OPTIMIZING ENDOCRINE THERAPY

Unravelling dosing strategies, pharmacology and adverse events in breast cancer

Sanne Buijs

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Unravelling dosing strategies, pharmacology and adverse events in breast cancer

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CHAPTER 1

General introduction

INTRODUCTION

Breast cancer is the most common cancer in women, accounting for 30% of new female cancer diagnoses each year.[1] With a lifetime risk of one in seven, its prevalence is substantial.[2] Although treatments are improving and breast cancer death rates have almost halved since 1989, still ~2.5% of all women will finally die from (complications from or progressive) breast cancer. These statistics underscore the critical need for continued advancement in our understanding of this disease.

Approximately 75% of breast cancers are estrogen receptor-positive, meaning that these breast cancer cells possess estrogen receptors.[3] Estrogen, a hormone that is naturally produced in the body, can bind to these receptors, thereby stimulating the growth and division of cancer cells.[4] However, this also means that anti-hormonal therapies that target estrogen receptors or estrogen can be effective treatments for this type of breast cancer.[5,6] This thesis focusses on two important anti-cancer drugs in the management of estrogen receptor-positive breast cancer: the selective estrogen receptor modulator tamoxifen and the class of CDK4/6 inhibitors (palbociclib, ribociclib and abemaciclib).

Tamoxifen

Tamoxifen is a selective estrogen receptor modulator, mainly deriving its efficacy in breast cancer from binding to the estrogen receptor competitively with estrogen. At this target, it acts as an estrogen receptor antagonist, suppressing the expression of estrogen-regulated genes, growth factors and angiogenic factors otherwise stimulated by estrogen **(Figure 1)**.[4-6] Despite being registered already since 1973, tamoxifen remains a cornerstone in the adjuvant treatment of estrogen receptor-positive breast cancer, typically administered following local or locoregional therapy, including surgery and often radiotherapy, to reduce the risk of breast cancer recurrence. The standard dose of 20 mg reduces the breast cancer recurrence rate by approximately 40% during the first ten years of follow-up, and the annual breast cancer death rate by one third.[7,8] Tamoxifen is especially important in the treatment of premenopausal women. For this sub-group, tamoxifen is recommended for a duration of five to ten years.[9-11] Post-menopausal women are mostly advised to use two to three years of adjuvant tamoxifen, followed by an aromatase inhibitor, another form of hormonal therapy, for a similar period of time.

Given its role as an estrogen receptor modulator, tamoxifen exhibits agonistic or antagonistic effects depending on the specific tissue it targets.[12] As estrogen receptors are expressed in various healthy tissues, the binding of tamoxifen or its metabolites to these receptors can lead to a spectrum of (endocrine) adverse effects. For instance, hot

flashes likely result from an estrogen receptor antagonistic effect in the central nervous system, where estrogen receptors are also present, which can lead to thermoregulatory dysfunction.[13] In contrast, tamoxifen's estrogen receptor agonistic effects in the endometrium can induce endometrial abnormalities and vaginal discharge **(Figure 1)**.[12] Other adverse effects are arthralgia, insomnia, mood alterations, weight gain and vaginal dryness.[14] These adverse effects significantly impact patient quality of life, particularly given the long treatment duration in the adjuvant setting, which can extend up to ten years. Remarkably, nearly half of all patients discontinue tamoxifen early due to adverse effects, with one-third of these patients already discontinuing within the first year of treatment.[15-18] Another substantial group of patients adhere to tamoxifen therapy but endure compromised health-related quality of life.[14,19]

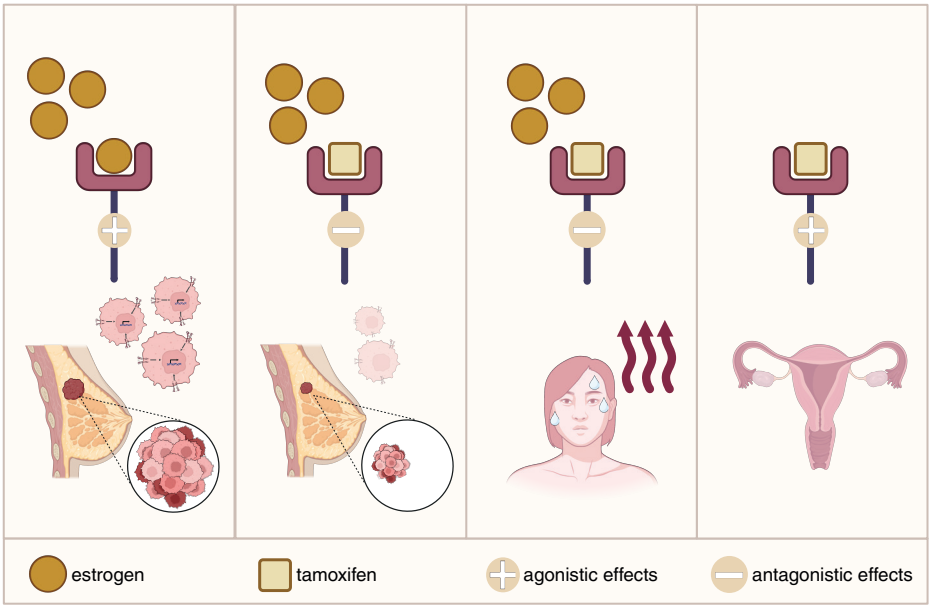

Figure 1. Working mechanism of tamoxifen
I. Estrogen stimulates growth and division of breast cancer cells by binding to the estrogen receptor; II. Tamoxifen prevents estrogen from binding to the estrogen receptor, thereby preventing potential microscopic residual disease from progressing; III. Tamoxifen works in the brain as an estrogen receptor antagonist, leading to hot flashes; IV. In the endometrium, tamoxifen works as an estrogen receptor agonist, thereby causing vaginal discharge
Figure created with Biorender.

Tamoxifen is known for its complex metabolism. It is metabolized into multiple metabolites, which is catalyzed by many phase I and phase II metabolizing enzymes.[20] Most importantly, tamoxifen is metabolized by cytochrome P450 (CYP) enzymes CYP2D6 and CYP3A4 to 4-hydroxy-tamoxifen, *N*-desmethyl-tamoxifen and endoxifen

(Figure 2). Among these metabolites, both endoxifen and 4-hydroxy-tamoxifen exhibit the highest affinity for the estrogen receptor, surpassing tamoxifen by more than 300 times.[21] However, endoxifen is considered the most important metabolite resulting from its higher plasma concentration compared to other metabolites.[22,23] Several retrospective studies have revealed an exposure-response relation between endoxifen levels and tamoxifen efficacy with suggested endoxifen thresholds ranging from 10 to 16 nM.[24-26] Among these thresholds, 16 nM is the most widely accepted, as demonstrated by the largest study conducted to date.[24] It is also the most conservative threshold, minimizing the likelihood of patients continuing to use an ineffective dose. Nevertheless, thus far, no prospective study has succeeded in confirming the 'definitive' endoxifen efficacy threshold, likely due to insufficient statistical power.[27-29]

Therapeutic drug monitoring (TDM) is a therapeutic strategy wherein drug plasma concentrations are measured and doses are adjusted based on these measurements in order to achieve a therapeutic threshold.[30] The threshold of 16 nM is determined while comparing different quantiles of endoxifen exposure. Therefore, approximately 20 percent of patients have endoxifen levels below the supposed threshold of 16 nM when using the standard dose of tamoxifen 20 mg. TDM can be used to increase the percentage of patients with endoxifen concentrations exceeding 16 nM. Earlier studies have demonstrated the feasibility of implementing TDM for tamoxifen in clinical practice.[31,32] By implementing dose-escalations to tamoxifen 30 or 40 mg in patients with endoxifen levels below 16 nM, the percentage of patients with 'too low' endoxifen levels was nearly halved.

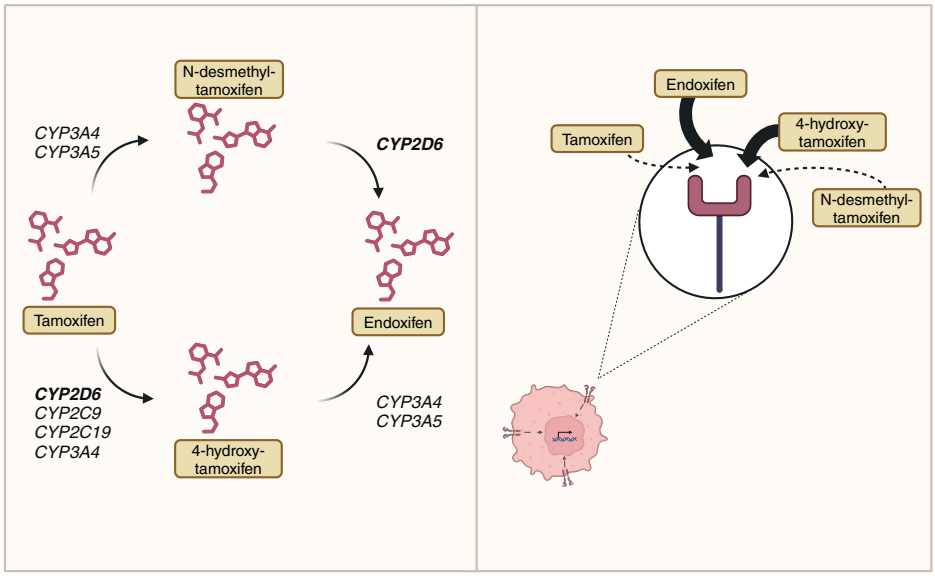

Figure 2. Metabolism of tamoxifen
I. A simplified, schematic overview of the metabolism of tamoxifen; II. Endoxifen and 4-hydroxy-tamoxifen have the highest affinity with the estrogen receptor, compared to tamoxifen and n-desmethyl-tamoxifen. However, endoxifen also has the highest concentration in the blood.
Figure created with Biorender.

PART I. Tamoxifen and adverse effects

In this thesis, we first have searched for possible solutions to decrease tamoxifen-related adverse effects because of the high incidence of adverse effects based non-adherence. A prerequisite for this solution was that sufficient endoxifen levels would be attained.

A potential approach to decrease tamoxifen-related adverse effects could be tamoxifen dose reduction. In **chapter 2,** an overview of existing research concerning lower dosages of tamoxifen and their impact on adverse effects and clinical efficacy is presented. Additionally, practical tools for implementing tamoxifen dose reductions in the adjuvant setting are provided and further research aimed at establishing optimal dosing strategies for individual patients is discussed.

While evidence supporting the clinical efficacy and enhanced tolerability of lower doses of tamoxifen is well-established in the primary and secondary prevention settings (e.g., among patients at higher risk for breast cancer or those with breast carcinoma-in-situ), there are almost no studies performed in the adjuvant setting. In **chapter 3,** we aimed to investigate whether endoxifen-guided tamoxifen dose reduction could lead to fewer adverse effects in the adjuvant setting while attaining endoxifen levels ≥16 nM. In this

clinical study, patients with bothersome tamoxifen-related adverse effects and endoxifen levels ≥32 nM underwent a dose reduction to tamoxifen 10 mg and before and after dose reduction adverse effects, quality of life and endoxifen levels were evaluated.

In **chapter 4,** we investigated the combination of tamoxifen and CBD-oil. Primarily, the possible pharmacokinetic interaction between tamoxifen and CBD, a main component of cannabis, was assessed. Since tamoxifen has a complex metabolism, it is prone for drug-drug or drug-herb interactions. As a secondary aim, we investigated whether the use of CBD-oil could be a solution for tamoxifen-related adverse effects. CBD is used frequently among patients with breast cancer in the hope to alleviate adverse effects.[33] CBD can indeed modulate receptors such as the opioid, dopamine, melatonin, serotonin and acetylcholine receptors.[34] Also the cannabinoid receptors, mostly present in the central nervous and immune system, are modulated by CBD.[35] However, CBD might also affect tamoxifen pharmacokinetics since it is a potential inhibitor of CYP2D6.[36,37] We investigated the pharmacokinetic interaction between CBD-oil and tamoxifen and whether there could be a beneficial effect of CBD-oil on tamoxifen-related adverse effects and health-related quality of life.

Tamoxifen can also lead to some more rare, but severe or even dangerous, adverse effects. For example, tamoxifen-treated patients face a 2-3.5 times elevated risk of venous thromboembolism compared to breast cancer patients without adjuvant tamoxifen treatment.[39,40] Recent studies have also associated tamoxifen use with a decline in cognitive functioning.[41,42] Estrogens, acting through estrogen receptor α and estrogen receptor β receptors, which are also present in the brain, exert various cognitive-enhancing effects in the brain.[43,44] Tamoxifen, by inhibiting estrogen action via estrogen receptor binding, potentially impacts cognitive function directly and could also indirectly affect cognition through other tamoxifen-related adverse effects such as fatigue and mood disturbances.[43,45] Consequently, cognitive decline is a plausible side effect of tamoxifen. Considering TDM and subsequent dose adjustments, it is important to know whether higher plasma concentrations of tamoxifen and endoxifen can influence the incidence of these (more long-term) adverse effects.

It is not clearly understood how tamoxifen increases the venous thromboembolism risk, but it is known that tamoxifen decreases anticoagulant proteins, including antithrombin, protein C and tissue factor pathway inhibitor, and enhances thrombin generation.[46-48] In **chapter 5,** we have investigated the levels of antithrombin, protein C, tissue factor and thrombin generation in tamoxifen users and assessed the potential relation of this tamoxifen-associated coagulation proteins with tamoxifen dose and tamoxifen and endoxifen plasma levels.

In **chapter 6,** we aimed to evaluate the effect of two years of tamoxifen treatment on subjective and objective cognitive function (measured using the *Amsterdam Cognition Scan,* a validated online neuropsychological test battery) in a large cohort of women with breast cancer. In addition, we investigated the association between tamoxifen and endoxifen plasma concentrations and objective and subjective cognitive function.

PART II. Tamoxifen Model-informed precision dosing

One drawback of TDM is that dose adjustments can only be performed once steady-state plasma concentrations are reached, which for tamoxifen is after three months of therapy. Consequently, patients requiring a tamoxifen dose-escalation after TDM might receive suboptimal treatment during the first three to six months of therapy.[49] Model-informed precision dosing (MIPD) offers a potential solution by forecasting the appropriate tamoxifen dose prior to treatment initiation. MIPD relies on population-pharmacokinetic (POP-PK) models, which can delineate and predict the drug's absorption, distribution, metabolism, and elimination based on various patient characteristics. Hereby, plasma concentrations can be forecasted during or even before treatment.[50]

A POP-PK model is developed using Non-linear Mixed Effects Modelling (NONMEM). NONMEM is a powerful mathematic approach used to describe pharmacokinetics and/or pharmacodynamics.[51] It describes the course of these outcomes using mathematical formulas. By doing so, these models can help explain complex relationships between drug doses, blood concentrations and physiological responses.[52] Additionally, these models quantify both inter- and intra-individual variability in pharmacokinetics acquiring individual-specific key pharmacokinetic parameters. This can be used to predict the correct dose and to explain variability between patients or dosing cycles by testing correlations of these parameters with patient characteristics. Thereby, NONMEM could be a tool to aid personalized medicine.

To date, six population POP-PK models have been developed to describe the pharmacokinetics of both tamoxifen and endoxifen.[53-58] These models showed that inter-individual variation in the rate of endoxifen formation was for the largest part explained by *CYP2D6* phenotype or CYP2D6 activity score groups. In **chapter 7** we developed a POP-PK model where CYP2D6 activity per allele was estimated on a continuous scale with the goal of developing a more sensitive POP-PK model for tamoxifen. Dense and sparse data from 3661 samples of 539 patients were used in this model. After inclusion of covariates, the model was subsequently validated using an independent external dataset (of in total 378 patients).

In **chapter 8,** the aforementioned PK-model was used in the implementation of MIPD before start of tamoxifen treatment. Age, body height, BMI and CYP2D6 activity (continuous scale) were used as predictors for steady-state endoxifen concentrations and based on these predictions, patients were prescribed a tamoxifen starting dose of 20, 30 or 40 mg. The aim of this study was to increase the proportion of patients achieving an endoxifen level ≥16 nM at the moment of steady-state and thereby potentially improve treatment outcomes.

PART III. CDK4/6 inhibitors

Cyclin-dependent kinase (CDK) 4/6 inhibitors are relatively new, targeted drugs in the treatment of advanced or metastatic estrogen receptor-positive breast cancer. As advanced or metastatic breast cancer is an incurable disease, the main purpose of treatment is to delay disease progression. CDK4/6 inhibitors target the cyclin-dependent kinases 4 and 6, which are crucial in cell cycle regulation **(Figure 3)**.[59,60] The CDK-RB1-E2F pathway is essential for progression through the cell cycle. CDK4 and CDK6 are normally kept in check by the protein p16 thereby inhibiting the binding between CDK4/6 and cyclin D, but this mechanism of cell cycle control is often disrupted in cancer.[59] In breast cancer specifically, activation of estrogen receptors and other proliferation-inducing signals further stimulate the complexation of CDK4/6 with cyclin D1.[59,61] This binding triggers phosphorylation of the retinoblastoma tumor suppressor protein (Rb1), releasing the binding with transcription factor E2F.[60] Consequently, the signal for (uncontrolled) cell division is initiated. However, when CDK4 and CDK6 are inhibited, Rb1 remains dephosphorylated.[61] In dephosphorylated state, Rb1 can stay bound to the transcription factor E2F which is then unable to function, thereby halting (cancer) cell cycle progression.

Palbociclib, ribociclib and abemaciclib are the three CDK4/6 inhibitors currently registered for the treatment of advanced or metastatic estrogen receptor-positive breast cancer. In both first and second line of treatments, all CDK4/6 inhibitors have demonstrated nearly doubling of progression free survival rates.[62-67] When used in first line, CDK4/6 inhibitors are administered alongside aromatase inhibitors, while in second line they are combined with fulvestrant, an estrogen receptor antagonist. Endocrine therapy effectively suppresses estrogen-dependent stimulation of cancer cells, leading to downregulation of cyclin D1 and reduced formation of cyclin D1 with CDK4 and CDK6 **(Figure 3)**.[61] Therefore, endocrine therapy and CDK4/6 inhibitors are hypothesized to work synergistically.

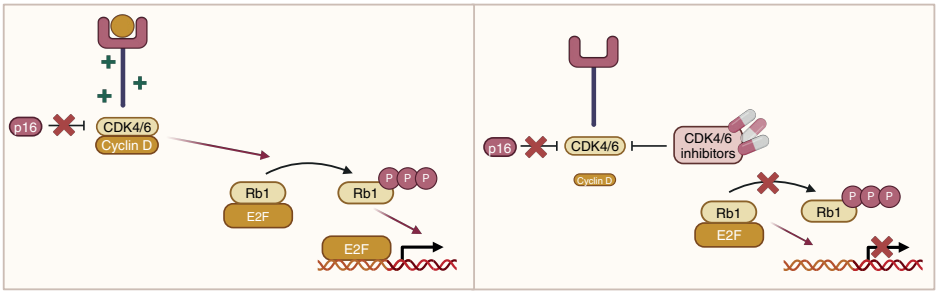

Figure 3. Working mechanism of CDK4/6 inhibitors
I. Disrupted CDK-RB1-E2F pathway in breast cancer. The protein p16 no longer inhibits the complexation of CDK4 and 6 with cyclin D. Activation of the estrogen receptor by estrogen even further stimulates this complexation. When CDK4 and 6 bind to cyclin D, this stimulates the phosphorylation of tumor suppressor protein Rb1. When phosphorylated, Rb1 releases transcription factor E2F which can cause uncontrolled cell division; II. Mechanism of CDK4/6 inhibitors. While p16 is still no longer inhibiting the complexation of CDK4 and 6 with cyclin D, CDK4/6 inhibitors can overtake this function. Also, due to the endocrine therapy there is less estrogen-dependent stimulation. Therefore, cyclin D is downregulated and cannot bind to CDK4/6 causing Rb1 to remain dephosphorylated. E2F will then remain bound to Rb1 and the cell division is inhibited. Figure created with Biorender.

Since CDK4/6 inhibitors demonstrated comparable effectiveness in both first- and second line treatment, it was unclear in which line CDK4/6 inhibitors could best be applied. To address this question, the SONIA-study was initiated.[68,69] This study compared the addition of a CDK4/6 inhibitor alongside an aromatase inhibitor in the first line with its addition alongside fulvestrant in the second line. No significant difference in progression-free survival after two lines of treatment was observed between patients who received a CDK4/6-inhibitor in the first versus the second line, while adverse events were higher among patients who received a CDK4/6-inhibitor in the first line. Consequently, for most patients, second line treatment may emerge as the preferred option.

The most frequent adverse effect in CDK4/6 inhibitors is neutropenia, with an incidence of 60% in patients using palbociclib or ribociclib (grade 3 or higher) and 20% of patients using abemaciclib.[63,65,73] This discrepancy is probably due to the fact that abemaciclib is a more potent inhibitor of CDK4 than CDK6 and CDK6 is more important in the hematopoesis.[74,75] On the contrary, diarrhoea is much more frequent in patients using abemaciclib (10% grade 3 or higher), since abemaciclib also inhibits CDK9, a kinase linked to intestinal toxicity.[65,76,77] Other frequently occurring adverse effects are anemia, thrombocytopenia, fatigue and nausea.[62,65,73] Moreover, prolongation of QTc interval is a specific concern for patients receiving ribociclib.[73]

In **chapter 9** we delved into another, less frequently described adverse event observed across all types of CDK4/6 inhibitors: the elevation of creatinine levels.[66,74,78,79] Such an increase could indicate a decline in renal function. However, it is important to note that creatinine levels can also rise if tubular secretion of creatinine is inhibited. Active tubular secretion accounts for 10-40% of creatinine clearance and is mediated trough the organic cation transporter 2 (OCT2) on the basolateral membrane of the proximal tubules and the multidrug and toxin extrusion (MATE) protein1 and 2 on the apical membrane.[80,81] *In vitro* research has demonstrated that abemaciclib can inhibit all three of these transporters, while ribociclib inhibits OCT2 and MATE1 and palbociclib solely inhibits OCT2.[82] When creatinine levels rise due to inhibition of tubular secretion, while kidney function actually remains intact, this is called pseudo-acute kidney injury. The incidence of pseudo-acute kidney injury in patients using CDK4/6 inhibitors is currently unknown. In clinical practice, encountering an elevated plasma creatinine level prompts an investigation into the underlying cause of the acute kidney injury (AKI). Also, CDK4/6 inhibitors or other important, but nephrotoxic medication might be interrupted or decreased in dose. However, if pseudo-AKI emerges as a common issue in patients using CDK4/6 inhibitors, incorporating the measurement of cystatin C could offer a solution. Therefore, we aimed to determine the incidence of pseudo-AKI in patients treated with CDK4/6 inhibitors by assessing both creatinine and cystatin C in plasma.

Among patients using CDK4/6 inhibitors there is a large variability in clinical efficacy and toxicity rates. This difference might be explained by a variability in pharmacokinetics. Indeed, all CDK4/6 inhibitors have a large inter-patient variability in, for example, trough plasma concentrations.[83,84] For palbociclib specifically, the IC_{50} concentration, which is the plasma concentration to inhibit the target for 50% *in vitro*, are 33.5 and 48.7 ng/mL for CDK4 and CDK6, respectively.[84,85] This concentrations are almost similar to the average trough concentration of 47 ng/mL which suggests that palbociclib might be very sensitive for changes in exposure.[83] If an efficacy or toxicity threshold for palbociclib (or other CDK4/6 inhibitors) can be found, this could guide in dose adjustments already at start of treatment.

To better understand pharmacokinetics of palbociclib, a POP-PK model could help. Therefore, in **chapter 10,** a POP-PK model of palbociclib was developed. Using this POP-PK model we aimed to investigate whether a relationship exists between palbociclib levels and progression free survival or adverse events.

Finally, **chapter 11** provides a summary of this thesis and discusses future perspectives and the possibilities for further research.

REFERENCES

1. American Cancer Society: "Breast Cancer Facts & Figures 2022-2024, "Cancer Facts & Figures 2023." https://www.cancer.org/content/dam/cancer-org/research/cancer-facts-and-statistics/breast-cancer-facts-and-figures/2022-2024-breast-cancer-fact-figures-acs.pdf (accessed April 22, 2024 2024).
2. Integraal Kankercentrum Nederland. Borstkanker in nederland. Kerncijfers uit de Nederlandse Kankerregistratie. https://iknl.nl/borstkankercijfers#:~:text=Borstkanker%20is%20daarmee%20de%20meest,van%20de%20COVID%2D19%20pandemie (accessed 21-05-2024 2024).
3. Perou CM, Sørlie T, Eisen MB, van de Rijn M, Jeffrey SS, Rees CA, Pollack JR, Ross DT, Johnsen H, Akslen LA, Fluge O, Pergamenschikov A, Williams C, Zhu SX, Lønning PE, Børresen-Dale AL, Brown PO, Botstein D. Molecular portraits of human breast tumours. Nature 2000; 406(6797): 747-52.
4. Osborne CK. Tamoxifen in the treatment of breast cancer. New England Journal of Medicine 1998; 339(22): 1609-18.
5. Jordan VC. Chemosuppression of breast cancer with tamoxifen: laboratory evidence and future clinical investigations. Cancer Investigation 1988; 6(5): 589-95.
6. Ellis PA, Saccani-Jotti G, Clarke R, Johnston SRD, Anderson E, Howell A, A'Hern R, Salter J, Detre S, Nicholson R, Robertson J, Smith IE, Dowsett M. Induction of apoptosis by tamoxifen and ICI 182780 in primary breast cancer. International Journal of Cancer 1997; 72(4): 608-13.
7. Early Breast Cancer Trialists' Collaborative G, Davies C, Godwin J, Gray R, Clarke M, Cutter D, Darby S, McGale P, Pan HC, Taylor C, Wang YC, Dowsett M, Ingle J, Peto R. Relevance of breast cancer hormone receptors and other factors to the efficacy of adjuvant tamoxifen: patient-level meta-analysis of randomised trials. Lancet 2011; 378(9793): 771-84.
8. Early Breast Cancer Trialists' Collaborative Group. Effects of chemotherapy and hormonal therapy for early breast cancer on recurrence and 15-year survival: an overview of the randomised trials. Lancet 2005; 365(9472): 1687-717.
9. Loibl S, André F, Bachelot T, Barrios CH, Bergh J, Burstein HJ, Cardoso MJ, Carey LA, Dawood S, Del Mastro L, Denkert C, Fallenberg EM, Francis PA, Gamal-Eldin H, Gelmon K, Geyer CE, Gnant M, Guarneri V, Gupta S, Kim SB, Krug D, Martin M, Meattini I, Morrow M, Janni W, Paluch-Shimon S, Partridge A, Poortmans P, Pusztai L, Regan MM, Sparano J, Spanic T, Swain S, Tjulandin S, Toi M, Trapani D, Tutt A, Xu B, Curigliano G, Harbeck N. Early breast cancer: ESMO Clinical Practice Guideline for diagnosis, treatment and follow-up☆. Annals of Oncology 2024; 35(2): 159-82.
10. Cardoso F, Kyriakides S, Ohno S, Penault-Llorca F, Poortmans P, Rubio IT, Zackrisson S, Senkus E. Early breast cancer: ESMO Clinical Practice Guidelines for diagnosis, treatment and follow-up†. Annals of Oncology 2019; 30(8): 1194-220.
11. Burstein HJ, Lacchetti C, Anderson H, Buchholz TA, Davidson NE, Gelmon KA, Giordano SH, Hudis CA, Solky AJ, Stearns V, Winer EP, Griggs JJ. Adjuvant Endocrine Therapy for Women With Hormone Receptor-Positive Breast Cancer: ASCO Clinical Practice Guideline Focused Update. Journal of Clinical Oncology 2019; 37(5): 423-38.
12. Martinkovich S, Shah D, Planey SL, Arnott JA. Selective estrogen receptor modulators: tissue specificity and clinical utility. Clinical Interventions in Aging 2014; 9: 1437-52.
13. Stearns V, Ullmer L, López JF, Smith Y, Isaacs C, Hayes D. Hot flushes. Lancet 2002; 360(9348): 1851-61.
14. Land SR, Wickerham DL, Costantino JP, Ritter MW, Vogel VG, Lee M, Pajon ER, Wade JL, Dakhil S, Lockhart JB, Wolmark N, Ganz PA. Patient-Reported Symptoms and Quality of Life During Treatment With Tamoxifen or Raloxifene for Breast Cancer PreventionThe NSABP Study of Tamoxifen and Raloxifene (STAR) P-2 Trial. JAMA 2006; 295(23): 2742-51.
15. Owusu C, Buist DSM, Field TS, Lash TL, Thwin SS, Geiger AM, Quinn VP, Frost F, Prout M, Yood MU, Wei F, Silliman RA. Predictors of Tamoxifen Discontinuation Among Older Women With Estrogen Receptor–Positive Breast Cancer. Journal of Clinical Oncology 2008; 26(4): 549-55.
16. Lailler G, Memoli V, Le Bihan Benjamin C, Ben Diane M-K, Lauzier S, Mancini J, Bousquet PJ, Bouhnik A-D. Five-Year Adjuvant Endocrine Therapy Adherence Trajectories Among Women With Breast Cancer: A Nationwide French Study Using Administrative Data. Clinical Breast Cancer 2021; 21(4): e415-e26.

17. Murphy CC, Bartholomew LK, Carpentier MY, Bluethmann SM, Vernon SW. Adherence to adjuvant hormonal therapy among breast cancer survivors in clinical practice: a systematic review. Breast Cancer Research and Treatment 2012; 134(2): 459-78.
18. Bosco-Lévy P, Jové J, Robinson P, Moore N, Fourrier-Réglat A, Bezin J. Persistence to 5-year hormonal breast cancer therapy: a French national population-based study. British Journal of Cancer 2016; 115(8): 912-9.
19. Boehm DU, Lebrecht A, Eckhardt T, Albrich S, Schmidt M, Siggelkow W, Kandelhardt E, Koelbl H. Quality of life and adjuvant tamoxifen treatment in breast cancer patients. European Journal of Cancer Care 2009; 18(5): 500-6.
20. Binkhorst L, Mathijssen RHJ, Jager A. Individualization of tamoxifen therapy: much more than just CYP2D6 genotyping. Cancer Treatment Reviews 2015.
21. Coezy E, Borgna JL, Rochefort H. Tamoxifen and metabolites in MCF7 cells: correlation between binding to estrogen receptor and inhibition of cell growth. Cancer Research 1982; 42(1): 317-23.
22. Stearns V, Johnson MD, Rae JM, Morocho A, Novielli A, Bhargava P, Hayes DF, Desta Z, Flockhart DA. Active tamoxifen metabolite plasma concentrations after coadministration of tamoxifen and the selective serotonin reuptake inhibitor paroxetine. Journal of the National Cancer Institute 2003; 95(23): 1758-64.
23. Mürdter TE, Schroth W, Bacchus-Gerybadze L, Winter S, Heinkele G, Simon W, Fasching PA, Fehm T, German T, Group AIC, Eichelbaum M, Schwab M, Brauch H. Activity levels of tamoxifen metabolites at the estrogen receptor and the impact of genetic polymorphisms of phase I and II enzymes on their concentration levels in plasma. Clinical Pharmacology & Therapeutics 2011; 89(5): 708-17.
24. Madlensky L, Natarajan L, Tchu S, Pu M, Mortimer J, Flatt SW, Nikoloff DM, Hillman G, Fontecha MR, Lawrence HJ, Parker BA, Wu AHB, Pierce JP. Tamoxifen Metabolite Concentrations, CYP2D6 Genotype, and Breast Cancer Outcomes. Clinical Pharmacology & Therapeutics 2011; 89(5): 718-25.
25. Helland T, Henne N, Bifulco E, Naume B, Borgen E, Kristensen VN, Kvaløy JT, Lash TL, Alnæs GIG, van Schaik RH, Janssen EAM, Hustad S, Lien EA, Mellgren G, Søiland H. Serum concentrations of active tamoxifen metabolites predict long-term survival in adjuvantly treated breast cancer patients. Breast Cancer Research 2017; 19(1): 125.
26. Saladores P, Mürdter T, Eccles D, Chowbay B, Zgheib NK, Winter S, Ganchev B, Eccles B, Gerty S, Tfayli A, Lim JS, Yap YS, Ng RC, Wong NS, Dent R, Habbal MZ, Schaeffeler E, Eichelbaum M, Schroth W, Schwab M, Brauch H. Tamoxifen metabolism predicts drug concentrations and outcome in premenopausal patients with early breast cancer. Pharmacogenomics Journal 2015; 15(1): 84-94.
27. Sanchez-Spitman A, Dezentjé V, Swen J, Moes DJAR, Böhringer S, Batman E, van Druten E, Smorenburg C, van Bochove A, Zeillemaker A, Jongen L, Los M, Neven P, Gelderblom H, Guchelaar H-J. Tamoxifen Pharmacogenetics and Metabolism: Results From the Prospective CYPTAM Study. Journal of Clinical Oncology 2019; 37(8): 636-46.
28. Sanchez-Spitman AB, Moes DAR, Swen JJ, Dezentjé VO, Lambrechts D, Neven P, Gelderblom H, Guchelaar HJ. Exposure-response analysis of endoxifen serum concentrations in early-breast cancer. Cancer Chemotherapy and Pharmacology 2020; 85(6): 1141-52.
29. Braal CL, Beijnen JH, Koolen SLW, Oomen-de Hoop E, Steeghs N, Jager A, Huitema ADR, Mathijssen RHJ. Relevance of Endoxifen Concentrations: Absence of Evidence Is Not Evidence of Absence. Journal of Clinical Oncology 2019; 37(22): 1980-1.
30. Kang JS, Lee MH. Overview of therapeutic drug monitoring. Korean Journal of Internal Medicine 2009; 24(1): 1-10.
31. Braal CL, Jager A, Hoop EO, Westenberg JD, Lommen K, de Bruijn P, Vastbinder MB, van Rossum-Schornagel QC, Thijs-Visser MF, van Alphen RJ, Struik LEM, Zuetenhorst HJM, Mathijssen RHJ, Koolen SLW. Therapeutic Drug Monitoring of Endoxifen for Tamoxifen Precision Dosing: Feasible in Patients with Hormone-Sensitive Breast Cancer. Clinical Pharmacokinetics 2022; 61(4): 527-37.
32. Fox P, Balleine RL, Lee C, Gao B, Balakrishnar B. Dose Escalation of Tamoxifen in Patients with Low Endoxifen Level: Evidence for Therapeutic Drug Monitoring—The TADE StudyTamoxifen Dose Escalation and.. Clinical Cancer Research 2016.
33. Weiss MC, Hibbs J, McHugh TW, Buckley M, Larson S, Green N, Kaklamani VG, Chlebowski RT, Martinez D. A survey of breast cancer patients' use of cannabis before, during, and after treatment. Journal of Clinical Oncology 2020; 38(15_suppl): e19210-e.
34. Kisková T, Mungenast F, Suváková M, Jäger W, Thalhammer T. Future Aspects for Cannabinoids in Breast Cancer Therapy. International Journal of Molecular Sciences 2019; 20(7).

35. Shahbazi F, Grandi V, Banerjee A, Trant JF. Cannabinoids and Cannabinoid Receptors: The Story so Far. iScience 2020; 23(7): 101301.

36. Brown JD, Winterstein AG. Potential Adverse Drug Events and Drug-Drug Interactions with Medical and Consumer Cannabidiol (CBD) Use. Journal of Clinical Medicine 2019; 8(7).

37. Graham M, Martin JH, Lucas CJ, Murnion B, Schneider J. Cannabidiol drug interaction considerations for prescribers and pharmacists. Expert Review of Clinical Pharmacology 2022; 15(12): 1383-97.

38. Fisher B, Costantino JP, Redmond CK, Fisher ER, Wickerham DL, Cronin WM. Endometrial cancer in tamoxifen-treated breast cancer patients: findings from the National Surgical Adjuvant Breast and Bowel Project (NSABP) B-14. Journal of the National Cancer Institute 1994; 86(7): 527-37.

39. Amir E, Seruga B, Niraula S, Carlsson L, Ocaña A. Toxicity of adjuvant endocrine therapy in postmenopausal breast cancer patients: a systematic review and meta-analysis. Journal of the National Cancer Institute 2011; 103(17): 1299-309.

40. Hernandez RK, Sørensen HT, Pedersen L, Jacobsen J, Lash TL. Tamoxifen treatment and risk of deep venous thrombosis and pulmonary embolism: a Danish population-based cohort study. Cancer 2009; 115(19): 4442-9.

41. Schilder CM, Seynaeve C, Beex LV, Boogerd W, Linn SC, Gundy CM, Huizenga HM, Nortier JW, van de Velde CJ, van Dam FS, Schagen SB. Effects of tamoxifen and exemestane on cognitive functioning of postmenopausal patients with breast cancer: results from the neuropsychological side study of the tamoxifen and exemestane adjuvant multinational trial. Journal of Clinical Oncology 2010; 28(8): 1294-300.

42. Collins B, Mackenzie J, Stewart A, Bielajew C, Verma S. Cognitive effects of hormonal therapy in early stage breast cancer patients: a prospective study. Psycho-Oncology 2009; 18(8): 811-21.

43. Arevalo MA, Azcoitia I, Garcia-Segura LM. The neuroprotective actions of oestradiol and oestrogen receptors. Nature Reviews Neuroscience 2015; 16(1): 17-29.

44. Fester L, Prange-Kiel J, Jarry H, Rune GM. Estrogen synthesis in the hippocampus. Cell and Tissue Research 2011; 345(3): 285-94.

45. Lien EA, Solheim E, Ueland PM. Distribution of tamoxifen and its metabolites in rat and human tissues during steady-state treatment. Cancer Research 1991; 51(18): 4837-44.

46. Mannucci PM, Bettega D, Chantarangkul V, Tripodi A, Sacchini V, Veronesi U. Effect of tamoxifen on measurements of hemostasis in healthy women. Archives of Internal Medicine 1996; 156(16): 1806-10.

47. Blondon M, Bodmer A, Thouvenin L, Lecompte T, Righini M, Fontana P, Casini A. Differential impact of tamoxifen and aromatase inhibitors on thrombin generation: the prospective HEMOBREAST cohort. Blood Advances 2022; 6(9): 2884-92.

48. Erman M, Abali H, Oran B, Haznedaroglu IC, Canpinar H, Kirazli S, Celik I. Tamoxifen-induced tissue factor pathway inhibitor reduction: a clue for an acquired thrombophilic state? Annals of Oncology 2004; 15(11): 1622-6.

49. Sanchez-Spitman AB, Swen JJ, Dezentje VO, Moes D, Gelderblom H, Guchelaar HJ. Clinical pharmacokinetics and pharmacogenetics of tamoxifen and endoxifen. Expert Review of Clinical Pharmacology 2019; 12(6): 523-36.

50. Aarons L. Physiologically based pharmacokinetic modelling: a sound mechanistic basis is needed. British Journal of Clinical Pharmacology 2005; 60(6): 581-3.

51. Bauer RJ. NONMEM Tutorial Part I: Description of Commands and Options, With Simple Examples of Population Analysis. CPT: Pharmacometrics & Systems Pharmacology 2019; 8(8): 525-37.

52. Sassen SDT, Zwaan CM, van der Sluis IM, Mathôt RAA. Pharmacokinetics and population pharmacokinetics in pediatric oncology. Pediatric Blood & Cancer 2020; 67(4): e28132.

53. Klopp-Schulze L, Mueller-Schoell A, Neven P, Koolen SLW, Mathijssen RHJ, Joerger M, Kloft C. Integrated Data Analysis of Six Clinical Studies Points Toward Model-Informed Precision Dosing of Tamoxifen. Fronties in Pharmacology 2020; 11: 283.

54. Puszkiel A, Arellano C, Vachoux C, Evrard A, Le Morvan V, Boyer JC, Robert J, Delmas C, Dalenc F, Debled M, Venat-Bouvet L, Jacot W, Dohollou N, Bernard-Marty C, Laharie-Mineur H, Filleron T, Roché H, Chatelut E, Thomas F, White-Koning M. Model-Based Quantification of Impact of Genetic Polymorphisms and Co-Medications on Pharmacokinetics of Tamoxifen and Six Metabolites in Breast Cancer. Clinical Pharmacology and Therapeutics 2021; 109(5): 1244-55.

55. ter Heine R, Binkhorst L, de Graan AJ, de Bruijn P, Beijnen JH, Mathijssen RH, Huitema AD. Population pharmacokinetic modelling to assess the impact of CYP2D6 and CYP3A metabolic phenotypes on the pharmacokinetics of tamoxifen and endoxifen. British Journal of Clinical Pharmacology 2014; 78(3): 572-86.

56. Min W, Yiyun G, Haitang X. Population pharmacokinetics of tamoxifen in patients with breast cancer. Chinese Journal of Clinical Pharmacology and Therapeutics 2019; 24(4): 418-23.

57. Klopp-Schulze L, Joerger M, Wicha SG, Ter Heine R, Csajka C, Parra-Guillen ZP, Kloft C. Exploiting Pharmacokinetic Models of Tamoxifen and Endoxifen to Identify Factors Causing Subtherapeutic Concentrations in Breast Cancer Patients. Clinical Pharmacokinetics 2018; 57(2): 229-42.

58. van der Lee M, Allard WG, Vossen R, Baak-Pablo RF, Menafra R, Deiman B, Deenen MJ, Neven P, Johansson I, Gastaldello S, Ingelman-Sundberg M, Guchelaar HJ, Swen JJ, Anvar SY. Toward predicting CYP2D6-mediated variable drug response from CYP2D6 gene sequencing data. Science Translational Medicine 2021; 13(603).

59. Hall M, Peters G. Genetic Alterations of Cyclins, Cyclin-Dependent Kinases, and Cdk Inhibitors in Human Cancer. In: Vande Woude GF, Klein G, eds. Advances in Cancer Research: Academic Press; 1996: 67-108.

60. Harbour JW, Luo RX, Santi AD, Postigo AA, Dean DC. Cdk Phosphorylation Triggers Sequential Intramolecular Interactions that Progressively Block Rb Functions as Cells Move through G1. Cell 1999; 98(6): 859-69.

61. Scott SC, Lee SS, Abraham J. Mechanisms of therapeutic CDK4/6 inhibition in breast cancer. Seminars in Oncology 2017; 44(6): 385-94.

62. Finn RS, Martin M, Rugo HS, Jones S, Im S-A, Gelmon K, Harbeck N, Lipatov ON, Walshe JM, Moulder S, Gauthier E, Lu DR, Randolph S, Diéras V, Slamon DJ. Palbociclib and Letrozole in Advanced Breast Cancer. New England Journal of Medicine 2016; 375(20): 1925-36.

63. Cristofanilli M, Turner NC, Bondarenko I, Ro J, Im S-A, Masuda N, Colleoni M, DeMichele A, Loi S, Verma S, Iwata H, Harbeck N, Zhang K, Theall KP, Jiang Y, Bartlett CH, Koehler M, Slamon D. Fulvestrant plus palbociclib versus fulvestrant plus placebo for treatment of hormone-receptor-positive, HER2-negative metastatic breast cancer that progressed on previous endocrine therapy (PALOMA-3): final analysis of the multicentre, double-blind, phase 3 randomised controlled trial. The Lancet Oncology 2016; 17(4): 425-39.

64. Hortobagyi GN, Stemmer SM, Burris HA, Yap YS, Sonke GS, Paluch-Shimon S, Campone M, Petrakova K, Blackwell KL, Winer EP, Janni W, Verma S, Conte P, Arteaga CL, Cameron DA, Mondal S, Su F, Miller M, Elmeliegy M, Germa C, O'Shaughnessy J. Updated results from MONALEESA-2, a phase III trial of first-line ribociclib plus letrozole versus placebo plus letrozole in hormone receptor-positive, HER2-negative advanced breast cancer. Annals of Oncology 2018; 29(7): 1541-7.

65. Johnston S, Martin M, Di Leo A, Im S-A, Awada A, Forrester T, Frenzel M, Hardebeck MC, Cox J, Barriga S, Toi M, Iwata H, Goetz MP. MONARCH 3 final PFS: a randomized study of abemaciclib as initial therapy for advanced breast cancer. npj Breast Cancer 2019; 5(1): 5.

66. Sledge GW, Jr., Toi M, Neven P, Sohn J, Inoue K, Pivot X, Burdaeva O, Okera M, Masuda N, Kaufman PA, Koh H, Grischke EM, Frenzel M, Lin Y, Barriga S, Smith IC, Bourayou N, Llombart-Cussac A. MONARCH 2: Abemaciclib in Combination With Fulvestrant in Women With HR+/HER2- Advanced Breast Cancer Who Had Progressed While Receiving Endocrine Therapy. Journal of Clinical Oncology 2017; 35(25): 2875-84.

67. Slamon DJ, Neven P, Chia S, Fasching PA, De Laurentiis M, Im SA, Petrakova K, Bianchi GV, Esteva FJ, Martín M, Nusch A, Sonke GS, De la Cruz-Merino L, Beck JT, Pivot X, Vidam G, Wang Y, Rodriguez Lorenc K, Miller M, Taran T, Jerusalem G. Phase III Randomized Study of Ribociclib and Fulvestrant in Hormone Receptor-Positive, Human Epidermal Growth Factor Receptor 2-Negative Advanced Breast Cancer: MONALEESA-3. Journal of Clinical Oncology 2018; 36(24): 2465-72.

68. Sonke GS, Van Ommen - Nijhof A, Wortelboer N, van der Noort V, Swinkels ACP, Blommestein HM, Beeker A, Beelen K, Hamming LC, Heijns JB, Honkoop AH, De Jong PC, Van Rossum-Schornagel QC, van Schaik-van de Mheen C, Tol J, Tromp-Van Driel C, Vrijaldenhoven S, Van Leeuwen-Stok AE, Konings I, Jager A. Primary outcome analysis of the phase 3 SONIA trial (BOOG 2017-03) on selecting the optimal position of cyclin-dependent kinases 4 and 6 (CDK4/6) inhibitors for patients with hormone receptor-positive (HR+), HER2-negative (HER2-) advanced breast cancer (ABC). Journal of Clinical Oncology 2023; 41(17_suppl): LBA1000-LBA.

69. van Ommen-Nijhof A, Konings IR, van Zeijl CJJ, Uyl-de Groot CA, van der Noort V, Jager A, Sonke GS, committee Sss. Selecting the optimal position of CDK4/6 inhibitors in hormone receptor-positive advanced breast cancer - the SONIA study: study protocol for a randomized controlled trial. BMC Cancer 2018; 18(1): 1146.

70. Johnston SRD, Toi M, O'Shaughnessy J, Rastogi P, Campone M, Neven P, Huang CS, Huober J, Jaliffe GG, Cicin I, Tolaney SM, Goetz MP, Rugo HS, Senkus E, Testa L, Del Mastro L, Shimizu C, Wei R, Shahir A, Munoz M, San Antonio B, André V, Harbeck N, Martin M, monarch ECM. Abemaciclib plus endocrine therapy for hormone receptor-positive, HER2-negative, node-positive, high-risk early breast cancer (monarchE): results from a preplanned interim analysis of a randomised, open-label, phase 3 trial. Lancet Oncology 2023; 24(1): 77-90.

71. Gnant M, Dueck AC, Frantal S, Martin M, Burstein HJ, Greil R, Fox P, Wolff AC, Chan A, Winer EP, Pfeiler G, Miller KD, Colleoni M, Suga JM, Rubovsky G, Bliss JM, Mayer IA, Singer CF, Nowecki Z, Hahn O, Thomson J, Wolmark N, Amillano K, Rugo HS, Steger GG, Hernando Fernández de Aránguiz B, Haddad TC, Perelló A, Bellet M, Fohler H, Metzger Filho O, Jallitsch-Halper A, Solomon K, Schurmans C, Theall KP, Lu DR, Tenner K, Fesl C, DeMichele A, Mayer EL, groups P, investigators. Adjuvant Palbociclib for Early Breast Cancer: The PALLAS Trial Results (ABCSG-42/AFT-05/BIG-14-03). Journal of Clinical Oncology 2022; 40(3): 282-93.

72. Slamon D, Lipatov O, Nowecki Z, McAndrew N, Kukielka-Budny B, Stroyakovskiy D, Yardley Denise A, Huang C-S, Fasching Peter A, Crown J, Bardia A, Chia S, Im S-A, Ruiz-Borrego M, Loi S, Xu B, Hurvitz S, Barrios C, Untch M, Moroose R, Visco F, Afenjar K, Fresco R, Severin I, Ji Y, Ghaznawi F, Li Z, Zarate Juan P, Chakravartty A, Taran T, Hortobagyi G. Ribociclib plus Endocrine Therapy in Early Breast Cancer. New England Journal of Medicine 2024; 390(12): 1080-91.

73. Hortobagyi GN, Stemmer SM, Burris HA, Yap Y-S, Sonke GS, Paluch-Shimon S, Campone M, Blackwell KL, André F, Winer EP, Janni W, Verma S, Conte P, Arteaga CL, Cameron DA, Petrakova K, Hart LL, Villanueva C, Chan A, Jakobsen E, Nusch A, Burdaeva O, Grischke E-M, Alba E, Wist E, Marschner N, Favret AM, Yardley D, Bachelot T, Tseng L-M, Blau S, Xuan F, Souami F, Miller M, Germa C, Hirawat S, O'Shaughnessy J. Ribociclib as First-Line Therapy for HR-Positive, Advanced Breast Cancer. New England Journal of Medicine 2016; 375(18): 1738-48.

74. Patnaik A, Rosen LS, Tolaney SM, Tolcher AW, Goldman JW, Gandhi L, Papadopoulos KP, Beeram M, Rasco DW, Hilton JF, Nasir A, Beckmann RP, Schade AE, Fulford AD, Nguyen TS, Martinez R, Kulanthaivel P, Li LQ, Frenzel M, Cronier DM, Chan EM, Flaherty KT, Wen PY, Shapiro GI. Efficacy and Safety of Abemaciclib, an Inhibitor of CDK4 and CDK6, for Patients with Breast Cancer, Non–Small Cell Lung Cancer, and Other Solid Tumors. Cancer Discovery 2016; 6(7): 740-53.

75. Malumbres M, Barbacid M. Cell cycle, CDKs and cancer: a changing paradigm. Nature Reviews in Cancer 2009; 9(3): 153-66.

76. Chen P, Lee NV, Hu W, Xu M, Ferre RA, Lam H, Bergqvist S, Solowiej J, Diehl W, He YA, Yu X, Nagata A, VanArsdale T, Murray BW. Spectrum and Degree of CDK Drug Interactions Predicts Clinical Performance. Molecular Cancer Therapeutics 2016; 15(10): 2273-81.

77. Thibault S, Hu W, Hirakawa B, Kalabat D, Franks T, Sung T, Khoh-Reiter S, Lu S, Finkelstein M, Jessen B, Sacaan A. Intestinal Toxicity in Rats Following Administration of CDK4/6 Inhibitors Is Independent of Primary Pharmacology. Molecular Cancer Therapeutics 2019; 18(2): 257-66.

78. Goetz MP, Toi M, Campone M, Sohn J, Paluch-Shimon S, Huober J, Park IH, Trédan O, Chen SC, Manso L, Freedman OC, Garnica Jaliffe G, Forrester T, Frenzel M, Barriga S, Smith IC, Bourayou N, Di Leo A. MONARCH 3: Abemaciclib As Initial Therapy for Advanced Breast Cancer. Journal of Clinical Oncology 2017; 35(32): 3638-46.

79. Rugo HS, Huober J, García-Sáenz JA, Masuda N, Sohn JH, Andre VAM, Barriga S, Cox J, Goetz M. Management of Abemaciclib-Associated Adverse Events in Patients with Hormone Receptor-Positive, Human Epidermal Growth Factor Receptor 2-Negative Advanced Breast Cancer: Safety Analysis of MONARCH 2 and MONARCH 3. The Oncologist 2021; 26(1): e53-e65.

80. Levey AS, Perrone RD, Madias NE. Serum creatinine and renal function. Annual review of medicine 1988; 39(1): 465-90.

81. Lepist EI, Ray AS. Renal drug-drug interactions: what we have learned and where we are going. Expert Opinion on Drug Metabolism and Toxicology 2012; 8(4): 433-48.

82. Chappell JC, Turner PK, Pak YA, Bacon J, Chiang AY, Royalty J, Hall SD, Kulanthaivel P, Bonventre JV. Abemaciclib Inhibits Renal Tubular Secretion Without Changing Glomerular Filtration Rate. Clinical Pharmacology & Therapeutics 2019; 105(5): 1187-95.

83. Braal CL, Jongbloed EM, Wilting SM, Mathijssen RHJ, Koolen SLW, Jager A. Inhibiting CDK4/6 in Breast Cancer with Palbociclib, Ribociclib, and Abemaciclib: Similarities and Differences. Drugs 2021; 81(3): 317-31.

84. Groenland SL, Martínez-Chávez A, van Dongen MGJ, Beijnen JH, Schinkel AH, Huitema ADR, Steeghs N. Clinical Pharmacokinetics and Pharmacodynamics of the Cyclin-Dependent Kinase 4 and 6 Inhibitors Palbociclib, Ribociclib, and Abemaciclib. Clinical Pharmacokinetics 2020; 59(12): 1501-20.

85. Fry DW, Harvey PJ, Keller PR, Elliott WL, Meade M, Trachet E, Albassam M, Zheng X, Leopold WR, Pryer NK, Toogood PL. Specific inhibition of cyclin-dependent kinase 4/6 by PD 0332991 and associated antitumor activity in human tumor xenografts. Molecular Cancer Therapeutics 2004; 3(11): 1427-38.

86. Molenaar-Kuijsten L, Braal CL, Groenland SL, de Vries N, Rosing H, Beijnen JH, Koolen SLW, Vulink AJE, van Dongen MGJ, Mathijssen RHJ, Huitema ADR, Steeghs N, Dutch Pharmacology Oncology G. Effects of the Moderate CYP3A4 Inhibitor Erythromycin on the Pharmacokinetics of Palbociclib: A Randomized Crossover Trial in Patients With Breast Cancer. Clinical Pharmacology and Therapeutics 2022; 111(2): 477-84.

87. Yu Y, Loi CM, Hoffman J, Wang D. Physiologically Based Pharmacokinetic Modeling of Palbociclib. Journal of Clinical Pharmacology 2017; 57(2): 173-84.

88. Fogli S, Del Re M, Curigliano G, van Schaik RH, Lancellotti P, Danesi R. Drug-drug interactions in breast cancer patients treated with CDK4/6 inhibitors. Cancer Treatment Reviews 2019; 74: 21-8.

89. Werk AN, Cascorbi I. Functional Gene Variants of CYP3A4. Clinical Pharmacology and Therapeutics 2014; 96(3): 340-8.

PART I

TAMOXIFEN AND ADVERSE EFFECTS

CHAPTER 2

Tamoxifen dose de-escalation: an effective strategy for reducing adverse effects?

Drugs, March 2024

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ABSTRACT

Tamoxifen, a cornerstone in the adjuvant treatment of estrogen receptor-positive breast cancer, significantly reduces breast cancer recurrence and breast cancer mortality; however, its standard adjuvant dose of 20 mg daily presents challenges due to a broad spectrum of adverse effects, contributing to high discontinuation rates. Dose reductions of tamoxifen might be an option to reduce treatment-related toxicity, but large randomized controlled trials investigating the tolerability and, more importantly, efficacy of low-dose tamoxifen in the adjuvant setting are lacking. We conducted an extensive literature search to explore evidence on the tolerability and clinical efficacy of reduced doses of tamoxifen. In this review, we discuss two important topics regarding low-dose tamoxifen: 1) the incidence of adverse effects and quality of life among women using low-dose tamoxifen; and 2) the clinical efficacy of low-dose tamoxifen examined in the preventive setting and evaluated trough the measurement of several efficacy derivatives. Moreover, practical tools for tamoxifen dose reductions in the adjuvant setting are provided and further research to establish optimal dosing strategies for individual patients are discussed.

INTRODUCTION

Tamoxifen is a selective estrogen receptor (ER) modulator frequently used in the treatment of ER-positive breast cancer. In the adjuvant setting, tamoxifen 20 mg daily for 5 years reduces the breast cancer recurrence rate by approximately 40% during the first 10 years of follow-up and decreases the annual breast cancer death rate by one third.[1,2] Tamoxifen is recommended for a duration of 5-10 years for premenopausal patients and for 2-3 years for postmenopausal patients followed by 3-7 years of an aromatase inhibitor.[3-5] Tamoxifen has been registered since 1973, but is still a cornerstone in the treatment of ER-positive breast cancer, especially for premenopausal women.[6]

As an ER-modulator, besides being an ER-agonist, tamoxifen also acts as an ER-antagonist, depending on the specific ER-containing tissue to which it binds.[7] Several healthy tissues express ER. As a consequence, a variety of (endocrine) adverse effects can occur after tamoxifen, or its metabolites, bind to these receptors. For example, hot flashes are probably caused by an ER-antagonistic effect in the central nervous system, since ERs are also present in the brain, which leads to thermoregulatory dysfunction.[8] In contrast, tamoxifen's ER-agonistic effect in the endometrium can cause endometrial abnormalities and vaginal discharge.[7] Other mentioned bothersome adverse effects are arthralgia, insomnia, mood alterations, weight gain and vaginal dryness.[9] Venous thromboembolism (VTE) and endometrial cancer can also occur and, although rare, are serious adverse effects of tamoxifen.[10-12] The aforementioned adverse effects can have a huge impact on the patient's quality of life, especially since the duration of treatment in the adjuvant setting can be up to 10 years.[13] This becomes painfully visible as almost half of the patients discontinue tamoxifen within 5 years due to adverse effects and one-third of these patients discontinue tamoxifen already within the first year of treatment.[14-17] Another substantial group of patients adheres to tamoxifen therapy while compromising on health-related quality of life.[9,13]

Tamoxifen is a prodrug and is metabolized by cytochrome P450 (CYP) enzymes CYP2D6 and CYP3A4 to 4-hydroxy-tamoxifen, *N*-desmethyl-tamoxifen and endoxifen.[18] Both endoxifen and 4-hydroxy-tamoxifen have the highest affinity for the ER (more than 300 times higher than tamoxifen); however, endoxifen is considered the most important metabolite because it also has the highest plasma concentrations of all metabolites.[19-21] Several retrospective studies among primary breast cancer patients using tamoxifen 20 mg have indicated an exposure-response relation between endoxifen levels and tamoxifen efficacy, with suggested endoxifen thresholds varying from 10 to 16 nM.[22-24] Of these thresholds, 16 nM is the most widely accepted, as shown in the largest study thus far (1370 patients[23] compared to 86[22] and 306 patients[24]). It is also the most

conservative threshold, minimizing the chance of patients inappropriately continuing to use an ineffective dose.[25,26] However, until now no prospective study was able to confirm the 'definitive' endoxifen efficacy threshold, possibly due to inadequate statistical power.[27-29] The effect of tamoxifen and metabolite levels on the occurrence of adverse effects remains largely unclear. While some studies found no association between tamoxifen, endoxifen, 4-hydroxy-tamoxifen, *N*-desmethyl-tamoxifen and adverse effects[30,31], others showed an association between elevated tamoxifen or endoxifen levels and increased adverse effects.[32,33] Notably, none of the patients in these studies were treated with tamoxifen doses that were lower than the standard dose of 20 mg.

The high incidence of tamoxifen-related adverse effects affecting quality of life, as well as the high discontinuation rate of tamoxifen among patients with ER-positive breast cancer, raises the question whether reducing the dose of tamoxifen could lead to a better toxicity profile without reducing its efficacy. In the primary (for those at increased risk for breast cancer) and the secondary (for patients with premalignant lesions) chemoprevention setting, tamoxifen 20 mg is also recommended in National Comprehensive Cancer Network (NCCN) and American Society of Clinical Oncology (ASCO) guidelines, as it can reduce the risk for breast cancer development by one-third.[34-37] After a successful randomized controlled trial (RCT), low-dose tamoxifen (5 mg) is also considered an option in the primary and secondary chemoprevention setting.[34,35,38] In the adjuvant setting, no RCT between tamoxifen 20 mg and lower doses of tamoxifen has been performed thus far. Given the impressively large number of patients needed, together with the long duration of follow-up that would be required to obtain firm conclusions [29,39], it is highly unlikely that such a study will ever be conducted. To determine whether there are other possibilities to solve this pressing question, the current literature was systematically reviewed to discuss two important topics: 1) tamoxifen-related adverse effects in women using low-dose tamoxifen compared with the standard adjuvant dose of 20 mg or placebo; and 2) clinical efficacy of low-dose tamoxifen compared with standard dose tamoxifen or placebo. Finally, based on these findings, we attempted to provide practical advice on how to respond when patients experience bothersome adverse effects of tamoxifen.

METHODS

We conducted a search of the Embase, Medline ALL, Web of Science Core Collection and the Cochrane Register of Controlled Trials databases using the following search terms: "(tamoxifen) AND (drug dose reduction OR drug underdose) OR (tamoxifen NEAR (dose OR dosage OR reduct OR decreas OR tapering OR low OR lower OR

regiment OR de-escalat OR adjustment OR modificat OR alter OR altered OR change OR dependent OR underdose OR underdosage)" up to December 1st 2023. We excluded reviews, guidelines and editorials, prequels from other published studies, studies where no lower doses of tamoxifen (i.e. below the standard adjuvant dose of 20 mg) were investigated, studies where tamoxifen was not continuously administered, and studies where no adverse effects, clinical efficacy or suitable derivatives for clinical efficacy of tamoxifen were assessed. To qualify as a 'suitable derivative for tamoxifen efficacy', the following criteria had to be met: 1) the derivative had to be associated with breast cancer risk; 2) the derivative could be influenced by tamoxifen and; 3) alteration of the derivative after tamoxifen could predict the long-term efficacy of tamoxifen.

RESULTS

Based on the systematic search, a total of 2081 results were found and screened by title or abstract for relevance, leading to 106 relevant abstracts; 19 articles were eventually included in this review. An overview of the article selection can be found in **Figure 1**, and the studies discussed in this review can be found in **Table 1**.

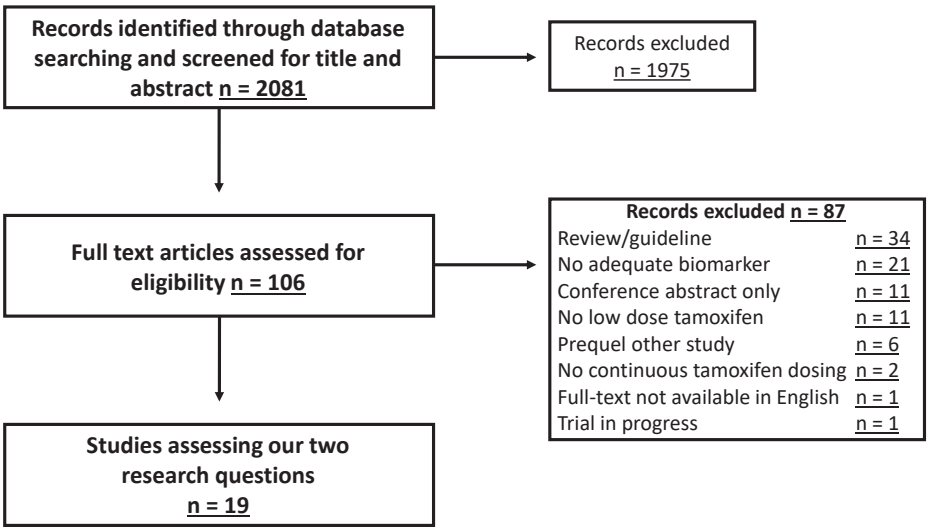

Figure 1. Article selection; articles found by systematic search up to December 1st 2023

Table 1. Overview of low-dose tamoxifen studies assessing toxicity or clinical efficacy, discussed in this review

Indication low-dose tam	First author, year	Study population	Type of study	Sample size	Treatment	Duration of tamoxifen (months)	Follow-up (months)
Women using HRT	Decensi, 2013[40]	Postmenopausal women	RCT	1884	Tamoxifen 5 mg/day OR placebo	60	74
	Decensi, 2007[41]	Postmenopausal women <60 years of age	RCT	210	Tamoxifen 1 mg/day, OR tamoxifen 10 mg/week, OR tamoxifen 5 mg/day OR placebo	12	12
High-risk women	Bhatia, 2021[42]	Chest-irradiated cancer survivors	RCT	72	Tamoxifen 5 mg/day OR placebo	24	24
	Eriksson, 2021[43] Hammarström, 2023[44]	Women 40-74 years of age with higher mammographic density	RCT	1230	Tamoxifen 1, 2.5, 5, 10, 20 mg/day OR placebo	6	6
Carcinoma in situ	Decensi, 2019[38] Buttiron Weber, 2021[45] Lazzeroni, 2023[46]	Women ≤75 years of age with atypical ductal/lobular hyperplasia, DCIS or LCIS	RCT	500	Tamoxifen 5 mg/day OR placebo	36	36-120
	Guerrieri-Gonzaga, 2013[47]; Guerrieri-Gonzaga, 2009[48]	Women with DCIS	Observational study	680-833	Tamoxifen 5 mg/day, OR tamoxifen 20 mg/week, OR tamoxifen 0 mg/day	22	66-120
	Guerrieri-Gonzaga, 2016[49]	Women with DCIS	Observational study	1091	Tamoxifen 10 mg q.o.d., OR tamoxifen 20 mg/week, OR tamoxifen 0 mg tam/day, AND/OR RTx	60	92
	Decensi, 2009[50] Serrano, 2018[51]	Women with DCIS/LCIS, 5-year Gail risk >1.3% or pT1mic/pT1a BC	RCT	235	Tamoxifen 5 mg/day + placebo, OR fenretinide 200 mg/day + placebo, OR both, OR 2 x placebo	24	12-144

Indication low-dose tam	First author, year	Study population	Type of study	Sample size	Treatment	Duration of tamoxifen (months)	Follow-up (months)
Preoperative for BC	Decensi, 2003[52], Kisanga, 2004[53]	Women >45 years with invasive BC	RCT	120	Tamoxifen 1, 5 OR 20 mg/day	1	1
	de Sousa, 2006[54]	Women with invasive BC	RCT	38	Tamoxifen 10 mg/day OR tamoxifen 0 mg/day	0.5	0.5
	Serrano, 2013[55]	Premenopausal women with invasive BC	RCT	125	Tamoxifen 10 mg/week, OR raloxifen 60 mg/day, OR placebo	1.5	1.5
Adjuvant for BC	Lee, 2019[56]	Patients with severe hot flashes	Intervention study	20	Tamoxifen dose reduction from 20 mg to 10 mg	2-month dose -reduction	2
	Buijs, 2023[57]	Patients with severe adverse effects and endoxifen level ≥32 nM	Intervention study	17	Tamoxifen dose reduction from 20 mg to 10 mg	3 months 20 mg – 3 months 10 mg	3

BC breast cancer, DCIS ductal carcinoma in situ, LCIS lobular carcinoma in situ, RCT randomized controlled trial, RTx radiotherapy, HRT hormone replacement therapy, qod every other day

Dose of tamoxifen and side effects

To determine whether taking a lower dose of tamoxifen can decrease the high incidence of adverse effects, we first investigated whether low-dose tamoxifen leads to fewer adverse effects. An overview of the results considering low-dose tamoxifen and menopausal symptoms can be found in **Table 2.** Low-dose tamoxifen is defined as all tamoxifen doses below the standard dose of 20 mg daily.

Menopausal symptoms

Low-dose tamoxifen (<20 mg once daily) compared with standard-dose tamoxifen (20 mg once daily)

Two studies compared the adverse effects of different levels of low-dose tamoxifen with that of a standard daily dose of tamoxifen 20 mg, and both showed a trend towards fewer adverse effects with low-dose tamoxifen.[43,44,52] The first study was a large RCT randomizing 1230 healthy women with high mammographic density between placebo and tamoxifen 1, 2.5, 5, 10 or 20 mg daily for 6 months. Adverse effects were self-reported using five-point Likert scale questionnaires assessing symptoms of anti-hormonal treatment of breast cancer. In this study, lower doses of tamoxifen led to fewer adverse effects, specifically in vasomotor and gynecologic symptoms as well as muscle cramps.[43,44] This reduction was however confined to premenopausal women.[44] In the other much smaller study (n = 120) tamoxifen 1 , 5 or 20 mg daily was administered for 4 weeks preoperatively to patients with invasive breast cancer. Patients in the 1 or 5 mg tamoxifen group experienced fewer hot flashes (32% and 36% in the 1 and 5 mg groups, respectively, vs 50% in the 20 mg group) and less vaginal discharge (26% and 22% in the 1 and 5 mg group, respectively, vs. 47% in the 20 mg group) compared with patients in the tamoxifen 20 mg group[52]; however, these differences were not statistically significant, likely because of the small numbers of patients under study (only 40 participants per dose group). From this data, it can be concluded that lower doses of tamoxifen seem to lead to fewer adverse effects than the standard dose.[43,44,52]

Low-dose tamoxifen (<20 mg once daily) compared with placebo

The use of hormonal replacement therapy (HRT) in healthy women is associated with an increased risk for breast cancer development compared with non-users.[58] In two primary prevention studies among healthy postmenopausal women using HRT for menopausal symptoms, women were randomized between low-dose tamoxifen or placebo.[40,41] Besides the incidence of invasive breast cancer, menopausal symptoms were assessed in detail in both studies. In the smaller study (n = 210) no difference was found in 12 menopausal symptoms between women taking low-dose tamoxifen for 1 year compared with placebo.[41] Women were randomized between tamoxifen doses of 1 mg daily, 5 mg daily, or 10 mg weekly, i.e. two-thirds of the women taking tamoxifen

received a very low tamoxifen dose (1 mg tamoxifen daily or 10 mg tamoxifen weekly). There was a trend towards more hot flashes, sweating and vaginal discharge when total weekly dose of tamoxifen increased. The second, much larger study (*n* = 1884) showed that using tamoxifen 5 mg daily for 5 years led to more hot flashes, nights sweats, vaginal discharge and vaginal dryness compared with placebo.[40] The question is how generalizable these findings are for the general population, since, in this study, there was a clear preselection of women who had already proven to have complaints related to the physiological menopause for which they used HRT.

In three prevention studies of patients with ductal carcinoma-in-situ (DCIS) or lobular carcinoma in situ (LCIS; *n* = 500[38,45] and *n* = 235[50,51]) and patients with a history of chest-irradiation (*n* = 72)[42], the use of tamoxifen 5 mg daily for a period of 2-3 years was compared with placebo. Studies assessed adverse effects using Common Terminology Criteria for Adverse Events (CTCAE), patient-reported symptoms, or menopause-related adverse effects questionnaires. In most of the over 40 evaluated adverse effects, no significant differences between tamoxifen and placebo were found. The same accounted for four menopausal quality-of-life domains. However, compared with placebo, tamoxifen did lead to increased frequency of hot flashes, but without an increase in the intensity of the hot flashes[38] as well as more fatigue and myalgia.[42] Unfortunately, in the latter study, no correction for multiple testing was done despite comparing 26 different adverse effects.

Overall, low doses of tamoxifen (≤5 mg daily) showed a good safety profile. Although some increase in adverse effects was found with low-dose tamoxifen compare with placebo in three of five prevention studies, this was in a minority of the evaluated adverse effects.[38,40,42]

Effect of lowering the tamoxifen dose

Two single-arm studies assessed the effect on adverse effects of a dose reduction of standard tamoxifen dose (20 mg) in the adjuvant setting in patients who experienced tamoxifen-related adverse effects.[56,57] In the first study, tamoxifen dose was decreased from 20 to 10 mg daily in 20 patients with invasive breast cancer experiencing severe hot flashes. The investigators evaluated the effects using a specific hot flash diary and measured subjective improvement in hot flashes after 8 weeks of taking the reduced dose of tamoxifen 10 mg.[56] Seventeen patients (85%) reported a subjective improvement in hot flashes after dose reduction. There was a numeric difference in hot flash score (131 points with a 20 mg dose vs. 47 points with a 10 mg dose), although this did not statistically differ. In a second study from our own group, the tamoxifen dose was reduced from 20 to 10 mg daily for 3 months in 17 patients with invasive breast

cancer experiencing bothersome tamoxifen-related adverse effects who also had an endoxifen level ≥32 nM (i.e. two times the conservative endoxifen efficacy threshold of 16 nM).[23,57] Endocrine symptoms (primary endpoint) and health-related quality of life, both measured using the FACT-ES questionnaire[59], were assessed at baseline and after 3 months of using a lower dose of tamoxifen. Both endocrine symptoms and health-related quality of life improved statistically significant and clinically meaningful in 41% and 65% of patients, respectively. Almost three-quarters of the patients graded the improvement in tamoxifen-related adverse effects after tamoxifen dose reduction as sufficient. Endocrine symptoms and health-related quality of life were also compared in 60 patients who continued to take tamoxifen 20 mg for 3 months. No improvements were seen in this group over time.[57] From these two studies, it seems that lowering the dose of tamoxifen compared with a standard dose improves tolerability by reducing menopausal symptoms, although performance bias due to the fact that patients were not blinded for dose reduction cannot be fully excluded.

Severe adverse effects: endometrial cancer and venous thromboembolism

Beside menopausal symptoms, tamoxifen can also lead to some rare but severe adverse effects, such as VTE and endometrial cancer. The rate of endometrial cancer increases approximately two to three times with tamoxifen compared with breast cancer patients not using tamoxifen, although the absolute incidence is very low (1.6/1000 patients).[60] The risk increases with a longer duration of tamoxifen therapy[61], likely due to increasing cumulative tamoxifen dose.[62] Endometrial polyps also occur more frequently with tamoxifen use compared with non-users (>10% incidence after 4 years of tamoxifen standard dose in postmenopausal patients compared with non-tamoxifen users) and can transform into endometrial cancer.[63,64] It would be very beneficial if reducing the dose of tamoxifen due to severe menopausal symptoms could also diminish these risks.

Five studies that investigated the influence of low-dose tamoxifen on endometrial polyps[38,40,50] or endometrial cancer were identified.[40,47,49] All three studies that investigated the incidence of endometrial cancer, using low-dose (20 mg weekly, 5 mg daily) tamoxifen for 2 to 5 years with a follow-up time of at least 5 years, included a large number of women (sample sizes reaching from 500 to 1884). Two studies included patients in a secondary chemoprevention setting[47,49] and one study investigated healthy women receiving HRT.[40] None of these studies found an increased incidence of endometrial cancer in the low-dose tamoxifen group compared with the placebo group.[40,47,49] Three studies investigated low-dose tamoxifen and the incidence of endometrial polyps.[38,50] In two small studies in the secondary chemoprevention setting (n = 500 and n = 235), a trend towards a higher incidence of endometrial polyps was found for women using tamoxifen 5 mg for 3 years compared with placebo, although

this was not statistically significant (11% vs. 7% $p = 0.62$; and 2.8% vs. 1.6% $p = 0.54$, respectively).[38,50] The third much larger study among women receiving HRT ($n = 1884$) found an almost five times higher significant increase in endometrial polyps among those taking a daily dose of tamoxifen 5 mg for 5 years compared with placebo (2.9% in the tamoxifen group vs. 0.6% in the placebo group; relative risk [RR] 4.74, 95% confidence interval [CI] 1.96-11.5).[40] These findings imply that there is an increased risk of developing endometrial polyps when using low-dose tamoxifen compared with placebo, although it is unknown how the low-dose tamoxifen polyp incidence compares with that of standard-dose tamoxifen.

A tamoxifen dose of 20 mg daily results in a RR for VTE ranging from 1.6 to 3.0.[65-68] The reported VTE incidence was 1-3% during standard dose tamoxifen treatment, and most events occur within the first 2 years of treatment.[69,70] Two studies compared the incidence of VTE between tamoxifen 5 mg daily for 3-5 years and placebo. One study was performed in healthy women receiving HRT ($n = 1884$) and the other study was performed in patients with carcinoma in situ ($n = 500$). No significant difference was found (0.5% for tamoxifen vs. 0.2% for placebo [RR 2.64, 95% CI 0.51-13.6] and 0.4% for both tamoxifen and placebo with a p-value of 1.0, respectively) over a follow-up period of 6-10 years.[40,46] Although the first mentioned study was in women receiving HRT,[40] which might have influenced the VTE incidence because HRT leads to a higher VTE risk itself,[71] the absolute incidence for VTE is very low. These findings support the idea that a reduction in tamoxifen dose may lead to a lower incidence of VTE than standard tamoxifen dosing, although this has not been directly investigated.

Dose of tamoxifen and clinical efficacy

Thus far, no RCT investigating the efficacy between a standard tamoxifen dose of 20 mg and lower doses of tamoxifen in the adjuvant setting has been conducted and is highly unlikely to be conducted given the impressively large number of patients needed, together with the long duration of follow-up that is required. Consequently, direct evidence elucidating the clinical efficacy of lower tamoxifen doses in the adjuvant setting is lacking. To answer the question whether lower doses of tamoxifen still have antitumor efficacy, a search was conducted for articles that evaluated lower doses of tamoxifen versus standard dose or placebo using derived measures of tamoxifen efficacy in the adjuvant setting. First, the efficacy of low-dose tamoxifen in preventing the development of breast cancer (primary and secondary chemoprevention) will be discussed. Second, two derived measures of tamoxifen efficacy are discussed: 1) the effect of tamoxifen on mammographic density; and 2) the effect of tamoxifen on the proliferation marker Ki67. An overview of the results of low-dose tamoxifen on the different clinical efficacy derivatives can be found in **Table 3**.

Table 2. Studies regarding toxicity of low-dose tamoxifen

	First author, year	Population	Type of study
Low-dose tamoxifen vs. standard dose	Eriksson, 2021[43]	Healthy women with high mammographic density >5%	RCT
	Hammarström, 2023[44]	Healthy women with high mammographic density >5%	RCT
	Decensi, 2003[52]	Women >45 years of age with ER and/or PR+ BC	RCT
Low-dose tamoxifen vs. placebo	Decensi, 2009[50] Serrano, 2018[51]	Premenopausal women with DCIS/LCIS, (*n* = 160), 5-year Gail Risk >1.3% (*n* = 54), pT1mic/PT1a BC (*n* = 21)	RCT
	Bhatia, 2021[42]	Healthy women with a history of chest radiation	RCT
	Decensi, 2019[38] Buttiron Weber, 2021[45]	Women ≤75 years with ER+ or unknown atypical ductal hyperplasia, DCIS or LCIS	RCT
	Decensi, 2007[41]	Healthy postmenopausal women <60 years using HRT or about to start it for menopausal symptom relief	RCT
	Decensi, 2013[40]	Healthy postmenopausal women using HRT for menopausal symptom relief	RCT
Low-dose tamoxifen in the adjuvant setting (without a control group)	Lee, 2019[56]	Patients using adjuvant tamoxifen for primary BC with severe hot flashes when taking tamoxifen 20 mg	Prospective intervention study
	Buijs, 2023[57]	Patients using adjuvant tamoxifen for primary BC with severe adverse effects and endoxifen levels ≥32 nM when taking tamoxifen 20 mg	Prospective intervention study

BC breast cancer, DCIS ductal carcinoma in situ, LCIS lobular carcinoma in situ, HRT hormone replacement therapy, ER estrogen receptor, PR progesterone receptor, RCT randomized controlled trial, sign: significant, HF hot flashes, QOL quality of life, RR relative risk, CI confidence interval, IQR interquartile range

Outcome	Strength
Significantly fewer vasomotor symptoms in the tamoxifen 1 mg (-15.5%, 95% CI -23.9 to -7.0), 2.5 mg (-13.5%, 95% CI -22.1 to -4.9) and 5 mg tam (-9.6%, 95% CI -18.4 to -0.8) groups compared with tamoxifen 20 mg (34%, 95% CI 27.8-40.7). Significantly fewer gynecologic symptoms in the tamoxifen 1 mg (-8.0, 95% CI -15.3 to -0.7) and 5 mg (-9.2, 95% CI -16.4 to -2.1) groups compared with tamoxifen 20 mg (21.2%, 95% CI 16.1-27.3)	Moderate, adverse effects were no primary endpoint but the study was an RCT and had a large sample size. However, adverse effects in different tamoxifen or placebo groups were only compared with tamoxifen 20 mg and not with each other
Significantly fewer adverse effects in premenopausal women taking tamoxifen 2.5/5 mg compared with women taking tamoxifen 10/20 mg (sum of mean Likert score change: 1.61 (95% CI 1.17-2.04) vs. 2.47 (95% CI 1.98-2.96)	Moderate, see Eriksson 2021.[43] Moreover, adverse effects In this study were compared between low (2.5/5mg) and high (10/20mg) tamoxifen doses although 10 mg should also be considered as low-dose tamoxifen
No significant difference between tamoxifen dose groups for HF (tamoxifen 1 mg, 32%; tamoxifen 5 mg, 36%; and tamoxifen 20 mg, 50%) or vaginal discharge (tamoxifen 1 mg, 26%; tamoxifen 5 mg, 22%; tamoxifen 20 mg, 47%)	Low, not powered for adverse effect analysis, small sample size and treatment for only 4 weeks
No significant difference between tamoxifen and placebo in HF (38% vs. 37%) and vaginal discharge (38% vs 23%). No significant difference in four menopausal QOL domains	Low, not powered for adverse effect analysis
No significant difference between tamoxifen and placebo in 26 patient-reported symptoms with the exception of myalgia (tamoxifen 21% vs. placebo 3%; $p = 0.02$) and fatigue (tamoxifen 29% vs. placebo 8%; $p = 0.03$)	Low, not powered for adverse effect analysis and small sample size. No correction for multiple testing has been performed despite testing 26 items
No significant difference between tamoxifen and placebo in vaginal discharge, dryness or pain with intercourse, musculoskeletal symptoms or arthralgia and HF score. Significantly higher daily number of HF with tamoxifen (RR 1.46, 90% CI 1.05-2.00)	High, adverse effects were no primary endpoint but the study was an RCT and had a large sample size
No significant difference between the tamoxifen groups and placebo in 12 menopausal symptoms	Low, not powered for adverse effect analysis, no comparison between placebo and different tamoxifen dose groups
Significantly more HF (RR 1.78, 95% CI 1.48-2.15), night sweats (RR 1.62, 95% CI 1.34-1.97), and vaginal dryness (RR 1.49, 95% CI 1.25-1.76) and discharge (RR 2.13, 95% CI 1.71-2.65) with tamoxifen	Moderate, tamoxifen was combined with HRT in patients with menopausal symptoms and is therefore less comparable with clinical practice, but the study has a large sample size and was an RCT
No significant difference after dose reduction from tamoxifen 20 mg (median HF score 131, IQR 22-1482) to 10 mg tam (median HF score 47, IQR 5-864); $p = 0.24$	Low, not powered to find a difference in hot flash score and no focus on other adverse effects; study was not blinded or randomized
Clinically relevant improvement in endocrine symptoms in 41% of patients after dose reduction from tamoxifen 20 mg to tamoxifen 10 mg (90% CI 21-65%; $p = 0.038$)	Moderate, powered for difference but study was not blinded or randomized

Table 3. Studies regarding clinical efficacy of low-dose tamoxifen

Clinical efficacy derivative	First author, year	Population	Study design
Breast cancer (invasive or in situ)	Decensi, 2009[50]	Premenopausal women with pT1mic/PT1a BC, DCIS or LCIS or 5-year Gail risk >1.3%	RCT
	Decensi, 2013[40]	Healthy postmenopausal women using HRT for menopausal symptom relief	RCT
	Guerrieri-Gonzaga, 2009[48]	Women who underwent surgery for ER+ DCIS	Observational study
	Guerrieri-Gonzaga, 2013[47]	Women who underwent surgery for ER+ DCIS	Observational study
	Guerrieri-Gonzaga, 2016[49]	Women who underwent surgery for ER+ DCIS	Observational study
	Decensi, 2019[38]	Women ≤75 years of age with ER+ or unknown atypical ductal hyperplasia, DCIS or LCIS	RCT
Mammographic density (MD)	Bhatia, 2021[42]	Healthy pre- (*n* = 44) and postmenopausal (*n* = 28) women with a history of chest radiation	RCT
	Decensi, 2007[41]	Healthy postmenopausal women receiving HRT for menopausal symptoms	RCT
	Decensi, 2009[50]	Premenopausal women with pT1mic/PT1a BC (*n* = 21), DCIS or LCIS (*n* = 160) or 5-year Gail risk >1.3% (*n* = 54)	RCT
	Eriksson, 2021[43]	Healthy pre- (*n* = 210) and postmenopausal (*n* = 290) women with high MD (>5%)	RCT
Ki67	Decensi, 2003[52]	Women >45 years of age with ER and/or PR+ BC	RCT
	de Sousa, 2006[54]	Women with invasive ER+ BC	RCT
	Serrano, 2013[55]	Premenopausal women with invasive ER+ BC	RCT

HRT hormone replacement therapy, BC breast cancer, DCIS ductal carcinoma in situ, LCIS lobular carcinoma in situ, ER estrogen receptor, PR progesterone receptor, MD mammographic density, IBC invasive breast cancer, HER2- HER2neu-negative, HR hazard ratio, CI confidence interval, RCT randomized controlled trial, RR relative risk

Outcome	Strength
No significant difference in IBC or DCIS between tamoxifen or placebo (HR 0.70, 95% CI 0.32-1.52). Breast cancer incidence: 3.5% in the tamoxifen arm and 5.2% in the placebo arm	Low, lack of power (n = 60 per treatment group)
No significant difference in IBC between tamoxifen or placebo (RR 0.80, 95% CI 0.44-1.46), subgroup analysis in HRT users <5 years showed a decrease in IBC (RR 0.35, 95% CI 0.15-0.82)	Low, lack of power (only 24 BC events when receiving placebo and 19 BC events when taking tamoxifen)
Tamoxifen decreased BC in ER+Her2- DCIS patients (HR 0.55, 95% CI 0.32-0.97)	Low, non-randomized design.
Tamoxifen decreased IBC or BC in situ compared with no treatment (HR 0.70, 95% CI 0.34-0.94), with greater benefit in postmenopausal women (HR 0.57, 95% CI 0.34-0.94) than premenopausal women (HR 0.79, 95% CI 0.54-1.17)	Low, non-randomized design
Tamoxifen decreased ipsilateral recurrence or DCIS (HR 0.70, 95% CI 0.54-0.91), with greater benefit ≥50 years (HR 0.51, 95% CI 0.33-0.77) than <50 years (HR 0.84, 95% CI 0.60-1.18)	Low, non-randomized design
Tamoxifen halved IBC or DCIS (HR 0.48, 95% CI 0.26-0.92). Less contralateral BC in the tamoxifen group (HR 0.25, 95% CI 0.07-0.88)	High
Tamoxifen significantly decreased MD compared with placebo (tamoxifen -10.2% vs. placebo -4.4% reduction)	High
No significant difference when the tamoxifen groups were compared with placebo. Significantly more decrease in MD with tamoxifen 5 mg/day compared with tamoxifen 1 mg/day or tamoxifen 10 mg/week (tamoxifen 5 mg/d, 15.4%; tamoxifen 1 mg/d, 3.7%; tamoxifen 10 mg/w, 2.4% reduction)	Medium, no comparison has been made between tamoxifen 5 mg and placebo separately, HRT use might confound results
Tamoxifen significantly decreased MD (mean -16.2%), while placebo did not significantly decreased MD (mean -8.9%)	Medium, no direct comparison between tamoxifen and placebo
Tamoxifen significantly decreased MD compared with placebo; tamoxifen ≥2.5 mg/day led to non-inferior MD reduction compared with 20 mg	High
Tamoxifen significantly decreased Ki67 compared with the control group, with no differences within the tamoxifen dose groups. Median % ΔKi67 was -14.0% (95% CI -38.8 to 0.0) with tamoxifen 1 mg; -11.7% (95% CI -32 to 8.5) with tamoxifen 5 mg, and -15.6% (95% CI -44.5 to 14.1) with tamoxifen 20 mg. In control group without treatment, Ki67 increased with 18.6% (95% CI -3.3% to 33.0%)	Medium, lack of power to find significant change in Ki67 expression in tamoxifen dose groups or control group / lack of power to find difference between dose groups
Tamoxifen significantly decreased Ki67 from 24.69% to 10.43% (p < 0.001), while the control group without tamoxifen did not	Medium, no direct comparison between tamoxifen and control group
No significant decrease in Ki67 after tamoxifen 10 mg/week in the total study cohort, only in normal CYP2D6 metabolizers	High, primary outcome was comparison between ΔKi67

Low-dose tamoxifen in preventing breast cancer development

A standard dose of tamoxifen is known to be effective in not only preventing breast cancer recurrence after invasive breast cancer but also in primary and secondary prevention, i.e. preventing (new) primary breast cancers in patients with high breast cancer risk or a history of breast carcinoma in situ, such as DCIS.[36] For example, in women with DCIS, tamoxifen 20 mg reduces the risk of developing invasive breast cancer by 36%.[36] These findings have resulted in ASCO and NCCN guidelines to consider a daily dose of tamoxifen 20 mg for women with high risk for breast cancer, DCIS or LCIS to prevent breast cancer development.[34,35] Aiming to increase the compliance for the primary and secondary prevention indication, studies with low-dose tamoxifen for this patient group were performed. Six prevention studies (three observational and three randomized) examined the clinical efficacy of low-dose tamoxifen in terms of preventing the development of breast cancer.[38,40,47-50]

In three large observational studies, low-dose tamoxifen (5 mg/day, 10 mg every other day, or 20 mg per week) for 2-5 years was compared with no tamoxifen (not placebo-controlled) in women who underwent surgery for DCIS.[47-49] They found approximately 30% reduction in breast cancer risk in women taking low-dose tamoxifen (independent of dose) compared with women who did not use tamoxifen.[47-49] In sub analyses, the significant breast cancer risk reduction of low-dose tamoxifen disappeared in women below 50 years of age.[49] The same trend was seen for premenopausal women.[47]

Three randomized, placebo-controlled trials were conducted in healthy women using HRT[40] and women with carcinoma in situ,[38,50] comparing tamoxifen 5 mg daily with placebo for 2-5 years. In two studies, only a numerical (but no statistical) lower incidence of invasive breast cancer or DCIS in tamoxifen-treated patients could be found.[40,50] One study had a small sample size of only 60 patients per treatment group[50] and the other study enrolled a significantly lower number of women than estimated (n = 1884 instead of 4500) due to challenges in recruitment and an earlier-than-expected cessation of inclusion, also leading to a lack of power.[40] In the third study (n = 500), taking tamoxifen for 3 years halved the incidence of breast cancer.[38,46] Consistent with the observational studies, the efficacy of low-dose tamoxifen was more pronounced in postmenopausal women than in premenopausal women.[46]

Taken together, these data provide evidence for efficacy of low doses of tamoxifen in the primary and secondary prevention setting, mainly in postmenopausal women. It is however not entirely clear whether the efficacy in preventing the development of primary breast cancer can simply be translated into efficacy in preventing breast

cancer recurrences in the adjuvant setting. Also, no direct comparisons were made with tamoxifen 20 mg daily.

Lowering mammographic density as derived measure of tamoxifen efficacy

Mammographic density is based on the distribution between stromal, epithelial and fat cells, where women with high mammographic density have relatively more stromal and epithelial cells and less adipocytes.[72] Several studies have shown that high breast tissue density, as assessed by mammography, is associated with an increased risk for developing breast cancer in both pre- and postmenopausal women compared with low breast density.[72-75] Although it is not completely understood why higher mammographic density is associated with higher breast cancer risk, it is hypothesized that a combination of higher cell proliferation of stromal and epithelial cells and genetic damage to these proliferating cells in dense breast tissue increases the risk of breast cancer.[76]

A standard dose of tamoxifen 20 mg daily can significantly reduce mammographic density compared with placebo after 1 year of treatment.[77] Interestingly, in the preventive setting, a reduction in mammographic density of ≥10% after 1 year of tamoxifen 20 mg daily led to a reduction in breast cancer risk of 63% compared with a group of women who received placebo.[78] This reduction in breast cancer risk was not seen in women treated with tamoxifen who experienced <10% reduction in mammographic density. Similar results were found in the adjuvant setting. Breast cancer patients with a 20% reduction in mammographic density after an average of 1 year of standard-dose tamoxifen had a 50% reduction in the risk for breast cancer-specific death compared with patients taking tamoxifen with no reduction in mammographic density.[79]

Low doses of tamoxifen (5 mg daily) also led to a significant reduction in mammographic density after 6-12 months in women with a high baseline mammographic density[43], HRT for menopausal symptoms[41], history of chest radiation[42] or carcinoma in situ[50] compared with placebo (or ultralow-dose tamoxifen)[41-43,50] and a non-inferior reduction compared with standard dose of tamoxifen.[43] Notably, the breast density reduction was predominantly seen in premenopausal women.[43,50]

Ki67 changes in response to endocrine therapy

Tamoxifen slows the proliferation of breast cancer cells by inhibiting the cell cycle progression from the G1-phase to the S-phase.[80] To express the degree of proliferation in cancer cells, Ki67 staining is often used. Ki67 is a nuclear marker expressed in all phases of the cell cycle other than the G0-phase, is absent in nuclei of resting cells, and is expressed in proliferating cells.[81,82] Ki67 is a well-known prognostic marker in

primary breast cancer.[81,83] More interestingly, changes in Ki67 expression in cancer cells in response to standard endocrine therapy have shown to be strong predictive markers for efficacy of endocrine therapy.[83,84]

Nearly twenty years ago, Dowsett et al. were the first to demonstrate, that only 2 weeks of endocrine therapy (tamoxifen or aromatase inhibitors) before surgery could lead to a decrease in proliferation (expressed as Ki67 decrease) of ER-positive breast cancer cells, and that this phenomenon might be predictive of recurrence-free survival.[85] In the POETIC study, a large, randomized, phase 3 study, it was confirmed that the effect of 2 weeks of preoperative aromatase inhibitors on ER-positive breast cancer cell proliferation was a strong predictor of time-to-recurrence and therefore could be used as a surrogate endpoint for the long-term efficacy of endocrine therapy.[83] These investigators came to the conclusion that there is efficacy of the endocrine therapy if the Ki67 falls below 10% after 2 weeks of treatment. If the value is already below 10% before the start of treatment, no reliable conclusion can be drawn as to whether or not the endocrine therapy is effective. Since then, this surrogate endpoint has been widely used in preoperative endocrine therapy studies (both tamoxifen and aromatase inhibitors) to answer important clinically relevant research questions, of which the ADAPT study is a perfect example.[86,87] In that study, breast cancer patients who had an adequate decrease in Ki67 after a short duration of neoadjuvant endocrine therapy were spared adjuvant chemotherapy.[86] In contrast, in the ongoing POETIC-A trial, breast cancer patients who did not have an adequate response in Ki67 after neoadjuvant endocrine therapy are offered additional adjuvant abemaciclib (Clinicaltrials.gov NCT04584853).[86]

The ability of low doses of tamoxifen to suppress the proliferation of ER-positive breast cancer, as a measure of efficacy, has been investigated in three studies, with somewhat conflicting results.[52,54,55] In the first study, three groups of patients (40 patients per group) with ER-positive breast cancer were randomized to treatment with tamoxifen 1, 5 or 20 mg compared with non-randomized breast cancer patients who were not treated preoperatively. After 4 weeks of tamoxifen treatment, Ki67 decreased similarly in all three treated groups (i.e. no dose-response relation) and the decrease was significantly lower than in the untreated patient group. This suggests that treatment with a lower dose of tamoxifen also shows antitumor activity. Furthermore, no evidence of an association between change in Ki67 expression and concentrations of tamoxifen or 4-hydroxy-tamoxifen in serum could be found.[53] Unfortunately, no endoxifen levels were measured. In a second smaller study, these results were confirmed.[52] Eighteen ER-positive breast cancer patients were treated with tamoxifen 10 mg daily for 2 weeks and showed a significant reduction of Ki67, from a mean expression index of 25% to

a mean expression of 10%, while in the control group who did not receive tamoxifen no significant reduction in Ki67 was seen.[54] In the third study, these results could not be confirmed for ultra-low dose tamoxifen. In that study, premenopausal patients with invasive breast cancer (*n* = 125) were randomized between an ultra-low dose tamoxifen of 10 mg/week or placebo for 6 weeks before surgery.[55] No significant decrease in Ki67 expression was seen after preoperative treatment with ultra-low dose tamoxifen among these premenopausal patients.

DISCUSSION

Our review shows that low-dose tamoxifen demonstrates a clinically relevant, better toxicity profile than standard-dose tamoxifen, and that there is strong indirect evidence that lower doses of tamoxifen also possess antitumor efficacy. This is important because it could allow dose reduction in those patients who experience bothersome adverse effects from tamoxifen at a standard dose of 20 mg daily. However, in the absence of randomized trials in the adjuvant setting, the challenge is to select the right patients with invasive breast cancer for whom dose reduction can potentially be used.

The ultimate goal of reducing the tamoxifen dose in case of severe adverse effects is to increase the adherence to tamoxifen and thus improve the prognosis for breast cancer patients. Unfortunately, there were no studies in patients with breast cancer that have examined whether lowering the adverse effects by reducing the tamoxifen dose also led to an increase in adherence. This has been investigated in prevention studies with tamoxifen in women at high risk of developing breast cancer. Patients preferred low-dose tamoxifen over standard-dose tamoxifen in the preventive setting.[88,89] Furthermore, adherence rates were numerically higher for low-dose tamoxifen (93.3% versus 85%) although this did not meet statistical significance.[88] Adherence between placebo and low-dose tamoxifen was equal in several prevention studies;[38,40,42,51] however, treatment compliance among study populations within prevention studies tends to be lower than in the adjuvant setting, with adherence rates often falling below 50%.[90] This can be partly attributed to adverse effects, but might also be influenced by lower intrinsic motivation of patients to use medication for primary or secondary prevention. Consequently, the findings from such studies may possess limited generalizability to the adjuvant setting.

A first evidence that lower doses of tamoxifen also have an antitumor effect comes from preventive studies that showed that low doses of tamoxifen compared with placebo also prevent the development of breast cancer. This evidence led to including tamoxifen 5 mg daily as an alternative option (compared with tamoxifen 20 mg daily)

for patients with high breast cancer risk, DCIS or other breast carcinoma in situ in ASCO an NCCN guidelines.[34,35] The effect of low-dose tamoxifen in primary prevention was mainly observed in postmenopausal women. The explanation of menopausal status as a possible effect-modifier must probably be sought in the working mechanism of tamoxifen, i.e. competitive inhibition of the ER with estradiol. In the studies that also included premenopausal women, these women did not receive gonadotropin hormone-releasing hormone (GnRH) agonists next to the tamoxifen treatment, and thus estradiol levels were much higher compared with the postmenopausal women. Moreover, in contrast with postmenopausal women, estradiol levels increase with tamoxifen use in premenopausal women.[91] The elevated estradiol levels might compete with the relatively low endoxifen levels for the ER. This could therefore explain the smaller preventive effect of low-dose tamoxifen in premenopausal women. Indeed, in one of the RCTs the effect of low-dose tamoxifen on breast cancer prevention also seemed more pronounced in women with lower than median, compared with higher than median estradiol levels.[46,92]

A second indirect indication that lower doses of tamoxifen have an antitumor effect comes from studies that looked at a decrease in mammographic density, which has been shown to be predictive of reducing the risk of breast cancer recurrence.[78,79] Although low-dose tamoxifen also reduces breast density, this was found to be mainly the case in premenopausal women. Although this seems in contrast with the efficacy of low-dose tamoxifen in primary prevention studies, the absence of an effect in postmenopausal women is probably caused by the much lower mammographic density at baseline found in postmenopausal women compared with premenopausal women.[93] Unfortunately, for now, mammographic density reduction does not seem to be practical to use as an individual test for tamoxifen efficacy because of the long duration of tamoxifen treatment (6-12 months) that is needed to influence the density of the breast. One small study (n = 42) showed significant mammographic density reduction after 3 months of tamoxifen, but more research to confirm this timing is needed.[94] Moreover, no clear limits of adequate or inadequate mammographic density reduction are known.

The results of the functional test used to determine the efficacy of endocrine treatments by measuring Ki67 after low-dose tamoxifen is probably the most compelling evidence for efficacy of low-dose tamoxifen for invasive breast cancer. Two studies showed this convincingly, although a third study was seemingly in contrast with these finding.[55] Seemingly, since three explanations could be given for these findings. First, a tamoxifen dose of 10 mg/week might be too low to be effective. A sub-analysis of patients with a normal CYP2D6 enzymatic function (in contrast to poor or intermediate metabolizers)

further supports this theory.[55] In this analysis, in normal CYP2D6 metabolizers Ki67 did show a significant reduction after tamoxifen 10 mg weekly, likely because patients with a normal CYP2D6 function reach higher endoxifen levels than poor or intermediate metabolizers. Second, the post-treatment breast cancer samples on which the Ki67 was measured were derived from resection material (core cuts). An additional analyses of the POETIC trial showed that in patients who underwent a core biopsy and a resection after a short duration of endocrine therapy preoperatively, the decrease in Ki67 found on core biopsies was not seen on the resection sample.[95] Although further research is needed to clarify these findings, it could have played a role in this study. Finally, as previously mentioned, treating premenopausal patients with low-dose tamoxifen without a GnRH agonist could result in inefficacy of tamoxifen due to the loss of competition with high plasma estradiol levels for the ER.

How can the findings described in this review be applied in the clinical setting for patients with bothersome adverse effects from standard doses of tamoxifen? One approach could be a dose reduction of tamoxifen based on endoxifen levels. For this approach, the precise threshold value for endoxifen, the most active metabolite of tamoxifen, must be known. Previous studies have shown different lower limits ranging between 10 and 16 nM.[22-24] Based on linear kinetics, it can be predicted that halving the tamoxifen dose approximately halves the level of endoxifen. If a conservative lower limit for endoxifen of 16 nM is used, a dose reduction can only be safely achieved in patients with an endoxifen level of 32 nM or higher with the standard tamoxifen dose. This seems to be the case for only 30% of patients using tamoxifen at a standard dose of 20 mg (**Figure 2**).[96] Although the minimal effective concentration of endoxifen is likely much lower, there is still too little evidence to recommend a safe dose reduction based on a much lower threshold of endoxifen. In addition, the efficacy of tamoxifen is not solely dependent on the dose but also on factors such as tamoxifen resistance mechanisms and, importantly, the expression of ER- and PR receptors on the breast tumor cells. Tamoxifen dose reduction based on endoxifen levels alone therefore appears to be an approach that is too limiting, as patient and tumor characteristics are not taken into account enough.

The use of a functional endocrine sensitivity test could be the fitting solution for individualized tamoxifen dosing in the future. The difference in Ki67 percentage before and after short exposure of tamoxifen treatment preoperatively could serve as an endocrine sensitivity test used on an individual base. This approach incorporates all individual patient and tumor characteristics and could be performed preoperatively without postponing breast cancer treatment. After all, after 2-3 weeks of preoperative treatment, often corresponding to the waiting time until surgery, this test already leads

to a result. Since endoxifen only reaches steady-state after 12 weeks, the endoxifen levels reached after 2-3 weeks will be specifically low and therefore useful for tamoxifen dose reduction in case of adverse effects when tamoxifen will be administered in the adjuvant setting. Although promising, there are still some challenges that need to be resolved before this test for tamoxifen sensitivity can be routinely used in clinical practice. These include the Ki67 staining on breast cancer cells causing high intra- and intervariability in inexperienced hands, and the fact that demonstration of the inefficacy of tamoxifen at a certain dose has not yet demonstrated the efficacy of a somewhat higher dose. This is likely the reason why, at the moment, this test is mainly used within innovative trials.

CONCLUSIONS

Our review shows that low-dose tamoxifen has an improved toxicity profile compared with standard-dose tamoxifen. In the primary and secondary chemoprevention setting, low-dose tamoxifen has already proven its clinical efficacy. Although there is growing evidence that a lower dose of tamoxifen may also have antitumor efficacy against ER-positive breast cancers, this cannot yet be translated into a generally accepted lower dose of tamoxifen at which efficacy is guaranteed in the adjuvant setting. Nevertheless, in one-third of patients with unacceptable adverse effects after receiving standard doses of tamoxifen, a dose reduction of tamoxifen can be performed based on endoxifen levels (**Figure 2**). For the remaining patients, further development of the functional test based on the Ki67 changes on ER-positive breast cancer after a short preoperatively treatment with tamoxifen is likely of great value.

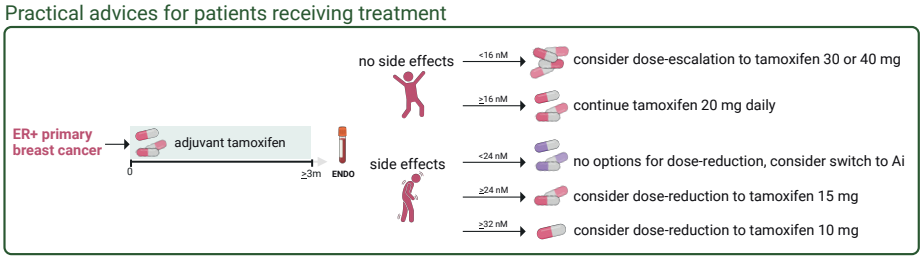

Figure 2. Practical advice for patients receiving treatment
Patients with ER-positive breast cancer who are treated with adjuvant tamoxifen reach steady-state levels of endoxifen after 3 months of treatment. From then on, the endoxifen concentration should be measured at least once. When a patient does not experience (bothersome) adverse effects, the standard dose of tamoxifen 20 mg can be continued if the endoxifen concentration is ≥16 nM. In case a patient experiences bothersome adverse effects, for some patients tamoxifen dose reduction can be considered using the conservative endoxifen threshold of 16 nM. AI aromatase inhibitor, ER+ estrogen receptor-positive. Figure created with Biorender

REFERENCES

1. Early Breast Cancer Trialists' Collaborative Group. Relevance of breast cancer hormone receptors and other factors to the efficacy of adjuvant tamoxifen: patient-level meta-analysis of randomised trials. Lancet 2011; 378(9793): 771-84.
2. Early Breast Cancer Trialists' Collaborative Group. Effects of chemotherapy and hormonal therapy for early breast cancer on recurrence and 15-year survival: an overview of the randomised trials. Lancet 2005; 365(9472): 1687-717.
3. Cardoso F, Kyriakides S, Ohno S, et al. Early breast cancer: ESMO Clinical Practice Guidelines for diagnosis, treatment and follow-up†. Annals of Oncology 2019; 30(8): 1194-220.
4. Burstein HJ, Lacchetti C, Anderson H, et al. Adjuvant Endocrine Therapy for Women With Hormone Receptor-Positive Breast Cancer: ASCO Clinical Practice Guideline Focused Update. Journal of Clinical Oncology 2019; 37(5): 423-38.
5. Gnant M, Fitzal F, Rinnerthaler G, et al. Duration of Adjuvant Aromatase-Inhibitor Therapy in Postmenopausal Breast Cancer. New England Journal of Medicine 2021; 385(5): 395-405.
6. Jordan VC. 50th anniversary of the first clinical trial with ICI 46,474 (tamoxifen): then what happened? Endocrine-Related Cancer 2021; 28(1): R11-R30.
7. Martinkovich S, Shah D, Planey SL, Arnott JA. Selective estrogen receptor modulators: tissue specificity and clinical utility. Clinical Interventions in Aging 2014; 9: 1437-52.
8. Stearns V, Ullmer L, López JF, Smith Y, Isaacs C, Hayes D. Hot flushes. Lancet 2002; 360(9348): 1851-61.
9. Land SR, Wickerham DL, Costantino JP, et al. Patient-Reported Symptoms and Quality of Life During Treatment With Tamoxifen or Raloxifene for Breast Cancer PreventionThe NSABP Study of Tamoxifen and Raloxifene (STAR) P-2 Trial. JAMA 2006; 295(23): 2742-51.
10. Swerdlow AJ, Jones ME, British Tamoxifen Second Cancer Study G. Tamoxifen treatment for breast cancer and risk of endometrial cancer: a case-control study. Journal of the National Cancer Institute 2005; 97(5): 375-84.
11. Cuzick J, Powles T, Veronesi U, et al. Overview of the main outcomes in breast-cancer prevention trials. Lancet 2003; 361(9354): 296-300.
12. Buijs SM, van Dorst DCH, Kruip MJHA, et al. The interplay between tamoxifen and endoxifen plasma concentrations and coagulation parameters in patients with primary breast cancer. Biomedicine & Pharmacotherapy 2024; 170: 115969.
13. Boehm DU, Lebrecht A, Eckhardt T, et al. Quality of life and adjuvant tamoxifen treatment in breast cancer patients. European Journal of Cancer Care 2009; 18(5): 500-6.
14. Owusu C, Buist DSM, Field TS, et al. Predictors of Tamoxifen Discontinuation Among Older Women With Estrogen Receptor–Positive Breast Cancer. Journal of Clinical Oncology 2008; 26(4): 549-55.
15. Lailler G, Memoli V, Le Bihan Benjamin C, et al. Five-Year Adjuvant Endocrine Therapy Adherence Trajectories Among Women With Breast Cancer: A Nationwide French Study Using Administrative Data. Clinical Breast Cancer 2021; 21(4): e415-e26.
16. Murphy CC, Bartholomew LK, Carpentier MY, Bluethmann SM, Vernon SW. Adherence to adjuvant hormonal therapy among breast cancer survivors in clinical practice: a systematic review. Breast Cancer Research and Treatment 2012; 134(2): 459-78.
17. Bosco-Lévy P, Jové J, Robinson P, Moore N, Fourrier-Réglat A, Bezin J. Persistence to 5-year hormonal breast cancer therapy: a French national population-based study. British Journal of Cancer 2016; 115(8): 912-9.
18. Mulder TAM, de With M, Del Re M, Danesi R, Mathijssen RHJ, van Schaik RHN. Clinical CYP2D6 Genotyping to Personalize Adjuvant Tamoxifen Treatment in ER-Positive Breast Cancer Patients: Current Status of a Controversy. Cancers (Basel) 2021; 13(4).
19. Stearns V, Johnson MD, Rae JM, et al. Active tamoxifen metabolite plasma concentrations after coadministration of tamoxifen and the selective serotonin reuptake inhibitor paroxetine. Journal of the National Cancer Institute 2003; 95(23): 1758-64.
20. Coezy E, Borgna JL, Rochefort H. Tamoxifen and metabolites in MCF7 cells: correlation between binding to estrogen receptor and inhibition of cell growth. Cancer Research 1982; 42(1): 317-23.
21. Mürdter TE, Schroth W, Bacchus-Gerybadze L, et al. Activity levels of tamoxifen metabolites at the estrogen receptor and the impact of genetic polymorphisms of phase I and II enzymes on their concentration levels in plasma. Clinical Pharmacology & Therapeutics 2011; 89(5): 708-17.
22. Helland T, Henne N, Bifulco E, et al. Serum concentrations of active tamoxifen metabolites predict long-term survival in adjuvantly treated breast cancer patients. Breast Cancer Research 2017; 19(1): 125.

23. Madlensky L, Natarajan L, Tchu S, et al. Tamoxifen Metabolite Concentrations, CYP2D6 Genotype, and Breast Cancer Outcomes. Clinical Pharmacology & Therapeutics 2011; 89(5): 718-25.
24. Saladores P, Mürdter T, Eccles D, et al. Tamoxifen metabolism predicts drug concentrations and outcome in premenopausal patients with early breast cancer. Pharmacogenomics Journal 2015; 15(1): 84-94.
25. Groenland SL, van Eerden RAG, Westerdijk K, et al. Therapeutic drug monitoring based precision dosing of oral targeted therapies in oncology: a prospective multicentre study. Annals of Oncology 2022 Oct; 33(10): 1071-82.
26. Groenland SL, Verheijen RB, Joerger M, et al. Precision Dosing of Targeted Therapies Is Ready for Prime Time. Clinical Cancer Research 2021; 27(24): 6644-52.
27. Neven P, Jongen L, Lintermans A, et al. Tamoxifen Metabolism and Efficacy in Breast Cancer: A Prospective Multicenter Trial. Clinical Cancer Research 2018; 24(10): 2312-8.
28. Sanchez-Spitman A, Dezentjé V, Swen J, et al. Tamoxifen Pharmacogenetics and Metabolism: Results From the Prospective CYPTAM Study. Journal of Clinical Oncology 2019; 37(8): 636-46.
29. Braal CL, Beijnen JH, Koolen SLW, et al. Relevance of Endoxifen Concentrations: Absence of Evidence Is Not Evidence of Absence. Journal of Clinical Oncology 2019; 37(22): 1980-1.
30. Jager NG, Koornstra RH, Vincent AD, et al. Hot flashes are not predictive for serum concentrations of tamoxifen and its metabolites. BMC Cancer 2013; 13: 612.
31. Peyrade F, Frenay M, Etienne MC, et al. Age-related difference in tamoxifen disposition. Clinical Pharmacology & Therapeutics 1996; 59(4): 401-10.
32. Gallicchio L, Lord G, Tkaczuk K, et al. Association of Tamoxifen (TAM) and TAM Metabolite Concentrations with Self-Reported Side Effects of TAM in Women with Breast Cancer. Breast Cancer Research and Treatment 2004; 85(1): 89-97.
33. Lorizio W, Wu AHB, Beattie MS, et al. Clinical and biomarker predictors of side effects from tamoxifen. Breast Cancer Research and Treatment 2012; 132(3): 1107-18.
34. Gradishar WJ, Anderson BO, Balassanian R, et al. Breast Cancer, Version 4.2017, NCCN Clinical Practice Guidelines in Oncology. Journal of the National Comprehensive Cancer Network 2018; 16(3): 310-20.
35. Visvanathan K, Fabian CJ, Bantug E, et al. Use of endocrine therapy for breast cancer risk reduction: ASCO clinical practice guideline update. Journal of Clinical Oncology 2019; 37(33): 3152-65.
36. Allred DC, Anderson SJ, Paik S, et al. Adjuvant Tamoxifen Reduces Subsequent Breast Cancer in Women With Estrogen Receptor–Positive Ductal Carcinoma in Situ: A Study Based on NSABP Protocol B-24. Journal of Clinical Oncology 2012; 30(12): 1268-73.
37. Landis-Piwowar KR, Iyer NR. Cancer chemoprevention: current state of the art. Cancer Growth Metastasis 2014; 7: 19-25.
38. DeCensi A, Puntoni M, Guerrieri-Gonzaga A, et al. Randomized placebo controlled trial of low-dose tamoxifen to prevent local and contralateral recurrence in breast intraepithelial neoplasia. Journal of Clinical Oncology 2019; 37(19): 1629-37.
39. de Vries Schultink AHM, Dolro TPC, Madlensky L, Pierce JP, Beijnen JH, Huitema ADR. Prospective evaluation of Therapeutic Drug Monitoring of endoxifen: feasibility of observational and randomized trials. Available online at: wwwpage-meetingorg/?abstract=9150 2019.
40. DeCensi A, Bonanni B, Maisonneuve P, et al. A phase-III prevention trial of low-dose tamoxifen in postmenopausal hormone replacement therapy users: The HOT study. Annals of Oncology 2013; 24(11): 2753-60.
41. Decensi A, Gandini S, Serrano D, et al. Randomized dose-ranging trial of tamoxifen at low doses in hormone replacement therapy users. Journal of Clinical Oncology 2007; 25(27): 4201-9.
42. Bhatia S, Palomares MR, Hageman L, et al. A Randomized Phase IIb Study of Low-dose Tamoxifen in Chest-irradiated Cancer Survivors at Risk for Breast Cancer. Clinical Cancer Research 2021; 27(4): 967-75.
43. Eriksson M, Eklund M, Borgquist S, et al. Low-dose tamoxifen for mammographic density reduction: A randomized controlled trial. Journal of Clinical Oncology 2021; 39(17): 1899-908.
44. Hammarström M, Gabrielson M, Crippa A, et al. Side effects of low-dose tamoxifen: results from a six-armed randomised controlled trial in healthy women. British Journal of Cancer 2023; 129(1): 61-71.
45. Buttiron Webber T, Marra D, Puntoni M, et al. Patient- versus physician-reported outcomes in a low-dose tamoxifen trial in noninvasive breast cancer. The Breast Journal 2021; 27(11): 817-23.

46. Lazzeroni M, Puntoni M, Guerrieri-Gonzaga A, et al. Randomized Placebo Controlled Trial of Low-Dose Tamoxifen to Prevent Recurrence in Breast Noninvasive Neoplasia: A 10-Year Follow-Up of TAM-01 Study. Journal of Clinical Oncology 2023; 41(17): 3116-21.

47. Guerrieri-Gonzaga A, Lazzeroni M, Botteri E, et al. Effect of low-dose tamoxifen after surgical excision of ductal intraepithelial neoplasia: Results of a large retrospective monoinstitutional cohort study. Annals of Oncology 2013; 24(7): 1859-66.

48. Guerrieri-Gonzaga A, Botteri E, Rotmensz N, et al. Ductal intraepithelial neoplasia: Postsurgical outcome for 1,267 women cared for in one single institution over 10 years. Oncologist 2009; 14(3): 201-12.

49. Guerrieri-Gonzaga A, Sestak I, Lazzeroni M, et al. Benefit of low-dose tamoxifen in a large observational cohort of high risk ER positive breast DCIS. International Journal of Cancer 2016; 139(9): 2127-34.

50. Decensi A, Robertson C, Guerrieri-Gonzaga A, et al. Randomized double-blind 2 X 2 trial of low-dose tamoxifen and fenretinide for breast cancer prevention in high-risk premenopausal women. Journal of Clinical Oncology 2009; 27(23): 3749-56.

51. Serrano D, Gandini S, Guerrieri-Gonzaga A, et al. Quality of life in a randomized breast cancer prevention trial of low-dose tamoxifen and fenretinide in premenopausal women. Cancer Prevention Research 2018; 11(12): 811-8.

52. Decensi A, Robertson C, Viale G, et al. A randomized trial of low-dose tamoxifen on breast cancer proliferation and blood estrogenic biomarkers. Journal of the National Cancer Institute 2003; 95(11): 779-90.

53. Kisanga ER, Gjerde J, Guerrieri-Gonzaga A, et al. Tamoxifen and Metabolite Concentrations in Serum and Breast Cancer Tissue during Three Dose Regimens in a Randomized Preoperative Trial. Clinical Cancer Research 2004; 10(7): 2336-43.

54. de Sousa JA, Facina G, da Silva BB, Gebrim LH. Effects of low-dose tamoxifen on breast cancer biomarkers Ki-67, estrogen and progesterone receptors. International seminars in surgical oncology 2006; 3.

55. Serrano D, Lazzeroni M, Gandini S, et al. A randomized phase II presurgical trial of weekly low-dose tamoxifen versus raloxifene versus placebo in premenopausal women with estrogen receptor-positive breast cancer. Breast Cancer Research 2013; 15(3).

56. Lee CI, Fox P, Balakrishnar B, et al. Tamoxifen-induced severe hot flashes and endoxifen levels: is dose reduction a safe and effective strategy? Breast 2019; 46: 52-7.

57. Buijs SM, Hoop EO, Braal CL, et al. The impact of endoxifen-guided tamoxifen dose reductions on endocrine side-effects in patients with primary breast cancer. ESMO Open 2023; 8(1): 100786.

58. Chlebowski RT, Hendrix SL, Langer RD, et al. Influence of Estrogen Plus Progestin on Breast Cancer and Mammography in Healthy Postmenopausal WomenThe Women's Health Initiative Randomized Trial. JAMA 2003; 289(24): 3243-53.

59. Fallowfield LJ, Leaity SK, Howell A, Benson S, Cella D. Assessment of quality of life in women undergoing hormonal therapy for breast cancer: validation of an endocrine symptom subscale for the FACT-B. Breast Cancer Research and Treatment 1999; 55(2): 189-99.

60. Fisher B, Costantino JP, Redmond CK, Fisher ER, Wickerham DL, Cronin WM. Endometrial cancer in tamoxifen-treated breast cancer patients: findings from the National Surgical Adjuvant Breast and Bowel Project (NSABP) B-14. Journal of the National Cancer Institute 1994; 86(7): 527-37.

61. Davies C, Pan H, Godwin J, et al. Long-term effects of continuing adjuvant tamoxifen to 10 years versus stopping at 5 years after diagnosis of oestrogen receptor-positive breast cancer: ATLAS, a randomised trial. Lancet 2013; 381(9869): 805-16.

62. Bernstein L, Deapen D, Cerhan JR, et al. Tamoxifen therapy for breast cancer and endometrial cancer risk. Journal of the National Cancer Institute 1999; 91(19): 1654-62.

63. Runowicz CD, Costantino JP, Wickerham DL, et al. Gynecologic conditions in participants in the NSABP breast cancer prevention study of tamoxifen and raloxifene (STAR). American Journal of Obstetrics & Gynaecology 2011; 205(6): 535 e1-5.

64. Cohen I, Bernheim J, Azaria R, Tepper R, Sharony R, Beyth Y. Malignant endometrial polyps in postmenopausal breast cancer tamoxifen-treated patients. Gynecologic Oncology 1999; 75(1): 136-41.

65. Fisher B, Costantino JP, Wickerham DL, et al. Tamoxifen for prevention of breast cancer: report of the National Surgical Adjuvant Breast and Bowel Project P-1 Study. Journal of the National Cancer Institute 1998; 90(18): 1371-88.

66. Cuzick J, Forbes JF, Sestak I, et al. Long-term results of tamoxifen prophylaxis for breast cancer--96-month follow-up of the randomized IBIS-I trial. Journal of the National Cancer Institute 2007; 99(4): 272-82.

67. Saphner T, Tormey DC, Gray R. Venous and arterial thrombosis in patients who received adjuvant therapy for breast cancer. Journal of Clinical Oncology 1991; 9(2): 286-94.

68. Deitcher SR, Gomes MP. The risk of venous thromboembolic disease associated with adjuvant hormone therapy for breast carcinoma: a systematic review. Cancer 2004; 101(3): 439-49.

69. Amir E, Seruga B, Niraula S, Carlsson L, Ocaña A. Toxicity of adjuvant endocrine therapy in postmenopausal breast cancer patients: a systematic review and meta-analysis. Journal of the National Cancer Institute 2011; 103(17): 1299-309.

70. Hernandez RK, Sørensen HT, Pedersen L, Jacobsen J, Lash TL. Tamoxifen treatment and risk of deep venous thrombosis and pulmonary embolism: a Danish population-based cohort study. Cancer 2009; 115(19): 4442-9.

71. Eisenberger A, Westhoff C. Hormone replacement therapy and venous thromboembolism. The Journal of Steroid Biochemistry and Molecular Biology 2014; 142: 76-82.

72. Nazari SS, Mukherjee P. An overview of mammographic density and its association with breast cancer. Breast Cancer 2018; 25(3): 259-67.

73. Boyd NF, Byng JW, Jong RA, et al. Quantitative classification of mammographic densities and breast cancer risk: results from the Canadian National Breast Screening Study. Journal of the National Cancer Institute 1995; 87(9): 670-5.

74. McCormack VA, dos Santos Silva I. Breast density and parenchymal patterns as markers of breast cancer risk: a meta-analysis. Cancer Epidemiology, Biomarkers & Prevention 2006; 15(6): 1159-69.

75. Boyd NF, Guo H, Martin LJ, et al. Mammographic density and the risk and detection of breast cancer. New England Journal of Medicine 2007; 356(3): 227-36.

76. Martin LJ, Boyd NF. Mammographic density. Potential mechanisms of breast cancer risk associated with mammographic density: hypotheses based on epidemiological evidence. Breast Cancer Research 2008; 10(1): 201.

77. Cuzick J, Warwick J, Pinney E, Warren RM, Duffy SW. Tamoxifen and breast density in women at increased risk of breast cancer. Journal of the National Cancer Institute 2004; 96(8): 621-8.

78. Cuzick J, Warwick J, Pinney E, et al. Tamoxifen-induced reduction in mammographic density and breast cancer risk reduction: a nested case-control study. Journal of the National Cancer Institute 2011; 103(9): 744-52.

79. Li J, Humphreys K, Eriksson L, Edgren G, Czene K, Hall P. Mammographic density reduction is a prognostic marker of response to adjuvant tamoxifen therapy in postmenopausal patients with breast cancer. Journal of Clinical Oncology 2013; 31(18): 2249-56.

80. Osborne CK. Tamoxifen in the treatment of breast cancer. New England Journal of Medicine 1998; 339(22): 1609-18.

81. Nielsen TO, Leung SCY, Rimm DL, et al. Assessment of Ki67 in Breast Cancer: Updated Recommendations from the International Ki67 in Breast Cancer Working Group. Journal of the National Cancer Institute 2020; 113(7): 808-19.

82. Uxa S, Castillo-Binder P, Kohler R, Stangner K, Müller GA, Engeland K. Ki-67 gene expression. Cell Death & Differentiation 2021; 28(12): 3357-70.

83. Smith I, Robertson J, Kilburn L, et al. Long-term outcome and prognostic value of Ki67 after perioperative endocrine therapy in postmenopausal women with hormone-sensitive early breast cancer (POETIC): an open-label, multicentre, parallel-group, randomised, phase 3 trial. The Lancet Oncology 2020; 21(11): 1443-54.

84. Dowsett M, Ebbs SR, Dixon JM, et al. Biomarker changes during neoadjuvant anastrozole, tamoxifen, or the combination: influence of hormonal status and HER-2 in breast cancer--a study from the IMPACT trialists. Journal of Clinical Oncology 2005; 23(11): 2477-92.

85. Dowsett M, Smith IE, Ebbs SR, et al. Short-term changes in Ki-67 during neoadjuvant treatment of primary breast cancer with anastrozole or tamoxifen alone or combined correlate with recurrence-free survival. Clinical Cancer Research 2005; 11(2 Pt 2): 951s-8s.

86. Nitz U, Gluz O, Kreipe HH, et al. The run-in phase of the prospective WSG-ADAPT HR+/HER2- trial demonstrates the feasibility of a study design combining static and dynamic biomarker assessments for individualized therapy in early breast cancer. Therapeutic Advances in Medical Oncology 2020; 12: 1758835920973130.

87. Harbeck N, Nitz UA, Christgen M, et al. De-Escalated Neoadjuvant Trastuzumab-Emtansine With or Without Endocrine Therapy Versus Trastuzumab With Endocrine Therapy in HR+/HER2+ Early Breast Cancer: 5-Year Survival in the WSG-ADAPT-TP Trial. Journal of Clinical Oncology 2023; 41(22): 3796-804.

88. Bychkovsky B, Laws A, Katlin F, et al. Initiation and tolerance of chemoprevention among women with high-risk breast lesions: the potential of low-dose tamoxifen. Breast Cancer Research and Treatment 2022; 193(2): 417-27.

89. Patel R, Jin C, Tiersten A. Low-dose tamoxifen for breast cancer prevention in patients with ductal carcinoma in situ (DCIS) and atypical lesions: A real-world experience. Journal of Clinical Oncology 2022; 40(16).

90. Zhao H, Hei N, Wu Y, et al. Initiation of and adherence to tamoxifen and aromatase inhibitor therapy among elderly women with ductal carcinoma in situ. Cancer 2017; 123(6): 940-7.

91. Ravdin PM, Fritz NF, Tormey DC, Jordan VC. Endocrine status of premenopausal node-positive breast cancer patients following adjuvant chemotherapy and long-term tamoxifen. Cancer Research 1988; 48(4): 1026-9.

92. De Censi A, Puntoni M, Guerrieri-Gonzaga A, et al. Effect modifiers in a randomized phase III trial of low-dose tamoxifen in breast preinvasive disease. Journal of Clinical Oncology 2019; 37.

93. Burton A, Maskarinec G, Perez-Gomez B, et al. Mammographic density and ageing: A collaborative pooled analysis of cross-sectional data from 22 countries worldwide. PLoS Med 2017; 14(6): e1002335.

94. Bäcklund M, Eriksson M, Hammarström M, et al. Time to Mammographic Density Decrease After Exposure to Tamoxifen. Oncologist 2022; 27(7): e601-e3.

95. Bliss JM. Polychemotherapy for early breast cancer: Results from the international adjuvant breast cancer chemotherapy randomized trial. Journal of the National Cancer Institute 2007; 99(7): 506-15.

96. Braal CL, Jager A, Hoop EO, et al. Therapeutic Drug Monitoring of Endoxifen for Tamoxifen Precision Dosing: Feasible in Patients with Hormone-Sensitive Breast Cancer. Clinical Pharmacokinetics 2022; 61(4): 527-37.

CHAPTER 3

The impact of endoxifen-guided tamoxifen dose reductions on endocrine side-effects in patients with primary breast cancer

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ABSTRACT

Background

Tamoxifen is important in the adjuvant treatment of hormone-sensitive breast cancer and substantially reduces recurrence; however, almost 50% of patients are non-compliant mainly due to side effects. The aim of this study was to investigate whether endoxifen-guided tamoxifen dose reduction could lead to fewer side-effects.

Methods

Effects of tamoxifen dose reduction were investigated in patients with bothersome side-effects and endoxifen levels ≥32 nM and compared to patients with side-effects who remained on tamoxifen 20 mg. Endocrine symptoms and health-related quality of life (HR-QOL) were assessed after 3 months with the Functional Assessment of Cancer Therapy – Endocrine Symptoms (FACT-ES) questionnaire.

Results

Tamoxifen dose was reduced in 20 patients, 17 of whom were evaluable for side-effect analyses. A clinically relevant improvement of >6 points was observed in endocrine symptoms and HR-QOL in 41% and 65% of the patients, respectively. In total, there was a significant and clinically relevant improvement in endocrine symptoms (5.7, 95% confidence interval (CI) -0.5–11.5) and HR-QOL (8.2, 95% CI 0.9-15.4) after dose reduction. This was not seen in patients whose doses were not reduced (n=60). In 21% of patients, endoxifen dropped slightly below the 16 nM threshold (12.8, 15.5, 15.8, 15.9 nM).

Conclusions

Endoxifen-guided dose reduction of tamoxifen significantly improved tamoxifen-related side-effects and HR-QOL. Nearly 80% of patients remained above the most conservative endoxifen threshold.

INTRODUCTION

Tamoxifen is currently recommended in the adjuvant treatment of early estrogen receptor-positive breast cancer for 5-10 years for premenopausal women and for 2-3 years for postmenopausal women.[1] Unfortunately, during this long treatment period many patients experience tamoxifen-related side-effects such as hot flashes, arthralgia, vaginal dryness, mood alterations and insomnia.[2] Therefore, almost twenty percent of all patients stop tamoxifen treatment already in the first year of therapy and another annual 5%-10% of patients are non-compliant for the remainder of the treatment period.[3,4] For patients remaining on tamoxifen therapy, a substantial proportion of them have side-effects impacting their quality of life.[5] Despite the high incidence of this problem, there is currently a lack of successful interventions in case patients experience tamoxifen-related side-effects.

A possible solution could be to carry out dose reductions in patients who experience many side-effects. From earlier research - where tamoxifen was prescribed to patients with high mammographic density - we already know that the incidence of tamoxifen-related side effects was ≈50% lower with tamoxifen 1-5 mg daily compared to tamoxifen 20 mg daily.[6] In another study with patients with ductal carcinoma *in situ*, tamoxifen 5 mg daily led to side-effects which were equal to placebo.[7] Also, a study with tamoxifen in the preventive setting showed that the tamoxifen discontinuation rate of tamoxifen 5 mg daily was almost two-thirds lower than when tamoxifen 20 mg was prescribed.[8] Thus, in the non-invasive setting, lower dosing of tamoxifen seems effective in reducing side-effects. However, surprisingly, in primary breast cancer patients on adjuvant tamoxifen, the effect of dose reduction on side-effects has hardly been studied.

Tamoxifen is a prodrug and is metabolized in, mainly, endoxifen, the metabolite that contributes for the most part to the antiestrogenic effect of tamoxifen.[9,10] Endoxifen can be measured easily in plasma.[11] There are several retrospective studies suggesting an exposure-response relationship for endoxifen.[12-14] An analysis including 1370 patients receiving adjuvant tamoxifen found that patients in the lowest endoxifen exposure quintile (up to 16 nM) had a 26% higher risk of recurrence than patients in the other four endoxifen exposure quintiles.[12] When exploring dichotomized cut-off points for a lower risk of recurrence, again an endoxifen threshold of 16 nM was found.[12] Two other small studies found a higher risk of recurrence in patients with endoxifen levels below 14 nM and below 9 nM, respectively.[13,14] The threshold of 16 nM (i.e. 5.97 ng/mL) is most generally accepted in the field of precision dosing of tamoxifen, since it has been found in the largest study thus far and is the most conservative threshold, at which the likelihood of patients being under dosed is assumed negligible.[12,15,16] This endoxifen threshold could be used to carry out a responsible tamoxifen dose reduction

in patients who experience bothersome tamoxifen-related side-effects. Only one study investigated halving the tamoxifen dose in the adjuvant setting before, but this study only focussed on severe hot flashes and was not guided by endoxifen levels.[17]

Therefore, the aim of the present study was to investigate whether endoxifen-guided dose reduction of tamoxifen in patients with bothersome tamoxifen-related side-effects could lead to fewer side-effects and better quality of life while retaining adequate endoxifen levels.

METHODS

The TOTAM (Therapeutic drug monitoring Of TAMoxifen) trial is a large intervention study coordinated by the Erasmus MC Cancer Institute in Rotterdam, the Netherlands. This study was approved by the local Medical Ethics Committee in January 2018 and registered in the International Clinical Trial Registry Platform (ICTRP; https://trialsearch.who.int/; NL6918). Informed consent was obtained from all participants. The main goal of the TOTAM study was to investigate the feasibility of therapeutic drug monitoring of tamoxifen. A secondary endpoint of this trail was to investigate the effect of reducing the tamoxifen dose in patients with bothersome side-effects and a steady-state endoxifen plasma level of 32 nM or higher (i.e. two times the threshold of 16 nM).

Patients and study design

Female patients who were using tamoxifen in the standard daily dose of 20 mg for 3 months were included in the TOTAM study. The design of the study and the main results have been described in detail elsewhere.[18,19] For the current research question, all patients had to fill in the Functional Assessment of Cancer Therapy – Endocrine Symptoms (FACT-ES) questionnaire at baseline (= 3 months of tamoxifen) and after 6 weeks (= 4.5 months of tamoxifen) and 3 months (= 6 months of tamoxifen). During the study, a dose reduction of tamoxifen from 20 mg to 10 mg was proposed to patients who experienced bothersome subjective side-effects which impacted their quality of life or wherefore they were considering discontinuation of tamoxifen. Simultaneously, they had to have an endoxifen level $\geq$32 nM. Also, the endocrine symptoms (ES19) score of the FACT-ES questionnaire had to be $\leq$72 points (maximum: 76 points) in order to be able to measure a clinically relevant difference in ES19 (i.e. 4 points) after dose reduction. Patients were seen at 3 months, 4.5 months and 6 months of tamoxifen use or, when this time points did not coincide, at 6 weeks and 3 months after tamoxifen dose reduction.

Pharmacokinetic analysis

Tamoxifen and endoxifen trough levels (C_{min} concentrations) were obtained during every study visit. Plasma levels were measured using a validated ultra-performance liquid chromatography with a tandem mass spectrometry method (UP-LCMS/MS).[11]

Quality of life and side-effect analysis

Also, during every study visit, the toxicity of tamoxifen was assessed using the US National Cancer Institute's Common Terminology Criteria for Adverse Events version 5 (CTCAEv5) and quality of life and tamoxifen-related side-effects were evaluated using the FACT-ES questionnaire. The FACT-ES is a validated and reliable questionnaire of in total 46 questions and is a measure of health-related quality of life (HR-QOL, 27 items) using physical, social, emotional and functional well-being questions and a measure of side-effects of endocrine treatments given in breast cancer patients (ES19, endocrine subscale of in total 19 items). Four other endocrine-related items (sleep, fatigue, nervousness and nausea) are already included in the HR-QOL items. The result of these four items in addition to the 19 items of the endocrine subscale can also be scored as an additional and more extended endocrine subscale (ES23, 23 items).[20] The different endocrine subscale items can be found in **the supplementary**. Higher scores of the FACT-ES equate with good quality of life and/or experiencing few side-effects, while lower scores indicate poorer quality of life and/or experiencing many/severe side-effects. As an additional insight into the effect of dose reduction, patients were asked to score the beneficial effect of their dose reduction on a 10-point Likert scale (range 1-10, 1: no improvement, 10: excellent improvement) 3 months after the tamoxifen dose was reduced.

Statistical analysis

The primary endpoint of this study was the individual difference in total ES19 score as part of the FACT-ES questionnaire in patients whose tamoxifen dose was reduced before and after 3 months of tamoxifen dose reduction. Change scores of >0.5 of the baseline standard deviation (SD) are considered clinically relevant changes and seen as more than a moderate effect size.[21] Before the start of the study, it was estimated that the SD would be 7-8 points and therefore a change score of minimally 4 points, also used in an earlier endocrine subscale validation study, was expected to be clinically relevant.[20] We hypothesized that the ES19 score would improve with at least 4 points in >50% of dose-reduced patients. To test this hypothesis against a null hypothesis of 20% with a one-sided α of 0.05 and a power of 80%, the tamoxifen dose had to be reduced in at least 13 patients.

Table 1. Patient characteristics

	Dose-reduction cohort (*n* = 17)	**Patients with side-effects who remained on tamoxifen 20 mg (*n* = 60)**
Age (years) median [IQR]	59 (49 – 64)	59 (49 – 66)
BMI (kg/m²) median [IQR]	25.8 (21.8 – 30)	26.2 (23.2 – 30.5)
Tumour stage, *n* (%)		
T1	10 (59%)	27 (45%)
T2	7 (41%)	23 (38.3%)
T3	-	8 (13.3%)
T4	-	1 (1.7%)
Tx	-	1 (1.7%)
Nodal stage, *n* (%)		
N0	11 (65%)	33 (55%)
N1	5 (29%)	19 (31.7%)
N2	1 (6%)	6 (10%)
N3	-	2 (3.3%)
Histologic classification, *n* (%)		
Ductal (NST)	15 (88%)	44 (73.3%)
Lobular	2 (12%)	13 (21.7%)
Other	-	3 (5%)
Histologic grade (Bloom Richardson), *n* (%)		
BR I	3 (18%)	5 (8.3%)
BR II	11 (65%)	44 (73.3%)
BR III	3 (18%)	11 (18.3%)
Progesterone receptor, *n* (%)		
Positive	13 (80%)	52 (86.7%)
Negative	4 (20%)	8 (13.3%)
Her2Neu receptor, *n* (%)		
Positive	1 (6%)	3 (5%)
Negative	16 (94%)	57 (95%)
Local treatment, *n* (%)		
Lumpectomy alone	1 (6%)	1 (1.7%)
Lumpectomy + RTx	14 (82%)	36 (60%)
Mastectomy	1 (6%)	11 (18.2%)
Mastectomy + RTx	1 (6%)	12 (20%)
(Neo)adjuvant chemotherapy, *n* (%)		
Yes	8 (47%)	30 (50%)
No	9 (53%)	30 (50%)
CYP2D6 phenotype, *n* (%)		
PM	-	-
IM	5 (29%)	18 (30%)
NM	10 (59%)	42 (70%)
UM	2 (12%)	-
Use of ovarian function suppression (OFS), *n* (%)		
Yes	2 (11.8%)	5 (8.3%)
No	15 (88.2%)	55 (91.7%)
Days of tam before dose reduction, median [IQR]	113 [101 – 153.5]	-

BMI, body mass index; IQR, interquartile range

The primary endpoint was analysed by means of the binomial probability test. The percentage of patients with a reduction in experienced side-effects of >0.5 of baseline SD will be given together with the binomial exact 90% confidence interval (CI).

As secondary endpoints, the individual differences in ES23 and HR-QOL measured with the FACT-ES before and after dose reduction were determined and compared with >0.5 of baseline SD that was found in this study. Also, the within-group difference before and 3 months after dose reduction was determined with a paired sample *t*-test or a Wilcoxon signed rank test when a sample was not normally distributed. To check for a potential effect of time on tamoxifen-related side-effects, analyses were repeated in the group of patients with side-effects who remained on the regular tamoxifen 20 mg dose between 3 and 6 months of treatment. Differences in specific side-effects (separate items of the extensive endocrine subscale) were analysed descriptively. In an exploratory way, we searched for a correlation between change in FACT-ES scores before and after dose reduction and endoxifen levels at 3 months of tamoxifen 10 mg use.

RESULTS

Patient selection

In total, 151 patients were included in this secondary aim of the TOTAM trial. Two patients were excluded due to not yet reaching a steady-state endoxifen level or accidentally using a lower tamoxifen dose. Of the 149 assessable patients, 125 (84%) patients experienced any tamoxifen-related side-effect and scored ≤72 points on the endocrine subscale. Of these 125 patients, 37 patients (30%) had an endoxifen level ≥32 nM and 18 of these patients experienced their side-effects as bothersome and thus were eligible for tamoxifen dose reduction. Later on in the study, two additional patients who at 3 months of therapy had endoxifen levels just below 32 nM but at the time of dose reduction had endoxifen levels ≥32 nM and bothersome side-effects underwent a reduction in tamoxifen dose. Eventually, in 20 out of 149 (13%) patients in this study, tamoxifen dose was reduced and of these patients, 17 were assessable for the primary endpoint. This number is slightly higher than the minimum number of patients required for this study (n=13). This difference occurred because eligible patients were offered dose-reduction until at least 13 evaluable patients used tamoxifen 10 mg tamoxifen for at least 3 months. Interestingly, also four patients who at baseline refused a dose reduction asked for dose reduction themselves after a longer duration of tamoxifen treatment. A flowchart visualizing the selection process can be found in **the supplementary.** All patients with side-effects, an endocrine subscale score ≤72 and endoxifen levels ≥16 nM who remained on tamoxifen 20 mg from baseline until 6 months of tamoxifen (n= 60) were used as a control group, which also can be found in **the supplementary.**

Patient characteristics

The baseline characteristics of the 17 assessable patients after dose reduction can be found in **Table 1**. For comparison, the baseline characteristics of the 60 patients with side-effects who remained on tamoxifen 20 mg from baseline until 6 months of tamoxifen use are also shown. Patients in both the 20-mg and 10-mg groups had a median age of 59 years. The incidence of (neo)adjuvant chemotherapy treatment was similar between both groups. The use of ovarian function suppression (OFS) was very low but slightly higher in the dose-reduction group. After a median of 113 days of tamoxifen use, patients underwent a reduction in their tamoxifen dose.

FACT-ES scores before and after 3 months of dose reduction

FACT-ES scores were assessed after a median of 91 days (interquartile range (IQR) 85-95) after dose reduction. The baseline SD of the ES19 was higher than expected and therefore an improvement of >0.5 SD (i.e. clinical relevant) was equal to 6 points. Out of the 17 assessable patients in whom the tamoxifen dose was reduced, 7 patients (41%, 90% CI 21 to 65%, P = 0.038) had an improvement in ES19 score of at least 6 points. The ES23 score had to improve with ≥7 points to be clinically relevant, which was achieved in 5 out of 17 patients (29%, 90% CI 12 to 52%). The HR-QOL improved with 6 points or more (>0.5 SD) in 11 out of 17 patients (65%, 90% CI 42 to 83%). Change scores of all individual patients in ES19, ES23 and HR-QOL can be found in **Table 2**. There was a significant and clinically relevant within-group improvement in HR-QOL after dose reduction compared with scores before dose reduction (**Table 3**). There was also a clinically relevant improvement in ES19 and ES23 and this improvement had a trend towards statistical significance (P = 0.053).

FACT-ES scores at 3 and 6 months of tamoxifen 20 mg use

To check for a potential effect of time on side-effects, the FACT-ES scores after 3 months of tamoxifen were compared with the FACT-ES scores after 6 months of tamoxifen in patients who had side-effects and remained on the standard dose of tamoxifen 20 mg. There was no statistically or clinically relevant within-group difference in FACT-ES scores at 6 months of tamoxifen compared to 3 months of tamoxifen. Cis and P values can be found in Table 3. Also, when making a sub-selection of patients with endoxifen levels ≥32 nM who remained on tamoxifen 20 mg (n = 16), no difference in FACT-ES scores between 3 and 6 months of tamoxifen was found (data not shown). In 11 out of 60 (18%, 90 CI 11 to 29%) patients, the HR-QOL improved with at least 6 points. In 13 out of 60 patients (22%, 90% CI 13 to 32%) the ES19 improved with at least 5 points and in 14 out of 60 patients (23%, 90% CI 15 to 34%) the ES23 improved with 6 points or more (i.e. clinical relevant improvements).

Table 2. Individual change scores in FACT-ES

	Change in scores after 3 months of dose reduction		
Subject	**HR-QOL**	**Endocrine subscale 19**	**Endocrine subscale 23**
1	18	23	26
2	2	-1	-3
3	11	3	2
4	-6	2	5
5	19	9	6
6	6	1	1
7	16	21	24
8	-5	-3	-2
9	45	14	23
10	18	10	11
11	-4	1	0
12	5	7	6
13	-21	-12	-13
14	8	-2	1
15	9	-9	-7
16	12	30	36
17	6	3	4
0.5 SD	**5.65**	**5.35**	**6.05**

Change in scores of FACT-ES questionnaire after 3 months of tamoxifen dose reduction compared to FACT-ES scores at steady-state tamoxifen 20 mg. Negative change scores stand for worsening of symptoms, while positive change scores stand for improvement of symptoms. Change scores of >0.5 baseline SD are considered clinically relevant.
FACT-ES, Functional Assessment of Cancer Therapy – Endocrine Symptoms; HR-QOL; health-related quality of life; SD, standard deviation

Side-effects before and after dose reduction

Adverse events before and after dose reduction are shown in **Table 4**. The majority of side-effects decreased after tamoxifen dose reduction. However, the incidence of muscle cramp and weight gain stayed the same and fatigue, anxiety, increased appetite and vaginal discharge all occurred in one additional patient despite dose reduction. Two patients had a CTCAE 2 and a CTCAE 3 thromboembolic event after tamoxifen was started but before tamoxifen dose was reduced and this was also partly the reason to decrease their tamoxifen dose.

Patients were asked to score the improvement of side-effects 3 months after dose reduction on a 10-point Likert scale. Twelve patients (71%) graded their improvement as sufficient whereof seven patients scored a 6-7 and five patients scored an 8 or higher.

Table 3. Within-group differences after dose reduction or between 3 and 6 months of tamoxifen treatment

Before and after dose reduction (N=17)	Mean before dose reduction	Mean 3 months after dose reduction	Difference before and after dose reduction (95% CI)	*P* value (two-sided)
Health-related QOL	74.3	82.5	8.2 (0.9 – 15.4)	0.03
Endocrine subscale 19	49.3	55.0	5.7 (-0.5 – 11.5)	0.053
Endocrine subscale 23	58.9	66.0	NA*	0.053
3 and 6 months of 20 mg tamoxifen use (N=60)	**Mean 3 months**	**Mean 6 months**	**Δ 6 months vs 3 months (95% CI)**	***P* value (two-sided)**
Health-related QOL	82.6	81.9	-0.6 (-2.8 – 1.5)	0.55
Endocrine subscale 19	56.2	56.7	0.5 (-1.4 – 2.4)	0.61
Endocrine subscale 23	68.0	68.5	0.5 (-1.9 – 2.8)	0.71

*Wilcoxon signed rank test

Finally, the effect of dose reduction on separate items of the ES23 was analysed descriptively. In contrast to the CTCAEv5 grading, the improvement was most frequently seen in the lack of energy item (*n* = 10; 59%). Other frequently improved items were hot flashes and cold sweats (*n* = 9; 53%) and night sweats, insomnia, bloated feeling, mood swings and lightheaded feeling (*n* = 8; 47%).

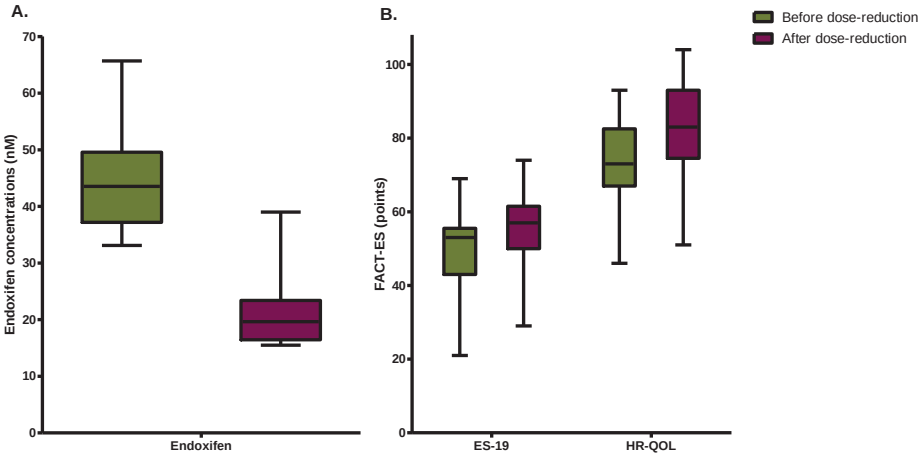


Figure 1. Effect of dose reduction on endoxifen concentrations, endocrine symptoms and quality of life
A. Median, IQR, minimum and maximum endoxifen concentrations before and after 3 months of dose reduction (n=18)
B. Median, IQR, minimum and maximum ES19 scores and HR-QOL scores before and after 3 months of dose reduction (n=17)
ES19, endocrine subscale of in total 19 items; FACT-ES, Functional Assessment of Cancer Therapy – Endocrine Symptoms; HR-QOL, health-related quality of life; IQR, interquartile range

Effect of dose-reduction on tamoxifen and endoxifen plasma levels

The median tamoxifen level of the patients at 20 mg daily who underwent a reduction in tamoxifen dose was 397 nM (IQR 343.7–478.8 nM) and the median endoxifen level was 43.2 nM (IQR 37.1-49.0 nM) before dose reduction. One patient quit tamoxifen treatment before 3 months of tamoxifen 10 mg were completed and was therefore not assessable for pharmacokinetic analysis. Endoxifen levels of 15 out of 19 patients (79%) remained above the supposed threshold of 16 nM after 3 months of tamoxifen 10 mg. One patient had already an endoxifen level below 16 nM (12.8 nM) after 26 days of dose reduction. Three patients had endoxifen levels ranging from 15.5 to 15.9 nM after 3 months of tamoxifen 10 mg use. All four patients whose endoxifen levels fell below 16 nM after the 50% dose reduction had baseline endoxifen levels just above the accepted lower boundary of 32 nM (endoxifen levels ranging from 35 to 37.7 nM). The median tamoxifen level in the 18 patients measured 3 months after 10 mg tamoxifen was 216.5 nM (IQR 163.3–285.3 nM) and the median endoxifen level was 19.7 nM (IQR 16.5–23.4 nM). A visual illustration of the effect of dose reduction on endoxifen levels and FACT-ES scores is shown in **Figure 1**.

Correlation between endoxifen levels and the effect of dose reduction on side-effects

There was a significant moderately negative correlation between change in ES19 scores after dose reduction and endoxifen levels after 3 months of tamoxifen 10 mg ($r = -0.68$, $P = 0.003$, $n = 17$) meaning that more improvement after dose reduction was seen in patients who attained lower endoxifen levels after dose reduction. Change in HR-QOL even showed a significant highly negative correlation with endoxifen levels after 3 months of tamoxifen 10 mg ($r = -0.72$, $P = 0.001$, $n = 17$).

Table 4. CTCAEv5 toxicity before and after dose reduction

	Before dose reduction at steady-state tamoxifen 20 mg (N=17)		3 months after dose reduction to tamoxifen 10 mg (N=17)
Side-effect	**CTCAE 1**	**CTCAE 2**	**CTCAE 1**
Hot flashes	13 (76%)	2 (12%)	12 (71%)
Insomnia	10 (59%)	1 (6%)	7 (41%)
Mood alterations	10 (59%)	0	4 (24%)
Arthralgia	7 (41%)	3 (18%)	8 (47%)
Muscle cramp	4 (24%)	0	4 (24%)
Nausea	2 (12%)	1 (6%)	1 (6%)
Headache	3 (18%)	0	1 (6%)
Vaginal dryness	3 (18%)	0	1 (6%)
Fatigue	2 (12%)	0	3 (18%)
Dizziness	2 (12%)	0	1 (6%)
Weight gain	2 (12%)	0	2 (12%)
Chills	1 (6%)	0	0
Bloated feeling	1 (6%)	0	0
Obstipation	1 (6%)	0	1 (6%)
Alopecia	1 (6%)	0	1 (6%)
Dry mouth	1 (6%)	0	0
Anorexia	1 (6%)	0	0
Decreased libido	1 (6%)	0	0
Amnesia	1 (6%)	0	0
Increased appetite	0	0	1 (6%)
Anxiety	0	0	1 (6%)
Vaginal discharge	0	0	1 (6%)

CTCAEv5, Common Terminology Criteria for Adverse Events version 5

DISCUSSION

To the best of our knowledge, this is the first study describing the effects of adjuvant endoxifen-guided tamoxifen dose reductions on a broad selection of therapy-related side-effects based on a validated questionnaire. We demonstrated that reducing the tamoxifen dose improves endocrine symptoms in almost half of patients and strongly increases health-related quality of life (even in two-third of patients). This improvement does not occur over time in patients with side-effects who remained on tamoxifen 20 mg. Of the patients who underwent a dose reduction, 79% retained endoxifen levels well above the conservative threshold of 16 nM, while 16% had endoxifen levels just below 16 nM (15.5 – 15.9 nM). Only one patient dropped ~20% below

the most conservative threshold of 16 nM. Thus, endoxifen-guided dose reduction of tamoxifen, with the aim of alleviating symptoms, lead to significant and clinically relevant conditions in a significant proportion of women.

In this study we chose to only carry out dose reductions in patients with endoxifen levels ≥32 nM. Since tamoxifen has linear pharmacokinetics, we tried to retain almost all patients above the 16 nM threshold. Considering the (low) intra-patient endoxifen variability of ~10%-20%, it is not surprising that some patients' endoxifen plasma levels still dropped slightly below 16 nM after halving the tamoxifen dose.[18,22] Indeed, all four patients whose endoxifen levels fell below 16 nM had baseline endoxifen levels only slightly above the accepted lower boundary of 32 nM. As mentioned before, the threshold of 16 nM is a conservative one and other threshold values have been mentioned before in studies as well. [12-14] However, since this is a curative setting, it is extremely important to maintain effective endoxifen levels. Therefore, if patients drop below 16 nM after dose reduction, a slight increase of the mean dose of tamoxifen to, for example, 15 mg daily should be considered.

Unfortunately, this study does not offer a solution for patients with endoxifen levels <32 nM in case they suffer from tamoxifen-related side-effects. But, if we take the intra-patient variability into account, dose reduction in case of severe side-effects could eventually also be tried when patients have endoxifen levels within 26 – 32 nM (20% range) and endoxifen levels could then possibly still remain above or around 16 nM. Another option might be to try dose reduction from 20 mg to 15 mg in patients with severe side-effects and endoxifen levels ranging between 21 and 32 nM. Consequently, 2-3 months after dose reduction, endoxifen levels should be measured again and the effect of dose reduction on side-effects should be assessed. If endoxifen levels with tamoxifen 20 mg are already far below 32 nM, endoxifen levels after dose reduction drop far below the conservative level of 16 nM or if patients do not benefit from tamoxifen dose-reduction, another strategy, like switching to aromatase inhibitors, should be considered.

The improvement in endocrine symptoms seen in this study was, although clinically relevant and significant, less than hypothesized in advance. Any improvement in ES19 was seen in 12 out of 17 (71%) of dose-reduced patients while a clinically relevant improvement (≥6 points) was seen in 41% of patients. A possible explanation for this lower than hypothesized improvement is the use of OFS next to tamoxifen in part of premenopausal women. OFS has quite similar side-effects as tamoxifen and when tamoxifen dose is reduced this clearly does not affect side-effects due to OFS. Two patients used OFS and both did not achieve clinically relevant improvements

in ES19 and ES23 scores after dose reduction. Secondly, patients could have other side-effect-inducing events during tamoxifen dose reduction. For instance, one patient used amoxicillin/clavulanic acid for 2 weeks because of an undefined infection during the third month of tamoxifen dose reduction and showed a decrease in ES19, ES23 and HR-QOL scores.

Most remarkably, we found a correlation between endoxifen levels after 3 months of 10 mg tamoxifen and change in HR-QOL and ES19 scores with more improvement in FACT-ES scores when endoxifen levels after 3 months of 10 mg were lower and less improvement in FACT-ES scores when endoxifen levels still remained relatively high. Possibly, patients whose endoxifen level remains relatively high after dose reduction could benefit from even further tamoxifen dose de-escalation.

In some patients the endocrine side-effects did not improve in a clinical relevant way while HR-QOL did improve. This is most likely the result of an improvement in HR-QOL resulting from causes other than the reduction of endocrine therapy-related symptoms, such as recovery from breast surgery and possibly chemotherapy. However, this improvement in HR-QOL was not seen in the group of patients who remained on standard dose of tamoxifen. An alternative explanation would be that a very small improvement of side-effects can significantly improve quality of life during long adjuvant treatment of tamoxifen.

In an earlier study from our group, the feasibility of therapeutic drug monitoring of tamoxifen was shown.[18] Here, tamoxifen dose escalation in patients with endoxifen levels <16 nM nearly halved the percentage of patients with endoxifen levels below threshold. Our current study complements nicely to this preceding research, offering a solution for patients on the other side of the spectrum (i.e. patients having high levels of endoxifen with sometimes severe side-effects). Our study highlights once again how important it is to know the 'true' threshold of endoxifen to implement this in therapeutic drug monitoring of tamoxifen in clinical practice. However, in order to confirm an endoxifen exposure-response relationship prospectively, large trials of over 3000 patients would be needed, making this kind of prove for an endoxifen threshold infeasible.[23,24] Until further evidence is attained, for example, from neo-adjuvant window-of-opportunity trials, the conservative endoxifen threshold of 16 nM could be used pragmatically for tamoxifen dose escalation in patients with endoxifen levels <16 nM and for dose de-escalating (dose reduction) in patients with severe side-effects.

Of course, our study has some limitations. Firstly, a placebo effect of lowering the tamoxifen dose cannot be ruled out as the dose adjustment was not blinded. Secondly, this was not a randomized study. Dose reduction was offered to all patients with bothersome side-effects and endoxifen levels $\geq$32 nM and patients were free in making a choice for dose reduction or remaining on 20-mg dose. This led to different time points of dose reduction and it could be possible that patients who had more belief in improvement from this intervention were more likely to choose for dose reduction, introducing potential bias. However, the different time points of dose reduction also account for more robustness of the study and make it more convertible to daily practice. In this study, a relatively limited number of dose-reduced cases are discussed. This might lead to less generalizability, although of course the linear pharmacokinetics of tamoxifen is the same for every patient. Another limitation is the duration of the study. Because we only evaluated side-effects after 3 months, this study does not provide information about durability of the improvement. Probably, side-effects could improve further over time making the impact of tamoxifen dose reduction even bigger. Last, we chose to only investigate dose reductions from 20 mg to 10 mg. Therefore, we lack interesting information about side-effects after dose adjustments to tamoxifen 15 mg.

Conclusions

In conclusion, we demonstrated that endoxifen-guided dose reduction in case of bothersome tamoxifen-related side-effects can improve endocrine symptoms in almost half of patients and strongly increase HR-QOL in two-third of these patients while keeping endoxifen levels mainly above or around the threshold. Therefore, it could be an effective strategy for patients who would otherwise quit their endocrine therapy or who are highly suffering from tamoxifen-related side-effects.

REFERENCES

1. Cardoso F, Kyriakides S, Ohno S, et al. Early breast cancer: ESMO Clinical Practice Guidelines for diagnosis, treatment and follow-up†. Annals of Oncology 2019; 30(8): 1194-220.
2. Land SR, Wickerham DL, Costantino JP, et al. Patient-Reported Symptoms and Quality of Life During Treatment With Tamoxifen or Raloxifene for Breast Cancer PreventionThe NSABP Study of Tamoxifen and Raloxifene (STAR) P-2 Trial. JAMA 2006; 295(23): 2742-51.
3. Owusu C, Buist DSM, Field TS, et al. Predictors of Tamoxifen Discontinuation Among Older Women With Estrogen Receptor–Positive Breast Cancer. Journal of Clinical Oncology 2008; 26(4): 549-55.
4. Murphy CC, Bartholomew LK, Carpentier MY, Bluethmann SM, Vernon SW. Adherence to adjuvant hormonal therapy among breast cancer survivors in clinical practice: a systematic review. Breast Cancer Research and Treatment 2012; 134(2): 459-78.
5. Boehm DU, Lebrecht A, Eckhardt T, et al. Quality of life and adjuvant tamoxifen treatment in breast cancer patients. European Journal of Cancer Care 2009; 18(5): 500-6.
6. Eriksson M, Eklund M, Borgquist S, et al. Low-Dose Tamoxifen for Mammographic Density Reduction: A Randomized Controlled Trial. Journal of Clinical Oncology 2021 Jun; 39(17): 1899-908.
7. DeCensi A, Puntoni M, Guerrieri-Gonzaga A, et al. Randomized Placebo Controlled Trial of Low-Dose Tamoxifen to Prevent Local and Contralateral Recurrence in Breast Intraepithelial Neoplasia. Journal of Clinical Oncology 2019; 37(19): 1629-37.
8. Bychkovsky B, Laws A, Katlin F, et al. Initiation and tolerance of chemoprevention among women with high-risk breast lesions: the potential of low-dose tamoxifen. Breast Cancer Research and Treatment 2022; 193(2): 417-27.
9. Mürdter TE, Schroth W, Bacchus-Gerybadze L, et al. Activity levels of tamoxifen metabolites at the estrogen receptor and the impact of genetic polymorphisms of phase I and II enzymes on their concentration levels in plasma. Clinical Pharmacology & Therapeutics 2011; 89(5): 708-17.
10. Coezy E, Borgna JL, Rochefort H. Tamoxifen and metabolites in MCF7 cells: correlation between binding to estrogen receptor and inhibition of cell growth. Cancer Research 1982; 42(1): 317-23.
11. Binkhorst L, Mathijssen RH, Ghobadi Moghaddam-Helmantel IM, et al. Quantification of tamoxifen and three of its phase-I metabolites in human plasma by liquid chromatography/triple-quadrupole mass spectrometry. J Pharmaceut Biomed 2011; 56(5): 1016-23.
12. Madlensky L, Natarajan L, Tchu S, et al. Tamoxifen Metabolite Concentrations, CYP2D6 Genotype, and Breast Cancer Outcomes. Clinical Pharmacology & Therapeutics 2011; 89(5): 718-25.
13. Saladores P, Mürdter T, Eccles D, et al. Tamoxifen metabolism predicts drug concentrations and outcome in premenopausal patients with early breast cancer. Pharmacogenomics Journal 2015; 15(1): 84-94.
14. Helland T, Henne N, Bifulco E, et al. Serum concentrations of active tamoxifen metabolites predict long-term survival in adjuvantly treated breast cancer patients. Breast Cancer Research 2017; 19(1): 125.
15. Groenland SL, van Eerden RAG, Westerdijk K, et al. Therapeutic drug monitoring based precision dosing of oral targeted therapies in oncology: a prospective multicentre study. Annals of Oncology 2022 Oct; 33(10): 1071-82.
16. Groenland SL, Verheijen RB, Joerger M, et al. Precision Dosing of Targeted Therapies Is Ready for Prime Time. Clinical Cancer Research 2021; 27(24): 6644-52.
17. Lee CI, Fox P, Balakrishnar B, et al. Tamoxifen-induced severe hot flashes and endoxifen levels: is dose reduction a safe and effective strategy? Breast 2019; 46: 52-7.
18. Braal CL, Jager A, Hoop EO, et al. Therapeutic Drug Monitoring of Endoxifen for Tamoxifen Precision Dosing: Feasible in Patients with Hormone-Sensitive Breast Cancer. Clinical Pharmacokinetics 2022; 61(4): 527-37.
19. Braal CL, Westenberg JD, Buijs SM, et al. Factors affecting inter-individual variability in endoxifen concentrations in patients with breast cancer: results from the prospective TOTAM trial. Breast Cancer Res Treat 2022 Aug; 195(1): 65-74.
20. Fallowfield LJ, Leaity SK, Howell A, Benson S, Cella D. Assessment of quality of life in women undergoing hormonal therapy for breast cancer: validation of an endocrine symptom subscale for the FACT-B. Breast Cancer Research and Treatment 1999; 55(2): 189-99.
21. Cohen J. Statistical power analysis for the behavioral sciences. 2nd edition, reprint: New York: Psychology Press 2009.

22. Jager NG, Rosing H, Schellens JH, Linn SC, Beijnen JH. Tamoxifen dose and serum concentrations of tamoxifen and six of its metabolites in routine clinical outpatient care. Breast Cancer Research and Treatment 2014; 143(3): 477-83.

23. Braal CL, Beijnen JH, Koolen SLW, et al. Relevance of Endoxifen Concentrations: Absence of Evidence Is Not Evidence of Absence. Journal of Clinical Oncology 2019; 37(22): 1980-1.

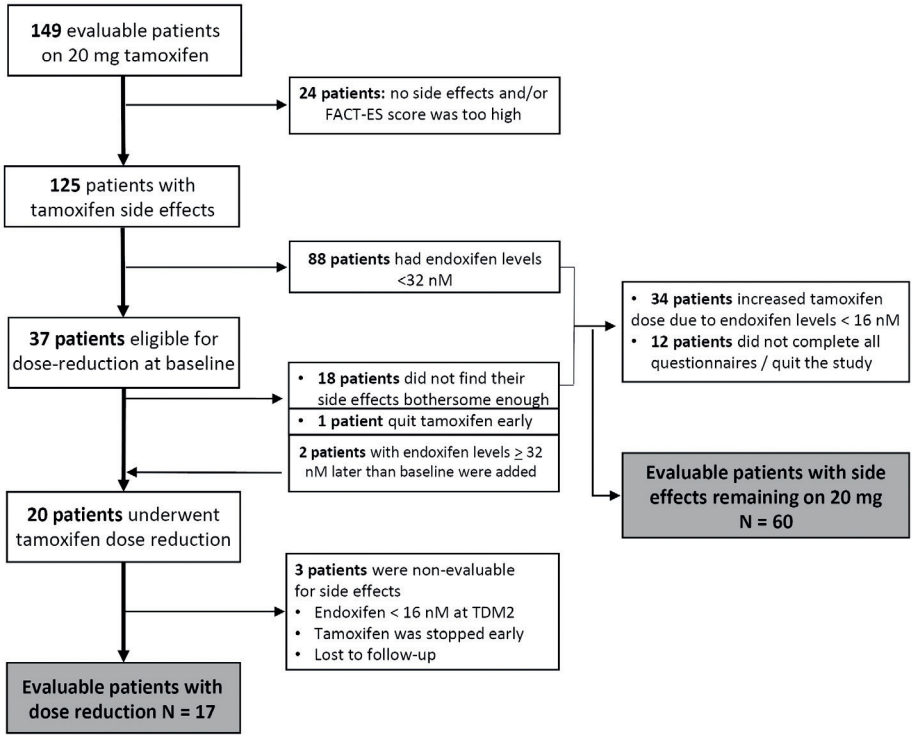
24. de Vries Schultink AHM. Pharmacometrics for Treatment Optimization and Drug Development in Oncology: Utrecht University; 2019.

SUPPLEMENTARY MATERIAL

FACT-ES items ES19 and ES23

Endocrine subscale items FACT-ES					
ES19 items	Not at all	A little bit	Some-what	Quite a bit	Very much
I have hot flashes	0	1	2	3	4
I have cold sweats	0	1	2	3	4
I have night sweats	0	1	2	3	4
I have vaginal discharge	0	1	2	3	4
I have vaginal itching/irritation	0	1	2	3	4
I have vaginal bleeding or spotting	0	1	2	3	4
I have vaginal dryness	0	1	2	3	4
I have pain or discomfort with intercourse	0	1	2	3	4
I have lost interest in sex	0	1	2	3	4
I have gained weight	0	1	2	3	4
I feel lightheaded/dizzy	0	1	2	3	4
I have been vomiting	0	1	2	3	4
I have diarrhea	0	1	2	3	4
I get headaches	0	1	2	3	4
I feel bloated	0	1	2	3	4
I have breast sensitivity/tenderness	0	1	2	3	4
I have mood swings	0	1	2	3	4
I am irritable	0	1	2	3	4
I have pain in my joints	0	1	2	3	4
ES23 items (ES19 + four items below)	0	1	2	3	4
I have a lack of energy	0	1	2	3	4
I have nausea	0	1	2	3	4
I feel nervous	0	1	2	3	4
I am sleeping well	0	1	2	3	4

Legend. The FACT-ES has a 5 point Likert-type response scale and measures four domains of health-related quality of life: physical, social, emotional and functional well-being (i.e. FACT-General). The additional endocrine-subscale specifically measures hormone therapy related side effects with 19 (ES19, i.e. the standard version) or 23 (ES23, i.e. the extended version) items. Some items were negatively framed and were therefore reversed for analysis.



Flowchart patient selection for dose reduction

Legend. From all patients undergoing therapeutic drug monitoring according to the TOTAM-study, 125 patients experienced tamoxifen-related side-effects. From this group 37 patients had endoxifen levels ≥32 nM and were eligible for dose reduction at baseline. Eighteen patients did not find their side-effects bothersome enough for dose reduction and remained on 20 mg, 1 patient quit tamoxifen shortly after start of study and two patients who at the first endoxifen measurement at baseline did not have endoxifen levels ≥32 nM were reduced later in the study when endoxifen levels became ≥32 nM. Eventually, 20 out 150 patients (13%) were reduced in tamoxifen dose. From these patients 1 was lost to follow-up for side-effect assessment, 1 quit tamoxifen 2 months after tamoxifen dose was reduced and in 1 patient the endoxifen level was already below 16 nM 26 days after the dose reduction and thus tamoxifen was increased to 15 mg.

The 88 patients with side-effects but endoxifen levels <32 nM and the 18 patients with endoxifen levels ≥32 nM but who refused dose reduction were used as a control group if they remained on a tamoxifen dose of 20 mg from 3 to 6 months of tamoxifen use. From these 106 patients, 34 patients were increased in tamoxifen dose due to endoxifen levels <16 nM and 12 patients did not complete all questionnaires or quit the study early. Therefore, 60 patients with side-effects who remained on 20 mg tamoxifen for at least 3 months after inclusion could be used as a group to check for a potential time-effect.

CHAPTER 4

CBD-oil as a potential solution in case of severe tamoxifen-related side effects

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ABSTRACT

Tamoxifen may lead to bothersome side effects contributing to non-compliance and decreased quality of life. Patients searching for relief are increasingly turning to cannabinoids such as CBD-oil. However, CBD-oil might affect tamoxifen pharmacokinetics (PK) through CYP2D6 inhibition. The aims of this open-label, single-arm study were 1) to determine the PK profile of tamoxifen when using CBD-oil, and 2) to subsequently investigate whether CBD-oil has a beneficial influence on side effects. Study patients had to have steady-state endoxifen concentrations ≥16 nM (conservative threshold). PK sampling and side effect assessment was done at initiation of CBD-oil and 28 days thereafter. Bio-equivalence could be concluded if the 90% confidence interval (CI) for the difference in endoxifen AUC fell within the [-20%; +25%] interval. The effect of CBD-oil on side effects was evaluated using the FACT-ES questionnaire. Endoxifen AUC decreased after CBD-oil by 12.6% (n = 15, 90% CI -18.7%, -6.1%) but remained within bio-equivalence boundaries. The endocrine subscale of the FACT-ES improved clinically relevant with 6.7 points (n = 26, $p < 0.001$) and health-related quality of life improved with 4.7 points after using CBD (95% CI +1.8, +7.6). We conclude that CBD-oil, if of good quality and with a dosage below 50 mg, does not have to be discouraged in patients using it for tamoxifen-related side effects.

INTRODUCTION

Tamoxifen is effective in the treatment of estrogen-receptor positive breast cancer[1,2] and is recommended for two to three years for postmenopausal patients and up to ten years for premenopausal patients.[3] Unfortunately, tamoxifen can lead to bothersome side effects such as hot flashes, arthralgia, insomnia and mood alterations. Forty percent of patients eventually discontinue tamoxifen therapy early, mainly due to side effects.[4-6]

Breast cancer patients searching for relief from side effects are increasingly turning to cannabinoids (CBs). CBs are a group of compounds found in the *Cannabis sativa* plant but interestingly, the human body also produces endocannabinoids.[7] One of the most active phytocannabinoids produced by Cannabis sativa is the non-psychoactive substance cannabidiol (CBD).[8,9] CBD acts as a negative allosteric modulator of the cannabinoid receptor 1 (CB1), mostly present in the central nervous system, and as an inverse agonist of the cannabinoid receptor 2 (CB2), mostly present in the immune system. CBD can also modulate other receptors such as: opioid, dopamine, melatonin, serotonin and acetylcholine receptors.[7,10] In the United States, more than 20% of breast cancer patients used CBs during their endocrine therapy hoping to reduce side effects.[11] A recent meta-analysis suggested a small improvement in pain and quality of sleep after CBD use.[12] Most recently, a RCT did not find any symptom relief after CBD compared to placebo in an advanced cancer population.[13] Whether CBD improves tamoxifen-related side effects in breast cancer patients has never been investigated.

Tamoxifen is a prodrug metabolised mostly by the cytochrome P450 (CYP) enzyme CYP2D6 in its main and most active metabolite endoxifen.[14] Several retrospective studies have shown an exposure-response relationship of endoxifen, which led to the suggestion of efficacy thresholds varying from 9-16 nM.[15-18] Due to the complex metabolism, tamoxifen is prone to drug-drug or drug-herb interactions.[19,20] CBD might also affect tamoxifen pharmacokinetics since it is known to be a potential inhibitor of CYP2D6.[21,22] Recently, a case report about a woman treated with tamoxifen for primary breast cancer showed lower endoxifen levels when tamoxifen was combined with CBD compared to tamoxifen monotherapy.[23] However, the bioavailability of sublingual CBD in the highest over-the-counter dosage should theoretically not be sufficient for significant CYP2D6 inhibition.[24]

If CBD can diminish tamoxifen-related side effects without negative impact on tamoxifen pharmacokinetics, this could be a solution for the high frequency of tamoxifen discontinuation. Therefore, the aims of our study were 1) to determine the pharmacokinetic interaction between CBD-oil and endoxifen and 2) to investigate

whether there is a beneficial effect of CBD-oil on tamoxifen-related side effects and health-related quality of life (HR-QOL) in primary breast cancer patients.

METHODS

This pharmacokinetic open-label single-arm, one-way cross-over study was performed at the Erasmus MC Cancer Institute in Rotterdam, The Netherlands, between November 2020 and September 2022. The study protocol was written conform the Declaration of Helsinki, approved by the Erasmus MC Medical Ethics Committee and registered at the International Clinical Trial Registry Platform (NL8786; https://www.who.int/clinical-trials-registry-platform).

Patients

We included patients who were treated with adjuvant tamoxifen for at least three months. Steady-state endoxifen plasma concentrations had to be ≥16 nM. Furthermore, patients had to experience at least one of the following tamoxifen related side effects, scored using US National Cancer Institute's Common Terminology Criteria for Adverse Events version 5 (CTCAEv5): hot flashes grade ≥2, arthralgia grade ≥2, mood alterations grade ≥2 or insomnia grade ≥1. Patients were excluded if they had used CBs within three months before inclusion or if they used strong CYP3A4, CYP2D6, UDP-glucuronosyltransferase or P-glycoprotein inhibitors or inducers. All included patients gave written informed consent.

Study design

The study started with continuation of tamoxifen monotherapy for 7 days. Patients were ordered to take their tamoxifen at 9 AM. Patients were then hospitalized for 24-h pharmacokinetic blood sampling of tamoxifen and endoxifen. Afterwards, patients started with 5 drops 10% CBD-oil sublingually three times daily for four weeks (i.e., ≈50 mg CBD per day, the highest over-the-counter dose) concomitantly to their tamoxifen treatment. The pharmaceutical grade CBD-oil was manufactured by a Dutch Pharmacy (Clinical Cannabis Care, Breukelen, the Netherlands, article number 16779517). After four weeks of concomitant CBD and tamoxifen, patients were again hospitalized for pharmacokinetic blood sampling of tamoxifen and endoxifen. Also, before and after start of CBD side effects were assessed and laboratory analysis (blood count, kidney- and liver function) was performed. Patients were asked to fill in a patient diary to verify patients' compliance.

After the intended 15 patients completed the study protocol, the study was amended to include 11 more patients. With this amendment we were able to investigate whether CBD-oil could have a beneficial effect on tamoxifen-related side effects. Hospitalization

for pharmacokinetic blood sampling was not required for these patients. A single endoxifen trough concentration (Cmin) was measured after four weeks of CBD-oil. Other design aspects of the extension part were identical to the main study.

Pharmacokinetic and pharmacogenetic analysis

Blood samples for determination of tamoxifen and endoxifen pharmacokinetics were obtained at 13 predefined time points (t=0 (before tamoxifen intake); and 0.5h; 1h; 1.5h; 2h; 2.5h; 3h; 3.5h; 4h; 6h; 8h; 10h and 24h after tamoxifen intake). Single measurements of plasma concentrations were performed on a validated liquid chromatography with a tandem mass spectrometry method (UP-LC-MS/MS).[25] Using Phoenix WinNonLin version 8.3 the following pharmacokinetic parameters were determined or calculated: area under the plasma concentration time curves (AUC), C_{min} and maximum observed plasma concentration (C_{max}) of tamoxifen and endoxifen.

CYP2D6 genotyping was performed on germline DNA using the Infiniti test (Autogenomics; Carlsblad, CA, USA) and the Quantstudio test (ThermoFisher Scientific; Waltham, MA, USA). Blood samples were assayed on the follow genetic variants: **2-10, *12, *13, *14, *17, *29*, and **41*.

Quality of life and side effect

Side effects of tamoxifen and CBD-oil were assessed using CTCAEv5. To evaluate the effect of CBD-oil on tamoxifen-related side effects and HR-QOL in more detail, patients were asked to fill in the Functional Assessment of Cancer Therapy – Endocrine Symptoms (FACT-ES) questionnaire before and four weeks after start of CBD.[26] The FACT-ES is a validated questionnaire of in total 46 questions and consists of physical, social, emotional and functional wellbeing subscales (together measuring the health-related quality of life) and an additional endocrine subscale measuring side effects of endocrine treatments given in breast cancer patients. The different endocrine subscale items and additional information about the questionnaire can be found in **Supplementary Table 2**.

Statistical analysis

The primary endpoint of the study was the AUC of endoxifen. Bio-equivalence of tamoxifen with and without CBD-oil could be concluded according to Food and Drug Administration (FDA) guidelines, which suggest that the 90% confidence interval (CI) of the ratio of geometric means of the AUC should be within 0.80-1.25.[27] Given a two-sided α of 5%, 80% power, assuming a standard deviation of the difference of 20% and a true ratio of AUC geometric means of 1.0 a sample size of 15 patients was required.

The AUC of endoxifen as well as all other pharmacokinetic endpoints were analysed by means of a paired t-test on log-transformed data.

As a secondary endpoint, the ES scores of the FACT-ES questionnaire before start of CBD-oil and four weeks after start of CBD-oil were compared. Change scores of more than 0.5 of the baseline SD are considered a clinically relevant change and seen as more than a moderate effect size.[28] Hence, we hypothesized that the ES score would improve with at least 0.5 of baseline SD after four weeks of CBD-oil, estimated at four points based on earlier research.[26] To test this hypothesis with a paired sample t-test a sample size of 26 patients was required (one-sided α 5%, 80% power, estimated within-patient correlation 0.7). Also, differences in HR-QOL before and after CBD-oil use were determined and tested with a paired sample t-test or Wilcoxon signed rank test in case the scores were not normally distributed. Differences before and after CBD in specific side effects measured with the endocrine subscale of the FACT-ES were analysed descriptively. The item 'I am sleeping well' is not part of the endocrine subscale but it is an item in the HR-QOL part. Because insomnia was frequently mentioned as a side effect of tamoxifen and CBD is known to potentially improve this, we also checked for changes in this item.

In an exploratory way, the difference in endoxifen AUC was analysed for each CYP2D6 phenotype separately. If a difference between CYP2D6 phenotypes was found, the endoxifen C_{min} would be analysed for different CYP2D6 phenotypes as well in all 26 patients.

RESULTS

Patient characteristics

In total, 35 patients were enrolled in the study. Four patients were excluded before start of study due to voluntary withdrawal (*n* = 2), disease progression (*n* = 1) and an endoxifen level <16 nM despite dose escalation (*n* = 1). Five other patients were excluded during the study due to protocol violation (*n* = 3), personal circumstances (*n* = 1) and poor venous access for blood withdrawal (*n* = 1). There were 26 evaluable patients whereof 15 for the primary pharmacokinetic endpoint. Patient characteristics can be found in **Table 1**.

Pharmacokinetics

Table 2 shows the main pharmacokinetic parameters of tamoxifen and endoxifen during tamoxifen monotherapy as compared to tamoxifen combined with CBD-oil.

For endoxifen, the $AUC_{0\text{-}24h}$ significantly decreased by 12.6% (90% CI -18.7%, -6.1%) when CBD-oil was used in addition to tamoxifen. However, the 90% CI was within the limits of bio-equivalence. Also, the C_{min} and C_{max} of endoxifen decreased significantly when using CBD-oil next to tamoxifen. There were no significant differences in tamoxifen $AUC_{0\text{-}24h}$, C_{min} and C_{max} between tamoxifen monotherapy or tamoxifen combined with CBD-oil. Moreover, the 90% CIs of all parameters were within bio-equivalence boundaries.

Table 1. Baseline characteristics

***n* = 26**	***n* (%) or median [IQR]**	
Age	49.5	[46.8 - 54]
BMI (kg/m2)	26.1	[23.8 – 30.9]
Biochemistry		
ALT (U/L)	18.5	[14.0 – 24.5]
Creatinine (µmol/L)	69	[64.8 – 75]
Hemoglobin (mmol/L)	8.0	[7.8 – 8.4]
Leucocytes (x10^9/L)	5.8	[4.9 – 7.0]
Thrombocytes (x10^9/L)	236.5	[193.0 – 294.5]
Duration of adjuvant tamoxifen use (months)	13	[5 - 24]
WHO Performance Score		
0	16	61.5%
1	10	38.5%
Local treatment		
Lumpectomy + radiotherapy	13	50%
Mastectomy only	4	15.4%
Mastectomy + radiotherapy	9	34.6%
(Neo)adjuvant chemotherapy		
Yes	20	76.9%
No	6	23.1%
(Neo)adjuvant anti-Her2Neu therapy		
Yes	1	3.8%
No	25	96.2%
Tamoxifen dose		
20 mg	18	69.2%
30 mg	4	15.4%
40 mg	4	15.4%
CYP2D6 phenotype		
Intermediate metabolizer (IM)	13	50%
Extensive metabolizer (EM)	12	46.2%
Ultrarapid metabolizer (UM)	1	3.8%

n number; IQR interquartile range

Table 2. Tamoxifen pharmacokinetics with or without CBD

Pharmacokinetic parameters (*n* = 15)	Tamoxifen monotherapy[a]	Tamoxifen + CBD[a]	Relative difference (%) with vs without CBD (90% CI)
Tamoxifen			
$AUC_{0\text{-}24h}$ (nmol · h · L^{-1})	7100 (669)	6900 (622)	-2.8% (-7.7, +2.4%)
C_{min}	272 (67)	252 (64)	-7.2% (-14.1, +0.4%)
C_{max}	397(69)	392 (61)	-1.2% (-8.2, +6.4%)
Endoxifen			
$AUC_{0\text{-}24h}$ (nmol · h · L^{-1})	621 (22)	542 (18)	-12.6% (-18.7, -6.1%)
C_{min}	28 (22)	23 (20)	-18.2% (-23.4, -12.7%)
C_{max}	33 (20)	27 (17)	-16.3% (-20.7, -11.7%)

AUC: area under the curve, C_{min}: minimum plasma concentration, C_{max}: maximum plasma concentration
[a]geometric mean (coefficient of variation %)

The AUCs of tamoxifen and endoxifen were also analyzed for the intermediate (IM) and extensive (EM) CYP2D6 phenotype patients separately (see **Supplementary Table 1**). In patients with an IM CYP2D6 phenotype, the AUC of tamoxifen and endoxifen decreased significantly when using CBD-oil, while in patients with an EM CYP2D6 metabolism, the AUC of both tamoxifen and endoxifen remained comparable with the 90% CIs within the bio-equivalence boundaries. There was a significant difference between $\Delta AUC_{PK2\text{-}PK1}$ of endoxifen in IM metabolizers compared with EM metabolizers (p = 0.004, independent samples *t*-test). To further study this difference, the C_{min} of tamoxifen and endoxifen was determined for IM and EM CYP2D6 phenotypes separately in the total study group (*n* = 25, one patient missing due to logistic reasons). Tamoxifen C_{min} was comparable in both IM and EM CYP2D6 phenotypes. Endoxifen C_{min} decreased in a comparable range in both groups and there was no statistical difference between $\Delta Cmin_{PK2\text{-}PK1}$ of endoxifen in IM metabolizers compared with EM metabolizers (*p* = 0.48, independent samples t-test).

FACT-ES scores

In **Figure 1** a visual representation of the effect of CBD-oil on ES and HR-QOL is shown. There was a clinical relevant (≥5 points i.e., >0.5 of SD of ES on baseline) and significant improvement in ES after four weeks of using CBD next to tamoxifen. The HR-QOL showed a significant improvement as well, but this change was not clinical relevant (<8 points i.e. <0.5 of SD of HR-QOL on baseline). Means, confidence intervals an *p*-values can be found in **Table 3.**

To rule out the effect of the (slight) decrease in endoxifen levels on tamoxifen-related side effects instead of the effect of using CBD-oil, a Spearman correlation test was performed between the difference in endoxifen C_{min} and the improvement in ES score. No significant correlation was found (r = 0.19, p = 0.37, n = 25).

Joint pain improved most frequently of all separate items of the endocrine subscale of the FACT-ES (n = 16; 62%). Also, hot flashes, cold sweats, night sweats and insomnia (n = 15; 58%) and bloated feeling (n = 13; 50%) were items that improved frequently (i.e. in at least half of the patients). Percentage of improvement of all side effects assessed with the FACT-ES questionnaire can be found in **Table 4.** Improvement is seen as at least one point improvement on the 5-point Likert scale after four weeks of CBD.

CTCAE-toxicity

Side effects with tamoxifen monotherapy and after four weeks of tamoxifen and CBD-oil concomitantly can be found in **Table 5.** Hot flashes and arthralgia improved with at least one grade in six out of 25 patients (24%) and insomnia improved with one grade in 11 out of 26 patients (42%). This is in line with the trend seen in improvement in separate endocrine subscale items.

Ten out of 26 patients (38%) experienced some kind of CBD-oil-related toxicity. Most frequented mentioned side effects were fatigue (n = 3, 12%) and dry mouth (n = 3, 12%). All side effects were grade 1. None of the patients quit CBD-oil because of side effects. Sixty-nine percent of patients wished to continue CBD-oil after the study was finished.

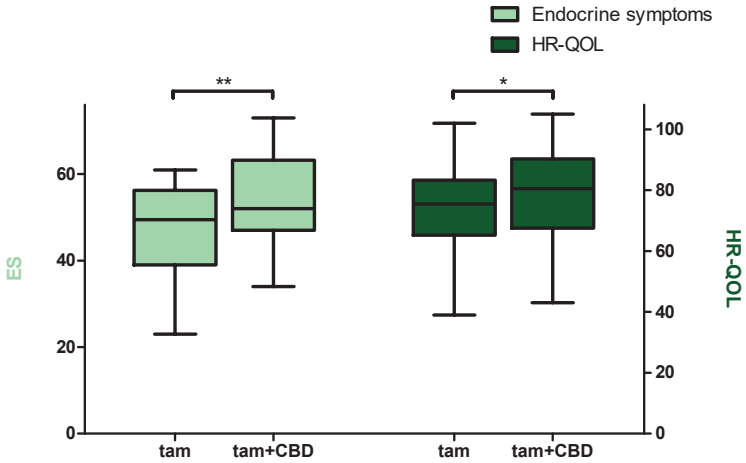

Figure 1. FACT-ES scores with tamoxifen monotherapy and after 4 weeks of CBD use
ES; endocrine symptoms (0-76), HR-QOL; health-related quality of life (0-108) * significant ** significant and clinical relevant *one-sided paired sample t-test **one-sided wilcoxon signed rank test. TAM mono: ES median: 49.5, IQR: 39-56, min-max: 23-61. HR-QOL median: 75.5, IQR: 65-73, min-max: 39-102. TAM + CBD: ES median: 52.0, IQR: 47-63, min-max: 34-73. HR-QOL median: 80.5, IQR: 68-90, min-max: 43-105

Table 3. FACT-ES scores with tamoxifen monotherapy and after 4 weeks of CBD use

FACT-ES scores (*n* = 26)	Mean without CBD	Mean after 4 weeks of CBD	Difference before and after CBD (95% CI)	*P* value (1-sided)
Health-related QOL	74.3	79.0	+4.7[a] (+1.8, +7.6)	0.001[b]
Endocrine symptoms	47.1	53.8	+6.7[a] (NA)	<0.001[c]

[a]Clinically relevant improvement when >0.5 of baseline SD, HR-QOL baseline SD 15.8, ES baseline SD 10.4
[b]Paired samples t-test [c]Wilcoxon signed rank test

Table 4. Side effects assessed with FACT-ES questionnaire

Side effect	Patients with side effect on baseline (*n*)	Patients in which side effect improved (*n*)	% improved (from total patient group *n* = 26)	% improved (from patients with side effect)
Hot flashes	26	15	58%	58%
Joint pain	25	16	62%	64%
Insomnia	25	15	58%	60%
Cold sweats	24	15	58%	63%
Night sweats	23	15	58%	65%
Mood swings	21	10	38%	48%
Irritable feeling	21	10	38%	48%
Vaginal discharge	19	7	27%	37%
Vaginal dryness	19	8	31%	42%
Lost interest in sex[a]	18	5	19%	28%
Weight gain	18	7	27%	39%
Bloated feeling	18	13	50%	72%
Headache	17	8	31%	47%
Lightheaded/dizziness	16	9	35%	56%
Pain/discomfort with intercourse[a]	15	6	23%	40%
Breast sensitivity/ tenderness[a]	13	9	34%	69%
Vaginal itching/irritation[a]	9	4	15%	44%
Diarrhea	6	4	15%	67%
Vomiting	3	3	12%	100%
Vaginal bleeding or spotting	2	1	4%	50%

[a]For this item some patients were missing, vaginal itching n = 1, pain/discomfort with intercourse n = 5, lost interest in sex n = 1, breast sensitivity/tenderness n = 2

Table 5. CTCAE toxicity of tamoxifen with and without concomitant CBD

	Tamoxifen-related side with tamoxifen only (*n* = 26)		Tamoxifen-related side effects with tamoxifen and CBD (*n* = 26)	
***n* (%)**	**Grade 1**	**Grade 2**	**Grade 1**	**Grade 2**
Insomnia	16 (62%)	10 (38%)	19 (73%)	3 (12%)
Hot flashes	20 (77%)	5 (19%)	22 (85%)	1 (4%)
Arthralgia	15 (58%)	10 (38%)	21 (81%)	3 (12%)
Mood alterations	19 (73%)	1 (4%)	17 (65%)	1 (4%)
Muscle cramp	7 (27%)	1 (4%)	8 (31%)	-
Fatigue	3 (12%)	3 (12%)	8 (31%)	1 (4%)
Headache	4 (15%)	-	4 (15%)	-
Vaginal dryness	2 (8%)	1 (4%)	1 (4%)	-
Amnesia	4 (15%)	-	2 (8%)	-
Weight gain	2 (8%)	-	1 (4%)	-
Dry mouth	-	-	3 (12%)	
Nausea			2 (8%)	

Toxicity is shown when it occurred in more than one patient

DISCUSSION

Although the combination of CBD-oil and tamoxifen lead to a significant decrease in endoxifen plasma concentrations, the decrease remained within bio-equivalence boundaries and is not considered clinically relevant. Furthermore, CBD-oil seems to improve tamoxifen-related side effects as measured by an endocrine symptoms quality-of-life questionnaire (FACT-ES) while CBD-oil itself has only mild side effects. Thus, in case of bothersome tamoxifen-related side effects CBD addition may reduce side effects and hopefully lower the high tamoxifen discontinuation rate. However, since this was an open-label, single arm study, it is not known how much of the improvement is due to a placebo effect. HR-QOL improved significantly but this improvement was too little to be clinically relevant. Since CBD-oil was used for only four weeks this may have been too short to achieve a clinical relevant improvement in HR-QOL.

In patients with an IM CYP2D6 phenotype the decrease in endoxifen AUC seemed more pronounced than in patients with an EM CYP2D6 phenotype. Because of the additional patients recruited for side effect analysis extra information about C_{min} concentrations could be obtained. With regard to C_{min} endoxifen concentrations, there was no significant difference between IM and EM subgroups. Although C_{min} is known to be a less robust pharmacokinetic parameter than AUC, this analysis, done with a much larger subgroup, makes a difference in CBD effects between CYP2D6 phenotypes less probable.

In our study mean scores of ES and HR-QOL were 47 and 74 points, respectively. ES scores were much lower than found in previous studies where ES were assessed in large groups of unselected patients using adjuvant tamoxifen (ES: 59-62 points; HR-QOL 79-83 points).[29-31] This is as expected, since, in contrast to our study, these populations were not selected for tamoxifen-related complaints. After four weeks of CBD, HR-QOL scores improved to an average of 79 points. While the improvement of ES scores was clinically relevant they remained below population average (54 points). Possibly, a longer period of CBD treatment can further improve ES scores.

Earlier studies suggested effects of CBD-oil on sleep and pain which was confirmed in our study.[32] Insomnia improved the most (i.e., in 42% of patients) and also, hot flashes and arthralgia improved in 24% of patients. But, a placebo effect cannot be ruled out. The most compelling study to date that placebo effect may play an important role in symptom relief from CBD-oil comes from the recently published study by Hardy et al. In a randomized study they showed beneficial effect of two weeks CBD use for symptom relief in an advanced cancer population.[13] However, this effect was not statistically significantly better than placebo, suggesting an important role for a placebo effect.[13] Although a placebo-controlled randomized study is the ultimate form of ruling out a placebo effect, some nuance should be made here. The questionnaire used had a large overlap with the well-known complaints associated with CBD use. Symptoms that were assessed in this study were pain, tiredness, drowsiness, nausea, lack of appetite, shortness of breath, depression, anxiety and wellbeing. Almost half of these symptoms (tiredness, drowsiness, nausea and lack of appetite) are known side effects of CBD.[33,34] Besides, this assessment scale is hardly overlapping with any of the endocrine side effects of tamoxifen. This, in combination with the very high dose of CDB used (eight-times higher than in our study), makes it difficult to make a definitive statement about the effect of CBD-oil in case of tamoxifen-related side effects. Overall, our findings of CBD use for reducing tamoxifen-related side effects in a well-defined breast cancer patient population are promising, but certainly need further investigation in a placebo-controlled study to demonstrate the real added value of CBD-oil.

Although it is known that CBD can interact with several G-protein coupled receptors such as CB1-, CB2-, opioid-, melatonin-, acetylcholine-, serotonin- and dopamine-receptors, it is not understood how this interplay of agonism and antagonism of receptors might lead to, for example, alleviation of pain or improvement of sleep.[7] However, CB1 is highly expressed in areas in the brain related to, among others, pain, anxiety, sensory and visceral perception, motor coordination and endocrine functions.[10] We hypothesize that this might be one of the reasons that CB1 receptor activation by CBD could lead to less hot flashes and a decrease in arthralgia. Also, it is presumable

that activation of other receptors as opioid- and melatonin-receptors could lead to a decrease in pain and an improvement in sleep, subsequently. This is most probably a separate mechanism not related to tamoxifen but which can coincidentally improve side effects that occur with tamoxifen. However, it is not ruled out that an interaction between the estrogen receptor and receptors where CBD engages occurs, for example in the brain where both are present.

Our study has several strengths. It is the first study investigating the pharmacokinetic interaction between tamoxifen and CBD-oil leading to highly requested knowledge for many breast cancer patients. Pharmaceutical grade CBD-oil without THC (i.e., THC <0.05%) was used next to tamoxifen for four weeks in the highest over-the-counter dose (i.e. ≈50 mg), also securing the safety of lower CBD-oil doses. Because patients were their own control and AUCs of tamoxifen and endoxifen were measured, this led to a robust answer to this pharmacokinetic question. However, it remains unclear if higher doses of CBD, non-pharmaceutical grade CBD or other formulations of CBD are equally safe. Also, this is the first study investigating the effect of CBD-oil on tamoxifen-related side effects using the validated and reliable FACT-ES questionnaire next to CTCAE toxicity grading. A limitation of the study is the lack of a control arm when it comes to the research question about the effect of CBD use on side effects reduction. However, this is no issue for our primary, pharmacological research question. Finally, if subsequent studies confirm our results, CBD use will unfortunately not be applicable worldwide. CBD-oil is seen as a supplement in the Netherlands and many other European countries, but is on the list of prohibited narcotics in several other countries.

In conclusion, endoxifen levels remained within bio-equivalence boundaries when CBD-oil was used in combination with tamoxifen. Therefore, sublingual CBD-oil, if of good quality and not higher than the highest over-the-counter dose (<50 mg per day), does not have to be discouraged in patients using it as complementary medication. In addition, the use of CBD-oil in this single arm study resulted in a promising improvement in endocrine symptoms and quality of life, but the real effect of CBD-oil has yet to be proven in a placebo-controlled study that is currently being set up.

REFERENCES

1. Early Breast Cancer Trialists' Collaborative Group. Relevance of breast cancer hormone receptors and other factors to the efficacy of adjuvant tamoxifen: patient-level meta-analysis of randomised trials. Lancet 378, 771-784, doi:10.1016/s0140-6736(11)60993-8 (2011).
2. Early Breast Cancer Trialists' Collaborative Group. Effects of chemotherapy and hormonal therapy for early breast cancer on recurrence and 15-year survival: an overview of the randomised trials. Lancet 365, 1687-1717, doi:10.1016/s0140-6736(05)66544-0 (2005).
3. Cardoso, F. et al. Early breast cancer: ESMO Clinical Practice Guidelines for diagnosis, treatment and follow-up†. Annals of Oncology 30, 1194-1220, doi:https://doi.org/10.1093/annonc/mdz173 (2019).
4. Owusu, C. et al. Predictors of Tamoxifen Discontinuation Among Older Women With Estrogen Receptor–Positive Breast Cancer. Journal of Clinical Oncology 26, 549-555, doi:10.1200/jco.2006.10.1022 (2008).
5. Lailler, G. et al. Five-Year Adjuvant Endocrine Therapy Adherence Trajectories Among Women With Breast Cancer: A Nationwide French Study Using Administrative Data. Clinical Breast Cancer 21, e415-e426, doi:https://doi.org/10.1016/j.clbc.2021.01.007 (2021).
6. Murphy, C. C., Bartholomew, L. K., Carpentier, M. Y., Bluethmann, S. M. & Vernon, S. W. Adherence to adjuvant hormonal therapy among breast cancer survivors in clinical practice: a systematic review. Breast Cancer Research and Treatment 134, 459-478, doi:10.1007/s10549-012-2114-5 (2012).
7. Shahbazi, F., Grandi, V., Banerjee, A. & Trant, J. F. Cannabinoids and Cannabinoid Receptors: The Story so Far. iScience 23, 101301 (2020).
8. Lucas, C. J., Galettis, P. & Schneider, J. The pharmacokinetics and the pharmacodynamics of cannabinoids. British Journal Clinical Pharmacology 84, 2477-2482, doi:10.1111/bcp.13710 (2018).
9. Atakan, Z. Cannabis, a complex plant: different compounds and different effects on individuals. Therapeutic Advances in Psychopharmacology 2, 241-254, doi:10.1177/2045125312457586 (2012).
10. Kisková, T., Mungenast, F., Suváková, M., Jäger, W. & Thalhammer, T. Future Aspects for Cannabinoids in Breast Cancer Therapy. International Journal of Molecular Sciences 20 (2019).
11. Weiss, M. C. et al. A survey of breast cancer patients' use of cannabis before, during, and after treatment. Journal of Clinical Oncology 38, e19210-e19210, doi:10.1200/JCO.2020.38.15_suppl.e19210 (2020).
12. Wang, L. et al. Medical cannabis or cannabinoids for chronic non-cancer and cancer related pain: a systematic review and meta-analysis of randomised clinical trials. British Medical Journal 374, n1034 (2021).
13. Hardy, J. et al. Phase IIb Randomized, Placebo-Controlled, Dose-Escalating, Double-Blind Study of Cannabidiol Oil for the Relief of Symptoms in Advanced Cancer (MedCan1-CBD). Journal of Clinical Oncology, JCO.22.01632, doi:10.1200/jco.22.01632 (2022).
14. Mulder, T. A. M. et al. Clinical CYP2D6 Genotyping to Personalize Adjuvant Tamoxifen Treatment in ER-Positive Breast Cancer Patients: Current Status of a Controversy. Cancers 13 (2021).
15. Helland, T. et al. Serum concentrations of active tamoxifen metabolites predict long-term survival in adjuvantly treated breast cancer patients. Breast Cancer Research 19, 125 (2017).
16. Madlensky, L. et al. Tamoxifen Metabolite Concentrations, CYP2D6 Genotype, and Breast Cancer Outcomes. Clinical Pharmacology & Therapeutics 89, 718-725, doi:https://doi.org/10.1038/clpt.2011.32 (2011).
17. Saladores, P. et al. Tamoxifen metabolism predicts drug concentrations and outcome in premenopausal patients with early breast cancer. Pharmacogenomics Journal 15, 84-94 (2015).
18. Yamaori, S., Okamoto, Y., Yamamoto, I. & Watanabe, K. Cannabidiol, a major phytocannabinoid, as a potent atypical inhibitor for CYP2D6. Drug Metabolism and Disposition 39, 2049-2056 (2011).
19. Binkhorst, L. et al. Effects of CYP induction by rifampicin on tamoxifen exposure. Clinical Pharmacology & Therapeutics 92, 62-67 (2012).
20. Hussaarts, K. et al. Impact of Curcumin (with or without Piperine) on the Pharmacokinetics of Tamoxifen. Cancers 11 (2019).
21. Brown, J. D. & Winterstein, A. G. Potential Adverse Drug Events and Drug-Drug Interactions with Medical and Consumer Cannabidiol (CBD) Use. Journal of Clinical Medicine 8 (2019).
22. Graham, M., Martin, J. H., Lucas, C. J., Murnion, B. & Schneider, J. Cannabidiol drug interaction considerations for prescribers and pharmacists. Expert Review of Clinical Pharmacology 15, 1383-1397 (2022).

23. Parihar, V. et al. Reduction in Tamoxifen Metabolites Endoxifen and N-desmethyltamoxifen With Chronic Administration of Low Dose Cannabidiol: A CYP3A4 and CYP2D6 Drug Interaction. Journal of Pharmacy Practice 35, 322-326 (2022).

24. Nelson, K. M. et al. The Essential Medicinal Chemistry of Cannabidiol (CBD). Journal of Medical Chemistry 63, 12137-12155 (2020).

25. Binkhorst, L. et al. Quantification of tamoxifen and three of its phase-I metabolites in human plasma by liquid chromatography/triple-quadrupole mass spectrometry. J Pharmaceut Biomed 56, 1016-1023 (2011).

26. Fallowfield, L. J., Leaity, S. K., Howell, A., Benson, S. & Cella, D. Assessment of quality of life in women undergoing hormonal therapy for breast cancer: validation of an endocrine symptom subscale for the FACT-B. Breast Cancer Research and Treatment 55, 189-199 (1999).

27. U.S. Department of Health and Human Services Food and Drug Administration Center for Drug Evaluation and Research (CDER). Bioequivalence Studies With Pharmacokinetic Endpoints for Drugs Submitted Under an ANDA Guidance for Industry. (2021).

28. Cohen, J. Statistical power analysis for the behavioral sciences. 2nd edition, reprint. (New York: Psychology Press 2009).

29. Ohsumi, S. et al. Health-related quality of life and psychological distress of breast cancer patients after surgery during a phase III randomized trial comparing continuation of tamoxifen with switching to anastrozole after adjuvant tamoxifen for 1–4 years: N-SAS BC 03. Breast Cancer Research and Treatment 127, 143-152, doi:10.1007/s10549-011-1400-y (2011).

30. Takei, H. et al. Health-related quality of life, psychological distress, and adverse events in postmenopausal women with breast cancer who receive tamoxifen, exemestane, or anastrozole as adjuvant endocrine therapy: National Surgical Adjuvant Study of Breast Cancer 04 (N-SAS BC 04). Breast Cancer Research and Treatment 133, 227-236, doi:10.1007/s10549-011-1943-y (2012).

31. Cella, D. et al. Quality of Life of Postmenopausal Women in the ATAC ("Arimidex", Tamoxifen, Alone or in Combination) Trial after Completion of 5 years' Adjuvant Treatment for Early Breast Cancer. Breast Cancer Research and Treatment 100, 273-284, doi:10.1007/s10549-006-9260-6 (2006).

32. Wang, L. et al. Medical cannabis or cannabinoids for chronic non-cancer and cancer related pain: a systematic review and meta-analysis of randomised clinical trials. British Medical Journal 374, n1034 (2021).

33. Iffland, K. & Grotenhermen, F. An Update on Safety and Side Effects of Cannabidiol: A Review of Clinical Data and Relevant Animal Studies. Cannabis and Cannabinoid Research 2, 139-154 (2017).

34. Pauli, C. S., Conroy, M., Vanden Heuvel, B. D. & Park, S. H. Cannabidiol Drugs Clinical Trial Outcomes and Adverse Effects. Frontiers in Pharmacology 11, 63 (2020)

SUPPLEMENTARY MATERIAL

Supplementary table 1. Tamoxifen pharmacokinetics with or without CBD per CYP2D6 phenotype

Pharmacokinetic parameters	Tamoxifen monotherapy[1]	Tamoxifen + CBD[1]	Relative difference (%) with vs without CBD (90% CI)
Intermediate metabolizers (IM)			
Tamoxifen $AUC_{0\text{-}24h}$ (N=8)[2]	8130(59)	7570 (64)	-6.9% (-13.2, -0.2%)
Tamoxifen C_{min} (N=13)[3]	321 (55)	309 (58)	-3.7% (-10.1, +3.1%)
Endoxifen $AUC_{0\text{-}24h}$ (N=8)[2]	623 (28)	494 (18)	-20.8% (-26.4, -14.8%)
Endoxifen C_{min} (N=13)[3]	26 (29)	22 (28)	-17.7% (-23.3, -11.7%)
Extensive metabolizers (EM)			
Tamoxifen $AUC_{0\text{-}24h}$ (N=7)[2]	6090 (76)	6210 (57)	+2.0% (-6.1, +10.8%)
Tamoxifen C_{min} (N=11)[3]	268 (59)	245 (51)	-8.5% (-18.6, +3.0%)
Endoxifen $AUC_{0\text{-}24h}$ (N=7)[2]	618 (15)	604 (13)	-2.2% (-11.1, +7.6%)
Endoxifen C_{min} (N=11)[3]	31 (46)	27 (34)	-13.7% (-21.9, -4.6%)

[1]geometric mean (coefficient of variation %) [2]all patients with IM and EM metabolism where AUC was determined [3]patients with IM and EM metabolism where AUC was determined AND all other patients with IM and EM metabolism where only C_{min} was determined

Supplementary table 2. FACT-ES items Endocrine subscale

Endocrine subscale items FACT-ES					
ES items	**Not at all**	**A little bit**	**Some-what**	**Quite a bit**	**Very much**
I have hot flashes	0	1	2	3	4
I have cold sweats	0	1	2	3	4
I have night sweats	0	1	2	3	4
I have vaginal discharge	0	1	2	3	4
I have vaginal itching/irritation	0	1	2	3	4
I have vaginal bleeding or spotting	0	1	2	3	4
I have vaginal dryness	0	1	2	3	4
I have pain or discomfort with intercourse	0	1	2	3	4
I have lost interest in sex	0	1	2	3	4
I have gained weight	0	1	2	3	4
I feel lightheaded/dizzy	0	1	2	3	4
I have been vomiting	0	1	2	3	4
I have diarrhea	0	1	2	3	4
I get headaches	0	1	2	3	4
I feel bloated	0	1	2	3	4
I have breast sensitivity/tenderness	0	1	2	3	4
I have mood swings	0	1	2	3	4
I am irritable	0	1	2	3	4
I have pain in my joints	0	1	2	3	4

Legend. The FACT-ES has a 5 point liker-type response scale and contains 46 questions in total. It measures four domains of health-related quality of life: physical, social, emotional and functional well-being in 27 items. Also, it is a measure of side effects of endocrine treatments given in breast cancer patients (ES, 19 items, see above). Some items were negatively framed and were therefore reversed for analysis. High scores of the FACT-ES equate with good quality of life and/or experiencing few side effects while lower scores indicate poorer quality of life and/or experiencing many/severe side effects.

CHAPTER 5

The interplay between tamoxifen and endoxifen plasma concentrations and coagulation parameters in patients with primary breast cancer

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ABSTRACT

Background

Tamoxifen is an effective treatment for primary breast cancer but increases the risk for venous thromboembolism. Tamoxifen decreases anticoagulant proteins, including antithrombin (AT), protein C (PC) and tissue factor (TF) pathway inhibitor, and enhances thrombin generation (TG). However, the relation between plasma concentrations of both tamoxifen and its active metabolite endoxifen and coagulation remains unknown.

Methods

Tamoxifen and endoxifen were measured in 141 patients from the prospective open-label intervention TOTAM-study after 3 months (m) and 6 m of tamoxifen treatment. Levels of AT and PC, the procoagulant TF, and TG parameters were determined at both timepoints if samples were available (n = 53-135 per analysis). Levels of coagulation proteins and TG parameters were correlated and compared between: 1) quartiles of tamoxifen and endoxifen levels, and 2) 3 m and 6 m of treatment.

Results

At 3 m, levels of AT, PC, TF and TG parameters were not associated with tamoxifen nor endoxifen levels. At 6 m, median TF levels were lower in patients in the 3rd (56.6 [33] pg/mL), and 4th (50.1 [19] pg/mL) endoxifen quartiles compared to the 1st (lowest) quartile (76 [69] pg/mL) (p = 0.027 and p = 0.018, respectively), but no differences in anticoagulant proteins or TG parameters were observed. An increase in TF levels (3m: 46.0 [15], 6m: 54.4 [39] pg/mL, $p < 0.001$) and TG parameters was observed at the 6 m treatment period, while AT and PC levels remained stable.

Conclusions

Our results indicate that higher tamoxifen and endoxifen levels are not correlated with an increased procoagulant state, suggesting tamoxifen dose escalation does not further promote hypercoagulability.

INTRODUCTION

Tamoxifen is indicated for the adjuvant treatment of estrogen-receptor (ER) positive breast cancer, effectively reducing the annual breast cancer death rate with almost one-third.[1] Tamoxifen and its metabolites act as selective ER modulators (SERM) and have antagonistic effects on the ER in breast cancer cells, yielding anti-tumor effects by prevention of estrogen-mediated tumor cell growth.[2] However, tamoxifen can act as an ER agonist in other tissues.[2] These tissue-specific ER agonistic or antagonistic effects of tamoxifen are determined by several factors including tissue-specific expression of the two ER subtypes (ERα and ERβ) and availability of intracellular coactivators and corepressors for ER-dependent target genes.[3]

Tamoxifen treatment is associated with various side effects, of which hot flashes, joint pain, vaginal dryness and insomnia are most commonly reported.[4] These side effects are caused by the ER agonistic or antagonistic effects of tamoxifen and its metabolites in tissues other than breast cancer cells. For example, tamoxifen treatment can stimulate endometrial cell growth by agonistic effects on endometrial tissue, whereas it can cause hot flashes by its antagonistic effects in the central nervous system. An alarming observation is that tamoxifen increases the risk of venous thromboembolism (VTE): tamoxifen-treated patients have a 2-3.5 fold increased risk of developing a VTE compared to breast cancer patients without adjuvant tamoxifen treatment. The reported VTE incidence is 1-3% during tamoxifen therapy and most events occur within the first 2 years of treatment.[5,6] Next to being potentially life-threatening in severe cases, VTE can lead to significant morbidity, a lower quality of life and psychological stress.[7,8] Moreover, anticoagulant therapy for treatment and secondary prevention of VTE can increase the risk of bleeding. Therefore, a better understanding of tamoxifen-associated VTE is essential to optimize patient treatment.

Currently, the mechanisms underlying the prothrombotic properties of tamoxifen treatment remain largely unclear. Some studies have shown that tamoxifen treatment is associated with a reduction in plasma levels of various anticoagulant proteins, including protein C, antithrombin and tissue factor pathway inhibitor (TFPI), and an increase in thrombin generation potential, suggestive of a procoagulant state.[9-11] Although there is currently no direct evidence for a dose-dependent effect of tamoxifen on VTE risk, one study found higher levels of the anticoagulant antithrombin in patients who received low daily tamoxifen doses (1 mg or 5 mg) compared with the standard of 20 mg.[12] While a higher tamoxifen dose is not associated with an increase in patient-reported side effects such as hot flashes and vaginal dryness[13-15], it is essential to determine if higher levels of tamoxifen and its metabolites are linked to an increased procoagulant state, which could possibly further increase VTE risk.

Tamoxifen itself has relatively low affinity for the ER and is converted into 4-hydroxytamoxifen or n-desmethyltamoxifen and subsequently to endoxifen by various hepatic cytochrome P450 (CYP) enzymes, mainly CYP2D6 and CYP3A4.[16] Endoxifen is considered the most important metabolite for treatment efficacy.[17] Endoxifen has a much higher affinity for the ER than n-desmethyltamoxifen[17,18] and a similar affinity as 4-hydroxytamoxifen, but endoxifen plasma concentrations are up to 14-fold higher than the latter.[17,19] Since low endoxifen plasma levels are associated with increased breast cancer recurrence rates[20], an efficacy threshold of minimally 16 nM endoxifen is generally accepted for tamoxifen precision dosing.[21,22] Given that one out of five patients do not reach this threshold on the standard daily dose of 20 mg tamoxifen, therapeutic drug monitoring (TDM) of tamoxifen and endoxifen plasma levels could be useful to select patients who require an increase in tamoxifen dose.[13] Particularly tamoxifen plasma levels often become significantly higher than population average upon tamoxifen dose escalation[14] and both tamoxifen and endoxifen levels have a high interpatient variability regardless of dose.[23] Therefore, it is essential to determine the possible implications of higher concentrations of both tamoxifen and its primary metabolite endoxifen on VTE risk.

Here we investigated whether higher plasma levels of tamoxifen and endoxifen are associated with a procoagulant state of the coagulation system. For this, we assessed if tamoxifen and endoxifen plasma levels correlated with 1) levels of various pro- and anti-coagulant proteins which were previously demonstrated to be affected by tamoxifen[9-11], and 2) thrombin generation parameters in patients undergoing TDM of adjuvant tamoxifen treatment for primary breast cancer. In addition, we investigated the time-dependent effects of tamoxifen on coagulation parameters.

MATERIAL AND METHODS

The current study was a secondary analysis from the TOTAM (Therapeutic drug monitoring Of TAMoxifen) study: a prospective intervention study on the feasibility of TDM of tamoxifen coordinated by the Erasmus MC Cancer Institute in Rotterdam, the Netherlands.[13] This study was approved by the local Medical Ethics Committee in January 2018 (MEC 2017-548) and registered in the International Clinical Trial Registry Platform (ICTRP; https://trialsearch.who.int; NL6918). Patients were included in this specific part from the study between November 2020 and November 2021. Informed consent was obtained from all participants.

Study design

As described in the original study, female patients who used adjuvant tamoxifen 20 mg daily for primary breast cancer were included after 3 months (3 m) of therapy.[13,24,25] Steady-state tamoxifen and endoxifen levels were measured at study inclusion. If endoxifen levels were below the treatment threshold of 16 nM, tamoxifen dose was increased to 30 mg or 40 mg daily. If endoxifen levels were above or equal to 32 nM and patients reported bothersome side effects, tamoxifen dose could be reduced to 10 mg daily. Tamoxifen and endoxifen levels were measured again after 6 months (6 m) of tamoxifen therapy. At both the 3m and 6m timepoints, coagulation analyses were performed. Patients who were diagnosed with recurrence of breast cancer or a new primary cancer within 1 year after start of tamoxifen were excluded to eliminate the effect of a (new) active malignancy on coagulation protein measurements. Also, measurements were excluded from analyses if patients were using anticoagulant therapy (direct oral anticoagulants, vitamin K antagonist or low molecular weight heparins) at the time of sampling. VTE events within 1 year of tamoxifen therapy initiation were identified by manual chart review of the electronic medical record and all VTE events were diagnosed using radiologic imaging.

Pharmacokinetic analysis

Tamoxifen and endoxifen trough (C_{min}) plasma concentrations were measured in blood samples after 3 m and 6 m of tamoxifen therapy, using a validated ultra-performance liquid chromatography with a tandem mass spectrometry method (UP-LCMS/MS).[26]

Coagulation analyses

In all available blood samples, protein C, antithrombin, tissue factor and thrombin generation parameters were determined after 3 m and 6 m of tamoxifen therapy. For protein C, antithrombin and thrombin generation analyses blood was collected in citrate tubes, while for tissue factor determination blood was sampled in lithium heparin tubes. Plasma levels of protein C and antithrombin were determined using a chromogenic assay (respectively Berichrom® Protein C and INNOVANCE® Antithrombin) on a Sysmex CS5100 (Siemens Healthineers). Circulating tissue factor was assessed using an enzyme-linked immunosorbent assay (ELISA) (Human Coagulation Factor III/ Tissue factor Quantikine ELISA; R&D systems). Thrombin generation was adapted from protocols using low plasma volumes as previously described.[27,28] Thrombin generation curves were obtained from reactions of patient plasma supplemented with either PPPlow reagent (Stago) containing tissue factor and phospholipids (i.e. with exogenous tissue factor) or with phospholipids only (phospholipid-TGT, Rossix; final concentration 4 µM; i.e. without exogenous tissue factor). Thrombin formation was initiated by the addition of substrate buffer (FluCa, Stago). The final reaction volume was 60 µL, of which 40 µL was

plasma. Thrombin formation was determined every 15 seconds for 90-120 minutes and corrected for the calibrator using Thrombinoscope software. The thrombin generation parameters determined were: endogenous thrombin potential (ETP or area under the curve), thrombin peak, lag time, time to peak, and velocity index.[29] The ETP represents the total amount of thrombin generated over time; the thrombin peak is the maximum concentration of thrombin generated; the lag time is defined as the time between the addition of the trigger until the initiation of thrombin generation; the time to peak is the time required to reach the peak of thrombin generation, and the velocity index is a composite index defined as [peak height / (time to peak – lag time)].

Statistical analysis

Normal distribution of the data was assessed using the Shapiro Wilk test. Patients were stratified to quartiles (Q1-Q4) based on their tamoxifen and endoxifen plasma levels at the 3 m and 6 m timepoints separately. Subsequently, levels of coagulation proteins and thrombin generation were compared between quartiles, with the lowest quartile (Q1) serving as the reference group, using ANOVA with Dunnett's test or Kruskall-Wallis with a Bonferroni correction approach (p-value times 3, i.e. the number of comparisons) to reduce the risk of type-1 error associated with multiple comparisons. Correlations between coagulation proteins and absolute tamoxifen and endoxifen concentrations were determined using Spearman's rank correlation. To assess the time-effect of tamoxifen treatment, levels of coagulation proteins and thrombin generation were compared between 3 m and 6 m with the paired sample t-test or Wilcoxon signed rank test. Also, coagulation parameters were compared between patients who received chemotherapy and patient who did not receive chemotherapy with unpaired samples t-test or Mann-Whitney U test. If data was missing for specific measurements patients were excluded from these analyses. Data were analysed using SPSS Statistics (IBM version 28.0.1.0) and p values < 0.05 were considered statistically significant.

RESULTS

From the total cohort of 144 patients, three patients were excluded because of the development of a second malignancy ($n = 2$) or metastatic breast cancer ($n = 1$) within one year after initiation of tamoxifen treatment. In total, 141 patients were eligible for this study. Patient characteristics are summarized in **Table 1**. Median age was 58 [IQR 49-67] and most patients had stage 1 or 2 disease with the no special type as the most common subtype (78%). The majority of patients had received both breast conserving surgery and radiotherapy prior to the start of tamoxifen treatment (60%), almost half received (neo)adjuvant chemotherapy (45%) and approximately 10% of patients received adjuvant anti-HER2 therapy.

Table 1. Baseline characteristics of the study participants

Baseline characteristics (*n* = 141)		**Median [IQR] or *n* (%)**
Age		58 [49-67]
BMI		26.4 [23.7-30.2]
Tumor stage		
	T1	70 (49.6)
	T2	57 (40.4)
	T3	12 (8.5)
	T4	2 (1.4)
Nodal stage		
	N0	80 (56.7)
	N1	45 (31.9)
	N2	13 (9.2)
	N3	3 (2.1)
Tumor pathology		
	NST	110 (78.0)
	Lobular	25 (17.7)
	Other	6 (4.3)
Histological grade (BR)		
	I	14 (9.9)
	II	101 (71.6)
	III	26 (18.4)
Local treatment		
	BCS only	2 (1.4)
	BCS + RTx	85 (60.3)
	Mastectomy only	28 (19.9)
	Mastectomy + RTx	26 (18.4)
(Neo)adjuvant chemotherapy		
	Yes	63 (44.7)
	No	78 (55.3)
(Neo)adjuvant anti-HER2 therapy		
	Yes	13 (9.2)
	No	128 (90.8)
Smoking status		
	Current smoker	13 (9.2)
	Former smoker	46 (32.6)
	Never smoker	79 (56.0)
	Unknown	3 (2.1)
History of VTE		3 (2.1)

Age and BMI were determined at the time of first blood sampling (after 3 months of tamoxifen therapy). Abbreviations: BMI body mass index, BCS breast conserving surgery, IQR interquartile range, NST no special type, RTx radiotherapy, VTE venous thromboembolism

VTE occurrence

VTE occurred in 7 (5.0%) of the included patients within one year after start of tamoxifen treatment. These VTE consisted of: deep venous thrombosis (*n* = 3), superficial thrombophlebitis (*n* = 3) and pulmonary embolism (*n* = 1). The characteristics of these VTE events are specified in **Supplementary Table A**. All three patients with a medical history of VTE in our cohort experienced a VTE event again. None of the patients used anticoagulation during tamoxifen therapy given that these previous events of VTE had occurred more than 5 years prior to initiation of tamoxifen treatment.

Correlation between tamoxifen or endoxifen levels and coagulation parameters

Coagulation parameters were available of 53-135 patients, depending on the specific parameter assessed and duration of tamoxifen treatment. The levels of coagulation parameters and tamoxifen and endoxifen plasma levels for the total study population can be found in **Supplementary table B**. The plasma tamoxifen levels ranged from 91 to 962 nM and correlated weakly with protein C at 3 m of treatment (r = 0.180, *p* = 0.039, **Figure 1A**), but not at 6 m of therapy (r = 0.090, *p* = 0.364, **Figure 1B**). When stratifying to tamoxifen plasma levels, no significant difference was observed when comparing protein C levels in the higher quartiles with those of the lowest quartile of patients at 3 m or 6 m of treatment (**Table 2**). No correlation was observed between the tamoxifen plasma levels and those of antithrombin or tissue factor (**Figures 1C-F**), and no significant difference was observed for the latter when comparing these based on quartiles of tamoxifen plasma levels (**Table 2**). In addition, tamoxifen levels did not correlate with parameters of thrombin generation triggered with either exogenously added or endogenously present tissue factor (**Table 3**). Overall, these data indicate that higher tamoxifen levels are weakly associated with higher levels of the anticoagulant protein C after 3 m of treatment, while no correlation indicative of a procoagulant state was observed.

The plasma endoxifen levels at 3 months ranged from 4 to 70 nM. For both 3 m and 6 m of tamoxifen therapy, no correlation was observed between plasma endoxifen levels and protein C or antithrombin levels (**Figure 2A-D**), neither when comparing these factors based on quartiles of endoxifen plasma levels (**Table 4**). In contrast, endoxifen levels correlated negatively with tissue factor at 6 m of treatment (r = -0.290, *p* = 0.004, **Figure 2F**). When stratified to quartiles of endoxifen levels, patients with higher endoxifen concentrations had lower tissue factor levels at 6 m of therapy (Q3: 56.6 [33] pg/ml and Q4: 50.1 [19] pg/mL versus Q1: 75.6 [69] pg/mL, adjusted *p* values of 0.027 and 0.018, respectively) (**Table 4**). No correlation with tissue factor levels was observed at 3 m of tamoxifen treatment. Thrombin generation parameters did

not correlate with endoxifen levels at any timepoint (**Table 5**). These data indicate that higher endoxifen levels are associated with lower circulating levels of the procoagulant tissue factor, which is not associated with a procoagulant state.

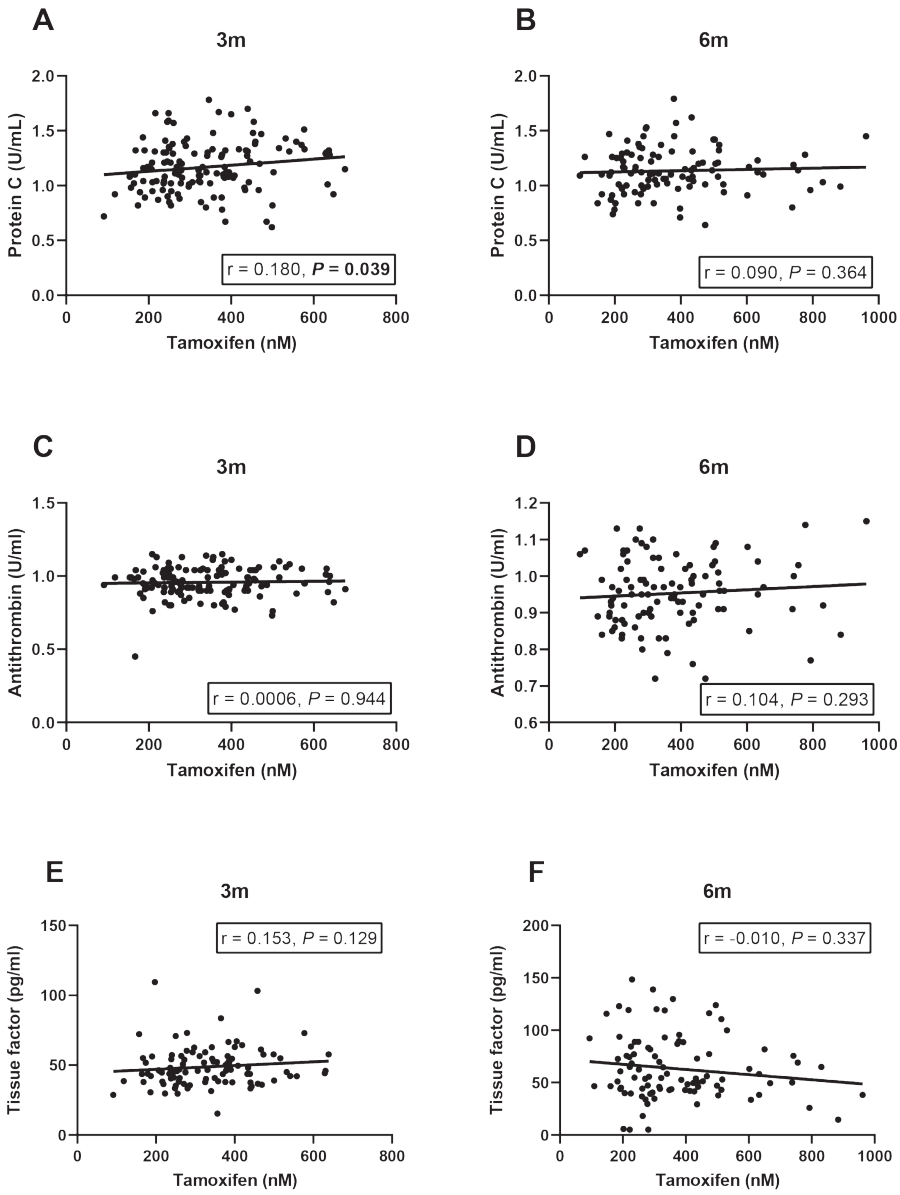

Figure 1. Correlation of tamoxifen plasma levels after 3 months and 6 months of tamoxifen treatment with **A+B,** protein C (n = 133 and n = 104, respectively), **C+D** antithrombin (n = 135 and n = 104, respectively), and **E+F** tissue factor (n = 100 and n = 95, respectively).

Table 2. Levels of coagulation factors by quartiles of tamoxifen plasma levels

3 months of tamoxifen				
	Q1	**Q2**	**Q3**	**Q4**
Tamoxifen (nM)	**91-246** ***n*** **= 34**	**246-325** ***n*** **= 35**	**325-432** ***n*** **= 33**	**432-676** ***n*** **= 33**
Protein C (U/mL)	1.122 (0.21) *n* = 34	1.169 (0.21) *n* = 34	1.153 (0.25) *n* = 33	1.233 (0.25) *n* = 32
Antithrombin (U/mL)	0.955 (0.12) *n* = 34	0.950 (0.08) *n* = 35	0.965 (0.11) *n* = 33	0.963 (0.09) *n* = 33
Tissue factor (pg/mL)	44.1 [14] *n* = 26	46.0 [21] *n* = 25	50.1 [13] *n* = 29	44.6 [20] *n* = 20
6 months of tamoxifen				
	Q1	**Q2**	**Q3**	**Q4**
Tamoxifen (nM)	**94-238** ***n*** **= 26**	**238-331** ***n*** **= 27**	**331-469** ***n*** **= 27**	**469-962** ***n*** **= 26**
Protein C (U/mL)	1.067 (0.19) *n* = 26	1.162 (0.20) *n* = 27	1.177 (0.24) *n* = 26	1.125 (0.20) *n* = 25
Antithrombin (U/mL)	0.947 (0.09) *n* = 26	0.953 (0.10) *n* = 27	0.938 (0.08) *n* = 26	0.974 (0.11) *n* = 25
Tissue factor (pg/mL)	66.6 [45] *n* = 24	53.5 [40] *n* = 25	52.0 [45] *n* = 24	55.6 [40] *n* = 22

Data are displayed as mean (SD) or median [IQR]. Data were missing for some participants in some subgroups. All comparisons were non-significant.

Table 3. Thrombin generation parameters by quartiles of tamoxifen plasma levels

3 months of tamoxifen				
	Q1	**Q2**	**Q3**	**Q4**
Tamoxifen (nM)	**91-246** ***n*** **= 34**	**246-325** ***n*** **= 35**	**325-432** ***n*** **= 33**	**432-676** ***n*** **= 33**
With exogenous tissue factor				
ETP (nM*min)	2238 (1070)	2139 (357)	2178 (388)	2040 (279)
Thrombin peak (nM)	236 (59)	267 (60)	260 (59)	238 (51)
Lag time (min)	7.2 (1.6)	6.6 (1.3)	7.1 (1.6)	7.3 (1.4)
Time to peak (min)	11.6 [3]	10.5 [2]	11.5 [3]	11.8 [3]
Velocity index (nM/min)	53.0 (19)	66.5 (23)	61.5 (21)	59.5 (21)
Without exogenous tissue factor				
ETP (nM*min)	1083 (563)	1170 (361)	1260 (343)	1118 (435)
Thrombin peak (nM)	41.4 (39)	67.5 (36)	73.7 (43)	58.2 (35)
Lag time (min)	37.2 [63]	37.4 [23]	35.0 [39]	36.8 [48]
Time to peak (min)	58.5 [50]	44.0 [21]	43.1 [29]	44.9 [29]
Velocity index (nM/min)	4.0 [9]	9.8 [10]	9.9 [18]	11.3 [15]
6 months of tamoxifen				
	Q1	**Q2**	**Q3**	**Q4**
Tamoxifen (nM)	**94-238** ***n*** **= 26**	**238-331** ***n*** **= 27**	**331-469** ***n*** **= 27**	**469-962** ***n*** **= 26**
With exogenous tissue factor				
ETP (nM*min)	2160 (450)	2128 (379)	2118 (504)	2028 (312)
Thrombin peak (nM)	248 (67)	257 (66)	253 (78)	248 (58)
Lag time (min)	6.1 [2]	6.0 [2]	6.8 [5]	6.1 [5]
Time to peak (min)	10.5 [2]	10.5 [2]	11.0 [3]	10.0 [2]
Velocity index (nM/min)	56 (20)	60 (23)	59 (26)	59 (22)
Without exogenous tissue factor				
ETP (nM*min)	1301 (442)	945 (467)	1433 (391)	1095 (550)
Thrombin peak (nM)	69 [69]	68 [62]	79 [110]	68 [127]
Lag time (min)	32.0 (13)	31.6 (13)	26.0 (14)	31.3 (19)
Time to peak (min)	43 [18]	39 [11]	33 [17]	40 [23]
Velocity index (nM/min)	12.8 [13]	10.4 [15]	15.1 [28]	11.7 [39]

Data are displayed as mean (SD) or median [IQR]. Data were missing for some participants in some subgroups. All comparisons were non-significant. Abbreviations: ETP endogenous thrombin potential.

Table 4. Levels of coagulation factors by quartiles of endoxifen

3 months of tamoxifen				
	Q1	**Q2**	**Q3**	**Q4**
Endoxifen (nM)	**4-17** ***n* = 34**	**17-26** ***n* = 35**	**26-37** ***n* = 33**	**37-70** ***n* = 33**
Protein C (U/mL)	1.155 (0.22) *n* = 34	1.162 (0.21) *n* = 34	1.155 (0.23) *n* = 32	1.202 (0.28) *n* = 33
Antithrombin (U/mL)	0.967 (0.09) *n* = 34	0.944 (0.12) *n* = 35	0.959 (0.08) *n* = 33	0.960 (0.11) *n* = 33
Tissue factor (pg/mL)	44.7 [16] *n* = 27	43.0 [10] *n* = 20	52.2 [19] *n* = 27	46.5 [19] *n* = 26
6 months of tamoxifen				
	Q1	**Q2**	**Q3**	**Q4**
Endoxifen (nM)	**11-20** ***n* = 26**	**20-25** ***n* = 27**	**25-34** ***n* = 27**	**34-70** ***n* = 26**
Protein C (U/mL)	1.144 (0.18) *n* = 25	1.137 (0.17) *n* = 27	1.032 (0.21) *n* = 27	1.230 (0.23) *n* = 25
Antithrombin (U/mL)	0.941 (0.09) *n* = 25	0.979 (0.08) *n* = 27	0.935 (0.09) *n* = 27	0.955 (0.10) *n* = 25
Tissue factor (pg/mL)	75.6 [69] *n* = 23	65.7 [46] *n* = 26	**56.6 [33]*** *n* = 25	**50.1 [19]*** *n* = 21

Data are displayed as mean (SD) or median [IQR]. Data were missing for some participants in some subgroups. *p-value <0.05 versus Q1, all other comparisons were non-significant. Abbreviations: ETP endogenous thrombin potential

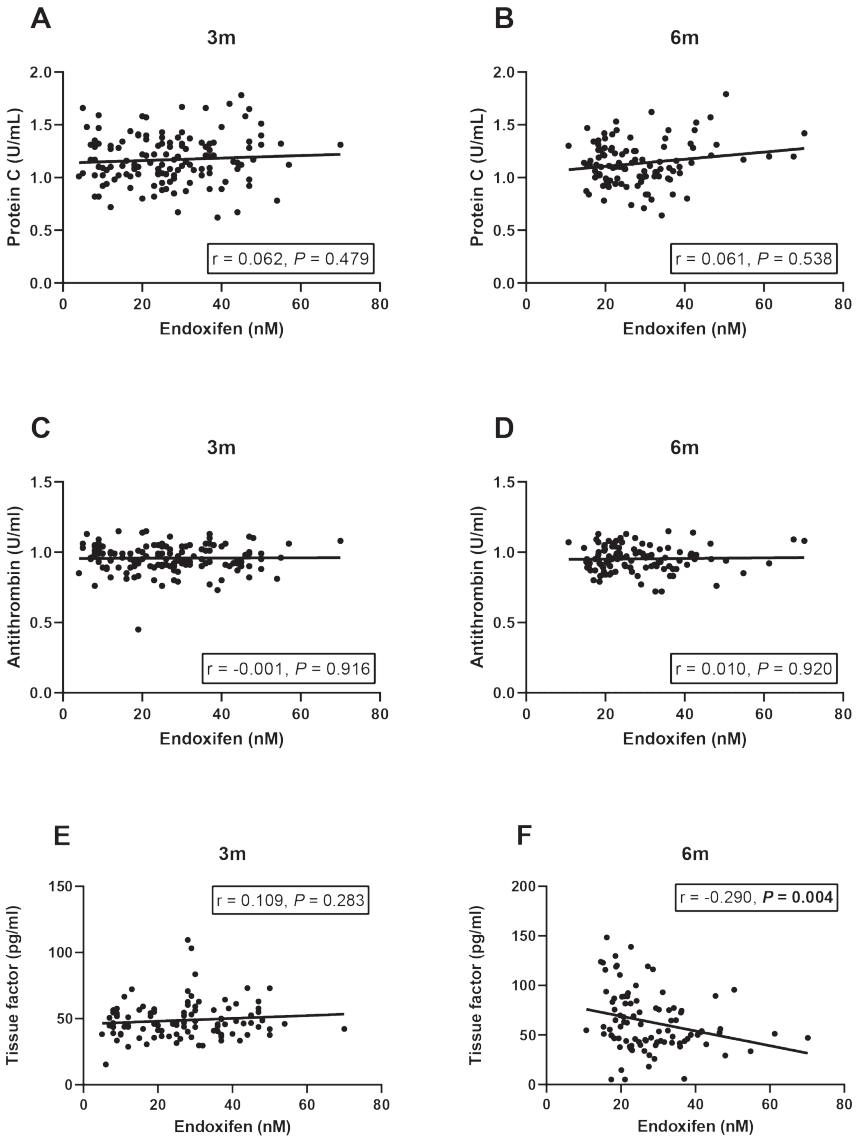

Figure 2. Correlation of endoxifen plasma levels after 3 months and 6 months of tamoxifen treatment with **A+B,** protein C (n = 133 and n = 104, respectively), **C+D** antithrombin (n = 135 and n = 104, respectively), and **E+F** tissue factor (n = 100 and n = 95, respectively).

Time-dependent effect of tamoxifen treatment on coagulation

The time-dependent effect of tamoxifen treatment on coagulation was determined in patients who remained on 20 mg tamoxifen daily (n = 80) during the study period (**Table 6**). Compared to the 3 m timepoint, median tissue factor levels were significantly higher after 6 m of therapy (46.0 vs. 54.4 pg/mL, respectively, $p < 0.001$). In line with this, thrombin generation initiated by endogenous tissue factor was enhanced at 6 m relative to 3 m of therapy, reflected by a significant increase in thrombin peak and velocity index, and shortened lag time and time to peak. Parameters of thrombin generation triggered by exogenous tissue factor and levels of protein C and antithrombin were similar between 3 m and 6 m of therapy. This significant increase in circulating tissue factor levels after 6 m of treatment was also observed in patients who received a tamoxifen dose increase to 30 or 40 mg daily (**Supplementary Table C**) as well as in patients in whom the tamoxifen dose was decreased to 10 mg daily (**Supplementary Table D**). This coincided with a significant increase in thrombin peak in endogenously triggered thrombin generation for patients who switched to 10 mg tamoxifen (**Supplementary Table D**). The levels of protein C and antithrombin remained similar in both patient groups.

Coagulation parameters in chemotherapy-treated patients versus patients who did not receive chemotherapy

All coagulation parameters were separately analysed in the group of patients that received (neo-)adjuvant chemotherapy for their breast cancer and in patients who did not receive chemotherapy (**Supplementary table E + F**). Patients who received chemotherapy did not demonstrate an increase in procoagulant parameters or decrease in anticoagulant parameters, except for a slightly shorter lag time after 6 m of treatment only.

Table 5. Thrombin generation parameters by quartiles of endoxifen

3 months of tamoxifen				
	Q1	**Q2**	**Q3**	**Q4**
Endoxifen (nM)	**4-17** ***n*** **= 34**	**17-26** ***n*** **= 35**	**26-37** ***n*** **= 33**	**37-70** ***n*** **= 33**
With exogenous tissue factor				
ETP (nM*min)	2133 (369)	2292 (1177)	2057 (282)	2206 (442)
Thrombin peak (nM)	261 (59)	245 (65)	244 (51)	255 (65)
Lag time (min)	7.0 (1.5)	7.3 (1.8)	6.8 (1.4)	7.0 (1.2)
Time to peak (min)	10.9 [2.0]	11.6 [3.7]	10.9 [2.3]	12.1 [2.4]
Velocity index (U/mL)	66 (25)	56 (21)	56 (18)	59 (20)
Without exogenous tissue factor				
ETP (nM*min)	1304 (339)	1351 (431)	977 (399)	1122 (387)
Thrombin peak (nM)	75 (45)	77 (47)	48 (32)	48 (26)
Lag time (min)	31 [44]	34 [21]	37 [36]	50 [28]
Time to peak (min)	45 [21]	43 [22]	48 [32]	62 [22]
Velocity index (U/mL)	11.3 [17]	14.5 [13]	5.5 [5.8]	6.3 [8.2]
6 months of tamoxifen				
	Q1	**Q2**	**Q3**	**Q4**
Endoxifen level (nM)	**11-20** ***n*** **= 26**	**20-25** ***n*** **= 27**	**25-34** ***n*** **= 27**	**34-70** ***n*** **= 26**
With exogenous tissue factor				
ETP (nM*min)	2156 (463)	1975 (396)	2109 (325)	2271 (436)
Thrombin peak (nM)	270 (84)	235 (65)	236 (49)	271 (52)
Lag time (min)	6.0 [6]	6.1 [3]	6.1 [2]	7.0 [6]
Time to peak (min)	9.9 [1]	10.9 [3]	10.9 [2]	10.8 [2]
Velocity index	67 (30)	54 (20)	50 (16)	62 (15)
Without exogenous tissue factor				
ETP (nM*min)	1252 (534)	996 (383)	1083 (478)	1463 (499)
Thrombin peak (nM)	87 [146]	51 [24]	79 [68]	80 [147]
Lag time (min)	26.8 (18)	34.9 (14)	32.3 (8.5)	26.7 (14)
Time to peak (min)	36 [21]	41 [10]	36 [17]	33 [24]
Velocity index (U/mL)	16.3 [42]	9.0 [7]	15.1 [15]	15.1 [34]

Data are displayed as mean (SD) or median [IQR]. Data were missing for some participants in some subgroups. All comparisons were non-significant. Abbreviations: ETP endogenous thrombin potential.

Table 6. Time-dependent effect of tamoxifen treatment on coagulation parameters in patients who remained on 20 mg tamoxifen daily during the study

		3 months	6 months
Protein C (U/mL)	*n* = 66	1.19 (0.25)	1.16 (0.22)
Antithrombin (U/mL)	*n* = 67	0.95 (0.08)	0.94 (0.09)
Tissue factor (pg/mL)	*n* = 55	46.0 [15.4]	**54.4 [38.8]*****
Thrombin generation parameters			
With exogenous tissue factor			
ETP (nM*min)	*n* = 40	2080 (369)	2170 (387)
Thrombin peak (nM)	*n* = 40	245 (58)	256 (65)
Lag time (min)	*n* = 40	6.9 (1.4)	9.0 (7.9)
Time to peak (min)	*n* = 40	11.3 [2.8]	10.5 [2.0]
Velocity index (nM/min)	*n* = 40	56.3 (21)	59.12 (19)
Without exogenous tissue factor			
ETP (nM*min)	*n* = 29	1137 (414)	1276 (463)
Thrombin peak (nM)	*n* = 30	59.5 (35)	**97.0 (66)*****
Lag time (min)	*n* = 30	38.7 [20]	**29.7 [19]*****
Time to peak (min)	*n* = 30	44.0 [21]	**38.8 [18]****
Velocity index (nM/min)	*n* = 30	9.10 [9.4]	**12.9 [23]****

Data are displayed as mean (SD) or median [IQR]. Data were missing for some participants in some subgroups. Abbreviations: ETP: endogenous thrombin potential. P value indicates results of paired t-test or Wilcoxon signed rank test. **p value <0.01, ***p value <0.001, all other comparisons were non-significant.

DISCUSSION

This study is the first to assess if the procoagulant effects of tamoxifen are associated with plasma levels of tamoxifen and its primary active metabolite endoxifen in a representative cohort of patients with primary breast cancer receiving adjuvant tamoxifen. By measurement of both various coagulation proteins previously shown to be affected by tamoxifen[9,10] and thrombin generation parameters, we demonstrate that higher plasma levels of tamoxifen and endoxifen are not associated with higher procoagulant or lower anticoagulant parameters. These findings provide a first indication that higher tamoxifen or endoxifen levels do not have an additional procoagulant effect and therefore might not lead to an further increased risk of tamoxifen-related VTE.

Levels of antithrombin and protein C were previously demonstrated to decrease during tamoxifen therapy, but these studies did not measure tamoxifen and endoxifen plasma levels.[9,10] Our study shows that endoxifen levels do not correlate with these anticoagulant factors. Protein C correlated positively with tamoxifen levels after 3

months of therapy. Despite this weak correlation, this observation points to a possible anticoagulant effect that did not persist at 6 months of treatment. Normal levels of protein C range from approximately 0.70 to 1.40 IU/mL and levels approximately below are associated with a significant increase in VTE risk.[30] Most protein C levels observed in our cohort fall within this normal range and are therefore not considered specifically low.[31] Although antithrombin levels in our cohort were slightly lower than earlier described in healthy controls [median 1.04 IU/mL], most still fell within the normal range of 0.80-1.20 IU/mL and were above the lower limit earlier associated with an increased VTE risk.[30,32] In addition, antithrombin did not have any association with higher concentrations of tamoxifen nor endoxifen.

To the best of our knowledge, the procoagulant protein tissue factor has not been directly measured in the context of tamoxifen therapy before. It has been shown that levels of the anticoagulant protein TFPI decrease during treatment with tamoxifen.[11] Given that this factor inhibits the activity of the tissue factor FVIIa complex in a FXa-dependent manner, this tamoxifen-induced TFPI decrease potentially leads to a hypercoagulable state.[33] Here we found that the endoxifen levels are negatively, albeit modestly, correlated with circulating tissue factor levels after 6 months of tamoxifen treatment. Thus, if tissue factor has any correlation with plasma levels during tamoxifen therapy at all, this is most likely in the direction of anti-coagulation. Importantly, the effect of tamoxifen and endoxifen levels on TFPI has not been studied yet, and the eventual net outcome on tissue factor / TFPI signalling remains therefore currently unclear.

To gain a better understanding of the possible effect of tamoxifen and endoxifen levels on a procoagulant state during tamoxifen, we performed thrombin generation assays which provide a more comprehensive evaluation of coagulation relative to the prothrombin time (PT) and activated partial thromboplastin time (APTT) clotting assays.[29] Given that the parameters of thrombin generation were similar between all patients stratified for tamoxifen and endoxifen plasma concentrations, this further indicates that an increase in plasma levels of tamoxifen and endoxifen plasma concentrations does not coincide with a procoagulant potential. Interestingly, we found increased thrombin generation after 6 months compared to 3 months of treatment, independent from any tamoxifen dose adjustments. This was only observed in the condition without exogenous addition of tissue factor suggesting that this enhanced thrombin generation is tissue factor-mediated. Indeed, tissue factor increased after 6 months compared to 3 months of treatment. Although our observed number of VTE events was small, the majority of patients (4 out of 7 patients) experienced an event between approximately 3 and 6 months after start of tamoxifen therapy. This could indicate that patients, within the first year of tamoxifen treatment, experience the

highest thrombotic risk during this time period. Of note, all patients with a previous history of VTE developed a VTE again during tamoxifen therapy. Although the small number of events has to be taken into account, this observation might suggest that patients with a history of VTE have the highest prothrombotic risk during tamoxifen treatment. Further studies on the effects of tamoxifen on tissue factor and tissue factor signalling at different timepoints would be interesting to gain a better insight in the general prothrombotic effects of tamoxifen and to study if tamoxifen increases VTE risk in a time-dependent manner. Moreover, in patients with a previous history of VTE, the possible extra risk of developing a VTE during tamoxifen therapy requires further study and warrants cautiousness in clinical practice.

In patients with breast cancer treated with adjuvant tamoxifen, there are other factors that can determine the prothrombotic risk. For example, chemotherapy, radiotherapy and surgery are all independent risk factors for VTE.[34-36] Since most patients had completed their chemotherapy and radiotherapy treatments before the start of tamoxifen, the influence of these treatments on VTE risk was probably minimal and even further diminished over time. Although these other treatments might directly affect various coagulation factors including a possible increase in the procoagulant tissue factor as well, we found an increase rather than a decrease in tissue factor levels over time (i.e. longer after completion of the of the other treatments). This makes it more likely that tamoxifen is directly responsible for the observed increase in tissue factor in this study. Also, no consistent trend towards an increase in procoagulant or a decrease in antiocoagulant factors was observed in patients who received chemotherapy compared to patients who did not receive chemotherapy. Lastly, although the presence of (recurrent) cancer is an independent risk factor for VTE,[37] the recurrence rate for ER-positive breast cancer is generally low, especially in the first year.[1] Also, patients who developed clinical breast cancer recurrence (n = 1) or a new primary cancer (n = 2) within one year after start of tamoxifen therapy were excluded from our analyses. Therefore, it is very unlikely that any of the included patients had (recurrent) cancer at time of measurements and status of cancer did thus probably not influence the observed time-dependent effect of tamoxifen treatment on tissue factor and thrombin generation levels.

Given that treatment with aromatase inhibitors, another adjuvant endocrine treatment for ER-positive breast cancer, does not predispose to VTE[38], we hypothesized that the pro-thrombotic effects of tamoxifen are predominately mediated via the ER, rather than estrogenic effects specifically. Endoxifen and 4-hydroxytamoxifen are the metabolites with the highest affinity for the ER.[17] However, tamoxifen and endoxifen reach up to respectively 14- and 40-fold higher plasma concentrations than 4-hydroxytamoxifen.[10,18]

Although n-desmethyltamoxifen reaches slightly higher plasma concentrations than tamoxifen, its affinity for the ER is 100 times lower and this metabolite is therefore considered to be of minor importance.[18] Hence, the inclusion of both tamoxifen and its primary metabolite endoxifen was a strength of the current study. Although more research is needed to definitely rule out a role for the other metabolites in the increased VTE risk, tamoxifen has linear pharmacokinetics, indicating that higher levels of tamoxifen and endoxifen were most likely paralleled by higher levels of 4-hydroxytamoxifen and n-desmethyltamoxifen as well. Unfortunately, our study was underpowered to investigate if higher tamoxifen or endoxifen levels predispose to VTE since the number of events was limited. However, our observations provide a first indication that levels of tamoxifen and endoxifen are not associated with increased VTE risk. Also, tamoxifen and endoxifen levels from patients who experienced a VTE did not differ substantially from the median tamoxifen and endoxifen levels in our total study population. In fact, in three out of five patients tamoxifen and endoxifen levels before the occurrence of the VTE belonged to the lowest quartiles. A previous phase I study in which endoxifen was administered as a drug itself (rather than tamoxifen) found that only one out of the 38 included patients with metastatic breast cancer developed a VTE (2.6%).[39] This low VTE incidence despite endoxifen plasma levels 10-100 times higher (360-5200 nM) than in our current study[39] and the fact that included patients had metastatic breast cancer, which is an independent VTE risk factor[40], further suggest that higher endoxifen levels do not predispose to higher VTE risk.

The current study has some limitations. First, samples before start of tamoxifen therapy were not available. Therefore, the direct relationship between the included coagulation proteins and tamoxifen treatment could not be validated. However, we focused on the relationship between tamoxifen and endoxifen concentrations and coagulation system activation. In addition, we performed intra-patient comparisons. Therefore, baseline samples were not essential to answer our primary research questions. Secondly, although we carefully selected the measured coagulation parameters based on previous studies and additionally performed thrombin generation assays, a selection of surrogate markers for a procoagulant state was used. In future studies, the direct correlation between tamoxifen and endoxifen plasma levels and VTE should be investigated. Thirdly, samples were missing for some measurements which could limit statistical power. Fourthly, no definite conclusions can be drawn for substantially higher or lower tamoxifen levels than observed in our study. As the standard dose of 20 mg daily is most frequently prescribed and is thus representative for the current clinical practice, the number of patients using higher or lower tamoxifen doses was limited here. Therefore, more research in patients using non-standard tamoxifen doses is required.

In conclusion, our study indicates that higher tamoxifen and endoxifen levels are not correlated with an increased procoagulant state. Although adequate monitoring of VTE remains important, this provides a first indication that a TDM-directed tamoxifen dose escalation does not additionally increase VTE risk.

REFERENCES

1. Early Breast Cancer Trialists Collaborative Group. Effects of chemotherapy and hormonal therapy for early breast cancer on recurrence and 15-year survival: an overview of the randomised trials. Lancet 2005; 365(9472): 1687-717.
2. Martinkovich S, Shah D, Planey SL, Arnott JA. Selective estrogen receptor modulators: tissue specificity and clinical utility. Clinical Interventions in Aging 2014; 9: 1437-52.
3. Riggs BL, Hartmann LC. Selective estrogen-receptor modulators -- mechanisms of action and application to clinical practice. New England Journal of Medicine 2003; 348(7): 618-29.
4. Land SR, Wickerham DL, Costantino JP, et al. Patient-Reported Symptoms and Quality of Life During Treatment With Tamoxifen or Raloxifene for Breast Cancer PreventionThe NSABP Study of Tamoxifen and Raloxifene (STAR) P-2 Trial. JAMA 2006; 295(23): 2742-51.
5. Amir E, Seruga B, Niraula S, Carlsson L, Ocaña A. Toxicity of adjuvant endocrine therapy in postmenopausal breast cancer patients: a systematic review and meta-analysis. Journal of the National Cancer Institute 2011; 103(17): 1299-309.
6. Hernandez RK, Sørensen HT, Pedersen L, Jacobsen J, Lash TL. Tamoxifen treatment and risk of deep venous thrombosis and pulmonary embolism: a Danish population-based cohort study. Cancer 2009; 115(19): 4442-9.
7. Goldhaber SZ, Visani L, De Rosa M. Acute pulmonary embolism: clinical outcomes in the International Cooperative Pulmonary Embolism Registry (ICOPER). Lancet 1999; 353(9162): 1386-9.
8. Simon N, Rhian L, Jodie W, Sarah L, Paul B. Long-term psychological consequences of symptomatic pulmonary embolism: a qualitative study. BMJ Open 2014; 4(4): e004561.
9. Mannucci PM, Bettega D, Chantarangkul V, Tripodi A, Sacchini V, Veronesi U. Effect of tamoxifen on measurements of hemostasis in healthy women. Arch Intern Med 1996; 156(16): 1806-10.
10. Blondon M, Bodmer A, Thouvenin L, et al. Differential impact of tamoxifen and aromatase inhibitors on thrombin generation: the prospective HEMOBREAST cohort. Blood Advances 2022; 6(9): 2884-92.
11. Erman M, Abali H, Oran B, et al. Tamoxifen-induced tissue factor pathway inhibitor reduction: a clue for an acquired thrombophilic state? Ann Oncol 2004; 15(11): 1622-6.
12. Decensi A, Robertson C, Viale G, et al. A randomized trial of low-dose tamoxifen on breast cancer proliferation and blood estrogenic biomarkers. Journal of the National Cancer Institute 2003; 95(11): 779-90.
13. Braal CL, Jager A, Hoop EO, et al. Therapeutic Drug Monitoring of Endoxifen for Tamoxifen Precision Dosing: Feasible in Patients with Hormone-Sensitive Breast Cancer. Clinical Pharmacokinetics 2022; 61(4): 527-37.
14. Dezentjé VO, Opdam FL, Gelderblom H, et al. CYP2D6 genotype- and endoxifen-guided tamoxifen dose escalation increases endoxifen serum concentrations without increasing side effects. Breast Cancer Res Treat 2015; 153(3): 583-90.
15. Hertz DL, Deal A, Ibrahim JG, et al. Tamoxifen Dose Escalation in Patients With Diminished CYP2D6 Activity Normalizes Endoxifen Concentrations Without Increasing Toxicity. Oncologist 2016; 21(7): 795-803.
16. Binkhorst L, Mathijssen RHJ, Jager A, van Gelder T. Individualization of tamoxifen therapy: Much more than just CYP2D6 genotyping. Cancer Treatment Reviews 2015; 41(3): 289-99.
17. Mürdter TE, Schroth W, Bacchus-Gerybadze L, et al. Activity levels of tamoxifen metabolites at the estrogen receptor and the impact of genetic polymorphisms of phase I and II enzymes on their concentration levels in plasma. Clinical Pharmacology & Therapeutics 2011; 89(5): 708-17.
18. Fabian C, Tilzer L, Sternson L. Comparative binding affinities of tamoxifen, 4-hydroxytamoxifen, and desmethyltamoxifen for estrogen receptors isolated from human breast carcinoma: correlation with blood levels in patients with metastatic breast cancer. Biopharm Drug Dispos 1981; 2(4): 381-90.
19. Stearns V, Johnson MD, Rae JM, et al. Active tamoxifen metabolite plasma concentrations after coadministration of tamoxifen and the selective serotonin reuptake inhibitor paroxetine. J Natl Cancer Inst 2003; 95(23): 1758-64.
20. Madlensky L, Natarajan L, Tchu S, et al. Tamoxifen Metabolite Concentrations, CYP2D6 Genotype, and Breast Cancer Outcomes. Clinical Pharmacology & Therapeutics 2011; 89(5): 718-25.
21. Groenland SL, van Eerden RAG, Westerdijk K, et al. Therapeutic drug monitoring based precision dosing of oral targeted therapies in oncology: a prospective multicentre study. Annals of Oncology 2022 Oct; 33(10): 1071-82.

22. Groenland SL, Verheijen RB, Joerger M, et al. Precision Dosing of Targeted Therapies Is Ready for Prime Time. Clinical Cancer Research 2021; 27(24): 6644-52.

23. Agema BC, Buijs SM, Sassen SDT, et al. Toward model-informed precision dosing for tamoxifen: A population-pharmacokinetic model with a continuous CYP2D6 activity scale. Biomedicine & Pharmacotherapy 2023; 160: 114369.

24. Braal CL, Westenberg JD, Buijs SM, et al. Factors affecting inter-individual variability in endoxifen concentrations in patients with breast cancer: results from the prospective TOTAM trial. Breast Cancer Res Treat 2022 Aug; 195(1): 65-74.

25. Buijs SM, Hoop EO, Braal CL, et al. The impact of endoxifen-guided tamoxifen dose reductions on endocrine side-effects in patients with primary breast cancer. ESMO Open 2023; 8(1): 100786.

26. Binkhorst L, Mathijssen RH, Ghobadi Moghaddam-Helmantel IM, et al. Quantification of tamoxifen and three of its phase-I metabolites in human plasma by liquid chromatography/triple-quadrupole mass spectrometry. J Pharmaceut Biomed 2011; 56(5): 1016-23.

27. Bloemen S, Kelchtermans H, Hemker HC. Thrombin generation in low plasma volumes. Thrombosis Journal 2018; 16: 10.

28. Hemker HC, Giesen P, Al Dieri R, et al. Calibrated automated thrombin generation measurement in clotting plasma. Pathophysiology of Haemostasis and Thrombosis 2003; 33(1): 4-15.

29. Tripodi A. Thrombin Generation Assay and Its Application in the Clinical Laboratory. Clinical Chemistry 2016; 62(5): 699-707.

30. Bucciarelli P, Passamonti SM, Biguzzi E, et al. Low borderline plasma levels of antithrombin, protein C and protein S are risk factors for venous thromboembolism. Journal of Thrombosis and Haemostasis 2012; 10(9): 1783-91.

31. Rodeghiero F, Tosetto A. The VITA Project: Population-based Distributions of Protein C, Antithrombin III, Heparin-cofactor II and Plasminogen -Relationship with Physiological Variables and Establishment of Reference Ranges. Thrombosis and Haemostasis 1996; 76(08): 226-33.

32. Croles FN, Van Loon JE, Dippel DWJ, De Maat MPM, Leebeek FWG. Antithrombin levels are associated with the risk of first and recurrent arterial thromboembolism at a young age. Atherosclerosis 2018; 269: 144-50.

33. Mast AE. Tissue Factor Pathway Inhibitor: Multiple Anticoagulant Activities for a Single Protein. Arteriosclerosis, Thrombosis, and Vascular Biology 2016; 36(1): 9-14.

34. Temraz S, Moukalled N, Gerotziafas GT, et al. Association between Radiotherapy and Risk of Cancer Associated Venous Thromboembolism: A Sub-Analysis of the COMPASS-CAT Study. Cancers (Basel) 2021; 13(5).

35. Maesaka JY, Reis YN, Elias LM, Akerman D, Baracat EC, Filassi JR. Venous thromboembolism incidence in postoperative breast cancer patients. Clinics 2023; 78: 100229.

36. Lyman GH, Eckert L, Wang Y, Wang H, Cohen A. Venous thromboembolism risk in patients with cancer receiving chemotherapy: a real-world analysis. Oncologist 2013; 18(12): 1321-9.

37. Caio JF, Luciana TKM, José L. Alves J, et al. Cancer-associated thrombosis: the when, how and why. European Respiratory Review 2019; 28(151): 180119.

38. Xu X, Chlebowski RT, Shi J, Barac A, Haque R. Aromatase inhibitor and tamoxifen use and the risk of venous thromboembolism in breast cancer survivors. Breast Cancer Research and Treatment 2019; 174(3): 785-94.

39. Goetz MP, Suman VJ, Reid JM, et al. First-in-Human Phase I Study of the Tamoxifen Metabolite Z-Endoxifen in Women With Endocrine-Refractory Metastatic Breast Cancer. Journal of Clinical Oncology 2017; 35(30): 3391-400.

40. Walker KJ, Price-Thomas JM, Candlish W, Nicholson RI. Influence of the antioestrogen tamoxifen on normal breast tissue. Br J Cancer 1991; 64(4): 764-8.

SUPPLEMENTARY MATERIAL

Supplementary Table A. Characteristics of venous thrombotic events that occurred within 1 year of tamoxifen treatment

No.	Event	Tamoxifen dose during VTE	Duration of tamoxifen at time of VTE	Tamoxifen level prior to VTE (nM)	Endoxifen level prior to VTE (nM)	Days between last clotting factor measurement and VTE	PC (U/mL)	AT (U/mL)	TF (pg/mL)	RTx	CTx	Medical history of VTE
1	DVT right subclavian vein	20 mg	15 days[1]	NA	NA	NA	NA	NA	NA	Y	Y	-
2	Superficial thromboflebitis lower extremity	20 mg	133 days	542	37	44 days	1.51	0.95	72.9	Y	N	-
3	DVT lower extremity	20 mg	119 days	167	18.7	29 days	1.10	0.45	55.0	Y	N	2x DVT
4	Superficial thromboflebitis lower extremity	20 mg	125 days	248	35.6	36 days	1.66	1.06	45.7	Y	N	DVT
5	DVT lower extremity	10 mg	348 days	187	19	152 days[2]	0.84	0.89	116	Y	N	-
6	Superficial thromboflebitis leg	20 mg	100 days	500	7.7	-1[3]	0.82	0.76	39.0	Y	N	Superficial thromboflebitis
7	Pulmonary embolism (subsegmental)	20 mg	70 days	NA	NA	NA	NA	NA	NA	N	Y	-

Abbreviations: AT antithrombin, CTx chemotherapy, DOAC direct oral anticoagulant, DVT deep venous thrombosis, LMWH low molecular weight heparin, N no, NA not assessed, PC protein C, RTx radiotherapy, TF tissue factor, VTE venous thromboembolism, Y:yes. [1]breast cancer surgery was performed more >12 weeks before VTE occurred; [2]clotting factors were measured after 6 months of tamoxifen treatment. All other clotting factors in this table were measured after 3m of tamoxifen treatment; [3]VTE was diagnosed the day before clotting factor measurement, but treatment was not yet initiated

Supplementary table B. Levels of tamoxifen and endoxifen and coagulation factors and in the total study cohort

	At 3 months		At 6 months	
Tamoxifen and endoxifen plasma levels				
Tamoxifen (nmol/L)	*n* = 135	325 [186]	*n* = 106	331 [231]
Endoxifen (nmol/L)	*n* = 135	26.2 [20]	*n* = 106	25.2 [15]
Coagulation factors				
Protein C (U/mL)	*n* = 133	1.19 (0.23)	*n* = 104	1.13 (0.21)
Antithrombin (U/mL)	*n* = 135	0.96 (0.10)	*n* = 104	0.95 (0.09)
Tissue factor (pg/mL)	*n* = 100	46.0 [17.2]	*n* = 95	54.4 [38.8]
Thrombin generation parameters				
With exogenous tissue factor				
ETP (nM*min)	*n* = 72	2160 (630)	*n* = 65	2115 (410)
Thrombin peak (nM)	*n* = 72	251 (58.7)	*n* = 65	252 (66.2)
Lag time (min)	*n* = 72	7.00 (1.48)	*n* = 65	9.28 (9.07)
Time to peak (min)	*n* = 72	11.3 [2.69]	*n* = 65	10.5 [2.20]
Velocity index (nM/min)	*n* = 72	58.0 [29.5]	*n* = 65	57.2 [35.9]
Without exogenous tissue factor				
ETP (nM*min)	*n* = 53	1169 (411)	*n* = 63	1172 (492)
Thrombin peak (nM)	*n* = 55	55.5 [46.2]	*n* = 63	70.1 [60.6]
Lag time (min)	*n* = 55	39.2 [21.2]	*n* = 63	31.0 [17.8]
Time to peak (min)	*n* = 55	46.9 [24.4]	*n* = 63	39.6 [17.6]
Velocity index (nM/min)	*n* = 55	9.13 [10.2]	*n* = 63	11.7 [14.2]

Data are displayed as mean (SD) or median [IQR]. Data were missing for some participants in some subgroups. Abbreviations: ETP endogenous thrombin potential.

Supplementary Table C. Time-dependent effect of tamoxifen treatment on coagulation parameters in patients in whom tamoxifen dose was increased to 30-40 mg daily during the study, n = 30)

		3 months	6 months
Protein C (U/mL)	*n* = 23	1.09 (0.21)	1.10 (0.19)
Antithrombin (U/mL)	*n* = 23	0.96 (0.08)	0.97 (0.02)
Tissue factor (pg/mL)	*n* = 18	48.5 [16]	**60.6 [38]****
Thrombin generation parameters			
With exogenous tissue factor			
ETP (nM*min)	*n* = 11	2117 (349)	2067 (520)
Thrombin peak (nM)	*n* = 11	248 (64)	246 (81)
Lag time (min)	*n* = 11	6.80 (1.4)	7.59 (4.1)
Time to peak (min)	*n* = 11	10.5 [3]	10.6 [2]
Velocity index (nM/min)	*n* = 11	59.2 (25)	56.5 (25)
Without exogenous tissue factor			
ETP (nM*min)	*n* = 8	1236 (382)	1095 (600)
Thrombin peak (nM)	*n* = 8	71.5 (42)	113 (72)
Lag time (min)	*n* = 8	37.9 [20]	23.7 [21]
Time to peak (min)	*n* = 8	46.1 [21]	29.8 [22]
Velocity index (nM/min)	*n* = 8	9.80 [16]	21.6 [36]

Data are displayed as mean (SD) or median [IQR]. Data were missing for some participants in some subgroups. p value indicates results of paired t-test or Wilcoxon signed rank test. **p value <0.01, all other comparisons were non-significant. Abbreviations: ETP endogenous thrombin potential.

Supplementary Table D. Time-dependent effect of tamoxifen treatment on coagulation parameters amongst patients in whom tamoxifen dose was decreased to 10 mg daily during the study, n = 16)

		3 months	6 months
Protein C (U/mL)	*n* = 10	1.15 (0.14)	1.15 (0.18)
Antithrombin (U/mL)	*n* = 10	0.98 (0.11)	0.96 (0.09)
Tissue factor (pg/mL)	*n* = 10	42.4 [18]	**68.7 [46]***
Thrombin generation parameters			
With exogenous tissue factor			
ETP (nM*min)	*n* = 5	2247 (453)	2176 (393)
Thrombin peak (nM)	*n* = 5	256 (72)	248 (61)
Lag time (min)	*n* = 5	6.47 (1.2)	11.5 (13)
Time to peak (min)	*n* = 5	11.5 [3]	11.5 [2]
Velocity index (nM/min)	*n* = 5	59.7 (23)	53.7 (20)
Without exogenous tissue factor			
ETP (nM*min)	*n* = 3	1116 (523)	1369 (358)
Thrombin peak (nM)	*n* = 3	68.4 (67)	**109 (81)***
Lag time (min)	*n* = 3	55.3 [NA] Range: 31.1	21.3 [NA] Range: 26
Time to peak (min)	*n* = 3	63.9 [NA] Range: 36.6	37.1 [NA] Range: 14.0
Velocity index (nM/min)	*n* = 3	5.49 [NA] Range: 30.4	14.5 [NA] Range: 40

Data are displayed as mean (SD) or median [IQR]. Data were missing for some participants in some subgroups. p value indicates results of paired t-test or Wilcoxon signed rank test. *p value <0.05, all other comparisons were non-significant. Abbreviations: ETP endogenous thrombin potential.

Supplementary Table E. Coagulation parameters in patients who received (neo-)adjuvant chemotherapy versus patients who did not receive chemotherapy at 3 months of tamoxifen therapy

		No chemotherapy		**Chemotherapy**
Protein C (U/mL)	*n* = 77	1.17 (0.24)	*n* = 56	1.17 (0.22)
Antithrombin (U/mL)	*n* = 78	0.96 [0.12]	*n* = 57	0.95 [0.13]
Tissue factor (pg/mL)	*n* = 59	46.8 [15]	*n* = 41	44.8 [15]
Thrombin generation parameters				
With exogenous tissue factor				
ETP (nM*min)	*n* = 40	2152 [668]	*n* = 32	1975 [514]*
Thrombin peak (nM)	*n* = 40	261.3 (61.9)	*n* = 32	238.7 (52.9)
Lag time (min)	*n* = 40	6.82 [2.3]	*n* = 32	6.63 [1.9]
Time to peak (min)	*n* = 40	11.8 (1.9)	*n* = 32	11.0 (1.6)
Velocity index (nM/min)	*n* = 40	61.6 (22.1)	*n* = 32	56.8 (19.7)
Without exogenous tissue factor				
ETP (nM*min)	*n* = 31	1382 [522]	*n* = 22	1002 [536]
Thrombin peak (nM)	*n* = 32	60.0 [52.0]	*n* = 23	52.4 [51.7]
Lag time (min)	*n* = 32	41.1 [20.4]	*n* = 23	38.5 [26.1]
Time to peak (min)	*n* = 32	47.7 [21.4]	*n* = 23	43.8 [28.3]
Velocity index (nM/min)	*n* = 32	9.5 [9.1]	*n* = 23	[10.5]

Data are displayed as mean (SD) or median [IQR]. Data were missing for some participants in some subgroups. p value indicates results of independent samples t-test or Mann Whitney-U test. *p value <0.05, all other comparisons were non-significant. Abbreviations: ETP endogenous thrombin potential.

Supplementary table F. Coagulation parameters in patients who received (neo-)adjuvant chemotherapy versus patients who did not receive chemotherapy at 6 months of tamoxifen therapy

		No chemotherapy		Chemotherapy
Protein C (U/mL)	*n* = 54	1.12 (0.20)	*n* = 50	1.15 (0.20)
Antithrombin (U/mL)	*n* = 54	0.94 (0.09)	*n* = 50	0.97 (0.09)
Tissue factor (pg/mL)	*n* = 49	54.8 [29.8]	*n* = 46	51.3 [50.2]
Thrombin generation parameters				
With exogenous tissue factor				
ETP (nM*min)	*n* = 30	2205 (359)	*n* = 35	2037 (440)
Thrombin peak (nM)	*n* = 30	267.7 (54.8)	*n* = 35	238.2 (72.5)
Lag time (min)	*n* = 30	8.3 [2]	*n* = 35	6.1 [2]*
Time to peak (min)	*n* = 30	9.9 [2]	*n* = 35	10.9 [2]*
Velocity index (nM/min)	*n* = 30	63.2 (20.1)	*n* = 35	54.2 (23.4)
Without exogenous tissue factor				
ETP (nM*min)	*n* = 29	1309 (515)	*n* = 34	1056 (446)
Thrombin peak (nM)	*n* = 29	72.3 [121]	*n* = 34	67.3 [57]
Lag time (min)	*n* = 29	29.6 (15.0)	*n* = 34	31.3 (13.8)
Time to peak (min)	*n* = 29	35 [21]	*n* = 34	40 [14]
Velocity index (nM/min)	*n* = 29	11.7 [33]	*n* = 34	11.7 [13]

Data are displayed as mean (SD) or median [IQR]. Data were missing for some participants in some subgroups. P value indicates results of independent samples t-test or Mann Whitney-U test. *p value <0.05, all other comparisons were non-significant. Abbreviations: ETP endogenous thrombin potential.

CHAPTER 6

Effects of tamoxifen on cognitive function in patients with primary breast cancer

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ABSTRACT

Introduction

Tamoxifen may adversely affect cognitive function by interfering with estrogen action in the brain. Despite growing evidence for a relationship between tamoxifen and cognitive problems, findings remain inconclusive. While some tamoxifen-related side effects seem exposure-dependent with concentrations of tamoxifen or its main metabolite, endoxifen, this has never been investigated for cognitive function. We investigated cognitive function after two years of tamoxifen and its association with tamoxifen and endoxifen exposure

Methods

135 women with breast cancer completed the Amsterdam Cognition Scan (ACS), an online neuropsychological test battery, after two years of tamoxifen. Test scores were converted to standardized Z-scores based on a matched 'no-cancer' control group. Tamoxifen and endoxifen concentrations and tamoxifen dose were regressed separately on cognitive functioning.

Results

Patients reported mild cognitive complaints and had worse verbal learning, processing speed, executive functioning, and motor functioning compared to matched controls. After correcting for age, mean tamoxifen and endoxifen levels, as well as tamoxifen dose, were associated with worse performance on several cognitive domains.

Conclusion

Tamoxifen is adversely associated with objective as well as self-reported cognitive function, which may depend on the level of exposure to tamoxifen and endoxifen. Further research is warranted to confirm this hypothesis.

INTRODUCTION

Breast cancer is the most commonly diagnosed cancer among women worldwide, accounting for approximately 25% of all cancer cases.[1] The standard of care for hormone-receptor positive breast cancer is often adjuvant endocrine therapy, such as tamoxifen. Especially for premenopausal patients, tamoxifen is the primary drug of choice, prescribed for a minimum duration of five years. For postmenopausal patients, tamoxifen is frequently prescribed for two to three years before switching to an aromatase-inhibitor.[2] However, tamoxifen can cause a variety of side effects, including hot flashes, arthralgia, vaginal discharge, and mood alterations,[3] and may also cause cognitive problems.

Tamoxifen is a selective estrogen receptor (ER) modulator, that acts as an agonist or antagonist depending on the specific tissue that it binds to.[4] Since ER are present in various other tissues in the female body, the binding of tamoxifen and its metabolites to these ER probably serves as the mechanism behind (most) side effects of tamoxifen.[4,5] In the brain, ERα and ERβ are distributed in varying ratios depending on the specific brain area. Through these receptors, estrogens play an important role in normal cognitive functioning.[6-11] Tamoxifen, able to pass the blood-brain barrier and bind to the ER, can interfere with the action of estrogen[6,12-14], potentially affecting cognitive function.

Cognitive problems are commonly observed during or after cancer treatment.[15] A recent meta-analysis of chemotherapy-treated patients with breast cancer revealed that a significant number of patients (44%) reported cognitive complaints, while a smaller but still notable percentage (21 to 34%) exhibited objective neuropsychological test-derived impairment.[16] Whereas the adverse effects of chemotherapy on cognitive function have been well-established in the past decades, knowledge about the potential adverse effects of endocrine therapies such as tamoxifen remains limited. Given the importance of cognitive functions for daily functioning and quality of life[15], research into the cognitive side effects of tamoxifen is highly relevant.

A growing number of studies supports the concerns regarding cognitive side effects of tamoxifen. In the TEAM-trial (n = 80) for example, postmenopausal breast cancer patients undergoing tamoxifen treatment performed significantly worse on verbal memory and executive functioning compared to no-cancer controls.[17] Even after transitioning to the aromatase inhibitor exemestane, a carryover effect of tamoxifen persisted.[18] A smaller study (n = 31) in postmenopausal patients on tamoxifen reported similar effects of tamoxifen on verbal memory and executive functioning.[19] A large meta-analysis of small cohort studies indicated decreased verbal learning and memory, executive functioning, and processing speed in patients treated with

tamoxifen or aromatase inhibitors.[20] In contrast, a study of van Dyk et al. (*n* = 58) did not find significant changes between baseline up to one year of tamoxifen treatment on standardized neuropsychological tests.[21] This warrants further research to elucidate the exact effect of tamoxifen on cognitive function.

It has never been investigated whether the potential effects of tamoxifen on cognitive function could depend on the level of exposure to tamoxifen or its metabolites. Tamoxifen is metabolized in the liver into endoxifen, 4-hydroxy-tamoxifen and n-desmethyl-tamoxifen primarily by cytochrome P450 (CYP) enzymes CYP2D6 and CYP3A4.[22] Endoxifen is considered the most important metabolite for tamoxifen efficacy.[23] Results from retrospective studies indeed indicated an exposure-response relation for endoxifen.[24-28] Therapeutic drug monitoring (TDM), in which plasma concentrations are measured and doses are adapted by the measured concentration in order to reach a therapeutic threshold, can increase the percentage of patients on tamoxifen with endoxifen concentrations above the supposed efficacy threshold of 16 nM.[29,30] However, up till now findings regarding the relationship between tamoxifen and metabolite concentrations and the occurrence of side effects remain conflicting.

In this study we aimed to evaluate the effect of two years of tamoxifen treatment on objective and subjective cognitive function in a large cohort of women with breast cancer. In addition, we investigated the association between tamoxifen and endoxifen plasma concentrations and objective and subjective cognitive function, as well as the association with tamoxifen dose.

MATERIAL AND METHODS

Study design

The current study was part of the TOTAM (Therapeutic drug monitoring Of TAMoxifen) trial; a large intervention study coordinated by the Erasmus MC Cancer Institute in Rotterdam, the Netherlands, which aimed to investigate the feasibility of TDM of tamoxifen. As a secondary endpoint, we examined the effect of tamoxifen on cognitive function. The study was approved by the local Medical Ethics Committee and registered in the International Clinical Trial Registry Platform (ICTRP; https://trialsearch.who.int: NL6918). All participants provided written informed consent.

The study's methodology, including eligibility criteria and primary findings, has been thoroughly documented elsewhere.[29,31] Briefly, female patients diagnosed with hormone-receptor positive primary breast cancer who were treated with the standard

daily dose of 20 mg tamoxifen for a period of 3 months were included in the TOTAM study. They were followed for the first two years of their tamoxifen treatment, during which they were seen six times at the out-patient clinic. Each visit, tamoxifen and endoxifen plasma concentrations were determined and side effects were assessed. If patients had endoxifen levels <16 nM, the daily tamoxifen dose was increased to 30 or 40 mg, if possible. Also, for patients with bothersome side effects and relatively high endoxifen levels, tamoxifen dose could be decreased to 10 mg daily. After the sixth and last visit (i.e., after two years of tamoxifen treatment) patients underwent an online neuropsychological assessment. Patients who preliminary went off study due to toxicity were also invited to complete the neuropsychological assessment.

Pharmacokinetics

Tamoxifen and endoxifen trough concentrations (C_{min}) were obtained during each study visit over two years of tamoxifen treatment. Plasma concentrations were measured using a validated ultra-performance liquid chromatography with a tandem mass spectrometry method (UP-LCMS/MS).[32] Because patients could use different tamoxifen doses for various durations during the study, their tamoxifen and endoxifen concentrations could be different at each study visit. To estimate the mean tamoxifen and endoxifen plasma levels over the two years of tamoxifen treatment as accurate as possible, an average C_{min} level per patient was simulated using Nonlinear Mixed Effects Modelling (NONMEM).[33] In addition, the mean dose over the two years interval was calculated by multiplying the tamoxifen dose (in mg) with the number of days per dose and then dividing the total dose exposure by the total number of days of tamoxifen treatment.

Neuropsychological assessment

Objective cognitive functioning was assessed using the Amsterdam Cognition Scan (ACS; www.cognitionscan.org)[34]; a validated online neuropsychological test battery which patients can complete on their own computer at home without supervision.[34,35] The ACS consists of 7 online cognitive tests covering a wide range of cognitive domains and takes approximately 1 hour to complete. An overview of the cognitive tests and corresponding cognitive domains can be found in **Table 1.** The ACS also contains 3 questionnaires: The MD Anderson Symptom Inventory for multiple myeloma (MDASI-MM) is a validated questionnaire on subjective cognitive function, that measures self-reported severity of memory and concentration problems and their interference with daily activities during the past 24 hours (classified as 0: none, 1–4: mild, 5–6: moderate, 7–10: severe).[36] These questions are not disease-specific and are applicable for various forms of cancer. The Hospital Anxiety and Depression Scale (HADS) measures the severity of anxiety and depression during the past four weeks, with higher scores

reflecting more severe symptoms.[37] The Multidimensional Fatigue Inventory (MFI-20) measures fatigue on five dimensions (general fatigue, physical fatigue, mental fatigue, reduced activity, and reduced motivation), with higher scores indicating more fatigue.[38] The 'no-cancer' control group that was included in the cognitive side-study of the SONIA trial[39] also served as the ACS norm group for the current study. These were female relatives or friends of participants of the SONIA study who also completed the ACS, including the HADS and MFI questionnaire. The MDASI-MM was not administered.

Additionally, tamoxifen side effects were evaluated using the Functional Assessment of Cancer Therapy-Endocrine Subscale (FACT-ES); a validated questionnaire for the assessment of endocrine treatment in patients with breast cancer.[40] It includes 27 health-related quality of life items (HR-QOL) covering physical, social, emotional, and functional well-being, with a 19-item endocrine subscale (ES19). Higher FACT-ES scores indicate better HR-QOL and fewer side effects.

Table 1. Cognitive tests of the Amsterdam Cognition Scan

Cognitive domain	ACS test	Main outcome measure
Learning and memory	Wordlist learning Wordlist recall Wordlist recognition	Total number of correct words (learning: trial 1 to 5)
Attention & working memory	Box tapping Digit sequences I Digit sequences II	Total number of correctly repeated sequences
Processing speed	Reaction speed Connect the dots I	Average reaction time (ms) Completion time in seconds
Executive functioning	Connect the dots II Place the beads	Completion time in seconds Total number of extra moves
Motor functioning	Fill the grid	Completion time in seconds

Statistical analysis

A power calculation indicated that with an effect size of 0.30 based on a previous study[18], alpha set at 0.05, a power of .80, and taking into account a potential dropout rate of 30%, at least 117 patients should be included.

Using nearest-neighbour propensity score matching, an equally-sized group of no-cancer controls was selected, matched on age, educational level, and computer usage. After matching, independent t-tests and a chi-square test were performed to check for remaining differences in age, computer usage, and educational level. Outliers and invalid ACS test scores were identified and excluded from analysis, as described by Feenstra et al. (using age groups ≤50, >50 and ≤65, and >65 years).[35] Scores on

wordlist recall were considered invalid and excluded if the retention interval exceeded 45 minutes. Raw test scores were converted to standardized Z-scores based on the matched control group. Z-scores (normally distributed with a mean of zero and a standard deviation of one) indicate to what extent a score deviates from the normative mean, with negative scores indicating worse performance while positive scores indicate better performance than expected.[41] Patients' average cognitive performance was visualized and considered significantly deviant if 0 fell outside of the 95% confidence interval. In addition, the prevalence of cognitive impairment was determined for both patients and no-cancer controls using the International Cognition and Cancer Task Force (ICCTF) criterion: two or more test scores at or below –1.5 SDs or a single test score at or below –2.0 SDs from the normative mean, or both.[42] This was compared to the family-wise error rate using a binomial test, which reflects the probability of a finding at least one deviating test score given the number of tests administered.[43] The above steps were repeated for the subgroups of patients treated only with tamoxifen and patients who also received chemotherapy prior to inclusion, to rule out that any observed effect would be driven solely by previously received chemotherapy.

Anxiety, depression, and fatigue were compared between patients and controls using Mann-Whitney U tests. The relation of these self-reported outcomes and endocrine symptoms to the ACS Z-scores for objective cognitive function and MDASI-MM severity and interference subscales for subjective cognitive function was determined through correlational analysis. To investigate the association between cognitive function and tamoxifen and endoxifen exposure, mean tamoxifen or endoxifen plasma levels were regressed on the ACS Z-scores or the MDASI-MM subscales in linear regression analyses. Age was included as a covariate, as both cognitive function[44] and tamoxifen and endoxifen concentrations[33,45-47] tend to vary with age. These regression analyses were repeated with mean tamoxifen dose to also investigate the association of tamoxifen dose and cognitive function. All analyses were performed in R Studio. P-values were considered significant at α=0.05.

RESULTS

Inclusion and participant characteristics

In total, 177 (89%) out of the 200 approached patients consented to undergo a neuropsychological assessment, of which 139 (78%) completed the ACS. Four patients only partly completed the ACS and were excluded due to missing demographic data. Reasons for not completing the ACS were: no computer(experience) (n = 11); no time (n = 12); no response (n = 11). As only three out of the 139 patients withdrew from the

study early (i.e., before completing two years of tamoxifen) due to toxicity, and one patient turned out to be non-compliant to the treatment, these patients were excluded from analysis, resulting in an evaluable sample of 135 patients. Matching resulted in a group of 135 no-cancer controls, with no significant differences in age ($t(266) = -0.44$, $p = 0.66$), educational level ($X^2(1) = 0.95$, $p = 0.33$), and computer usage ($t(268) = -0.45$, $p = 0.66$) after matching. Demographic and clinical characteristics of patients and matched controls can be found in **Table 2.** At the time of cognitive assessment (i.e., after two years of tamoxifen) the majority of patients was postmenopausal. Nine (60%) of the 15 premenopausal patients (7% of all patients) were treated with ovarian function suppression.

Pharmacokinetics

Median trough levels of tamoxifen and endoxifen over two years of tamoxifen treatment were 331.1 nM (*IQR* = 264.5-424.2) and 26.1 nM (*IQR* = 21.5-32.4), respectively. Tamoxifen levels showed a weak correlation with age ($r_s(133) = 0.17$, $p = 0.04$), with older individuals having higher tamoxifen levels. No correlation with age was found for endoxifen levels ($r_s(133) = 0.09$, $p = 0.32$). Mean tamoxifen dose over two years was 22.7 mg (*SD* = 7.1).

Cognitive function

Average ACS Z-scores of the patients standardized based on the matched control group are visualized in **Figure 1**. The subtest Wordlist Recognition was not analysed because the variance in test scores was limited due to a ceiling effect. Patients performed worse than the matched controls on tests measuring verbal learning, processing speed, executive functioning, and motor functioning. In total, 47% of the patients were classified as cognitively impaired based on the ICCTF criterion, significantly higher than the 28% in the matched control group ($p = 0.002$) and the family-wise error rate of 23% ($p < 0.001$). The prevalence in the control group was not different from the family-wise error rate ($p = 0.10$).

Table 2. Demographic & clinical characteristics

	Patients (*n* = 135)	Matched HCs (*n* = 135)
Participant characteristics		
Age, *M* ±SD [range] *median [IQR]*	58 ±11 [27-84] 58 [45-68]	57 ±10 [25-81]
Educational level, *N* (%)		
Low	3 (2)	0
Middle	64 (47)	58 (43)
High	68 (50)	77 (57)
Computer usage, *M* ±SD [range]	16 ±13 [0-50]	17 ±13 [1-42]
BMI, *median [IQR]*	26.6 [23.1-31.6]	
Menopausal status		
Premenopausal	15 (11)	
Postmenopausal	109 (81)	
Perimenopausal	10 (7)	
Unknown	1 (1)	
Tumor and treatment characteristics		
Tumour stage, *N* (%)		
T1	60 (44)	
T2	55 (41)	
T3	16 (12	
T4	3 (2)	
Tx	1 (1)	
Nodal stage, *N* (%)		
N0	68 (50)	
N1	47 (35)	
N2	14 (10)	
N3	5 (4)	
Nx	1 (1)	
Metastatic stage, *N* (%)		
M0	35 (26)	
M1	3 (2)	
Mx	97 (72)	
Her2neu receptor, *N* (%)		
Positive	16 (12)	
Negative	119 (88)	
Local treatment, *N* (%)		
Lumpectomy alone	2 (1)	
Lumpectomy + radiotherapy	84 (62)	
Mastectomy alone	28 (21)	
Mastectomy + radiotherapy	21 (16)	
(Neo)adjuvant chemotherapy, *N* (%)		
Yes	72 (53)	
No	63 (47)	
Tamoxifen dose, *N* (%)		
10 mg	11 (8)	
15 mg	2 (1)	
20 mg	93 (69)	
30 mg	8 (6)	
40 mg	21 (16)	

Abbreviations. BR: Bloom Richardson

To evaluate whether the effect on cognitive function in this study is tamoxifen-specific or could be explained by (neo)-adjuvant chemotherapy, subgroup analysis was performed in patients treated with and without chemotherapy. Patients who did not receive chemotherapy (n = 63, M_{age} = 61) showed a similar pattern of test scores as found in the whole sample, with worse verbal learning, processing speed, executive functioning, and motor functioning. Patients who received (neo-)adjuvant chemotherapy prior to the study (n = 72, M_{age} = 56) only showed worse processing speed compared to the no-cancer control group. 52% of patients who did not receive chemotherapy were classified as cognitively impaired compared to 41% of the patients who were also treated with chemotherapy. Notably, patients who received chemotherapy were on average five years younger than those who did not.

Patient-reported outcomes

In **Table 3**, results from the self-report questionnaires on cognitive function, anxiety, depression, fatigue, and endocrine therapy side effects are reported. Patients reported cognitive complaints of mild severity and mild interference with daily activities. No significant differences in self-reported anxiety or depression were found between patients and controls (anxiety: W = 9187, p = 0.91; depression: W = 9233, p = 0.85). However, patients reported significantly higher levels of fatigue on all five domains (total fatigue: W = 6474, p <0.001; general fatigue: W = 6496, p <0.001; physical fatigue: W = 6811, p <0.001; mental fatigue: W = 6623, p <0.001; reduced activity: W = 7581, p = 0.02; reduced motivation: W = 7594, p = 0.02). For endocrine side effects, a mean score of 57 points for FACT-ES19 was found. This coincides with scores from earlier studies in patients treated with tamoxifen (59-62 points).[48-50]

Anxiety, depression, fatigue, and endocrine side effects were all correlated with self-reported cognitive function. More severe symptomatology was consistently associated with more severe self-reported cognitive complaints and greater interference of these complaints with daily functioning. In contrast, no associations were found between objective cognitive function and anxiety, depression, physical and mental fatigue, and endocrine symptoms. Only reduced activity and reduced motivation were weakly associated with two of the ten ACS outcomes. A correlogram visualizing the association between objective and subjective cognitive function and the self-reported outcome measures can be found in **Supplementary A.**

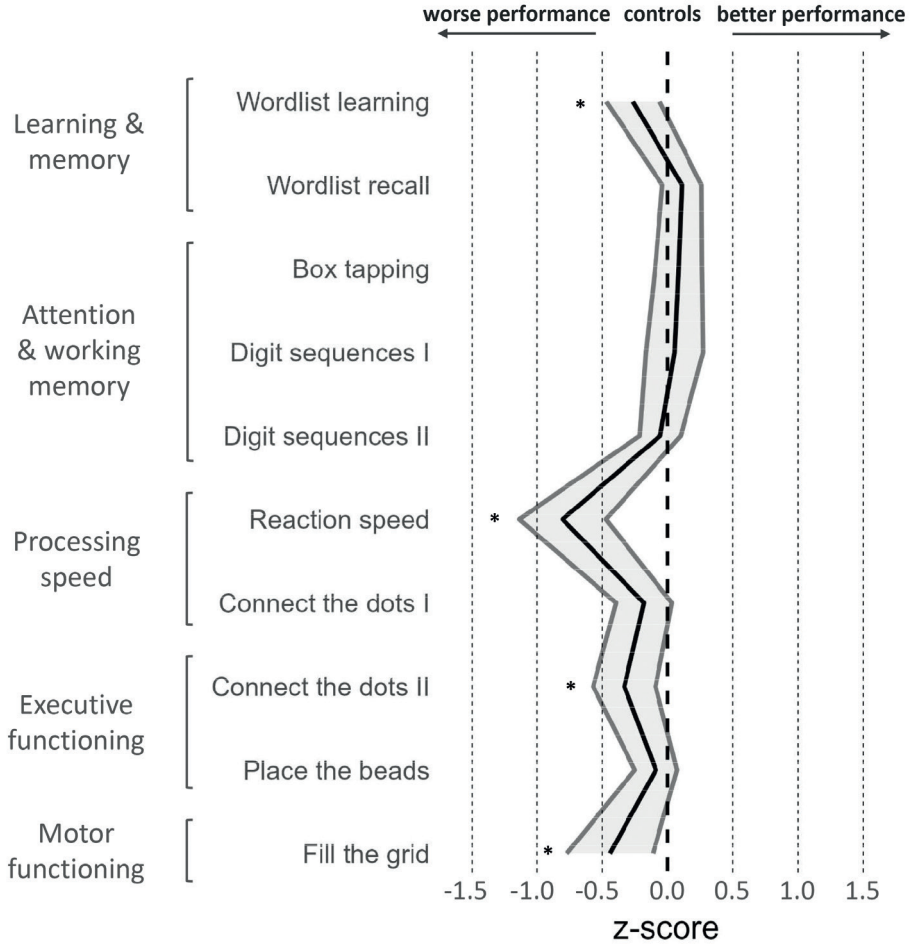


Figure 1. Cognitive test performance
Visualized are patients' average ACS Z-scores, standardized based on the matched control group. The shaded area indicates the 95% confidence interval. * indicates significant deviation from 0.

Table 3. Patient-reported outcomes

	Patients (*n* = 135)	Matched HCs (*n* = 135)
Subjective cognitive functioning (MDASI-MM)		
Severity	**4.4 ±2.3**	
Memory problems	4.4 ±2.3	
Concentration problems	4.4 ±2.4	
Interference	**4.8 ±1.9**	
General activity	2.9 ±2.1	
Mood	2.9 ±2.2	
Work	3.3 ±2.5	
Relationships	2.3 ±2.1	
Walking	3.0 ±2.7	
Enjoying life	2.4 ±2.0	
Mood (HADS)		
Anxiety	**5.5 ±4.1**	**5.3 ±3.2**
Depression	**3.5 ±2.9**	**3.4 ±2.6**
Fatigue (MFI-20)		
Total fatigue	**49.6 ±17.3**	**40.9 ±14.2**
General fatigue	11.5 ±4.6	9.2 ±4.2
Physical fatigue	10.2 ±4.7	8.2 ±3.8
Mental fatigue	11.0 ±4.8	8.7 ±3.7
Reduced activity	8.8 ±3.8	7.7 ±3.4
Reduced motivation	8.0 ±3.6	7.1 ±3.3
Tamoxifen side effects (FACT-ES)		
ES19	**57 ±11**	

Abbreviations. MDASI-MM: MD Anderson Symptom Inventory for Multiple Myeloma. HADS: Hospital Anxiety and Depression Scale. MFI-20: Multidimensional Fatigue Inventory. FACT-ES: Functional Assessment of Cancer Therapy-Endocrine Subscale.
All outcomes are reported as mean ±SD.

Tamoxifen exposure and cognitive function

In **Figure 2**, results of the linear regression analyses of tamoxifen and endoxifen plasma levels and tamoxifen dose on objective and subjective cognitive function are illustrated in a forest plot (see **Supplementary B-D** for detailed regression models). Tamoxifen plasma levels were negatively associated with cognitive test performance on 5 out of the 10 ACS outcomes (i.e., Connect the dots I&II, Wordlist learning, Box tapping, and Fill the grid). After including age as a covariate, the associations remained significant for 2 ACS outcomes (i.e., Box tapping and Fill the grid). A difference of 100 nM in tamoxifen plasma levels corresponded to a difference of Z = 0.14 in visuospatial working memory and a difference of Z = 0.24 in motor functioning. Self-reported cognitive function was associated with tamoxifen levels as well. Higher exposure was associated with less self-reported cognitive complaints on the MDASI-MM severity and interference scale. After including age as a covariate, the association between tamoxifen levels and self-reported severity of memory and attention complaints disappeared.

Endoxifen plasma levels were negatively associated with performance on 3 ACS outcomes (i.e., Wordlist learning, Box tapping, and Fill the grid). After including age, the association remained significant on 2 ACS outcomes (i.e., Box tapping and Fill the grid). A difference of 10 nM in endoxifen plasma levels corresponded to a difference of Z = 0.24 in visuospatial working memory and a difference of Z = 0.38 in motor functioning. Endoxifen plasma levels were not associated with self-reported cognitive function.

Tamoxifen dose was negatively associated with performance on 2 ACS outcomes (i.e., Wordlist recall and Fill the grid). Including age as a covariate did not alter the results. Specifically, a difference in tamoxifen dose of 10 mg corresponded to a difference of Z = 0.24 in memory recall and a difference of Z = 0.48 in motor functioning. Tamoxifen dose was associated with self-reported cognitive function. Again, higher dose was associated with less severe cognitive complaints on the MDASI-MM severity scale. However, after including age, this association disappeared.

To further explore the effect of age on cognitive function and the association with tamoxifen exposure, a median-split was performed based on age (median = 57 years). Z-scores were recalculated based on no-cancer controls from the same age group and the regressions were repeated. Patients aged 57 years or older showed relatively poorer cognitive function compared with controls of the same age than the group below 57 years, with worse performance on tests of verbal learning, processing speed, executive functioning, and motor functioning, whereas the younger group only showed slower reaction speed (**Supplementary E**). In the patients with an age below 57 years, the negative associations between cognitive function and tamoxifen and endoxifen levels remained or reached significance for other ACS outcomes as well (i.e., Connect the dots I, Wordlist learning, Reaction speed, and Place the beads), but the associations with tamoxifen dose disappeared. In patients aged 57 years or older the associations with tamoxifen and endoxifen levels disappeared, while the associations with tamoxifen dose remained (**Supplementary F**).

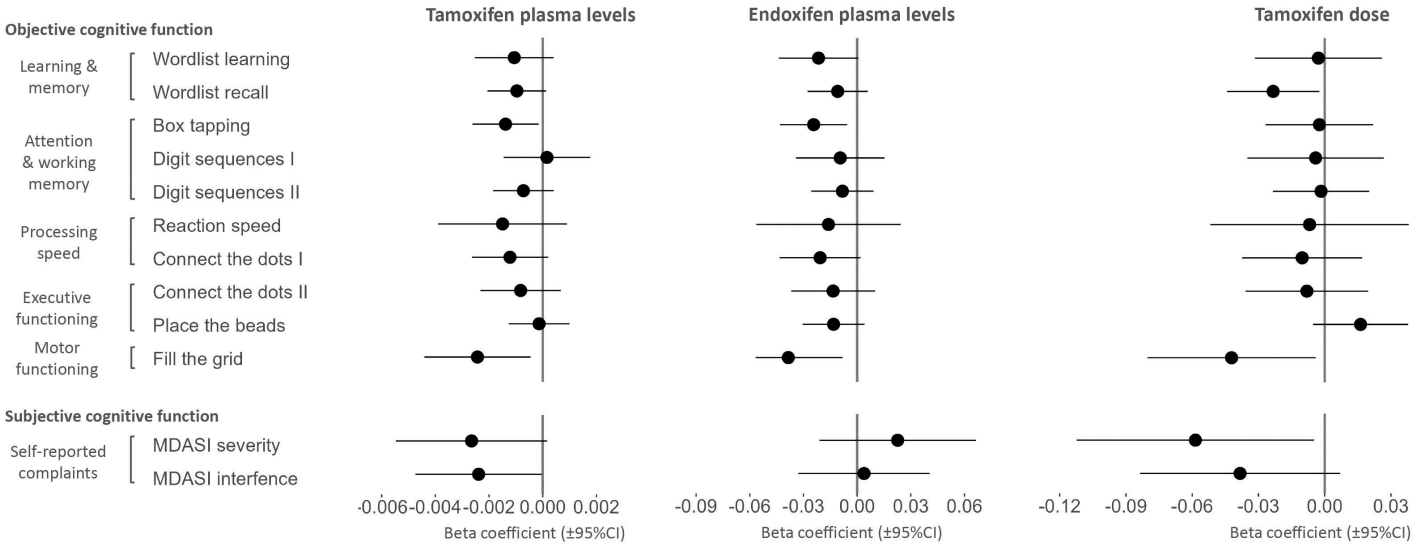


Figure 2. Tamoxifen exposure and cognitive function
Forest plot of unstandardized beta coefficients from linear regressions of mean tamoxifen or endoxifen concentrations and tamoxifen dose on cognitive outcome measures, adjusted for age. For objective cognitive function, negative coefficients indicate worse cognitive performance. For subjective cognitive function, positive coefficients indicate more self-reported problems

DISCUSSION

In the largest study conducted thus far, we investigated the effect of tamoxifen on self-reported and objectively measured cognitive function. After two years of tamoxifen treatment, women with breast cancer report mild cognitive complaints and perform worse than matched no-cancer controls on the cognitive domains of verbal learning, processing speed, executive functioning, and motor functioning. Moreover, this study was the first to investigate a potential exposure-dependent association and found that performance was associated with tamoxifen and endoxifen plasma levels and tamoxifen dose on several cognitive domains.

Older women appear particularly vulnerable for cognitive problems across multiple cognitive domains. It is well known that cognitive function tends to decline with healthy aging.[44] However, our study indicates that tamoxifen affects cognitive function to a greater extent in older women (>57 years of age), compared to women without cancer of the *same* age, than in younger women (below 57 years). These findings align with results from the TEAM trial, where effects of tamoxifen on verbal memory and executive functioning were most evident in older women.[17] Consequently, extra attention for this serious side effect is required especially in the older population.

Interestingly, the exposure-dependent association found in this study was more prominent in younger women, whereas it mostly disappeared in older women. This may seem counterintuitive at first but may be explained by the vulnerability at older age. Older women perform relatively poor already at lower exposure levels. Younger women, on the other hand, may be able to maintain their cognitive function at low exposure but decline after exposure to higher levels of tamoxifen, in line with theories about cognitive reserve.[51] Menopausal status might also explain why younger and older women are differently affected. It could be possible that tamoxifen's effect is different in women with different hormonal backgrounds. Estrogen is known to affect cognition during the menstrual cycle and menopause in healthy women.[52] Alterations in estrogen level that occur during the menopause could underlie the increased vulnerability to cognitive effects at older age. How exactly tamoxifen and endoxifen affect the brain and what the precise role of menopause is remains yet to be understood.

Tamoxifen seems to have the potential to influence performance in all cognitive domains assessed, depending on age and level of exposure. This may be explained by the widespread distribution of estrogen receptors throughout the brain. While most domains coincide with those found in the literature previously described[17,19,20], we also found exposure-dependent effects of tamoxifen on tests of visuospatial memory and motor functioning, which have not been reported before. As mentioned previously,

brain areas vary in the ratio of their expression of ERα and ERβ. Ratios of ERβ, hypothesized to underlie tamoxifen's potential effects on cognition, are highest in the hippocampus and temporal cortex, important for verbal and visuospatial memory.[53] ERβ are also found in the prefrontal cortex, sensorimotor, and cerebellum[54], involved amongst others in executive function[55] and motor coordination.[56] This could explain why certain domains seem more sensitive to varying levels of exposure to tamoxifen.

Neuroimaging could help us to better understand how tamoxifen influences cognitive function. Although widely used in imaging of chemotherapy-induced brain changes[57], only a handful of studies have applied imaging to examine the effects of endocrine therapy in breast cancer patients. These studies are often limited by small samples of patients who received mixed treatment of both endocrine therapy and chemotherapy, making it difficult to disentangle individual treatment effect. Nevertheless, the results of these studies are suggestive of structural and functional changes associated with endocrine therapy.[58,59] Future research should employ neuroimaging methods to further improve knowledge about the biological mechanism through which tamoxifen affects the brain.

The discovered exposure-dependent association of tamoxifen demands caution but also offers possibilities in the field of therapeutic drug monitoring. Our findings suggest that alterations in a patient's administered tamoxifen dose and consequent changes in their tamoxifen and endoxifen plasma level may have small --yet potentially impactful-- effects on their cognitive function. For clinical practice, this implies that in case of dose-escalation, possible adverse effects on cognition should be taken into account. Likewise, dose reduction could potentially also be considered in patients who suffer from cognitive impairment, in particular in younger women with high tamoxifen and endoxifen plasma levels.[31] Although the current study design only allowed for cross-sectional comparison, our hypothesis-generating findings warrant further investigation of the effects of dose-escalation, reduction, or discontinuation of tamoxifen on cognition.

Tamoxifen might also affect cognition indirectly through other tamoxifen-related side effects including fatigue, insomnia, and mood disturbances.[60] In this study, we indeed found that endocrine side effects, anxiety, depression, and fatigue were strongly associated with self-reported cognitive functioning. However, these factors could not explain the effect of tamoxifen on objective cognitive function. Objectively-measured and subjective cognitive function are conceptually different constructs.[61] Self-report questionnaires on subjective cognitive function measure perceived difficulties in daily life and are known to be strongly associated with psychological factors,[61] as also evident in our study. Standardized neuropsychological tests are less sensitive

to these psychological factors. As such, self-reported complaints may rather reflect psychological distress instead of cognitive impairment. This may also explain why the exposure-dependent association was found for objective cognitive function but not for self-reported complaints.

This study offers several clinical implications. Clinicians must be aware of the high prevalence of cognitive problems among tamoxifen users. Patients should be adequately informed regarding cognitive changes that may occur when starting tamoxifen therapy. When a patient presents with cognitive complaints, it is important to assess what underlies these complaints to refer to appropriate care, in collaboration with neuropsychologists or rehabilitation specialists. Patients with cognitive complaints but without objectifiable cognitive impairments may benefit most from interventions targeting underlying psychological factors to alleviate their distress. In case of cognitive impairments, patients may benefit more from compensatory strategies to cope with them. Our findings underscore the need for further research and personalized clinical approaches to optimize tamoxifen therapy outcomes while mitigating cognitive side effects.

Our study has several limitations. First, no correction for multiple comparisons was performed, resulting in an increased risk of type I errors. Although this was partly taken into account by comparing the incidence of cognitive impairment to the family-wise error rate, and the exposure-response analyses were primarily hypothesis-generating, our results should be interpreted with caution. Second, only patients who tolerated tamoxifen well enough, and maintained tamoxifen therapy for two years, were evaluated. Those who discontinued tamoxifen prematurely due to severe tamoxifen-related side-effects were not evaluated, potentially limiting our findings and underestimating tamoxifen's effects, as these patients could also be (most) affected cognitively. However, objective cognitive function did not seem to be associated with the severity of endocrine side-effects in our study. Moreover, a previous study found no difference in cognition based on a cognitive screener between women who discontinued endocrine therapy within two years and those who continued.[62] Third, the hypothesized role of menopausal status could unfortunately not be further investigated. Due to the small number of premenopausal patients in our study, we were unable to compare the effects of tamoxifen on cognitive functioning between pre- and postmenopausal patients. Finally, as cognitive function was assessed only once and we lacked a baseline measurement in our study, no conclusions about cognitive decline over time could be drawn. Currently, a follow-up study is ongoing in which cognitive function is assessed longitudinally, with assessments at the start and after two years of tamoxifen (clinicaltrials.gov; NCT05525481).

CONCLUSIONS

In the largest study thus far, we assessed cognitive function using a validated, online neuropsychological test battery after two years of tamoxifen treatment for breast cancer, and found mild cognitive complaints and worse cognitive function across several domains, especially in older women. Moreover, we are the first to study and suggest a potential exposure-dependent effect. Further research is needed to investigate the effects of dose-escalation, dose-reduction, or cessation of therapy on cognitive function.

REFERENCES

1. Sung H, Ferlay J, Siegel RL, et al. Global Cancer Statistics 2020: GLOBOCAN Estimates of Incidence and Mortality Worldwide for 36 Cancers in 185 Countries. CA: A Cancer Journal for Clinicians 2021; 71(3): 209-49.
2. Loibl S, André F, Bachelot T, et al. Early breast cancer: ESMO Clinical Practice Guideline for diagnosis, treatment and follow-up☆. Annals of Oncology 2024; 35(2): 159-82.
3. Land SR, Wickerham DL, Costantino JP, et al. Patient-Reported Symptoms and Quality of Life During Treatment With Tamoxifen or Raloxifene for Breast Cancer PreventionThe NSABP Study of Tamoxifen and Raloxifene (STAR) P-2 Trial. JAMA 2006; 295(23): 2742-51.
4. Martinkovich S, Shah D, Planey SL, Arnott JA. Selective estrogen receptor modulators: tissue specificity and clinical utility. Clinical Interventions in Aging 2014; 9: 1437-52.
5. Stearns V, Ullmer L, López JF, Smith Y, Isaacs C, Hayes D. Hot flushes. Lancet 2002; 360(9348): 1851-61.
6. Arevalo MA, Azcoitia I, Garcia-Segura LM. The neuroprotective actions of oestradiol and oestrogen receptors. Nature Reviews Neuroscience 2015; 16(1): 17-29.
7. Fester L, Prange-Kiel J, Jarry H, Rune GM. Estrogen synthesis in the hippocampus. Cell and Tissue Research 2011; 345(3): 285-94.
8. Irwin RW, Yao J, Hamilton RT, Cadenas E, Brinton RD, Nilsen J. Progesterone and estrogen regulate oxidative metabolism in brain mitochondria. Endocrinology 2008; 149(6): 3167-75.
9. Sunday L, Osuna C, Krause DN, Duckles SP. Age alters cerebrovascular inflammation and effects of estrogen. American Journal of Physiology-Heart an Circulatory Physiology 2007; 292(5): H2333-40.
10. Švob Štrac D, Pivac N, Mück-Šeler D. The serotonergic system and cognitive function. Translational Neuroscience 2016; 7(1): 35-49.
11. Everitt BJ, Robbins TW. Central cholinergic systems and cognition. Annual Review of Psychology 1997; 48: 649-84.
12. Lien EA, Solheim E, Ueland PM. Distribution of tamoxifen and its metabolites in rat and human tissues during steady-state treatment. Cancer Research 1991; 51(18): 4837-44.
13. Novick AM, Scott AT, Neill Epperson C, Schneck CD. Neuropsychiatric effects of tamoxifen: Challenges and opportunities. Frontiers in Neuroendocrinology 2020; 59: 100869.
14. Buwalda B, Schagen SB. Is basic research providing answers if adjuvant anti-estrogen treatment of breast cancer can induce cognitive impairment? Life Sciences 2013; 93(17): 581-8.
15. Wefel JS, Kesler SR, Noll KR, Schagen SB. Clinical characteristics, pathophysiology, and management of noncentral nervous system cancer-related cognitive impairment in adults. CA: A Cancer Journal for Clinicians 2015; 65(2): 123-38.
16. Whittaker AL, George RP, O'Malley L. Prevalence of cognitive impairment following chemotherapy treatment for breast cancer: a systematic review and meta-analysis. Scientific Reports 2022; 12(1): 2135.
17. Schilder CM, Seynaeve C, Beex LV, et al. Effects of tamoxifen and exemestane on cognitive functioning of postmenopausal patients with breast cancer: results from the neuropsychological side study of the tamoxifen and exemestane adjuvant multinational trial. Journal of Clinical Oncology 2010; 28(8): 1294-300.
18. Lee Meeuw Kjoe PR, Kieffer JM, Small BJ, et al. Effects of tamoxifen and exemestane on cognitive function in postmenopausal patients with breast cancer. JNCI Cancer Spectrum 2023; 7(2).
19. Collins B, Mackenzie J, Stewart A, Bielajew C, Verma S. Cognitive effects of hormonal therapy in early stage breast cancer patients: a prospective study. Psycho-Oncology 2009; 18(8): 811-21.
20. Underwood EA, Rochon PA, Moineddin R, et al. Cognitive sequelae of endocrine therapy in women treated for breast cancer: a meta-analysis. Breast Cancer Research and Treatment 2018; 168(2): 299-310.
21. Van Dyk K, Crespi CM, Bower JE, Castellon SA, Petersen L, Ganz PA. The cognitive effects of endocrine therapy in survivors of breast cancer: A prospective longitudinal study up to 6 years after treatment. Cancer 2019; 125(5): 681-9.
22. Mulder TAM, de With M, Del Re M, Danesi R, Mathijssen RHJ, van Schaik RHN. Clinical CYP2D6 Genotyping to Personalize Adjuvant Tamoxifen Treatment in ER-Positive Breast Cancer Patients: Current Status of a Controversy. Cancers 2021; 13(4).
23. Stearns V, Johnson MD, Rae JM, et al. Active tamoxifen metabolite plasma concentrations after coadministration of tamoxifen and the selective serotonin reuptake inhibitor paroxetine. Journal of the National Cancer Institute 2003; 95(23): 1758-64.

24. Madlensky L, Natarajan L, Tchu S, et al. Tamoxifen Metabolite Concentrations, CYP2D6 Genotype, and Breast Cancer Outcomes. Clinical Pharmacology & Therapeutics 2011; 89(5): 718-25.

25. Saladores P, Mürdter T, Eccles D, et al. Tamoxifen metabolism predicts drug concentrations and outcome in premenopausal patients with early breast cancer. Pharmacogenomics Journal 2015; 15(1): 84-94.

26. Helland T, Henne N, Bifulco E, et al. Serum concentrations of active tamoxifen metabolites predict long-term survival in adjuvantly treated breast cancer patients. Breast Cancer Research 2017; 19(1): 125.

27. Sanchez-Spitman AB, Moes DAR, Swen JJ, et al. Exposure-response analysis of endoxifen serum concentrations in early-breast cancer. Cancer Chemotherapy and Pharmacology 2020; 85(6): 1141-52.

28. Braal CL, Beijnen JH, Koolen SLW, et al. Relevance of Endoxifen Concentrations: Absence of Evidence Is Not Evidence of Absence. Journal of Clinical Oncology 2019; 37(22): 1980-1.

29. Braal CL, Jager A, Hoop EO, et al. Therapeutic Drug Monitoring of Endoxifen for Tamoxifen Precision Dosing: Feasible in Patients with Hormone-Sensitive Breast Cancer. Clinical Pharmacokinetics 2022; 61(4): 527-37.

30. Fox P, Balleine RL, Lee C, et al. Dose Escalation of Tamoxifen in Patients with Low Endoxifen Level: Evidence for Therapeutic Drug Monitoring-The TADE Study. Clinical Cancer Research 2016; 22(13): 3164-71.

31. Buijs SM, Oomen-de Hoop E, Braal CL, et al. The impact of endoxifen-guided tamoxifen dose reductions on endocrine side-effects in patients with primary breast cancer. ESMO Open 2023; 8(1): 100786.

32. Binkhorst L, Mathijssen RH, Ghobadi Moghaddam-Helmantel IM, et al. Quantification of tamoxifen and three of its phase-I metabolites in human plasma by liquid chromatography/triple-quadrupole mass spectrometry. Journal of Pharmaceutical and Biomedical Analysis 2011; 56(5): 1016-23.

33. Agema BC, Buijs SM, Sassen SDT, et al. Toward model-informed precision dosing for tamoxifen: A population-pharmacokinetic model with a continuous CYP2D6 activity scale. Biomedicine & Pharmacotherapy 2023; 160: 114369.

34. Feenstra HEM, Murre JMJ, Vermeulen IE, Kieffer JM, Schagen SB. Reliability and validity of a self-administered tool for online neuropsychological testing: The Amsterdam Cognition Scan. Journal of Clinical and Experimental Neuropsychology 2018; 40(3): 253-73.

35. Feenstra HE, Vermeulen IE, Murre JM, Schagen SB. Online Self-Administered Cognitive Testing Using the Amsterdam Cognition Scan: Establishing Psychometric Properties and Normative Data. Journal of Medical Internet Research 2018; 20(5): e192.

36. Jones D, Vichaya EG, Wang XS, et al. Validation of the M. D. Anderson Symptom Inventory multiple myeloma module. Journal of Hematology & Oncology 2013; 6: 13.

37. Zigmond AS, Snaith RP. The hospital anxiety and depression scale. Acta Psychiatrica Scandinavica 1983; 67(6): 361-70.

38. Smets EM, Garssen B, Bonke B, De Haes JC. The Multidimensional Fatigue Inventory (MFI) psychometric qualities of an instrument to assess fatigue. Journal of Psychosomatic Research 1995; 39(3): 315-25.

39. Luijendijk M, Lee Meeuw Kjoe P, Wortelboer N, et al. Cognitive function in patients with HR+ advanced breast cancer treated with endocrine therapy with or without CDK4/6 inhibitors in the SONIA trial. Journal of Clinical Oncology 2024; 42(16_suppl): 1016-.

40. Fallowfield LJ, Leaity SK, Howell A, Benson S, Cella D. Assessment of quality of life in women undergoing hormonal therapy for breast cancer: validation of an endocrine symptom subscale for the FACT-B. Breast Cancer Research and Treatment 1999; 55(2): 189-99.

41. Lezak MD. Neuropsychological assessment: Oxford University Press, USA; 2004.

42. Wefel JS, Vardy J, Ahles T, Schagen SB. International Cognition and Cancer Task Force recommendations to harmonise studies of cognitive function in patients with cancer. Lancet Oncology 2011; 12(7): 703-8.

43. Ingraham L, Aiken C. An Empirical Approach to Determining Criteria for Abnormality in Test Batteries With Multiple Measures. Neuropsychology 1996; 10: 120-4.

44. Salthouse TA. Selective review of cognitive aging. Journal of the International Neuropsychological Society 2010; 16(5): 754-60.

45. Souwer ETD, Sanchez-Spitman A, Moes D, et al. Tamoxifen pharmacokinetics and pharmacodynamics in older patients with non-metastatic breast cancer. Breast Cancer Research and Treatment 2023; 199(3): 471-8.

46. Lien EA, Søiland H, Lundgren S, et al. Serum concentrations of tamoxifen and its metabolites increase with age during steady-state treatment. Breast Cancer Research and Treatment 2013; 141(2): 243-8.

47. Mueller-Schoell A, Klopp-Schulze L, Schroth W, et al. Obesity Alters Endoxifen Plasma Levels in Young Breast Cancer Patients: A Pharmacometric Simulation Approach. Clinical Pharmacology & Therapeutics 2020; 108(3): 661-70.

48. Ohsumi S, Shimozuma K, Ohashi Y, et al. Health-related quality of life and psychological distress of breast cancer patients after surgery during a phase III randomized trial comparing continuation of tamoxifen with switching to anastrozole after adjuvant tamoxifen for 1–4 years: N-SAS BC 03. Breast Cancer Research and Treatment 2011; 127(1): 143-52.

49. Takei H, Ohsumi S, Shimozuma K, et al. Health-related quality of life, psychological distress, and adverse events in postmenopausal women with breast cancer who receive tamoxifen, exemestane, or anastrozole as adjuvant endocrine therapy: National Surgical Adjuvant Study of Breast Cancer 04 (N-SAS BC 04). Breast Cancer Research and Treatment 2012; 133(1): 227-36.

50. Cella D, Fallowfield L, Barker P, et al. Quality of Life of Postmenopausal Women in the ATAC ("Arimidex", Tamoxifen, Alone or in Combination) Trial after Completion of 5 years' Adjuvant Treatment for Early Breast Cancer. Breast Cancer Research and Treatment 2006; 100(3): 273-84.

51. Ahles TA, Saykin AJ, McDonald BC, et al. Longitudinal assessment of cognitive changes associated with adjuvant treatment for breast cancer: impact of age and cognitive reserve. Journal of Clinical Oncology 2010; 28(29): 4434-40.

52. Luine VN. Estradiol and cognitive function: Past, present and future. Hormones and Behavior 2014; 66(4): 602-18.

53. Zwart W, Terra H, Linn SC, Schagen SB. Cognitive effects of endocrine therapy for breast cancer: keep calm and carry on? Nat Rev Clin Oncol. 2015;12:597–606.

54. Boyle CP, Raji CA, Erickson KI, Lopez OL, Becker JT, Gach HM, et al. Estrogen, brain structure, and cognition in postmenopausal women. Hum Brain Mapp. 2021;42:24–35.

55. Shanmugan S, Epperson CN. Estrogen and the prefrontal cortex: towards a new understanding of estrogen's effects on executive functions in the menopause transition. Hum Brain Mapp. 2014;35:847–65.

56. Dieni CV, Contemori S, Biscarini A, Panichi R. De Novo Synthesized Estradiol: A Role in Modulating the Cerebellar Function. Int J Mol Sci. 2020;21:3316.

57. Li M, Caeyenberghs K. Longitudinal assessment of chemotherapy-induced changes in brain and cognitive functioning: A systematic review. Neurosci Biobehav Rev. 2018;92:304–17.

58. Chen X, He X, Tao L, Li J, Wu J, Zhu C, et al. The Working Memory and Dorsolateral Prefrontal-Hippocampal Functional Connectivity Changes in Long-Term Survival Breast Cancer Patients Treated with Tamoxifen. Int J Neuropsychopharmacol. 2017;20:374–82.

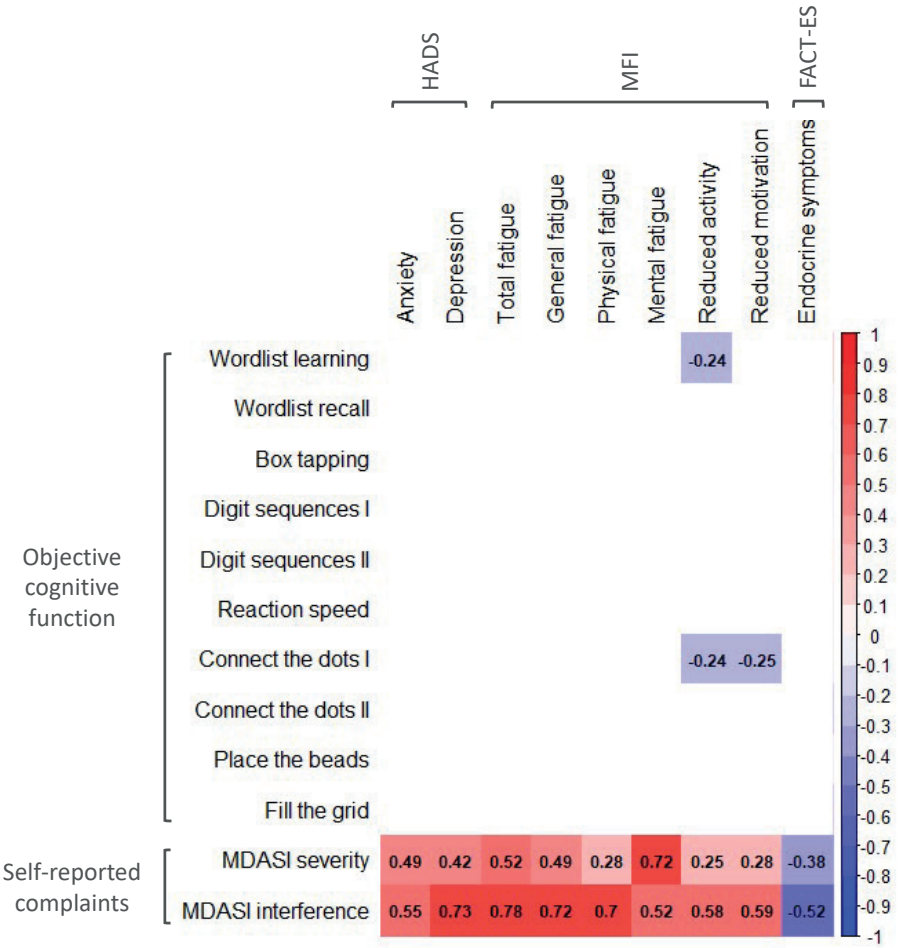
59. McDonald BC, Van Dyk K, Deardorff RL, Bailey JN, Zhai W, Carroll JE, et al. Multimodal MRI examination of structural and functional brain changes in older women with breast cancer in the first year of antiestrogen hormonal therapy. Breast Cancer Res. Treat. 2022;194:113–26.

60. Kjoe P, van der Wall E, Schagen SB. Endocrine Therapy With or Without CDK4/6 Inhibitors in Women With Hormone-receptor Positive Breast Cancer: What do we Know About the Effects on Cognition? Clin Breast Cancer 2022; 22(3): 191-9.

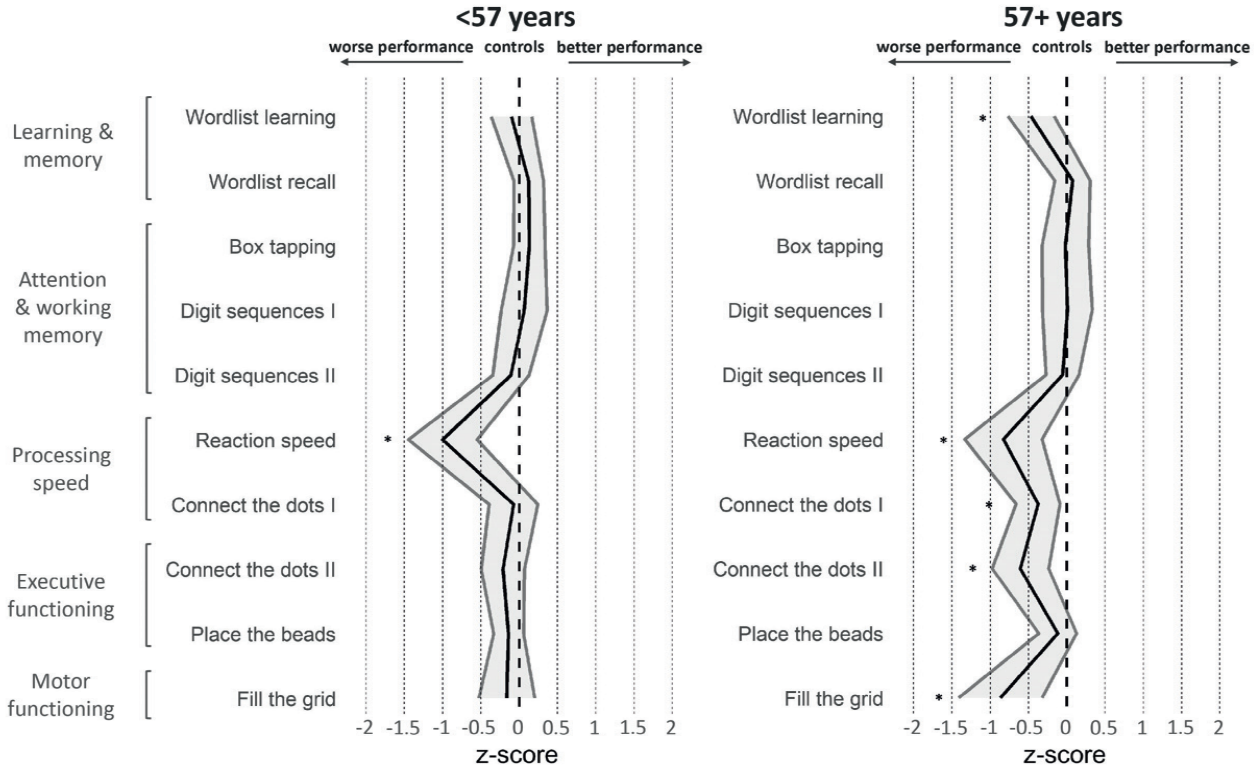
61. Hutchinson AD, Hosking JR, Kichenadasse G, Mattiske JK, Wilson C. Objective and subjective cognitive impairment following chemotherapy for cancer: a systematic review. Cancer Treatment Reviews 2012; 38(7): 926-34.

62. Baltussen JC, Derks MGM, Lemij AA, et al. Association between endocrine therapy and cognitive decline in older women with early breast cancer: Findings from the prospective CLIMB study. European Journal of Cancer 2023; 185: 1-10.

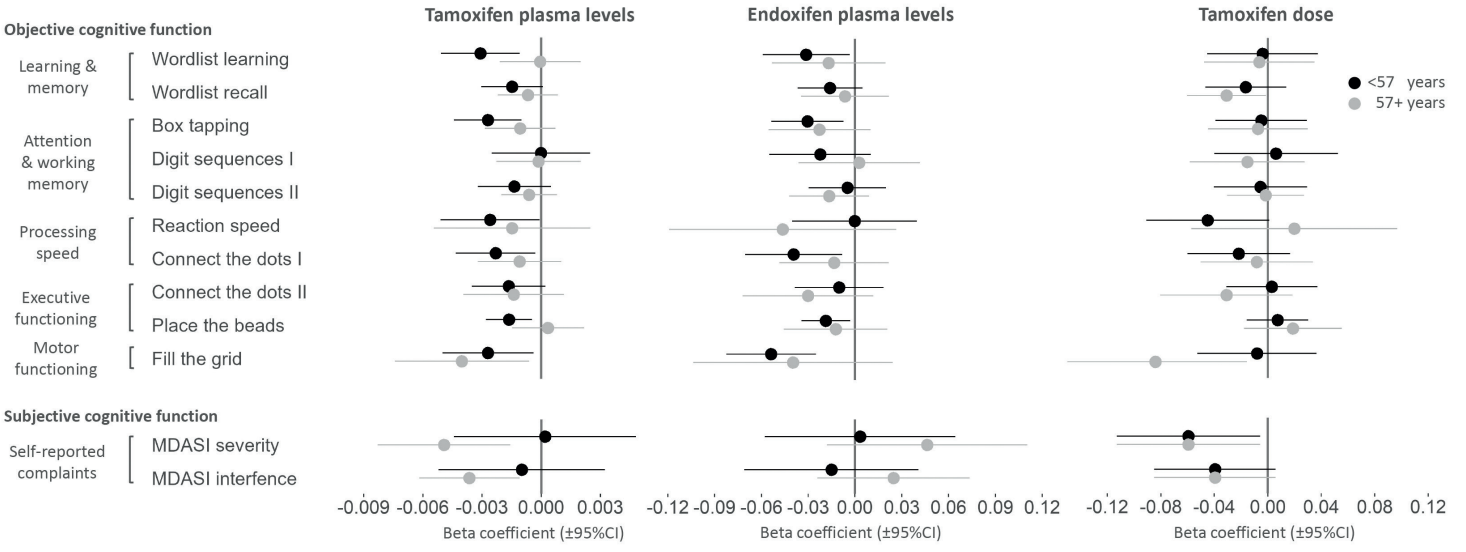
SUPPLEMENTARY MATERIAL



Supplementary A. Cognitive function and self-reported outcomes
Correlogram of the pairwise correlations between objective and subjective cognitive function and other self-reported outcomes. Only significant Pearson's correlation coefficients are visualized.



Supplementary E. Cognitive test performance after median age-split (<57 years versus 57 years or older)
Visualized are patients' average ACS Z-scores for patients aged below 57 years and patients aged 57 years or older, standardized based on controls of the same age. The shaded area indicates the 95% confidence interval. * indicates significant deviation from 0.



Supplementary F. Tamoxifen exposure and cognitive function after median age-split (<57 years versus 57 years or older)
Forest plot of unstandardized beta coefficients from linear regressions of mean tamoxifen or endoxifen concentrations and tamoxifen dose on cognitive outcome measures, for patients aged below 57 years (black) and patients aged 57 years or older (grey). For objective cognitive function, negative coefficients indicate worse cognitive performance. For subjective cognitive function, positive coefficients indicate more self-reported problems.

PART II

TAMOXIFEN AND MODEL-INFORMED PRECISION DOSING

CHAPTER 7

Toward model-informed precision dosing for tamoxifen: a population-pharmacokinetic model with a continuous CYP2D6 activity scale

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ABSTRACT

Background

Tamoxifen is important in the adjuvant treatment of breast cancer. A plasma concentration of the active metabolite endoxifen of ≥16 nM is associated with a lower risk of breast cancer-recurrence. Since inter-individual variability is high and >20% of patients do not reach endoxifen levels ≥16 nM with the standard dose tamoxifen, therapeutic drug monitoring is advised. However, ideally, the correct tamoxifen dose should be known prior to start of therapy. Our aim is to develop a population pharmacokinetic (POP-PK) model incorporating a continuous CYP2D6 activity scale to support model informed precision dosing (MIPD) of tamoxifen to determine the optimal tamoxifen starting dose.

Methods

Data from eight different clinical studies were pooled (539 patients, 3661 samples) and used to develop a POP-PK model. In this model, CYP2D6 activity per allele was estimated on a continuous scale. After inclusion of covariates, the model was subsequently validated using an independent external dataset (378 patients). Thereafter, dosing cut-off values for MIPD were determined.

Results

A joint tamoxifen/endoxifen POP-PK model was developed describing the endoxifen formation rate. Using a continuous CYP2D6 activity scale, variability in predicting endoxifen levels was decreased by 37% compared to using standard *CYP2D6* genotype predicted phenotyping. After external validation and determination of dosing cut-off points, MIPD could reduce the proportion of patients with subtherapeutic endoxifen levels from 22.1% toward 4.8%.

Conclusion

Implementing MIPD from the start of tamoxifen treatment with this POP-PK model can reduce the proportion of patients with subtherapeutic endoxifen levels at steady-state to less than 5%.

INTRODUCTION

Tamoxifen is being used for decades to prevent disease recurrence and mortality in patients suffering from estrogen receptor-positive breast cancer. In prior research, five years of adjuvant tamoxifen therapy reduced breast cancer recurrence with 42% and breast cancer specific death by 22%.[1] However, despite this effective treatment, breast cancer still recurred in 30% of patients within 15 years of follow-up.[2,3]

Tamoxifen is a prodrug which is metabolized by CYP2D6 and CYP3A4 into 4-hydroxytamoxifen, N-desmethyl-tamoxifen (NDM-tamoxifen) and is subsequently metabolized into its most clinically relevant metabolite; endoxifen.[4] Endoxifen competes with estrogen for the estrogen receptor and thereby inhibits the stimulating effect of estrogen on breast cancer cells.[5] An exposure – response relationship of endoxifen was found in a large retrospective cohort and an activity threshold of 16 nM endoxifen plasma concentration was reported.[6] Patients with endoxifen levels below this threshold showed 26% higher breast cancer recurrence rates compared to patients with endoxifen levels above the 16 nM threshold. The endoxifen exposure – response relation has been validated in a different study showing that patients with endoxifen concentrations <14 nM had an almost two-fold higher risk of distant recurrence.[7] Therefore, it is hypothesized that applying therapeutic drug monitoring (TDM) of tamoxifen to verify that patients are above the 16 nM endoxifen threshold could further decrease disease recurrence.

A large cohort study showed that approximately 21% of patients did not reach sufficient steady-state endoxifen levels (<16 nM), using the standard dose of 20 mg tamoxifen once daily. When TDM was applied, the proportion of patients below the threshold was reduced to 11%.[8] However, as endoxifen reaches steady-state after three months, six months was needed to assign the correct personalized dose to each patient. Ideally, the appropriate dose to reach the endoxifen threshold concentration should be known prior to starting tamoxifen treatment. If the appropriate dose is not known, patients could be exposed to insufficient endoxifen levels and thus, suboptimal treatment. Selecting the optimal dose, from the start of treatment, using model-informed precision dosing (MIPD) may solve these problems.

To date, six population pharmacokinetic (pop-PK) models have been developed to describe both tamoxifen and endoxifen PK.[9-14] These models found that inter-individual variation (IIV) on the rate of endoxifen formation was best explained by *CYP2D6* phenotype or CYP2D6 activity score with scores of 0, 0.5 or 1 per allele. However, recent findings showed that CYP2D6 activity could also be described on a more sensitive continuous scale.[15] In this study we use a continuous CYP2D6 activity scale

to develop a more sensitive pop-PK model. This model may, in turn, be used in MIPD to treat the patient with the correct tamoxifen dose when starting tamoxifen treatment.

MATERIALS AND METHODS

Clinical database

Data was pooled from multiple datasets originating from eight different clinical studies conducted in the Erasmus Medical Center Cancer Institute. Both, sparse data describing tamoxifen/endoxifen PK over multiple years and dense data describing PK during a single-dose interval were available. Sparse PK data was provided by the TOTAM study, a prospective open-label intervention study.[8] Female patients treated with adjuvant tamoxifen for breast cancer were eligible for participation. In this study, blood samples were obtained from patients at 3, 4.5, 6, 12, 18 and 24 months after starting tamoxifen treatment. Dense PK data was available from seven studies which were studying possible interacting agents (*i.e.* rifampicin, curcumin, green tea, probenecid, and cannabidiols)[16-20], the effect of circadian rhythm on tamoxifen PK[21], or using dextromethorphan as phenotyping test to predict endoxifen plasma concentrations.[22] All PK samples which were taken during co-administration with a potent interacting agent were excluded to ensure model and covariate stability. In total, 37 participants of the mentioned dense sampling studies also participated in the TOTAM study. All patients provided written informed consent prior to participation and all studies were conducted according to the declaration of Helsinki.

All PK samples in the clinical database were analyzed in the laboratory of translational pharmacology at the Erasmus MC Cancer Institute using a validated LC-MS/MS method.[23] *CYP2D6* and *CYP3A4* genotyping analyses were performed using both the Quantstudio (ThermoFisher Scientific; Waltham, MA) and the Infiniti (Autogenomics; Carlsbad, CA) machines.

Population PK model

All PK data was converted into molar values and subsequently logarithmically transformed prior to modelling. Initially, tamoxifen PK data was modelled to a one-compartmental model with first order absorption and first order elimination. Thereafter, multi-compartmental models, different absorption models (lag-time, transit compartments, Weibull absorption, and zero-order absorption), different elimination models (zero-order, nonlinear clearance) and introduction of exponentially modelled IIV, on different parameters were tested. Subsequently, the available NDM-tamoxifen and 4-hydroxytamoxifen samples were added to the model. Thereafter, endoxifen was

included in the model using a first-order metabolic rate with IIV. Residual error in plasma concentrations was estimated with a proportional error model. As two types of data were available, the residual error was separately estimated for each data type (*i.e.* dense, sparse) and each compound. In addition, for the dense data, inter-occasional variability (IOV) was introduced to account for differences between dense sampling occasions.

The effect of *CYP2D6* genotype on the endoxifen formation rate was incorporated as a continuous scale into the model. When an allele was present in less than two patients, the activity score was fixed to the categorical activity score of Pharmvar (activity of 0, 0.5 or 1).[24] If the *CYP2D6* genotype was unknown, the genotype was assigned to a distinct variable for unknown CYP2D6 activity so that known alleles were not affected by this group. The allele showing the lowest activity expressed by at least five patients was fixed to 0 (no activity) and the *1 genotype was fixed to 1 (full activity). An additional parameter estimated the relative amount of the formation rate to be dependent of *CYP2D6* genotype activity. All patients in the model development dataset were tested for *CYP2D6* *1 - *7, *9, *10, *17, *29, *31, *41 and duplications.

To further explain variability in the endoxifen formation rate, weight, height, age, body mass index (BMI), lean body mass (LBM), and body surface area (BSA) were tested as continuous covariates. These were centered on the median and tested as power models. Missing values were replaced by the carry-forward method or if no data was known the median value was imputed. *CYP3A4*22* genotype and radiation therapy were tested as categorical covariates. Covariates models were included using a stepwise forward inclusion ($p < 0.05$) with backward elimination ($p < 0.01$) procedure.

Model validation

The final model was internally evaluated using visual predictive checks (VPCs). External validation was performed using data from the Margarete Fischer-Bosch-Institute of Clinical Pharmacology in Stuttgart, Germany.[25] Patient characteristics of this population are depicted in the **Supplementary Table S1**. In contrast to the model-development dataset, patients were not screened for harboring *CYP2D6* *2 (normal activity), *17 (decreased activity), *29 (decreased activity) or *31 (no activity) alleles, whereas patients in the validation set were screened for the *35 allele (normal activity), which was not present in the model development dataset. These patients were excluded from the validation. Using the final model the median prediction error (MDPE) (<20%), the median absolute prediction error (MAPE) (<30%), and the fraction within 30% ($F_{30\%}$) (>50%) and 20% ($F_{20\%}$) (>35%) were calculated to test the accuracy and precision of the final model.[26,27]

MIPD simulations

Cut-off values for each dosing interval (20, 30 or 40 mg) were determined using receiver operating characteristic (ROC) curves. The population prediction was used as a predictor whereas the first measured endoxifen trough concentration at steady-state was used as true value. The optimal cut-off point was determined, as proposed by Perkins and Schisterman, using the prevalence of subtherapeutic endoxifen concentrations in the dataset and a cost which was set to 0.35 in consultation with clinicians.[28] After determining the cut-off points, this dosing strategy was implemented on both the development dataset as well as the validation dataset.

RESULTS

Clinical database

The model-development dataset constituted of 539 patients and 3613 plasma samples in which both endoxifen and tamoxifen were quantified. Almost half of these samples were steady-state trough levels (n = 1655), whereas the other half (n = 1958) constituted of dense PK data from one of 165 24 h-cycles on steady-state. In total, data from 25 patients and 11 additional samples were excluded due to concomitant CYP2D6 inhibitor use (12 patients), missing dosing information (seven patients), tamoxifen non-adherence (six patients and three additional samples) or samples that were accidentally taken after discontinuation of tamoxifen therapy (six samples), two samples were excluded as both the tamoxifen and endoxifen concentrations from a patient raised by 50% after hospitalization for allopurinol induced drug induced rash with eosinophilia and systemic symptoms. The endoxifen and tamoxifen concentrations thereafter returned to normal steady-state concentrations. Patient characteristics are depicted in **Table 1.** All patients were treated in the adjuvant setting for primary breast cancer (stage I to stage III breast cancer). Patient characteristics per study population and the validation dataset are shown in **Supplementary Table S1** .

Population PK model

The final model is schematically presented in **Figure 1**. A two-compartmental model with an additive error model best described simultaneous tamoxifen and endoxifen plasma concentrations. As NDM-tamoxifen and 4-hydroxytamoxifen were quantified in only 37% of all samples, inclusion of these samples introduced significant instability and high shrinkage to the model and were therefore excluded. In the base model, IIV was modelled on tamoxifen clearance and the transformation rate of tamoxifen to endoxifen. Endoxifen distribution volume was fixed to 400 L as reported earlier in literature and also used in previous models describing tamoxifen/endoxifen PK.[10,29]

Absorption was best described by a combined absorption lag time followed by first order absorption. The addition of a peripheral tamoxifen compartment introduced model instability and was hence discarded. For endoxifen, the additional compartment did not lead to a significantly improved model. The clearance of both endoxifen and tamoxifen was best described by a first-order rate. Introduction of IOV on tamoxifen clearance or endoxifen formation rate between different 24-hour cycles introduced a shrinkage >30% and was therefore not incorporated into the final structural model.

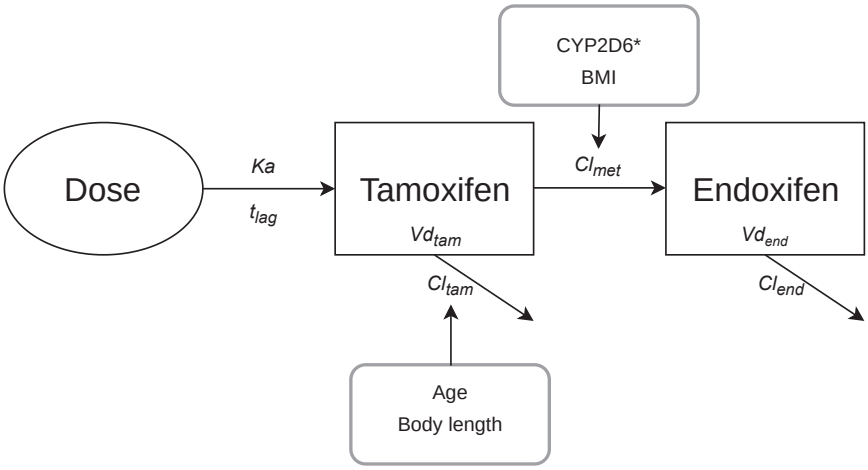

Figure 1. Schematic representation of the final population PK model structure and the incorporated covariate relationships represented in the blue boxes. * The influence of CYP2D6 on the endoxifen formation rate was modelled as seen in Eq. 1. Ka absorption rate, t_{lag} lag time, Vd_{tam} apparent distribution volume of tamoxifen, Cl_{tam} apparent clearance of tamoxifen, Cl_{met} apparent endoxifen formation rate, BMI body mass index, Vd_{end} apparent distribution volume of endoxifen, Cl_{end} apparent clearance of endoxifen.

The influence of *CYP2D6* genotype on endoxifen formation rate was modelled on a continuous scale and multiplied by a parameter estimating the percentage of the endoxifen formation rate to be CYP2D6 dependent. Duplicate fully active (*1 and *2) alleles were pooled as the CYP2D6 activity score predictions were similar. Inclusion of CYP2D6 phenotypes decreased the inter-individual variability (IIV) of the endoxifen formation rate from 59.0% to 42.8%. Inclusion of known CYP2D6 activity score subgroups instead of phenotypes decreased the IIV from 59.0% to 33.4% and a model-predicted continuous CYP2D6 activity scale decreased the IIV further to 26.8%. After careful evaluation of the residual unexplained IIV a power term was added to the equation ensuring a good fit over all values of CYP2D6 activity, which decreased unexplained IIV by 0.6% (Eq. 1) (**Supplementary Figure S1**).

Table 1. Patient characteristics of the model-development cohort

Patient characteristic	**Median**	**IQR**
Age (years)	56	47 – 65
Height (cm)	168	164 – 173
Weight (kg)	74	66 – 84
BMI (kg/m^2)	26.1	23.0 – 29.9
BSA (m^2)	1.87	1.75 – 1.99
LBM (kg)	45.3	41.9 – 49.1
Data type		
Dense	134	24.4%
Sparse	415	75.6%
	No.	%
CYP2D6 alleles		
*1/*2	83	15.1%
*1/*4	76	13.8%
*1/*1	72	13.1%
*1/*41	36	6.6%
*2/*2	33	6.0%
*2/*41	30	5.5%
*2/*4	28	5.1%
*4/*4	22	4.0%
*1/*9	15	2.7%
*1/*5	11	2.0%
*4/*41	10	1.8%
*4/*5	8	1.5%
*2/*5	8	1.5%
*2/*3	8	1.5%
*2/*9	7	1.3%
*1/*10	7	1.3%
*4/*10	6	1.1%
Unknown	10	1.8%
Other	123	13.5%
CYP3A4 alleles		
*1/*1	366	66.7%
*1/*22	16	2.9%
*22/*22	4	0.7%
Unknown	163	29.7%

BMI body mass index, BSA body surface area, IQR inter-quartile range, LBM lean body mass

$$CYP2D6\ activity = \left(Allele_1 + Allele_2/2\right)^{\theta} \quad (1)$$

In this equation, *Allele1* and *Allele2* represent the activity of each allele. θ is the exponent which was estimated by NONMEM (**Table 2**). Model-predicted CYP2D6 activity scores of the most common allele combinations are depicted in **Table 2**. A visual comparison of the categorical phenotyping scale, the gene activity score[30], and the model-estimated activity scale, of CYP2D6 is shown in **Figure 2**.

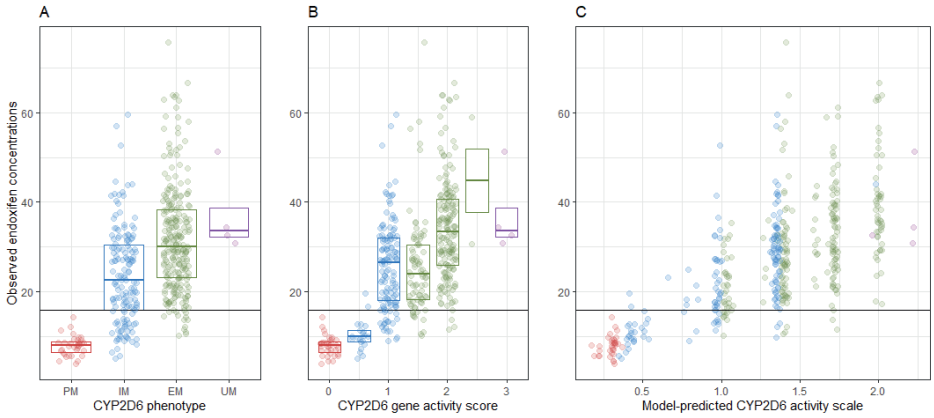

Figure 2. Comparison of conventional and the model-predicted CYP2D6 activity scales. The observed endoxifen concentration was stratified on conventional CYP2D6 predicted phenotype (A), gene activity score (B) and the model-predicted CYP2D6 activity score (C). Data comprised of the first endoxifen trough observations at steady-state. The horizontal line represents the 16nM effectivity threshold. The colors represent the predicted phenotype where red dots represent poor metabolizers, blue represents the intermediate metabolizers, green represents the extensive metabolizers and purple dots represent ultrarapid metabolizers. PM poor metabolizer, IM intermediate metabolizer, EM extensive metabolizer, UM ultrarapid metabolizer.

In addition to *CYP2D6* genotype, patients' BMI significantly influenced the endoxifen formation rate. Including these covariates in the model, diminished unexplained variability in the endoxifen formation rate from 26.8% to 25.1%. Tamoxifen clearance was influenced by both age and patient height. Inclusion of these covariates reduced IIV on this parameter from 34.7% to 32.1%. CYP3A4*22 genotype, radiation therapy, LBM, BSA and weight did not affect endoxifen or tamoxifen PK to a significant extent. The effect of each covariate on the steady-state endoxifen concentrations is shown in **Figure 3**. All parameter estimates and their corresponding 95% confidence intervals and shrinkages are depicted in **Table 2**.

Model validation

Six patients were excluded from the external validation dataset as these patients harboured a CYP2D6 allele which was not present in the model-development dataset. As patient height was missing in 61% of cases, it was imputed in the validation dataset when missing, using a reference dataset from the Dutch central bureau for statistics which contained the estimated height for each age group depending on their birth year.[31] External model validation showed that the model adequately described the data by meeting the criteria mentioned in the methods section (MDPE, -1.53%; MAPE, 34.25%; F_{20}, 39.06%; F_{30}, 55.21%).

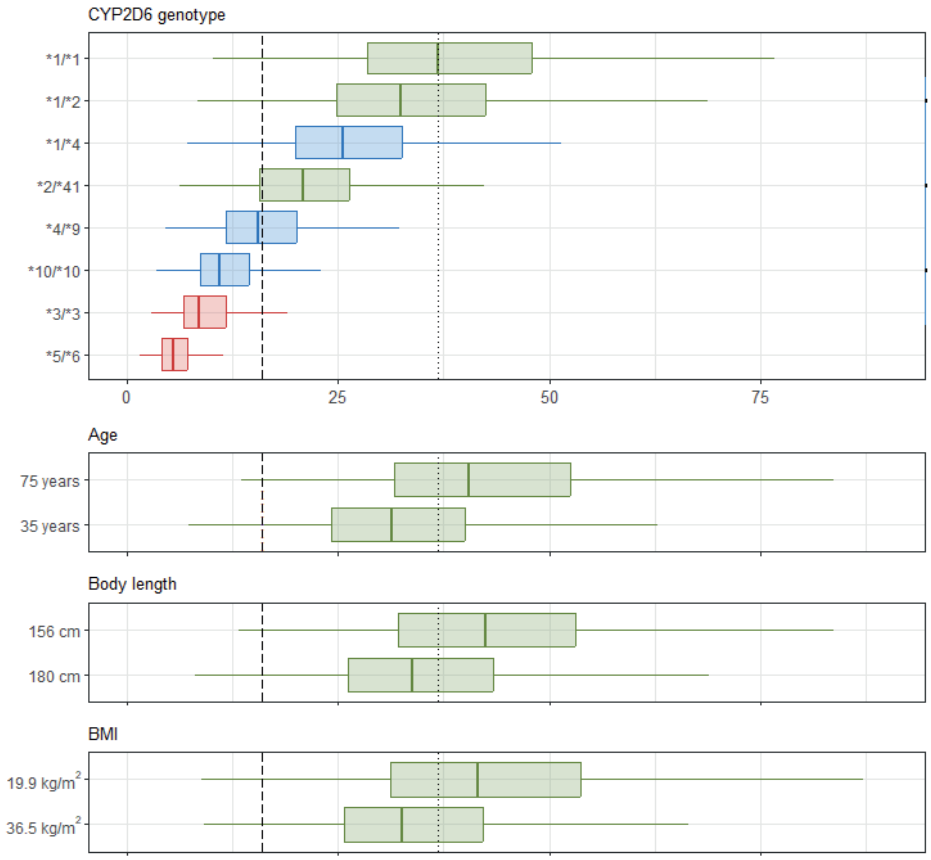

Figure 3. Effect of incorporated covariates on the predicted steady-state endoxifen concentration. For every situation, 1000 simulations were run. For CYP2D6, different genotypes were depicted based on prevalence and activity score. In the CYP2D6 simulations, other covariates were set to the median. In the other simulations, the *1/*1 genotype was used. CYP2D6 phenotypes are represented by colours where red is poor metabolizer, blue is intermediate metabolizer, and green represents normal/extensive metabolizer. The 16 nM threshold is shown as a dashed-line. The dotted line represents the median of a person with median age (56 years), patient height (168 cm), BMI (26.03 kg/m^2) and a *1/*1 genotype. BMI body mass index

Table 2. Final model parameter estimates

Parameter	Estimate	95% CI	Shrinkage
K_a (h)	1.45	1.13 – 1.77	
t_{lag} (h)	0.44	0.37 – 0.50	
Vd_{tam}/F (L)	880	729 - 1031	
Cl_{tam}/F (L/h)	7.38	7.18 – 7.58	
Cl_{met}/F (L/h)	0.155	0.113 – 0.197	
Vd_{end}/F (L)	400	FIX	
Cl_{end}/F (L/h)	1.23	0.91 – 1.56	
CYP2D6 alleles			
*1	1.000	FIX	
*2	0.560	0.476 – 0.644	
*3	0.066	0.024 – 0.108	
*4	0.047	0.018 – 0.076	
*5	0.040	0.007 – 0.073	
*6	0.000	FIX	
*7	0.000	FIX	
*9	0.378	0.267 – 0.489	
*10	0.103	0.028 – 0.178	
*17	0.156	0.064 – 0.248	
*29	0.490	0.278 – 0.702	
*31	0.000	FIX	
*41	0.110	0.052 – 0.168	
duplicate *1/*2	1.400	0.806 – 1.994	
Unknown	0.589	0.369 – 0.809	
Exponent (eq.1)	0.606	0.466 – 0.746	
% CYP2D6 mediated*	0.946	0.892 – 1.000	
Covariates			
Age (Cl_{tam})†	-0.414	-0.535 - -0.293	
Patient height (Cl_{tam})†	1.460	0.670 – 2.250	
BMI (Cl_{met}) †	-0.394	-0.511 - -0.277	
Additive error model			
Dense data			
Tamoxifen	0.153	0.143 – 0.161	3.1%
Endoxifen	0.161	0.151 – 0.171	3.1%
Sparse data			
Tamoxifen	0.188	0.178 – 0.198	11.0%
Endoxifen	0.186	0.177 – 0.195	11.4%

Parameter	Estimate	95% CI	Shrinkage
Residual IIV			
IIV Cl_{tam}	32.0%		3.7%
IIV Cl_{met}	25.3%		11.0%

* This parameter estimated the percentage of the endoxifen formation rate to be CYP2D6 dependent. † Power model. ‡ Proportional model. Ka absorption rate, t_{lag} lag time, Vd_{tam} apparent distribution volume of tamoxifen, Cl_{tam} apparent clearance of tamoxifen, Cl_{met} apparent endoxifen formation rate, BMI body mass index, Vd_{end} apparent distribution volume of endoxifen, Cl_{end}, apparent clearance of endoxifen, FIX parameter was not estimated but set to this value.

MIPD simulations

Dosing cut-off points were determined using ROC curves (**Supplementary Figure S2**). The optimal cut-off point was determined with prevalence set to 23% and cost set to 0.35. The cut-off point for receiving 40 mg tamoxifen was a model-predicted steady-state level of 11.40 nM endoxifen when treated with 20 mg tamoxifen. When the model predicted that a patient will not reach 20.23 nM endoxifen at steady-state when using 20 mg tamoxifen, this patient should be given 30 mg. Patients with a predicted endoxifen concentration above 20.23 nM will be treated with the standard dose of 20 mg. Simulations showed that instead of 22.1% not reaching sufficient endoxifen levels, using these model-informed dosing recommendations could diminish this proportion to 9.9%. When also switching patients that were identified to be at risk for not being capable of reaching endoxifen thresholds even at the maximum registered dose of 40 mg (steady state endoxifen <8.56 nM), to aromatase inhibitors, the proportion of patients that does not reach 16 nM endoxifen decreases further toward 4.8%. The results of imposing this dosing strategy on the first endoxifen samples in the model development dataset is visualized in **Figure 4A**. Out of all patients with a simulated dose-increase, 19.7% showed endoxifen plasma concentrations >32 nM and could have been treated with a lower dose. In addition, from all patients which were recommended to be switched, 30.0% could manage to obtain endoxifen plasma levels >16 nM with 40 mg.

When imposing these dosing cut-off points on the external validation set, a similar reduction in patients with endoxifen plasma concentrations <16 nM is seen. Whereas with a one-dose-fits-all dosing regimen 17.9% of patients do not reach endoxifen levels >16 nM, with MIPD this could be reduced to 9.5% (**figure 4B**) and further toward 6.5% when also switching patients at risk for underexposure on 40 mg to aromatase inhibitors.

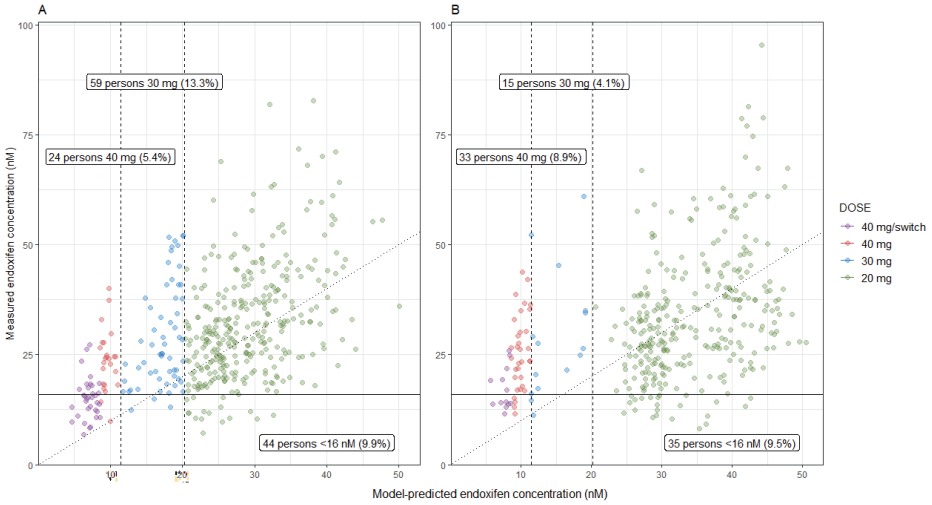

Figure 4. Simulation of model-informed prediction dosing for tamoxifen/endoxifen.
(A) Data comprised of the first endoxifen trough concentrations at steady-state in patients in the model development dataset (n = 443). (B) The MIPD cut-off points simulated in the external dataset (n = 369). The horizontal line represents the 16 nM effectivity threshold and the vertical lines represent the 40 mg and the 30 mg dosing cut-off values. The dotted line is the unity line.

DISCUSSION

Using a continuous individual CYP2D6 allele activity score, the model's accuracy to predict endoxifen trough concentrations was significantly improved compared to using CYP2D6 phenotypes. Therefore using this model for MIPD could reduce the number of patients being below the threshold of 16 nM from 22.1% toward 4.8% immediately after reaching steady-state. Similar results were seen when simulating the MIPD in an external dataset. The addition of MIPD using this model at the start of tamoxifen treatment could help to ensure a fast and safe determination of the right tamoxifen dose.

Continuous-scale CYP2D6 activity assignment has been previously performed in one study.[15] The assignments of our predicted activity scores per genotype using NONMEM are comparable to this study. However, in this previous research, an additive model with an addition from a neural network when a patient harboured a single *1 genotype was used. As no machine learning was implemented in this research, we described the relation between genotypes and CYP2D6 activity using a power model.

Although most of the IIV was explained, 25% still remains unexplained. This could be due to patients harbouring SNPs which were not included in our panel. However, the minor allele frequency of these are low (<1%) or, like *CYP2D6*35,* are known to minimally affect CYP2D6 activity compared to a fully functioning variant.[24]

Therefore, we feel this should not significantly affect the results of our activity scale. Besides, non-genetic causes such as treatment adherence, which was not quantified in every dataset, or unknown interactions are more likely explaining this residual variability.

In addition to the more sensitive CYP2D6 activity scale, age and patient height affected tamoxifen clearance, whereas BMI influenced endoxifen formation rate. Although age has been described in previous tamoxifen models[10], patient height has not been described as an influential covariate. However, as IIV was only modelled on tamoxifen clearance, patient height may be affecting distribution volume as tamoxifen is mostly distributed into organs, which size are affected by patient height.[32] The effect of BMI or body weight has already been described in previous papers.[9,10,33] As BMI is mostly affected by body weight instead of height and the covariate analysis was performed using forward inclusion, including both in the model is feasible.

Because the validation cohort was not specifically tailored for this study some covariate information was missing. The uncertainty of harbouring the *2, *17, *29 or *31 alleles could have influenced the results from the external validation as these alleles were present in the development dataset. Patients harbouring these genotypes will be falsely interpreted as fully functioning *1 alleles. In addition, although the imputation of patients' height corrected for the influence of age, the approximation still leads to loss of data. Although the effect of patient height on the steady-state endoxifen levels is clinically irrelevant, this could have affected the validation. However, most importantly, the validation showed that the model adequately described the data despite these impediments.

In addition to developing a model using a more sensitive CYP2D6 activity scale, cut-off points which can be used for MIPD were identified. Using these cut-off values, simulations showed that the proportion of patients not reaching >16 nM endoxifen after three months of treatment could be diminished. As endoxifen reaches steady-state concentrations after three months of tamoxifen use, using TDM guided dosing could take six to nine months to get patients on the ideal dose to reach sufficient endoxifen trough levels.[8] Using MIPD with a simple knowledge of patients' age, patient height and weight and determining CYP2D6 genotype could decrease the proportion of patients with insufficient endoxifen levels to less than 5% when steady-state is first reached. Rapid achievement of sufficient endoxifen levels may translate into better outcomes for tumor relapse. In addition, patients with a high risk of not reaching sufficient tamoxifen steady-state concentrations can be identified before starting therapy and could be treated with an aromatase inhibitor and sooner receive adequate treatment.

However, in most cases, subsequent TDM at steady-state should still be used to identify the small amount patients that do not reach sufficient steady-state endoxifen concentrations. As shown in **Figure 4**, a small proportion of patients in the 20 mg group do not reach endoxifen concentrations >16 nM at first TDM. However, in some cases TDM does not have to be necessary, an old patient with a *1/*1 genotype or patients with a duplicate *1 allele have an approximate 99% chance of reaching adequate endoxifen concentrations at 20 mg. In addition, using MIPD, less TDM samples will be necessary as more patient will be sufficiently exposed at the first TDM occasion. When more research is performed explaining variability in tamoxifen and endoxifen pharmacokinetics, over time MIPD might wholly replace TDM.

In conclusion, applying MIPD with the developed model incorporating the influence of CYP2D6 activity on a continuous scale could diminish the amount of patients with insufficient endoxifen levels to less than 5%. Applying MIPD may therefore improve outcomes for women with estrogen-receptor positive breast cancer. Prospective implementation of this dosing strategy will further ensure the feasibility of MIPD for tamoxifen in clinical practice.

REFERENCES

1. Tamoxifen for early breast cancer: an overview of the randomised trials. Early Breast Cancer Trialists' Collaborative Group. Lancet 1998; 351(9114): 1451-67.
2. Early Breast Cancer Trialists' Collaborative G. Effects of chemotherapy and hormonal therapy for early breast cancer on recurrence and 15-year survival: an overview of the randomised trials. Lancet 2005; 365(9472): 1687-717.
3. Early Breast Cancer Trialists' Collaborative G, Davies C, Godwin J, et al. Relevance of breast cancer hormone receptors and other factors to the efficacy of adjuvant tamoxifen: patient-level meta-analysis of randomised trials. Lancet 2011; 378(9793): 771-84.
4. Binkhorst L, Mathijssen RH, Jager A, van Gelder T. Individualization of tamoxifen therapy: much more than just CYP2D6 genotyping. Cancer Treatment Reviews 2015; 41(3): 289-99.
5. Coezy E, Borgna JL, Rochefort H. Tamoxifen and metabolites in MCF7 cells: correlation between binding to estrogen receptor and inhibition of cell growth. Cancer Research 1982; 42(1): 317-23.
6. Madlensky L, Natarajan L, Tchu S, et al. Tamoxifen metabolite concentrations, CYP2D6 genotype, and breast cancer outcomes. Clinical Pharmacology & Therapeutics 2011; 89(5): 718-25.
7. Saladores P, Murdter T, Eccles D, et al. Tamoxifen metabolism predicts drug concentrations and outcome in premenopausal patients with early breast cancer. Pharmacogenomics J 2015; 15(1): 84-94.
8. Braal CL, Jager A, Hoop EO, et al. Therapeutic Drug Monitoring of Endoxifen for Tamoxifen Precision Dosing: Feasible in Patients with Hormone-Sensitive Breast Cancer. Clinical Pharmacokinetics 2022; 61(4): 527-37.
9. Klopp-Schulze L, Mueller-Schoell A, Neven P, et al. Integrated Data Analysis of Six Clinical Studies Points Toward Model-Informed Precision Dosing of Tamoxifen. Frontiers in Pharmacology 2020; 11: 283.
10. Mueller-Schoell A, Klopp-Schulze L, Schroth W, et al. Obesity Alters Endoxifen Plasma Levels in Young Breast Cancer Patients: A Pharmacometric Simulation Approach. Clinical Pharmacology & Therapeutics 2020; 108(3): 661-70.
11. Puszkiel A, Arellano C, Vachoux C, et al. Model-Based Quantification of Impact of Genetic Polymorphisms and Co-Medications on Pharmacokinetics of Tamoxifen and Six Metabolites in Breast Cancer. Clinical Pharmacology & Therapeutics 2021; 109(5): 1244-55.
12. ter Heine R, Binkhorst L, de Graan AJ, et al. Population pharmacokinetic modelling to assess the impact of CYP2D6 and CYP3A metabolic phenotypes on the pharmacokinetics of tamoxifen and endoxifen. British Journal of Clinical Pharmacology 2014; 78(3): 572-86.
13. Min W, Yiyun G, Haitang X. Population pharmacokinetics of tamoxifen in patients with breast cancer. Chinese Journal of Clinical Pharmacology and Therapeutics 2019; 24(4): 418-23.
14. Klopp-Schulze L, Joerger M, Wicha SG, et al. Exploiting Pharmacokinetic Models of Tamoxifen and Endoxifen to Identify Factors Causing Subtherapeutic Concentrations in Breast Cancer Patients. Clinical Pharmacokinetics 2018; 57(2): 229-42.
15. van der Lee M, Allard WG, Vossen R, et al. Toward predicting CYP2D6-mediated variable drug response from CYP2D6 gene sequencing data. Science Translational Medicine 2021; 13(603).
16. Binkhorst L, van Gelder T, Loos WJ, et al. Effects of CYP induction by rifampicin on tamoxifen exposure. Clinical Pharmacology & Therapeutics 2012; 92(1): 62-7.
17. Braal CL, Hussaarts K, Seuren L, et al. Influence of green tea consumption on endoxifen steady-state concentration in breast cancer patients treated with tamoxifen. Breast Cancer Research and Treatment 2020; 184(1): 107-13.
18. Hussaarts K, Hurkmans DP, Oomen-de Hoop E, et al. Impact of Curcumin (with or without Piperine) on the Pharmacokinetics of Tamoxifen. Cancers (Basel) 2019; 11(3).
19. Buck SAJ, Braal CL, Hofman MM, et al. Influence of probenecid on endoxifen systemic exposure in breast cancer patients on adjuvant tamoxifen treatment. Therapeutic Advances in Medical Oncology 2022; 14: 17588359221081075.
20. Buck SAJ, Braal CL, Kuijper-Tissot van Patot H, et al. Study on the potential pharmacokinetic interaction between cannabidiol (CBD) and tamoxifen in patients with primary breast cancer. Study Protocol [Unpublished Work] 2022.

21. Binkhorst L, Kloth JSL, de Wit AS, et al. Circadian variation in tamoxifen pharmacokinetics in mice and breast cancer patients. Breast Cancer Research Treatment 2015; 152(1): 119-28.

22. de Graan AJ, Teunissen SF, de Vos FY, et al. Dextromethorphan as a phenotyping test to predict endoxifen exposure in patients on tamoxifen treatment. Journal of Clinical Oncology 2011; 29(24): 3240-6.

23. Binkhorst L, Mathijssen RH, Ghobadi Moghaddam-Helmantel IM, et al. Quantification of tamoxifen and three of its phase-I metabolites in human plasma by liquid chromatography/triple-quadrupole mass spectrometry. Journal of Pharmaceutical and Biomedical Analysis 2011; 56(5): 1016-23.

24. Consortium PV. CYP2D6. 31 May 2022 2022. https://www.pharmvar.org/gene/CYP2D6 (accessed 21 Juni 2022).

25. Murdter TE, Schroth W, Bacchus-Gerybadze L, et al. Activity levels of tamoxifen metabolites at the estrogen receptor and the impact of genetic polymorphisms of phase I and II enzymes on their concentration levels in plasma. Clinical Pharmacology and Therapeutics 2011; 89(5): 708-17.

26. Sheiner LB, Beal SL. Some suggestions for measuring predictive performance. Journal of Pharmacokinetics and Biopharmaceutics 1981; 9(4): 503-12.

27. Zhao CY, Jiao Z, Mao JJ, Qiu XY. External evaluation of published population pharmacokinetic models of tacrolimus in adult renal transplant recipients. British Journal of Clinical Pharmacology 2016; 81(5): 891-907.

28. Perkins NJ, Schisterman EF. The inconsistency of "optimal" cutpoints obtained using two criteria based on the receiver operating characteristic curve. American Journal of Epidemiology 2006; 163(7): 670-5.

29. Ahmad A, Shahabuddin S, Sheikh S, et al. Endoxifen, a new cornerstone of breast cancer therapy: demonstration of safety, tolerability, and systemic bioavailability in healthy human subjects. Clinical Pharmacology and Therapeutics 2010; 88(6): 814-7.

30. Gaedigk A, Simon SD, Pearce RE, Bradford LD, Kennedy MJ, Leeder JS. The CYP2D6 activity score: translating genotype information into a qualitative measure of phenotype. Clinical Pharmacology and Therapeutics 2008; 83(2): 234-42.

31. Reep C. Een studie naar de lengteontwikkeling van Nederlanders. 2021. https://www.cbs.nl/nl-nl/longread/statistische-trends/2021/een-studie-naar-de-lengteontwikkeling-van-nederlanders/3-resultaten (accessed 21 June 2022).

32. Lien EA, Solheim E, Ueland PM. Distribution of tamoxifen and its metabolites in rat and human tissues during steady-state treatment. Cancer Research 1991; 51(18): 4837-44.

33. Braal CL, Westenberg JD, Buijs SM, et al. Factors affecting inter-individual variability in endoxifen concentrations in patients with breast cancer: results from the prospective TOTAM trial. Breast Cancer Research and Treatment 2022; 195(1): 65-74.

34. Braal CL, Jager A, Hoop EO, et al. Therapeutic Drug Monitoring of Endoxifen for Tamoxifen Precision Dosing: Feasible in Patients with Hormone-Sensitive Breast Cancer. Clinical Pharmacokinetics 2021.

SUPPLEMENTARY MATERIAL

Table S1: Patient characteristics stratified by study cohort

Data type					**Dense**			**Sparse**	**Total**	**Validation**
Study	**De Graan 2011 (1)**	**Binkhorst 2012 (2)**	**Binkhorst 2015 (3)**	**Hussaarts 2019 (4)**	**Braal 2020 (5)**	**Buck 2022 (6)**	**Buck 2022(7)**	**Braal 2021 (8)**		**Murdter 2011 (9)**
No. of patients	36	8	31	16	14	11	14	445	539	369
No. of samples per cycle	9	13	9	13	13	13	13	1		1
Sampling cycles	1	1	2	1	2	1	1	3 - 6		1
Dose at start of study										
20 mg	26	6	30	16	13	5	12	445	525	369
30 mg	0	0	0	0	0	0	1	0	1	0
40 mg	10	2	1	0	1	6	1	0	13	0
Patient characteristic (median)										
Age (years)	52	50	51	44.5	58.5	58.5	49	57	56	64.25
Height (cm)	168	164	167.5	171	167.5	171	166.5	168	168	163
Weight (kg)	71.75	66.2	73.8	72.85	77	68	76	75	74	70
BMI (kg/m^2)	26.06	25.35	25.99	25.14	27.01	23.51	26.23	26.12	26.08	25.81
CYP2D6 alleles										
*1	25	6	22	15	11	2	14	315	387	445
*2	21	0	8	5	10	3	4	207	241	1
*3	2	0	1	1	0	0	0	13	17	12
*4	14	1	12	7	3	9	5	147	182	129
*5	3	0	2	0	1	4	1	31	37	21
*6	1	0	0	1	0	1	0	14	16	11

Data type	Dense							Sparse	Total	Validation
Study	**De Graan 2011 (1)**	**Binkhorst 2012 (2)**	**Binkhorst 2015 (3)**	**Hussaarts 2019 (4)**	**Braal 2020 (5)**	**Buck 2022 (6)**	**Buck 2022(7)**	**Braal 2021 (8)**		**Murdter 2011 (9)**
*7	0	0	0	0	0	0	1	1	1	2
*9	2	0	6	0	0	0	1	23	31	21
*10	0	0	1	0	0	1	1	22	23	18
*17	1	0	1	0	1	0	1	9	11	0
*29	0	0	0	0	0	0	0	5	5	0
*31	0	0	0	0	0	0	0	1	1	0
*41	5	1	7	0	2	2	0	86	99	68
duplicate *1	0	0	0	0	0	0	0	2	2	6
duplicate *2	0	0	0	1	0	0	0	4	5	4
Unknown	4	8	2	2	0	0	0	4	20	0
CYP2D6 phenotypes										
Poor metabolizer	0	0	0	1	0	4	0	35	36	22
Intermediate metabolizer	15	1	9	7	3	7	7	151	185	41
Extensive metabolizer	19	3	21	6	11	0	7	253	303	303
Ultrarapid metabolizer	0	0	0	1	0	0	0	4	5	1
Unknown	2	4	1	1	0	0	0	2	10	0
Participated in Braal 2021 [7]	0	0	0	0	14	11	12	445	37	0

CHAPTER 8

Implementation of model-informed precision dosing for tamoxifen therapy in patients with breast cancer: A prospective intervention study

The Breast, January 2025

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ABSTRACT

Tamoxifen is an estrogen-receptor (ER) antagonist, used as adjuvant treatment of ER-positive breast cancer. It is converted by CYP2D6 into endoxifen, its most active metabolite. Patients with endoxifen plasma concentrations <16 nM face a higher risk of recurrence. The use of *a priori* model-informed precision dosing (MIPD) may lead to faster target attainment and thus potentially improve patient outcomes. In total, 106 evaluable patients were prospectively included in this single-arm MIPD-intervention study. Patients received a model-predicted tamoxifen dose when starting tamoxifen-treatment (65.1% of patients received 20mg, 16.0% received 30mg and 18.9% received 40mg). Seventy-five percent of the 40mg group was predicted to be unable to reach the threshold 16 nM despite receiving the highest registered dose. After attaining steady-state, 84.0% of patients reached endoxifen levels ≥16 nM, which was not significantly higher compared to a historical control cohort (77.9%, $p = 0.17$). The model showed adequate performance and correctly identified patients requiring 40mg tamoxifen. Endoxifen samples that were acquired 4 – 6 weeks after treatment initiation, are informative of steady-state endoxifen levels and can be used to inform MIPD and adjust tamoxifen dosing prior to steady-state attainment. In this first MIPD implementation study for patients treated with tamoxifen, MIPD did lead to more patients achieving endoxifen levels ≥16 nM as compared to the one-dose-fits-all strategy, albeit insignificant. This may partly be explained by a larger proportion of patients who were recommended to switch to an aromatase inhibitor (AI) in the intervention cohort. In conclusion, MIPD seems beneficial compared to one-size-fits-all-dosing, but TDM still remains an important addition.

INTRODUCTION

Tamoxifen is a selective estrogen-receptor (ER) modulator and is important in the adjuvant treatment of ER-positive breast cancer where it reduces the breast cancer recurrence rate.[1,2] Tamoxifen is a prodrug and exerts its effect primarily through its active metabolite endoxifen.[3,4] Tamoxifen is converted into endoxifen by cytochrome P450 (CYP) iso-enzymes, particularly by CYP2D6. Polymorphisms of the *CYP2D6* gene can hamper CYP2D6 activity and lead to lower endoxifen concentrations.[5,6] Madlensky *et al.* found an association between endoxifen concentrations and breast cancer recurrence in a large retrospective analysis of a prospective study.[7] Patients with endoxifen concentrations <16 nmol/L (5.97 ng/mL) were exposed to a 30% higher risk of breast cancer recurrence compared to patients with endoxifen concentrations above this threshold.[7]

Approximately 20-24% of tamoxifen patients do not reach the 16 nmol/L endoxifen threshold while treated with the standard tamoxifen dose of 20 mg.[7-10] When applying Therapeutic Drug Monitoring (TDM), doses are adapted to measured plasma concentrations in order to reach the therapeutic threshold. In past studies, implementation of TDM resulted in approximately 90% of patients with endoxifen levels above the 16 nM threshold after 6 months of tamoxifen therapy.[8,11] One drawback of TDM of tamoxifen in its current form, however, is that the dose adjustments can only be performed after reaching steady-state plasma concentrations, which for tamoxifen is reached after 3 months of therapy.[12] Consequently, patients requiring a tamoxifen dose adjustment after TDM are potentially undertreated during the first 3 to 6 months of therapy. Model-informed precision dosing (MIPD) may counter this problem by both predicting the adequate tamoxifen dose per patient before the start of treatment and identifying patients will not reach the 16 nM threshold using the highest registered dose and therefore may profit from a switch toward an aromatase inhibitor (AI). Although it is under debate on what falls within the term of MIPD, we refer to using a population-pharmacokinetic (popPK) model, capable of describing and predicting patient-specific absorption, distribution, metabolism, and elimination of a drug based on several patient characteristics, to forecast plasma concentrations before treatment.[13] However, it has never been prospectively investigated whether MIPD leads to an increase in the proportion of patients inside the therapeutic interval while avoiding unnecessary dose increases. Therefore, the primary aim of this study was to investigate whether implementation of MIPD could increase the proportion of patients achieving an endoxifen level >16 nM at steady-state.

MATERIALS AND METHODS

Study Type and Population

The PREDICTAM (PREDICtion of TAMoxifen) trial was conducted as an open-label, single-arm intervention study at the Erasmus MC Cancer Institute in Rotterdam, the Netherlands. It received approval from the institutional review board (MEC-2022-0437) and was registered in the U.S. National Library of Medicine (clinicaltrials.gov; NCT05525481). Patient were eligible for inclusion when starting adjuvant treatment with tamoxifen for primary breast cancer who were able and willing to abstain from moderate and strong CYP2D6 and CYP3A4 inhibitors. Exclusion criteria were ongoing tamoxifen treatment exceeding two weeks prior to the moment of inclusion (i.e. baseline visit), previous quantification of endoxifen levels and the male sex. The control arm was a cohort derived from the TOTAM-study (MEC-2017-548), with which the POP-PK model was developed.[14] This cohort was followed-up prospectively at the Erasmus MC Cancer Institute using the same catchment area and in- and exclusion criteria as the interventional arm. It consisted of 443 patients, all treated with the standard dose of 20 mg tamoxifen during the first 3 months, of whom 345 (77.9%) reached the endoxifen threshold of 16 nM at 3 months of treatment (steady-state).[14] Randomization was not performed in this study as the intervention arm consisted of the entire PREDICTAM cohort and the control arm of the entire cohort derived from the TOTAM study, which rendered the study an open-label study.

Study design

This study used a previously described validated popPK model to predict a patient's steady-state endoxifen plasma concentration and determine the appropriate tamoxifen dose at baseline.[14] Details regarding the prediction model and for which patient each dose was predicted can also be found in the **Supplementary (Section I).** The study consisted of three visits: at baseline (before or within 2 weeks after start of tamoxifen), after 4-6 weeks of tamoxifen therapy, and after 3 months of tamoxifen therapy or, when performed, after a dose-escalation (i.e. steady-state). At baseline, informed consent was obtained and all patient covariates needed for dose prediction were collected (CYP2D6 genotype, body mass index (BMI), age, and body height. Also, patients were asked to fill in the Functional Assessment of Cancer Therapy – Endocrine Symptoms (FACT-ES) questionnaire regarding endocrine side effects and health-related quality of life (HR-QOL). In case patients had already started tamoxifen treatment, an endoxifen plasma sample was collected. After determination of the CYP2D6 genotype **(Supplementary Section II)**, a model-informed tamoxifen dose of either 20, 30, or 40 mg was determined and prescribed. During the second visit, after 4-6 weeks of treatment, another plasma sample was taken to investigate whether pre-steady-state endoxifen plasma samples could be indicative of steady-state endoxifen levels. As the early plasma samples were analyzed

after completion of the trial, this data did not influence the advised dose during the study. At the last visit, steady-state endoxifen concentrations **(Supplementary Section II)** were measured and patients filled in the FACT-ES questionnaire for the second time.

Statistical analysis

The primary endpoint of this study was the proportion of patients with an endoxifen level ≥16 nM at steady-state, *i.e.* 3 months after start of therapy or dose-escalation using MIPD. It was hypothesized that the proportion of patients with endoxifen levels ≥16 nM would be at least 90% and, therefore, higher than the 78% observed in the historical cohort. To test this hypothesis, with a two-sided alpha of 5% and a power of 80%, at least 106 patients were needed in this new cohort. Comparison between the proportions of the historical and new cohorts was conducted using a chi-squared test. Also, the proportions of patients with endoxifen levels ≥16 nM in the different dosing categories were compared with the historical cohort using a chi-squared test or Fisher's exact test when appropriate. All secondary endpoints and corresponding statistical methods can be found in the **Supplementary (Section III)**

RESULTS

Patient selection

Between November 2022 and September 2023, 117 patients with breast cancer who received or were about to receive adjuvant tamoxifen treatment were enrolled in this study. Eight participants withdrew their consent before reaching the primary endpoint. Five patients withdrew due to experiencing tamoxifen-related side-effects, two patients because of other health complications and one patient because of the requested time burden. Three participants were excluded from the analysis. One participant was excluded due to documented and self-reported poor tamoxifen treatment compliance. Two other patients were excluded before analysis, due to a screening failure (i.e. male sex). Finally, 106 patients were evaluable for the analysis of the primary endpoint (**Figure 1**).

Baseline characteristics control and intervention cohort

An overview of the most relevant baseline characteristics of the control and intervention cohort is provided in **Table 1**. Patient height and age differed significantly, although not clinically relevant between the intervention and control cohort. For comparison purposes, it was retrospectively determined which model-informed doses would have been predicted in the control group at baseline. Remarkably, significantly more patients in the intervention cohort were predicted in need for an AI, because they would not achieve endoxifen levels >16 nM with tamoxifen while treated with a tamoxifen dose

of 40 mg, in the intervention cohort compared to the control cohort. Also, a higher percentage of patients in the intervention group had CYP2D6 activity scores below 0.6, although this difference did not meet significance due to small numbers.

Endoxifen threshold

Of the 106 patients undergoing MIPD, 89 patients (84%) reached an endoxifen level ≥16 nM at steady-state. This was not significantly higher compared with the control group (78%, $X^2 = 1.91$; $p = 0.167$). The distribution of steady-state endoxifen levels is depicted in **Figure 2**. If patients who were predicted to not reach 16 nM, despite receiving 40 mg, were actually switched to an AI, 91.5% of the population would have reached endoxifen levels ≥ 16 nM. The incidence of endoxifen levels <16 nM was also stratified per dose group **(Table 2)**. This showed that the proportion of patients with endoxifen levels <16 nM was significantly reduced in the 40 mg and switch to AI groups in the intervention cohort, compared to the dose groups in the control group where no MIPD was performed **(Table 2)**. A list with characteristics of each individual patient with subtherapeutic endoxifen levels <16 nM in the intervention cohort, was given in **Supplementary Table 1**. Notably, in the 20 mg category, four patients had endoxifen levels <16 nM, despite having favorable CYP2D6 genotypes (**1/*1, *1/*1, *1/*2* and **1/*9,* all corresponding with a CYP2D6 activity > 0.75).

Model performance

The model performance is depicted in **Figure 2** and **Table 2 & 3**. Both the relative bias and MAPE are beneath the prespecified external evaluation thresholds (**Supplementary Section III**). The model slightly overpredicts the steady-state endoxifen concentrations within the intervention cohort. Upon stratification by dose, the MAPE remained relatively consistent whereas the relative bias showed variability. Especially the 40 mg group was underpredicted by the model, although this group comprised of a group of 5 patients.

When focusing on the therapeutic interval, the model significantly increased the proportion of patients inside the therapeutic interval for patients with CYP2D6 activity ≤0.3 (**Table 2**).[14] Only one patient in this group had a steady-state endoxifen of <16 nM in case the patients predicted to need a switch were actually switched. This one patient was treated with 30 mg tamoxifen after the model predicted the patient to reach endoxifen levels 0.02nM above the 40 mg threshold. Furthermore, patients with a CYP2D6 activity ranging between 0.3 and 0.75 also benefit from a model-informed dose, albeit without statistical significance. In patients harboring a more active CYP2D6 genotype (>0.75), the model did not perform better compared to the control group as these patients were already adequately dosed with the standard 20 mg dose. A more detailed definition and explanation of CYP2D6 activity presented in the **Supplementary (Section I)**.

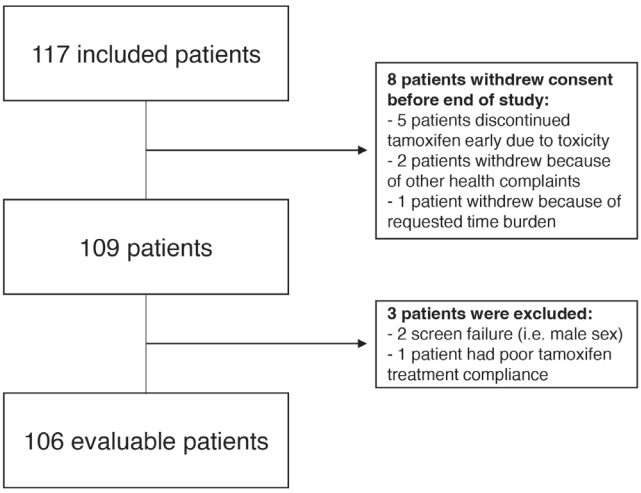

Figure 1. Flowchart for patient selection

Table 1. Baseline characteristics of the evaluable intervention

	Intervention cohort (*n* = 106)		**Control cohort (*n* = 443)**		**p-value[c]**
Age, *years*	60	[50, 68]	57	[48, 66]	0.049
Height, *cm*	166	[161, 170]	168	[163, 173]	0.001
Weight, *kg*	74	[68, 84]	75	[66, 84]	0.831
BMI, $kg \cdot m^{-2}$	27.3	[24.0, 31.3]	26.2	[23.0, 30.0]	0.087
CYP2D6 activity	0.68	[0.48, 0.86]	0.68	[0.51, 0.86]	0.325
Predicted dose					
20 mg	69	(65.0%)	326[a]	(74.0%)	0.080
30 mg	17	(16.0%)	59[a]	(13.0%)	0.467
40 mg	5	(4.7%)	24[a]	(5.4%)	0.772
(potential) switch to AI[b]	15	(14.0%)	34[a]	(7.7%)	0.036
CYP2D6 activity					
0.0 – 0.30	21	(19.8%)	62	(14.0%)	0.133
0.30 – 0.60	23	(21.6%)	83	(18.7%)	0.488
0.60 – 0.75	25	(23.6%)	152	(34.3%)	0.034
0.75 – 0.90	19	(17.9%)	90	(20.3%)	0.579
0.90 – 1.5	18	(17.0%)	56	(12.6%)	0.240

All values, except for the predicted dose and CYP2D6 activity groups, were given as medians[IQR]. The combination of two patient alleles was scored using a continuous CYP2D6 activity scale, with 1.0 being a fully active *1/*1 genotype. The predicted dose and CYP2D6 activity groups were given as n(%). [a]The predicted dose at baseline in the control group was retrospectively determined for comparison purposes. [b]This group was treated with 40 mg to evaluate the chance of reaching endoxifen 16 nM.
[c]t-test for continuous variables and chi-squared test for distributions of categorical variables.

Table 2. Patients with an endoxifen concentration of <16 nM in the control and intervention cohorts

	Patients with endoxifen <16 nM				
	Intervention cohort (MIPD)		Control cohort (20 mg)		
	n	*%/cohort*[a]	*n*	*%/cohort*[a]	*p-value*
Predicted dose					
20 mg	5	(7.2%)	17[a]	(5.2%)	0.504[b]
30 mg	4	(23.5%)	26[a]	(44.1%)	0.164[c]
40 mg	0	(0%)	21[a]	(87.5%)	<0.001[c]
(potential) switch to AI	8	(53.3%)	34[a]	(100%)	<0.001[b]
CYP2D6 activity					
0.0 – 0.30	9	(42.9%)	59	(95.2%)	<0.001[b]
0.30 – 0.60	4	(17.4%)	25	(30.1%)	0.295[c]
0.60 – 0.75	0	(0.0%)	10	(6.6%)	0.361[c]
0.75 – 0.90	3	(15.8%)	4	(4.4%)	0.100[c]
0.90 – 1.5	1	(5.6%)	0	(0.0%)	0.244[c]

All patients in the control cohort received 20 mg while in the intervention cohort patients received the predicted dose.[a] The proportion represents the percentage of patients with <16 nM at steady state per group. [b] chi-squared test. [c] Fisher's exact test

Among patients predicted to fall short of adequate endoxifen plasma concentrations, a majority indeed failed to attain adequate endoxifen plasma concentrations (>16 nM). Those achieving adequate levels showed a median endoxifen level of 18.2 nM and a maximum of 20.4 nM. The model-informed doses led to overexposure in six patients, which all but one occurred in the group treated with 30 mg tamoxifen.

Table 3. Metrics evaluating the predictive value of the model predictions

Predictions	MAPE (%)	Relative bias (%)[a]	RMSE (nM)	$F_{80-125\%}$
A priori	21.19	2.99	9.72	47.17
20 mg	*22.25*	*6.44*	*11.01*	*47.83*
30 mg	*21.33*	*11.63*	*8.21*	*47.06*
40 mg	*20.27*	*-20.27*	*7.31*	*40.00*
(potential) switch to AI	*21.65*	*-5.41*	*3.89*	*46.67*
<14 days sample[b]	27.46	0.13	10.68	37.21
4-6 weeks sample[c]	14.97	-10.91	7.36	63.73
Both samples	17.47	-12.16	7.91	53.49

Metrics evaluating the predictive value of the model predictions informed with no PK-information (a priori), stratified per dose group, or with either one or both of the samples obtained prior to reaching steady state. [a] negative percentage represents underprediction of the model. [b] Available in 43 patients. [c] Available in 102 patients. MAPE: median absolute prediction error. RMSE: root mean squared error. $F_{80-125\%}$: The amount of patients inside the 80 – 125% prediction/observation ratio.

The predictive value of pre-steady-state endoxifen samples is shown in **Table 3**. As the model-development dataset did not contain pre-steady state PK samples, these samples could not be used to change dosing. A post-hoc analysis using the samples was used to impute the pre-steady state endoxifen levels in the model and was further discussed in **Supplementary** (**Section IV**).

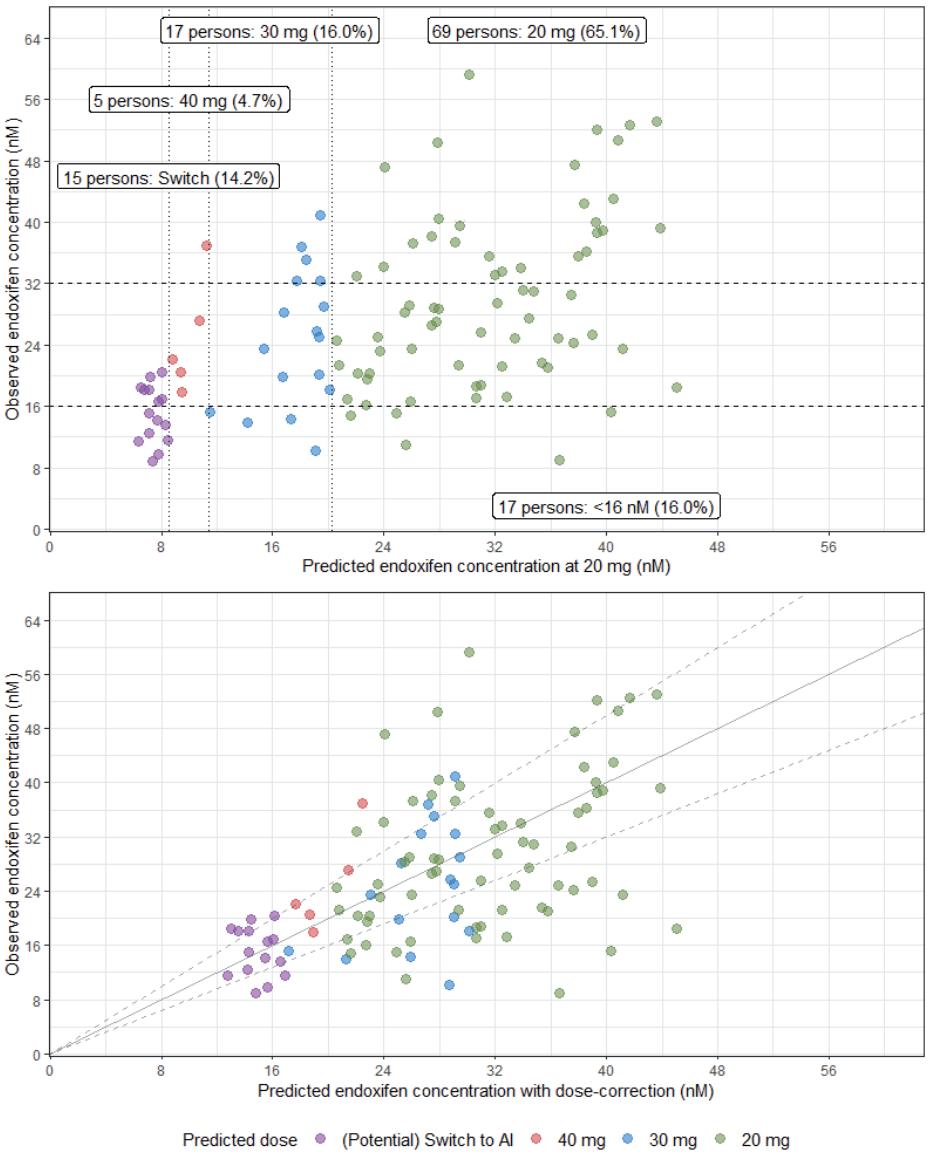

Figure. 2. Distribution of steady-state endoxifen plasma concentrations in the intervention cohort.

Quality of life and tamoxifen-related side effects results were missing for one patient, assigned to the 20 mg dose group. No significant or clinically relevant differences (>4.4 points for endocrine symptoms and >6.98 points for HR-QOL) between baseline and after 3 months in side-effects or quality of life were observed overall or in any individual dosing group (**Table 4**). At patient-level, 25 out of 68 (37%) patients had a clinically relevant increase in side-effects in the 20 mg dosing group. This applied to 6 out of 17 (35%) patients in the 30 mg group and 8 out of 20 (40%) in the 40 mg group.

Table 4. Difference in side-effects overall and in the different dosing groups.

	Baseline	**3 months**
	Total intervention cohort (*n* = 105)	
HR-QOL	86.1	87.0
ES19	64.8	61.3
	20 mg dose group (*n* = 68)	
HR-QOL	88.6	88.4
ES19	66.7	62.8
	30 mg dose group (*n* = 17)	
HR-QOL	79.6	80.8
ES19	59.5	57.0
	40 mg dose group (*n* = 20)	
HR-QOL	82.8	87.6
ES19	62.8	59.8

Each group shows the mean score at baseline on the left and mean score after three months on the right. [b]ES-19 comprises of 19 questions about specific hormone therapy related side-effects. [c]HR-QOL includes questions from 4 domains about quality-of-life. None of the differences shown were significant or clinically relevant. Higher scores equate less side effects or better quality of life.

DISCUSSION

This study aimed to implement MIPD for oncology practice. A fundamental study by Joerger et al. proved MIPD could play an important role in individualized therapy for oncology patients.[15] In this study, initial paclitaxel therapy for patients with non-small-cell lung cancer (NSCLC) in a palliative setting was dosed, adjusted to several patient characteristics. Subsequent doses were directed by neutropenia levels and previous-cycle paclitaxel exposure, therefore combining MIPD and TDM to improve patient outcomes, such as treatment side-effects and overall survival (OS).[15] The PREDICTAM study focused on putting MIPD into practice, by replacing TDM, specifically in adjuvant treatment for patients with hormone sensitive breast cancer. In contrast to the pharmacodynamic endpoints of the study by Joerger et al., this study mainly centered around pharmacokinetic objectives, such as attaining target endoxifen concentrations.

Although the difference in patients with endoxifen levels >16 nM in the intervention cohort was not significantly different from the control cohort, MIPD did result in less undertreatment when separate dose groups were compared. The model adequately identified patients who were at risk of being undertreated and identified patients who were unable to reach the therapeutic interval. In the intervention cohort, more patients than expected had low CYP2D6 activity scores, likely explaining the lower-than-expected percentage of patients reaching endoxifen levels >16 nM. Notably, a significant proportion of these patients belonged to the 'potential' switch AI group. More than half of these patients did not reach the therapeutic window and those who did, were mostly within the error margin of the model of 18.6%. When excluding these patients, 91.5% of the remaining individuals achieved endoxifen levels exceeding 16 nM, more accurately showing the potential of MIPD. In a post-hoc analysis early endoxifen samples were imputed into the model, informing unexplained inter-individual variability in the conversion rate from tamoxifen to endoxifen (**Supplementary Section IV**). If this was used to adjust dosing, the primary endpoint showed a significant improvement in the proportion of patients achieving adequate endoxifen exposure.

MIPD showed promise compared to conventional one-size-fits-all dosing of 20 mg tamoxifen. For example, patients for whom a tamoxifen dose of 20 mg was predicted, were at a low risk of undertreatment (endoxifen <16 nM in 7% of these patients in our study). In cases where non-adherence is not suspected, omitting TDM for this dose-group may be considered. Also, for patients who were predicted in the 'potential switch to AI' group more than 50% did not reach endoxifen levels ≥16 nM despite being treated with the highest dose of tamoxifen (40 mg). Especially among postmenopausal patients, initiating treatment with an AI may offer a more efficacious alternative in such cases. However, for other dose-groups, TDM still remains critical to verify adequate steady-state endoxifen levels and adjust tamoxifen doses when necessary. Nonetheless, giving a model-informed starting dose increased the adequate exposure significantly in the 40 mg group when reaching steady-state endoxifen exposure. Overall, this tailored approach reduces the need for TDM samples. While this was not an aim of our study, this approach increases the efficiency of accurate tamoxifen treatment due to fewer hospital visits being needed for TDM in this specific dose group. Additionally, this method may also facilitate earlier attainment of on-treatment endoxifen levels for all dose groups or prompt consideration for commencing treatment with an AI.

The mean scores for tamoxifen-related symptoms and HR-QOL in our study were 61.3 and 87.0 points after 3 months of tamoxifen. These scores were comparable, or even slightly better, than in previous studies where tamoxifen toxicity was assessed in larger groups of patients (ES19: 59-62 points; HR-QOL 79-83 points).[16-18] This may

be explained by the short period of tamoxifen treatment in our study. No significant or clinically relevant changes in toxicity or quality of life were observed either overall or in any individual dosing study group. These results are in contrast with previous tamoxifen studies, that commonly reported tamoxifen-related toxicity.[19,20] An explanation for this could be that our study was performed in patients who recently started tamoxifen therapy and were still recovering from other anti-cancer therapies such as surgery, radiotherapy and chemotherapy. However, although sample sizes per dose group were small, these results also imply that prescribing a dose of 30 or 40 mg tamoxifen to selected patients using MIPD, instead of the usual 20 mg dose, does not increase the risk of self-reported side-effects in the first three months of treatment. Probably, this can be explained by the fact that endoxifen levels of these patients remain within normal limits, despite using higher dosages. Although this offers reassurance regarding the risk at increased toxicity when using MIPD, caution is still warranted to avoid administering higher doses than necessary, particularly considering late and more rare side effects such as venous thromboembolism or endometrial abnormalities.[21,22]

Endoxifen plasma samples obtained prior to steady-state may add to the early detection of patients unable to reach adequate endoxifen levels. While samples collected within the first 21 days of treatment lack informative value, samples taken after 4 – 6 weeks add predictive value to the model. When imputed in the model, more than half of patients who could benefit from a dose increase at steady-state are identified. These samples were not used to adjust dosing as the model was not developed using pharmacokinetic samples prior to reaching steady-state and could therefore lead to incorrect dose adjustments. However, in post-hoc analyses the ability of the model to use these samples to correctly recommend dose adjustments is shown. Other benefits of obtaining a pre-steady-state sample is that it informs the treating physician of possible treatment non-adherence. By addressing adherence issues early on, patients can be educated about the critical importance of consistent treatment compliance. Even though the model-development dataset of the PK model did not incorporate samples taken prior to steady state, it adequately estimates empirical Bayes estimates (EBE) leading to an adequate prediction of steady-state. The underprediction after imputation of the samples in this model might result in more false positive cases of patients that might need a dose increase, although this was not the case in our cohort. However, although the results obtained show the predictive value of pre-steady-state samples, the popPK model should be updated with these samples to allow precise prediction of endoxifen concentrations and therefore allowing to change the tamoxifen dose prior to achieving steady-state.

To date, several tamoxifen population PK models have been developed[14, 23-27], yet only two of these models have been externally validated, of which one is used in this research. Thus far, the model described in this research is the only model that has been prospectively validated. In the externally validated model by Klopp-Schulze et al., the researchers showed that 92.8 percent of a simulation cohort can reach the therapeutic window.[26] In this research, we show that implementing MIPD in a prospectively followed cohort, 91.5% of patients reach the therapeutic window, when physicians adhere to the proposed switch to an AI. The similarity in this proportion indicates that implementing other models will probably not lead to a significant increase in patients reaching the therapeutic window compared to the presented model.

The presented study is not without limitations. Most notably, the differences in CYP2D6 activity in the intervention and control cohort affected the primary endpoint. Therefore, the primary endpoint does not fully reflect the ability of the model to predict an adequate dose for each individual patient. Although the number of patients being adequately exposed is a clinically relevant endpoint, being the most suitable comparison for TDM, it only partly captures the ability of the model to adequately predict endoxifen plasma levels and, subsequently, the correct tamoxifen dosage. Especially not when the most influential covariate is differently distributed in the intervention cohort compared to the control cohort. Nevertheless, numerical model evaluation techniques confirm adequate predictive performance, especially with pre-steady-state plasma samples informing EBEs. Naturally, evaluating the effect using clinical outcomes such as recurrence should be used when feasible. However, considering the relatively low recurrence rate of breast cancer, such a study would take multiple years and many more patients rendering it unfeasible. Additionally, therapy adherence was not regularly assessed in the study. Several patients had lower endoxifen plasma concentrations at steady-state compared to the pre-steady-state samples, which indicates that treatment adherence may have affected the results of the primary outcome. Lastly, in this study we used the endoxifen threshold of 16 nM, a threshold determined in a large retrospective cohort study.[7] This threshold has not been confirmed prospectively and there are studies that found lower efficacy limits for endoxifen efficacy (i.e. 9 and 14 nM).[28,29] Moreover, in studies with patients with a high risk for breast cancer or a history of breast carcinoma-in-situ, low doses of tamoxifen (5-10 mg) were proven effective in preventing breast cancer.[30,31] However, an advantage of MIPD is that it can be applied for endoxifen thresholds of whatever desired value.

This MIPD implantation study is the first in the landscape of solid breast tumors to adjust tamoxifen doses before the start of treatment. Implementation of MIPD did not result in a significantly higher proportion of patients achieving endoxifen levels ≥16 nM

compared to the one-dose-fits-all strategy. This can be explained by a larger proportion of patients with impaired CYP2D6 activity in the intervention cohort compared to the control cohort. Implementation of MIPD showed promise compared to one-size-fits-all dosing 20 mg tamoxifen, especially in a subgroup recommended to be treated with 40 mg. Particularly when the dose recommendation is informed with a pre-steady-state sample at 4-6 weeks after treatment start, MIPD may ensure swift attainment of adequate steady-state endoxifen plasma levels. Moving forward, refining the early sampling strategy and showing its predictive value across diverse patient populations will be pivotal in optimizing tamoxifen treatment outcomes.

REFERENCES

1. Systemic treatment of early breast cancer by hormonal, cytotoxic, or immune therapy. 133 randomised trials involving 31,000 recurrences and 24,000 deaths among 75,000 women. Early Breast Cancer Trialists' Collaborative Group. Lancet. 1992;339(8784):1-15.
2. Fisher B, Dignam J, Bryant J, DeCillis A, Wickerham DL, Wolmark N, et al. Five versus more than five years of tamoxifen therapy for breast cancer patients with negative lymph nodes and estrogen receptor-positive tumors. Journal of the National Cancer Institute. 1996;88(21):1529-42.
3. Lim YC, Li L, Desta Z, Zhao Q, Rae JM, Flockhart DA, et al. Endoxifen, a secondary metabolite of tamoxifen, and 4-OH-tamoxifen induce similar changes in global gene expression patterns in MCF-7 breast cancer cells. Journal of Pharmacology and Experimental Therapeutics. 2006;318(2):503-12.
4. Johnson MD, Zuo H, Lee KH, Trebley JP, Rae JM, Weatherman RV, et al. Pharmacological characterization of 4-hydroxy-N-desmethyl tamoxifen, a novel active metabolite of tamoxifen. Breast Cancer Research and Treatment. 2004;85(2):151-9.
5. Borges S, Desta Z, Li L, Skaar TC, Ward BA, Nguyen A, et al. Quantitative effect of CYP2D6 genotype and inhibitors on tamoxifen metabolism: implication for optimization of breast cancer treatment. Clinical Pharmacology and Therapeutics. 2006;80(1):61-74.
6. Jin Y, Desta Z, Stearns V, Ward B, Ho H, Lee KH, et al. CYP2D6 genotype, antidepressant use, and tamoxifen metabolism during adjuvant breast cancer treatment. Journal of the National Cancer Institute. 2005;97(1):30-9.
7. Madlensky L, Natarajan L, Tchu S, Pu M, Mortimer J, Flatt SW, et al. Tamoxifen metabolite concentrations, CYP2D6 genotype, and breast cancer outcomes. Clinical Pharmacology and Therapeutics. 2011;89(5):718-25.
8. Braal CL, Jager A, Hoop EO, Westenberg JD, Lommen K, de Bruijn P, et al. Therapeutic Drug Monitoring of Endoxifen for Tamoxifen Precision Dosing: Feasible in Patients with Hormone-Sensitive Breast Cancer. Clinical Pharmacokinetics. 2022;61(4):527-37.
9. Martinez de Dueñas E, Ochoa Aranda E, Blancas Lopez-Barajas I, Ferrer Magdalena T, Bandrés Moya F, Chicharro García LM, et al. Adjusting the dose of tamoxifen in patients with early breast cancer and CYP2D6 poor metabolizer phenotype. Breast. 2014;23(4):400-6.
10. Hertz DL, Deal A, Ibrahim JG, Walko CM, Weck KE, Anderson S, et al. Tamoxifen Dose Escalation in Patients With Diminished CYP2D6 Activity Normalizes Endoxifen Concentrations Without Increasing Toxicity. Oncologist. 2016;21(7):795-803.
11. Fox P, Balleine RL, Lee C, Gao B, Balakrishnar B, Menzies AM, et al. Dose Escalation of Tamoxifen in Patients with Low Endoxifen Level: Evidence for Therapeutic Drug Monitoring-The TADE Study. Clinical Cancer Research. 2016;22(13):3164-71.
12. Sanchez-Spitman AB, Swen JJ, Dezentje VO, Moes D, Gelderblom H, Guchelaar HJ. Clinical pharmacokinetics and pharmacogenetics of tamoxifen and endoxifen. Expert Review of Clinical Pharmacology. 2019;12(6):523-36.
13. Kluwe F, Michelet R, Mueller-Schoell A, Maier C, Klopp-Schulze L, van Dyk M, et al. Perspectives on Model-Informed Precision Dosing in the Digital Health Era: Challenges, Opportunities, and Recommendations. Clin Pharmacol Ther 2021 Ja;109(1):29–36.
14. Agema BC, Buijs SM, Sassen SDT, Mürdter TE, Schwab M, Koch BCP, et al. Toward model-informed precision dosing for tamoxifen: A population-pharmacokinetic model with a continuous CYP2D6 activity scale. Biomedicine & Pharmacotherapy. 2023;160:114369.
15. Joerger M, von Pawel J, Kraff S, Fischer JR, Eberhardt W, Gauler TC, et al. Open-label, randomized study of individualized, pharmacokinetically (PK)-guided dosing of paclitaxel combined with carboplatin or cisplatin in patients with advanced non-small-cell lung cancer (NSCLC). Ann Oncol 2016 Oc;27(10):1895–1902.
16. Cella D, Fallowfield L, Barker P, Cuzick J, Locker G, Howell A, et al. Quality of life of postmenopausal women in the ATAC ("Arimidex", tamoxifen, alone or in combination) trial after completion of 5 years' adjuvant treatment for early breast cancer. Breast Cancer Res Treat 2006 De;100(3):273–284.
17. Takei H, Ohsumi S, Shimozuma K, Takehara M, Suemasu K, Ohashi Y, et al. Health-related quality of life, psychological distress, and adverse events in postmenopausal women with breast cancer who receive tamoxifen, exemestane, or anastrozole as adjuvant endocrine therapy: National Surgical Adjuvant Study of Breast Cancer 04 (N-SAS BC 04). Breast Cancer Res Treat 2012 Ma;133(1):227–236.

18. Ohsumi S, Shimozuma K, Ohashi Y, Shinji M, Hozumi Y, Mukai H, et al. Health-related quality of life and psychological distress of breast cancer patients after surgery during a phase III randomized trial comparing continuation of tamoxifen with switching to anastrozole after adjuvant tamoxifen for 1-4 years: N-SAS BC 03. Breast Cancer Res Treat 2011 Ma;127(1):143–152.

19. Land SR, Wickerham DL, Costantino JP, Ritter MW, Vogel VG, Lee M, et al. Patient-Reported Symptoms and Quality of Life During Treatment With Tamoxifen or Raloxifene for Breast Cancer PreventionThe NSABP Study of Tamoxifen and Raloxifene (STAR) P-2 Trial. JAMA. 2006;295(23):2742-51.

20. Murphy CC, Bartholomew LK, Carpentier MY, Bluethmann SM, Vernon SW. Adherence to adjuvant hormonal therapy among breast cancer survivors in clinical practice: a systematic review. Breast Cancer Research and Treatment. 2012;134(2):459-78.

21. Deitcher SR, Gomes MP. The risk of venous thromboembolic disease associated with adjuvant hormone therapy for breast carcinoma: a systematic review. Cancer. 2004;101(3):439-49.

22. Swerdlow AJ, Jones ME, British Tamoxifen Second Cancer Study G. Tamoxifen treatment for breast cancer and risk of endometrial cancer: a case-control study. Journal of the National Cancer Institute. 2005;97(5):375-84.

23. Kanji CR, Nyabadza G, Nhachi C, Masimirembwa C. Pharmacokinetics of Tamoxifen and Its Major Metabolites and the Effect of the African Ancestry Specific CYP2D6*17 Variant on the Formation of the Active Metabolite, Endoxifen. Journal of Personalized Medicine [Internet]. 2023; 13. Available from: https://mdpi-res.com/d_attachment/jpm/jpm-13-00272/article_deploy/jpm-13-00272-v2.pdf?version=1675213202.

24. Puszkiel A, Arellano C, Vachoux C, Evrard A, Le Morvan V, Boyer JC, et al. Model-Based Quantification of Impact of Genetic Polymorphisms and Co-Medications on Pharmacokinetics of Tamoxifen and Six Metabolites in Breast Cancer. Clinical Pharmacology and Therapeutics. 2021;109(5):1244-55.

25. Mueller-Schoell A, Klopp-Schulze L, Schroth W, Mürdter T, Michelet R, Brauch H, et al. Obesity Alters Endoxifen Plasma Levels in Young Breast Cancer Patients: A Pharmacometric Simulation Approach. Clinical Pharmacology and Therapeutics. 2020;108(3):661-70.

26. Klopp-Schulze L, Mueller-Schoell A, Neven P, Koolen SLW, Mathijssen RHJ, Joerger M, et al. Integrated Data Analysis of Six Clinical Studies Points Toward Model-Informed Precision Dosing of Tamoxifen. Frontiers in Pharmacology. 2020;11:283.

27. ter Heine R, Binkhorst L, de Graan AJ, de Bruijn P, Beijnen JH, Mathijssen RH, et al. Population pharmacokinetic modelling to assess the impact of CYP2D6 and CYP3A metabolic phenotypes on the pharmacokinetics of tamoxifen and endoxifen. British

28. Helland T, Henne N, Bifulco E, Naume B, Borgen E, Kristensen VN, et al. Serum concentrations of active tamoxifen metabolites predict long-term survival in adjuvantly treated breast cancer patients. Breast Cancer Res 2017 Nov 28;19(1):125–4.

29. Saladores P, Mürdter T, Eccles D, Chowbay B, Zgheib NK, Winter S, et al. Tamoxifen metabolism predicts drug concentrations and outcome in premenopausal patients with early breast cancer. Pharmacogenomics J 2015 Fe;15(1):84–94.

30. Buijs SM, Koolen SLW, Mathijssen RHJ, Jager A. Tamoxifen Dose De-Escalation: An Effective Strategy for Reducing Adverse Effects? Drugs 2024 Ap;84(4):385–401.

31. Placebo Controlled Trial of Low-Dose Tamoxifen to Prevent Local and Contralateral Recurrence in Breast Intraepithelial Neoplasia. J Clin Oncol 2019 Jul 1;37(19):1629–1637.

SUPPLEMENTARY MATERIAL

Supplementary section I

The development and details of the used popPK model have been presented in a previous article.[1] In that research several predictors for steady-state endoxifen concentrations were identified, including age, body height, body mass index (BMI), and most importantly, continuous CYP2D6 activity, based on CYP2D6 genotype (eq.1). Individual activity scores for each allele can be found in Table S1 whereas significant covariate-PK relations have been presented in previous research.[1] Other factors such as liver function were not proven to affect tamoxifen PK and were therefore not obtained.

$$Activity_{CYP2D6} = \left(\frac{Activity_{Allele1} + Activity_{Allele2}}{2}\right)^{0.606} \quad .^{1}$$

The dosing predictions were confined to 20, 30 or 40 mg tamoxifen. The model predicted the endoxifen level for each patient under the assumption of using the standard tamoxifen dose of 20 mg. Dosing cut-off points for predicted endoxifen levels were determined using ROC-curves. Patients with predicted endoxifen levels surpassing 20.23 nM were prescribed tamoxifen 20 mg, while those with predicted levels between 11.40 nM and 20.23 nM received 30 mg, and those with predicted levels between 8.56 nM and 11.40 nM were given 40 mg. Additionally, for patients anticipated to have endoxifen levels below 8.56 nM while on 20 mg tamoxifen, it was expected that they would not reach the therapeutic threshold of 16 nM when using tamoxifen 40 mg. These patients might benefit more from adjuvant aromatase inhibitors (AI). To test this hypothesis, in this study, these patients were treated with tamoxifen 40 mg. This subgroup of patients was defined as '(potential) switch to AI' group.

Supplementary section II

Pharmacokinetic and pharmacogenetic analyses

A validated liquid chromatography-tandem mass spectrometry method (UPLC-MS/MS) was used for the quantification of all tamoxifen and endoxifen measurements.[2] Blood withdrawals were performed at the moment of trough levels of tamoxifen and endoxifen.

CYP2D6 genotyping was performed using the Infiniti (Autogenomics; Carlsblad, CA, USA) and the Quantstudio machines (ThermoFisher Scientific; Waltham, MA, USA) at the Clinical Chemistry laboratory of the Erasmus University Medical Center. The patients' blood samples were assayed on the following genetic variants: *2-10, *12, *13, *14, *17, *29, and *41. When the activity of specific CYP2D6 alleles was unknown, the website (pharmvar.org/gene/CYP2D6) was consulted. The literature was searched for its activity and a comparable allele in the model was filled in.

Supplementary section III

To elucidate the potential of the POP-PK model, the patient-specific predicted endoxifen levels were compared with the observed steady-state endoxifen levels by quantifying the mean absolute prediction error (MAPE), relative bias and the root mean squared error (RMSE). A model is generally seen as potent when the relative bias is < 20% and the MAPE is < 35% and more than 30% of all prediction errors is within 80 – 125% limit.[3] Moreover, the POP-PK model's efficacy in identifying patients failing to achieve the 16 nM endoxifen threshold despite receiving the maximum tamoxifen dose of 40 mg, i.e. the '(potential) switch to AI' group, was evaluated. Additionally, it was evaluated whether the model informed dose resulted in overexposure, which was defined as surpassing an endoxifen level of 32 nM while treated with a model-initiated dose increase. Both secondary endpoints were analyzed descriptively.

The predictive efficacy of early endoxifen levels (measured within 14 days or between 4-6 weeks after the initiation of tamoxifen treatment) was assessed in a post-hoc analysis by integrating them into the predictive model. The early endoxifen levels were not used to change tamoxifen doses during the study. Subsequently, the MAPE, relative bias, and RMSE were computed to evaluate the accuracy of predictions based on these early samples. Furthermore, an exploration was conducted to assess the potential impact of possessing this information, particularly in the context of acting if the early samples indicated levels below a certain threshold.

To investigate whether dose adjusting according to MIPD would lead to more toxicity, the difference in tamoxifen-related side effects and HR-QOL between start of therapy and after reaching steady-state endoxifen plasma levels was evaluated, stratified on separate dosing categories. The difference in tamoxifen-related side effects and quality of life between baseline and 3 months of therapy was compared using a paired sample t-test or a Wilcoxon signed-rank test when appropriate. Changes in ES or HR-QOL were seen as clinically relevant when they surpassed 0.5 of standard deviation at baseline.[4] The percentage of patients with clinically relevant changes in side effects per dose group were analyzed descriptively.

Supplementary section IV

During the study endoxifen samples prior to steady-state were obtained at the time of inclusions (<14 days of tamoxifen treatment, n=43) and after 4 – 6 weeks of treatment (n=102). Distribution of samples over time is provided in **Figure S1**. These samples were obtained to assess the predictive value of these samples for predicting steady-state concentrations. As the used model was not developed using pre-steady-state samples these were not used to affect the tamoxifen dose during treatment. In this post-hoc

analysis, the samples were imputed in the popPK model used in this research.[1] A sample at the start of treatment (<14 days) deteriorated the predictive value of the model compared to the predictions prior to the start of treatment. Conversely, samples obtained between 4 – 6 weeks of treatment improved model predictions, although it introduced underprediction. Model evaluation by a visual predictive check, showed slight overprediction for the pre-steady-state samples, explaining the underprediction at steady-state (**Figure S2**). When imputing both pre-steady-state samples in the model, this did not improve the model predictions compared to only the sample taken after 4 – 6 weeks.

Remarkably, in 13.2% of patients, the endoxifen plasma concentration decreased at steady-state endoxifen concentrations compared to the 4-6 week sample. In more than half of these patients the endoxifen plasma concentration dropped over 10%. For two patients in the 20 mg group, this meant that their endoxifen level dropped under the critical 16 nM threshold at steady-state.

When using the early samples obtained at 4 – 6 weeks after treatment, 20 patients were expected to not reach endoxifen levels ≥16 nM at steady state. Fourteen out of fifteen patients identified as necessitating a switch to an AI fell within this group. Six of these patients will eventually achieve adequate endoxifen plasma levels (range 16.6 – 20.5 nM). The six other patients were treated with 40 mg (1 patient), 30 mg (4 patients) or 20 mg (1 patient). None of the five patients in this group that were treated with less than the maximum registered dose of 40 mg reached an endoxifen level ≥16 nM. Therefore, these patients would have profited from dose escalation. Four out of these five patients would have achieved adequate exposure with dose escalation toward 40 mg, assuming therapy compliance would remain similar. The addition of samples obtained between 4-6 weeks after treatment initiation did not identify any false positives that would have been unrightfully dose increased. Alternatively, it did not recognize three patients treated with 20 mg that did not reach ≥16 nM levels at steady state. These patients had 4 – 6 weeks endoxifen levels of 15.7, 16.4, 16.6 nM (**Table S2)**. For one patient who did not reach 16 nM treated with 20 mg, no pre-steady-state sample was available. If the early samples were used to change dose predictions, this would have changed the primary outcome. In that case 87.7% of the population would have achieved adequate endoxifen levels corresponding with a $X^2 = 5.15$; $p = 0.023$.

References

1. Agema BC, Buijs SM, Sassen SDT, Mürdter TE, Schwab M, Koch BCP, et al. Toward model-informed precision dosing for tamoxifen: A population-pharmacokinetic model with a continuous CYP2D6 activity scale. Biomedicine & Pharmacotherapy. 2023;160:114369.
2. Binkhorst L, Mathijssen RH, Ghobadi Moghaddam-Helmantel IM, de Bruijn P, van Gelder T, Wiemer EA, Loos WJ. Quantification of tamoxifen and three of its phase-I metabolites in human plasma by liquid chromatography/triple-quadrupole mass spectrometry. J Pharm Biomed Anal. 2011;56(5):1016-23.
3. Zhao C-Y, Jiao Z, Mao J-J, Qiu X-Y. External evaluation of published population pharmacokinetic models of tacrolimus in adult renal transplant recipients. Br J Clin Pharmacol. 2016;81(5):891-907.
4. Fallowfield LJ, Leaity SK, Howell A, Benson S, Cella D. Assessment of quality of life in women undergoing hormonal therapy for breast cancer: validation of an endocrine symptom subscale for the FACT-B. Breast Cancer Research and Treatment. 1999;55(2):189-99.

Table S1. Overview of the activity levels of the different CYP2D6

***CYP2D6* allel**	**Activity level in POP-PK model**
*1	1
*2	0.560
*3	0.066
*4	0.047
*5	0.040
*6	0
*7	0
*9	0.378
*10	0.103
*17	0.156
*29	0.490
*31	0
*41	0.110
*1/*2 duplicate	1.400

Table S2. Overview of patients not reaching endoxifen levels ≥ 16 nM at steady-state

CYP2D6 allel 1	CYP2D6 allel 2	CYP2D6 activity score	Weight (kg)	Height (cm)	BMI (kg/m^2)	Age (years)	Predicted endoxifen with 20 mg tamoxifen (nM)	Predicted dose (mg)	(Potential) switch to AI' (Yes/ No)	Predicted endoxifen level at predicted dose (nM)	Observed endoxifen level (nM)	Observed tamoxifen level (nM)	Predicted endoxifen after pre-SS sample	Pre-SS sample
*4	*4	0.157	106.6	181	32.54	57	6.38	40	Yes	12.76	11.5	866	8.81	6.22
*4	*4	0.157	73.8	172	24.95	48	7.10	40	Yes	14.2	12.5	376	9.64	8.40
*4	*7	0.103	53	158	21.23	62	7.13	40	Yes	14.26	15.1	433	11.2	11.6
*4	*4	0.157	76.7	160.5	29.77	50	7.38	40	Yes	14.76	8.89	596	9.44	5.05
*4	*4	0.157	73.4	175	23.97	60	7.71	40	Yes	15.42	14.2	666	13.8	11.9
*6	*41	0.172	100.1	170	34.64	67	7.82	40	Yes	15.64	9.76	531	9.58	5.03
*4	*41	0.214	109.5	173	36.59	58	8.29	40	Yes	16.58	13.6	422	9.92	6.82
*4	*41	0.214	116.5	161	44.94	57	8.43	40	Yes	16.86	11.6	319	7.99	6.37
*41	*41	0.262	82.1	165	30.16	60	11.45	30	No	17.175	15.2	434	13.4	12.5
*2	*4	0.486	136	188	38.48	56	14.16	30	No	21.24	13.9	253	15.7	13.2
*2	*5	0.482	90.1	164	33.5	50	17.29	30	No	25.935	14.3	342	12.0	8.93
*2	*41	0.515	81.9	173	27.36	55	19.10	30	No	28.65	10.2	302	11.6	8.08
*2	*5	0.482	63	169.5	21.93	65	21.65	20	No	21.65	14.8	245	15.2	14.0
*1	*2	0.860	113	166.5	40.76	43	24.90	20	No	24.9	15.1	206	18.1	16.6
*1	*9	0.798	108.4	171.5	36.86	55	25.60	20	No	25.6	11.0	145	18.0	16.4
*1	*1	1.000	82.8	162	31.55	56	36.62	20	No	36.62	9.02	387	-	-
*1	*2	0.860	45.9	149.5	20.54	50	40.29	20	No	40.29	15.2	245	21.4	15.6

Pre-SS sample: sample obtained 4 – 6 weeks after treatment initiation, - : pre-SS sample was not obtained

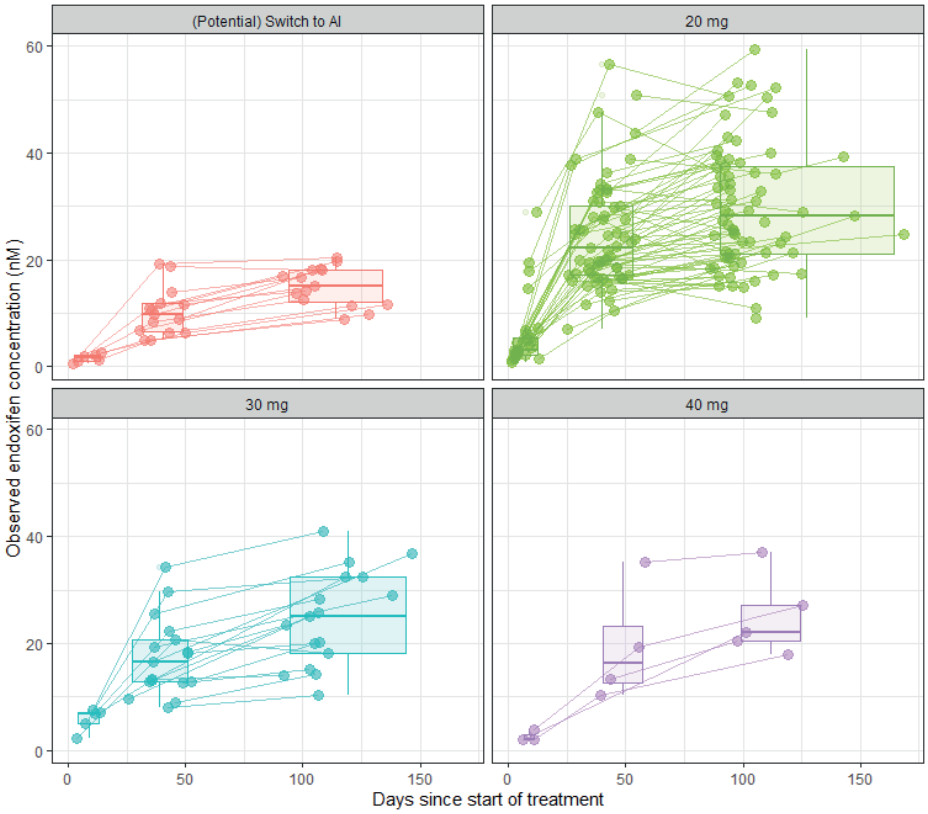

Figure S1. Distribution of all samples stratified on predicted dose group

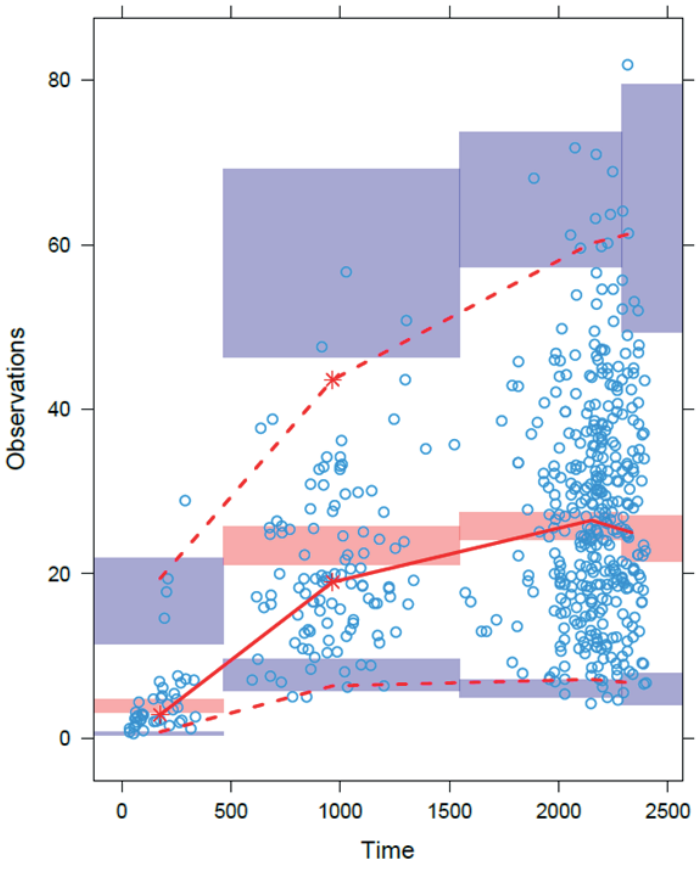

Figure S2. Visual Predictive Check for endoxifen samples obtained in the study
y-axis endoxifen nM, x-axis time in hou

PART III

CDK4/6 INHIBITORS

CHAPTER 9

Pseudo acute kidney injury in patients receiving CDK4/6 inhibitors

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ABSTRACT

Introduction

CDK4/6 inhibitors (CDK4/6i) improve progression-free survival in patients with advanced oestrogen-receptor positive breast cancer. However, all CDK4/6i may increase creatinine levels, which can indicate kidney injury. *In vitro* research has shown that CDK4/6i can also inhibit tubular secretion of creatinine, thereby causing the phenomenon 'pseudo-acute kidney injury (pseudo-AKI)'. The incidence of pseudo-AKI is, however, unknown. We aimed to determine this incidence by assessing cystatin C, a protein filtered in the glomerulus without being subject to tubular secretion, in patients with creatinine increase during CDK4/6i treatment.

Methods

In this retrospective single-center cohort study patients with breast cancer who received CDK4/6 inhibitors between January 1st 2017 and December 29th 2023 were screened for the incidence of creatinine increases suggesting potential kidney injury in the first six months of treatment. A significant creatinine increase was defined as 1) a creatinine plasma level of >90 µmol/L in women or >115 µmol/L in men and >10% increase from baseline creatinine plasma level or 2) a creatinine plasma level >1.5 times baseline creatinine or 3) an increase in creatinine plasma level from baseline with >26 µmol/L. Pseudo-AKI was diagnosed if the estimated glomerular filtration rate (eGFR) using cystatin C at the moment of creatinine increase was 1) equal or higher than eGFR using creatinine at baseline and/or 2) at least 25% higher than eGFR using creatinine at the moment of creatinine increase. The primary endpoint was the percentage of patients with pseudo-AKI analysed by means of the binomial probability test.

Results

From the 234 patients treated with a CDK4/6i, 41 (17.5%) had creatinine levels indicating an AKI. From 22 of these 41 patients, cystatin C could be determined in retrospectively available serum. Pseudo-AKI was found in 16 out of 22 patients (73%, 95% CI 50-89%). In 5 out of 41 patients (12%) the CDK4/6i dose was unjustly adjusted or the drug was stopped due to creatinine increase.

Conclusion

Pseudo-AKI has a high incidence in patients treated with CDK4/6i. Determining an eGFR based on the cystatin C value should therefore be considered as the first step when creatinine increases during CDK4/6i treatment.

INTRODUCTION

CDK4/6 inhibitors are protein kinase inhibitors that inhibit the cyclin-dependent kinases (CDK) 4 and 6; both important kinases for progression of the cell cycle.[1] Currently, three CDK4/6 inhibitors are registered for the treatment of advanced breast cancer: palbociclib, ribociclib and abemaciclib. The addition of CDK4/6 inhibitors to endocrine therapy has led to an improvement of both progression-free survival and overall survival in patients with advanced oestrogen-receptor positive (ER+)/HER2-negative breast cancer when applied in first- or second line of treatment.[2-9] Recently, abemaciclib, was also approved for treatment of patients with ER+/HER2-negative early breast cancer to prevent recurrence of disease.[10]

Despite the advantages of CDK4/6 inhibitors, these drugs can also lead to adverse events. Among the most frequently reported are neutropenia, anemia, fatigue, and diarrhea.[4,5,8] An adverse event, observed across all types of CDK4/6 inhibitors, which so far has received less attention, is an elevation in creatinine which (by definition) leads to a lowered estimated glomerular filtration rate (eGFR). Abemaciclib showed an incidence range of 11-44%[5,6,11,12], palbociclib of 13-22%[13,14], and ribociclib of 22-28%.[13,15]

An increase in creatinine during the use of CDK4/6 inhibitors can be the result of nephrotoxicity of these drugs leading to kidney injury and a true decrease in GFR. The precise mechanism behind this form of kidney injury is unclear, but acute tubular necrosis and acute tubulo-interstitial nephritis have both been (rare) observations in patients treated with CDK4/6 inhibitors and may be caused by tubular damage due to cycle inhibition or hypersensitivity reactions, respectively.[16] However, an increase in creatinine can also be caused by inhibition of the tubular secretion of creatinine. Active tubular secretion accounts for 10-40% of creatinine clearance depending on the stage of chronic kidney disease.[17,18] This secretion is mediated trough the organic cation transporter 2 (OCT2) on the basolateral membrane of proximal tubule cells and the multidrug and toxin extrusion (MATE) protein 1 and 2 on the apical membrane.[19,20]

In vitro research showed that abemaciclib, palbociclib and ribociclib can inhibit these transporters.[21] Indeed, few case-reports[22-24] and a case series[25] demonstrated that in patients receiving CDK4/6 inhibitors in whom an increase in creatinine occurred, plasma concentrations of cystatin C, a protein filtered in the glomerulus but not subject to tubular secretion[26], remained similar. This suggests that an elevation in creatinine is not always indicative of a reduction in renal function; it can also be caused by the inhibition of active tubular secretion of creatinine. This phenomenon is known as pseudo-acute kidney injury (pseudo-AKI). The incidence of pseudo-AKI in patients using CDK4/6 inhibitors is, however, currently unknown.

In clinical practice, encountering an elevated creatinine level prompts an investigation into the underlying cause. Also, CDK4/6 inhibitors or other important, but potentially nephrotoxic medication might be interrupted or decreased in dose. However, if pseudo-AKI indeed appears a frequent issue in patients using CDK4/6 inhibitors, incorporating the measurement of cystatin C in plasma could present a non-invasive and inexpensive solution for detecting pseudo-AKI. Therefore, the aim of our study is to determine the incidence of pseudo-AKI in patients treated with CDK4/6 inhibitors by assessing both creatinine and cystatin C in plasma. In addition, diagnostic and treatment consequences of creatinine increase in clinical practice were inventoried.

METHODS

This retrospective single-centre cohort study was conducted among patients treated with CDK4/6 inhibitors at the Erasmus MC Cancer Institute, Rotterdam, the Netherlands. On December 12th 2023 the study was approved by the local Medical Ethics Committee (Erasmus Medical Center, MEC 2023-0715). The study did not fall under the scope of the Medical Research Involving Human Subjects Act (WMO) and has been performed according to the "Code of Conduct for Responsible Use (2011)". Therefore, written informed consent was not indicated. The study was registered on Clinicaltrials.gov (register.clinicaltrials.gov, MEC-2023-0715).

All patients with breast cancer who received CDK4/6 inhibitors at our hospital between January 1st 2017 and December 29th 2023 were screened for the incidence of creatinine increases suggesting potential kidney injury. Palbociclib and ribociclib are prescribed in cycles of three weeks followed by one week of rest, while abemaciclib is prescribed continuously. The inclusion criteria were as follows: 1) Patients must have used the CDK4/6 inhibitor for a minimum duration of one month, and 2) a baseline value of creatinine had to be available within 30 days prior to the start of CDK4/6 inhibitor treatment. There was no upper limit of the baseline creatinine plasma level. Patients were screened for the incidence of AKI in the first six months of CDK4/6 inhibitor treatment. This defined period of six months was used to limit the presence of other factors potentially influencing the kidney function. Creatinine values were measured following standard clinical practice, mostly after 2, 4, 6, 8 and 12 weeks after start of treatment and then every three months thereafter. If a significant creatinine increase was found, creatinine levels were followed for a period of one year after start of treatment to observe the course of creatinine levels. A significant creatinine increase was defined as 1) a creatinine plasma level of >90 µmol/L in women or >115 µmol/L in men (according Common Terminology Criteria for Adverse Events (CTCAE) grade 1) and >10% increase from baseline creatinine plasma level and/or 2) a creatinine plasma

level >1.5 times baseline creatinine (according ≥CTCAE grade 2) and/or 3) an increase in creatinine plasma level from baseline with >26 µmol/L (AKIN grade 1).[27] If patients used multiple CDK4/6 inhibitors, they were screened for creatinine increases in the six months after start of each individual CDK4/6 inhibitor. However, each patient could be included only once in the database.

For the patients in whom an increase in creatinine occurred, patient- and tumour characteristics were collected and potential clinical consequences of the increase were inventoried. Patient charts were searched for influential factors for cystatin C at the moment of creatinine increase, namely hypo- or hyperthyroidism[28], inflammation or infection[29], weight loss or weight gain[30], diabetes mellitus[31] or use of corticosteroids.[32]

At the time-points of creatinine increase, it was retrospectively checked whether cystatin C levels had been measured or if serum samples from stored blood from the exact same blood withdrawal at the time of creatinine increase were available. In patients from whom serum was available at the moment of AKI, it was also checked if serum was available at baseline. Serum samples used in this study were collected as part of standard diagnostic work-up. Remaining serum was stored in liquid nitrogen freezers. In this serum, cystatin C was measured.[33] Subsequently, the eGFR according cystatin C levels were determined (**Supplementary**)**.** Increase in creatinine levels was defined as pseudo-AKI if eGFR using cystatin C plasma levels was:

1. equal or higher at the moment of creatinine increase than eGFR using creatinine plasma levels at baseline; and/or
2. at least 25% higher than eGFR using creatinine plasma levels at the moment of creatinine increase.

The primary endpoint was the percentage of patients with pseudo-AKI, determined by dividing the number of patients with a pseudo-AKI, identified through cystatin C measurement, by the total number of patients with available cystatin C levels. This endpoint was analysed by means of the binomial probability test and calculated together with the binomial exact 90% confidence interval (CI). Cystatin C levels at baseline, if available, were analysed descriptively, as was the incidence of clinical consequences of creatinine rise.

RESULTS

In total, 234 patients diagnosed with advanced ER+/Her2-negative breast cancer were administered a CDK4/6 inhibitor between January 1st 2017 and December 29th 2023. Among these 234 patients, 41 (17.5%) were identified as having a significant increase in creatinine levels according to our definition (**Figure 1**)**.** Baseline characteristics can be found in **Table 1**.

The median time until a first increase in creatinine was 57 days (IQR 14-99) after start of treatment. In 22 of 41 patients, cystatin C was already prospectively measured (*n* = 3) and/or serum samples for measurement of cystatin C were available (*n* = 20). From 9 of these 22 patients, cystatin C could also be determined in serum levels taken at baseline, making it possible to compare cystatin C levels with pretreatment concentrations. In **Table 1** is shown that no difference exists between patients with a cystatin C level determined and patients without a cystatin level determined.

Pseudo-AKI was found in 16 out of 22 patients (70%, 95% CI 50-89%) and AKI was confirmed by cystatin C levels in the other six patients (**Table 2, Figure 2**). In the 22 patients in which cystatin C measurements were performed, pseudo-AKI occurred in 7 out of 8 (88%) patients on abemaciclib, 8 out of 11 (73%) patients on palbociclib and 2 out of 4 (50%) patients on ribociclib. All patients in whom cystatin C was measured were screened for the presence of influential factors of cystatin C. These were present in four patients (i.e. one patient with hypothyroidism, one patient with an infection and two patients with weight loss) and might have affected the cystatin C level in one of them (subject 2, **Table 2**).

Twelve of 41 patients with significant creatinine rise during CDK4/6 inhibitor use had used multiple types of CDK4/6 inhibitors. One of these 12 patients had an increase in creatinine during the use of all three types of CDK4/6 inhibitors. For this patient, we could measure cystatin C levels during the use of palbociclib and ribociclib. During both types of CDK4/6 inhibitors, pseudo-AKI was observed.

An overview of cystatin C and creatinine levels before the start of CDK4/6 inhibitor treatment can be found in **Table 3.** The eGFRs obtained through cystatin C levels were (substantially) lower compared to those derived from creatinine levels, with one exception. For eight of these nine patients the baseline value was taken at a moment of observed cancer progression.

Of the 41 patients with creatinine values according to our definition of AKI, additional diagnostics (*n* = 4) and/or medication adjustments (*n* = 11) were made in 15 patients (36%)

(**Table 4**). In five of the 41 cases, the CDK4/6 inhibitor was stopped or lowered in dose. Patient characteristics of these patients can be found in the **Supplement**.

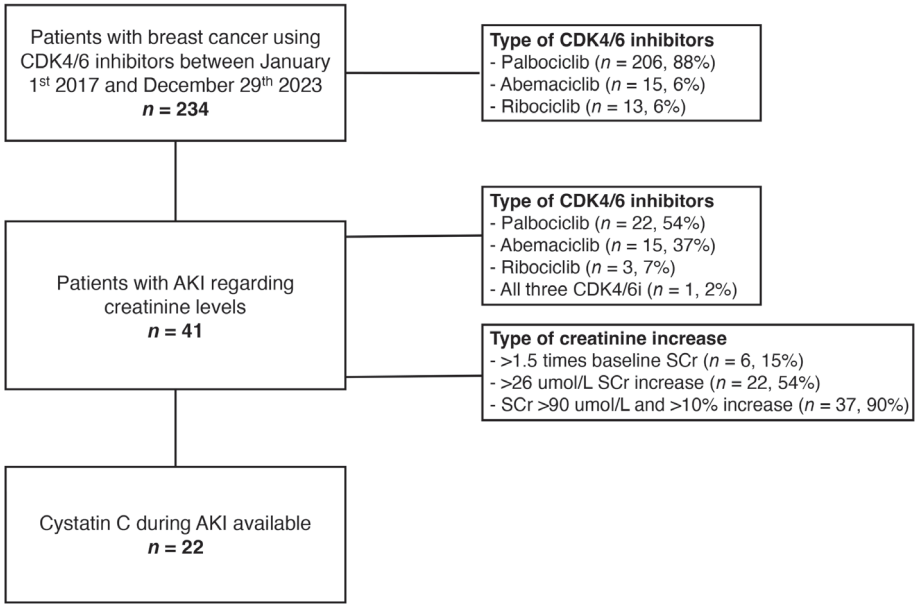

Figure 1. Flowchart patient selection
SCr: serum creatinine, CDK4/6i: CDK 4/6-inhibitors

Table 1. Baseline characteristics

	Baseline characteristics (n =4 1)	No Cystatine C (n = 19)	Cystatin C (n = 22)	p value
Age at start CDK4/6 inhibitor (years)	58 [54-61]	58 [56-60]	57 [53-64]	0.77
Timing of metastatic disease				1
Synchronous	5 (12%)	2 (11%)	3 (14%)	
Metachronous	36 (88%)	17 (89%)	19 (86%)	
Localisation of metastasis				
Visceral	31 (76%)	14 (74%)	17 (77%)	1
Bone only	6 (15%)	2 (11%)	4 (18%)	0.8
Bone and lymph node only	4 (10%)	3 (16%)	1 (5%)	0.5
Number of treatment lines before CDK4/6 inhibitor				0.52
0	12 (29%)	6 (32%)	6 (27%)	
1	16 (39%)	9 (47%)	7 (32%)	
2	8 (20%)	2 (11%)	6 (27%)	
≥3	5 (12%)	2 (11%)	3 (14%)	
CDK4/6 inhibitor used				0.28
Palbociclib	21 (51%)	12 (63%)	9 (41%)	
Abemaciclib	5 (12%)	2 (11%)	3 (14%)	
Ribociclib	3 (7%)	0 (0%)	3 (14%)	
Multiple	12 (29%)	5 (26%)	7 (32%)	
Endocrine backbone				0.18
Aromatase inhibitor	12 (29%)	8 (42%)	4 (18%)	
Fulvestrant	29 (71%)	11 (58%)	18 (82%)	
Median duration of CDK4/6 inhibitor treatment (days)	184 [85-277]	128 [76-215]	233 [104-309]	0.38
Baseline renal values				0.4
Normal values (eGFR ≥90 ml/min/m^2)	8 (20%)	5 (26%)	3 (14%)	
Chronic kidney disease grade 1 (eGFR 60-89 ml/min/m^2)	30 (73%)	12 (63%)	18 (82%)	
Chronic kidney disease grade 2 (eGFR 30-59 ml/min/m^2)	3 (7%)	2 (11%)	1 (5%)	
Comorbidity				
Hypertension	4 (10%)	2 (11%)	2 (9%)	1
Coronary artery disease	0 (0%)	0 (0%)	0 (0%)	1
Medication				
Angiotensin receptor inhibitor	2 (5%)	1 (5%)	1 (5%)	1
NSAID	3 (7%)	1 (5%)	2 (9%)	1
ACE inhibitor	1 (2%)	0 (0%)	1 (5%)	1

	Baseline characteristics (n =4 1)	No Cystatine C (n = 19)	Cystatin C (n = 22)	p value
Cystatin C relevant variables				
Diabetes Mellitus	1 (2%)	1 (5%)	0 (0%)	1
Inflammation during AKI timepoint	1 (2%)	0 (0%)	1 (5%)	1
Corticosteroid use during AKI timepoint	0 (0%)	0 (0%)	0 (0%)	1
Thyroid dysfunction	1 (2%)	0 (0%)	1 (5%)	1

Table 2. Glomerular filtration rate (ml/min/m^2) using creatinine and cystatin C

Subject	CDK4/6i	Baseline		Moment of AKI according creatinine				
		creatinine (µmol/L)	eGFR	creatinine (µmol/L)	eGFR	cystatinC (mg/L)	eGFR	Difference eGFR[1]
1	Abemaciclib	80	70	100	53	0.62	111	109%*
2	Abemaciclib	76	73	113	45	0.94	78	74%*
3	Abemaciclib	79	75	96	60	0.78	103	71%*
4	Abemaciclib	81	75	92	64	0.7	109	71%*
5	Abemaciclib	120	45	151	34	1.34	50	47%*
6	Abemaciclib	70	79	93	55	0.93	78	42%*
7	Abemaciclib	51	90	95	57	0.93	80	40%*
8	Abemaciclib	75	78	93	60	1.16	61	2%
9	Palbociclib/ Ribociclib	90	63	109	43	1.04	70	63%*
10	Palbociclib	82	62	104	45	0.96	72	60%*
11	Palbociclib	62	90	89	66	0.8	102	55%*
12	Palbociclib	75	70	99	50	0.97	72	45%*
13	Palbociclib	81	70	92	60	0.88	87	45%*
14	Palbociclib	81	70	99	55	0.98	76	38%*
15	Palbociclib	86	66	96	58	0.96	78	35%*
16	Palbociclib	79	68	93	56	1	71	26%*
17	Palbociclib	67	93	94	61	1.01	74	22%
18	Palbociclib	86	60	125	38	1.84	31	-18%
19	Palbociclib	71	81	94	57	1.58	40	-30%
20	Ribociclib	70	74	110	43	1.03	66	53%*
21	Ribociclib	69	86	92	61	1.07	68	11%
22	Ribociclib	79	72	91	60	1.16	60	0%

Table 3. Creatinine and cystatin C at baseline

	Creatinine (µmol/L)	eGFR creatinine (ml/min)	Cystatin C (mg/L)	eGFR cystatin C (ml/min)	Difference in eGFR (ml/min)
3	79	75	0.98	78	3
8	75	78	1.16	61	-17
9	90	63	1.22	57	-6
15	86	66	1.17	60	-6
16	79	68	1.27	52	-16
18	86	60	1.48	42	-18
19	71	81	1.6	39	-42
20	70	74	1.16	56	-18
22	79	72	1.28	53	-19

Table 4. Clinical consequences of increased creatinine levels

Consequences	AKI cohort (*n* = 41)
CDK4/6 inhibitor stopped/interrupted	5 (12%)
CDK4/6 inhibitor reduced in dose	1 (2%)
Diagnostic evaluation for AKI performed	4 (12%)
Ultrasound abdomen	2
Urinalysis	2
Other medication that was altered[1]	9 (22%)
Bisphosphonates	6
NSAIDs	2
Diuretics	1

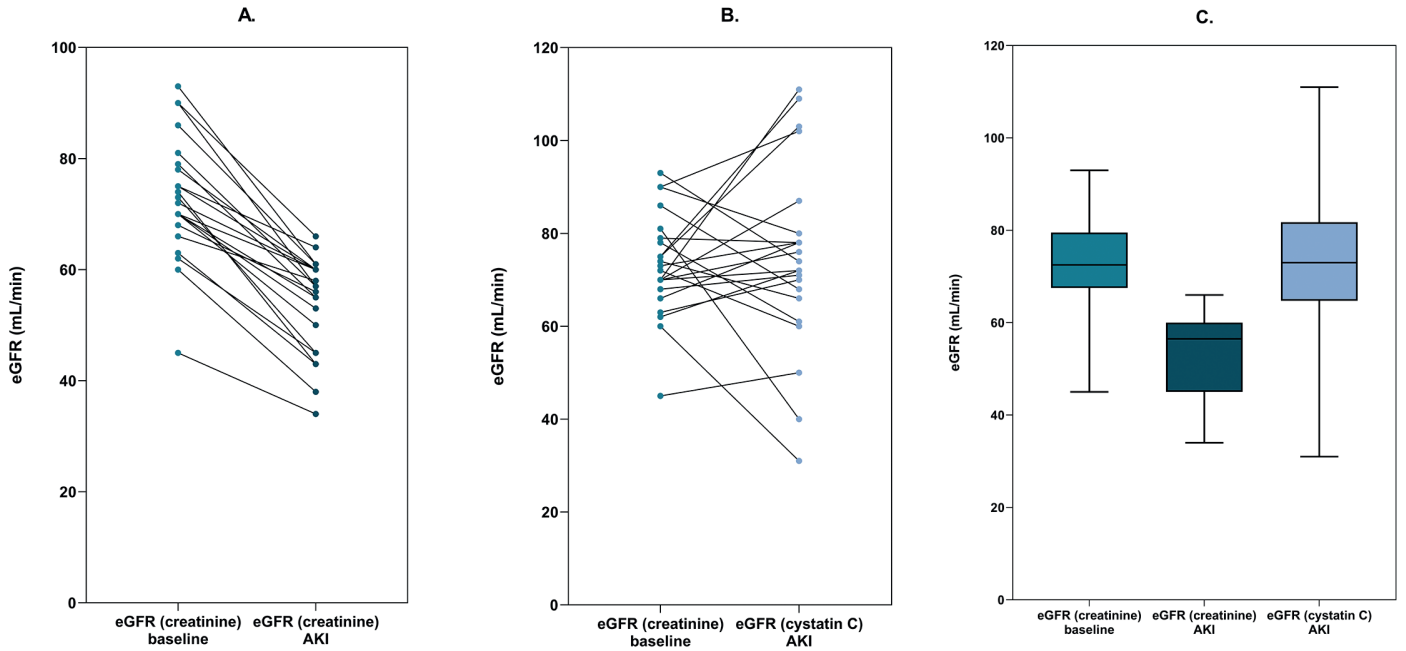

Figure 2. eGFR at baseline and moment of AKI

Legend. A. The course of eGFR derived from creatinine levels between baseline and at the moment of AKI; B. The course of eGFR derived from creatinine levels at baseline and from cystatin C levels at the moment of AKI; C. Boxplot of eGFR derived from creatinine on baseline and the moment of AKI and cystatin C at the moment of AKI (median, IQR, minimum and maximum).

DISCUSSION

This is the first study investigating the incidence of pseudo-AKI in a large real-life cohort of patients with advanced breast cancer using CDK4/6 inhibitors. Eighteen percent of the patients had a sufficient and clinically relevant rise in creatinine in the first six months of CDK4/6 inhibitor treatment. In these patients, we found a surprisingly high incidence of pseudo-AKI (73%), especially in patients using abemaciclib (88%). Furthermore, we observed that in 10% of patients additional diagnostics to the cause of AKI were performed (ultrasound abdomen or urinalysis) and in 21% of patients potentially nephrotoxic drugs (i.e. bisphosphonates, NSAIDs or diuretics) were altered. Most importantly, in 12% of patients the CDK4/6 inhibitor dose was unjustly adjusted or the drug was stopped due to the creatinine increase. Consequently, eGFR determination based on cystatin C levels upon creatinine increases should be encouraged before proceeding with such interventions. This could potentially prevent unnecessary diagnostic procedures or drug interruptions.

The incidence of creatinine elevations in our study (18%) is comparable with earlier studies.[5,6,11-15] We found that in only 27% of patients with a rise in creatinine levels, the diagnosis of AKI persisted when eGFR was determined using cystatin C levels. An earlier study investigated six patients on palbociclib (*n* = 3), abemaciclib (*n* = 2) and ribociclib (*n* = 1) with biopsy-proven AKI, among whom five patients were diagnosed with acute tubular necrosis and one patient with acute tubulointerstitial nephritis.[16] Interestingly, the median time from CDK4/6 initiation to AKI in these patients was 278 days, which is substantially longer than the median time of 57 days from CDK4/6 inhibitor initiation to creatine rise in our cohort. Also, the median creatinine value was 265 µmol/L, three patients had stage 3 AKI and one patient underwent renal replacement therapy. All patients had proteinuria on dipstick. Therefore, in severe cases of creatinine level increases, especially in patients with normal eGFR before start of CDK4/6 inhibitor treatment[18], the chance for a real AKI should always be considered.

In our study, pseudo-AKI was defined as an increase in creatinine values resulting in a decrease in eGFR calculated using creatinine plasma levels, while the eGFR calculated with cystatin C plasma levels was 1) equal to or higher than the baseline eGFR using creatinine plasma level; and/or 2) at least 25% higher than eGFR based on creatinine plasma levels at the time of creatinine increase. Although there is no universally accepted definition of pseudo-AKI, prior studies have explored this phenomenon. Our definition is stricter than that used in a recent study by Chen et al where pseudo-AKI was assessed for Tyrosine Kinase Inhibitors.[34] In this study creatinine increase was seen as pseudo-AKI when the eGFR measured with cystatin C was higher than the eGFR measured with creatinine. In another study involving abemaciclib in healthy

subjects, pseudo-AKI was evaluated using metformin.[21] Metformin is a known substrate of OCT2, MATE1 and MATE2. Coadministration of metformin and abemaciclib led to increased exposure to metformin. Interestingly, in the same study, the coadministration of abemaciclib and iohexol did not alter iohexol plasma concentrations, which counts as the gold standard for measuring GFR, Although not directly clinically applicable, these findings confirm the underlying theory of pseudo-AKI. Additionally, cystatin C concentrations remained stable between the periods when patients were administered abemaciclib and placebo. Unfortunately, in our study design we were not able to compare cystatin C levels within patients.

The incidence of pseudo-AKI seemed to differ in our study per CDK4/6 inhibitor (88% for abemaciclib, 73% for palbociclib and 50% for ribociclib), but absolute numbers were small, in particular for ribociclib (n = 3). *In vitro* studies have shown that abemaciclib can inhibit all three tubular transporters (OCT2, MATE1 and MATE2)[21], while ribociclib exhibits a potentially inhibitory effect on OCT2 and MATE1[35] and palbociclib only affects OCT2.[36] This pathophysiology can clarify the difference in incidence of pseudo-AKI among the different CDK4/6 inhibitors. Also, patients treated with palbociclib or ribociclib receive the drug for three weeks and then have a rest week before the next cycle, in contrast to abemaciclib which is given continuously. Taken the half-life of palbociclib (i.e. 29 hours) and ribociclib (i.e. 30-55 hours) into account, it is expected that the potential inhibiting effect of palbociclib and ribociclib on the transporters OCT2 and MATE1 should disappear during the rest week. Since laboratory tests including creatinine levels are usually performed at the end of the rest week, the incidence of pseudo-AKI might even be underestimated for palbociclib and ribociclib in this study.

In our study, we found that the cystatin C levels at baseline were much higher than expected (when compared with creatinine levels) and therefore eGFR derived from cystatin C levels was worse compared to eGFR derived from creatinine levels. There is evidence that cystatin C levels are higher in patients with cancer compared to healthy subjects.[37,38] Also, in studies including patients with melanoma, cystatin C levels were higher in patients with metastatic disease compared to patients with primary melonama.[39,40] Another study found that cystatin C was evident in cancer cells (and not in benign tumors).[41] The timing of baseline values in our study was just before the start of CDK4/6 inhibitors, i.e. the time of cancer progression. Possibly, cystatin C levels are also higher at time of cancer progression, which might explain the impaired eGFR derived from cystatin C levels before start of CDK4/6 inhibitors. This could lead to an underestimation of the eGFR based on the cystatin C in this study. However, if this would be the case, it would not affect the current finding of a percentage of pseudo-AKI, but would instead increase that percentage.

This study has some limitations. First, it concerns a retrospective study with the majority of cystatin C determined from stored residual serum samples. Given that cystatin C levels remain stable in serum for at least seven years, this factor has, at most, a very limited impact on the results.[33] Also, although cystatin C levels are not influenced by tubular secretion or muscle mass like creatinine, other factors such as inflammation, glucocorticoid use, diabetes, thyrotoxicosis, higher weight, and male sex could lead to higher cystatin C levels.[28,30-32] All patients were checked for the presence of these factors and only one patient lost more than 10% weight within ttwo months which may have influenced the results of that single patient. Last, eGFR based on cystatin C (or creatinine) is less reliable in a non-steady-state situation as AKI.[42] Indeed, in almost all cases only one blood withdrawal was available during AKI in which both creatinine and cystatin C was determined. There may be substantial individual variation in the eGFR measurement based on either creatinine or cystatin C which can only be accounted for by measuring the GFR by e.g. inulin clearance.[43] However, this is a costly and time-consuming procedure and not routinely available in most hospitals.

In conclusion, the additional use of cystatin C in case of a rise in serum creatinine may reveal pseudo-AKI in 73% of patients and could prevent unnecessary diagnostic interventions or drug alterations. In patients taking CDK4/6 inhibitors who develop a decreased eGFR based on creatinine levels, we therefore recommend determining eGFR based on serum cystatin C levels to make a pseudo-AKI the most likely cause.

REFERENCES

1. Braal CL, Jongbloed EM, Wilting SM, Mathijssen RHJ, Koolen SLW, Jager A. Inhibiting CDK4/6 in Breast Cancer with Palbociclib, Ribociclib, and Abemaciclib: Similarities and Differences. Drugs 2021; 81(3): 317-31.
2. Cristofanilli M, Turner NC, Bondarenko I, et al. Fulvestrant plus palbociclib versus fulvestrant plus placebo for treatment of hormone-receptor-positive, HER2-negative metastatic breast cancer that progressed on previous endocrine therapy (PALOMA-3): final analysis of the multicentre, double-blind, phase 3 randomised controlled trial. The Lancet Oncology 2016; 17(4): 425-39.
3. Turner NC, Slamon DJ, Ro J, et al. Overall Survival with Palbociclib and Fulvestrant in Advanced Breast Cancer. N Engl J Med 2018; 379(20): 1926-36.
4. Finn RS, Martin M, Rugo HS, et al. Palbociclib and Letrozole in Advanced Breast Cancer. New England Journal of Medicine 2016; 375(20): 1925-36.
5. Sledge GW, Jr., Toi M, Neven P, et al. MONARCH 2: Abemaciclib in Combination With Fulvestrant in Women With HR+/HER2- Advanced Breast Cancer Who Had Progressed While Receiving Endocrine Therapy. Journal of Clinical Oncology 2017; 35(25): 2875-84.
6. Goetz MP, Toi M, Campone M, et al. MONARCH 3: Abemaciclib As Initial Therapy for Advanced Breast Cancer. J Clin Oncol 2017; 35(32): 3638-46.
7. Hortobagyi GN, Stemmer SM, Burris HA, et al. Updated results from MONALEESA-2, a phase III trial of first-line ribociclib plus letrozole versus placebo plus letrozole in hormone receptor-positive, HER2-negative advanced breast cancer. Annals of Oncology 2018; 29(7): 1541-7.
8. Slamon DJ, Neven P, Chia S, et al. Phase III Randomized Study of Ribociclib and Fulvestrant in Hormone Receptor-Positive, Human Epidermal Growth Factor Receptor 2-Negative Advanced Breast Cancer: MONALEESA-3. Journal of Clinical Oncology 2018; 36(24): 2465-72.
9. Slamon DJ, Neven P, Chia S, et al. Ribociclib plus fulvestrant for postmenopausal women with hormone receptor-positive, human epidermal growth factor receptor 2-negative advanced breast cancer in the phase III randomized MONALEESA-3 trial: updated overall survival. Ann Oncol 2021; 32(8): 1015-24.
10. Johnston SRD, Toi M, O'Shaughnessy J, et al. Abemaciclib plus endocrine therapy for hormone receptor-positive, HER2-negative, node-positive, high-risk early breast cancer (monarchE): results from a preplanned interim analysis of a randomised, open-label, phase 3 trial. Lancet Oncol 2023; 24(1): 77-90.
11. Patnaik A, Rosen LS, Tolaney SM, et al. Efficacy and Safety of Abemaciclib, an Inhibitor of CDK4 and CDK6, for Patients with Breast Cancer, Non–Small Cell Lung Cancer, and Other Solid Tumors. Cancer Discovery 2016; 6(7): 740-53.
12. Rugo HS, Huober J, García-Sáenz JA, et al. Management of Abemaciclib-Associated Adverse Events in Patients with Hormone Receptor-Positive, Human Epidermal Growth Factor Receptor 2-Negative Advanced Breast Cancer: Safety Analysis of MONARCH 2 and MONARCH 3. The Oncologist 2021; 26(1): e53-e65.
13. Keskinkılıç M, Semiz HS, Yavuzsen T, Karaoglu A. 194P Increased blood creatinine level due to CDK 4/6 inhibitor treatments: Single center experience. Annals of Oncology 2022; 33: S216.
14. Hanna PE, Strohbehn IA, Moreno D, et al. Adverse kidney outcomes of CDK 4/6 inhibitors for metastatic breast cancer. NPJ Breast Cancer 2023; 9(1): 70.
15. Wilson BE, Mok K, Kiely BE, Nguyen R, Moylan E. Association between ribociclib and changes in creatinine in patients with hormone receptor positive metastatic breast cancer. Internal Medicine Journal 2019; 49(11): 1438-42.
16. Gupta S, Caza T, Herrmann SM, Sakhiya VC, Jhaveri KD. Clinicopathologic Features of Acute Kidney Injury Associated With CDK4/6 Inhibitors. Kidney Int Rep 2022; 7(3): 618-23.
17. Levey AS, Perrone RD, Madias NE. Serum creatinine and renal function. Annual review of medicine 1988; 39(1): 465-90.
18. Rose BD, Post TW. Clinical Physiology of Acid Base and Electrolyte Disorders: Columbus; 2000.
19. Lepist EI, Ray AS. Renal drug-drug interactions: what we have learned and where we are going. Expert Opin Drug Metab Toxicol 2012; 8(4): 433-48.
20. Ciarimboli G, Lancaster CS, Schlatter E, et al. Proximal tubular secretion of creatinine by organic cation transporter OCT2 in cancer patients. Clin Cancer Res 2012; 18(4): 1101-8.

21. Chappell JC, Turner PK, Pak YA, et al. Abemaciclib Inhibits Renal Tubular Secretion Without Changing Glomerular Filtration Rate. Clinical Pharmacology & Therapeutics 2019; 105(5): 1187-95.
22. Sy-Go JPT, Yarandi N, Schwartz GL, Herrmann SM. Ribociclib-Induced Pseudo-Acute Kidney Injury. Journal of Onco-Nephrology 2022; 6(1-2): 64-9.
23. Mach T, Qi A, Bouganim N, Trinh E. Targeted Cancer Therapies Causing Elevations in Serum Creatinine Through Tubular Secretion Inhibition: A Case Report and Review of the Literature. Can J Kidney Health Dis 2022; 9: 20543581221106246.
24. Bonilla M, Bashir KA, Jhaveri KD. An elevated serum creatinine in a patient receiving palbociclib. Journal of Onco-Nephrology 2021; 5(2): 133-5.
25. Guchelaar NAD, Buck SAJ, van Doorn L et al. The OCT2/MATE1 Interaction Between Trifluridine, Metformin and Cimetidine: A Crossover Pharmacokinetic Study. Clin Pharmacokinet 2024; **63**(7): 1037-44.
26. Grubb A. Diagnostic value of analysis of cystatin C and protein HC in biological fluids. Clin Nephrol 1992; **38 Suppl 1**: S20-7.
27. Lopes JA, Jorge S. The RIFLE and AKIN classifications for acute kidney injury: a critical and comprehensive review. Clin Kidney J 2013; 6(1): 8-14.
28. Kimmel M, Braun N, Alscher MD. Influence of Thyroid Function on Different Kidney Function Tests. Kidney and Blood Pressure Research 2011; 35(1): 9-17.
29. Vanhoutte T, Sprangers B. Pseudo-AKI associated with targeted anti-cancer agents-the truth is in the eye of the filtration marker. Clin Kidney J 2023; 16(4): 603-10.
30. Knight EL, Verhave JC, Spiegelman D, et al. Factors influencing serum cystatin C levels other than renal function and the impact on renal function measurement. Kidney Int 2004; 65(4): 1416-21.
31. Stevens LA, Schmid CH, Greene T, et al. Factors other than glomerular filtration rate affect serum cystatin C levels. Kidney International 2009; 75(6): 652-60.
32. Zhai JL, Ge N, Zhen Y, Zhao Q, Liu C. Corticosteroids Significantly Increase Serum Cystatin C Concentration without Affecting Renal Function in Symptomatic Heart Failure. Clin Lab 2016; 62(1-2): 203-7.
33. Karger AB, Eckfeldt JH, Rynders GP, et al. Long-Term Longitudinal Stability of Kidney Filtration Marker Measurements: Implications for Epidemiological Studies and Clinical Care. Clinical Chemistry 2021; 67(2): 425-33.
34. Chen MF, Harada G, Liu D, DeMatteo R, Falcon C, Wilhelm C, et al. Brief Report: Tyrosine Kinase Inhibitors for Lung Cancers That Inhibit MATE-1 Can Lead to False Decreases in Renal Function. Journal of Thoracic Oncology 2024; **19**(1): 153-9.
35. US Food ad Drug Administration. Center for Drug Evaluation and Research. Clinical Pharmacology Review Ribociclib. 2017 https://www.accessdata.fda.gov/drugsatfda_docs/nda/2017/209092orig1s000multidiscipliner.pdf2024).
36. Pabla N, Gibson AA, Buege M, Ong SS, Li L, Hu S, et al. Mitigation of acute kidney injury by cell-cycle inhibitors that suppress both CDK4/6 and OCT2 functions. Proc Natl Acad Sci U S A 2015; **112**(16): 5231-6.
37. Jones M, Denieffe S, Griffin C, Tinago W, Fitzgibbon MC. Evaluation of cystatin C in malignancy and comparability of estimates of GFR in oncology patients. Pract Lab Med 2017; 8: 95-104.
38. Nakai K, Kikuchi M, Fujimoto K, et al. Serum levels of cystatin C in patients with malignancy. Clin Exp Nephrol 2008; 12(2): 132-9.
39. Kos J, Stabuc B, Cimerman N, Brünner N. Serum cystatin C, a new marker of glomerular filtration rate, is increased during malignant progression. Clin Chem 1998; 44(12): 2556-7.
40. Kos J, Stabuc B, Schweiger A, et al. Cathepsins B, H, and L and their inhibitors stefin A and cystatin C in sera of melanoma patients. Clin Cancer Res 1997; 3(10): 1815-22.
41. Nishikawa H, Ozaki Y, Nakanishi T, et al. The role of cathepsin B and cystatin C in the mechanisms of invasion by ovarian cancer. Gynecol Oncol 2004; 92(3): 881-6.
42. Niemantsverdriet MSA, Pieters TT, Hoefer IE et al. GFR estimation is complicated by a high incidence of non-steady-state serum
43. Rahn KH, Heidenreich S, Brückner D. How to assess glomerular function and damage in humans. J Hypertens 1999; 17(3): 309-17.

SUPPLEMENTARY MATERIAL

Additional information

eGFR according cystatin C levels were determined using the following equations:

1. 133 * (cystatin C level/0.8)$^{-0.499}$ * 0.996^{Age}[*0.932 if female] when cystatin C levels were equal to or below 0.8 mg/L and
2. 133 * (cystatin C level/0.8)$^{-1.328}$ * 0.996^{Age}[*0.932 if female] when cystatin C levels were above 0.8 mg/L.[44]

Supplementary table

Supplementary Table 1. Patients in which CDK4/6 inhibitor was stopped or interrupted

CDK4/6 inhibitor	Baseline SCr	SCr at interruption	Duration interruption	eGFR restored?	Other toxicity (CTCAE)
Abemaciclib	57	99	1 week	Yes	Neutropenia grade 2 Headache grade 1
Palbociclib	69	106	2 weeks	Yes	Neutropenia grade 3
Palbociclib	86	125	No restart[1]	Yes	Diarrhea grade 1
Abemaciclib	77	104	1 week	Yes	None
		102	No restart[2]	Yes	None
Abemaciclib	80	101	4 weeks	Yes	Neutropenia grade 3 Diarrhea grade 1
		100	No restart[3]	Yes	Neutropenia grade 3

SCr in µmol/L, CTCAE; Common Terminology Criteria for Adverse Events
1. After discontinuation of palbociclib; progression of disease was discovered
2. After discontinuation of abemaciclib, patient developed severe neutropenia and therefore abemaciclib was definitely halted
3. No reintroduction of abemaciclib because of severe neutropenia

CHAPTER 10

Palbociclib exposure in relation to efficacy and toxicity in patients with advanced breast cancer

ESMO Open, In press

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ABSTRACT

Background

Data on exposure-response or exposure-toxicity relationships of CDK4/6 inhibitors (CDK4/6i) are limited and inconclusive. We aimed to investigate whether there is an association between palbociclib exposure and progression-free survival (PFS), adverse events (AEs), and dose reductions.

Methods

Data were retrieved from the prospective, multicenter SONIA-trial in which patients with advanced estrogen receptor-positive, HER2 neu receptor-negative breast cancer were randomized to receive CDK4/6i treatment in first versus second-line. Blood for pharmacokinetics (PK) was taken at day 15 of cycles 1 and 2 during CDK4/6i treatment. Individual trough concentrations and plasma area under the curves of palbociclib were constructed using a population-PK model. Associations with palbociclib exposure were tested using Cox regression for PFS and chi-squared tests for AEs or dose reductions.

Results

PK data were available for 344 patients. No association between palbociclib exposure and PFS was found. Although patients with higher palbociclib exposure had more dose reductions during their entire CDK4/6i treatment course, this was not reflected by a higher incidence of grade 3-4 AEs in the first three months.

Conclusion

The absence of an association between palbociclib exposure and PFS and the presence of the association between palbociclib exposure and dose reductions suggest that dose reductions may safely be performed in case of palbociclib-related toxicity.

INTRODUCTION

Since 2017, cyclin-dependent kinases 4 and 6 (CDK4/6) inhibitors are available for patients with hormone receptor positive (HR+) advanced breast cancer. The addition of CDK4/6 inhibitors (i.e. palbociclib, ribociclib, and abemaciclib) to endocrine therapy has nearly doubled progression free survival (PFS) in both first- and second line treatment.[1-6] CDK4/6 inhibitors use is also associated with increased toxicity. Grade 3-4 neutropenia occurs in over 60% of patients treated with palbociclib or ribociclib, and in almost one-quarter of patients treated with abemaciclib.[2,4,7] Other frequently reported adverse events include anemia, thrombocytopenia, nausea and fatigue.[1,4,7] Adverse events can lead to treatment interruption, dose modification, or discontinuation of the drug, potentially compromising treatment outcomes. Indeed, more than one-third of patients receiving CDK4/6 inhibitors require a dose reduction due to adverse events and almost one-quarter discontinue CDK4/6 inhibitor treatment early.[8,9] Conversely, some patients tolerate the administered dose very well but may not achieve optimal benefit from CDK4/6 inhibitor therapy as higher doses may be more effective. Given the variability in response and tolerability to CDK4/6 inhibitors, patients may benefit from individualized dosing approaches like dose-escalations or dose-reductions. High inter-patient variability in trough plasma levels (C_{min}) is known.[10-12] However, data on exposure-response or exposure-toxicity relationships of CDK4/6 inhibitors are limited and inconclusive to date.

In order to reach a greater understanding of the pharmacokinetics (PK) of palbociclib, a population pharmacokinetic model (popPK model) incorporating important predictors for exposure is helpful to predict C_{min} and plasma area under the curve (AUC) concentrations from random PK samples. Different popPK models have already been developed to describe the PK and pharmacodynamics of palbociclib.[13-16] These models were either based on PK data from clinical trials (Phase I, II and III trials) or from real-world data with limited sampling. Interestingly, these different sources of PK data resulted in different popPK model structures to describe palbociclib PK: one-compartment models for real-world data compared to two-compartment models for clinical trial data.[13-16] These differences between clinical trial and real-world data may be due to differences in the number of the PK samples available for model development. Based on limited samples, it is often difficult to estimate all PK parameters of a (rather complex) popPK model. An approach taking into account PK information from a previously developed popPK model is beneficial in case of such sparse datasets. This can be achieved using a frequentist Bayesian approach ($PRIOR approach), which enables the estimation of PK parameters based on previous PK data and sparse data collected.[17] PK data derived from this informed popPK model could help to further delineate the variability in response and tolerability to palbociclib in our cohort.

In this paper, we developed a popPK model of palbociclib using the $PRIOR approach. With this popPK model we predicted individual C_{min} and AUC concentrations from random PK samples taken from patients in the SONIA study. The SONIA study evaluated the efficacy of CDK4/6 inhibitors added to either first- or second-line endocrine therapy in patients with HR+, her2neu receptor-negative (Her2-) breast cancer. We aimed to investigate the potential relationship between palbociclib exposure and clinical response or toxicity and dose-reductions in the SONIA-study.

METHODS

All data were derived from patients participating in the SONIA (**S**electing the **O**ptimal positio**n** of CDK4/6 **I**nhibitors in HR+ **A**dvanced breast cancer) study. The SONIA study is a multicenter randomised phase 3 study evaluating the efficacy, safety, quality-of-life and cost-effectiveness of CDK4/6 inhibitors added to either first- or second line endocrine therapy in patients with HR+, Her2-advanced breast cancer.[18] The study was approved by the Medical Ethics committee from the Netherlands Cancer Institute in March 2017 and registered in the European Clinical Trials database (2015-000617-43) and in the ClinicalTrials.gov database (NCT03425838). Patients were included in the study between November 2017 and September 2021 in the Netherlands.

Study design

Patients were eligible for the study if they were diagnosed with advanced breast cancer and were planned to receive aromatase inhibitors as first line treatment. A total of 1050 patients were randomised 1:1 to receive either first line treatment with an aromatase inhibitor plus a CDK4/6 inhibitor, followed at progression by fulvestrant monotherapy in second line or first line treatment with monotherapy aromatase inhibitor followed at progression by fulvestrant plus a CDK4/6 inhibitor in second line. A detailed description of the study and the main results has been published earlier.[19] Patients started with the standard dose of 125 mg palbociclib. Dose reductions were performed according study protocol/ SmPC, i.e. dose reduction was recommended for the subsequent cycles in case a non-haematological adverse event Common Terminology Criteria for Adverse Events (CTCAE) grade 3 or higher occurred. In case of a grade 3 haematological adverse event, palbociclib dose was only reduced in case of prolonged (> 1 week) recovery to grade 2 or lower, recurrent grade 3 haematological or need for transfusions. Palbociclib dose was always reduced in case of a grade 4 haematological adverse event. In general, a first dose reduction would be to 100 mg/day and a second dose reduction to 75 mg/day. Dose re-escalation was not allowed.

Since February 2018, patients in the SONIA study could consent separately to participate in the PK part of the study. Extra blood samples were taken at at random time points on day 15 +/- 5 days of cycle 1 and day 15 +/- 5 days of cycle 2 during the treatment with a CDK4/6 inhibitor. Time of blood withdrawal and time of last CDK4/6 inhibitor intake before blood withdrawal were noted for every patient. Blood was collected in EDTA tubes and processed at the day of collection by centrifugation at 1500 – 2000 g for 10 minutes. Plasma was stored at -20˚C until analyses. Plasma concentrations were quantified using a validated liquid chromatography-tandem mass spectrometry method.[20]

Population pharmacokinetic model

A popPK model was developed using NONMEM (version 7.5.0, ICON development Solutions, Ellicott City, MD, USA) software. Since only limited sampling data were available for each patient, the $PRIOR subroutine as Bayesian modelling was used to inform poorly informed parameters of our data (based on the distribution of the prior parameter) according to Chan Kwong *et al*.[17] This is an elegant alternative to fixing PK parameters in case of sparse datasets due to difficulties estimating all PK parameters. Using the $PRIOR approach enables modelling of sparse data. The previously developed two-compartment model by Courlet *et al*. was used as prior information model.[13] Model development using the prior subroutine is further specified in the **Supplementary data**. Finally, a brief covariate analysis was performed to potentially further improve the model fit, where allometric scaling and differences between capsules or tablets were investigated (see **Supplementary data methods** for details).

To discriminate and select between models physiological plausibility, goodness-of-fit (GOF) plots, precision of parameter estimates and change in objective function were assessed. A significant improvement of the fit for hierarchical nested models was considered at a drop of ≥3.84 points, corresponding to a $p<0.05$ (χ^2-distribution with 1 degree of freedom). The GOF plots and prediction corrected visual predictive checks (pcVPC) were assessed to evaluate model fits.[21,22]

Pharmacokinetic analyses

For each patient, area under the curve ($AUC_{0\text{-}tau}$) plasma concentration and trough levels (C_{min}) at cycle 1 and 2 were constructed from random samples using the PK model. $AUC_{0\text{-}tau}$ describes the palbociclib exposure during the treatment period of three weeks, since palbociclib is prescribed in cycles of three weeks followed by one week off. $AUC_{0\text{-}tau}$ was derived from the PK model by dividing the dose with the individual estimated clearance, that was derived using Maximum a Posteriori Bayesian estimation. Individual PK parameter estimates (i.e. empirical Bayes PK estimates) were also used to obtain individual predicted C_{min}.[23] When PK data for both cycles

were available, an average $AUC_{0\text{-}tau}$ and C_{min} for both cycles together was calculated per patient. If PK data were available for only one cycle, $AUC_{0\text{-}tau}$ and C_{min} from this cycle were used for analyses. At least one sample should be above the lower limit of quantification in order for a patient to be included. If a patient switched to another CDK4/6 inhibitor after the first cycle, only PK data from the initial CDK4/6 inhibitor (i.e. palbociclib) were used. Since there was only a maximum of two PK samples per patient (spare data sampling), it was not possible to simulate other PK end-points such as time until maximum concentration (T_{max}) or peak plasma concentration (C_{max}) due to the high risk of shrinkage towards the population mean.

Response analyses

Radiologic response evaluation was performed every 12 weeks according Response Evaluation Criteria in Solid Tumors (RECIST v.1.1).[24] The relation between exposure and PFS was analysed separately per treatment strategy. First progression since start of palbociclib treatment, either in first- or second-line depending on when the patient received CDK4/6i treatment, was used for exposure-response analysis. Progression was defined as objective disease progression according to RECIST, clinical deterioration on palbociclib leading to discontinuation of therapy, initiation of chemotherapy for breast cancer or death, whatever occurred first.

Toxicity analyses

Laboratory assessment (hematology and chemistry) was performed every two weeks in the first two cycles, every four weeks during cycle three and four and every three months in the cycles thereafter in patients receiving CDK4/6 inhibitors. Grade 3 and 4 adverse events were assessed at every visit according the CTCAE. To exclude as many competing risks as possible, only grade 3 and 4 adverse events in the first three months after start of CDK4/6 inhibitor were included for analyses of the relation between exposure and toxicity. Adverse events were analysed separately when occurring in at least 10% of patients in the first three months of treatment. In patients in whom palbociclib was reduced in dose in the second cycle due to adverse events in the first cycle, only the PK data of the first cycle were included in the exposure-toxicity analyses. Also, when analysing the relationship between exposure and dose reduction, for all patients who underwent dose reduction in the second cycle, only PK data of the first cycle were included.

Statistical analyses

Baseline characteristics of patients were analysed using descriptive statistics. The primary endpoint for exposure-response analyses was time from randomisation to first progression for patients treated with palbociclib in the first line and time from start

palbociclib until first progression in second line for patients treated with palbociclib in second line. Univariable Cox regression was performed to test the association between PFS and palbociclib exposure by means of C_{min} and $AUC_{0\text{-}tau}$. For further insight, palbociclib exposure was split above or below median and above or below first quartile level. Hazard ratios were estimated by means of a Cox proportional hazard model. Survival curves were generated by Kaplan-Meier analysis. The occurrence of adverse events or dose reductions was compared between different quartiles of palbociclib exposure. Chi-squared test for trend in proportions was used to test whether a trend was seen for more adverse events or dose reductions with higher palbociclib exposure.

RESULTS

A total of 652 samples of 366 patients could be used for development of the PK-model. For analyses of the exposure-response and exposure-toxicity, only data of patients who initiated palbociclib at least three months before the data cut-off (December 1st 2022) were included. Therefore, PK data were available for 344 patients of whom 235 patients were treated with palbociclib in first line and 109 patients, those who progressed to second line before data cut-off, were treated with palbociclib in second line. Baseline characteristics can be found in **Table 1.**

PK model development

Palbociclib PK was adequately captured by a two-compartment model with first-order absorption (including a lag time), where the final popPK model was informed based on a prior model.[13] From the 652 samples, 51 samples (7.8%) were derived from patients using palbociclib tablets instead of capsules. No difference in bioavailability between capsules and tablets was identified. However, allometric scaling improved the goodness of fit (GOF) plots and was added to all relevant model parameters (clearance [CL], central compartment [V1], peripheral compartment [V2], and intercompartment clearance between V1 and V2 [Q]) with fixed allometric scaling components (0.75 for CL and Q and 1 for V1 and V2). CL was estimated at 62.6 L/h (relative standard error (RSE): 2%) and a high volume of distribution was estimated for both compartments (2370 (RSE: 7%) and 682 (RSE: 8.5%) L for V1 and V2, respectively). All PK parameters were estimated with good precision (relative standard errors (RSEs) <31%). Final parameter estimates and further information regarding model development is provided in the **Supplementary data** results. In addition, the GOF plots and pcVPC of the final model are shown in **Supplementary Figure 1 and 2**.

Table 1. Baseline characteristics

median [IQR] or *n* (%)	Palbociclib in first line (*n* = 235)	Palbociclib in second line (*n* = 109)
Age[a]	63 [54-70]	60 [53-71]
Weight[b]	73 [65-84]	75 [64-88]
BMI[b]	26 [24-30]	26 [24-30]
WHO performance[a]		
0	106 (45%)	55 (50%)
1	116 (49%)	48 (44%)
2	13 (6%)	6 (6%)
Menopausal status		
Pre- or perimenopausal	37 (16%)	19 (17%)
Postmenopausal	198 (84%)	90 (83%)
Visceral disease		
Yes	129 (55%)	62 (57%)
No	106 (45%)	47 (43%)
Bone only disease		
Yes	40 (17%)	19 (17%)
No	195 (83%)	90 (83%
Aromatase inhibitor[c]		
Anastrozole	57 (24%)	-
Letrozole	178 (76%)	-

age in years, BMI in kg/m^2
[a]at the moment of inclusion in the SONIA-study; [b]at the start of CDK4/6 inhibitor; [c]during CDK4/6 inhibitor treatment

Table 2. Adverse events and dose reduction in different palbociclib exposure groups

Palbociclib C_{min}	**Q1 <50.8 ng/mL**	**Q2 50.8–60.8 ng/mL**	**Q3 60.9-74.7 ng/mL**	**Q4 ≥74.8 ng/mL**	***p* for trend**
All AEs *≥grade 3*	45/86 (52%)	52/85 (61%)	46/85 (54%)	48/85 (56%)	0.88
Neutropenia *≥grade 3*	35/86 (41%)	39/85 (46%)	40/85 (47%)	41/86 (48%)	0.36
Dose reduction	25/83 (30%)	39/87 (45%)	36/85 (42%)	44/86 (51%)	0.01*
Palbociclib $AUC_{0\text{-}tau}$	**Q1 <1660 ng*h/mL**	**Q2 1660–1900 ng*h/mL**	**Q3 1900-2220 ng*h/mL**	**Q4 ≥2220 ng*h/mL**	***p* for trend**
All AEs *≥grade 3*	43/86 (50%)	50/85 (59%)	52/85 (61%)	46/86 (53%)	0.59
Neutropenia *≥grade 3*	31/86 (36%)	38/85 (45%)	46/85 (54%)	40/86 (47%)	0.09
Dose reduction	20/83 (24%)	41/87 (47%)	40/85 (47%)	43/86 (50%)	0.001*

C_{min}: trough concentration, AUC: area under the curve plasma concentration
*statistically significant

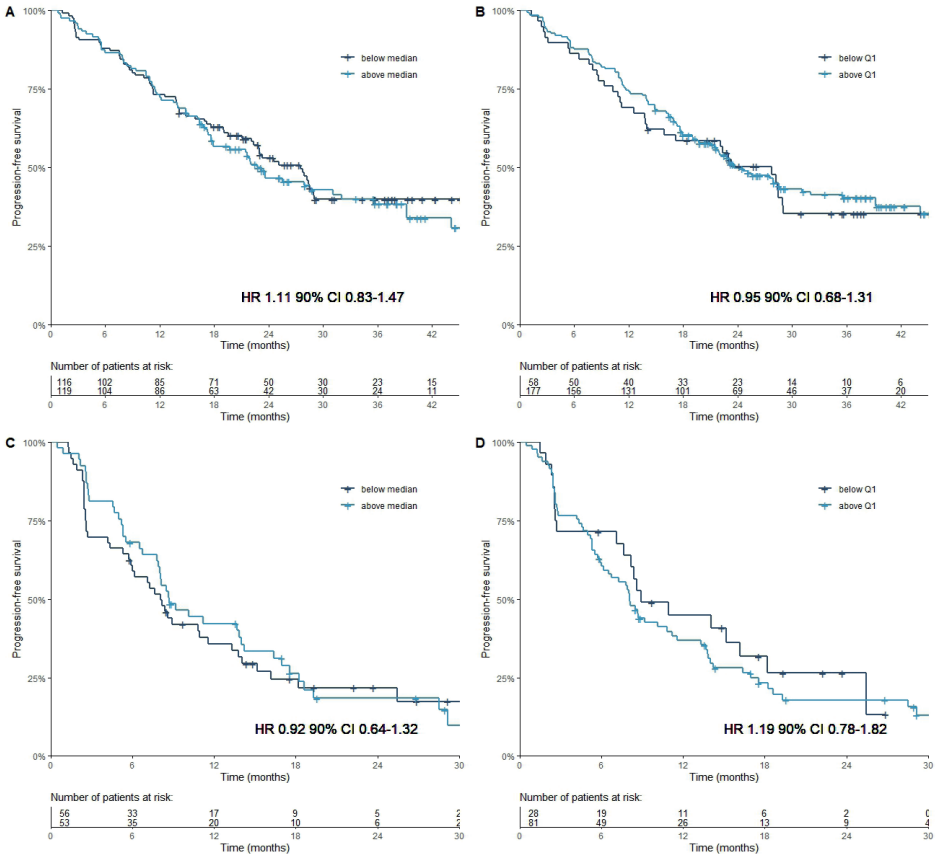

Figure 1. Kaplan Meier curves exposure-response relationship palbociclib
Palbociclib trough levels were included in the analyses. Panels A/B. Progression free survival when palbociclib was given as 1st line treatment; Panels C/D. Progression free survival when palbociclib was given as 2nd line treatment.
HR: hazard ratio, CI: confidence interval, Q1: first quartile
C_{min}: trough concentration, AUC: area under the curve plasma concentration *statistically significant

Exposure-respons analyses

For 270 patients, PK data of both treatment cycles were available, for 55 patients only PK data of cycle 1 were available and for 19 patients only PK data of cycle 2 were available. Thirty patients used a lower dose of palbociclib in cycle 2 (100 mg; *n* = 26, 75 mg, *n* = 4). The median C_{min} of palbociclib during cycle 1 and 2 in our cohort was 60 ng/mL (IQR 50 – 74 ng/mL, min-max 16.1-130.8 ng/mL) and the median $AUC_{0\text{-}tau}$ was 1886 ng*h/mL (IQR 1650 – 2210 ng*h/mL, min-max 1007 – 3567 ng*h/mL). In first line, there was no significant association between PFS and palbociclib exposure (HR 0.98 per 10 units (ng/mL) increase, 90% CI 0.90-1.06, *p* = 0.60 for C_{min} and HR 1.00, 90% CI 0.96-1.03 per 100 units (ng*h/mL) increase, *p* = 0.91 for $AUC_{0\text{-}tau}$). Also, PFS did not differ significantly

between patients with palbociclib C_{min} above or below median (HR 1.11, 90% CI 0.83-1.47) or between patients with palbociclib C_{min} above or below first quartile limit, i.e. 50 ng/mL (HR 0.95, 90% CI 0.68-1.31). For the latter analysis, the Kaplan-Meier curves crossed, but seemed to show an effect early on. This was checked by means of the restricted mean survival time method, yet again no effect could be found. Similar results were found for patients treated with palbociclib in second line **(Supplementary Table 1)**. Kaplan Meier curves and hazard ratios can be found in **Figure 1.** When the analyses were repeated using $AUC_{0\text{-}tau}$, similar results were found **(Supplementary Table 1)**.

Exposure-toxicity analyses

Twenty-four of 344 patients experienced toxicity grade 3-4 during cycle 1 leading to a dose reduction in cycle 2. For these patients, only PK data of cycle 1 were included. Since for two patients no PK data at cycle 1 were available, toxicity analyses were performed using 342 patients. 191/342 (56%) of patients experienced any toxicity grade 3-4 during the first three cycles. Neutropenia was the only individual adverse event occurring in >10% of patients during the first three cycles of treatment, occurring in 155/342 (45%) of patients. Patients were divided in quartiles according to C_{min} and $AUC_{0\text{-}tau}$ concentrations. Because of exclusion of some PK data for the toxicity endpoint, quartiles of palbociclib PK were slightly different compared to the exposure-response analyses. The occurrence of adverse events was compared between different quartiles and can be found in **Table 2**. No significant trend for more adverse events with higher palbociclib exposure could be found. No other individual toxicities could be analysed separately because of too low incidence in the first three months of treatment.

Additionally, the incidence of dose reductions during the entire treatment period with palbociclib was compared across different quartiles of exposure. Here, for all patients who had already received a dose reduction in cycle 2, only PK data from cycle 1 were used. This applied to 30 patients, three of whom had no PK data available for cycle 1, resulting in 341 patients available for analysis. All but three dose reductions were due to some form of toxicity (not further specified). There was a significant relationship between higher palbociclib C_{min} ($p = 0.01$) and $AUC_{0\text{-}tau}$ ($p = 0.001$) and the occurrence of dose reductions **(Table 2)**.

DISCUSSION

In the largest clinical PK study conducted thus far, we found no relationship between palbociclib exposure and PFS. Interestingly, no relationship between palbociclib exposure and grade 3-4 adverse events in the first three months was found. However, patients with higher palbociclib exposure more frequently underwent dose reduction.

For the development of the palbociclib popPK model used in our study the $PRIOR subroutine was used (adding PK information of a previously developed popPK model), as an alternative to fixing parameters or fitting an unrealistic simplified model due to limited sampling. Different studies have shown that using a prior approach results in a better fit than fixing parameters.[25,26] However, a disadvantage of the prior approach is that a covariate analysis cannot be performed on prior informed parameters.[17] Since it is known that the time until maximum plasma concentration of palbociclib capsules is longer than palbociclib tablets[27], we tested the effect of formulation on the relative bioavailability (with no prior weight) to further improve the model fit. However, no effect of differences between capsules and tablets on this relative bioavailability was identified indicating that formulation does not influence the bioavailability in patients.

Earlier research regarding the relationship between palbociclib exposure and PFS remained inconclusive. For instance, in the PALOMA-1 study, a trend for longer PFS in patients with palbociclib concentration >60 ng/mL, the same cut-off point as in our study, was found but this was not statistically significant and this study was performed in a small subgroup of patients (n = 81, median PFS of 24.5 months vs. 17 months).[28] Another study used popPK modelling to elucidate the relationship between palbociclib plasma concentration and PFS and found no difference between PFS in patients with palbociclib plasma concentrations above or below 78 ng/mL, coinciding with the third quartile limit in our study, in the PALOMA-3 study.[29] In vitro, the concentrations of palbociclib required to achieve 50% inhibition (IC50) of CDK4 and CDK6 were found to be 33.5 ng/mL and 48.7 ng/mL, respectively.[11,30] Given that these levels are comparable to the average plasma C_{min} concentrations observed clinically, we hypothesized that palbociclib efficacy could be influenced by variations in exposure. However, the lack of association between palbociclib exposure and PFS in our and other studies demonstrates that in vitro findings do not always directly translate to clinical outcomes.

In contrast with our findings, earlier research regarding the relationship between palbociclib exposure and toxicity did suggest more adverse events with higher palbociclib levels. Phase 1 studies and PK modelling suggested that higher palbociclib concentrations were associated with a higher incidence of neutropenia grade 3-4.[15] Also, a small prospective study (n = 58) found a trend for higher palbociclib C_{min} in patients with neutropenia grade 3-4 compared with patients without neutropenia grade 3-4 in the first two cycles (76.7 vs. 66.7 ng/mL, p = 0.06).[31] Compared to our study, these studies were small, which might be the reason for the conflicting results.

We showed that patients with higher palbociclib exposure more frequently underwent dose reductions. Almost all dose reductions were caused by some form of toxicity.

However, there was no higher incidence of grade 3-4 adverse events in the first three months of treatment in patients with higher palbociclib exposure. There are several potential explanations for this apparent contradiction. For instance, a substantial burden of grade 1-2 adverse events might have prompted the need for dose reduction over time. Unfortunately, these lower grade adverse events were not systematically monitored during the study. Furthermore, our toxicity analyses did not include grade 3-4 adverse events occurring beyond the initial three months of therapy. Although it is known that most adverse events occur in the first three months[32], it remains unknown if grade 3-4 adverse events occurring after this timeframe have influenced the likelihood of dose reduction.

Our results regarding a relationship between palbociclib exposure and dose reduction and the absence of a relationship between palbociclib exposure and PFS suggest that it is safe to reduce the dose in patients suffering from palbociclib-related toxicity. Indeed, a real world study involving 70 patients, of whom 40 underwent a dose reduction of palbociclib, found no differences in PFS between patients who underwent a dose reduction compared to those who did not.[33] Similar results were found in real-world studies of ribociclib and abemaciclib.[9,34,35] However, this study does not address whether dose reductions could be safely applied to all patients, including those without toxicity or even from start of treatment, which warrants further investigation. Additionally, it would be interesting to explore a potential relationship between palbociclib exposure and lower-grade or long-term adverse events.

Unfortunately, we only had PK data of cycle 1 and cycle 2 in this study. Therefore, dose reductions, interruptions or early discontinuation of treatment occurring after cycle 2 could not be taken into account when comparing PFS across different palbociclib exposure groups. This limitation may have introduced some bias, but it aligns with clinical practice where therapeutic drug monitoring would be most beneficial around start of treatment to consider early dose adjustments. Another limitation is that we had to predict C_{min} and $AUC_{0\text{-}tau}$ concentrations instead of taking blood samples at the time of minimum concentration or conducting more frequent sampling for each patient.

In conclusion, our study did not find a relationship between palbociclib exposure and PFS. Patients with higher palbociclib exposure more frequently underwent dose reductions. Yet, this was not reflected by a higher incidence of grade 3-4 adverse events in the first three months. Although more research regarding lower grade adverse events and the effect of dose reductions on efficacy is needed, our results suggest that patients who experience palbociclib-related toxicity can safely be reduced in dose.

REFERENCES

1. Finn RS, Martin M, Rugo HS, et al. Palbociclib and Letrozole in Advanced Breast Cancer. New England Journal of Medicine 2016; 375(20): 1925-36.
2. Cristofanilli M, Turner NC, Bondarenko I, et al. Fulvestrant plus palbociclib versus fulvestrant plus placebo for treatment of hormone-receptor-positive, HER2-negative metastatic breast cancer that progressed on previous endocrine therapy (PALOMA-3): final analysis of the multicentre, double-blind, phase 3 randomised controlled trial. The Lancet Oncology 2016; 17(4): 425-39.
3. Hortobagyi GN, Stemmer SM, Burris HA, et al. Updated results from MONALEESA-2, a phase III trial of first-line ribociclib plus letrozole versus placebo plus letrozole in hormone receptor-positive, HER2-negative advanced breast cancer. Annals of Oncology 2018; 29(7): 1541-7.
4. Johnston S, Martin M, Di Leo A, et al. MONARCH 3 final PFS: a randomized study of abemaciclib as initial therapy for advanced breast cancer. npj Breast Cancer 2019; 5(1): 5.
5. Sledge GW, Jr., Toi M, Neven P, et al. MONARCH 2: Abemaciclib in Combination With Fulvestrant in Women With HR+/HER2- Advanced Breast Cancer Who Had Progressed While Receiving Endocrine Therapy. Journal of Clinical Oncology 2017; 35(25): 2875-84.
6. Slamon DJ, Neven P, Chia S, et al. Phase III Randomized Study of Ribociclib and Fulvestrant in Hormone Receptor-Positive, Human Epidermal Growth Factor Receptor 2-Negative Advanced Breast Cancer: MONALEESA-3. Journal of Clinical Oncology 2018; 36(24): 2465-72.
7. Hortobagyi GN, Stemmer SM, Burris HA, et al. Ribociclib as First-Line Therapy for HR-Positive, Advanced Breast Cancer. New England Journal of Medicine 2016; 375(18): 1738-48.
8. Ismail RK, van Breeschoten J, Wouters M, et al. Palbociclib dose reductions and the effect on clinical outcomes in patients with advanced breast cancer. Breast 2021; 60: 263-71.
9. Burris HA, Chan A, Bardia A, et al. Safety and impact of dose reductions on efficacy in the randomised MONALEESA-2, -3 and -7 trials in hormone receptor-positive, HER2-negative advanced breast cancer. British Journal of Cancer 2021; 125(5): 679-86.
10. Braal CL, Jongbloed EM, Wilting SM, Mathijssen RHJ, Koolen SLW, Jager A. Inhibiting CDK4/6 in Breast Cancer with Palbociclib, Ribociclib, and Abemaciclib: Similarities and Differences. Drugs 2021; 81(3): 317-31.
11. Groenland SL, Martínez-Chávez A, van Dongen MGJ, et al. Clinical Pharmacokinetics and Pharmacodynamics of the Cyclin-Dependent Kinase 4 and 6 Inhibitors Palbociclib, Ribociclib, and Abemaciclib. Clinical Pharmacokinetics 2020; 59(12): 1501-20.
12. Groenland SL, Mathijssen RHJ, Beijnen JH, Huitema ADR, Steeghs N. Individualized dosing of oral targeted therapies in oncology is crucial in the era of precision medicine. European Journal of Clinical Pharmacology 2019; 75(9): 1309-18.
13. Courlet P, Cardoso E, Bandiera C, et al. Population Pharmacokinetics of Palbociclib and Its Correlation with Clinical Efficacy and Safety in Patients with Advanced Breast Cancer. Pharmaceutics 2022; 14(7).
14. Sun W, O'Dwyer PJ, Finn RS, et al. Characterization of Neutropenia in Advanced Cancer Patients Following Palbociclib Treatment Using a Population Pharmacokinetic-Pharmacodynamic Modeling and Simulation Approach. Journal of Clinical Pharmacology 2017; 57(9): 1159-73.
15. Marouille AL, Petit E, Kaderbhaï C, et al. Pharmacokinetic/Pharmacodynamic Model of Neutropenia in Real-Life Palbociclib-Treated Patients. Pharmaceutics 2021; 13(10).
16. Royer B, Kaderbhaï C, Fumet JD, et al. Population Pharmacokinetics of Palbociclib in aReal-World Situation. Pharmaceuticals 2021; 14(3).
17. Chan Kwong AHP, Calvier EAM, Fabre D, Gattacceca F, Khier S. Prior information for population pharmacokinetic and pharmacokinetic/pharmacodynamic analysis: overview and guidance with a focus on the NONMEM PRIOR subroutine. Journal of Pharmacokinetics and Pharmacodynamics 2020; 47(5): 431-46.
18. van Ommen-Nijhof A, Konings IR, van Zeijl CJJ, et al. Selecting the optimal position of CDK4/6 inhibitors in hormone receptor-positive advanced breast cancer - the SONIA study: study protocol for a randomized controlled trial. BMC Cancer 2018; 18(1): 1146.
19. Sonke GS, Van Ommen - Nijhof A, Wortelboer N, et al. Early versus deferred use of CDK4/6 inhibitors in advanced breast cancer. Nature 2024; 636: 474-480.
20. Janssen JM, de Vries N, Venekamp N, Rosing H, Huitema ADR, Beijnen JH. Development and validation of a liquid chromatography-tandem mass spectrometry assay for nine oral anticancer drugs in human plasma. J Pharmaceut Biomed 2019; 174: 561-6.

21. Karlsson MO, Savic RM. Diagnosing model diagnostics. Clinical Pharmacology & Therapeutics 2007; 82(1): 17-20.
22. Bergstrand M, Hooker AC, Wallin JE, Karlsson MO. Prediction-corrected visual predictive checks for diagnosing nonlinear mixed-effects models. The AAPS Journal 2011; 13(2): 143-51.
23. Janssen JM, Dorlo TPC, Beijnen JH, Huitema ADR. Evaluation of Extrapolation Methods to Predict Trough Concentrations to Guide Therapeutic Drug Monitoring of Oral Anticancer Drugs. Therapeutic Drug Monitoring 2020; 42(4): 532-9.
24. Eisenhauer EA, Therasse P, Bogaerts J, et al. New response evaluation criteria in solid tumours: revised RECIST guideline (version 1.1). European Journal of Cancer 2009; 45(2): 228-47.
25. Knebel W, Gastonguay MR, Malhotra B, El-Tahtawy A, Jen F, Gandelman K. Population pharmacokinetics of atorvastatin and its active metabolites in children and adolescents with heterozygous familial hypercholesterolemia: selective use of informative prior distributions from adults. Journal of Clinical Pharmacology 2013; 53(5): 505-16.
26. Knøsgaard KR, Foster DJ, Kreilgaard M, Sverrisdóttir E, Upton RN, van den Anker JN. Pharmacokinetic models of morphine and its metabolites in neonates:: Systematic comparisons of models from the literature, and development of a new meta-model. European Journal of Pharmaceutical Sciences 2016; 92: 117-30.
27. Pfizer. Summary of Product Characteristics IBRANCE
28. US Food and Drug Administration. Center for Drug Evaluation and Research. Clinical pharmacology and biopharmaceutics review palbociclib. Accessed 15 May 2024 2014. https://www.accessdata.fda.gov/drugsatfda_docs/nda/2015/207103Orig1s000ClinPharmR.pdf.
29. Sun W, Yu Y, Hoffman J, Turner NC, Cristofanilli M, Wang DD. Palbociclib exposure-response analyses in second-line treatment of hormone-receptor positive advanced breast cancer (ABC). Journal of Clinical Oncology 2017; 35(15_suppl): 1053-.
30. Fry DW, Harvey PJ, Keller PR, et al. Specific inhibition of cyclin-dependent kinase 4/6 by PD 0332991 and associated antitumor activity in human tumor xenografts. Molecular Cancer Therapeutics 2004; 3(11): 1427-38.
31. Leenhardt F, Fiteni F, Gauthier L, et al. Pharmacokinetic Variability Drives Palbociclib-Induced Neutropenia in Metastatic Breast Cancer Patients: Drug–Drug Interactions Are the Usual Suspects. Pharmaceutics 2022; 14(4): 841.
32. Thill M, Schmidt M. Management of adverse events during cyclin-dependent kinase 4/6 (CDK4/6) inhibitor-based treatment in breast cancer. Therapeutic Advances in Medical Oncology 2018; 10: 1758835918793326.
33. Wilkie J, Schickli MA, Berger MJ, et al. Progression-Free Survival for Real-World Use of Palbociclib in Hormone Receptor-Positive Metastatic Breast Cancer. Clinical Breast Cancer 2020; 20(1): 33-40.
34. Rugo HS, Huober J, García-Sáenz JA, et al. Management of Abemaciclib-Associated Adverse Events in Patients with Hormone Receptor-Positive, Human Epidermal Growth Factor Receptor 2-Negative Advanced Breast Cancer: Safety Analysis of MONARCH 2 and MONARCH 3. Oncologist 2021; 26(1): e53-e65.
35. Fernández-Cuerva C, Chinchilla-Alarcón T, Alcaraz-Sánchez JJ. Real-world effectiveness of ribociclib in metastatic breast cancer patients: Does dose affect survival? Journal of Oncology Pharmacy Practice 2023; 29(7): 1619-27.
36. Chan Kwong AHP, Calvier EAM, Fabre D, Gattacceca F, Khier S. Prior information for population pharmacokinetic and pharmacokinetic/pharmacodynamic analysis: overview and guidance with a focus on the NONMEM PRIOR subroutine. Journal of Pharmacokinetics and Pharmacodynamics 2020; 47(5): 431-46.

SUPPLEMENTARY MATERIAL

Supplementary Table 1. Hazard ratios palbociclib exposure and progression-free survival

	Palbociclib in first line	Palbociclib in second line
C_{min}		
Continuous (per 10 units)	HR 0.98, 90% CI 0.90-1.06	HR 0.95, 90% CI 0.88-1.03
Median split	HR 1.11, 90% CI 0.83-1.47	HR 0.92, 90% CI 0.64-1.32
Q1 split	HR 0.95, 90% CI 0.68-1.31	HR 1.19, 90% CI 0.78-1.82
AUC		
Continuous (per 100 units)	HR 1.00, 90% CI 0.96-1.03	HR 0.98, 90% CI 0.95-1.02
Median split	HR 1.19, 90% CI 0.90-1.58	HR 1.01, 90% CI 0.71-1.44
Q1 split	HR 1.04, 90% CI 0.75-1.46	HR 1.07, 90% CI 0.70-1.61

C_{min}: trough level, AUC: area under the curve plasma concentration, HR: hazard ratio, CI: confidence interval

PART IV

CONCLUSIONS

CHAPTER 11

Summary

General discussion & Future perspectives

SUMMARY

This thesis explored the pharmacokinetics of two important (classes of) drugs in the treatment of estrogen-receptor positive breast cancer: tamoxifen and CDK4/6 inhibitors. We investigated how the pharmacokinetics of these drugs relate to adverse effects and efficacy. Additionally, we aimed to elucidate less commonly known adverse effects associated with tamoxifen and CDK4/6 inhibitors. In this chapter, the key findings of our research will be summarized and discussed.

PART I. Tamoxifen and adverse effects

In **chapter 2**, the effects of lower tamoxifen doses on adverse effects and clinical efficacy was summarized in a narrative review. Several randomized controlled trials (RCTs) found fewer adverse effects with low dose tamoxifen compared to 20 mg tamoxifen and equal adverse effects between low dose tamoxifen and placebo. No studies assessing clinical efficacy of low dose tamoxifen were performed in the adjuvant setting. However, several studies (RCTs and observational studies) showed efficacy of low dose tamoxifen in decreasing the incidence of invasive breast cancer or carcinoma in situ in the primary (women with high breast cancer risk) or secondary prevention setting (women with a history of carcinoma in situ). Also, studies using derivatives for clinical efficacy, i.e. mammographic density and proliferation marker Ki67, were included. Mammographic density is based on the distribution between stromal, epithelial and fat cells and higher mammographic density has been associated with increased breast cancer risk. On the contrary, a reduction in mammographic density after a longer period of tamoxifen 20 mg daily, has been associated with a lower risk at breast cancer recurrence. We found that low dose tamoxifen also significantly reduced mammographic density compared to placebo and led to a non-inferior reduction compared with tamoxifen 20 mg. Second, Ki67, a nuclear marker expressed in all phases of the cell cycle other than the G0-phase, is expressed in proliferating cells in breast cancer and has proven to be a valuable predictive marker. A decrease in Ki67 expression after a short period of endocrine therapy has shown to be a strong predictor for efficacy of this treatment. We found that low dose tamoxifen can also decrease Ki67 expression. In this review, we have shown that low dose tamoxifen has a clinically relevant, improved toxicity profile compared with standard-dose tamoxifen and summarized strong, albeit indirect, evidence that lower doses of tamoxifen also possess anti-tumor efficacy.

To investigate whether dose reduction can also improve adverse effects in the adjuvant setting we set up the clinical study as described in **chapter 3**. In this study, tamoxifen dose reduction was proposed to patients with bothersome tamoxifen-related adverse effects and endoxifen levels above 32 nM (i.e. two times the supposed threshold of 16

nM). Eventually, 17 evaluable patients were reduced from a tamoxifen dose of 20 mg (i.e. standard dose) to a dose of 10 mg. A clinically relevant improvement in adverse effects and quality of life was observed in 41% and 65% of the patients, respectively. These improvements were not seen in patients whose doses were not reduced (*n* = 60). In 21% of patients, endoxifen dropped slightly below 16 nM (e.g. 12.8, 15.5, 15.8, 15.9 nM, respectively), stressing the importance of reassessing endoxifen levels after altering the tamoxifen dose. This study demonstrates that dose reduction can be an effective strategy for patients who would otherwise quit anti-hormonal therapy or who are highly suffering from tamoxifen-related adverse effects. However, no placebo arm was included in this study so a potential placebo effect of lowering the tamoxifen dose on side-effects cannot fully be excluded.

In **chapter 4**, we have investigated the combination of tamoxifen and cannabidiol (CBD); a component of the *Cannabis Sativa* plant. CBD is increasingly used among patients with breast cancer in the hope to alleviate adverse events. However, CBD might affect tamoxifen pharmacokinetics since it is known to be a potential inhibitor of the most important enzyme in tamoxifen metabolism; CYP2D6. Therefore, we investigated the potential pharmacokinetic interaction between CBD and tamoxifen. Plasma levels of endoxifen, the most important metabolite of tamoxifen, decreased when CBD was used concomitantly with tamoxifen but remained within bio-equivalence boundaries (*n* = 15, 90% confidence interval (CI) -18.7%, -6.1%). As a secondary aim, we investigated whether CBD could indeed alleviate tamoxifen-related adverse events. The use of CBD, in the maximum over-the-counter dosage of 50 mg, decreased adverse events and improved quality of life (*n* = 26). Whether CBD should be recommended to patients with bothersome tamoxifen-related adverse effects has yet to be proven in a placebo-controlled study. Meanwhile, patients do not have to be discouraged if they want to try CBD next to their tamoxifen therapy.

Venous thromboembolism (VTE) is a rare but severe adverse effect associated with tamoxifen use. Whether there is a dose- or exposure-dependent effect of tamoxifen on this adverse effects remains currently unknown. In **chapter 5** we investigated the relationship between tamoxifen and endoxifen plasma levels and various coagulation proteins which are presumably involved in tamoxifen-related VTE. At both 3 and 6 months of tamoxifen therapy, higher plasma levels of tamoxifen and endoxifen were not associated with higher levels of the procoagulant tissue factor or thrombin generation parameters nor with lower levels of the anticoagulant proteins antithrombin and protein C. Also, tamoxifen dose decrease or increase did not seem to be associated with adjustments of coagulation protein levels, although these comparisons were limited as the majority of patients in this study stayed on the standard dose of tamoxifen 20

mg. The outcome of this study is reassuring and provides a first indication that higher tamoxifen (or endoxifen) levels do not have an additional procoagulant effect and therefore might not lead to a further increased risk of tamoxifen-related VTE. However, a study with more patients who underwent tamoxifen dose-adjustments could give a more clear answer on whether therapeutic drug monitoring (TDM)-directed tamoxifen dose-escalation could additionally increase VTE risk. Also, evident VTE events would be a better end point but since VTE has a relatively low incidence of ~3% among tamoxifen users, this would require a very large number of patients and such a study seems not feasible.

In **chapter 6**, we have investigated a less frequently studied adverse effect of tamoxifen; cognitive functioning, and the impact of tamoxifen and endoxifen plasma levels on this important adverse effect. A total of 135 women completed the *Amsterdam Cognition Scan,* an online neurophysiological test assessing both subjective and objective cognition functioning after two years of tamoxifen treatment. Women who were treated with tamoxifen reported mild cognitive complaints and performed worse on verbal learning, processing speed, executive functioning, and motor functioning compared to matched no-cancer controls. The cognitive functions of older women (≥57 years of age) were more severely affected by tamoxifen than the cognitive functions of younger women. Remarkably, higher tamoxifen and endoxifen levels, as well as a higher tamoxifen dose, were associated with worse performance on several cognitive domains. This relationship was especially present in younger women. This study prompts additional questions regarding tamoxifen and cognition. As a secondary endpoint of the PREDICTAM-study (NCT05036278), performance on the *Amsterdam Cognition Scan* will be compared before start of tamoxifen and after two years of tamoxifen in patients using different tamoxifen doses. These results must confirm the effect of tamoxifen on cognitive functioning and the potential relationship between tamoxifen doses and plasma levels with the effect of tamoxifen on cognitive functioning. Subsequently, it should be investigated whether discontinuing tamoxifen, or even reducing the dose, could improve cognitive functioning in affected patients.

PART II. Tamoxifen Model Informed Precision Dosing

In **chapter 7,** we developed a population-pharmacokinetic (POP-PK) model for tamoxifen. In contrast with earlier POP-PK models, in our model, CYP2D6 activity per allele was estimated on a continuous scale. Compared with using CYP2D6 phenotypes, inter-individual variability (IIV) in predicting endoxifen levels was decreased by 37% when using the continuous CYP2D6 activity scale. Age, body length and BMI were identified as other influential factors for tamoxifen clearance (age and length) and endoxifen formation (BMI). Higher age and shorter length lead to less tamoxifen

clearance and thus higher endoxifen levels. Higher BMI leads to less metabolization from tamoxifen to endoxifen and thus lower endoxifen levels. Including age, BMI and length, unexplained IIV in endoxifen formation was decreased to 25.1% and IIV in tamoxifen clearance was decreased to 32.1%. The model was successfully validated in an external validation dataset. Using this model, dosing cut-off points could be determined in order to predict a tamoxifen dose before start of treatment with which a patient would reach an endoxifen level >16 nM.

In **chapter 8**, the POP-PK model that was developed in **chapter 7** was used to prospectively implement model-informed precision dosing (MIPD) in order to achieve adequate endoxifen exposure prior to TDM. In this study, 106 patients received a predicted tamoxifen dose at start of treatment among which 65% received 20 mg, 16% received 30 mg and 19% received 40 mg tamoxifen. After attaining steady-state, 84.0% of patients reached endoxifen levels ≥16 nM, which was not significantly higher compared to a historical control cohort where all patients were treated with tamoxifen 20 mg (77.9.%, p=0.17). However, the model showed adequate performance according several external evaluation thresholds and correctly identified patients requiring the maximum registered dose before starting treatment (significantly less endoxifen levels <16 nM in the 40 mg groups). The lower-than-expected percentage of patients reaching endoxifen levels >16 nM can be explained by a larger proportion of patients with impaired CYP2D6 activity in the intervention cohort compared to the control cohort. Additionally, the model correctly identified patients who were unable to reach the therapeutic threshold. In conclusion, MIPD showed promise compared to conventional one-size-fits-all dosing of 20 mg tamoxifen, particularly in certain subgroups, but TDM still remains an important addition. The model is practical for use in the clinic since it includes the predictors age, length, BMI and CYP2D6 genotype, which are all easy factors to assess. Another advantage is that MIPD can be applied for endoxifen thresholds of whatever desired value. Therefore, when in the future a definite endoxifen threshold will be implemented in clinical practice, a combination of MIPD and TDM should be used.

PART III. CDK4/6 inhibitors

In **chapter 9**, we investigated the incidence of pseudo-acute kidney injury (AKI) in patients with advanced breast cancer treated with CDK4/6 inhibitors. Pseudo-AKI is a phenomenon in which creatinine plasma levels are increased without a reduction in renal function. This can be caused by inhibition of tubular secretion of creatinine and abemaciclib, palbociclib and ribociclib can all induce this inhibition. Cystatin C is another protein which can be used to assess renal function and is not subject to tubular secretion. From the 234 patients treated with a CDK4/6 inhibitor (palbociclib 88%, ribociclib 6%, abemaciclib 6%) between 2017 and 2024 at the Erasmus MC

Cancer Institute, 41 (17.5%) of the 234 patients had a significant increase in creatinine levels in the first six months of treatment. From 22 of these 41 patients, cystatin C levels could be determined. Pseudo-AKI was found in 16 out of 22 patients (73%, 95% CI 50-89%). Pseudo-AKI occurred most frequently in patients using abemaciclib. In 15 out of 41 patients (36%) additional diagnostics (n = 4) and/or medication adjustments (n = 11) were performed because of increasing creatinine levels. Therefore, cystatin C levels should be measured in patients on CDK4/6 inhibitors treatment with an impaired kidney function based on creatinine levels since it can reveal pseudo-AKI and thereby prevent unnecessary diagnostic interventions or drug alterations.

In **chapter 10** we assessed whether palbociclib exposure was related to response or toxicity in patients with advanced breast cancer. We included 344 patients from the SONIA-study, of whom 235 patients were treated with palbociclib in the first line, and 109 patients were treated with this CDK4/6 inhibitor in the second line. Trough plasma levels and area under the curve (AUC) plasma concentrations were derived from a pharmacokinetic POP-PK model. In first line, progression free survival did not differ between palbociclib levels above or below the median trough plasma level of 60 ng/mL, nor between palbociclib levels below or above the 1st quartile limit of 50 ng/mL. Similar results were found for patients treated with palbociclib in second line. Also, when analyses were repeated using $AUC_{0\text{-}tau}$, similar results were found Adverse events were assessed in the first 3 months of treatment. Any toxicity Common Terminology Criteria for Adverse Events (CTCAE) grade 3 or higher occurred in the first three months in 191/342 (56%) of patients. Neutropenia CTCAE grade 3 or higher occurred in 155/342 (45%) of patients. No significant trend for more adverse events or neutropenia with higher palbociclib levels was found. However, there was a trend towards more dose reductions in patients with higher palbociclib levels (p = 0.01). This apparent contradiction may be explained by a substantial burden of grade 1-2 adverse events which might have prompted the need for dose reduction over time but were unfortunately not routinely assessed during our study. Although more research regarding lower grade adverse events and the effect of dose reductions on efficacy is needed, our results suggest that patients who experience palbociclib-related toxicity can safely be reduced in dose.

GENERAL DISCUSSION & FUTURE PERSPECTIVES

In this thesis, we have discussed the impact of tamoxifen-related adverse effects. Generally, tamoxifen-related adverse effects are scored as low grade, and therefore these side-effects are often considered less impactful than, for example, those experienced during chemotherapy. However, since patients are treated with adjuvant anti-hormonal therapy for at least five to ten years[1-3], even more low-grade adverse effects can become very distressing, especially since they can severely affect daily life activities, work, physical activities and (sexual) relationships during all these years of treatment.[4,5] The high non-adherence rate among adjuvant tamoxifen users (~40%) underscores the significant burden of tamoxifen-related adverse events.[6-8] Moreover, non-adherence to tamoxifen also results in higher breast cancer recurrence rates.[9,10] Correct management of anti-hormonal therapy-related adverse events is crucial for improving therapy outcomes and quality of life among breast cancer survivors. Unfortunately, there are few evidence-based approaches for the treatment of tamoxifen-related adverse events. As discussed in **chapters 2 and 3** tamoxifen dose reduction could be an option for managing adverse events. However, to ensure tamoxifen efficacy, an endoxifen threshold to which tamoxifen doses can be titrated should be established.

Conversely, as discussed in (mainly) **chapters 7 and 8,** patients with 'too low' endoxifen levels may require a tamoxifen dose-escalation to achieve optimal tamoxifen efficacy. To minimize potential adverse effects, tamoxifen dose-escalation should only be done in patients for whom it is really needed. An endoxifen efficacy threshold is also needed to select those patients in whom the tamoxifen dose should be increased.

Endoxifen efficacy threshold

Until now, endoxifen thresholds are derived from retrospective studies. Madlensky et al. (2011) divided endoxifen levels of 1370 adjuvant treated patients with breast cancer into quintiles and found that patients with endoxifen levels in the lowest quintile (<16 nM) had a 30% higher breast cancer recurrence risk than patients with endoxifen levels >16 nM (hazard ratio (HR) 1.35, 95% CI 1.00-1.82).[11] When exploring dichotomized optimal cut-off points, the threshold was again determined at 16 nM. A smaller study in 2015 from Saladores et al. divided endoxifen levels of 306 patients in quartiles and found a higher recurrence rate (HR 1.94, 95% CI 1.04 – 4.14) for patients with endoxifen levels in the lowest quartile (endoxifen <14 nM) compared to patients with endoxifen levels in the highest quartile (endoxifen >35 nM).[12] Lastly, Helland et al. (2017) searched for a cut-off value in endoxifen levels from 86 patients who were treated with adjuvant tamoxifen and were followed for a median of 14 years.[13] They found a breast cancer specific survival of 57% in patients with endoxifen levels below 9 nM compared to a

breast cancer specific survival of 84% in patients with endoxifen levels above 9 nM (HR 3.73, 95% CI 1.05 – 13.22). A strength of the studies of Helland and Madlesnky is the search for a cut-off value without dividing endoxifen levels in different subgroups, which can lead to loss of valuable information. A limitation of the work by Helland et al. is, however, the sample size of only 86 patients. Although the relationship between endoxifen exposure and response has been found in all three studies, the precise thresholds could be subject to chance, because of the retrospective nature of the studies and potentially unknown confounders.

To obtain stronger evidence, prospective studies tried to confirm the exposure-response relationship of endoxifen. In the CYPTAM study, 662 patients were prospectively followed and after a median follow-up of six years the effect of endoxifen levels on relapse-free survival was determined. No association between continuous endoxifen levels, endoxifen levels below or above 16 nM or endoxifen levels divided in quartiles with relapse-free survival could be found.[14] In another paper from the same group, the thresholds of 10 and 14 nM endoxifen were also investigated, but again, no association with breast cancer recurrence was found.[15] However, it was discussed that the CYPTAM study – with 662 patients and only 40 events – was probably underpowered to draw final conclusions, especially considering the short follow-up time and the censoring of two-third of the patients after switching to an aromatase inhibitor after 2.5 years.[16-18] It was estimated that - to adequately determine the added value of therapeutic drug monitoring and subsequent dose adjustment of tamoxifen - 1500-3200 patients should be randomized and followed for at least 15 years.[19,20] Such large studies with a long-follow up duration are probably not deemed feasible to be performed in this field. Consequently, the best available evidence currently comes from retrospective studies.

Determining an endoxifen threshold using surrogate endpoints

In the metastatic setting, events of breast cancer progression occur earlier and more frequent than in the adjuvant setting. Two prospective studies investigated the effect of endoxifen levels on progression-free survival or objective response rate.[21,22] The latter study also included patients treated with tamoxifen in the neoadjuvant setting, accounting for one third of the study population, and patients treated with tamoxifen as second-line metastatic treatment.[22] Both studies did not find an association between endoxifen levels and progression-free survival or objective response rate. However, tamoxifen is not the drug-of-choice for endocrine neo-adjuvant treatment, as aromatase inhibitors results in a higher proportion of pathologic complete responses.[23] Also, the chance for tamoxifen resistance is higher in the metastatic setting and after multiple treatment lines.[24,25] Consequently, studies in the advanced breast cancer setting do not seem appropriate for assessing a potential exposure-response relation of tamoxifen.

Although there is strong retrospective evidence that an exposure-response relationship for endoxifen exists, this cannot adequately be assessed in prospective studies. Accordingly, the measurement of endoxifen levels guiding the treatment with tamoxifen is currently not mentioned in both ESMO (European Society of Medical Oncology) and ASCO (American Society of Clinical Oncology) early breast cancer guidelines.[1,3] In the most recent guidelines of the Dutch Pharmacogenetics Working Group and Royal Dutch Pharmacist Association however, dose increase or alternative medication is advised for patients with poor and intermediate CYP2D6 metabolism, who frequently have low endoxifen levels.[26,27]

Probably, additional retrospective studies will not contribute much to expanding our knowledge regarding an endoxifen threshold, which is essential to optimize tamoxifen therapy, balancing both adverse effects and efficacy. Moreover, in such studies, the possibility of an individual endoxifen threshold cannot be taken into account. Other patient factors determining tamoxifen efficacy are, for example, higher expression of mainly the estrogen receptor, but also the progesterone receptor, both associated with increased tamoxifen efficacy.[28,29] Probably, estradiol plasma levels also play an important role, since tamoxifen is more effective in premenopausal women when combined with ovarian function suppression as Gonadotropin-Releasing Hormone (GnRH) agonists.[30] It could be hypothesized that patients with different estradiol levels or estrogen or progesterone receptor expression, require different levels of endoxifen exposure. Also, intratumoral endoxifen levels vary in patients, probably due to different expression levels of several drug transporters on the breast cancer cell.[31-33] Given the variability in individual factors potentially influencing endoxifen requirements, I advocate for the use of an *in vivo* endocrine therapy sensitivity test in the preoperative setting, to improve the personalization of tamoxifen therapy.

In-vivo endocrine therapy sensitivity tests in the preoperative setting

Since adjuvant therapy is administered when the tumor is removed, it is not possible to individually assess the efficacy of an adjuvant drug in the adjuvant setting. However, before breast surgery, the effect of a specific drug on the tumor can be assessed in the waiting-time for surgery, the so-called 'window-of-opportunity'. Ki67 is a proliferation marker in breast tumors that can be used as an efficacy marker in this setting. As mentioned in **chapter 2**, the percentage of Ki67 is used as a prognostic marker in breast cancer, in which a low Ki67% predicts a more favorable outcome compared to a high Ki67%.[34] Ki67 dynamics also appear to be a competent predictive marker. For example, the difference in early endocrine response (i.e. adequate suppression of Ki67 below 10%) between anastrozole and tamoxifen in the small neo-adjuvant

IMPACT-trial[35] predicted the long-term adjuvant results from the large ATAC-trial.[36] The same effect accounted for the BIG 1-98 trial where letrozole and tamoxifen for postmenopausal women were compared.[37,38] Also, in the POETIC-trial, patients with a high baseline Ki67% that decreased sufficiently after neo-adjuvant endocrine therapy had a breast cancer recurrence rate comparable to that of patients with a low baseline Ki67 and much lower than that of patients in which Ki67 remained high.[39] These studies show that Ki67 suppression after a short period of neo-adjuvant endocrine therapy can predict long-term adjuvant endocrine therapy response.[35,39,40] From a short period (two weeks) of neo-adjuvant tamoxifen in a daily dose of 20 mg it is known that it decreases Ki67 from ≥10% to <10% in approximately 40% of patients.[41,42] Therefore, this marker can be used as an early surrogate endpoint to predict long term outcome and therefore tamoxifen sensitivity.

The preoperative setting can be a window-of-opportunity to establish (individual) endoxifen thresholds for efficacy. If patients are treated preoperatively with tamoxifen, it can be investigated above which endoxifen level tumors show an adequate response in Ki67 (**Figure 1**). In patients who are treated with preoperative tamoxifen their specific response of Ki67 can be assessed, which also takes their personal ER- and PR-expression, estradiol level and intra-tumoral tamoxifen and endoxifen levels into consideration. This approach enables the determination of tumor sensitivity for specific endoxifen levels in each individual patient However, the assessment of individual tamoxifen sensitivity is only applicable for patients with luminal B breast cancer (i.e. tumors with Ki67% ≥10%) and for patients who do not have an indication for neoadjuvant chemotherapy. For patients with luminal A breast cancer or patients who have to undergo neoadjuvant chemotherapy the general threshold shall be applicable. Currently, a study to investigate this principle in a clinical setting has been set up and will enroll patients soon.

When a (personalized) endoxifen efficacy threshold can be established, this will improve tamoxifen treatment in multiple ways. First, the general endocrine therapy efficacy could be improved by increasing the tamoxifen dose or switching to aromatase inhibitors in patients in which endoxifen levels are too low. Second, menopausal adverse effects can be reduced in patients where tamoxifen dose reduction is feasible, as performed in a subset of patients in **chapter 3**. Second, although in **chapter 5** a reassuring lack of association between tamoxifen and endoxifen levels and a further increased procoagulant state was found, it remains uncertain whether the absence of this association will hold when assessing venous thromboembolism events. Increasing tamoxifen doses only for patients who require it based on their individual endoxifen threshold could minimize the additional risk of venous thromboembolism as much

as possible. Third, as discovered in **chapter 6**, cognitive problems after tamoxifen therapy may be influenced by the amount of tamoxifen and endoxifen exposure. Dose reduction, while attaining endoxifen efficacy, could potentially improve cognitive functioning in affected patients. Concurrently, in patients with endoxifen levels which are deemed too low, dose escalation of tamoxifen or a switch to aromatase inhibitors should be performed. As discussed in **chapter 8**, it is feasible to predict endoxifen steady-state levels before start of tamoxifen and dose-escalation could thus - when needed - already be performed at the moment of treatment initiation.

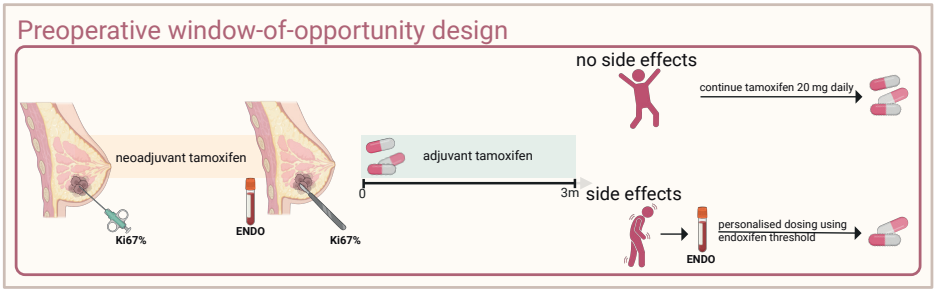

Figure 1. Pre-operative window-of-opportunity design
Patients with ER+ breast cancer who have an indication for adjuvant endocrine therapy receive preoperative tamoxifen. After a short period of 20 mg tamoxifen a tumor biopsy for Ki67 determination will be done and the concurrent endoxifen level will be determined in plasma. Another 2 weeks of 40 mg tamoxifen will result in the steady-state concentration of endoxifen. At this moment the patient will undergo breast surgery and the endoxifen concentration will be determined again. Using the combination of Ki67 response and the corresponding endoxifen level, dose adjustments of tamoxifen can be performed in the adjuvant setting in case of side effects. If patients do not respond to tamoxifen at all (Ki67 remained high) another adjuvant treatment could be considered.

CDK4/6 inhibitors in the advanced setting

The efficacy of endocrine therapy can also be optimized by the addition of other drugs, for example CDK4/6 inhibitors. In ER-positive, advanced breast cancer the addition of a CDK4/6 inhibitor to letrozole in first line or to fulvestrant in second line is standard-of-care and improves progressive-free and overall survival.[43-48] In **chapter 10,** we have investigated whether the exposure of palbociclib, one of the most frequently prescribed CDK4/6 inhibitors, is associated with progression free survival, adverse events or dose-reductions. We did not find an exposure-response relation between palbociclib exposure and progression free survival or severe adverse events. Contrarily, dose reductions were more common in patients with higher palbociclib concentrations, suggesting a higher incidence of (possibly low grade) adverse events in these patients. These findings imply that current palbociclib dosing reaches a therapeutic plateau. However, this study does not determine how far above the plateau current palbociclib levels are, nor whether all patients could safely undergo dose reduction.

For interpretation of these findings, it is important to know that anti-cancer drugs are traditionally dosed according the maximum tolerated dose (MTD), a familiar, time-honored method to determine the dose of investigational drugs on phase II and III trials.[49] The MTD is established in phase I trials, where increasing doses are administered until unacceptable adverse effects occur. However, the maximum dose that could be tolerated may not be the minimum dose that is needed for optimal efficacy. Also, the MTD is mostly determined in the first weeks of treatment and does not take into account more long-term adverse effects that occur over time.

Earlier real world studies regarding palbociclib found no differences in progression-free survival between patients who underwent a dose reduction due to toxicity compared to those who did not.[50] Similar results were found in real-world studies of ribociclib and abemaciclib.[51-53] An important adverse event of ribociclib is QTc prolongation. Recently, the AMALEE study is performed as a post-approval commitment to determine whether reducing the starting dose of ribociclib from 600 mg to 400 mg could decrease QTc prolongation without compromising efficacy in advanced breast cancer treatment.[54] In this phase II study 376 patients were randomized in first line between 400 mg ribociclib or 600 mg ribociclib next to an aromatase inhibitor. Results were presented at the San Antonio Breast Cancer Conference 2022. Overall response rate, the primary endpoint, was 41.5% in the patient group treated with 400 mg ribociclib and 45.3% in the group treated with 600 mg ribociclib. Although the overall response rates were not statistically different between the two dosage groups, non-inferiority for the 400 mg dose could not be established. However, immature results for progression free survival did not indicate any difference between 400 mg and 600 mg ribociclib treatment groups and QTc prolongation occurred less in the 400 mg dose group. The full manuscript of this trial is eagerly awaited. In the meantime, these study results confirm the potential overdosing in CDK4/6 inhibitors and encourage research regarding the potential similar efficacy between lower and standard dosing in targeted drugs, especially in CDK4/6 inhibitors.

CDK4/6 inhibitors in the adjuvant setting

Currently, CDK4/6 inhibitors are also considered for adjuvant treatment. However, the efficacy of CDK4/6 inhibitors added to adjuvant endocrine therapy is controversial. Abemaciclib was the first CDK4/6 inhibitor for which efficacy in the adjuvant treatment of patients with estrogen-receptor positive, her2neu-negative breast cancer was shown.[46] In the monarchE study, patients with high risk early breast cancer were randomized between two years of abemaciclib after breast surgery, in addition to standard of care endocrine therapy as tamoxifen or aromatase inhibitor, or standard of care endocrine therapy only. The addition of abemaciclib to endocrine therapy decreased the risk of breast cancer recurrence with a HR of 0.66 (95% CI 0.58-

0.76, 12% versus 17.6%) after a median follow-up of 42 months. Recently, benefit of ribociclib in the adjuvant setting was found in the NATALEE study.[55] Addition of ribociclib for 3 years reduced breast cancer recurrence within 3 years after breast surgery with a HR of 0.75 (95% CI 0.62-0.91, 6.9% versus 9.6%) in high risk patients. On the contrary, in both the PALLAS study and PENELOPE-B study, no benefit of adjuvant palbociclib could be found.[56,57]

Following the above mentioned results, adjuvant abemaciclib has been approved by both the U.S. Food and Drug Administration (FDA) and European Medicines Agency (EMA) and has been included in ESMO and ASCO guidelines.[58-61] The FDA and EMA mainly assess quality, safety and efficacy of a new drug. For the reimbursement of anti-cancer drugs in the Netherlands, a positive advice of the Dutch Committee for the Evaluation of Oncological Agents ("Commissie ter Beoordeling van Oncologische Middelen – commissie BOM") is required. The commissie BOM evaluates anti-cancer drugs for clinical relevance according the PASKWIL-criteria (i.e., Palliative, Adjuvant, Specific side-effects, Quality of Life, Impact of treatment and Level of Evidence-criteria).[62,63] The goal of commissie BOM is to ensure that anti-cancer drugs provide real benefits to patients by using the PASKWIL criteria to assess their added value, prioritizing overall survival improvement. Since proving survival benefits can be time-consuming, progression-free survival can be used as a surrogate endpoint but must meet stringent criteria. This strict evaluation aims to enhance the likelihood of eventual survival benefits. By differentiating between highly effective treatments and those with limited benefits and significant side effects, commissie BOM aims to ensure that only the most beneficial drugs become available to patients. However, such strict approval of anti-cancer drugs might also lead to delayed access to potentially efficacious drugs. In 2023, the PASKWIL criteria have been updated and, among others, the hazard ratio for disease free survival benefit in the adjuvant setting is adjusted from 0.7 to 0.6. Therefore, adjuvant abemaciclib is not yet reimbursed in the Netherlands.[64]

Endocrine therapy sensitivity tests might also guide the use of adjuvant CDK4/6 inhibitors

In the current setting, sixteen patients need to be treated with adjuvant abemaciclib for two years to be able to prevent one event of breast cancer recurrence. These numbers suggest that the specific patient group for which adjuvant CDK4/6 inhibitors are most beneficial, could (or should) be further specified. An endocrine therapy sensitivity test could guide in the selection of patients in need for extended adjuvant therapy. For example, patients in whom Ki67% remains high after preoperative endocrine therapy with the adjuvant endocrine drug of choice, might have extra benefit from the addition of adjuvant abemaciclib. In such a study, high risk patients according the

monarch-E population in whom Ki67% remains high after short preoperative endocrine therapy with an aromatase inhibitor, should be randomized between adjuvant treatment with or without abemaciclib. Possibly, such a study design could lead to less patients being overtreated while simultaneously abemaciclib becomes already available in the Netherlands for those in need for additional treatment. A similar study is currently being performed in countries where adjuvant abemaciclib is approved in certain patient groups with a high breast cancer risk. In the POETIC-A study (NCT04584853), patients with breast cancer who currently do not apply for adjuvant abemaciclib (according to the ASCO guidelines) are treated pre-operatively with an aromatase inhibitor. If Ki67 percentages remain high after this treatment, patients are randomized between addition of adjuvant abemaciclib to endocrine therapy or endocrine therapy only. In contrast with the above mentioned study idea, the POETIC-A mainly focusses on expanding instead of narrowing the indication for adjuvant CDK4/6 inhibitors.

In conclusion, this thesis explored various approaches to address tamoxifen-related side effects, an important problem in the adjuvant treatment of hormone-sensitive breast cancer. In addition to the well-known side effects, we further investigated less common adverse effects linked to tamoxifen exposure, like cognitive decline and venous thrombosis. Whether dose-adjustments can also mitigate these adverse effects warrants further investigation. Moreover, our research indicates that palbociclib exposure is not related to efficacy, although dose reductions are more frequently performed in patients with higher palbociclib exposure. This implies that dose reduction can safely be performed in case of adverse effects and high plasma levels. However, whether dose reductions can be applied to all patients, including those without toxicity or even from start of treatment, requires additional research. As highlighted in this discussion, in the future, the use of an *in vivo* sensitivity test may guide (endocrine) therapy choices in breast cancer by selecting the right therapy (dose) for the right patient and easily show (early) benefit, or absence of benefit, and should be used more often in clinical research and practice.

REFERENCES

1. Loibl S, André F, Bachelot T, et al. Early breast cancer: ESMO Clinical Practice Guideline for diagnosis, treatment and follow-up☆. Annals of Oncology 2024; 35(2): 159-82.
2. Cardoso F, Kyriakides S, Ohno S, et al. Early breast cancer: ESMO Clinical Practice Guidelines for diagnosis, treatment and follow-up†. Annals of Oncology 2019; 30(8): 1194-220.
3. Burstein HJ, Lacchetti C, Anderson H, et al. Adjuvant Endocrine Therapy for Women With Hormone Receptor-Positive Breast Cancer: ASCO Clinical Practice Guideline Focused Update. Journal of Clinical Oncology 2019; 37(5): 423-38.
4. Land SR, Wickerham DL, Costantino JP, et al. Patient-Reported Symptoms and Quality of Life During Treatment With Tamoxifen or Raloxifene for Breast Cancer PreventionThe NSABP Study of Tamoxifen and Raloxifene (STAR) P-2 Trial. JAMA 2006; 295(23): 2742-51.
5. Boehm DU, Lebrecht A, Eckhardt T, et al. Quality of life and adjuvant tamoxifen treatment in breast cancer patients. European Journal of Cancer Care 2009; 18(5): 500-6.
6. Lailler G, Memoli V, Le Bihan Benjamin C, et al. Five-Year Adjuvant Endocrine Therapy Adherence Trajectories Among Women With Breast Cancer: A Nationwide French Study Using Administrative Data. Clinical Breast Cancer 2021; 21(4): e415-e26.
7. Murphy CC, Bartholomew LK, Carpentier MY, Bluethmann SM, Vernon SW. Adherence to adjuvant hormonal therapy among breast cancer survivors in clinical practice: a systematic review. Breast Cancer Research and Treatment 2012; 134(2): 459-78.
8. Bosco-Lévy P, Jové J, Robinson P, Moore N, Fourrier-Réglat A, Bezin J. Persistence to 5-year hormonal breast cancer therapy: a French national population-based study. British Journal of Cancer 2016; 115(8): 912-9.
9. Pistilli B, Paci A, Ferreira AR, et al. Serum Detection of Nonadherence to Adjuvant Tamoxifen and Breast Cancer Recurrence Risk. Journal of Clinical Oncology 2020; 38(24): 2762-72.
10. Chirgwin JH, Giobbie-Hurder A, Coates AS, et al. Treatment Adherence and Its Impact on Disease-Free Survival in the Breast International Group 1-98 Trial of Tamoxifen and Letrozole, Alone and in Sequence. Journal of Clinical Oncology 2016; 34(21): 2452-9.
11. Madlensky L, Natarajan L, Tchu S, et al. Tamoxifen Metabolite Concentrations, CYP2D6 Genotype, and Breast Cancer Outcomes. Clinical Pharmacology & Therapeutics 2011; 89(5): 718-25.
12. Saladores P, Mürdter T, Eccles D, et al. Tamoxifen metabolism predicts drug concentrations and outcome in premenopausal patients with early breast cancer. Pharmacogenomics Journal 2015; 15(1): 84-94.
13. Helland T, Henne N, Bifulco E, et al. Serum concentrations of active tamoxifen metabolites predict long-term survival in adjuvantly treated breast cancer patients. Breast Cancer Research 2017; 19(1): 125.
14. Sanchez-Spitman A, Dezentjé V, Swen J, et al. Tamoxifen Pharmacogenetics and Metabolism: Results From the Prospective CYPTAM Study. Journal of Clinical Oncology 2019; 37(8): 636-46.
15. Sanchez-Spitman AB, Moes DAR, Swen JJ, et al. Exposure-response analysis of endoxifen serum concentrations in early-breast cancer. Cancer Chemotherapy and Pharmacology 2020; 85(6): 1141-52.
16. Braal CL, Beijnen JH, Koolen SLW, et al. Relevance of Endoxifen Concentrations: Absence of Evidence Is Not Evidence of Absence. Journal of Clinical Oncology 2019; 37(22): 1980-1.
17. Goetz MP, Suman VJ, Nakamura Y, Kiyotani K, Jordan VC, Ingle JN. Tamoxifen Metabolism and Breast Cancer Recurrence: A Question Unanswered by CYPTAM. Journal of Clinical Oncology 2019; 37(22): 1982-3.
18. Brauch H, Schroth W, Mürdter T, Schwab M. Tamoxifen Pharmacogenetics and Metabolism: The Same Is Not the Same. Journal of Clinical Oncology 2019; 37(22): 1981-2.
19. de Vries Schultink AHM. Pharmacometrics for treatment optimazation and drug development in oncology. Amsterdam 2019.
20. de Vries Schultink AHM, Dorlo TPC, Madlensky L, Pierce JP, Beijnen JH, ADR H. Prospective evaluation of Therapeutic Drug Monitoring of endoxifen: feasibility of observational and randomized trials. PAGE 2019: Clinical Applications; 2019.
21. Tamura K, Imamura CK, Takano T, et al. CYP2D6 Genotype-Guided Tamoxifen Dosing in Hormone Receptor-Positive Metastatic Breast Cancer (TARGET-1): A Randomized, Open-Label, Phase II Study. Journal of Clinical Oncology 2020; 38(6): 558-66.

22. Neven P, Jongen L, Lintermans A, et al. Tamoxifen Metabolism and Efficacy in Breast Cancer: A Prospective Multicenter Trial. Clinical Cancer Research 2018; 24(10): 2312-8.
23. Korde LA, Somerfield MR, Carey LA, et al. Neoadjuvant Chemotherapy, Endocrine Therapy, and Targeted Therapy for Breast Cancer: ASCO Guideline. Journal of Clinical Oncology 2021; 39(13): 1485-505.
24. Kim H, Whitman AA, Wisniewska K, et al. Tamoxifen Response at Single Cell Resolution in Estrogen Receptor-Positive Primary Human Breast Tumors. bioRxiv 2023.
25. Ahmed NS, Samec M, Liskova A, Kubatka P, Saso L. Tamoxifen and oxidative stress: an overlooked connection. Discover Oncology 2021; 12(1): 17.
26. Royal Dutch Pharmacist Association. Tamoxifen. https://kennisbank.knmp.nl/article/Informatorium_Medicamentorum/S1636.html (accessed 20-06-2024 2024).
27. Dutch Pharmacogenetics Working Group. Annotation of DPWG Guideline for tamoxifen and CYP2D6. https://www.pharmgkb.org/guidelineAnnotation/PA166104966 (accessed 20-06-2024 2024).
28. De Placido S, De Laurentiis M, Carlomagno C, et al. Twenty-year Results of the Naples GUN Randomized Trial: Predictive Factors of Adjuvant Tamoxifen Efficacy in Early Breast Cancer1. Clinical Cancer Research 2003; 9(3): 1039-46.
29. Early Breast Cancer Trialists' Collaborative Group. Relevance of breast cancer hormone receptors and other factors to the efficacy of adjuvant tamoxifen: patient-level meta-analysis of randomised trials. Lancet 2011; 378(9793): 771-84.
30. Francis PA, Fleming GF, Láng I, et al. Adjuvant Endocrine Therapy in Premenopausal Breast Cancer: 12-Year Results From SOFT. Journal of Clinical Oncology 2022; 41(7): 1370-5.
31. Gjerde J, Gandini S, Guerrieri-Gonzaga A, et al. Tissue distribution of 4-hydroxy-N-desmethyltamoxifen and tamoxifen-N-oxide. Breast Cancer Research and Treatment 2012; 134(2): 693-700.
32. Krisnamurti DG, Louisa M, Anggraeni E, Wanandi SI. Drug Efflux Transporters Are Overexpressed in Short-Term Tamoxifen-Induced MCF7 Breast Cancer Cells. Advances in Pharmacological and Pharmaceutical Sciences 2016; 2016: 6702424.
33. Gao CM, Pu Z, He C, et al. Effect of OATP1B1 genetic polymorphism on the uptake of tamoxifen and its metabolite, endoxifen. Oncology Reports 2017; 38(2): 1124-32.
34. Nielsen TO, Leung SCY, Rimm DL, et al. Assessment of Ki67 in Breast Cancer: Updated Recommendations from the International Ki67 in Breast Cancer Working Group. Journal of the National Cancer Institute 2020; 113(7): 808-19.
35. Dowsett M, Smith IE, Ebbs SR, et al. Short-Term Changes in Ki-67 during Neoadjuvant Treatment of Primary Breast Cancer with Anastrozole or Tamoxifen Alone or Combined Correlate with Recurrence-Free Survival. Clinical Cancer Research 2005; 11(2): 951s.
36. Howell A, Cuzick J, Baum M, et al. Results of the ATAC (Arimidex, Tamoxifen, Alone or in Combination) trial after completion of 5 years' adjuvant treatment for breast cancer. Lancet 2005; 365(9453): 60-2.
37. Ruhstaller T, Giobbie-Hurder A, Colleoni M, et al. Adjuvant Letrozole and Tamoxifen Alone or Sequentially for Postmenopausal Women With Hormone Receptor-Positive Breast Cancer: Long-Term Follow-Up of the BIG 1-98 Trial. Journal of Clinical Oncology 2019; 37(2): 105-14.
38. Ellis MJ, Coop A, Singh B, et al. Letrozole inhibits tumor proliferation more effectively than tamoxifen independent of HER1/2 expression status. Cancer Research 2003; 63(19): 6523-31.
39. Smith I, Robertson J, Kilburn L, et al. Long-term outcome and prognostic value of Ki67 after perioperative endocrine therapy in postmenopausal women with hormone-sensitive early breast cancer (POETIC): an open-label, multicentre, parallel-group, randomised, phase 3 trial. The Lancet Oncology 2020; 21(11): 1443-54.
40. Ellis MJ, Coop A, Singh B, et al. Letrozole Inhibits Tumor Proliferation More Effectively than Tamoxifen Independent of HER1/2 Expression Status. Cancer Research 2003; 63(19): 6523.
41. Nitz U, Gluz O, Kreipe HH, et al. The run-in phase of the prospective WSG-ADAPT HR+/HER2- trial demonstrates the feasibility of a study design combining static and dynamic biomarker assessments for individualized therapy in early breast cancer. Therapeutic Advances in Medical Oncology 2020; 12: 1758835920973130.
42. Gluz O, Christgen M, Nitz U, et al. Abstract LBO1-05: Impact of age and ovarian function suppression (OFS) on endocrine response to short preoperative endocrine therapy (ET): Results from the multicenter ADAPTcycle trial (n=4,334). Cancer Research 2024; 84(9_Supplement): LBO1-05-LBO1-.
43. Finn RS, Martin M, Rugo HS, et al. Palbociclib and Letrozole in Advanced Breast Cancer. New England Journal of Medicine 2016; 375(20): 1925-36.

44. Cristofanilli M, Turner NC, Bondarenko I, et al. Fulvestrant plus palbociclib versus fulvestrant plus placebo for treatment of hormone-receptor-positive, HER2-negative metastatic breast cancer that progressed on previous endocrine therapy (PALOMA-3): final analysis of the multicentre, double-blind, phase 3 randomised controlled trial. The Lancet Oncology 2016; 17(4): 425-39.

45. Hortobagyi GN, Stemmer SM, Burris HA, et al. Updated results from MONALEESA-2, a phase III trial of first-line ribociclib plus letrozole versus placebo plus letrozole in hormone receptor-positive, HER2-negative advanced breast cancer. Annals of Oncology 2018; 29(7): 1541-7.

46. Johnston SRD, Toi M, O'Shaughnessy J, et al. Abemaciclib plus endocrine therapy for hormone receptor-positive, HER2-negative, node-positive, high-risk early breast cancer (monarchE): results from a preplanned interim analysis of a randomised, open-label, phase 3 trial. Lancet Oncology 2023; 24(1): 77-90.

47. Sledge GW, Jr., Toi M, Neven P, et al. MONARCH 2: Abemaciclib in Combination With Fulvestrant in Women With HR+/HER2- Advanced Breast Cancer Who Had Progressed While Receiving Endocrine Therapy. Journal of Clinical Oncology 2017; 35(25): 2875-84.

48. Slamon DJ, Neven P, Chia S, et al. Phase III Randomized Study of Ribociclib and Fulvestrant in Hormone Receptor-Positive, Human Epidermal Growth Factor Receptor 2-Negative Advanced Breast Cancer: MONALEESA-3. Journal of Clinical Oncology 2018; 36(24): 2465-72.

49. Kurzrock R, Lin C-C, Wu T-C, Hobbs BP, Pestana RC, Hong DS. Moving Beyond 3+3: The Future of Clinical Trial Design. American Society of Clinical Oncology Educational Book 2021; (41): e133-e44.

50. Wilkie J, Schickli MA, Berger MJ, et al. Progression-Free Survival for Real-World Use of Palbociclib in Hormone Receptor-Positive Metastatic Breast Cancer. Clinical Breast Cancer 2020; 20(1): 33-40.

51. Rugo HS, Huober J, García-Sáenz JA, et al. Management of Abemaciclib-Associated Adverse Events in Patients with Hormone Receptor-Positive, Human Epidermal Growth Factor Receptor 2-Negative Advanced Breast Cancer: Safety Analysis of MONARCH 2 and MONARCH 3. Oncologist 2021; 26(1): e53-e65.

52. Fernández-Cuerva C, Chinchilla-Alarcón T, Alcaraz-Sánchez JJ. Real-world effectiveness of ribociclib in metastatic breast cancer patients: Does dose affect survival? Journal of Oncology Pharmacy Practice 2023; 29(7): 1619-27.

53. Burris HA, Chan A, Bardia A, et al. Safety and impact of dose reductions on efficacy in the randomised MONALEESA-2, -3 and -7 trials in hormone receptor-positive, HER2-negative advanced breast cancer. British Journal of Cancer 2021; 125(5): 679-86.

54. Cardoso F, Jacot W, Küemmel S, et al. Abstract PD17-12: Primary efficacy and safety results from the AMALEE trial evaluating 600 mg vs 400 mg starting doses of first-line ribociclib in patients with HR+/HER2– advanced breast cancer. Cancer Research 2023; 83(5_Supplement): PD17-2-PD-2.

55. Slamon D, Lipatov O, Nowecki Z, et al. Ribociclib plus Endocrine Therapy in Early Breast Cancer. New England Journal of Medicine 2024; 390(12): 1080-91.

56. Gnant M, Dueck AC, Frantal S, et al. Adjuvant Palbociclib for Early Breast Cancer: The PALLAS Trial Results (ABCSG-42/AFT-05/BIG-14-03). Journal of Clinical Oncology 2022; 40(3): 282-93.

57. Loibl S, Marmé F, Martin M, et al. Palbociclib for Residual High-Risk Invasive HR-Positive and HER2-Negative Early Breast Cancer-The Penelope-B Trial. Journal of Clinical Oncology 2021; 39(14): 1518-30.

58. Freedman RA, Caswell-Jin JL, Hassett M, et al. Optimal Adjuvant Chemotherapy and Targeted Therapy for Early Breast Cancer—Cyclin-Dependent Kinase 4 and 6 Inhibitors: ASCO Guideline Rapid Recommendation Update. Journal of Clinical Oncology 2024; 42(18): 2233-5.

59. Freedman RA, Graff SL, Somerfield MR, Telli ML, Wolff AC, Giordano SH. Adjuvant Abemaciclib Plus Endocrine Therapy in the Treatment of High-Risk Early Breast Cancer: ASCO Guideline Rapid Recommendation Update Q and A. JCO Oncology Practice 2022; 18(7): 516-9.

60. U.S. Food and Drug Administration. FDA expands early breast cancer indication for abemaciclib with endocrine therapy. 03-03-2023 2023. https://www.fda.gov/drugs/resources-information-approved-drugs/fda-expands-early-breast-cancer-indication-abemaciclib-endocrine-therapy (accessed 20-06-2024 2024).

61. Agostinetto E, Arecco L, de Azambuja E. Adjuvant CDK4/6 Inhibitors for Early Breast Cancer: How to Choose Wisely? Oncology and Therapy 2024; 12(1): 19-29.

62. Nederlandse Vereniging voor Medische Oncologie. BOM Over de adviezen. 2023. https://www.nvmo.org/over-de-adviezen/ (accessed 20-06-2024.

63. Nederlandse Vereniging voor Medische Oncologie. NVMO-commissie BOM. Systematische beoordeling van oncologische middelen 14-03-2012. https://medischeoncologie.nl/artikelen/2012/maart/editie-1/systematischebeoordelingvanoncologischemiddelen (accessed 20-06-2024.

64. Nederlandse Vereniging voor Medische Oncologie. NVMO-commissie BOM. Resultaten monarchE studie beoordeeld: Abemaciclib toegevoegd aan endocriene therapie als adjuvante behandeling bij hoogrisico hormoonreceptorpositief mammacarcinoom. 11-07-2023. https://medischeoncologie.nl/artikelen/2023/augustus/2023-6/abemaciclib-toegevoegd-aan-endocriene-therapie-als-adjuvante-behandeling-bij-hoogrisico-hormoonreceptorpositief-mammacarcinoom (accessed 20-06-2024.

Appendices

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NEDERLANDSE SAMENVATTING

Borstkanker is de meest voorkomende vorm van kanker bij vrouwen en grofweg 1 op de 7 vrouwen zal in haar leven borstkanker krijgen. Behandelingen worden gelukkig steeds beter dankzij het uitvoeren van wetenschappelijk onderzoek. Daarmee is de kans om aan borstkanker te overlijden sinds 1989 bijna gehalveerd. Helaas overlijdt wereldwijd nog steeds 2.5% van alle vrouwen aan (complicaties van of door uitgezaaide) borstkanker. Deze indrukwekkende getallen benadrukken het belang van het nog beter kunnen begrijpen van deze ziekte en het nog verder verbeteren van de behandelmogelijkheden.

Borstkanker kan worden ingedeeld in verschillende subtypes. Op basis van de aanwezigheid van bepaalde receptoren, bindingsplekken waarop bepaalde hormonen of eiwitten kunnen aangrijpen, kan de volgende indeling gemaakt worden:

- hormoongevoelig: bij de aanwezigheid van oestrogeen en/of progesteron receptoren
- Her2neu-positief: bij de aanwezigheid van her2neu receptoren
- triple-negatief: bij afwezigheid van de receptoren: oestrogeen-, progesteron- en Her2-receptor

Een grote meerderheid van de patiënten met borstkanker (ongeveer 75%) heeft het hormoongevoelige subtype. De groei van deze vorm van borstkanker wordt meestal met name, via de oestrogeen receptoren, gestimuleerd door het hormoon oestrogeen. Om deze groei tegen te gaan bieden de oestrogeen receptoren een goede mogelijkheid om doelgerichte behandelingen te geven. In dit proefschrift ligt de focus op twee belangrijke (groepen) medicijnen in de behandeling van hormoongevoelige borstkanker: tamoxifen en CDK4/6 remmers. Er is met name onderzoek gedaan naar de relatie tussen de farmacokinetiek van beide geneesmiddelen met de bijwerkingen of effectiviteit van de therapie.

Farmacokinetiek van een geneesmiddel beschrijft wat het lichaam van de patiënt doet met het geneesmiddel. Farmacokinetiek beschrijft de mate van absorptie (opname) van een geneesmiddel, bijvoorbeeld in de maag als het geneesmiddel oraal wordt ingenomen, maar ook de distributie (verdeling) van een geneesmiddel door het lichaam. Ook het metabolisme van een geneesmiddel (de omzetting van het geneesmiddel in actieve of juist inactieve stoffen; meestal in de lever) en de uitscheiding van een geneesmiddel via bijvoorbeeld de urine of de ontlasting, behoren tot de farmacokinetiek. De mate van absorptie, distributie, metabolisatie en uitscheiding van een geneesmiddel bepalen, samen met de eigenschappen van de patiënt die het geneesmiddel inneemt, de concentratie van het geneesmiddel in het bloed.

Tamoxifen

Tamoxifen is een ‘selectieve oestrogeen receptor modulator’. Het geneesmiddel kan binden aan de oestrogeen receptor van een tumorcel en zorgt er met deze binding voor dat oestrogeen niet meer kan binden en daarmee de tumorcel niet verder gestimuleerd kan worden door oestrogenen tot celdeling (leidend tot de groei van de kanker) en zelf uiteindelijk leidt tot het ten gronde gaan van de kankercellen. Tamoxifen wordt al gebruikt sinds 1973 en is nog steeds een enorm belangrijk medicijn in de behandeling van hormoongevoelige borstkanker. Het wordt meestal toegepast als ‘adjuvante’ behandeling. Dit is een type behandeling die wordt gegeven na lokale en/of locoregionale behandeling zoals borstoperaties en radiotherapie, om de kans op terugkeer van de borstkanker zo klein mogelijk te maken. Tamoxifen, in de standaard dosering van 20 mg per dag voor een periode van 5 jaar na de operatie, vermindert de kans op terugkeer van de borstkanker gemiddeld met 40%.

Doordat oestrogeen receptoren ook op andere organen voorkomen, kan tamoxifen helaas ook tot bijwerkingen leiden. Bijwerkingen van tamoxifen zijn onder andere opvliegers, gewrichtspijn, slapeloosheid, vaginale afscheiding, gewichtstoename en stemmingswisselingen. Hoewel de bijwerkingen van tamoxifen meestal beschreven worden als mild, zeker ten opzichte van bijvoorbeeld de bijwerkingen die kunnen optreden ten tijde van chemotherapie, hebben patiënten die tamoxifen gebruiken jarenlang last van deze bijwerkingen en kunnen de bijwerkingen zeker ook negatieve effecten hebben op de dagelijkse bezigheden zoals werk, lichamelijke activiteit, seksualiteit en relaties. Dit kan daarmee ok een grote invloed hebben op de kwaliteit van leven en zorgt er voor dat bijna de helft van de patiënten binnen vijf jaar (door eigen keuze) stopt met deze behandeling, waarvan 1/3 van deze patiënten zelfs al in het eerste jaar. Uiteraard zorgt het niet innemen van tamoxifen ook voor een grotere kans op terugkeer van de borstkanker. Het verminderen van de bijwerkingen van tamoxifen is dan ook van groot belang.

Met betrekking tot de farmacokinetiek is het belangrijk om te weten dat tamoxifen een complex metabolisme heeft. Tamoxifen wordt in de lever omgezet in een aantal andere bestanddelen (‘metabolieten’), waarvan *endoxifen* de belangrijkste is. Deze omzetting wordt geregeld door (met name) het enzym CYP2D6. Endoxifen heeft de sterkste binding met de oestrogeen receptor en de hoogste concentratie van alle bestandsdelen in het bloed. Verschillende retrospectieve studies hebben een relatie gevonden tussen de concentratie van endoxifen in het bloed en de effectiviteit van tamoxifen. De drempelwaarde van 16 nM heeft de sterkste wetenschappelijke basis.

Op basis van deze bestaande drempelwaardes van endoxifen kunnen voor de individuele patiënt zo nodig aanpassingen in de doseringen gemaakt worden mocht de concentratie onder die drempelwaarde liggen. Dit wordt *therapeutic drug monitoring* (TDM) van tamoxifen genoemd. Er zijn meerdere onderzoeken waarin TDM van tamoxifen is toegepast en dit halveert het percentage patiënten met een te lage endoxifen concentratie in het bloed van ongeveer 20 naar 10%.

Deel I. Tamoxifen en bijwerkingen

In dit proefschrift zijn we op zoek gegaan naar eventuele oplossingen voor bijwerkingen van tamoxifen, waarbij we uiteraard zoveel mogelijk de effectiviteit van tamoxifen willen behouden. Daarnaast hebben we gekeken naar zeldzamere lange termijn bijwerkingen van tamoxifen, waaronder veneuze trombose (bloedstolsels in de kuiten of stolsels die doorgeschoten zijn naar de longen) en cognitieve stoornissen en de eventuele relatie van deze bijwerkingen met tamoxifen en endoxifen concentraties in het bloed.

Een mogelijke oplossing voor het verminderen van de bijwerkingen van tamoxifen zou een dosisverlaging kunnen zijn. In **hoofdstuk 2** hebben we literatuuronderzoek gedaan om te achterhalen wat er al bekend is over de bijwerkingen en effectiviteit van lagere doseringen tamoxifen. Er zijn meerdere studies gedaan met lagere doseringen tamoxifen in de primaire (bij patiënten met een verhoogd risico op borstkanker) en secundaire (bij patiënten met een voorstadium van borstkanker) preventieve setting. In deze studies bleken de bijwerkingen van lage dosering tamoxifen minder te zijn ten opzichte van de bijwerkingen met de standaard dosering van 20 mg tamoxifen per dag en ongeveer gelijk aan de bijwerkingen die patiënten ervaren met een placebo. Tamoxifen in een lage dosering zorgt in deze populaties ook voor een lagere kans op borstkanker of een voorstadium van borstkanker ten opzichte van placebo. Er werd met betrekking tot de effectiviteit geen vergelijking gemaakt met 20 mg tamoxifen per dag. Ook zijn er helaas geen studies gedaan naar de effectiviteit van lagere doseringen tamoxifen in de adjuvante setting. Om meer inzicht te krijgen, hebben we ook gekeken naar studies waar afgeleide maten die mogelijk de klinische effectiviteit van tamoxifen weergeven werden onderzocht. Een van deze afgeleiden is de mammografische dichtheid. We weten al dat in patiënten waarbij de mammografische dichtheid vermindert na eenmaal daags 20 mg tamoxifen, de kans op borstkanker ook lager is. Lagere doseringen tamoxifen bleken mammografische dichtheid ook te kunnen verminderen, mogelijk in een zelfde mate als dagelijks 20 mg tamoxifen. Een andere afgeleide is de proliferatiemarker Ki67, te meten in delende borstkanker cellen. Een verlaging van Ki67 na kortdurende tamoxifen behandeling (voorafgaand aan de operatie) is voorspellend voor de lange termijn effectiviteit van tamoxifen na de operatie. Ook lage doseringen tamoxifen bleken Ki67 te kunnen verlagen. Dus, lagere

doseringen tamoxifen hebben een klinisch relevant, verbeterd bijwerkingenprofiel vergeleken met tamoxifen in standaarddosering en er zijn sterke, hoewel indirecte, aanwijzingen dat lagere doseringen tamoxifen eveneens een anti-tumoreffect hebben.

Omdat er vrijwel geen studies in de adjuvante setting zijn naar de bijwerkingen van lagere doseringen tamoxifen, hebben wij in **hoofdstuk 3** de tamoxifen dosering verlaagd van 20 mg naar 10 mg per dag bij patiënten die veel last ervaarden van hun bijwerkingen en endoxifen concentraties in het bloed hadden van ten minste 32 nM (twee keer de drempelwaarde van 16 nM). Van de 17 patiënten die een dosisverlaging hebben ondergaan, had na 3 maanden 41% van de patiënten een klinisch relevante verbetering in bijwerkingen en 65% van de patiënten in kwaliteit van leven. Deze verbetering werd niet gezien in een groep van 60 patiënten die de tamoxifen behandeling in een dosering van 20 mg per dag continueerden en leek dus niet te verklaren door een tijdseffect. Bij 21% van de patiënten daalde de endoxifen concentratie licht onder de 16 nM (12.8, 15.5, 15.8, 15.9 nM), wat het belang benadrukt van het opnieuw bepalen van endoxifen concentraties na het aanpassen van de tamoxifen dosering. Deze studie toont aan dat dosisverlaging een effectieve strategie kan zijn voor patiënten die sterk lijden onder of zouden stoppen met anti-hormonale therapie vanwege tamoxifen-gerelateerde bijwerkingen. Er is in deze studie helaas geen vergelijking gemaakt met een placebo, waardoor een mogelijk placebo-effect van het verlagen van de tamoxifendosering niet volledig kan worden uitgesloten.

In **hoofdstuk 4** hebben we de combinatie van tamoxifen en CBD-olie onderzocht. CBD is een van de twee belangrijkste bestandsdelen van cannabis maar, in tegenstelling tot THC, niet psychoactief of potentieel verslavend. CBD wordt in toenemende mate gebruikt door patiënten met borstkanker in de hoop bijwerkingen te verminderen. Hoewel CBD verschillende receptoren in het menselijk lichaam kan beïnvloeden en eerder in verband is gebracht met een vermindering van slapeloosheid en pijn, is het onbekend of CBD de bijwerkingen van tamoxifen zou kunnen verbeteren. Daarnaast zou CBD de concentraties van endoxifen in het bloed kunnen verlagen, doordat CBD een potentiële remmer is van CYP2D6 dat tamoxifen in endoxifen omzet. In ons onderzoek gebruikten 15 patiënten voor 4 weken lang CBD-olie in een dosering van driemaal daags tien druppels onder de tong (~50 mg) naast hun tamoxifen behandeling. Hoewel de plasma concentraties van endoxifen daalden bij gelijktijdig gebruik van CBD, bleven ze binnen bio-equivalentie grenzen. Dit betekent dat ondanks enige afname de concentraties van endoxifen met of zonder CBD nog steeds als vergelijkbaar kunnen worden gezien. Als secundair doel hebben we onderzocht of CBD daadwerkelijk tamoxifen-gerelateerde bijwerkingen kan verlichten. Het gebruik van CBD in de maximaal vrij verkrijgbare dosis is onderzocht in 26 patiënten en

verminderde bijwerkingen en verbeterde de kwaliteit van leven. Of CBD echt gunstig is en aanbevolen kan worden aan patiënten met hinderlijke bijwerkingen moet nog worden bewezen in een placebo-gecontroleerde studie. Momenteel hoeven patiënten in ieder geval niet meer ontmoedigd te worden als ze CBD willen proberen naast hun tamoxifen behandeling.

Het is niet volledig duidelijk hoe tamoxifen het risico op veneuze trombose verhoogt, maar het is bekend dat tamoxifen bloed-verdunnende eiwitten zoals antitrombine, proteine C en tissue factor pathway remmer kan verlagen en vorming van stollingseiwitten juist kan stimuleren. In **hoofdstuk 5** hebben we de concentratie van antitrombine, proteine C, tissue factor en trombine vorming parameters gemeten in patiënten die behandeld werden met tamoxifen en de mogelijke relatie van deze tamoxifen-geassocieerde stollingseiwitten met de tamoxifen dosis en concentraties van tamoxifen en endoxifen in het bloed onderzocht. Bij zowel 3 als 6 maanden tamoxifen behandeling bleken hogere concentraties van tamoxifen en endoxifen niet geassocieerd met hogere concentraties van het bloedstollende tissue factor of parameters voor trombine vorming noch met lagere concentraties van de bloed verdunnende eiwitten antitrombine en proteine C. Ook leek verhoging of verlaging van de tamoxifen dosis geen invloed te hebben op de stollingseiwit concentraties, hoewel deze vergelijkingen werden beperkt door het feit dat de meerderheid van de patiënten in deze studie de standaarddosis van 20 mg per dag gebruikte. Deze studie biedt geruststelling en geeft een eerste indicatie dat hogere tamoxifen- of endoxifen concentraties geen extra bloedstollend effect hebben en dus mogelijk niet leiden tot een verder verhoogd risico op tamoxifen-gerelateerde trombose. Een grotere studie met meer patiënten die tamoxifen dosisaanpassingen ondergaan, zou een duidelijker antwoord kunnen geven over het risico op trombose bij TDM-gestuurde tamoxifen dosisverhoging.

Recente studies hebben het gebruik van tamoxifen ook in verband gebracht met een afname in cognitief functioneren. Ook in de hersenen zitten oestrogeen-receptoren en oestrogenen kunnen in de hersenen dan ook verschillende cognitie verbeterende effecten hebben. Tamoxifen kan via binding aan de oestrogeen-receptoren in de hersenen deze effecten beïnvloeden. Over deze potentiële bijwerking is echter nog maar weinig bekend. In **hoofdstuk 6** hebben we door middel van afname van de *Amsterdam Cognition Scan* score (een gevalideerde online neuropsychologische test) gekeken naar het effect van twee jaar tamoxifen gebruik op het objectief en subjectief cognitief functioneren van in totaal 135 vrouwen met hormoongevoelige borstkanker. Tamoxifen gebruikers presteerden slechter op verbale leerprocessen, verwerkingssnelheid, uitvoerende functies en motorische vaardigheden in vergelijking met gezonde vrouwelijke controles. Patiënten die tamoxifen gebruiken rapporteerden

zelf milde cognitieve klachten. Het cognitief functioneren van vrouwen ouder dan 57 jaar was meer aangedaan door tamoxifen dan het cognitief functioneren van vrouwen onder de 57 jaar. Een opvallende bevinding was dat hogere tamoxifen en endoxifen concentraties en een hogere tamoxifendosering geassocieerd waren met slechtere prestaties op verschillende cognitieve domeinen. Verder onderzoek is nodig om de effecten van tamoxifen dosisverhoging, dosisverlaging of het stopzetten van de therapie op cognitief functioneren te onderzoeken.

Deel II. Predictie van tamoxifen en endoxifen blootstelling

In **hoofdstuk 7** hebben we een predictie-model voor de farmacokinetiek van tamoxifen ontwikkeld. Voor een dergelijk model wordt er onderzocht welke variabelen de concentraties van tamoxifen en endoxifen kunnen voorspellen. Met behulp van 3661 gemeten concentraties van meer dan 500 patiënten hebben we leeftijd, lengte, body-mass index (BMI) en CYP2D6 genotype geïncludeerd in het predictie-model. Leeftijd en lengte hebben invloed op de eliminatie van tamoxifen waarbij een hogere leeftijd leidt tot minder eliminatie van tamoxifen (en hogere concentraties) en een grotere lengte juist leidt tot meer eliminatie van tamoxifen (en lagere concentraties). BMI en CYP2D6 genotype hebben invloed op de omzetting van tamoxifen in endoxifen. Een hoger BMI zorgt voor verminderde omzetting en dus lagere concentraties van tamoxifen. Het CYP2D6 genotype is verreweg de belangrijkste factor in het predictiemodel. Het genotype staat voor de genen die je van je beide ouders hebt gekregen en die in dit geval samen de activiteit van het CYP2D6 eiwit bepalen. Deze activiteit kan ook ingedeeld worden in groepen, maar dit is minder nauwkeurig dan kijken naar activiteit op een continue schaal. Na ontwikkeling is het model succesvol gevalideerd in een extern validatie-cohort.

Met behulp van het model uit hoofdstuk 7 kunnen dosering drempels worden bepaald om de tamoxifen startdosering te voorspellen waarmee een patiënt een endoxifen concentratie >16 nM kan bereiken. Het toepassen van deze dosering drempels hebben we prospectief gevalideerd in **hoofdstuk 8**. Het doel van dit hoofdstuk was dat een hoger percentage patiënten een endoxifen concentratie >16 nM zou bereiken door al bij start van de behandeling de juiste dosering voor te schrijven. In 106 patiënten werd door middel van het model een startdosering van tamoxifen 20 mg (65%), 30 mg (16%) of 40 mg (19%) per dag voorspeld. Hiermee bereikte 84% van de patiënten een endoxifen concentratie >16 nM, wat niet significant hoger was dan een historische controlegroep waarin alle patiënten met 20 mg tamoxifen daags werden behandeld (77.9%). Het model voorspelde echter adequaat de toekomstige endoxifen concentraties en identificeerde correct patiënten die 40 mg tamoxifen per dag nodig hadden (significant minder patiënten met endoxifen concentratie <16

nM in de 40 mg-groep). Een verklaring voor het lager dan verwachte percentage patiënten met endoxifen concentratie >16 nM is het grotere aandeel van patiënten met verminderde CYP2D6-activiteit in de predictie-groep versus de controlegroep. Deze patiënten kunnen zelfs met de hoogste geregistreerde tamoxifen dagdosering van 40 mg geen endoxifen concentratie van 16 nM bereiken en kunnen daarom waarschijnlijk beter behandeld worden met een andere anti-hormonale therapie. Samenvattend lijkt doseren volgens het predictiemodel veelbelovend vergeleken met de conventionele 'one-size-fits-all' dosering van 20 mg tamoxifen per dag -- vooral in bepaalde subgroepen -- maar TDM blijft een belangrijke aanvulling.

Deel III. CDK4/6 remmers

CDK4/6 remmers zijn relatief nieuwe geneesmiddelen in de behandeling van uitgezaaide borstkanker. In dit geval is de borstkanker verspreid naar andere organen, zoals de botten, lever of lymfeklieren, en kan de borstkanker helaas niet meer genezend behandeld worden. Gelukkig kan de borstkanker met behulp van verschillende behandelingen nog wel afgeremd worden. Voor hormoongevoelige borstkanker zijn CDK4/6 remmers (waaronder palbociclib, ribociclib en abemaciclib) één van deze behandelingen. CDK4/6 remmers worden gecombineerd met anti-hormonale therapie en remmen (oestrogeen afhankelijke) celdeling en zorgen op deze manier voor een afname van de kanker celgroei.

Ook CDK4/6 remmers kunnen bijwerkingen veroorzaken, met neutropenie (een te laag aantal witte bloedcellen) als meest voorkomende bijwerking. Een minder bekende, maar ook vaak voorkomende, bijwerking is een hogere creatinine concentratie in het bloed. Een stijging van creatinine duidt meestal op verslechterde nierfunctie, maar kan ook komen doordat verschillende geneesmiddelen de uitscheiding van creatinine kunnen remmen. In dit geval is het creatinine wel verhoogd maar is de nierfunctie nog intact, en worden artsen misleid door de hoge creatinine waarden. Er zijn een paar zeer kleine studies die aantonen dat ook CDK4/6 remmers de uitscheiding van creatinine kunnen remmen, maar het is niet bekend hoe vaak een creatinine stijging wordt veroorzaakt door dit fenomeen en hoe vaak er echt een nierfunctiestoornis speelt bij patiënten die CDK4/6 remmers gebruiken. In **hoofdstuk 9** hebben we een retrospectief onderzoek gedaan bij alle patiënten die in het Erasmus MC behandeld werden met CDK4/6 remmers. Bij de patiënten waar een creatinine stijging optrad gedurende de behandeling hebben we cystatine C in het bloed gemeten. Dit is een eiwit dat net als creatinine gefilterd wordt in de nier en waarbij een stijging een verslechtering van de nierfunctie aanduidt. In tegenstelling tot creatinine, kan de uitscheiding van cystatine C niet beïnvloed worden door CDK4/6 remmers. Bij 41 van de 234 patiënten (17.5%) bleek sprake van een significante stijging van de creatinine concentratie. Bij 22 van

deze 41 patiënten konden cystatine C concentraties gemeten worden. In 16 van de 22 patiënten waar de creatinine concentraties op nierfunctiestoornissen duidden, waren de cystatine C concentraties normaal (73%). In 36% van de patiënten waren naar aanleiding van de gestegen creatinine concentraties aanvullende diagnostische onderzoeken of medicatie aanpassingen gedaan, die mogelijk achteraf dus niet nodig waren geweest. Naar aanleiding van ons onderzoek adviseren we om bij patiënten die behandeld worden met CDK4/6 remmers en creatinine concentraties hebben die duiden op een nierfunctie stoornis, eerst cystatine C te meten zodat onnodige diagnostiek, medicatie aanpassingen of bezorgdheid kunnen worden voorkomen.

In **hoofdstuk 10** hebben we naar de relatie gekeken tussen palbociclib concentraties en effectiviteit en toxiciteit van de behandeling. Wij konden geen relatie aantonen tussen progressie vrije overleving en palbociclib concentraties. Er was ook geen significante trend voor meer ernstige bijwerkingen of ernstige neutropenie bij hogere palbociclib concentraties. Er was wel een significante trend naar meer dosisverlagingen bij patiënten met hogere palbociclib concentraties. Hoewel dit tegenstrijdig lijkt, zou het kunnen dat mildere bijwerkingen, die helaas niet werden bijgehouden in deze studie, hebben geleid tot de noodzaak tot dosisreductie. Mogelijk bereiken sommige patiënten onnodig hoge palbociclib concentraties met de huidige standaard dosis van 125 mg per dag. De afwezigheid van een relatie tussen effectiviteit en concentraties suggereert dat het mogelijk is om de dosis veilig te verlagen in het geval van palbociclib-gerelateerde bijwerkingen.

LIST OF PUBLICATIONS

Buijs SM, Hendriksz TR, van Bommel EFH. Retroperitoneal Fibrosis After Spinal Surgery: A Report of 2 Cases. *JBJS Case Connect.* October 2021;27;11(4).

Braal CL, Westenberg JD, **Buijs SM**, Abrams S, Mulder TAM, van Schaik RHN, Koolen SLW, Jager A, Mathijssen RHJ. Factors affecting inter-individual variability in endoxifen concentrations in patients with breast cancer: results from the prospective TOTAM trial. *Breast Cancer Research and Treatment.* August 2022;195(1):65-74.

Buijs SM, Oomen – de Hoop E, Braal CL, van Rosmalen MM, Drooger JC, van Rossum – Schornagel QC, Vastbinder MB, Koolen SLW, Jager A, Mathijssen RHJ. The impact of endoxifen-guided tamoxifen dose reductions on endocrine side-effects in patients with primary breast cancer. *ESMO Open*. February 2023;8(1):100786.

Agema BC, **Buijs SM**, Sassen Sebastian DT, Mürdter TE, Schwab M, Koch BCP, Jager A, van Schaik, RHN, Mathijssen, RHJ, Koolen SLW. Toward model-informed precision dosing for tamoxifen: A population-pharmacokinetic model with a continuous CYP2D6 activity scale*. Biomedicine and Pharmacotherapy*. April 2023;160:114369.

Luyendijk M, Jager A, **Buijs SM**, Siesling S, Uyl – de Groot CA Blommestein HM. Cost-Effectiveness Analysis of MammaPrint® to Guide the Use of Endocrine Therapy in Patients with Early-Stage Breast Cancer. *Pharmacoeconomics.* May 2023;41(8):981-997.

Buijs SM, Braal CL, Buck SAJ, van Maanen NF, van der Meijden – Erkelens LM, Kuijper – Tissot van Patot HA, Oomen – de Hoop E, Saes L, van den Boogerd SJ, Struik LEM, van Rossum – Schornagel QC, Mathijssen RHJ, Koolen SLW, Jager A. CBD-oil as a potential solution in case of severe tamoxifen-related side effects. npj *Breast Cancer*. August 2023;9(1):63.

van Eerden RAG, de Boer NL, van Kooten JP, Bakkers C, Dietz MV, Creemers GM, **Buijs SM**, Bax R, de Man FM, Lurvink RJ, Diepeveen M, Brandt – Kerkhof ARM, van Meerten E, Koolen SLW, de Hingh IHJT, Verhoef C, Mathijssen RHJ, Burger JWA. Phase I study of intraperitoneal irinotecan combined with palliative systemic chemotherapy in patients with colorectal peritoneal metastases. *British Journal of Surgery*. October 2023;110(11):1502-1510.

Buijs SM*, van Dorst DCH*, Kruip MJHA, van de Akker RFP, Cheung KL, Porrazzo R, Oomen – de Hoop E, Jager A, Koolen SLW, Versmissen J, Danser JAH, Versteeg HH, Bos MHA, Mathijssen RHJ. The interplay between tamoxifen and endoxifen plasma concentrations and coagulation parameters in patients with primary breast cancer. *Biomedicine and Pharmacotherapy*. December 2023;170:115969.

Buijs SM, Koolen SLW, Mathijssen RHJ, Jager A. Tamoxifen dose de-escalation: an effective strategy for reducing adverse effects? *Drugs*. March 2024.

Buijs SM*, Luijendijk MJ*, Jager A, Koolen SLW, van der Wall E, Schagen SB, Mathijssen RHJ. Effects of tamoxifen on cognitive function in patients with primary breast cancer. *British Journal of Cancer*. November 2024;84(4):385-401.

van Nijnatten RYM, **Buijs SM***, Agema BC*, Fischer RMJ, Ghobadi Moghaddam-Helmantel I, Contant CME, de Jongh FE, Huijben AMT, van der Padt-Pruijsten A, Zuetenhorst HJM, van Schaik RHN, Koch BCP, Jager A, Koolen SLW, Mathijssen RHJ. Implementation of model-informed precision dosing for tamoxifen therapy in patients with breast cancer: a prospective intervention study. *The Breast*. January 2025;79:103880.

Buijs SM*, Jongbloed EM*, van Bergen LEM, Ramakers CRB, Koolen SLW, Mathijssen RHJ, Betjes MGH, Jager A. Pseudo acute kidney injury in patients receiving CDK4/6 inhibitors. *British Journal of Cancer*. In press.

Buijs SM, Mohmaed Ali MI, Oomen - de Hoop E, Braal CL, Wortelboer N, van Ommen-Nijhof A, Sonke GS, Konings IR, Jager A, Steeghs N, Siebinga H, Mathijssen RHJ, Huitema ADR, Koolen SLW. Palbociclib exposure in relation to efficacy and toxicity in patients with advanced breast cancer. *ESMO Open*. In press

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PhD PORTFOLIO

Summary of PhD training and teaching

Name PhD Candidate: Sanne Buijs

Erasmus University MC: Department of Medical Oncology

PhD period: 2020-2024

Promotor: Prof. dr. A.H.J. Mathijssen and prof. dr. A. Jager

Copromotor: Dr. S.L.W. Koolen

	Year	Workload (ECTS)
1. PhD training		
General courses		
BROK course	2021	1.5
Research Integrity	2022	0.3
Biostatistical Methods NIHES[1]	2021	4.5
Specific courses		
Excel Basic & Advanced course	2021	0.7
Basic course on 'R'	2021	1.8
Basic Introduction course on SPSS	2022	1.0
Survival Analysis course	2022	0.6
Biomedical English Writing and Communication	2022	2.0
Castor Study Building and Data Management	2021	0.1
Guidance Skills course	2021	0.1
PhD Intervision meetings	2022-2023	1.0
(Inter)national conferences		
Annual European Society Medical Oncology (ESMO) Congress, Paris, Madrid	2022, 2023	4.0
European Breast Cancer Conference (EBCC), Barcelona	2022	1.0
San Antonio Breast Cancer Symposium (SABCS), San Antonio	2022	2.0
Borstkanker Behandeling Beter Symposium	2021, 2022	1.0
Translational Pharmacology meetings	2020-2024	1.0
Dutch Society for Clinical Pharmacology & Biopharmacy (NVKFB) Scientific Meeting	2023	0.3
ACE-ABC (Academic Center for Breast Cancer) Annual Research days	2021-2024	1.0
Dutch Breast Cancer Research Group (BOOG) Plenary Meetings	2021-2023	1.0
NABON-BOOG Symposium	2022	0.2
Oncology Education meetings	2020-2024	1.0
Cancer Retraite Erasmus MC Cancer Institute	2023, 2024	1.0

	Year	Workload (ECTS)
Presentations		
Poster presentations ESMO Congress	2022, 2023	0.6
Poster presentation EBCC	2022	0.3
Poster presentation SABCS	2022	0.3
Poster presentation Medical Oncology Research day	2021	0.3
Poster presentation NVKFB Scientific Meeting	2023	0.3
Poster presentation Cancer Retraite	2023	0.3
Borstkanker Behandeling Beter Symposium	2021, 2022	0.8
BOOG Plenary meeting	2021	0.4
ACE-ABC Research Meeting	2020	0.4
BOOG-SABCS meeting	2022	0.4
Medical Oncology Research Meeting	2023	0.4
Clinical Pharmacology Meeting	2024	0.4
2. Teaching		
Medical school training		
Tutorial class first-year medical student	2021	1.0
Medical school bachelor phase coaching program	2022-2024	1.0
Supervising Master thesis		
Noud van Maanen	2021	1.0
Ruben van Nijnatten	2022-2023	1.5
Famke Weeterings	2023	1.0
3. Other		
Organizational activities		
BOOG Young Investigator Day	2023	1.0
Medical Oncology Scientific Day, Erasmus MC Cancer Institute	2021, 2022	1.0
Breast Cancer Journal Club	2022-2023	1.0
Total	**40.5 ECTS**	

CURRICULUM VITAE

Sanne Buijs werd op 27 augustus 1993 geboren in Capelle aan den IJssel. Als oudste dochter van Annemarie Rozendaal en Ronald Buijs groeide zij op samen met haar broer Peter en zus Madelief. Na het behalen van haar VWO diploma begon zij in 2011 aan de studie Psychologie aan de Universiteit Leiden. Na een jaar besloot zij echter haar passie te volgen en werd via de decentrale selectie toegelaten tot de studie Geneeskunde aan het Erasmus MC in Rotterdam. Tijdens haar studie haalde Sanne veel voldoening uit organisatorische nevenactiviteiten, wat haar ertoe bracht om na haar bachelor een jaar lang fulltime het bestuur van de Medische Faculteitsvereniging Rotterdam (MFVR) te versterken.

Gedurende haar verpleegstage in de Daniel den Hoed werd Sanne gegrepen door de oncologische zorg en deze interesse werd tijdens de minor Oncologie en de coschappen verder bevestigd. Zij besloot haar masteronderzoek te verrichten op de afdeling Interne Oncologie bij de Personalised Medicine groep van professor Mathijssen. Tijdens deze stage werd haar enthousiasme voor onderzoek doen aangewakkerd en na een bijzondere en hele leuke tijd als ANIOS Interne Geneeskunde in het Albert Schweitzer ziekenhuis in Dordrecht, keerde zij eind 2020 terug voor een promotietraject. Gedurende 3,5 jaar heeft Sanne zich verdiept in de behandeling van hormoongevoelige borstkanker onder begeleiding van professsor Agnes Jager, dr. Stijn Koolen en professor Ron Mathijssen. Vervolgens heeft zij met veel plezier op de afdeling Interne Oncologie in het Erasmus MC gewerkt. Op 1 januari 2025 is zij gestart met de opleiding tot internist in het Amphia Ziekenhuis in Breda.

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In ruim 4 jaar onderzoek werk je met heel veel verschillende collega's samen en komen er veel belangrijke mensen op je pad. Graag wil ik hier de gelegenheid nemen om mijn dankbaarheid uit te spreken.

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Dr. Koolen, beste Stijn, het was heel erg fijn om jou als copromotor bij mijn team te hebben. Ik heb veel gehad aan onze inhoudelijke discussies en kon altijd weer verder met een project waarmee ik was vastgelopen als ik bij jou was geweest. Jij bleef rustig (juist als ik soms heel druk was), gaf een weloverwogen advies en zorgde ervoor dat ik weer met vertrouwen door kon. Gelukkig hebben we ook veel lol gehad, bijvoorbeeld tijdens het onbeperkt chocolademousse eten in Parijs en op de SS-Rotterdam tijdens de retraites.

Graag wil ik professor Sabine Linn, professor Hugo van der Kuy, professor Henk-Jan Guchelaar, professor Birgit Koch, professor John Martens en dr. Linetta Koppert bedanken voor de interesse in mijn proefschrift en de bereidheid om plaats te nemen in de oppositie.

Linetta, jij hebt tijdens de oncologie minor mijn interesse voor de oncologie verder aangewakkerd en zowel tijdens mijn coschap chirurgie als gedurende de momenten dat wij elkaar tegenkwamen tijdens mijn promotie mij altijd enorm gemotiveerd met jouw onuitputtelijke enthousiasme. Bedankt daarvoor en ik vind het een grote eer dat je nu in mijn commissie plaatsneemt.

Ik wil alle co-auteurs en betrokkenen bij de verschillende manuscripten bedanken voor de prettige samenwerking. Ik heb van ieders eigen expertise enorm veel mogen leren.

Tijdens mijn promotie heb ik in een heel fijn team gewerkt en ik wil alle collega's van het Farmacolab enorm bedanken voor de samenwerking, altijd gezellige lunches, leuke labuitjes, padellen, barbecues, vrijmibo's, hulp met SPSS/R/research-manager/pipetteren en noem maar op. Ik ging echt (bijna) elke dag met een grote lach naar werk.

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Louwrens & Ruben, jullie fijne begeleiding tijdens mijn masteronderzoek heeft er mede toe geleid dat ik zo enthousiast over onderzoek ben geworden. Bedankt voor het vertrouwen dat jullie in mij hadden en Louwrens, ik vond het een eer om jouw onderzoekslijn door te mogen zetten.

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Niels G, wat was jij een aanwinst voor de 19e verdieping! Ondanks dat je onze jongste collega was, met toch (toen nog) de minste ervaring, was jij op de één of andere manier binnen no-time dé collega waarbij je voor zo ongeveer elke vraag terecht kon. En daarnaast

bracht je ook enorm veel gezelligheid. Gelukkig kan ik je straks in het Amphia nog heel vaak tegenkomen als co-collega!

Steef, wat bijzonder om naast vrienden ook collega's te zijn. Gelukkig was je de samenwerking nog niet zat en mag ik dit jaar zelfs helpen met het organiseren van jullie prachtige bruiloft.

Daan, na onze samenwerking in het Albert Schweitzer ziekenhuis kon het natuurlijk niet anders dan dat we op zoek gingen naar een gezamenlijk project om tijdens onze promoties te doen. We zijn er een aantal keer voor naar Leiden gereisd en hebben heel veel tijd gezamenlijk achter SPSS gezeten, maar ik vind het erg leuk dat we samen het artikel over tamoxifen en stolling hebben kunnen schrijven. Extra leuk dat we nu ook collega-aios zijn.

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Lieve Mir, van vriendinnen naar collega's naar vriendinnen, ik vond het zo fijn om al die jaren met jou samen te werken en elkaar zo vaak te spreken. Jij hebt altijd alles tot in de puntjes georganiseerd en ik (iedereen?) gebruik nog steeds jouw formats als voorbeeld.

Lieve Leni, het was altijd gezellig als jij er op donderdag was en je kan met jou dan ook over alles praten. Ik heb grote bewondering voor hoe jij jouw proefschrift hebt geschreven en jouw ontwikkeling als onderzoeker. Ook toen ik in de kliniek stond, kon ik af en toe even bij jou op de 5e komen buurten en mijn ei kwijt. Bedankt daarvoor!

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Lieve Lisa, wij hebben samen het meest intensief samen gewerkt en ik heb daar echt hele mooie herinneringen aan. Toen we gingen fietsen voor start van ons congres in Barcelona nam jij mij op sleeptouw en vervolgens mocht ik hetzelfde doen bij jou tijdens ons hardlooprondje. Zo ging het eigenlijk ook tijdens onze samenwerkingen, we vulden elkaar perfect aan. Zo heb ik van jou geleerd om nooit meer handmatig iets op te zoeken in Excel.. En was met jou samen zelfs uren in de stikstofvriezer samples uitzoeken leuk om te doen. Het is voor mij echt de kers op de taart dat ons onderzoek dat we samen opgezet hebben nu gepubliceerd is. Ik hoop nog heel veel vaker urenlang met elkaar over alles te kunnen praten, want ik heb er een vriendin bij.

Professor Martens, beste John, dit zie ik ook als de plek om jou te bedanken, want ook jij hebt mij zo af en toe in jouw groep opgenomen. Bedankt voor de gezelligheid tijdens San Antonio, de inhoudelijke discussies en het heerlijke etentje. Wat een bijzondere afsluiter dat jij ook in mijn commissie plaats neemt.

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