L	32.0 ± 26.0	30.2 ± 25.2	0.34
Total bilirubin, µmol/L	5.6 ± 2.6	4.7 ± 2.4	<0.001***
ALP, U/L	63.9 ± 18.7	58.7 ± 18.7	0.004*
Triglycerides, mmol/L	1.5 ± 0.7	1.9 ± 1.5	0.001**
Total Cholesterol, mmol/L	4.9 ± 1.0	4.8 ± 0.9	0.59
HDL, mmol/L	1.7 ± 0.5	1.8 ± 0.5	0.02*
LDL, mmol/L	2.9 ± 0.9	2.6 ± 0.7	<0.001***
APO-A1, g/L	1.7 ± 0.3	1.7 ± 0.3	0.09
APO-B1, g/L	0.8 ± 0.2	0.8 ± 0.2	0.02*

*[a],CAP value based on validation reports of the manufacturer of the FibroScan Touch 502; Apo-A, apolipoprotein A; Apo-B, apolipoprotein B; AST, aspartate aminotransferase; ALT, alanine aminotransferase; ALP, alkaline phosphatase; BMI, body mass index; CAP, controlled attenuation parameter; DM, diabetes mellitus; GGT, gamma-glutamyltransferase; HDL, high density lipoprotein; LDL, low density lipoprotein. LSM; liver stiffness measurement. T1; assessment 3 months after start with tamoxifen treatment, T2; assessment 2 years after start with tamoxifen. *, p-value <0.05; **, p-value <0.01; ***, p-value <0.001.*

The mechanism of development of fatty liver disease in (Asian) tamoxifen users is not fully elucidated, although there are indications of disturbance of the lipid homeostasis due to antagonism of the estrogen receptor. (8) The fibroscan was performed 3 months after initiation of tamoxifen due to practical considerations. Generally, development of liver steatosis progresses slowly, however, a rapid development (within a few months after tamoxifen initiation) may not be excluded in rare cases. In line with historical data, 48% of our patients were diagnosed with liver steatosis at T1. (9) Amongst a study population in the United States, liver steatosis prevalence was low in Asian patients (18%) and high amongst Mexican Americans (48%). (10) Therefore, apart from traditional risk factors ("lifestyle") and adaption of the Western culture, ethnic factors seem to play a significant role in the development of liver steatosis. The absence of lifestyle-related risk factors (e.g. hip-waist circumference and alcohol consumption) is a minor limitation of our study. Also a follow-up of 2 years is limited to identify serious complications of steatosis, such as non-alcoholic steatohepatitis (NASH) or liver fibrosis. The data of this study may not be generalizable to other populations that are more ethnically diverse (non-Caucasians) or have a higher mean body mass index.

In conclusion, in this prospective longitudinal study in Caucasian breast cancer patients, no clinical relevant alterations in liver steatosis in terms of CAP values and liver/lipid parameters were observed after 2 years of tamoxifen treatment. This study therefore demonstrates an absence of severe tamoxifen-related liver toxicity such as steatosis, fibrosis or cirrhosis. As recent data advocate longer adjuvant treatment periods, these data give no reason to doubt about long-term safety.

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CHAPTER 8

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.

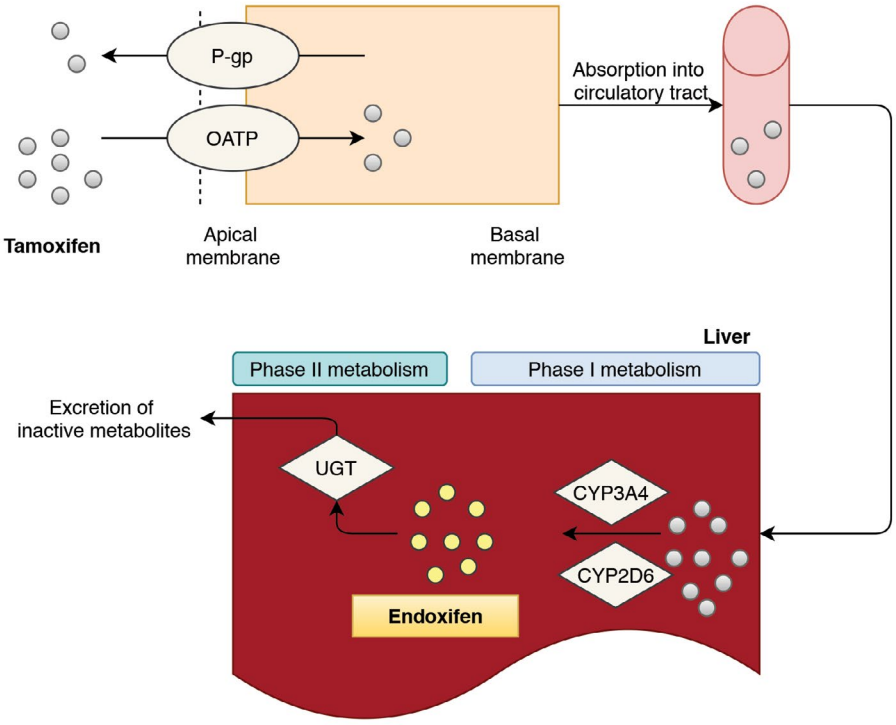

Figure 1 – Main metabolic pathway of tamoxifen. After absorption tamoxifen is metabolized mainly by CYP2D6 in its active metabolite endoxifen. Tamoxifen relies on phase II metabolism before it can be excreted from the body. Endoxifen is ultimately glucuronidated into endoxifen-glucuronide mainly by UGTs. 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.

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.

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.

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_{0-24h} (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_{0-24h} (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).

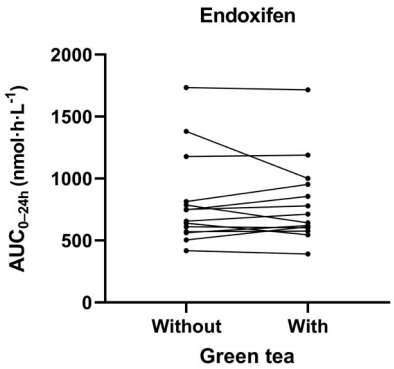

Figure 2 – Pharmacokinetics of endoxifen without and with concomitant green tea supplements.

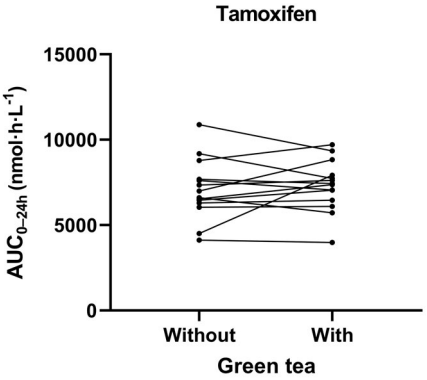

Figure 3 – Pharmacokinetics of tamoxifen without and with concomitant green tea supplements.

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.

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 9

Influence of probenecid on endoxifen systemic exposure in breast cancer patients on adjuvant tamoxifen treatment

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Abstract

Introduction: In breast cancer patients treated with the antiestrogen tamoxifen, low concentrations of the active metabolite endoxifen are associated with disease recurrence. We hypothesized that we could increase endoxifen concentrations by induction of its formation and inhibition of its metabolism by co-administration of probenecid.

Methods: We conducted a crossover study and measured endoxifen concentrations in patients on steady state tamoxifen monotherapy and after 14 days of combination treatment with probenecid. Eleven evaluable patients were included.

Results: Treatment with tamoxifen and probenecid resulted in a 26% increase of endoxifen area under the plasma-concentration-time curve from 0 to 24 hours ($AUC_{0\text{-}24h}$) compared to tamoxifen monotherapy (95% CI 8 to 46%; $P < 0.01$), while the maximum observed endoxifen concentration increased with 24% (95% CI 7 to 44%; $P < 0.01$). The metabolic ratio of endoxifen to tamoxifen increased with 110% (95% CI 82 to 143%; $P < 0.001$) after the addition of probenecid.

Conclusions: Probenecid resulted in a clinically relevant increase of endoxifen concentrations in breast cancer patients treated with adjuvant tamoxifen. This combination therapy could provide a solution for patients with a CYP2D6 poor metabolizer phenotype or endoxifen concentrations below the threshold despite earlier tamoxifen dose.

Introduction

Tamoxifen is a selective estrogen receptor modulator, frequently used in the adjuvant treatment of estrogen receptor positive breast cancer [1]. It is a prodrug that undergoes metabolization to its most active metabolite endoxifen by cytochrome P450 (CYP) 2D6 and 3A4 enzymes [2]. Despite five years of adjuvant treatment with tamoxifen, one third of patients develops disease recurrence within 15 years [3]. Importantly, systemic endoxifen concentrations are correlated with breast cancer relapse. Patients with endoxifen concentrations above the therapeutic threshold were found to have a 26% lower chance of disease recurrence [4].

The therapeutic threshold value for endoxifen has been defined at 14 to 16 nM, which is achieved by 75 to 80% of tamoxifen users [4, 5]. This large variance in endoxifen concentrations is mainly the result of interpatient variability in CYP2D6 activity, due to a high prevalence of functional polymorphisms in the *CYP2D6* gene [6, 7]. Enzyme activity is based on the presence of functional alleles. Patients with an extensive metabolizer phenotype have normal CYP2D6 activity, whereas intermediate metabolizers (IM) and poor metabolizers (PM) have reduced and little or no enzyme function, respectively. Hence, the biotransformation of tamoxifen to endoxifen is compromised in patients with an IM and – to a greater extent – a PM CYP2D6 phenotype. This results in lower endoxifen plasma concentrations compared with CYP2D6 extensive metabolizers [8]. While patients with an IM CYP2D6 phenotype usually reach therapeutic endoxifen concentrations after tamoxifen dose escalation, this is rarely the case for patients with a PM CYP2D6 phenotype. This subgroup is consequently more prone to disease recurrence [5].

Therefore, we sought a solution to increase systemic endoxifen exposure in this population by interfering with tamoxifen metabolism. After endoxifen formation by CYP2D6 and 3A4 (phase 1 metabolism), endoxifen undergoes glucuronidation to the inactive endoxifen-glucuronide by UDP-glucuronosyltransferases (UGTs) in order to be excretable [9, 10]. Aside from the impact of CYP activity on endoxifen concentrations, it has been demonstrated that its concentration is also influenced by functional UGT variants [9].

We hypothesized that administration of the CYP3A4 inducer and pan-UGT inhibitor probenecid would result in increased endoxifen concentrations by a mechanism of a twofold nature. Namely, by means of induction of tamoxifen to endoxifen transformation and inhibition of endoxifen glucuronidation (**Figure 1**). Probenecid is a uricosuric agent, nowadays seldom used in the treatment of gout [11]. It has a mild and predictable toxicity profile (i.e. gastrointestinal complaints, headache and rash), which does not overlap with that of tamoxifen [12]. Probenecid has already been demonstrated to alter drug exposure by both CYP3A4 induction and UGT inhibition in

several *in vitro* and clinical studies [13-17]. Here, we report the results of a prospective crossover study on the influence of probenecid on endoxifen concentrations in breast cancer patients treated with adjuvant tamoxifen with an impaired CYP2D6 phenotype.

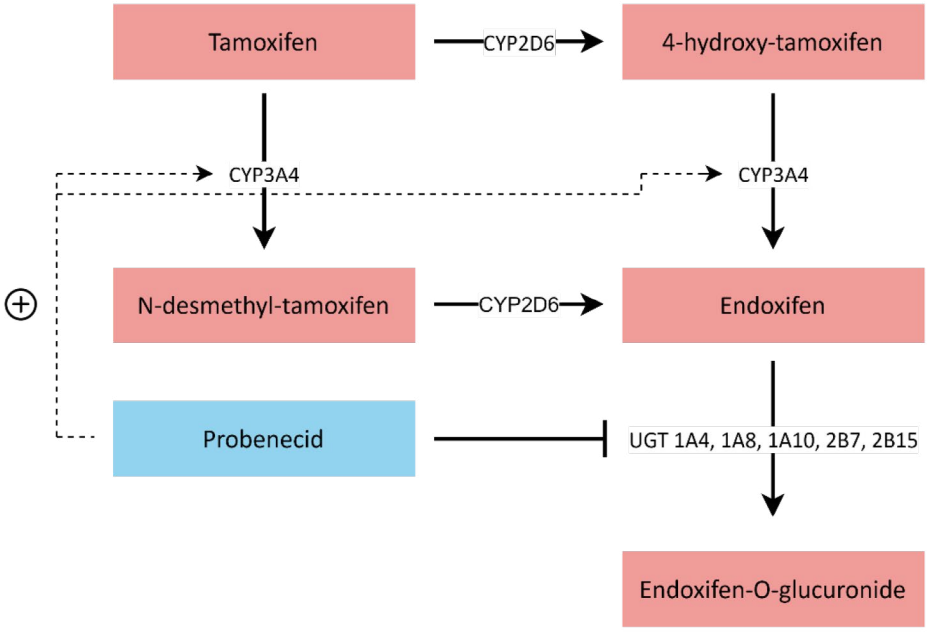

Figure 1 – Tamoxifen metabolism and hypothesized mechanism of CYP3A4 induction and UGT inhibition by probenecid. After administration, tamoxifen is metabolized to N-desmethyl-tamoxifen and 4-hydroxy-tamoxifen, mainly by CYP3A4 and CYP2D6, respectively. Next, N-desmethyl-tamoxifen and 4-hydroxy-tamoxifen are metabolized to endoxifen, mainly by CYP2D6 and CYP3A4, respectively. Endoxifen gets glucuronidated by UGTs to the inactive endoxifen-glucuronide. CYP: Cytochrome P450; UGT: UDP-glucuronosyltransferase.

Materials and Methods

Study design

The primary objective of this trial was to compare the area under the plasma-concentration-time curve from 0 to 24 hours ($AUC_{0\text{-}24h}$) of endoxifen with and without concomitant use of probenecid. A relative difference in $AUC_{0\text{-}24h}$ of endoxifen of at least 25% was considered clinically relevant [18]. Assuming a standard deviation of the difference of 25%, a total of 11 evaluable patients were required to detect a difference, given 90% power and a two sided alpha of 0.05 [19].

Secondary objectives were to compare the $AUC_{0\text{-}24h}$ of tamoxifen, the maximum observed plasma concentration (C_{max}) of endoxifen and tamoxifen and the $AUC_{0\text{-}24h}$ based metabolic

ratios endoxifen to tamoxifen, n-desmethyl-tamoxifen (NDM) to tamoxifen, endoxifen to 4-OH-tamoxifen (4-OH), 4-OH to tamoxifen, endoxifen to NDM and 4beta-hydroxycholesterol (4β-OHC) to cholesterol with and without concomitant use of probenecid. Adverse events were graded using the Common Terminology Criteria for Adverse Events version 5.0 (CTCAEv.5, National Cancer Institute, Bethesda, MD).

We conducted a one-way crossover study consisting of two phases. All patients entered the study on tamoxifen monotherapy and crossed over to combination treatment with probenecid after seven days, which lasted for 14 days. Probenecid (Biokanol Pharma GmbH, Rastatt, Germany) was administered at a dose of 1000 mg twice daily. Tamoxifen was administered once daily at a fixed dose of 20 mg according to standard of care or 40 mg because of prior dose escalation due to endoxifen concentrations below the threshold. In order to ensure steady state concentrations, medication adherence was assessed from three months before start until end of study. At the end of each phase, patients were hospitalized for 24 hours after drug administration to obtain 14 blood samples at predefined time points for pharmacokinetic analysis. Blood samples were processed to plasma and stored at -80°C until analysis.

Patients

Eligible patients had a confirmed diagnosis of breast cancer and were on adjuvant tamoxifen treatment for at least three months to guarantee steady-state concentrations. Patients had to have a PM or IM CYP2D6 phenotype based on *CYP2D6* genotype screening [6]. For complete inclusion and exclusion criteria, see the Supplementary Materials and Methods.

The study protocol (MEC 20-0188) was approved by the institutional review board (METC Erasmus MC) and was registered on March 9th 2020 in the Netherlands Trial Register (NL8444). All patients provided written informed consent before study entry.

Pharmacogenetic and pharmacokinetic analysis

CYP2D6 genotype was assessed by the Infiniti test (Autogenomics, Carlsbad, CA) and the Quantstudio test (Thermo Fisher Scientific, Waltham, MA). Plasma samples were analyzed for tamoxifen, NDM, 4-OH and endoxifen concentrations 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 [20, 21]. A non-compartmental pharmacokinetic analysis of concentrations was performed using Phoenix WinNonlin version 8.1 (Certara, Princeton, NJ). 4β-OHC to cholesterol ratios were determined as described previously [22].

Statistical analysis

Analyses of $AUC_{0\text{-}24h}$, metabolic ratios and C_{max} observations were performed on log-transformed data, since these are assumed to follow a log-normal distribution. Estimates for the mean differences were obtained using a paired t-test and are shown as ratios of the geometric means with corresponding 95% confidence intervals (CIs) by taking the exponent of the results from the paired t-test. The relation between the ratio 4β-OHC to cholesterol and the ratio NDM to tamoxifen was analyzed using the Pearson correlation coefficient. Analysis of treatment-related adverse events was of descriptive nature.

Results

Patients

A total of 11 evaluable patients taking tamoxifen on steady state, with a median age of 54 years (range 34 to 77 years) were enrolled between May 2020 and October 2020. Four patients had a PM phenotype and seven patients an IM phenotype for CYP2D6. Six patients, including all patients with a PM phenotype, used tamoxifen at a dose of 40 mg daily at the time of inclusion. Patient characteristics at baseline are listed in **Table 1**.

Table 1 – Patient characteristics at baseline.

Characteristic	n (%) or median [range]
Female	11 (100)
Age, years	54 [34-77]
Body mass index, kg/m^2	24.0 [20.8-32.8]
WHO Performance status	
0	11 (100)
Tamoxifen dose	
20 mg	5 (45)
40 mg	6 (55)
Time on adjuvant tamoxifen, months	6.7 [3.7-17.7]
Time since dose escalation, months	3.1 [3.0-6.7]
CYP2D6 phenotype	
Intermediate metabolizer	7 (64)
Poor metabolizer	4 (36)
Previous treatment	
Surgery	10 (91)
Radiotherapy	8 (73)
Chemotherapy	4 (36)
Ethnic origin	
Caucasian	11 (100)

Endoxifen concentrations

We measured endoxifen concentrations in all patients, treated with tamoxifen at steady state and compared these concentrations to endoxifen concentrations after 14 days of concomitant use of probenecid (1000 mg twice daily). Treatment with tamoxifen and probenecid resulted in a 26% increase of endoxifen $AUC_{0\text{-}24h}$ compared to tamoxifen monotherapy (95% CI 8 to 46%; *P* < 0.01; geometric mean 505 versus 402 nmol·h/L; **Figure 2A**). The C_{max} of endoxifen was 24% higher when patients used concomitant probenecid (95% CI 7 to 44%; *P* < 0.01; geometric mean 27.4 versus 22.0 nM; **Table 2**).

In patients with a CYP2D6 PM phenotype, endoxifen $AUC_{0\text{-}24h}$ increased with 41% (95% CI 2 to 95%; *P* = 0.04; geometric mean 404 versus 287 nmol·h/L) during combined treatment with probenecid. While in patients with a CYP2D6 IM phenotype, endoxifen $AUC_{0\text{-}24h}$ increased with 18% (95% CI -4 to 44%; *P* = 0.09; geometric mean 573 versus 487 nmol·h/L).

Tamoxifen and other metabolite concentrations

Tamoxifen $AUC_{0\text{-}24h}$ during concomitant use of probenecid decreased with 40% (95% CI -47 to -33%; *P* < 0.001; geometric mean 5,286 versus 8,844 nmol·h/L) compared to tamoxifen monotherapy (**Figure 2B**). Tamoxifen C_{max} decreased with 33% (95% CI -42 to -22%; *P* < 0.001; geometric mean 357 versus 532 nM) due to the addition of probenecid. The ratio endoxifen to tamoxifen during combination therapy increased with 110% (95% CI 82 to 143%; *P* < 0.001; geometric mean 0.10 versus 0.05) compared to tamoxifen monotherapy (**Table 2**).

The ratio NDM to tamoxifen and the ratio endoxifen to 4-OH (as a measure for CYP3A4 activity; **Figure 1**) increased with 36% (95% CI 23 to 50%; *P* < 0.001; geometric mean 3.26 versus 2.39) and 43% (95% CI 27 to 63%; *P* < 0.001; geometric mean 5.69 versus 3.97), respectively (**Table 2**).

The ratio endoxifen to NDM and the ratio 4-OH to tamoxifen (as a measure for CYP2D6 activity; **Figure 1**) increased with 55% (95% CI 41 to 70%; *P* < 0.001; geometric mean 0.03 versus 0.02) and 47% (95% CI 33 to 61%; *P* < 0.001; geometric mean 0.02 versus 0.01), respectively (**Table 2**).

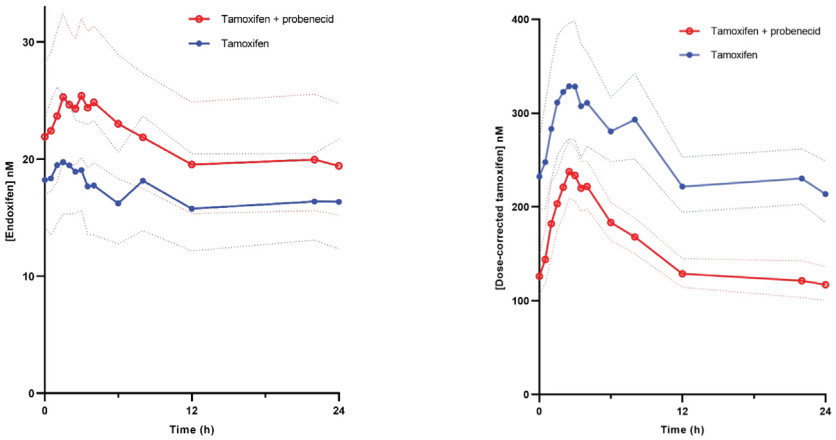

Figure 2 – Plasma concentrations of endoxifen and tamoxifen with and without probenecid. Geometric mean plasma concentration vs. time profiles of endoxifen (**A**) and dose corrected tamoxifen (**B**) are shown for tamoxifen monotherapy (blue) and tamoxifen with probenecid combination therapy (red). Confidence bands indicate the 95% CI.

Table 2 – Pharmacokinetic parameters of endoxifen and tamoxifen (n = 11).

Pharmacokinetic parameter	Tamoxifen monotherapy (CV%)	Tamoxifen with probenecid (CV%)	Relative difference (%) (95% CI)	*P*
Endoxifen				
$AUC_{0\text{-}24h}$ (nmol·h/L)	402 (43)	505 (41)	26 (8 to 46)	< 0.01
C_{max} (nM)	22.0 (46)	27.4 (41)	24 (7 to 44)	< 0.01
Tamoxifen				
$AUC_{0\text{-}24h}$ (nmol·h/L)	8,844 (45)	5,286 (46)	-40 (-47 to -33)	< 0.001
C_{max} (nM)	532 (48)	357 (47)	-33 (-42 to -22)	< 0.001
Metabolic ratios				
Endoxifen / tamoxifen	0.05 (72)	0.10 (58)	110 (82 to 143)	< 0.001
NDM / tamoxifen	2.39 (16)	3.26 (12)	36 (23 to 50)	< 0.001
Endoxifen / 4-OH	3.97 (41)	5.69 (36)	43 (27 to 63)	< 0.001
4-OH / tamoxifen	0.01 (32)	0.02 (32)	47 (33 to 61)	< 0.001
Endoxifen / NDM	0.02 (80)	0.03 (64)	55 (41 to 70)	< 0.001
4β-OHC / cholesterol	13.94 (60)	13.66 (84)	-2 (-46 to 77)	0.94

4β-OHC: 4β-hydroxy-cholesterol, 4-OH: 4-hydroxy-tamoxifen, AUC_{0-24h}: area under the plasma-concentration-time curve from 0 to 24 hours, CI: confidence interval, C_{max}: maximum observed plasma concentration, CV%: coefficient of variation, NDM: n-desmethyl-tamoxifen. AUC_{0-24h} and C_{max} are displayed as geometric mean. Metabolic ratios are ratios of the geometric mean.

CYP3A4 activity

To determine CYP3A4 metabolic activity with and without concomitant probenecid, we determined the ratio 4β-OHC to cholesterol, an established endogenous marker of CYP3A4 activity [23]. The ratio 4β-OHC to cholesterol did not change (2%; 95% CI -46 to 77%; *P* = 0.94; 13.66 versus 13.93). The fold change of the ratio 4β-OHC to cholesterol was correlated with the fold change of the ratio NDM to tamoxifen (Pearson correlation coefficient r = 0.67; *P* = 0.02; **Figure S1**).

Treatment related adverse events

Observed adverse events during combination treatment were relatively mild. Probenecid treatment-related adverse effects included hypokalemia, neutropenia, nausea, headache, dizziness, increased creatinine and leukopenia, and were all grade 1 or 2 (**Table S1**). Except for muscle cramps, which occurred two times more, tamoxifen related adverse events did not increase during combination therapy, compared to monotherapy. There were no severe or serious adverse events (CTCAE grade ≥3) observed.

Discussion

In this study, we demonstrated that probenecid causes a clinically relevant increase in endoxifen plasma concentrations in breast cancer patients treated with tamoxifen. This finding was accompanied by a decrease of tamoxifen concentrations during concomitant administration of probenecid. We determined concentrations of other tamoxifen metabolites in order to elucidate the mechanisms involved in these changes. Analysis of the tamoxifen metabolites NDM and 4-OH showed an increase of all CYP mediated tamoxifen-to-metabolite or metabolite-to-endoxifen conversions occurring in the phase 1 metabolism of tamoxifen. These findings implicated at least an induction of CYP3A4 and/or CYP2D6, causing the reported shifts in endoxifen and tamoxifen plasma concentrations. Therefore, we subsequently determined 4β-OHC to cholesterol ratios with and without probenecid. The 4β-OHC to cholesterol ratio is an endogenous marker of CYP3A4/5 activity, which has previously proven utility in confirming CYP3A4 induction by rifampicin, administered in combination with tamoxifen [24]. However, in the present study, no significant alterations could be detected in CYP3A4 functionality with or without probenecid administration. Yet, the fold change of the 4β-OHC-to-cholesterol ratio was correlated with the fold change of the NDM to tamoxifen ratio; both a CYP3A4 mediated conversion. This confirms the value of the 4β-OHC to cholesterol ratio as a genuine marker for CYP3A4 functional activity. Potential upregulation of CYP2D6 could not be investigated due to lack of an endogenous plasma marker for CYP2D6. The observed alterations in tamoxifen and endoxifen concentrations indicate a major effect of probenecid on the phase 1 metabolism of tamoxifen, but cannot assess an effect on endoxifen glucuronidation.

To our knowledge, this is the first study to show the feasibility of increasing endoxifen concentrations by a pharmacological intervention, which could be especially important for patients with a PM phenotype for CYP2D6. A meta-analysis of 29 studies, including 13,000 tamoxifen users, demonstrated that PM patients on average had endoxifen concentrations of 8.8 ± 7.2 nM [25]. Furthermore, patients with a PM phenotype on average benefit the least of tamoxifen dose escalation, due to a lower increase of endoxifen concentrations per fixed increase of tamoxifen dose. It was found that on average for each 10 mg increase in tamoxifen dosage, patients with a PM phenotype only had a 1.2 nM increase of endoxifen, compared to a population average increase of 7.8 nM [26]. In a population of 145 tamoxifen users, 100% of PM patients and 34% of IM patients had endoxifen concentrations below the threshold of 16 nM. Despite tamoxifen dose escalation, only 36% and 79% of these patients reached the threshold, respectively [27]. Moreover, another study in 353 tamoxifen users demonstrated that dose escalation is not feasible for patients with a PM phenotype [28]. These observations stress the need for a solution, other than a dose escalation, to increase endoxifen concentrations in these patients to therapeutic concentrations.

Here, we demonstrated that in patients with a PM phenotype for CYP2D6, endoxifen concentrations increased to a greater extent compared to patients with an IM phenotype. Therefore, the currently proposed intervention is of greatest interest for this subgroup of patients. Although patients in the present study were not selected on sub-therapeutic endoxifen concentrations, all patients with a PM phenotype had endoxifen trough concentrations below the therapeutic threshold at baseline. These concentrations increased to borderline therapeutic concentrations after co-treatment with probenecid, demonstrating the effectiveness of this intervention. In addition, as no serious side-effects occurred after 14 days of combination treatment, the feasibility of the intervention was also shown. In clinical practice, probenecid is administered as a uricosuric drug up to 1000 mg twice daily for several years [29]. Absolute contraindications for probenecid are scarce and despite long term administration, toxicity is generally mild [12]. This reflects our own observations on low drug toxicity and warrants further investigation of this likely tolerable combination in long term treatment.

Our study is limited by the short duration of the treatment intervention. However, the main goal of the present study was assessment of pharmacokinetics for proof of concept, for which the parameters used were sufficient. Validation of our findings in a larger group of patients is required prior to implementation of this intervention in clinical practice. A second limitation is the quantification of relevant metabolites. Although we determined several metabolites of tamoxifen and performed a phenotypical analysis of drug metabolism, we could not analyze all concentrations of relevant metabolites and activity of all conversions involved in the complex

metabolism of tamoxifen. A third limitation is the purely systemic measurement of endoxifen concentrations performed in this study, contrarily to measurements in the target cancer cell. However, the study was performed according to current guidelines in the pharmacological field, since such targeted measurements are practically impossible in this population, which is being treated in the adjuvant setting. Nonetheless, differences between systemic and intra-tumoral drug exposure is a relevant topic.

This study shows that probenecid can be used to increase endoxifen concentrations in breast cancer patients treated with tamoxifen. This combination therapy could provide a solution for patients with endoxifen concentrations below the threshold despite earlier tamoxifen dose escalation or in case of tamoxifen related toxicity at lower doses.

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Supplementary Information

Supplementary materials and methods

Full inclusion and exclusion criteria

Inclusion criteria

1. Age ≥ 18 years.
2. Patients with a confirmed diagnosis of primary or advanced breast cancer, who are on tamoxifen treatment for at least three months (steady state concentration).
3. A CYP2D6 poor metabolizer or intermediate metabolizer phenotype.
4. WHO performance ≤ 1.
5. Able and willing to sign the informed consent form prior to screening evaluations
6. Willing to abstain from strong CYP3A4, CYP2D6, CYP2C9, CYP2C19, UGT and P-gp inhibitors or inducers, herbal or dietary supplements or other over-the-counter medication besides paracetamol.
7. Adequate kidney function defined as: GFR > 50 ml/min/1.73 m^2.

Exclusion criteria

1. Pregnant or lactating patients.
2. Patients with known impaired drug absorption (e.g. gastrectomy and achlorhydria).
3. Use of drugs which may show an increased systemic exposure when taken concomitantly with probenecid e.g. methotrexate, penicillin, cephalosporin or chinolon antibiotics or NSAIDs.
4. Patients with known blood dyscrasias, porphyria, uric acid kidney stones or until an acute gouty attack has subsided.
5. Known serious illness or medical unstable conditions that could interfere with this study requiring treatment (e.g. HIV, hepatitis, varicella zoster or herpes zoster, organ transplants, kidney failure (GFR<30 ml/min/1.73 m^2), serious liver disease (e.g. severe cirrhosis), cardiac and respiratory diseases).

Supplementary figures

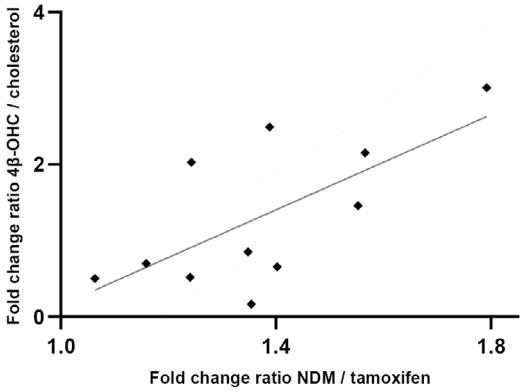

Figure S1 – Scatter plot of the fold change of the ratio 4β-OHC to cholesterol versus the fold change of the ratio NDM to tamoxifen. The fold change of each ratio is calculated by dividing the metabolic ratio with probenecid to the metabolic ratio without probenecid. The line of best fit is depicted. Confidence bands indicate the 95% CI.

Supplementary tables

Table S1 – Treatment related adverse events.

Adverse event	Tamoxifen monotherapy (n)	Tamoxifen with probenecid (n)
Grade 1		
Nausea	0	4
Headache	1	3
Dizziness	0	1
Hot flashes	3	3
Muscle cramp	1	3
Leukopenia	1	1
Creatinine increased	0	2
Grade 2		
Neutropenia	0	1
Hypokalemia	0	1
Grade ≥ 3	0	0

CHAPTER 10

Inhibiting CDK4/6 in breast cancer with palbociclib, ribociclib, and abemaciclib: similarities and differences

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Abstract

The cyclin dependent kinase (CDK) 4/6 inhibitors belong to a new class of drugs which interrupt proliferation of malignant cells. Three drugs in this class; palbociclib, ribociclib, and abemaciclib were recently approved for breast cancer treatment in various settings and combination regimens. On the basis of their impressive efficacy all three CDK4/6 inhibitors have an important role in the treatment of patients with HR+, HER2- breast cancer, however their optimal role still needs to be established. The three drugs have many similarities in both pharmacokinetics as pharmacodynamics. However, there are some differences on the basis of which the choice for a particular CDK4/6 inhibitor for an individual patient can be important. In this review the clinical pharmacokinetic and pharmacodynamic profiles of the three CDK4/6 inhibitors are discussed and important future directions of the clinical applicability of CDK4/6 inhibitors will be discussed.

1. Introduction

Of all patients with stage IV breast cancer, approximately 75% is hormone receptor-positive (HR+) and human epidermal growth factor receptor 2 negative (HER2-).[1–3] As advanced or metastatic breast cancer represents an incurable disease, the main purpose of treatment is to delay disease progression, preferably with anticancer drugs that are patient friendly in its use and its toxicity.[4] Patients with HR+, HER2- breast cancer are often treated with single agent endocrine therapy. However, finally resistance always develops, resulting in the need for cytotoxic chemotherapy.[5–7]

Recently a novel drug class, the cyclin dependent kinase (CDK) 4/6 inhibitors, has been introduced as treatment option for patients with HR+, HER2- advanced breast cancer either as first line therapy combined with an aromatase inhibitor or as second line therapy in combination with fulvestrant.[8–13] The CDK-RB1-E2F pathway targeted by CDK4/6 inhibitors is essential for progression through the cell cycle and disrupted in the majority of cancers.[14–18] In breast cancer, the activation of estrogen receptors as well as other proliferation inducing signals stimulate the complexation of CDK4/6 with Cyclin D1.[19] Binding of CDK4/6 to Cyclin D1 induces phosphorylation of the retinoblastoma (Rb) tumor suppressor protein, releasing its inhibitory effect and thereby providing the starting signal for cell division.(16,18,20–22) Normally, CDK4 and CDK6 are inhibited by the protein p16. However in cancer, this mechanism of cell cycle control is often disrupted.[19,23] Furthermore, Cyclin D1, the binding partner of CDK4/6, is often overexpressed in patients with HR+, HER2- breast cancer leading to continuous activation of the Cyclin D1 – CDK4/6 complex.[24–26] Inhibition of CDK4/6 induces complete dephosphorylation of Rb, resulting in sequestration of the transcription factor E2F and subsequent inhibition of cell cycle progression.[27–29]

The effectiveness of CDK4/6 inhibitors can be increased by combining them with drugs that prevent the downstream estrogen dependent stimulation of the cancer cell. Inhibition of the estrogen pathway – by endocrine therapy - results in downregulation of cyclin D1 and reduced complexation of CDK4 and CDK6.[30] Therefore, the selective CDK4/6 inhibitors palbociclib, ribociclib, and abemaciclib are given in combination with endocrine therapy (aromatase inhibitors or fulvestrant) in the treatment of HR+, HER2- breast cancer.[31–33]

So far, no head-to-head comparison has been carried out between the three different CDK4/6 inhibitors in HR+, HER2- advanced breast cancer. At this moment, CDK4/6 inhibitors are prescribed based on individual physician's experience with these CDK4/6 inhibitors, differences in toxicity profiles, costs, or preference policy of the hospital. Insights into pharmacological

profiles of these three CDK4/6 inhibitors may help to rationalize the selection of the most optimal inhibitor for the individual patient. In this review, the pharmacokinetics, pharmacodynamics, efficacy and tolerability of palbociclib, ribociclib and abemaciclib for the treatment of breast cancer are discussed. Furthermore, future directions of the clinical applicability of the CDK4/6 inhibitors are discussed such as the potential role of biomarkers in determining treatment strategy in specific patient groups, the combination with other drug modalities and the use of these therapies in other types of (breast) cancer (**Figure 1**).

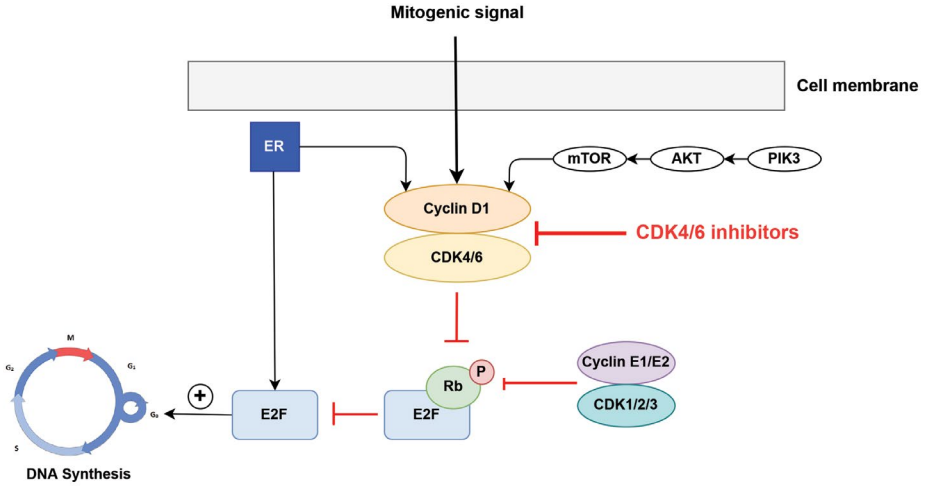

Figure 1 – Mechanism of action of CDK4/6 inhibitors. The CDK4/6-Cyclin D1 complex induces phosphorylation of the retinoblastoma (Rb) tumor-suppressor protein. Free transcription factor E2F stimulates cell transition from the G1 to the S phase and cell division. *AKT, protein kinase B; CDK4/6, cyclin dependent kinase 4 and 6; ER, estrogen receptor; E2F, transcription factor family; HER2, human epidermal growth factor receptor-2; PIK3, phosphoinositide 3-kinase; Rb, retinoblastoma tumor suppressor protein; mTOR, mammalian target of rapamycin.*

2. Pharmacokinetics

2.1 Pharmacokinetic profiles

Palbociclib (Ibrance®, Pfizer, New York, USA) received US FDA approval in 2015, with a recommended starting dosage of 125 mg once daily in a '3 weeks on and 1 week off' schedule in combination with an non-steroidal aromatase inhibitor or the selective estrogen receptor degrader (SERD) fulvestrant.[32] After rapid absorption of palbociclib, the maximum concentration (C_{max}) is achieved within 6-12 hours and the drugs are being eliminated with a elimination half-life of 24–34 hours. Steady-state will be achieved after 4-5 elimination half-lives, which is important to predict the one set of action or improvement of toxicity after treatment discontinuation. Ribociclib (Kisqali®, Novartis, Basel, Switzerland) received US FDA approval in 2017, with a

recommended starting dosage of 600 mg once daily in a '3 weeks on/ 1 week off' schedule in combination with an aromatase inhibitor or fulvestrant.[33] Ribociclib has a high absorption rate and reaches C_{max} within 1-4 hours upon intake. The elimination half-life of ribociclib is 30-55 hours. Finally, abemaciclib (Verzenios®, Eli Lilly, Indianapolis, USA) received US FDA approval in 2017, with a recommended starting dosage of 150 mg twice daily in a continuous dosing schedule combined with an aromatase inhibitor or fulvestrant. In addition, abemaciclib is also approved for administration as monotherapy with a starting dosage of 200 mg twice daily.[31] Abemaciclib achieves its C_{max} within 8 hours upon intake and the drug elimination half-life is 17–38 hours. Pharmacokinetic parameters of palbociclib, ribociclib and abemaciclib are depicted in **Table 1**.

2.2 Similarities and differences in pharmacokinetics

2.2.1 Absorption

The pharmacokinetic profiles of the three inhibitors are strikingly similar. After rapid absorption and distribution, all three CDK4/6 inhibitors are metabolized mainly by CYP3A4. Abemaciclib is given in a continuous schedule of two doses per day, where palbociclib and ribociclib are given once daily in '3 weeks on/ 1 week off' schedules. Only for abemaciclib, preclinical studies have displayed saturation of drug absorption, which supported the development and registration of a twice daily dosing regimen to improve drug absorption.[34,35] Furthermore, preclinical studies showed that continuous administration of abemaciclib reduced tumor growth more efficiently compared to an intermittent schedule.[34–36] The efficacy of palbociclib and ribociclib in continuous dosing schedules compared to the efficacy of the current intermittent schedule is unknown, but hematological toxicities urged the development of an intermittent schedule.

2.2.2 Distribution

From a biopharmaceutical point of view, a distinguishing feature of abemaciclib in comparison with the other CDK4/6 inhibitors is its theoretical ability to penetrate breast tissue and the blood brain barrier more efficiently due to its higher lipophilicity (cLog P 5.5 versus 2.7 and 2.3, see **Table 1**). In support of this theoretical advantage, preclinical data from abemaciclib in human xenograft models showed decreased tumor growth in the brain, suggesting an effective penetration.[38] In addition, a clinical study indicated that systemic treatment with abemaciclib resulted in similar concentrations of abemaciclib in both plasma and cerebrospinal fluid.[39] When compared to palbociclib and ribociclib, abemaciclib was demonstrated in a xenograft model to have the highest unbound brain to plasma ratio, which is an indication for a higher penetration potential in the central nerve system.[38] In addition, both palbociclib and ribociclib are substrates for breast cancer resistance protein (BCRP; ABCG2) and P-glycoprotein (P-gP; ABCB1) which

Table 1 – Pharmacokinetics of palbociclib, ribociclib and abemaciclib.

	Palbociclib (Ibrance®) **PD-0332991** [10,32,42,50,105–108]	**Ribociclib (Kisqali®)** **LEE011** [33,50,71,115]	**Abemaciclib (Verzenios®)** **LY2835219** [31,34,34,39,77,116,116–118]
US FDA approval In combination with an aromatase inhibitor or fulvestrant	2015	2017	2017
Molecular weight (g/mol)	447.54	434.55	506.59
cLog P	2.7	2.3	5.5
Route of administration	Oral	Oral	Oral
Dose	125 mg qd; 3 weeks on/1 week off	600 mg qd; 3 weeks on/ 1 week off	150 mg bid; continuously
Dosage form and strengths	Capsules 75, 100, 125 mg	Tablets 200 mg	Tablets 50, 100, 150, 200 mg
C_{trough} (ng/mL)	47 (CV; 48%)	457 (CV; 67%)	176 (CV; 89%)
T_{max} (h)	6 – 12	1 – 4	8
C_{max} (ng/mL)	97 (CV; 41%)	1680 (CV; 31%)	249 (CV; 64%)
$T_{1/2}$ (h)	24 – 34	30 – 55	17 – 38
Bioavailability (%)	46	NR	45
Accumulation ratio	2.4 (1.5 – 4.2)	2.51 (0.97 – 6.40)	3.2 (CV; 59%)
Protein binding (%)	~85	~70	93 – 98
Absorption Food effect	No	No	No
Distribution (L)	2583	1090	690.3
Metabolism	CYP3A4 + SULT2A1	CYP3A4	CYP3A4
Metabolites	Yes Palbociclib-glucuronide: 1.5%	Yes M13 (N-hydroxylation): 22% M4 (N-demethylation): 20% M1 (Secondary glucuronide): 18%	Yes M2 (N-desethylation): 25% M20 (Hydroxylation): 26% M18 (Hydroxy-N-desthylation): 13%
Excretion (%)	Faeces: 74 Urine: 18	Faeces: 69 Urine: 23	Faeces: 81 Urine: 3
Age, weight, gender, race, mild hepatic/ renal impairment	No effect on exposure	No effect on exposure	No effect on exposure

bid, twice daily; cLog P, calculated Log P (lipophilicity); C_{max}, maximum concentration; CV, coefficient of variation; M, metabolite; NR, not reported; qd, once daily; T_{max}, time to reach maximum observed concentration.

is an additional explanation for their limited brain penetration capacity observed in preclinical studies.[40,41] The efficacy of the three different CDK4/6 inhibitors in treating brain metastases is an important unanswered question in daily clinical practice. Prospective trials are ongoing to evaluate brain penetration and efficacy of palbociclib (NCT02774681), ribociclib (NCT02933736) and abemaciclib (NCT02308020) in the treatment of brain metastases.

2.2.3.1 Metabolism and excretion

For all three CDK4/6 inhibitors metabolization occurs hepatically, and was shown by both *in vitro* and *in vivo* studies to be primarily CYP3A4 mediated. Concomitant administration of CDK4/6 inhibitors and strong CYP3A4 inhibitors (*i.e.* itraconazole, ketoconazole and ritonavir) can lead to an increase in exposure of CDK4/6 inhibitors in the blood and an increased chance of toxicity. The administration of palbociclib in combination with the strong CYP3A4 inhibitor itraconazole resulted in an increase in $AUC_{0\text{-}inf}$ and C_{max} of 87% and 34%, respectively; ribociclib in combination with ritonavir increased ribociclib exposure up to 3.2-fold; and based on an animal model ketoconazole is predicted to increase the exposure of abemaciclib by up to 16-fold. The FDA therefore advises to avoid concomitant use of CDK4/6 inhibitors and strong CYP3A4 inhibitors.[31–33]

Yu *et al.* published a physiologically based pharmacokinetic model – based on silico, *in vitro*, and *in vivo* pharmacokinetic data – in which they simulated the effects of the moderate CYP3A4 inhibitors verapamil and diltiazem on palbociclib pharmacokinetics. They estimated an increase in C_{max} and AUC of 22% and 38% for verapamil and 23% and 42% for diltiazem, respectively. The authors concluded that the risk of drug-drug interactions for palbociclib co-administered with moderate CYP3A4 inhibitors is relatively modest and that no upfront dose adjustment is needed.[42] However, a 40% increase in exposure could still be clinically relevant, especially since a higher palbociclib exposure is associated with increased toxicity.[42] A clear relationship between exposure and toxicity is not described in literature for both ribociclib and abemaciclib. More research is needed to elucidate the plausible relationship between exposure and adverse events. Currently, a randomized pharmacokinetic cross-over trial is ongoing to evaluate the effect of the moderate CYP3A4 inhibitor erythromycin on the pharmacokinetics of palbociclib (Netherlands Trial Register NL7549) and results are expected in 2021.

2.2.3.2 Metabolism and pharmacogenetics

Besides CYP3A4 mediated metabolism, palbociclib is also hepatically metabolized by the sulfotransferase enzyme SULT2A1.[43] SULTs, such as SULT2A1, highly expressed in the small intestine, liver and adrenal cortex metabolize orally administered drugs through sulfate conjugation. Pharmacogenetic variation in SULT2A1 activity could affect the drug's biotransformation

and thus its pharmacokinetics.[44] Variability in CYP3A4 and SULT2A1 drug metabolization can often be partly explained by genetic polymorphisms. The extent to which this applies for all three CDK4/6 inhibitors is unclear and requires further research. Sequencing of CYP3A4 and SULT2A1 genes in patients treated with CDK4/6 inhibitors may potentially identify subpopulations requiring dose adjustments. The incidence of any neutropenia and grade 3 neutropenia specifically is higher in the Asian population compared with non-Asian patients treated with palbociclib (10)(45), concordant with the observed higher mean steady-state concentration of palbociclib.[45] The reason for differences in pharmacokinetics between Asian and non-Asian populations is unknown, but could be related to genetic predispositions influencing metabolism or higher numbers of CDK receptors and/or sensitivity of the receptors.[46]

3. Pharmacodynamics

CDK4 is a prominent oncogenic driver in breast cancer, while CDK6 plays a crucial role in differentiation of hematopoietic stem cells.[47,48] Palbociclib, ribociclib and abemaciclib display subtle differences in kinase selectivity. Abemaciclib is the most potent CDK4/6 inhibitor and is approximately 5 times more potent against CDK4 than CDK6, which leads to the expectation that abemaciclib gives less hematological toxicity (**Table 2**).(34,37,49) Preclinical drug-exposure experiments showed inhibition of CDK4 and CDK6 with half maximal inhibitory concentrations (IC_{50}) of 9-11 nM and 15 nM (ratio IC_{50} CDK4:CDK6 - 1:1.5) for palbociclib, 10 nM and 39 nM (1:4) for ribociclib, and 2 nM and 9.9 nM (1:5), for abemaciclib, respectively.[50,51] In contrast to palbociclib and ribociclib, abemaciclib was shown to be a potent inhibitor of CDK9 as well.[50] CDK9 inhibition could potentially modify the cascade of glycogen synthase kinase 3 (GSK3) mediated effects of abemaciclib leading to the specific intestinal toxicity observed.[52,53]

4. Clinical efficacy

4.1 Advanced breast cancer

The effects of CDK4/6 inhibitors palbociclib, ribociclib, and abemaciclib in addition to non-steroidal aromatase inhibitors were studied in postmenopausal ER+, HER2- advanced breast cancer in first line setting in the three large randomized phase 3 trials; PALOMA-2 , MONALEESA-2 and MONARCH 3.[8–10] All three CDK4/6 inhibitors significantly prolonged the progression free survival (PFS) with almost identical hazard ratios (HR) for PFS (**Table 2**). Overall survival (OS) data for these studies are not available yet. However, OS benefit was found in the MONALEESA 7 study, which investigated the addition of ribociclib to the gonadotropin-releasing hormone (GnRH) agonist goserelin with either tamoxifen or a non-steroidal aromatase inhibitor in pre- or perimenopausal women (HR 0.71; 95% CI: 0.54 – 0.95).[54]

Table 2 – (Pre)clinical pharmacodynamics and efficacy of palbociclib, ribociclib and abemaciclib.

	Palbociclib (Ibrance®) **PD-0332991** [8,11,50,55,63,119]	**Ribociclib (Kisqali®)** **LEE011** [9,13,50,119,120]	**Abemaciclib (Verzenios®)** **LY2835219** [10,50,58,62,119]
-CDK4, IC_{50}	9-11 nM	10 nM	2 nM
-CDK6, IC_{50}	15 nM	39 nM	9.9 nM
-CDK9, IC_{50}	NR	NR	57 nM
Advanced setting			
Efficacy first line			
Median PFS vs aromatase inhibitor alone (months)	24.8 vs 14.5 (HR 0.58; p<0.001)	25.3 vs 16.0 (HR 0.56; p<0.001)	Not reached vs. 14.7 (HR 0.54; p<0.001)
Median OS (months)	NR	NR	NR
Efficacy second line			
Median PFS vs fulvestrant alone (months)	9.5 vs 4.6 (HR 0.46; *p<0.001*)	20.5 vs 12.8 (HR 0.59; *p*<0.001)	16.4 vs 9.3 (HR 0.55; *p<0.001)*
Median OS (months)	34.9 vs 28.0 (HR 0.81; *p=0.09*)	Not reached vs 40.0 (HR 0.72; *p=0.005*)	46.7 vs 37.3 (HR 0.76; *p=0.014*)
Toxicity advanced setting all/grade 3-4, (%)	Neutropenia (80/66) Anemia (24/5) Thrombocytopenia (16/1) Diarrhea (26/1) Fatigue (37/2) ALT increased (43/2) Creatinine increased (NR/NR) Infections (60/7)	Neutropenia (75/60) Anemia (18/1) Thrombocytopenia (29/1) Diarrhea (35/1) Fatigue (37/2) ALT increased (46/10) Creatinine increased (20/1) Infections (50/4)	Neutropenia (41/22) Anemia (28/6) Thrombocytopenia (10/2) Diarrhea (81/9) Fatigue (46/3) ALT increased (48/6) Creatinine increased(98/2) Infections (39/5)
Dosing patterns advanced setting	Dose reduction: 36% Dose interruptions: 67% Permanent discontinuation: 7.4%	Dose reduction: 54% Dose interruptions: 76% Permanent discontinuation: 7.5%	Dose reduction: 43% Dose interruptions: 56% Permanent discontinuation: 20%

Table 2 (continued)

	Palbociclib (Ibrance®) **PD-0332991** [8,11,50,55,63,119]	**Ribociclib (Kisqali®)** **LEE011** [9,13,50,119,120]	**Abemaciclib (Verzenios®)** **LY2835219** [10,50,58,62,119]
Adjuvant setting			
Efficacy adjuvant setting Proportion with IDFS event (%)	5.9% in the palbociclib arm vs. 6.3% in control arm (HR 0.93; p>0.05)	NR	4.8% in the abemaciclib arm vs. 6.6% in control arm (HR 0.75; p<0.05)
Toxicity adjuvant setting (all/grade 3-4), (%)	Neutropenia (83/61) Anemia (23/1) Thrombocytopenia (21/1) Diarrhea (17/1) Fatigue (41/2) Upper respiratory infections (28/1)	NR	Neutropenia (45/19) Anemia (23/2) Thrombocytopenia (12/1) Diarrhea (82/8) Fatigue (38/3) Upper respiratory infections (10/0)
Dosing patterns adjuvant setting	Dose reduction: NR Discontinued prematurely: 42%	NR	Dose reduction: 41% Discontinuation prematurely: 17%

NR, not reported.

The effects of CDK4/6 inhibitors in addition to fulvestrant mainly during the second line of treatment in patients with HR+, HER2- advanced breast cancer was studied in the three large randomized phase 3 trials; PALOMA-3, MONALEESA-3 and MONARCH-2.[12,13,55] All phase 3 trials showed prolongation of the PFS in the CDK4/6 inhibitor group as well as an OS benefit.[56–58]

Based on the above described results CDK4/6 inhibitors are registered for first- and second line treatment in advanced HR+, HER2- breast cancer patients. Their most optimal position during treatment (i.e. first or second line) is a matter of debate,[59–61] and is being investigated in the currently ongoing randomized phase 3 SONIA trial (NCT03425838).[61] Due to the unique study design, this study will also allow for a head-to-head comparison of the three FDA approved CDK4/6 inhibitors to identify the differences in clinical pharmacology.

4.2 Adjuvant setting

The positive results in the advanced breast cancer setting raised interest in CDK4/6 inhibitors as potential treatment in the neo-adjuvant and adjuvant setting. For all three CDK4/6 inhibitors large randomized phase 3 trials are currently ongoing to investigate the addition of a CDK4/6 inhibitor to standard adjuvant endocrine therapy (NCT03701334).[62,63] In the MonarchE study the addition of abemaciclib treatment for 2 years in postmenopausal, high risk HR+, HER2- early breast cancer (EBC) patients is explored.[62] After a median follow-up of 15.5 months 136 of the 2,808 patients (4.8%) experienced recurrence of invasive disease in the abemaciclib arm compared to 187 of the 2,829 patients (6.6%) in the control arm (HR 0.75; 95% CI: 0.60 – 0.93).[62] Almost all patients experienced adverse events in the abemaciclib arm (97.9%), with grade ≥3 adverse events reported in 45.9% of patients compared to 86.1% and 12.9% in the endocrine monotherapy group, respectively. In total 463 patients (16.6%) discontinued abemaciclib due to these treatment-related adverse events. These results are in contrast with those of the PALLAS trial, which was terminated early following the interim analysis since no effect of additional palbociclib to adjuvant endocrine therapy was found. After a median follow-up of 23.7 months 170 of the 2,883 patients (5.9%) in the palbociclib arm experienced recurrence of invasive disease compared to 181 of the 2,877 patients (6.3%) in the control arm (HR 0.93; 95% CI: 0.75 – 1.14). A remarkably high premature discontinuation rate of 42% was observed in the palbociclib arm of this study, which was attributed to adverse events in 64% of these patients.

These contradictory results in effectiveness raise questions. One important difference between these two trials is the study population. Whereas the PALLAS study included all patients with stage 2-3 EBC, the MonarchE study specifically included patients with high risk EBC, defined as patients with ≥4 positive pathologic lymph nodes or patients with 1 to 3 pathologic lymph nodes combined with either tumor size ≥5 cm, histologic grade 3, or centrally assessed KI-67

proliferation index of ≥20%. Subgroup analyses of only patients with N2-N3 disease and patients with clinically high risk (≥4 nodes or 1-3 nodes with either T3-T4 and/or grade 3 disease) disease in the PALLAS study still did not show a significant effect in recurrence rate with addition of palbociclib, suggesting differences in study population may not be the main cause for the difference in study outcome. However, cross study comparison should be interpreted with caution. Furthermore, follow-up imaging for the presence of recurrence was not a protocol requirement, but was performed at the discretion of the treating medical oncologist. In combination with the open-label design of the MonarchE trial, this could introduce confounding by indication bias by a likely tendency to more often perform a scan in high risk EBC patients in the placebo arm compared to the CDK4/6 inhibitor arm. This could lead to more frequently detected recurrences in patients in the placebo arm compared to patients in the abemaciclib arm.

Longer follow-up data of trials are needed to confirm the clinical benefit of CDK4/6 inhibitors in the adjuvant setting, including the impact on overall survival. Several other studies in the adjuvant setting are still ongoing. The NATALEE trial (NCT03701334) is an ongoing trial in the adjuvant setting in which ribociclib is added to endocrine treatment for 3 years. Recruitment is ongoing and no results are presented or published yet. The PENELOPE-B trial (NCT01864746) studies the addition of palbociclib to endocrine therapy for 1 year in patients with a high risk of recurrence based on the CPS-EG score, a risk score based on clinical stage before neoadjuvant treatment, pathological stage after neoadjuvant treatment, nuclear grade and estrogen receptor status. The results are eagerly awaited, since when comparable effectivity is reached with additional palbociclib for one year compared to two years in the MonarchE this would be advantageous to patients and decrease healthcare costs.

4.3 Neoadjuvant setting

The addition of CDK4/6 inhibitors in the neoadjuvant setting could potentially increase antiproliferative effects on the primary tumor before surgery will take place and ultimately result in an improved overall survival. Although no long term clinical outcomes have been published yet in the neoadjuvant setting, several studies have shown an increase in cell cycle arrest with the addition of a CDK4/6 inhibitor to endocrine treatment in the neoadjuvant setting. In the NeoPalAna trial a complete cell cycle arrest, as determined by the Ki67 index, was reached in a significantly higher proportion of the patients who received palbociclib and anastrazole compared to patients who received anastrazole monotherapy.[64] The addition of ribociclib to letrozole during 6 cycles in the neoadjuvant setting was explored in the FELINE study. It showed a higher number of patients with a complete cell cycle arrest after 2 weeks of treatment in the ribociclib group compared to the placebo group, although at surgery no difference in proportion of patients with complete cell cycle arrest was found.[65] The NeoMonarch showed a reduction

in Ki67 when abemaciclib was added to anastrazole compared to anastrazole monotherapy after two weeks of treatment.[66] In all studies the number patients with a complete cell cycle arrest decreased after discontinuation of the CDK4/6 inhibitor, which indicates the importance of continuation of CDK4/6 inhibition until surgery takes place.

5. Tolerability

The main side effects of CDK4/6 inhibitors are bone marrow suppression – such as neutropenia, anemia and thrombopenia – and gastrointestinal toxicities. However, there seem to be some distinct differences between the three drugs. Interestingly, despite these differences in toxicity profiles, all three CDK4/6 inhibitors showed comparable EORTC QLQ-C30 quality of life scores.[67–69] Approximately 40-50% of patients treated in phase 3 trials needed a dose reduction in cycle 1 or 2 (**Table 2**). Importantly, permanent discontinuation was more reported with abemaciclib treatment (20%) compared with palbociclib and ribociclib (both 7.5%).[8–10] The most common reasons for dose modification were myelosuppression for palbociclib and ribociclib and diarrhea for abemaciclib.

5.1 Neutropenia

Grade 3-4 neutropenia – scored by the common terminology criteria for adverse events (CTCAE) – was the most reported adverse event for palbociclib (66%) and ribociclib (60%) and was the second most reported adverse event in patients treated with abemaciclib (22%). Neutropenia is usually observed in the first cycles of both palbociclib and ribociclib. Median time from first dose to onset of first episode of neutropenia grade ≥3 is set at 28.0 (12-854) days with a median duration of 7 days.[70] Also for abemaciclib neutropenia often occurs in the first cycle, however in some cases a more delayed onset of neutropenia was observed in cycle 2 or higher. (10,32,71,72) Febrile neutropenia was observed at low frequency in patients treated with a CDK4/6 inhibitor (2% for both palbociclib and ribociclib and lower than 1% for abemaciclib). (8,10,13) A pharmacokinetic and pharmacodynamic model suggested that a higher palbociclib exposure was associated with a lower absolute neutrophil counts.[73] However, in general no clear correlation between race and toxicity was found in all phase 3 trials.[8–11,13,58] Therefore, more research is needed to identify pharmacokinetic targets for the three CDK4/6 inhibitors.

5.2 Gastro-intestinal toxicity

Grade 3 diarrhea, *i.e.* an increase of seven or more stools a day, was more frequently reported in patients treated with abemaciclib (9%) compared to palbociclib and ribociclib (both 1%) (**Table 2**).(8–10,52,74,75) Median time to onset of diarrhea was 6 days with a median duration of 6-8 days. The highest rate of diarrhea was noticed in the first cycle and decreased in following

cycles.[76] Prevention of diarrhea is important, since this side effect can negatively impact drug absorption. A population pharmacokinetic and pharmacodynamic model demonstrated that administration of 200 mg twice daily abemaciclib resulted in proportionally less drug absorption than administration of 150 mg twice daily.[77] An explanation for this phenomenon is the increasing incidence of gastro-intestinal toxicity and diarrhea at higher abemaciclib doses, resulting in decreased absorption as a consequence of disrupted intestinal endothelial cells.[39,77] In most patients this side effect does not lead to drug discontinuation. If there are no signs of infections, diarrhea can be treated with dietary modifications, hydration, and loperamide or other antidiarrheal agents. After recovery, a dose reduction can be considered.[31]

5.3 QTc prolongation

Following ribociclib administration, the QTc interval might be prolonged, whereas the other two drugs are not associated with a clinically relevant QTc time prolongation (>20 ms) at the recommended dosing schedules.[31,79] Therefore, QTc prolongation seems to be rather drug specific, instead of a class effect of CDK4/6 inhibitors.[80] QTc prolongation (on average 22.9 ms) was mainly observed in the first cycle of ribociclib in combination with endocrine therapy. In the MONALEESA-2 trial, 11 patients (3.3%) were diagnosed with a QTc prolongation up to >480 ms in combination with letrozole. In the MONALEESA-7 trial a higher incidence of QTc prolongation (>60 ms from baseline) was reported in patients receiving ribociclib in combination with tamoxifen (16%) compared with the combination of ribociclib and a non-steroidal aromatase inhibitor (7%).[81] Based on the foregoing, concomitant intake of ribociclib and drugs with a known potential for QTc prolongation – such as anti-arrhythmic drugs– should be avoided or closely monitored.[80]

6. Future perspectives

6.1 Refining CDK4/6 inhibitor therapy by using biomarkers

Despite the fact that many HR+, HER2- advanced BC patients respond well to CDK4/6 inhibitors in the first line of treatment, in subsequent lines the response period is evidently shorter and in the end all patients will develop resistance to CDK4/6 inhibitors. Consequently, predictive and prognostic biomarkers are needed to further refine CDK4/6 inhibitor treatment of advanced HR+/HER2- BC patients. In the large randomized controlled phase 3 SONIA trial (NCT03425838) the optimal position of CDK4/6 inhibitors in HR+, HER2- advanced breast cancer in first- or in second line treatment setting will be determined. Important secondary aims of this phase 3 trial are to investigate the possible associations between pharmacokinetic, pharmacodynamic and circulating tumor DNA (ctDNA) markers for each CDK4/6 inhibitor in relationship to clinical

outcome.[61] The identification of biomarkers will be of importance both for selection of responsive patients and the optimization of the therapeutic response within a patient.

6.1.1 Biomarkers reflecting sensitivity or resistance to CDK4/6 inhibitors

Biomarkers reflecting increased activity of the CDK-RB1-E2F pathway in tumor cells could help to identify patients sensitive to CDK4/6 inhibitor treatment. However, within the PALOMA-1 study the use of CCND1 amplification or p16 loss as additional biomarkers for patient selection did not result in an improved PFS compared to all ER+/HER2- patients.[84] Vice versa, identification of resistance mechanisms may yield biomarkers to identify tumors resistant to CDK4/6 inhibitors. Whole exome sequencing of CDK4/6 inhibitor exposed breast cancer tissues identified 8 potential resistance mechanisms, including RB1 loss, activating alterations in AKT1, RAS, AURKA, CCNE2, ERBB2, and FGFR2, and loss of ESR1 expression, together explaining 66% of the observed resistance.[85] In the PALOMA-3 trial RB1 mutations were acquired in 5% of patients after treatment with palbociclib, RB1 deletions were observed more frequently but no evidence was found for selection of RB1 deletion during treatment.[87,88] Next to RB1, particularly the E-type cyclins, encoded by the CCNE1 and CCNE2 genes, appear to represent promising markers of resistance. High cyclin E expression was observed in preclinical models with acquired resistance to CDK4/6 inhibitors (88) and gene expression analyses of breast cancer tissues from the PALOMA-3 study showed that low expression of CCNE1 before treatment start was associated with a longer PFS.[89] In the NeoPalAna trial described above, expression of CCNE1, CCND3, and CDKN2D genes remained elevated during treatment with CDK4/6 inhibitors only in non-responding patients.[64] Large clinical trials have to confirm this potential value of cyclin E proteins for identification of CDK4/6 resistant patients.

6.1.2 Biomarkers in liquid biopsies

Liquid biopsies, usually referring to detection of tumor-specific DNA fragments in a patient's blood, represent an promising means for real-time monitoring of treatment response. Targeted next generation sequencing analyses on blood-derived cell-free DNA (cfDNA) from baseline samples were performed in the PALOMA-3 study and showed that copy number gains of FGFR1, CDK4, MYC, CCNE1, and MCL1 as well as p53 mutations were associated with a decreased PFS in patients treated with fulvestrant and palbociclib. However, in multivariable analysis FGFR1 gain, TP53 mutations, and estimated ctDNA tumor fraction were significantly associated with PFS irrespective of treatment arm. Results of this study demonstrate that no specific alteration at baseline was associated with PFS specifically in the palbociclib group.[85] In the same cohort, PIK3CA and ESR1 mutations were determined at baseline and after 2 weeks of treatment only in the palbociclib arm. Interestingly, a decrease in the level of detected mutant PIK3CA molecules, but not ESR1 mutations, within the first two weeks of CDK4/6 inhibitor treatment was associated

with a longer PFS. Although this phenomenon is probably not CDK4/6 inhibitor specific and is likely to reflect ctDNA tumor fraction (i.e. the amount of tumor-derived DNA in the blood) it could provide a potential tool for early monitoring of treatment response irrespective of treatment.[86]

6.1.3 Therapeutic Drug Monitoring (TDM)

For many drugs it has been shown that optimization of drug dosage by plasma concentration within a predefined therapeutic window – also known as therapeutic drug monitoring (TDM) – improves clinical outcome or reduced toxicity.[92–94] For CDK4/6 inhibitors this represents an interesting concept as well. However, presently a minimal effective concentration has not been established for either of these three agents. On the other hand, the correlation between palbociclib exposure and toxicity suggests that we can improve the tolerability by dose reductions.[42,73] Further research is needed to investigate whether the current practice of toxicity guided dose adaptations can be further improved by implementing TDM.

6.2 Widening the application of CDK4/6 inhibitors

6.2.1 HER2+ breast cancer

Several studies have shown a crosstalk exists between HER2 signaling and estrogen receptor pathways in a bidirectional way which make CDK4/6 inhibitors an attractive therapeutic option in this breast cancer subtype.[95] In a preclinical study it has been shown that the CDK4/6 pathway could mediate resistance against HER2- targeted therapies.[96] A phase 2 randomized study explored the safety and efficacy of the combination of abemaciclib plus fulvestrant with trastuzumab in HR+, HER2+ breast cancer patients.[97] Patients were included after at least 2 lines of therapy in the advanced setting. The experimental combination trastuzumab plus abemaciclib and fulvestrant in comparison to trastuzumab plus standard-of-care chemotherapy of physicians choice showed a PFS of 8.3 months versus 5.7 months in the standard-of-care arm (HR 0.673; 95% CI: 0.45 – 1.00). This study confirms preclinical data and is promising since a significant increase in PFS is present, even after 2 or more lines of treatment. However, results of phase 3 studies are needed to confirm this effectiveness.

6.2.2 Triple negative breast cancer

Triple-negative breast cancer (TNBC) is a heterogeneous and aggressive type of breast cancer with limited therapeutic options. Currently CDK4/6 inhibitors do not play a role in the treatment since efficacy is expected to be limited. Several preclinical and clinical studies examine a role for CDK4/6 inhibitors in TNBC in which diverse biological rationales and hypotheses are tested. TNBC can be divided in subgroups by gene expression profiling: luminal-AR, mesenchymal, basal-like immune-suppressed and basal-like immune-activated.[98] Experiments in TNBC

cell lines have shown these subtypes differ in their sensitivity to CDK4/6 inhibition, with the luminal-AR subtype being more sensitive to CDK4/6 inhibitors, whereas the basal-like subtype is highly insensitive. These results, indicate that patients with TNBC should be selected based on their subgroup for treatment with CDK4/6 inhibitors.[99]

Research is ongoing into new developed CDK4/6 inhibitors in combination with other drug modalities in breast cancer. A phase 2 trial explored the benefit of addition of trilaciclib, a novel CDK4/6 inhibitor, to chemotherapy (gemcitabine and carboplatin) in patients with metastatic TNBC. An improvement of antitumor effect and in the tolerability of chemotherapy by myelopreservation was observed in patients who received additional trilaciclib.[100] One of the hypotheses to explain the observed effect is the enhancement of antitumor immunity by trilaciclib,(101) an effect of CDK4/6 inhibitors which has recently raised more interest. Based on the foregoing information phase 3 trials in which TNBC patients are selected based on their underlying subtype are needed to further explore the potential effects of adding CDK4/6 inhibitors to chemotherapy.

6.2.3 CDK4/6 inhibitors and chemotherapy

CDK4/6 inhibitors and chemotherapy target different phases of the cell cycle. CDK4/6 inhibitors induce cell cycle arrest in the G0-G1 phase preventing cells to enter subsequent phases of the cell cycle. This antagonizes the effect of classic chemotherapeutics of which the main mode of action takes place in these subsequent phases. Several preclinical studies in breast cancer explored the effect of treatment with CDK4/6 inhibitors followed by chemotherapy in which monotherapy was superior to the combination of these therapies.[102,103] Interestingly, a preclinical study in pancreatic cancer showed that reversing the order of treatments by giving CDK4/6 inhibitors after chemotherapy could increase the effect of chemotherapy by repressive effects on homologous recombination proteins.[104] Based on these studies sequence and timing of therapies seems to be essential to reach optimal antitumor effects. Further preclinical investigations potentially followed by clinical studies in breast cancer are warranted to explore the implications for future clinical use of CDK4/6 inhibitors after DNA-damaging therapies.

6.2.4 HR+, HER2- breast cancer

Next to CDK4/6-RB1 signaling, PI3K/AKT/mTOR signaling is an important growth stimulatory pathway in HR+, HER2- metastatic breast cancer. One of the described mechanisms of resistance to CDK4/6 inhibition is the PI3K dependent activation of Cyclin D1/CDK2 complex. This complex could phosphorylate pRb leading to S-phase entry independent of CDK4/6. Blocking the activation of cyclin D1 by inhibiting PIK3CA could be a strategy to overcome this resistance, which was effective in cell line experiments.[88] Similarly, mTORC1/2 inhibitors have shown potency to restore sensitivity to CDK4/6 inhibitors in resistant ER+ breast cancer cell

lines.[105] Combining of fulvestrant, a CDK4/6 inhibitor and a PIK3CA inhibitor, which was explored breast cancer patients with advanced disease in a phase 1 trial, results in promising efficacy and safety. A retrospective clinical study found a limited effect of palbociclib when it was given after progression on everolimus, which suggests a role for mTOR inhibitors during or after CDK4/6 inhibitor treatment.[106] These studies implicate that additional treatments which block the PI3K/AKT/mTOR signaling pathway could be of value in overcoming resistance and prolonging the anti-tumor effects of CDK4/6 inhibitors. Sequence and timing will be essential and biomarkers are needed to determine the optimal sequence and timing of treatment on individual patient level.

6.2.5 Other tumor types

Since aberrations in the CDK-RB1-E2F pathway are frequently observed in many types of tumors it is expected that CDK4/6 inhibitors have a wider applicability than breast cancer alone. Amplification of cyclin D (CCND1) for example is described in non-small lung cancer, melanoma and endometrial cancer among others. Several clinical studies in these tumor types are ongoing and the first encouraging results are currently presented.[39] A recently performed phase 2 trial in patients with HR+ recurrent or advanced endometrial cancer showed a prolongation in PFS with the addition of palbociclib to letrozole.[107]

In addition, several preclinical studies suggest other potential mechanisms of action of CDK4/6 inhibitors which could be of interest for the application in other types of tumors as well. For example the ability of CDK4/6 inhibitors to increase the capacity of tumor cells to express antigens and enhance T cell infiltration was shown *in vitro*.[108] This suggests CDK4/6 in combination with immune checkpoint blockades could be of interest in specific tumor types. However, possible applications first need to be explored further in clinical trials.

6.3 New types of CDK4/6 inhibitors

Research is ongoing into the novel CDK4/6 inhibitors lerociclib (NCT03455829; NCT02983071) and trilaciclib (NCT02978716; NCT03041311; NCT02514447) in combination with other targeted therapies or chemotherapy in lung and breast cancer. Preclinical work showed a different pharmacokinetic and pharmacodynamic profile for lerociclib compared to the other CDK4/6 inhibitors since lerociclib accumulated in xenograft tumors but not in plasma.[109] This resulted in less inhibition of myeloid progenitor cells. A recently performed phase 1/2 study of lerociclib in patients with HR+, HER2- MBC showed the potential clinical benefits of lerociclib since a low rate of both neutropenia grade 3 or 4 and gastro-intestinal adverse events was seen compared to the other CDK4/6 inhibitors.[110] These results await confirmation in phase 3 trials. Trilaciclib differs from the clinically available CDK4/6 inhibitors since it is administered intravenously

and is developed to be combined with chemotherapy, mainly to reduce side effects. As earlier described, the first phase 1/2 trial showed promising results in terms of efficacy.

7. Conclusions

CDK4/6 inhibitors – palbociclib, ribociclib and abemaciclib – play an eminent role in the treatment of advanced breast cancer. The pharmacokinetics, -dynamics and efficacy of the three CDK4/6 inhibitors seem to be comparable, although there are also interesting differences such as ability for brain penetration, side effects and dosing schedules. These differences between the three CDK4/6 inhibitors can be used to optimize selection of treatment for individual patients. Further research is needed to investigate the optimal treatment sequence of CDK4/6 inhibitors in different breast cancer settings and different subtypes and to develop (pharmacodynamic) biomarkers for selecting patients, predicting response, and to optimize the treatment schedule.

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CHAPTER 11

Effects of the moderate CYP3A4 inhibitor erythromycin on the pharmacokinetics of palbociclib: a randomized cross-over trial in patients with breast cancer

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Abstract

Palbociclib is an oral inhibitor of cyclin-dependent kinases 4 and 6 used in the treatment of locally advanced and metastatic breast cancer, and is extensively metabolized by cytochrome P450 enzyme 3A4 (CYP3A4). A pharmacokinetic/pharmacodynamic relationship between palbociclib exposure and neutropenia is well known. This study aimed to investigate the effects of the moderate CYP3A4 inhibitor erythromycin on the pharmacokinetics of palbociclib. We performed a randomized crossover trial comparing the pharmacokinetics of palbociclib monotherapy 125 mg once daily (QD) with palbociclib 125 mg QD plus oral erythromycin 500 mg three times daily for seven days. Pharmacokinetic sampling was performed at steady-state for both dosing schedules. Eleven evaluable patients have been enrolled. For palbociclib monotherapy, geometric mean area under the plasma concentration-time curve from zero to infinity ($AUC_{0\text{-}24h}$), maximum plasma concentration (C_{max}), and minimum plasma concentration (C_{min}) were $1.46*10^3$ ng*h/mL (coefficient of variation (CV) 45.0%), 80.5 ng/mL (CV 48.5%), and 48.4 ng/mL (CV 38.8%), respectively, compared with $2.09*10^3$ ng*h/mL (CV 49.3%, p=0.000977), 115 ng/mL (CV 53.7%, p=0.00562), and 70.7 ng/mL (CV 47.5%, p=0.000488) when palbociclib was administered concomitantly with erythromycin. Geometric mean ratios (90% confidence intervals) of $AUC_{0\text{-}24h}$, C_{max}, and C_{min} for palbociclib plus erythromycin versus palbociclib monotherapy were 1.43 (1.24-1.66), 1.43 (1.20-1.69), and 1.46 (1.30-1.63). Minor differences in adverse events were observed, and only one grade ≥ 3 toxicity was observed in this short period of time. To conclude, concomitant intake of palbociclib with the moderate CYP3A4 inhibitor erythromycin resulted in an increase in palbociclib $AUC_{0\text{-}24h}$ and C_{max} of both 43%. Therefore, a dose reduction of palbociclib to 75 mg QD is rational, when palbociclib and moderate CYP3A4 inhibitors are used concomitantly.

Introduction

Palbociclib is an orally administered inhibitor of cyclin-dependent kinases 4 and 6, and is currently approved in combination with an aromatase inhibitor or fulvestrant for the treatment of hormone receptor positive, human epidermal growth factor receptor 2 (HER2)-negative, locally advanced or metastatic breast cancer.[1–3] In the pivotal PALOMA2 (palbociclib (PD 0332991) combined with letrozole versus letrozole for first-line treatment of postmenopausal women with ER-positive, HER2-negative advanced breast cancer) study, patients receiving palbociclib plus letrozole as a first-line treatment had a significantly longer median progression-free survival compared with patients treated with letrozole alone (24.8 months versus 14.5 months, hazard ratio 0.58, p<0.001).[4] Similarly, the addition of palbociclib to fulvestrant was superior to fulvestrant alone in second or subsequent treatment lines in the PALOMA3 (palbociclib (PD 0332991) combined with fulvestrant versus fulvestrant in hormone receptor-positive, HER2-negative metastatic breast cancer after endocrine failure) study (median progression-free survival 9.2 versus 3.8 months, hazard ratio 0.42, p<0.001).[5] The approved dose of palbociclib is 125 mg once daily (QD) in a 3-weeks-on/1-week-off dosing schedule.

As palbociclib is extensively metabolized by cytochrome P450 enzyme 3A4 (CYP3A4), its exposure can be markedly affected by concomitant administration with CYP3A4 modulators.[1,3] In a previous drug-drug interaction study in twelve healthy male volunteers, co-administration of itraconazole, a strong CYP3A4 inhibitor, in a dose of 200 mg for 5 days resulted in an increase in the area under the plasma concentration-time curve from zero to infinity ($AUC_{0-\infty}$) and maximum plasma concentration (C_{max}) of 87% and 34%, respectively, after a single dose of palbociclib on day 5.[1,3,6,7] Based on these data, it is recommended in the drug label of both U.S. Food and Drug Administration (FDA) and the European Medicines Agency (EMA) to avoid concomitant use of strong CYP3A4 inhibitors, or otherwise to reduce the palbociclib dose to 75 mg QD instead of the standard dose of 125 mg QD.[1,3]

No clinical studies have been performed to study the effects of moderate CYP3A4 inhibitors on palbociclib pharmacokinetics. Simulations with a physiologically based pharmacokinetic (PBPK) model of palbociclib predicted that concomitant administration of palbociclib with moderate CYP3A4 inhibitors diltiazem and verapamil would lead to an increase in palbociclib AUC and C_{max} of 38% and 22% for verapamil, and 42% and 23% for diltiazem, respectively.[7] It has been concluded that the risk of drug-drug interactions for palbociclib co-administered with moderate CYP3A4 inhibitors is modest and that dose adjustments are thus not needed. However, we argue that a 40% increase in exposure could be clinically relevant, as higher palbociclib exposure is related to an increased risk of toxicity (i.e. neutropenia).[8,9] In the pivotal studies, 30 to 40% of

patients needed a dose reduction due to toxicity.[4,5,10] Especially in these patients, concomitant administration with moderate CYP3A4 inhibitors could lead to increased adverse events. Based on these simulations, dose reductions to 75 mg QD or 100 mg QD (60% or 80% of the standard dose) might be a strategy to reduce the risk of toxicities, while maintaining adequate exposure. The effect of drug-drug interactions via CYP3A4 has thus far only been evaluated in single dose studies in healthy male volunteers and PBPK simulations. Therefore, a drug-drug interaction study at steady-state concentration in real-life patients treated with palbociclib, would provide the most essential and clinically relevant information. Moreover, this could serve as a showcase for other oral targeted therapies metabolized by CYP3A4 and other moderate CYP3A4 inhibitors. Based on the above, we conducted a randomized pharmacokinetic crossover trial to study the effects of the moderate CYP3A4 inhibitor erythromycin on the pharmacokinetics of palbociclib in female breast cancer patients.

Methods

Study design

We performed a prospective, multi-center, randomized clinical trial with a crossover design, according to the guideline of the FDA for drug-drug interaction studies.[11] **Figure 1** provides a schematic overview of the study design. Patients were randomized to start with either palbociclib 125 mg QD combined with erythromycin 500 mg three times daily (TID)(arm A) or palbociclib monotherapy 125 mg QD (arm B). Pharmacokinetic exposure was determined at both dosing schedules. Erythromycin was selected as a moderate CYP3A4 inhibitor, because this drug shows few side effects compared with other moderate CYP3A4 inhibitors. The selected dose was within the therapeutic range and the dose was in agreement with other DDI studies where erythromycin was used.[12–15] Taking into account the duration-dependent inhibition of CYP3A4 by erythromycin and the mean elimination half-life of palbociclib of 29 hours, one week was considered to be sufficient to reach steady-state concentrations.[1,15] As erythromycin is inhibiting CYP3A4 irreversibly, it can take up to one week until CYP3A4 function is returned to baseline function after discontinuation of erythromycin.[16,17] Therefore, a washout period of one week followed by one week to reach new steady-state concentrations has been chosen. The crossover design of the study was chosen to evaluate potential effects of this washout on outcome. Erythromycin was administered for seven days on either day 1–7 or day 15–21, depending on randomization. Randomization was performed by block randomization in ALEA (FormsVision BV, Abcoude, The Netherlands). The block size was four, and blocks were only visible for a system administrator. Patients were instructed to take palbociclib at 8.00 AM, and erythromycin three times daily at 8.00 AM, 4.00 PM, and 12.00 AM, both palbociclib and erythromycin together with food (diet of own choice of the patient). Patients requiring a dose

interruption or dose reduction or who discontinued treatment during the study were considered non-evaluable for the pharmacokinetic analyses and were replaced. At the end of the trial, palbociclib treatment was continued as part of standard care.

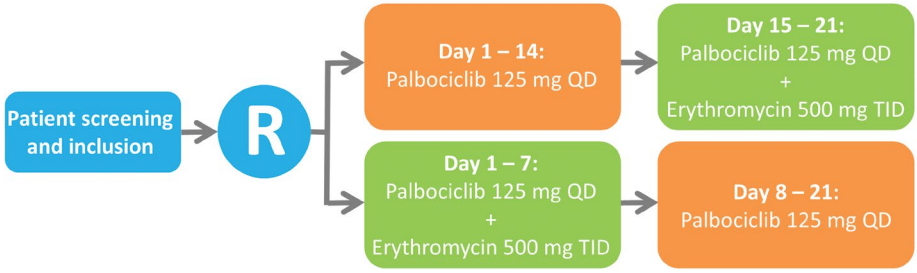

Figure 1 – Pharmacokinetic sampling was performed at day 7 and day 21 of the study. R = randomization, QD = once daily, TID = three times daily.

Patient population

Patients with histological or cytological proof of cancer with an indication for treatment with palbociclib (i.e., advanced breast cancer) at the standard dose of 125 mg QD were eligible for inclusion. Further inclusion criteria were age ≥ 18 years, World Health Organization performance status of 0, 1, or 2, and adequate organ function per judgment of the treating physician.

Exclusion criteria were concomitant use of other medication that could influence the pharmacokinetics of palbociclib within 14 days or five half-lives of the drug (whichever was shorter) before start of the study, including (but not limited to) CYP3A4 inhibitors or inducers, or a QT duration corrected for heart rate (QTc) > 450 milliseconds (or > 480 milliseconds for patients with bundle branch block) because erythromycin may potentially prolong the QTc interval. Therefore, an electrocardiogram was performed at screening.

Pharmacokinetics

At day 7 and day 21 of the study, patients were admitted to the hospital and blood samples were collected for pharmacokinetic analyses. Time points were before dosing (directly before ingestion of palbociclib) and 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, and 24 hours post dosing (just before ingestion of a new palbociclib dose). At each time point, a blood sample was collected in a 3-mL K_2 EDTA tube and centrifuged directly after collection (1,500 *g*, 5 min, 4°C). Plasma was stored at -20°C until analysis. Plasma palbociclib concentrations were quantified using a validated liquid chromatography-tandem mass spectrometry method.[18] This method was validated according to the EMA and FDA guidelines on bioanalytical method validation over a linear range of 50–1000 ng/mL.[19,20]

Study endpoint

The primary objective of this trial was to study the effect of the moderate CYP3A4 inhibitor erythromycin on the pharmacokinetics of palbociclib, measured as $AUC_{0\text{-}24h}$, C_{max}, and minimum plasma concentration (C_{min}). As a secondary objective, the incidence and severity of adverse events (AEs) with and without erythromycin was compared, according to Common Terminology Criteria for Adverse Events (CTCAE), version 5.0.[21]

Safety assessments

Patients were instructed to use a diary to keep track of AEs. Recording of AEs, vital signs, and hematology and blood chemistry assessments were performed at day 7 and day 21 of the study. The incidence, severity and start and end dates of all AEs were recorded and graded according to the CTCAE version 5.0.

Statistics

For the sample size calculation, it was assumed that concomitant administration with erythromycin would result in a 40% increase in palbociclib exposure, based on previous simulations. By assuming an intra-individual standard deviation of the difference of 50% between the two dosing schedules, eleven evaluable patients had to be included to obtain 80% power (one-sided α=0.05) to detect an increase of ≥ 40% in exposure. Pharmacokinetic parameters were calculated using non-compartmental analysis. $AUC_{0\text{-}24h}$ was calculated using the linear/log trapezoidal method. C_{max} was defined as the highest measured concentration. C_{min} was defined as the mean value of the pre-dose and 24 hours post-dose concentration. $AUC_{0\text{-}24h}$, C_{max} and C_{min} of palbociclib monotherapy and combined with erythromycin were compared using one-sided Wilcoxon signed rank tests because of the small sample size. The relative difference was calculated by dividing the value for the treatment with palbociclib plus erythromycin by the value for palbociclib monotherapy. Statistical analyses were performed using R version 3.6.3 (R Project, Vienna, Austria), and the geometric mean and confidence intervals were calculated using the Gmean function in the DescTools package.[22]

Ethics approval and consent to participate

The study was approved by the Medical Ethics Committee of The Netherlands Cancer Institute, Amsterdam. Participating centers were The Netherlands Cancer Institute and the Erasmus Medical Center (MC) Cancer Institute. Local approval was obtained in each participating center. The study was conducted in compliance with Good Clinical Practice and the Declaration of Helsinki. All patients provided written informed consent prior to inclusion in the trial. This study was registered in the Netherlands Trial Register (www.trialregister.nl, NL7549) and the EudraCT database (2018-004032-29). The full trial protocol can be accessed upon reasonable request by contacting the corresponding author.

Results

Patient characteristics

Twelve female patients were enrolled in the study from April 2019 until May 2021. One patient withdrew informed consent before pharmacokinetic (PK) sampling at the second dosing schedule, and was excluded. Baseline characteristics of the evaluable patients are provided in **Table 1**. Median age was 59 years and the median time on palbociclib treatment before enrollment in the study was 10.1 months.

Table 1 – Baseline characteristics (n=11).

Characteristic	n (%) or median [range]
Gender, female	11 (100%)
Age (years)	59 [36-79]
Tumor type	
Breast cancer	11 (100%)
Combination therapy	
Fulvestrant	9 (82%)
Anastrozole	1 (9%)
Letrozole	1 (9%)
WHO performance status	
0	8 (73%)
1	3 (27%)
Previous lines of systemic treatment in metastatic setting (number)	1 [0 – 6]
Previous systemic treatment	
Chemotherapy	11 (100%)
Anti-hormonal therapy	11 (100%)
Time on palbociclib at study inclusion (months)	10.1 [1.2-22.8]

WHO, World Health Organization.

Pharmacokinetics

Palbociclib exposure was higher, for all but one patient, when administered concomitantly with erythromycin (**Figure 2, Figure 3, Table 2**) (no differences were observed between arms). For palbociclib monotherapy, geometric mean $AUC_{0\text{-}24h}$, C_{max}, and C_{min} were $1.46*10^3$ ng*h/mL (coefficient of variation (CV) 45.0%), 80.5 ng/mL (CV 48.5%), and 48.4 ng/mL (CV 38.8%), respectively. When palbociclib was administered in combination with erythromycin, this resulted in an increase in $AUC_{0\text{-}24h}$, C_{max}, and C_{min} to $2.09*10^3$ ng*h/mL (CV 49.3%, p=0.000977), 115 ng/mL (CV 53.7%, p=0.00562), and 70.7 ng/mL (CV 47.5%, p=0.000488), respectively. Geometric mean ratios (90% confidence intervals) of $AUC_{0\text{-}24h}$, C_{max}, and C_{min} for palbociclib plus erythromycin versus palbociclib monotherapy were 1.43 (1.24-1.66), 1.43 (1.20-1.69), and 1.46 (1.30-1.63), respectively. The elimination half-life of palbociclib was 29.8 hours (CV 42.0%) for palbociclib monotherapy, compared with 42.6 hours (CV 39.4%) for palbociclib plus erythromycin (p=0.00928).

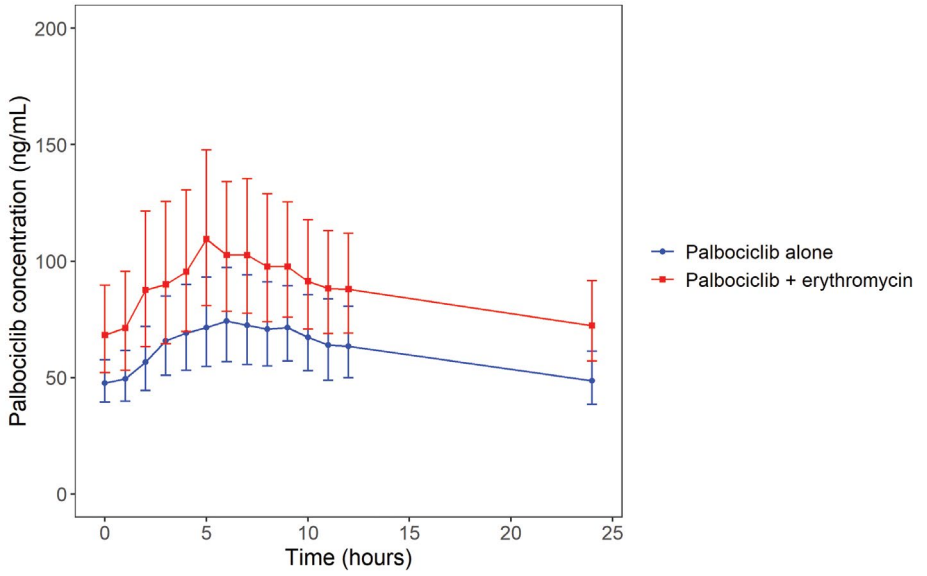

Figure 2 – Palbociclib plasma concentration-time curves of palbociclib monotherapy or combined with the moderate CYP3A4 inhibitor erythromycin.Data are represented as geometric mean + 90% confidence interval. CYP3A4, cytochrome P450 enzyme 3A4.

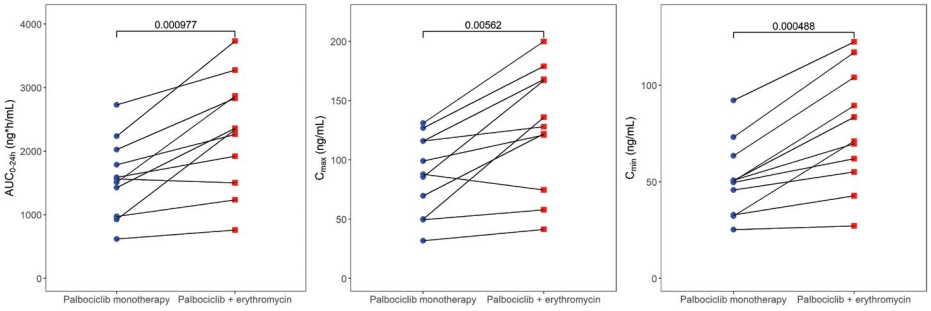

Figure 3 – Plots of palbociclib AUC0-24h, Cmax and Cmin, for palbociclib monotherapy and combined with the moderate CYP3A4 inhibitor erythromycin for each individual patient.

Wilcoxon signed rank tests were performed to calculate p-values (printed above the brackets)
AUC0-24h was calculated using the linear/log trapezoidal method
Cmax was defined as the highest measured concentration for each dosing schedule
Cmin was defined as the median value of the pre-dose and 24 hours post-dose sample
AUC0-24h = area under the plasma concentration-time curve from 0 to 24 hours, Cmax = maximum plasma concentration, Cmin = minimum plasma concentration; CYP3A4, cytochrome P450 enzyme 3A4

Table 2 – Pharmacokinetic parameters of palbociclib with and without the moderate CYP3A4 inhibitor erythromycin.

PK parameter	Palbociclib monotherapy	Palbociclib + erythromycin	Geometric mean ratio [90% CI]	*p*-value [a]
AUC_{0-24h} (ng*h/mL)[b]	$1.46*10^3$ (45.0%)	$2.09*10^3$ (49.3%)	1.43 [1.24-1.66]	0.000977
C_{max} (ng/mL)[c]	80.5 (48.5%)	115 (53.7%)	1.43 [1.20-1.69]	0.00562
C_{min} (ng/mL)[d]	48.4 (38.8%)	70.7 (47.5%)	1.46 [1.30-1.63]	0.000488
$T_{1/2}$ (h)	29.8 (42.0%)	42.6 (39.4%)	1.43 [1.14-1.79]	0.00928

Pharmacokinetic parameters are expressed as geometric mean (CV%).
Administered doses were 125 mg QD for palbociclib and 500 mg TID for erythromycin.
[a] Wilcoxon signed rank tests were performed to calculate *p*-values
[b] AUC_{0-24h} was calculated using the linear/log trapezoidal method
[c] C_{max} was defined as the highest measured concentration for each dosing schedule
[d] C_{min} was defined as the median value of the pre-dose and 24 hours post-dose sample
AUC_{0-24h} = area under the plasma concentration-time curve from time zero to 24 hours, C_{max} = maximum plasma concentration, C_{min} = minimum plasma concentration, CV = coefficient of variation, QD = once daily, TID = three times daily, $t_{1/2}$ = elimination half-life. PK, pharmacokinetic; CI, confidence interval; CYP3A4; cytochrome P450 enzyme 3A4.

Table 3 – Treatment-related adverse events (AEs) according to CTCAE v5.0.

Adverse event	Palbociclib monotherapy		Palbociclib plus erythromycin	
	Any grade (n)	Grade ≥ 3 (n)	Any grade (n)	Grade ≥ 3 (n)
All patients				
Diarrhea	0	0	4	0
Nausea	0	0	2	0
Vomiting	0	0	1	0
Neutropenia	3	0	2	1
Total number of patients experiencing AEs	4	0	7	1
Patients in arm A				
Diarrhea	0	0	3	0
Nausea	0	0	1	0
Vomiting	0	0	0	0
Neutropenia	3	0	0	0
Total number of patients experiencing AEs	3	0	3*	0
Patients in arm B				
Diarrhea	0	0	1	0
Nausea	0	0	1	0
Vomiting	0	0	1	0
Neutropenia	0	0	2	1
Total number of patients experiencing AEs	1	0	4**	1

*One patient experiencing both diarrhea and nausea, therefore total number of patients is lower than number of adverse events.
**One patient experiencing both diarrhea and neutropenia, therefore total number of patients is lower than number of adverse events.
AE = adverse event, CTCAE = Common Terminology Criteria for Adverse Events, arm A = started with palbociclib combined with erythromycin, arm B = started with palbociclib monotherapy

Treatment-related adverse events

An overview of all treatment-related AEs is provided in **Table 3**. Nine patients experienced one or more treatment-related AEs. No patients discontinued treatment and none required a dose reduction. Only one grade 3 toxicity (neutropenia) occurred during the treatment with palbociclib plus erythromycin.

Discussion

Here, we reported the results of a prospective randomized crossover study assessing the effects of the moderate CYP3A4 inhibitor erythromycin on the pharmacokinetics of palbociclib. Concomitant administration resulted in a significantly higher palbociclib exposure, with increases in $AUC_{0\text{-}24h}$, C_{max}, and C_{min} of 43%, 43%, and 46%, respectively, which is of clinical relevance. Minor differences in adverse events were observed, and only one grade 3 toxicity was observed, in this short period of time.

The observed effect size in the current study was in line with previous simulations for $AUC_{0\text{-}24h}$, but substantially larger for C_{max}, as earlier simulations with diltiazem and verapamil predicted an increase of ± 40% in AUC and ± 23% in C_{max}.[23] Notably, the effect on C_{max} as found in our study is even higher than the effect on C_{max} of the strong CYP3A4 inhibitor itraconazole (i.e., 34%).[1,6] Although no full explanation could be found for this discrepancy in effect size, this may partly be explained by the applied sampling schedule in the drug-drug interaction study with itraconazole (i.e., 2, 4, 6, 8, 12 h postdose, instead of each hour up to 12 h postdose in the current study, which may have missed the true C_{max}).[1,6]

The fact that a similar increase in $AUC_{0\text{-}24h}$, C_{max} and C_{min} was observed (**Table 2**), suggests that the effect of erythromycin is -for an important part- determined by an increased bioavailability (i.e., via inhibition of intestinal CYP3A4). Yet, the elimination half-life was also significantly longer for palbociclib plus erythromycin compared with palbociclib monotherapy, which means that a lower clearance (i.e., via inhibition of hepatic CYP3A4) plays a role as well. The prolonged half-life of palbociclib when it is combined with erythromycin, may imply that the washout period was shorter than five times the half-life. Still, the washout period was at least four times the half-life, which allowed for 94% of new steady-state. Most importantly, no difference in palbociclib PK when given as monotherapy was observed between treatment arms, and therefore, it could be concluded that the washout period was sufficient. Apart from being a moderate CYP3A4 inhibitor, erythromycin also inhibits P-glycoprotein (P-gp).[24] Theoretically, inhibition of P-gp could also explain the observed increase in bioavailability, as palbociclib is a substrate of P-gp.[25,26] However, a previous study in mice demonstrated that P-gp mainly restricted the

brain penetration of palbociclib, whereas its oral bioavailability was only marginally affected.[26] Therefore, we expect the effect of P-gp inhibition on the palbociclib plasma concentrations in the current study to be minimal.

An important advantage of the drug-drug interaction study described here, is that it was performed in the target population of (female) breast cancer patients. In the pivotal drug-drug interaction study with the strong CYP3A4 inhibitor itraconazole, only male healthy volunteers were included.[7] The subsequently performed PBPK simulations to predict the effect of moderate CYP3A4 inhibitors were based on the results found in the male subjects. To exclude the possibility of a gender effect, e.g., on CYP3A4 enzyme activity, this study was conducted in female patients, which are the patients using palbociclib in clinical practice.

Because of pharmacogenetic differences, the exposure to palbociclib could be different between patients. For CYP3A4 the polymorphism *CYP3A4*22* has been described by Wang *et al.*[27] In liver samples with a *CYP3A4*22* polymorphism ~ 15% of total CYP3A4 was non-functional, compared to 6% in wildtype liver samples. Because, in case of this polymorphism, still the majority of CYP3A4 will be functional, the genotype will have little effect on the extent of drug inhibition. Therefore, a meaningful comparison could be made between palbociclib monotherapy and palbociclib plus erythromycin combination therapy, without the need of prior pharmacogenetic analyses.

Neutropenia is the most common adverse event during palbociclib treatment. Higher palbociclib exposure has been related to an increased risk of neutropenia in previous studies.[8,9] It is, therefore, logical to assume that concomitant administration of palbociclib and moderate CYP3A4 inhibitors will result in a higher incidence of neutropenia, depending on dose and duration of concomitant administration of the inhibitor. The secondary outcome of the current study was to compare toxicities between the two dosing schedules (i.e., palbociclib monotherapy and palbociclib plus erythromycin). However, neutropenia is a cumulative toxicity that is most pronounced at the end of each palbociclib cycle. Therefore, comparisons of neutropenia between day 7 and day 21 of a cycle are not meaningful. Instead, comparisons could be made with previous palbociclib cycles, in which no moderate CYP3A4 inhibitors were used. However, only one grade 3 neutropenia was observed in our study, probably as a result of the short duration of erythromycin treatment of seven days. The patient who experienced a grade 3 neutropenia at the end of the studied period, had a grade 2 neutropenia at the end of her previous treatment cycles. Because of the short duration of concomitant use of a moderate CYP3A4 inhibitor, a meaningful comparison of toxicity could not be performed. However, as these patients had no indication to use a moderate CYP3A4 inhibitor, it was considered unethical to prescribe these drugs longer than necessary to reach steady-state concentrations. Since an exposure-toxicity

relationship for palbociclib has already been described, the comparison between palbociclib monotherapy and palbociclib plus erythromycin based on PK was considered sufficient to give a dose recommendation for the interaction.

As palbociclib exposure increased by more than 40% when administered concomitantly with erythromycin, and palbociclib pharmacokinetics change in a dose-proportional manner[1,3], it is rational to reduce the palbociclib dose by 40%, i.e., to 75 mg QD, in case of concomitant administration with moderate CYP3A4 inhibitors, without fear for underdosing. For patients who already received prior dose reductions, e.g., due to toxicity, it could be considered to reduce the dose even further by switching to an every other day dosing schedule (as no smaller capsule size than 75 mg is currently available). Adjusting the dosing schedule to 5 days on/2 days off every 7 days with no weeks off therapy might also be possible, since it has been described that this alternative schedule leads to a better tolerability.[28] For strong CYP3A4 inhibitors, a dose reduction to 75 mg QD was recommended as well, while $AUC_{0-\infty}$ was increased by 87% in that case.[1,3,6] However, first of all that combination should be avoided according to the drug label. Secondly, 75 mg capsules are the lowest dose currently available in the market.

Next to palbociclib, there are other cyclin-dependent kinases 4 and 6 (CDK4/6) inhibitors available for the treatment of breast cancer.[2,29–32] However, these CDK4/6 inhibitors are also substrates of CYP3A4.[29,30] Combination with a strong CYP3A4 inhibitor increased the AUC of palbociclib by 1.9-fold, compared with an increase of 3.2-fold for ribociclib, and 1.7 to 2.5-fold for abemaciclib plus active metabolites (potency adjusted).[1,29–32] Complicating factors are the auto-inhibition of CYP3A4 by ribociclib, and the metabolism of abemaciclib to active metabolites.[29–32] Since the effect of CYP3A4 inhibition on ribociclib is much larger than on palbociclib, the use of palbociclib is preferred if concomitant administration with a CYP3A4 inhibitor is necessary.[1,30] The effect of CYP3A4 inhibition on abemaciclib exposure seems comparable to the effect on palbociclib, but the effect of a moderate inhibitor on palbociclib is now studied in a clinical trial. Therefore, we recommend to use palbociclib if concomitant administration with a CYP3A4 inhibitor is necessary.

To conclude, concomitant intake of palbociclib and the moderate CYP3A4 inhibitor erythromycin results in an increase in AUC_{0-24h} and C_{max} of palbociclib of both 43%, which is clinically relevant. Therefore, in case of concomitant use of palbociclib and moderate CYP3A4 inhibitors, it is rational to reduce the palbociclib dose to 75 mg QD, without fear for underexposure. This is especially relevant for the 30 to 40% of patients who need a dose reduction of palbociclib during regular treatment due to toxicity.[4,5,10] It should be considered to update the drug label of palbociclib to include these findings and recommendations, and add moderate CYP3A4 inhibitors to the list of potentially interacting drugs for CDK4/6 inhibitors.

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CHAPTER 12

Quantification of ribociclib in dried blood spots by LC-MS/MS: method development and clinical validation

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Abstract

A reliable, specific, selective and robust liquid chromatography-tandem mass spectrometry (LC-MS/MS) method was developed for the determination of ribociclib in both dried blood spot (DBS) samples and potassium EDTA plasma. DBS samples were obtained simultaneously with a plasma sample in advanced breast cancer patients treated with ribociclib. A 6 mm disk from the central part of the dried blood spot sample was punched, followed by extraction of ribociclib using liquid-liquid extraction spiked with ribociclib-d6 as internal standard. Concentrations of ribociclib in DBS samples were correlated with corresponding plasma concentrations. From the blood sample also hematocrit was determined. The method was validated for selectivity, sensitivity, precision, lower limit of detection, linearity, stability and accuracy according to the food and drug administration (FDA) guideline. The within- and between-run precisions were ≤10.6 and ≤1.07%, respectively; while the average accuracy ranged from 100 to 103%. The influence of hematocrit on validation parameters was tested in the range of 0.20 – 0.40 L/L. No influence of hematocrit on validation parameters was observed. Regression analysis and a Bland-Altman plot indicated correlation between the results obtained from DBS and plasma samples. A strong correlation (R^2 >0.97) between DBS samples and plasma concentration from 17 breast cancer patients was found. A number of 12 out of 17 processed DBS samples (71%) fell inside the acceptable range of 20% difference of simultaneously obtained plasma samples. The lower limit of quantification in DBS is 10.0 ng/mL and linearity was demonstrated up to 1,000 ng/mL. In conclusion, the newly developed assay met the required standard for validation. The methods were used to study ribociclib disposition in patients with advanced breast cancer.

1. Introduction

Worldwide, hormone sensitive breast cancer is the most common cancer diagnosis among women. In the US and Europe, about 6% of women are diagnosed with *de novo* metastatic breast cancer.[1] Endocrine therapy is an effective, well-tolerated treatment option for metastatic and advanced breast cancer, however almost all patients develop resistance.[2] In recent years, a novel treatment option, i.e. the class of oral cyclin dependent kinase (CDK) 4/6 inhibitors, has been introduced for patients with advanced or metastatic hormone sensitive breast cancer.[3] Clinical trials demonstrated that CDK4/6 inhibitors in combination with endocrine therapy lead to a statistically significant improvement in progression-free survival.[4]

There are currently three CDK4/6 inhibitors – palbociclib, ribociclib and abemaciclib – approved by the Food and Drug Administration (FDA) for hormone sensitive metastatic or advanced breast cancer in combination with endocrine therapy.[3] Furthermore, clinical trials are ongoing to potentially extend the indication area to other solid tumour types (NCT02933736; NCT04000529; NCT03673124; NCT02555189).

In oncology, many pharmacokinetic targets for therapeutic drug monitoring (TDM) are being identified to individualise the dosage of oral anticancer agents.[5] A randomized phase III trial is ongoing to identify the pharmacokinetic targets and exposure-response relationship for the three CDK4/6 inhibitors.[6] For TDM, preferably a trough concentration is measured for interpretation of the systemic drug concentration.[7] This is difficult to achieve since conventional blood sampling often takes place immediately before a hospital visit at a random time point. For that reason and to offer a more patient friendly sampling technique, dried blood spot (DBS) sampling has been developed. With this technique a patient collects a drop of blood from his/her finger (<50 µL) on a small blood filter card at home and sends it by regular mail to the laboratory for analysis. Advantages of the DBS method are a better stability because of less enzymatic degradation compared to plasma; easier to sample a trough concentration by self-sampling and patients with phlebitis are no longer excluded from frequent blood sampling.[8–10]

Currently, some assays for ribociclib are available in human plasma and brain tumour tissue.[11–13] However, a DBS method for ribociclib has not yet been developed. Considering the advantages of a DBS method for patients and the possibility of applicability in pharmacokinetic research, the availability of a DBS method is desirable. The collection of a DBS sample is a more patient friendly method and is therefore useful for therapeutic drug monitoring in the near future. Therefore, the aim of this study was to develop and validate a DBS method for advanced breast cancer patients treated with ribociclib.

2. Materials and methods

2.1 Chemicals and materials

Ribociclib ($C_{23}H_{30}N_8O$) was obtained from TRC (Toronto, ON, Canada) and ribociclib-d6 was obtained from Clearsynth (Mississauga, ON, Canada). Acetonitrile, methanol and water were purchased from Biosolve (Valkenswaard, The Netherlands). Ammonium formate was obtained from Honeywell GmbH (Seelze, Germany). Dimethyl sulphoxide (DMSO) was obtained from Sigma-Aldrich (Zwijndrecht, The Netherlands). Formic acid was obtained from J.T. Baker (Deventer, The Netherlands). 2-propanol was obtained from Merck GmbH (Darmstadt, Germany). Nitrogen was purchased from Linde gas (Schiedam, the Netherlands). The Whatman 903™ protein saver cards were supplied by GE Healthcare companies (Cardiff, UK). Sterile safety 1.8 mm lancets were obtained from Vitrex Medical A/S (Herlev, Denmark). A regular puncher (Fiskars, Helsinki, Finland) was used for punching the DBS disks out of the spotting card. Blank human plasma was supplied by Biological Specialty Corporation (Colmar, PA, USA). In all experiments ribociclib-free human whole blood (Dutch blood donation center Sanquin, Rotterdam, The Netherlands) was used.

2.2 Preparation of stock solution, calibrators and QC samples

Ribociclib working stock solution (100,000 ng/mL) was prepared in DMSO and stored at T <-70°C. Calibration curve working solutions were prepared in acetonitrile/DMSO (1:1, *v/v*). The internal standard ribociclib-d6 (100 ng/mL) was prepared in methanol (DBS) or acetonitrile (plasma) and stored in a fridge T= 3-7 ˚C. Calibration curve standards were diluted in human ribociclib-free EDTA whole blood (DBS) or lithium heparinized plasma (plasma) to construct the calibration curves yielding amounts of: 10.0, 25.0, 100, 250, 500, 750, 900 and 1,000 ng/mL. The stock solution was diluted in organic solvent followed by preparation of individual levels from corresponding organic solvent dilutions. Four levels of quality controls (QCs) were prepared in EDTA blood (DBS) or lithium heparinized plasma (plasma), including the lower limit of quantification (LLOQ) (10.0 ng/mL), low-level (30.0 ng/mL), middle-level (400 ng/mL) and high-level (800 ng/mL). Calibration curves prepared in plasma were freshly prepared on the day of analysis, while pools of QC samples prepared in plasma were aliquotted and stored at T<-70°C until analyses. 50 µL calibration curve standards and pools of QC-samples prepared in EDTA blood were spotted onto Whatman 903™ Protein Saver Cards in the center of the spot and dried for 24 hours at ambient temperature. Hereafter they were stored in a sealed back at ambient temperature, protected from light, until analyses.

2.3 Study set-up and sample collection

A cross-sectional observational study was set up to determine ribociclib concentrations in both DBS and plasma samples. In this study, patients were eligible to enroll when they use ribociclib on a dose according to the prescription of the physician. On a random hospital visit at day 7 – 21 of a ribociclib cycle a DBS sample was obtained simultaneously with the peripheral venous sample. After an instruction from a nurse, a fingertip sample was performed by the patient. The blood sample was spotted onto a Whatman 903™ Protein Saver Card. The spotted cards were stored (protected from light in a sealed bag) at room temperature until analyses. From the peripheral venous blood sample used to generate plasma also hematocrit was determined by a DxH 500 hematology analyzer (Beckman Coulter Nederland B.V., Woerden, the Netherlands). Our study protocol was approved by the ethics committee of the Erasmus Medical Center (MEC 19-0467) and registered in the Netherlands Trial Register (www.trialregister.nl; number NL8197). Prior to collection of both plasma and DBS samples all patients provided written informed consent.

2.4 Plasma sample preparation and extraction

Ribociclib in human plasma was quantified by a validated LC-MS/MS method in our laboratory (unpublished data; Erasmus MC Cancer Institute, the Netherlands). An amount of 25 µL plasma was mixed with 100 µL of internal standard (200 ng/mL ribociclib-d6 in acetonitrile). After vortex mixing for 5 seconds and centrifugation for 10 minutes at 12,000**g*, an amount of 50 µL of the clear supernatant was transferred to a 96-well plate and mixed with 100 µL of 5 mM ammonium formate/water/formic acid (100:0.1, v/v) from which 10 µL was injected into the LC-MS/MS system.

2.5 DBS sample preparation and extraction

Using a manual disk puncher in the center of the spot, a 6 mm punch of the DBS sample was transferred to a 2-mL safe lock vial containing 200 µL internal standard working solution. After mixing on a vortex for 10 seconds, samples were treated with ultrasound for 20 minutes at T = 40 ± 5 ˚C. An aliquot of 50 µL supernatant was transferred into a 350-µL 96-well plate and 100 µL of 5 mM ammonium formate/water/formic acid (100:0.1 v/v) was added where after the plate was shaken for 5 minutes on a rocked platform. Aliquots of 10 µL were injected onto the UPLC column.

2.6 Apparatus and chromatographic system

The LC-MS/MS system (Waters Chromatography B.V. Etten-Leur, the Netherlands) consisted of a UPLC sample Manager (Waters Acquity), coupled to a Waters TQ mass spectrometer. Separation was achieved using a 2.1 mm x 50 mm, 1.8 µm UPLC column (Waters, Acquity

UPCL® HSS T3). Data was processed with MassLynx software V4.1 SCN627 and concentrations were calculated by using an integrator (QuanLynx software). The mobile phase A consisted of 5 mM ammonium formate/water/formic acid (*0.5:99.5:0.1 v/v/v*) and 5 mM ammonium formate/methanol/formic acid (*0.5:99.5:0.1 v/v/v*) for mobile phase B. A linear gradient setting was used with 90-50% mobile phase A (*i.e.* 10-50% mobile phase B) from 0 to 1 min, 50-0% from 1 to 2 min, 0-45% from 2 to 2.5 min and held for 0.5 min and back to 0% mobile phase A from 3 to 4 min and held for 0.5 min and back to 45% at 5.5 min. In 1 min, mobile phase was returned to initial situation and re-equilibrate for 0.5 min. The overall run-time was 7 min. The temperature of the column was set at T = 40 ˚C with a flow rate of 0.400 mL/min.

2.7 Mass spectrometry

Quantification was conducted with the positive multiple reaction monitoring (MRM+) mode, with use of argon gas collision induced dissociation, which resulted in the subsequent m/z ion transitions (*435>322*) for ribociclib and (*442>322*) for the internal standard. Primary to secondary ion ratios, *435>322 / 435>367* for ribociclib and *442>322 / 442>373* for the internal standard were used to show the observed peaks confirm identity. In **Figure 1** a typical mass spectrum for ribociclib is shown which was obtained with a cone voltage and collision energy of 50 V and 35 V, respectively. The following internal parameters of the device were used: capillary voltage 3.50 kV; source temperature 120 ˚C; desolvation temperature 350 ˚C; cone gas (nitrogen) 25L/h; desolvation gas (nitrogen) 800 L/h and a collision cell pirani pressure of ~$5.5e^{-3}$ mbar (measure for organic gas flow). Calibration curves were constructed by linear-regression analysis in a range of 10.0 to 1,000 ng/mL. Weighted Linear regression (1/concentration²) was performed in the range of 10 to 1000 ng/mL with peak area ratio (Analyte/IS) as dependent variable.

2.8 Method validation

The analytical method validation was performed at a standardized blood hematocrit value (0.40 L/L, *i.e.* 40%). Selectivity, accuracy (ACC), within-run precision (WRP), between-run precision (BRP), extraction recovery, matrix effect, carry-over and stability for both DBS and plasma samples were assessed. The method validation was based on the recommendations and criteria for bioanalytical method validation of the Food and Drug Administration (FDA) and the draft of ICH M10 Only matrix effect and recovery of DBS samples were tested on five lots of individual donors instead of the recommended six lots.

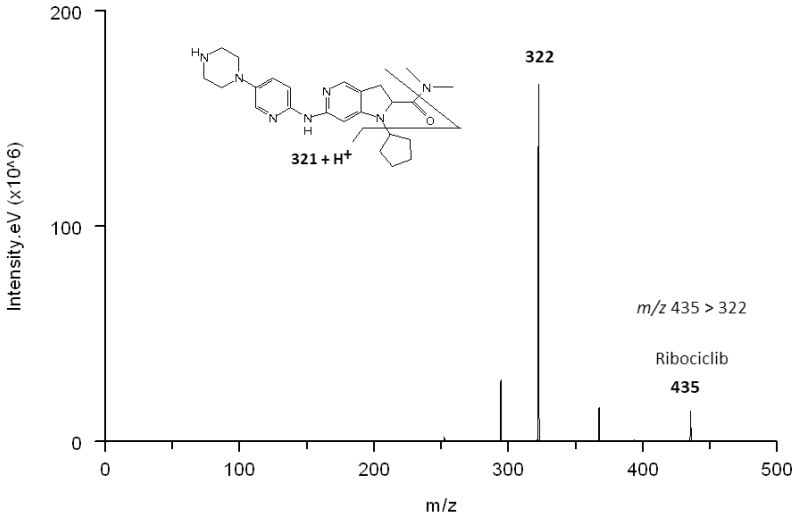

Figure 1 – Mass spectrum and chemical structure of ribociclib.

2.8.1 Extraction recovery and matrix effect

Extraction recovery was determined by comparing the MS/MS response of ribociclib at QC-Low and QC-High in six different lots of human potassium EDTA plasma before extraction versus extracts of six different lots of blank human potassium EDTA plasma after extraction. The influence of matrix components on the ribociclib ionization was evaluated by comparing the MS/MS response of ribociclib at QC-Low and QC-High spiked concentrations to the MS/MS responses of ribociclib spiked in triplicate into extracts of six human potassium EDTA plasma samples. The matrix effect for DBS samples was assessed by spiking five different lots of blank human whole blood (of individual donors) with ribociclib at concentrations of 30.0 ng/mL (QC-Low) and 800 ng/mL (QC-High). Amounts of 50 µL of blood was applied on the Whatman 903™ protein saver card and dried for 24 hours at room temperature. Hereafter, the samples were further prepared according to section 2.5. Matrix and recovery were determined as described earlier in literature.[14]

2.8.2 Stability

The stability of ribociclib in plasma during three freeze-thaw cycles was tested in triplicate at the concentrations of QC-Low, QC-High and QC-Diluted. The stability in human potassium EDTA plasma of ribociclib at ambient temperature was tested in triplicate at the concentrations of QC-Low and QC-High. The stability of ribociclib in DBS samples were tested in triplicate at concentrations of 30.0 ng/mL (QC-low) and 800 ng/mL (QC-high) ribociclib. The DBS samples were stored – protected from light – in a controlled cabin at 20 ± 5 °C, in a fridge at T= 3-7 °C (in a sealed bag of 2 gram including silica gel desiccant) and at room temperature for at least 5 months.

2.8.3 Hematocrit effect

In general, a point of attention in dried blood spot methods is the inter-individual variability in hematocrit concentration; as this can greatly influence the spreadability and rheological properties of the blood spot on the filter paper. This is especially relevant for compounds with large differences between plasma and whole blood concentrations.[15–17] In addition, hematocrit levels can vary greatly in certain (oncological) diseases. Therefore, a potential haematocrit effect should be considered during the clinical validation of a DBS method.[18] To investigate the potential influence of hematocrit on the concentration measurement of ribociclib, DBS samples were prepared in whole blood with different relevant hematocrit concentrations (0.20, 0.35 and 0.40 L/L) and spiked at concentrations of 30.0 ng/mL (accuracy 94-104% and RSD 2.7 – 8.3%, respectively) and 800 ng/mL (accuracy 90-108% and RSD 2.2 - 4.8%, respectively) ribociclib; with an acceptance criterion %RSD ≤15%.

2.9 Data analysis

To evaluate the correlation between ribociclib concentrations in different biological matrices a correlation coefficient was calculated (Microsoft Excel 2016). The correlation coefficient measures the strength and direction of a relationship between two variables. Bland-Altman analyses were performed to evaluate the correlation between the ribociclib concentrations in both matrices.[19] At least 67% of samples should have a prediction error of <20%, in accordance with to the criteria for validation of the European guideline on bioanalytical method validation for industry.[17]

3. Results and discussion

3.1 LC-MS/MS conditions

For the quantification of ribociclib MRM transition, cone voltage and collision energy were optimized by direct infusion. The mass spectrum of ribociclib is displayed in **Figure 1**. The lower limit of quantification (LLOQ) was 10.0 ng/mL and calibration curves were linear with concentrations in the range of 10.0 – 1,000 ng/mL (weighting factor of 1/concentration^2), as shown by the mean correlation coefficient of 0.97 (n=17; Y-intercept = 0.066; mean RSD 94.3%). Ribociclib was also validated for quantification in human potassium EDTA plasma on our laboratory in the range of 10.0 – 1,000 ng/mL (**Table 1**). In **Figure 2**, representative chromatograms are shown which were obtained after processing of a blank DBS sample and DBS spiked with Internal Standard and 10.0 ng/mL (LLOQ) ribociclib. Also a DBS sample collected from a representative breast cancer patient – containing 15.0 ng/mL ribociclib – was included.

Table 1 – Calculations of the grand mean, accuracy and within-run and between-run precisions of the LLOQ and QC samples[a].

Sample	Spiked (ng/mL)	GM (ng/mL)	ACC (%)	WRP (%)	BRP (%)	n[b]
Plasma						
LLOQ	10	10	100	8.60	3.58	15 of 15
QC Low	30	29.5	98.3	3.64	1.25	15 of 15
QC Middle	400	395	98.8	3.94	2.04	15 of 15
QC High	800	779	97.4	2.21	0.904	15 of 15
QC Diluted	8000	7628	95.4	3.51	2.64	15 of 15
DBS						
LLOQ	10	10.3	103	10.6	1.07	14 of 15
QC Low	30	30.6	102	6.52	#	15 of 15
QC Middle	400	400	100	4.10	#	15 of 15
QC High	800	810	101	4.52	#	15 of 15

Abbreviations: GM, grand mean; ACC, average accuracy; WRP, within-run precision; BRP, between-run precision; DBS, dried blood spot. Symbol: #: no additional variation observed by performing the assay in different runs. [a] n=5 in 3 separate runs. [b] Number of individual samples falling within acceptable range of accuracy of 85-115% (80-120% at LLOQ).

3.2 General method validation

The method fulfilled the acceptance criteria of the bioanalytical method validation FDA and ICH Q2 (R1) guidelines regarding accuracy and precision of the calibrators and QCs. The mean accuracy ranged from 100 to 103%. For ribociclib, the within- and between-run precisions at four tested concentrations were ≤10.6% and ≤1.07% including the LLOQ. The absolute deviations from the nominal value of all analyzed calibrators met the acceptance criteria of ≤15% for nominal concentrations (≤20% for the LLOQ). The mean accuracy, within- and between-run precision – assayed in quintuplicate on three occasions with the calibrators in duplicate – for both plasma and DBS samples are depicted in **Table 1**. No interferences from endogenous compounds in human potassium EDTA whole blood and carry-over effects were observed. Also no major matrix effect (mean 126 ± 5.42 (QC-Low) and 112 ± 2.12 (QC-High), respectively) was observed and the mean extraction efficiency was 72.0 ± 7.29 (QC-Low) an 64.8 ± 2.73 (QC-High), respectively. Ribociclib DBS samples were proven to be stable for at least 5 months when stored in a controlled cabin (T = 20 °C; relative air humidity (RH) 25%), at ambient temperature (T = 20 °C) protected from light or in a fridge (T = 3–7 °C); with mean percentages to control of 104%, 101% and 105%, respectively (**Table 2**). The validation parameters were not influenced by hematocrit in a relevant range for patients with cancer of 0.20–0.40 L/L. The bias from nominal value of hematocrit on spot volume was maximal 5% and 10% for QC-Low and QC-High, respectively.

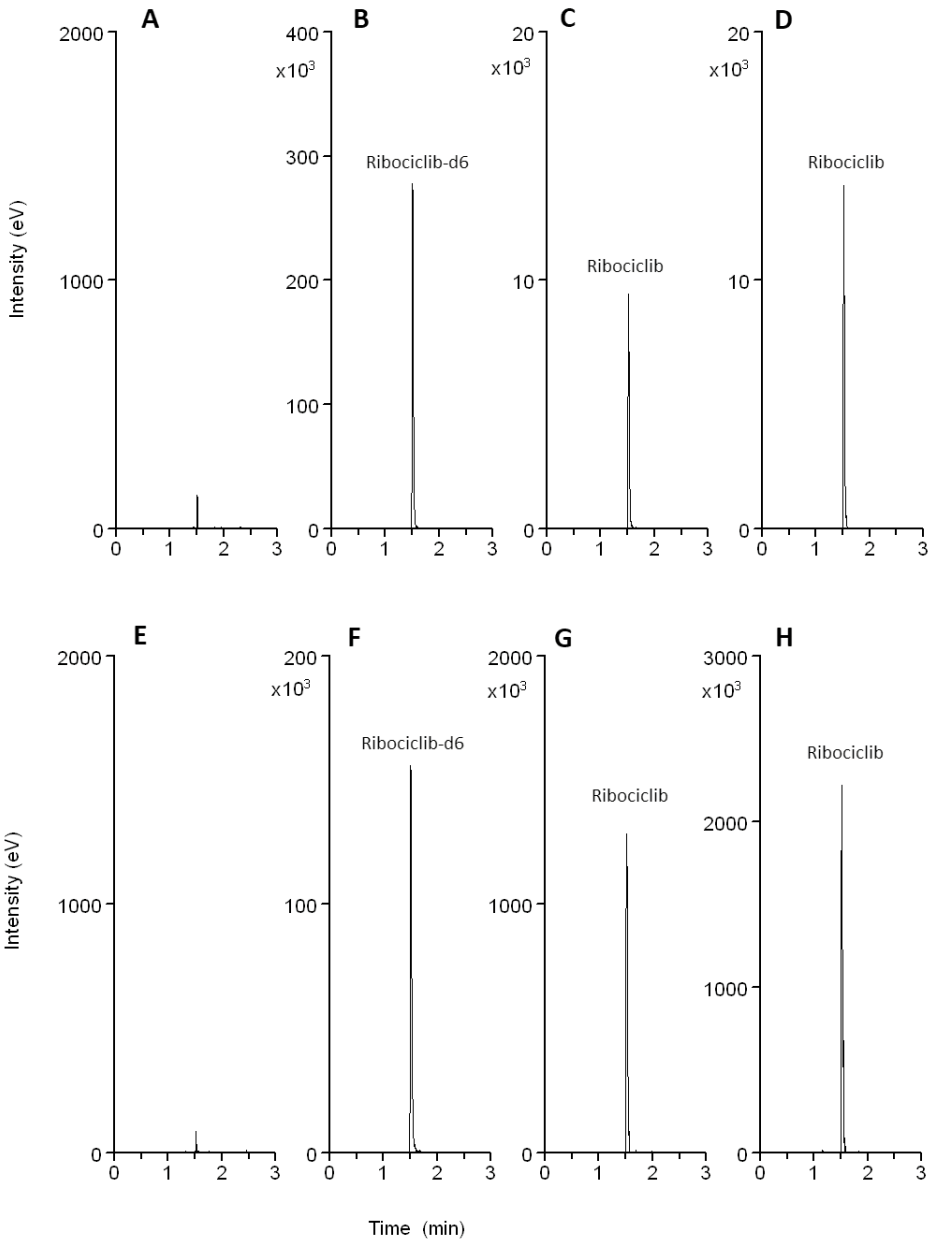

Figure 2 - Representative chromatograms of (A) double blank processed plasma sample, (B) blank processed plasma sample with Internal Standard, (C) spiked plasma sample containing 10.0 ng/mL ribociclib (LLOQ), (D) plasma sample collected prior to the administration of a fixed oral dose of 600 mg ribociclib once daily, containing 15.1 ng/mL ribociclib, (E) blank processed DBS sample, (F) blank processed DBS sample with Internal Standard, (G) DBS sample spiked with 10.0 ng/mL ribociclib (LLOQ) and (H) DBS sample collected simultaneously with a regular plasma sample (See D) containing 15.0 ng/mL ribociclib.

Table 2 – Stability of ribociclib in DBS samples.

Condition	% to control Concentration Low (RSD%)	High (RSD%)
Plasma ribociclib (n=3)		
5 days Ambient temperature[1]	91 (1.0)	97 (6.0)
3 freeze-thaw-cycles[1]	108 (12)	99 (2.7)
DBS ribociclib (n=3)		
Controlled cabin, T = 20 ˚C, RH 25% (5 months) [1]	106 (7.7)	102 (2.9)
Fridge, T= 3–7 ˚C (5 months)[1]	107 (9.8)	94 (3.7)
Ambient Temperature (5 months)[1]	106 (7.8)	103 (8.2)

[1] *Versus value non-treated.*

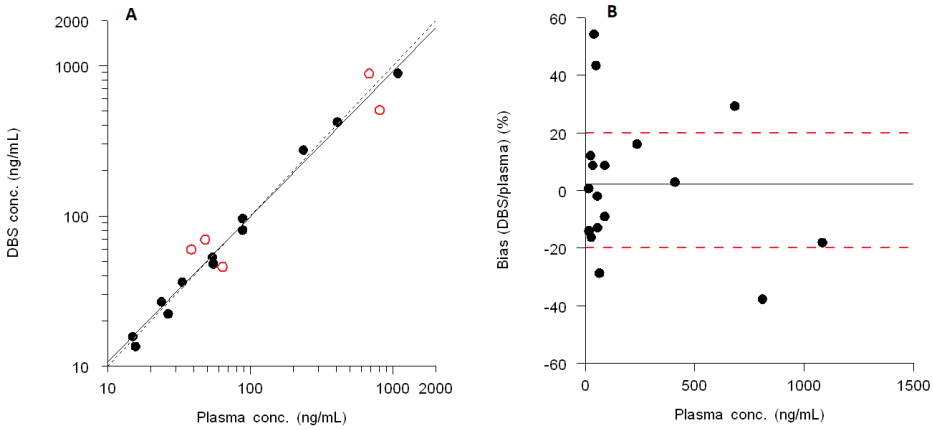

Figure 3 – (A) Correlation (R^2 >0.97) between ribociclib concentration in dried blood spot (DBS) and plasma samples from 17 breast cancer patients treated with ribociclib. Linear regression line (black lines) and the identity line (dashed line) are provided, while open dots represent DBS sample with a %DEV >20% compared with corresponding plasma sample. (B) Bland-Altman plot for total plasma and DBS. The dotted lines indicate the limits of agreement, and the solid line the mean ratio bias of 0.996.

3.3 Clinical application

Clinical validation has been performed by analyzing DBS samples and corresponding plasma samples. Our analysis showed that in 17/19 (89.5%) of the patients plasma as well DBS samples could be determined. In one patient, the concentration in the DBS sample was above the highest calibration curve standard; and another patient was excluded due to an error in sampling of the DBS sample. In our clinical validation trial both DBS and plasma samples of ribociclib concentrations could be determined in the range of 10.0 – 1,000 ng/mL. A strong correlation – with a coefficient of determination of R^2 >0.97 – between ribociclib concentration in DBS and plasma

concentration from 17 advanced breast cancer patients is shown in **Figure 3**. Bland-Altman analysis showed that a number of 12 out of 17 processed DBS samples (71%) fall inside the acceptable range of 20% difference of simultaneously obtained plasma samples (acceptance criterion ≥67%). Ribociclib concentrations of DBS and plasma samples were similar, as the bland-Altman plot showed a mean ratio of nearly one (mean ratio 0.996). A limitation of our study is the relatively small study population with 17 evaluable patients.[17] However, in our analysis no influence of hematocrit was found, therefore the expected variation in DBS sampling is considerably smaller than in other DBS studies with a clear hematocrit effect. The method meets the FDA requirements on clinical validation of this sampling approach.[20] Therefore, our method is suitable for clinical studies investigating the pharmacokinetic profile of ribociclib in an outpatient setting.

4. Conclusion

In conclusion, the assay was successfully applied to quantify dried blood spot and plasma samples of breast cancer patients treated with ribociclib. In order to investigate the disposition of ribociclib a reliable, reproducible, selective and sensitive dried blood spot method was developed. The dried blood spot sampling methods had been validated for whole-blood in both DBS and plasma samples over a range of 10.0 – 1,000 ng/mL. No influence of hematocrit (range 0.20 – 0.40 L/L) on validation parameters was observed. Therefore, both plasma and DBS method are suitable for a pharmacokinetic study to determine ribociclib concentrations.

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CHAPTER 13

Summary and Discussion

Summary

Breast cancer is the most frequently diagnosed type of cancer among women in the Western world.[1–3] Pharmacotherapy plays an essential role in the treatment of breast cancer. In this thesis, several examples are presented to provide guidance on individualising pharmacotherapy from both a pharmacokinetic and pharmacodynamic perspective. In the mentioned chapters below, results of clinical studies are described to optimise treatment with tamoxifen (**Section I**) or a CDK4/6 inhibitor (**Section II**) in patients with hormone sensitive breast cancer.

Tamoxifen is considered one of the first targeted therapies in oncology.[4–8] Tamoxifen is mainly used in the adjuvant setting of hormone sensitive breast cancer and reduces the risk of disease recurrence.[9,10] CDK4/6 inhibitors – palbociclib, ribociclib and abemaciclib – are used in the treatment of hormone receptor (HR)-positive, human epidermal growth factor receptor 2 (HER2)-negative advanced or metastatic breast cancer.[11,12] Both tamoxifen and the various CDK4/6 inhibitors are administered orally. Despite considerable differences between patients, tamoxifen and CDK4/6 inhibitors are prescribed at a fixed dose.

The difference in absorption, distribution, metabolism or elimination (ADME) of an orally administered drug results in a high interindividual variability of drug concentrations.[13] Patients with low exposure to a drug have an increased risk of reduced efficacy, while patients with a high exposure are more susceptible to suffer from treatment-related adverse events. With orally administered anticancer drugs (targeted therapies), it is estimated that ± 30% of patients are underdosed and ± 15% are overdosed, despite dose adjustments for renal or hepatic impairment.[14,15]

Section I: Tamoxifen

Tamoxifen is a drug with a complex metabolite profile. Endoxifen has the highest affinity for the estrogen receptor (ER) and is considered the major metabolite.[16,17] Retrospective studies associate a minimum endoxifen concentration of 14-16 nM with a lower risk of disease recurrence.[18,19] Several factors may contribute to interindividual variability, including genetic factors, comedication, compliance, body composition (weight and BMI) and fluctuations in pathophysiological conditions.[20–24]

Chapter 2 illustrated a high degree of interindividual variability of endoxifen concentration in 303 patients with tamoxifen therapy. Based on various patient characteristics, a prediction model for endoxifen concentration was developed. CYP2D6 activity, age and weight were identified as

the main predictors of endoxifen concentration. In total, 57% of the interindividual variability was explained by this combination of predictors. CYP2D6 activity contributed 54% to this variability, which is in line with the results (range 39-58%) of previous studies.[25–27] Model-based dosing was found to be insufficiently suitable for application in clinical practice. Follow-up research should demonstrate whether mathematical modelling software (i.e. pharmacometric simulation using NONMEM) can increase predictability.

Chapter 3 outlines a written response to a published clinical study by Sanchez-Spitman et al.[28] The researchers of this study examined whether there was a correlation between endoxifen concentration or CYP2D6 genotype and the clinical outcomes in 667 patients treated adjuvant with tamoxifen. Our response discussed shortcomings of this clinical study and subsequently made recommendations to gather conclusive evidence in follow-up studies.[29] The various submitted responses exemplified that there is currently still much debate concerning the need for dose individualization of tamoxifen.[30,31]

Chapter 4 evaluates the feasibility of TDM-guided dose individualisation of tamoxifen. Based on available literature a minimum endoxifen concentration of 16 nM was used.[19] In our prospective, open label, interventional study, 145 patients were included. Trough drug levels were serially collected following initiation after 3, 4.5 and 6 months, respectively. Based on the measured endoxifen concentration and toxicity profile, a dosage recommendation was formulated by a clinical pharmacologist and oncologist. The dose of tamoxifen was escalated to a maximum of 40 mg once daily (corresponding to the drug label of tamoxifen). The primary endpoint is the percentage of patients with an endoxifen level ≥16 nM. CYP2D6 status, tamoxifen-related side effects as well as the use of comedication were meticulously registered. At baseline, 79% of patients had achieved an endoxifen level ≥16 nM. After the TDM intervention an increase up to 89% was observed. CYP2D6 status was highly predictive of achieving the target concentration of endoxifen (≥16 nM). After dose escalation, target concentration was achieved in all CYP2D6 extensive metabolizers (EMs), 79% of intermediate metabolizers (IMs) and 36% of poor metabolizers (PMs). Hot flashes (61%), joint discomfort/arthralgia (19%), fatigue (11%), vaginal dryness (8%) and mood swings (6%) were the most commonly reported side effects. No increase in toxicity was observed after dose escalation. In conclusion, TDM-guided dosing of tamoxifen is feasible in clinical practice. This tool safely reduces the number of patients with subtherapeutic endoxifen levels.

The evaluation of (medical) costs is becoming increasingly important in the assessment of (new) drugs or interventions in oncology. On the basis of evaluations policymakers are able to make decisions that aim to keep healthcare costs manageable.[32–34] Earlier studies demonstrated

the cost-effectiveness of the intervention TDM-guided dosing of oral anticancer agents, such as abiraterone, imatinib and tamoxifen.[35–37]

Chapter 5 evaluates the cost-effectiveness of TDM-guided tamoxifen dosing in a model-based manner. A model was developed based on literature and data from our prospective TOTAM trial (Chapter 4). To deal with uncertainties scenarios were calculated and the model was subjected to a range of sensitivity analysis. A probabilistic sensitivity analysis showed that the intervention TDM led to a reduction of costs in 92% of cases. Dose individualization based on TDM leads to a cost reduction of over 15% of the total treatment costs in patients with breast cancer. These results are important to take into consideration during the decision-making process concerning implementation of TDM in the near future.

The effectiveness of drugs plays an eminent role in policy making. As survival rates for breast cancer patients continue to improve, the assessment of quality of life becomes more important.[38–42] **Chapter 6** describes the health-related quality of life and productivity of tamoxifen patients. Questionnaires were distributed to 145 patients three (T1) and six months (T2) after initiation with tamoxifen therapy. The response rates of the questionnaires were >80%. In our study population, utility scores of 0.87 ± 0.20 (T1) and 0.88 ± 0.22 (T2) were observed. Age and employment status were positively correlated with quality of life. Productivity increased significantly during the first six months. The presented description of the relatively high quality of life is valuable to use in disease models for economic (re)evaluation of (new) treatment algorithms.

The prognosis for hormone-sensitive breast cancer has improved significantly in recent decades. As a result, the medium- and long-term effects of a drug are becoming increasingly important when choosing a specific pharmacological treatment. In an Asian clinical trial, tamoxifen was shown to be associated with the development of liver steatosis. After three years, the cumulative incidence of hepatic steatosis was evaluated by computed tomography for with anastrozole and tamoxifen (14.6% versus 41.1%, p<0.0001; relative risk 0.30; 95% CI: 0.21-0.45)). No differences in liver function were observed during the study period.[43] Liver steatosis may cause serious complications, such as liver fibrosis, liver cirrhosis and hepatocellular carcinoma.[44,45] Despite of these data, no well-designed prospective studies on potential tamoxifen-related liver steatosis have been conducted in Caucasian patients so far.

In **Chapter 7**, the incidence of tamoxifen-related hepatic steatosis in a Caucasian population was studied. In this observational study, 95 Caucasian patients who had been treated with tamoxifen for at least two years were included. The presence of hepatic steatosis was determined by

transient elastography (Fibroscan).[46] The non-invasive fibroscan emits sound waves and a shock wave into the liver. The amount of feedback received from the sound waves (expressed as controlled attenuation parameter) provides an estimate of the degree of hepatic steatosis.[46,47] Two-year treatment with tamoxifen was not associated with an increase in the degree or severity of hepatic steatosis. Based on this prospective study, tamoxifen was shown to be safe in patients of Caucasian ethnicity. This result is relevant as there is a tendency to prescribe tamoxifen for a longer period of time (extended therapy) in mainly younger patients with breast cancer.[48,49]

Tamoxifen is frequently combined with food supplements,[50–52] such as green tea capsules. The main active pharmaceutical ingredient of green tea – the antioxidant epigallocatechin gallate (EGCG) – has been associated with anti-cancer effects in preclinical studies.[53,54] Additionally, there are also studies that show an interaction when a drug is combined with green tea.[55,56] **Chapter 8** presents a randomized, cross-over study which portrays that green tea consumption does not interact with the pharmacokinetics of tamoxifen. Therefore, the use of green tea by patients with tamoxifen does not have to be discouraged.

Drugs can also be combined to rationally induce specific pharmacokinetic interactions. In the past, for example, probenecid was used in times of shortage to inhibit renal excretion of penicillin. Probenecid has also been administered for gout.[57–59] Based on literature, there are indications that the metabolism and excretion of tamoxifen may be influenced by probenecid.[60–62]

Chapter 9 describes the potential pharmacokinetic interaction between tamoxifen and probenecid. Tamoxifen was combined with probenecid for 14 days. The results showed that probenecid increased the concentration of endoxifen by approximately 26%, which may be considered clinically relevant. It was concluded that the interaction between tamoxifen and probenecid is presumably based on the influence of the phase I and phase II metabolism of tamoxifen. No relevant differences in toxicity were observed during the study period. This interaction is relevant for patients who have a subtherapeutic endoxifen level despite the maximum dose of tamoxifen (40 mg once daily). A trial with a longer follow-up is necessary to determine whether this combination of drugs can safely be combined over a longer period of time.

Section II: CDK4/6 inhibitors

In **Chapter 10** a pharmacological literature review of palbociclib, ribociclib and abemaciclib in the treatment of breast cancer is given. In addition to similarities in properties, some relevant differences in pharmacokinetics, pharmacodynamics and toxicity profiles were described. Differences between these inhibitors may give rise to personalized treatment. All three CDK4/6 inhibitors are absorbed relatively quickly into the blood stream and metabolised by CYP3A4. The inhibitors differ in dosage schedules. Palbociclib and ribociclib are given on an intermittent '3 weeks on and 1 week off' schedule, whereas abemaciclib is given on a continuous schedule. Abemaciclib shows the highest degree of lipophilicity. This suggests that, in theory, abemaciclib is mainly absorbed in lipophilic tissue, such as the central nervous system. Preclinical and clinical studies confirm that passage of the blood-brain barrier is possible. Clinical studies are currently being held to further investigate the blood-brain barrier passage of the various CDK4/6 inhibitors. In both first line and second line treatment, the CDK4/6 inhibitors are associated with an improved progression free survival rates. The optimal position of CDK4/6 inhibitors in first or second line treatment is currently being investigated further in the SONIA trial (NCT03425838).[63]

While the efficacy of the three CDK4/6 inhibitors are broadly comparable, there are some relevant differences in toxicity profiles. Palbociclib and ribociclib are particularly associated with hematological toxicity (neutropenia), whereas abemaciclib is particularly associated with gastrointestinal toxicity (diarrhea). Abemaciclib-related diarrhea is generally short-lived and well-managed. QTc prolongation was observed in approximately 3% of ribociclib patients, whereas palbociclib and abemaciclib were not associated with clinically relevant differences in QTc prolongation.

Given the differences in the expected side effects, the choice of a CDK4/6 inhibitor could be based on a higher risk of QTc prolongation – due to medication or a congenital disorder – or on comorbidities, such as existing gastrointestinal problems or a predisposition to neutropenia. Based on the literature findings, it was recommended that follow-up studies be carried out to i) broaden the indication range of CDK4/6 inhibitors in the treatment of different types of breast cancer ii) examine biomarkers that may play a role in patient selection, prediction of response, or optimisation of the dosing regimen.

CDK4/6 inhibitors are primarily metabolised by CYP3A4. Previous studies have illustrated that the combination of a strong CYP3A4 inhibitor with a CDK4/6 inhibitor causes a clinically relevant drug interaction.[64–68] In **Chapter 11**, the interaction between palbociclib and erythromycin – a model substance for a moderate CYP3A4 inhibitor – is described. In a cohort of 11 patients

with breast cancer, erythromycin was added to the standard dose of palbociclib for one week. During the 24-hour hospitalization, palbociclib concentrations were determined 14 times and compared with blood samples taken during palbociclib monotherapy in the same patients. After administration of erythromycin tablets, the palbociclib concentration increased on average by about 40 percent. Despite this, minor differences in toxicity were observed. The pharmacokinetic interaction between palbociclib and erythromycin is based on inhibition of the cytochrome P450 system, and in particular the CYP3A4 enzyme. The consequence of this inhibition is that palbociclib is metabolized slower in the gut and liver, resulting in an increase in palbociclib concentration in the blood. The clinical relevance of this interaction was emphasized. This interaction possibly plays a part in the other CDK4/6 inhibitors (ribociclib and abemaciclib) as well, given the great similarities in metabolism and physical and chemical properties. Based on the aforementioned findings, a dose reduction of palbociclib to 75 mg once daily was recommended in patients with this drug combination. It should be considered to update the drug label of palbociclib to include these findings and recommendations, and add moderate CYP3A4 inhibitors to the list of potentially interacting drugs for CDK4/6 inhibitors.

The measurement and monitoring of drug concentrations will play an increasingly important role in oncology. There are currently a number of ongoing TDM feasibility studies with targeted therapies.[69] It is therefore useful to think about alternative, patient-friendly methods of measuring drug concentrations in the home environment to speed up implementation of this intervention in the (near) future.

Chapter 12 describes methods for determining ribociclib in plasma and in a dried blood spot (DBS). In a DBS method, blood is drawn by a prick of the fingertip. The venous and DBS blood samples were taken simultaneously. After liquid-liquid extraction, liquid chromatography-tandem mass spectrometry (LC-MS/MS) was used to quantify ribociclib concentrations. The analytical methods developed, and the associated validation parameters complied with the Food and Drug Administration (FDA) guideline for the validation of bioanalytical methods. Regression and Bland-Altman analyses showed a strong correlation between paired plasma and DBS samples. The assay was validated in a range of 10 – 1,000 ng/mL ribociclib. Haematocrit is an important measure of the rheological properties (i.e. viscosity) of blood. The haematocrit level had no influence on the validation parameters. Finally, the usability and accuracy of the analytical methods developed were tested in a pharmacokinetic study in patients treated with ribociclib. It was concluded that this patient-friendly DBS method can be used as an alternative method for measuring ribociclib concentrations.

Future perspectives

Considerations and consequences of the investigations for tamoxifen and CDK4/6 inhibitors
In this thesis three pharmacological tools to optimize pharmacotherapy with these agents were applied: a) pharmacogenetics b) Therapeutic Drug Monitoring and c) drug-drug and drug-food interactions. Considerations and consequences of the investigations for both tamoxifen and CDK4/6 inhibitors are discussed in the paragraphs below.

A) Pharmacogenetics and anti-cancer drugs
The cytochrome P450 system plays an important role in the metabolism of anti-cancer drugs. Therefore, genetic variation partially explains the interindividual variability in drug concentrations at steady state. In order to make optimal use of pharmacogenetics in daily clinical practice, it is necessary that every patient has the possibility to generate a genetic passport. For example, it is desirable to anticipate on the *CYP2D6* status before starting tamoxifen. Based on our study data, it is advisable to start with the maximum registered tamoxifen dose in patients with an intermediate or poor metabolism for *CYP2D6*. This offers opportunities to achieve the desired endoxifen concentration at an early stage of treatment.

Palbociclib, ribociclib and abemaciclib are primarily metabolized by CYP3A4. The prospective SONIA trial (clinicaltrial.gov number NCT03425838) – selecting the optimal position of CDK4/6 inhibitors in advanced breast cancer – offers opportunities to further investigate a possible pharmacokinetic-pharmacogenetic relationship.[63] Although pharmacogenetics can only partially explain the interindividual variability in drug concentrations, it is a powerful method to personalize the dosage at the start of treatment.

B) Therapeutic Drug Monitoring in oncology
A pharmacokinetic threshold for efficacy or toxicity has been identified for many anti-cancer drugs. Meanwhile, also feasibility trials are ongoing.[69,70] The SONIA trial offers an excellent opportunity to identify exposure-response-toxicity relationships for palbociclib, ribociclib and abemaciclib in the near future.[63] Also positive results of our tamoxifen feasibility study and cost-effectiveness evaluation are encouraging for multidisciplinary discussions about implementation in clinical practice. Ideally, to demonstrate the clinical benefit of TDM it is desirable to perform a randomized controlled trial. However, the feasibility of such a study is very low given the extreme high number of patients required and the long follow-up period in the case of tamoxifen.[29] Implementation requires a more pragmatic approach for most anti-cancer drugs [71], just like dosage recommendations in patients with renal impairment, liver impairment or in patients with potential drug-drug or food-drug interactions. Therefore, if the results of feasibility studies and cost-effectiveness analyses are positive, consid-

eration should be given to implement TDM in clinical practice, as recently mentioned by Groenland et al.[71] It is recommended that TDM-guided dose individualization should also be examined for other orally administered anti-cancer drugs which have a clear 'exposure-response' relationship. Guidelines can be useful in this respect to promote harmonization of the implementation process.

C) Drug-drug and food-drug interactions

Clinically relevant interactions can potentially lead to more adverse effects or reduced effectiveness. However, also positive effects of drug combinations may be observed. In this thesis, our proof of principle study with probenecid and tamoxifen observed an increase of 26% in endoxifen levels in patients with diminished *CYP2D6* activity. More research is needed on the long-term safety of this specific combination. Possibly, if safe, probenecid could be given to future CYP2D6 poor metabolizers who are treated with the maximum dose of tamoxifen, and who may also have a relative contraindication for an aromatase inhibitor.

Palbociclib in combination with the moderate CYP3A4 inhibitor erythromycin clearly demonstrated an increase in concentration of palbociclib. An exposure-toxicity relationship has been demonstrated for the CDK4/6 inhibitors, indicating a relative small therapeutic window. Interpretation of interactions of anti-cancer drugs must be seen in light of the small or large therapeutic window of the specific drug.

Tour d'horizon: individualized "bespoke" cancer drug therapy in the next decades

As was described previously, the role of the clinical pharmacist and his/her contribution to optimized and individualized therapy in patients with cancer was until recently mainly at the level of pharmacokinetics: on the one hand, dose adjustments to find the optimal therapeutic range in terms of prevention of adverse events and good tolerability, on the other hand to obtain maximally efficacy. In other words, they had to balance both survival duration and quality of life using the available tools, including therapeutic drug monitoring, pharmacogenetic profiling, knowledge of disposition in special populations, and drug-drug and drug-food interactions. In this thesis, multiple aspects were taken into consideration and a large armamentarium of pharmacological tools was applied to tamoxifen and CDK4/6 inhibitors. For me, it is without doubt however that in the near future the role of the clinical pharmacist will change considerably and will move from the pharmacokinetic level towards the pharmacodynamic level. In my opinion, an important thing what will change in the oncology field is the diagnosis of the cancer at an earlier stage and with different techniques, with more specific, selective, and sensitive methods. Automatically the early detection of cancer will lead to different approaches and the use of different types of medications will be possible and needed. Cancer will no longer be treated by a general drug given at an optimal dose, but will change to an individualized drug, individually titrated towards the optimally balanced therapy in terms of life prolongation and quality of life.

Overall conclusions and recommendations

This thesis exemplified how pharmacological research can contribute to the optimization of treatment for individual patients with breast cancer. This is illustrated – from a multidimensional approach – for a drug that has been available for decades (tamoxifen) and for more recently approved drugs (the CDK4/6 inhibitors). We used several pharmacological tools of which therapeutic drug monitoring is relatively new in the field of oncology. Positive results of our tamoxifen feasibility study and cost-effectiveness evaluation are encouraging for multidisciplinary discussions about implementation in clinical practice. Hopefully this research will lead to better treatment outcomes and quality of life for individual breast cancer patients treated with tamoxifen; and also offers opportunities and inspiration for optimization of the CDK4/6 inhibitors in the near future. In the future the individualised therapy will be of bespoke quality and the input of the clinical pharmacist will diverse further. In this way the future will hold a promise for all those who will suffer from severe illnesses like breast cancer, and pharmacological strategies will pave the way towards a future with less pain, less sorrows and better treatment outcomes for the most vulnerable: our patients.

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APPENDICES

1. Nederlandse samenvatting
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Appendix 1. Nederlandse samenvatting

Kanker is een complex ziektebeeld en gaat gepaard met een hoge mortaliteit. Onder vrouwen is borstkanker de meest voorkomende gediagnostiseerde vorm van kanker. Geneesmiddelen spelen een essentiële rol bij de behandeling van borstkanker. Tamoxifen is een relatief oud geneesmiddel en wordt beschouwd als een van de eerste doelgerichte therapieën binnen de oncologie. Tamoxifen wordt voornamelijk in de adjuvante behandeling toegepast, dus als nabehandeling na het operatief verwijderen van de borstkanker, en resulteert in een lagere kans op ziekteterugkeer.

Recentelijk zijn de *cyclin-dependent kinase* 4 en 6 (CDK4/6) remmers – palbociclib, ribociclib en abemaciclib – toegevoegd aan het farmacotherapeutisch arsenaal voor de behandeling van lokaalgevorderde of gemetastaseerde (uitgezaaide) borstkanker. CDK4/6 remmers grijpen aan op de celdeling van kankercellen en remmen daarmee specifiek de groei van kankercellen.

Zowel tamoxifen als de verschillende CDK4/6 remmers worden toegediend in de orale toedieningsvorm als tablet of capsule. Een verschil in absorptie, distributie, metabolisme of eliminatie van een oraal geneesmiddel resulteert in een hoge interindividuele variabiliteit in geneesmiddelconcentratie. Het is wenselijk om geneesmiddelen binnen een bepaalde bandbreedte te doseren. Patiënten met een lage blootstelling aan een geneesmiddel hebben een verhoogd risico op verminderde effectiviteit, terwijl patiënten met een hoge blootstelling juist meer kans hebben op (ernstige) bijwerkingen. Ondanks grote verschillen tussen patiënten worden deze geneesmiddelen in de standaardzorg in een vaste dosis gegeven.

In het algemeen was er beperkte informatie op het gebied van toegepaste individuele farmacotherapie van zowel tamoxifen als de CDK4/6 remmers beschikbaar bij de start van dit onderzoek. In dit proefschrift staat de optimalisatie van de farmacotherapie voor individuele patiënten met borstkanker centraal. In onderstaande hoofdstukken van dit proefschrift worden resultaten van klinische studies beschreven om medicamenteuze behandeling met tamoxifen (**deel 1**) of een CDK4/6 remmer (**deel 2**) te optimaliseren voor de individuele patiënt met borstkanker. In dit proefschrift worden verschillende voorbeelden genoemd om zowel vanuit een farmacokinetisch als een farmacodynamisch perspectief handreikingen te doen om farmacotherapie in meerdere mate te individualiseren voor patiënten.

Deel 1: Tamoxifen

Tamoxifen is een zogenaamde selectieve oestrogeen receptor modulator (SERM) en wordt met name in de adjuvante setting voorgeschreven om ziekteterugkeer van borstkanker te voorkomen. De *World Health Organization* (WHO) heeft tamoxifen opgenomen in de lijst van de meest essentiële geneesmiddelen die er bestaan. Tamoxifen is een zogenaamde *prodrug* en wordt gemetaboliseerd tot verschillende actieve afbraakproducten (metabolieten). Endoxifen is de voornaamste metaboliet van tamoxifen. In de literatuur wordt in een grote retrospectieve analyse een minimale endoxifen plasmaconcentratie van 14-16 nmol/L geassocieerd met een lagere kans op ziekteterugkeer. **Hoofdstuk 2** van dit proefschrift toont aan dat er een hoge mate van inter-individuele variabiliteit in endoxifen concentratie waarneembaar is onder steady-state condities. Het doel van deze studie was om een predictiemodel voor de endoxifen concentratie te ontwikkelen op basis van verschillende patiënten karakteristieken, zoals verschil in het cytochroom P450 enzymsysteem (*CYP2D6* en *CYP3A4*22* genotype status), leeftijd, body mass index (BMI), gewicht en comedicatie. In de studie werden 303 patiënten die adjuvant met tamoxifen werden behandeld geïncludeerd. Drie maanden na start met de tamoxifentherapie werd een dalspiegel (gemeten concentratie vlak voor de volgende gift) afgenomen om de endoxifen concentratie te bepalen. De voornaamste predictoren om de endoxifen concentratie te voorspellen waren activiteit van CYP2D6, leeftijd en gewicht. In totaal werd 57% van de inter-individuele variabiliteit verklaard door deze combinatie van predictoren. CYP2D6 activiteit had hierin een bijdrage van 54% en de overige 3% werd toegeschreven aan leeftijd en gewicht. Geconcludeerd werd dat op basis van het ontwikkelde model de voorspelbaarheid van endoxifen concentraties in individuele patiënten relatief beperkt is.

Hoofdstuk 3 is een korte wetenschappelijke reactie op een gepubliceerde klinische studie. De onderzoekers van deze studie bestudeerden of er een verband was tussen endoxifen concentratie of *CYP2D6* genotype en klinische uitkomsten in patiënten die adjuvant behandeld werden met tamoxifen. In onze reactie werden tekortkomingen van deze klinische studie besproken en eveneens werden er aanbevelingen gedaan hoe vervolgonderzoek er mogelijk uit zou kunnen zien.

Hoofdstuk 4 evalueert dosisindividualisatie van tamoxifen op basis van de bloedspiegel, beter bekend als *therapeutic drug monitoring* (TDM). Een minimale endoxifen streefconcentratie van 16 nM (5.97 ng/mL) werd gekozen op basis van beschikbare literatuurgegevens bij aanvang van de studie. In onze prospectieve, open label, interventie studie werden 145 adjuvante tamoxifen patiënten geïncludeerd. Dalspiegels werden serieel afgenomen, respectievelijk 3, 4.5 en 6 maanden na initiatie van tamoxifen therapie. Op basis van de gemeten endoxifen concentratie en het klinische beeld van de patiënt werd een doseeradvies voor tamoxifen geformuleerd. De

dosering tamoxifen werd geëscaleerd naar maximaal 40 mg per dag. Het primaire eindpunt van de studie werd gedefinieerd als het percentage patiënten met een endoxifenspiegel ≥16 nM zes maanden na initiatie van de behandeling met tamoxifen. Dit percentage werd vergeleken met historische data uit de literatuur. Patiënten werden CYP2D6 gefenotypeerd en eveneens werden tamoxifen gerelateerde toxiciteit en het gebruik van comedicatie nauwkeurig geregistreerd gedurende de gehele studieperiode. Op baseline (3 maanden na start van de tamoxifen therapie) had 79% van de patiënten een endoxifenspiegel ≥16 nM behaald. Na de interventie TDM-gestuurde dosisindividualisatie werd een statistisch significante stijging tot 89% geobserveerd. Bij 9 patienten die na dosisescalatie geen adequate endoxifenspiegel behaalden vond een succesvolle therapeutische substitutie plaats naar een zogenaamde aromatase remmer (bijvoorbeeld anastrozol, letrozol of exemestaan). CYP2D6 status was in hoge mate voorspellend voor het behalen van de endoxifen streefconcentratie. Na dosisescalatie werd de streefconcentratie behaald in alle *extensive CYP2D6 metabolizers*, 79% van de *intermediate CYP2D6 metabolizers* en slechts 36% van de *poor metabolizers van het CYP2D6*. Na dosisescalatie naar de maximale dosering tamoxifen werd een lineariteit in farmacokinetiek geobserveerd. Opvliegers (61%), gewrichtsklachten (19%), vermoeidheid (11%), vaginale veranderingen (8%) en stemmingswisselingen (6%) waren de meest gerapporteerde bijwerkingen. Na dosisescalatie werd geen toename in ernst of mate van tamoxifen gerelateerde toxiciteit geobserveerd. Geconcludeerd werd dat TDM-gestuurde dosisindividualisatie van tamoxifen haalbaar is in de klinische praktijk. Deze tool biedt de mogelijkheid om op een veilige manier het aantal patiënten met een endoxifenspiegel onder streefniveau te halveren.

Een evaluatie van medische kosten wordt steeds belangrijker bij nieuwe geneesmiddelen of interventies binnen de oncologie. Op basis van evaluaties kunnen beleidsmakers keuzes maken, waardoor de kosten in de gezondheidszorg beheersbaar blijven. In **hoofdstuk 5** worden de kosten van de TDM-gestuurde dosisindividualisatie modelmatig geëvalueerd vanuit een gezondheidsperspectief. Op basis van literatuurgegevens en gegevens uit de TOTAM studie (hoofdstuk 4) werd een model ontwikkeld om de kosteneffectiviteit van de interventie TDM-gestuurde dosisindividualisatie nader te bestuderen. Aannames in een model gaan altijd gepaard met een zekere mate van onzekerheid. Om met onzekerheden om te gaan werden verschillende scenario's doorgerekend en werd het model onderworpen aan sensitiviteitsanalyses. Een probabilistische sensitiviteitsanalyse liet vanuit een gezondheidsperspectief zien dat TDM-gestuurde dosisindividualisatie van tamoxifen in 92% van de gevallen leidt tot een reductie van kosten. Geconcludeerd werd dat dosisindividualisatie op basis van de endoxifenspiegel leidt tot een kostenbesparing van ruim 15% per patiënt op de totale behandelkosten.

In **hoofdstuk 6** worden de kwaliteit van leven en productiviteit van tamoxifen patiënten gemonitord gedurende het eerste half jaar van de therapie. Drie (tijdstip T1) en zes (tijdstip T2) maanden na start van de tamoxifen therapie werden gevalideerde vragenlijsten uitgereikt aan 145 geïncludeerde patiënten. De response percentages van de vragenlijsten waren hoger dan 80 procent. In onze studiepopulatie werden utiliteitsscores (algemene maat om de gezondheidstoestand te evalueren) van 0.87 ± 0.20 (op T1) en 0.88 ± 0.22 (op T2) geobserveerd. Leeftijd en arbeidsstatus waren positief gecorreleerd met kwaliteit van leven. Dosisescalatie van tamoxifen had geen significante invloed op de utiliteitsscore. De productiviteit – zowel betaald als onbetaald werk – van patiënten steeg significant gedurende het eerste halfjaar van de tamoxifen therapie. De gepresenteerde beschrijving van de kwaliteit van leven bij tamoxifen patiënten is waardevol om te gebruiken in ziektemodellen voor economische (re)evaluaties van (nieuwe) behandelingen.

De prognose voor hormoongevoelige borstkanker is de afgelopen decennia sterk verbeterd. Hierdoor gaan langetermijneffecten van een geneesmiddel zwaarder wegen bij de keuze voor een specifieke medicamenteuze behandeling. In een Aziatische studie werd eerder aangetoond dat tamoxifen geassocieerd is met de ontwikkeling van leververvetting (steatose). Leversteatose kan in zeldzame gevallen ernstige complicaties veroorzaken, zoals bijvoorbeeld leverfibrose, -cirrose en het hepatocellulair carcinoom. In **hoofdstuk 7** werd de incidentie van tamoxifen gerelateerde leversteatose in onze TOTAM-studie populatie nader bestudeerd. In deze observationele studie werden 95 Kaukasische patiënten geïncludeerd die minimaal 2 jaar met tamoxifen werden behandeld. De aanwezigheid van leversteatose werd bepaald middels transiënte elastografie (de zogenaamde fibroscan). De fibroscan zendt geluidsgolven en een schokgolf uit in de lever. De mate van uitdoving van de geluidsgolven – wordt softwarematig uitgedrukt als *'continued attenuation parameter'* – geeft op basis van 10 achtereenvolgende metingen een inschatting van de mate van leversteatose. Een tweejarige behandeling met tamoxifen werd niet geassocieerd met een toename in mate of ernst van leversteatose. Op basis van deze prospectieve longitudinale studie werd aangetoond dat tamoxifen gebruik veilig is wat betreft leversteatose. Deze uitkomst is relevant aangezien er een tendens is om tamoxifen voor een langere periode voor te schrijven in voornamelijk jongere patiënten; en er geen aanwijzingen zijn dat er een verhoogd risico is op tamoxifen gerelateerde leversteatose in patiënten met een Kaukasische achtergrond.

Tamoxifen wordt in de dagelijkse praktijk frequent gecombineerd met voedingssupplementen, zoals bijvoorbeeld groene thee capsules. Het voornaamste bestanddeel uit groene thee – de antioxidant epigallocatechine gallaat (EGCG) – wordt in preklinische studies geassocieerd met een anti-kanker werking. Daarnaast zijn er ook studies die een wisselwerking laten zien als een geneesmiddel wordt gecombineerd met consumptie van groene thee. **Hoofdstuk 8** laat in een gerandomiseerde, cross-over opgezette studie zien dat gelijktijdig gebruik van een hoge

dosering groene thee capsules geen kinetische wisselwerking heeft met tamoxifen en de actieve metaboliet endoxifen. Op grond van de genoemde bevindingen werd geconcludeerd dat vanuit farmacokinetisch perspectief deze combinatie veilig gebruikt kan worden in de klinische praktijk.

In **hoofdstuk 9** wordt de interactie beschreven tussen tamoxifen en probenecide. Probenecide is een oud geneesmiddel dat werd gebruikt bij de behandeling van jicht. Tamoxifen werd gedurende 14 dagen gecombineerd met probenecide tabletten. Uit de resultaten van de klinische studie bleek probenecide de concentratie van de actieve metaboliet van tamoxifen (endoxifen) met ongeveer 26% te verhogen, wat significant en klinisch relevant is. De conclusie werd getrokken dat de interactie tussen tamoxifen en probenecide waarschijnlijk berust op beïnvloeding van het fase I en fase II metabolisme van tamoxifen. Gedurende de studieperiode werden er geen relevante verschillen in tamoxifen gerelateerde bijwerkingen geobserveerd. De relevantie van deze interactie voor patiënten met een lage blootstelling endoxifen, ondanks eerdere dosis-ophoging van de tamoxifen, werd benadrukt. Vervolgonderzoek is nodig om te bestuderen of deze combinatie van geneesmiddelen op een veilige wijze langdurig gecombineerd kan worden.

Deel 2 – CDK4/6 remmers

In **hoofdstuk 10** wordt een literatuuroverzicht gegeven van de tot dusverre bekende farmacologische gegevens van palbociclib, ribociclib en abemaciclib bij de behandeling van lokaalgevorderde of gemetastaseerde borstkanker. Naast overeenkomsten in eigenschappen werden er enkele relevante verschillen in farmacokinetiek, farmacodynamiek en toxiciteit beschreven. Verschillen tussen deze remmers kunnen aanleiding geven voor een gepersonaliseerde behandeling van patiënten met borstkanker. Alle drie de CDK4/6 remmers worden relatief snel in het bloed worden opgenomen en door het cytochroom P450-systeem (CYP3A4) gemetaboliseerd. Verder verschillen de remmers in doseerschema. Palbociclib en ribociclib worden volgens een intermitterend schema gegeven (drie weken behandeling gevolgd door een week rust), terwijl abemaciclib volgens een continu schema wordt gegeven. Abemaciclib vertoont de hoogste mate van vetoplosbaarheid (lipofiliciteit) van de drie CDK4/6 remmers. Dit suggereert dat in theorie vooral abemaciclib wordt opgenomen in lipofiele weefsels, zoals het centraal zenuwstelsel. Preklinische en klinische studies bevestigen dat passage van de bloed-hersenbarrière mogelijk is. Momenteel zijn er klinische studies gaande om de bloed-hersenbarrièrepassage van de verschillende CDK4/6 remmers nader te onderzoeken. Zowel in eerste- als latere lijns behandeling zijn de CDK4/6 remmers geassocieerd met een significant verbeterde (progressievrije) overleving. Waar de werkzaamheid van de drie CDK4/6 remmers in grote lijnen vergelijkbaar lijkt, zijn er daarentegen enkele relevante verschillen in toxiciteit. Palbociclib en ribociclib worden met name geassocieerd met hematologische toxiciteit (neutropenie), terwijl abemaciclib met name geassocieerd is gastro-intestinale toxiciteit (diarree). Abemaciclib gerelateerde diarree is over

het algemeen van korte duur en goed te behandelen. Bij ongeveer 3% van de ribociclib patiënten werd een QTc-tijd verlenging op het ECG geconstateerd, terwijl palbociclib en abemaciclib niet geassocieerd werden met klinisch relevante QTc-verlenging. Gezien de verschillen in de te verwachten bijwerkingen, zou de keuze van een CDK4/6 remmer gebaseerd kunnen worden op het een hoger risico op QTc-verlenging (door medicijngebruik of een aangeboren afwijking) of op basis van comorbiditeiten, zoals bestaande gastro-intestinale problemen of een predispositie voor neutropenie. Op grond van de literatuurbevindingen werd aanbevolen om vervolgonderzoek te verrichten naar i) verbreding van het indicatiegebied van CDK4/6 remmers bij de behandeling van verschillende typen borstkanker en ii) biomarkers die een rol kunnen spelen bij selectie van patiënten, predictie van respons of optimalisatie van het doseerschema.

CDK4/6 remmers worden voornamelijk gemetaboliseerd door CYP3A4. Eerder onderzoek heeft aangetoond dat de combinatie van een sterke CYP3A4 remmer met een CDK4/6 remmers een klinische relevante geneesmiddelinteractie veroorzaakt. In **hoofdstuk 11** wordt de interactie tussen palbociclib en erytromycine – een modelstof voor een matige CYP3A4 remmer – beschreven onder steady-state omstandigheden. In een cohort van 11 patiënten werd erytromycine gedurende 1 week toegevoegd aan de standaarddosering met palbociclib. Gedurende de 24-uurs ziekenhuisopname werden op 14 tijdstippen palbociclib concentraties bepaald en vergeleken met bloed afnames tijdens palbociclib monotherapie in dezelfde patiënten. Na het toevoegen van de erytromycine tabletten steeg de palbociclib concentratie gemiddeld met ongeveer 40%. Er werden desondanks gedurende de studieperiode nauwelijks verschillen geobserveerd in palbociclib gerelateerde bijwerkingen. De farmacokinetische interactie tussen palbociclib en erytromycine berust op remming van het cytochroom P450 systeem, en dan in het bijzonder het CYP3A4 enzym. Het gevolg van deze remming is dat palbociclib langzamer wordt gemetaboliseerd in de darmen en lever met als gevolg een stijging van de palbociclib concentratie in het bloed. De klinische relevantie van deze interactie werd benadrukt. Deze interactie speelt vermoedelijk ook een rol bij de andere CDK4/6 remmers (ribociclib en abemaciclib) gelet op de grote overeenkomsten in metabolisme en fysisch-chemische eigenschappen. Op grond van de genoemde bevindingen werd een vermindering van de dosis aanbevolen in patiënten met deze combinatie van geneesmiddelen.

In **hoofdstuk 12** worden methoden beschreven voor de bepaling van ribociclib in plasma en middels een zogenaamde *dried blood spot* (DBS) bloedafname. In de DBS methode wordt bloed door een prik in de vingertop afgenomen. De veneuze en DBS bloedafname werden gelijktijdig afgenomen onder steady-state condities. Na vloeistof-vloeistof extractie werd gebruikt gemaakt van de techniek '*liquid chromatography tandem mass spectrometry*' om ribociclib concentraties te kwantificeren. De ontwikkelde analysemethoden en de daarbij behorende validatieparameters

voldeden aan de richtlijn van de *Food & Drug Administration* (FDA) voor de validatie van bioanalytische methoden. Regressie en Bland-Altman analyses toonden een sterke correlatie tussen gepaarde plasma en DBS monsters. De assay werd gevalideerd in een range van 10 – 1000 ng/mL ribociclib. Hematocriet is een belangrijke maat voor de reologische eigenschappen (onder andere viscositeit) van bloed. Op de validatie parameters had het hematocriet gehalte geen invloed. Tenslotte werd de bruikbaarheid en juistheid van de ontwikkelde analysemethoden getoetst in een farmacokinetische studie in patiënten die behandeld werden met ribociclib. Geconcludeerd werd dat deze patiëntvriendelijke DBS methode gebruikt kan worden als alternatief voor het meten van ribociclib concentraties.

Deel 3 : Conclusies

Samengevat laat dit proefschrift zien hoe klinisch farmacologisch onderzoek kan bijdragen aan de optimalisatie van de behandeling van individuele patiënten met (borst)kanker. Dit proefschrift laat verder zien dat er zowel voor een geneesmiddel dat al decennia beschikbaar is (tamoxifen) als voor recentelijk geïntroduceerde geneesmiddelen (CDK4/6 remmers) nieuwe mogelijkheden zijn om de farmacotherapie (nog) beter toe te spitsen op de individuele patiënt.

Appendix 2. Author affiliations

Affiliations at the time the research was conducted. Presented in alphabetic order.

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Appendix 3. List of publications

S.A.J. Buck, C.L. Braal, M.M. Hofman, E. Oomen-de Hoop, P. de Bruijn, I.M. Ghobadi Moghaddam-Helmantel, K.G.A.M. Hussaarts, M.B. Vastbinder, Q.C. van Rossum-Schornagel, R. H.N. van Schaik, A. Jager, S.L.W. Koolen, R.H.J. Mathijssen. **Influence of probenecid on endoxifen systemic exposure in breast cancer patients on adjuvant tamoxifen treatment**
Therapeutic Advances in Medical Oncology (Jan 2022)

C.L. Braal, A. Kleijburg, A. Jager, S.L.W. Koolen, R.H.J. Mathijssen, I. Corro Ramos, P. Wetzelaer, C.A. Uyl-de Groot. **Therapeutic drug monitoring-guided adjuvant tamoxifen dosing in early breast cancer patients: a cost-effectiveness analysis from the prospective TOTAM trial**
Clinical Drug Investigation (Dec 2021)

C.L. Braal, Agnes Jager, Esther Oomen–de Hoop, Justin D. Westenberg, Koen M.W.T. Lommen, Peter de Bruijn, Mijntje B. Vastbinder, Quirine C. van Rossum–Schornagel, Martine F. Thijs–Visser, Robbert J. van Alphen, Liesbeth E.M. Struik, Hanneke J.M. Zuetenhorst, Ron H.J. Mathijssen, Stijn L.W. Koolen. **Therapeutic drug monitoring of endoxifen for tamoxifen precision dosing: feasible in patients with hormone-sensitive breast cancer**
Clinical Pharmacokinetics (Sept 2021)

L. Molenaar-Kuijsten, C.L. Braal, S.L. Groenland, N. de Vries, H. Rosing, J.H. Beijnen, S.L.W. Koolen, A.J.E. Vulink, M.G.J. van Dongen, R.H.J. Mathijssen, A.D.R. Huitema, N. Steeghs. **Effects of the moderate CYP3A4 inhibitor erythromycin on the pharmacokinetics of palbociclib: a randomized cross-over trial in patients with breast cancer**
Clinical Pharmacology and Therapeutics (Sept 2021)

C.L. Braal, M.H. Lam, T. Rienks, C.J. van Tilborg, W. Heuts, J.B. Heijns, M.E.M.M. Bos, R.H.J. Mathijssen, P. de Bruijn, S.L.W. Koolen. **Quantification of ribociclib in dried blood spots by LC-MS/MS: method development and clinical validation**
J Pharm Biomed Anal (Jul 2021)

C.L. Braal, E.M. Jongbloed, S.M. Wilting, R.H.J. Mathijssen, S.L.W. Koolen, A. Jager. **Inhibiting CDK4/6 in breast cancer with palbociclib, ribociclib, and abemaciclib: similarities and differences**
Drugs (Feb 2021)

C.L. Braal, K.G.A.M. Hussaarts, L. Seuren, E. Oomen-de Hoop, P. de Bruijn, S.A.J. Buck, M.E.M.M. Bos, M.F. Thijs-Visser, H.J.M. Zuetenhorst, D. Mathijssen-van Stein, M.B. Vastbinder, R.W.F. van Leeuwen, T. van Gelder, S.L.W. Koolen, A. Jager, R.H.J. Mathijssen. **Influence of green tea consumption on endoxifen steady-state concentration in breast cancer patients treated with tamoxifen**
Breast Cancer Res Treat (Nov 2020)

C.L. Braal, J.H. Beijnen, S.L.W. Koolen, E. Oomen-de Hoop, N. Steeghs, A. Jager, A.D.R. Huitema, R.H.J. Mathijssen. **Relevance of endoxifen concentrations: absence of evidence is not evidence of absence**
J Clin Oncol (Aug 2019)

C.L. Braal, G.D.M. Veerman, R. Peric, J.G.J.V. Aerts, R.H.J. Mathijssen, S.L.W. Koolen, P. de Bruijn. **Quantification of the tyrosine kinase inhibitor erlotinib in human scalp hair by liquid chromatography-tandem mass spectrometry: pitfalls for clinical application**
J Pharm Biomed Anal (Aug 2019)

C.L. Braal, P. de Bruijn, F. Atrafı, M. van Geijn, C.J.F. Rijcken, R.H.J. Mathijssen, S.L.W. Koolen. **A new method for the determination of total and released docetaxel from docetaxel-entrapped core-crosslinked polymeric micelles (CriPec®) by LC-MS/MS and its clinical application in plasma and tissues in patients with various tumours**
J Pharm Biomed Anal (Nov 2018)

C.L. Braal, A. Jager, A.H.J. Mathijssen, S.L.W. Koolen. **Therapeutic drug monitoring van tamoxifen.**
Ned Tijdschr Oncol (Jun 2017)

Appendix 4. Dankwoord

In de afgelopen vier jaar zijn er veel mensen betrokken geweest bij de totstandkoming van dit proefschrift. Dit werk is alleen mogelijk geweest door intensieve samenwerking en steun van velen. Het is dan ook onmogelijk om iedereen persoonlijk te benoemen, maar graag wil ik een aantal welgemeende woorden – zonder wetenschappelijke stijlregels – aan het papier toevertrouwen.

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Gedurende het promotieonderzoek heeft het begeleiden van masterstudenten een belangrijke rol gespeeld. Marlou, Koen, Lieke, Anne en Justin dank voor jullie enorme inzet gedurende jullie masteronderzoek!

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Studies in dit proefschrift zijn mogelijk geweest door een nauwe samenwerking in de regio Rotterdam. Voor alle klinische studies met tamoxifen wil ik in het bijzonder een woord van dank uitspreken voor de samenwerking aan: dr. Vastbinder, dr. Kehrer en Manon (IJsselland ziekenhuis); dr. Drooger, dr. Thijs-Visser, dr. de Jongh en Liesbeth (Ikazia ziekenhuis); dr. van Alphen (Elizabeth Tweestede ziekenhuis); dr. Mathijssen, dr. Zuetenhorst en dr. van Rossum (Sint Franciscus Gasthuis & Vlietland). Prof. dr. van Schaik en Tessa, bedankt voor het mogelijk maken van de CYP2D6 en CYP3A4*22 bepalingen in het grote tamoxifen cohort.

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Prof. dr. Uyl-de Groot, beste Carin, Pim en Anne, dank dat jullie mij enthousiast hebben gemaakt voor de wondere wereld van de gezondheidseconomie. Anne, met jouw biofarmaceutische achtergrond klikte het al snel tussen ons. Dank voor je onuitputtelijke energie, zeer plezierige samenwerking en dat je me hebt meegenomen in dit voor mij nieuwe vakgebied in de wetenschap! Ik had me geen betere collega kunnen wensen en ik ben dan ook trots op het eindresultaat. Heel veel succes met je promotieonderzoek bij het Trimbos Instituut. Pim, dank voor je sublieme begeleiding – we hebben inmiddels al heel wat Zoom-meetings erop zitten – en Limburgse gezelligheid! Hannah, jouw kennis en passie voor kwaliteit van leven onderzoek is ongeëvenaard. Dank voor de samenwerking en je hulp bij de revisie ondanks de gammele internetverbinding tijdens jouw reis door Sri Lanka.

Dr. de Knegt, beste Rob, jij leerde mij hoe er een FibroScan van de lever gemaakt kon worden. Dank voor het vertrouwen dat je me gaf, de stoomcursus echografie, de leuke gesprekken en het superviseren van de uitslagen! Dr. Eechoute, beste Karel, aan het begin van het promotietraject tipte je me al dat het interessant is om te onderzoeken of tamoxifen leidt tot leversteatose. Dank voor het uitdenken van dit onderzoek en de telefonische begeleiding bij het schrijven van het manuscript.

Alle overige co-auteurs, die niet specifiek bij naam zijn genoemd, wil ik ook hartelijk danken voor de positieve inbreng op de manuscripten.

Daan en Justin, heel erg fijn dat jullie mijn paranimf willen zijn!

Lieve familie – in het bijzonder Kees, dank voor je goede adviezen – en vrienden dank voor jullie afleiding en betrokkenheid in de afgelopen jaren.

Lieve oma, bedankt voor alle warme belangstelling voor het onderzoek. Het boekje is nu eindelijk af en dat gaan we vieren!

Lieve pa en ma, Adriëtte, Hans en Rik, de liefdevolle basis die jullie mij geven heeft ervoor gezorgd, dat ik met plezier dit promotie-traject heb kunnen doen. Hartelijk dank voor jullie onvoorwaardelijke liefde, ondersteuning en interesse tijdens deze fase, maar ook in de jaren daarvoor!

Soli Deo Gloria

22-02-2022 Louwrens Braal

Appendix 5.
Portfolio – Summary of PhD training and teaching

Name PhD Candidate: Louwrens Braal
Erasmus University MC: Department of Medical Oncology
PhD period: 2017-2021
Promotor: Prof. Dr. A.H.J. Mathijssen
Copromotor: Dr. S.L.W. Koolen and Dr. A. Jager

1. PhD training

	Year	Workload (ECTS)
General courses		
BROK course	2017	1.5
Research Integrity	2017	0.3
Biostatistical Methods NIHES[1]	2019	0.3
Specific courses		
Basic course on 'R'	2017	2.0
Basic and Translational Oncology	2019	1.8
Basic introduction course on SPSS	2019	1.0
Pharmaceutical pricing ESHPM, Erasmus University Rotterdam	2020	5.0
Decision Modelling for Health Economic Evaluation, ESHPM, Erasmus University Rotterdam	2020	5.0
Advanced Research methods Health, Policy and Law course, ESHPM Erasmus University Rotterdam	2020	5.0
Health Technology Assessment and International Health Law, ESHPM, Erasmus University Rotterdam	2020	5.0
Presentations		
Translational Pharmacology meetings, Erasmus MC	2017-2021	2.0
NVFZ Symposium 'Oncologie in breed perspectief', Culemborg	2018	0.3
International Workshop on Clinical Pharmacology of Anticancer Drugs, Amsterdam	2018, 2019	0.8
Borstkankervereniging Nederland 'Geneesmiddelinteracties in de oncologie', Rotterdam	2018	0.1
Clinical pharmacology meeting 'Therapeutic Drug Monitoring of tamoxifen', Erasmus MC	2019	0.1
Breast Cancer Meeting, Franciscus Gasthuis Schiedam	2019	0.4
A cost effectiveness analysis of the TOTAM trial, Erasmus University Rotterdam, Institute for Medical Technology Assessment	2020	0.4
European Society of Medical Oncology Congress -191P 'Therapeutic drug monitoring of tamoxifen to improve adjuvant treatment of hormone sensitive breast cancer: the TOTAM study' -205P 'Influence of green tea consumption on endoxifen steady-state concentration in breast cancer patients treated with tamoxifen'	2020	2.0
Breast Cancer Scientific Meeting, Erasmus MC	2021	0.4

(Inter)national conferences		
Annual meeting NVKFB	2018-2020	2.5
Translational Pharmacology meetings	2017-2021	1
Clinical Pharmacology meetings	2017-2021	1
Symposium Therapeutic Drug Monitoring of anti-cancer drugs	2018	0.1
Young Oncologist evening, Erasmus MC	2018-2019	0.1
International Workshop on Clinical Pharmacology of Anticancer Drugs	2018-2019	1.0
Erasmus MC Liver Day, Erasmus MC	2020	0.3
2. Teaching		
Lecturing		
Department of Medical Oncology, Erasmus MC	2020-2021	0.2
Supervising Master's thesis		
Marlou Jongkees (Erasmus MC)	2018	1.0
Sanne Buijs (Erasmus MC)	2019	1.0
Lieke Seuren (Erasmus MC)	2020	1.0
Koen Lommen (Erasmus MC)	2020	1.0
Anne Kleijburg (Erasmus University)	2020	1.0
Justin Westenberg (University of Antwerpen, Belgium)	2020-2021	1.5
3. Other		
Chair of Medical Oncology Research Meeting (MORM), Erasmus MC	2018-2021	5.0
Peer review of manuscripts Journal of Pharmaceutical and Biomedical Analysis Therapeutic Drug Monitoring Breast Cancer Research and Treatment European Journal Pharmaceutical Sciences	2018-2021	1.0

[1] Netherlands Institute for Health Sciences

Appendix 6. Curriculum vitae

Louwrens Braal werd geboren op 29 december 1992 in Gouda, Nederland. In 2011 voltooide hij de middelbare school (VWO) aan het Driestar College te Gouda. In 2017 behaalde Louwrens de master farmacie aan de Universiteit Utrecht. Aansluitend werd er gestart met het promotieonderzoek op de afdeling Interne Oncologie in het Erasmus Medisch Centrum, onder supervisie van Prof. Dr. A.H.J. Mathijssen, Dr. S.L.W. Koolen en Dr. A. Jager. Zijn onderzoek heeft zich gefocust op de farmacologie en personalisatie van orale geneesmiddelen bij de behandeling van hormoongevoelige borstkanker.