From the Department of Clinical Science and Education at Södersjukhuset,
Karolinska Institutet, Stockholm, Sweden

# CYP2D6-POLYMORPHISM AND EFFECT OF ADJUVANT TAMOXIFEN IN BREAST CANCER PATIENTS

Linda Thorén


Karolinska Institutet

Stockholm 2023

Published by Karolinska Institutet.
Printed by Universitetsservice US-AB, 2023

ISBN 978-91-8017-053-6

# CYP2D6-polymorphism and effect of adjuvant tamoxifen in breast cancer patients
Thesis for Doctoral Degree (Ph.D.)

By

**Linda Thorén**

The thesis will be defended in public at Aulan, elevator C, floor 6 at Södersjukhuset, Stockholm, on the 29th of September 2023.

**Principal Supervisor:**
Associate Professor Sara Margolin
Karolinska Institutet
Department of Clinical Science and Education, Södersjukhuset

**Co-supervisors:**
Professor Erik Eliasson
Karolinska Institutet
Department of Laboratory Medicine

Associate Professor Jonatan D Lindh
Karolinska Institutet
Department of Laboratory Medicine

PhD Mikael Eriksson
Karolinska Institutet
Department of Medical Epidemiology and Biostatistics

**Opponent:**
Professor Lisa Rydén
Lund University
Department of Surgery
Institution of Clinical Sciences

**Examination Board:**
Associate Professor
Annelie Liljegren
Karolinska Institutet
Department of Oncology-Pathology

Professor Janne T. Backman
University of Helsinki
Department of Clinical Pharmacology

Professor Olle Stål
University of Linköping
Department of Biomedical and Clinical Sciences
Division of Surgery, Orthopedics and Oncology

Till min familj

# Popular science summary of the thesis

Tamoxifen is a treatment that blocks estrogen from attaching to hormone receptors in cancer cells, preventing them from growing. Tamoxifen has been used for several decades to reduce the risk of recurrence in patients operated due to hormone sensitive breast cancer, where estrogen stimulates the growth of the cancer cells. The effect of tamoxifen however varies and unfortunately no method is currently available that can detect a poor effect of the treatment at an early stage. Moreover, side effects to the standard treatment of tamoxifen, 20 mg daily for five to ten years, are common. Further personalization of tamoxifen treatment is therefore important.

The general aim of this thesis is to study different aspects of treatment with tamoxifen to contribute to improved personalized antihormonal treatment for patients operated due to breast cancer. Our goal is individualized dosing of tamoxifen, to improve quality of life and adherence to the treatment.

The body uses an enzyme in the liver called cytochrome P4502D6 (CYP2D6) to convert tamoxifen into more potent estrogen blocking substances. The CYP2D6 enzyme is made by the CYP2D6 gene. Due to genetic variation in the CYP2D6 gene different individuals generate varying amounts of active estrogen blocking substances when taking tamoxifen. Women with variants of the CYP2D6 gene that do not function, so tamoxifen is not activated as it should, have been suspected to get less benefit of the treatment. We have previously investigated a smaller group of women operated due to breast cancer more than 20 years ago who were treated with tamoxifen for five years. We found that younger women who were still menstruating and had a poor ability to activate tamoxifen had a poorer prognosis, compared to the women who had a normal or increased ability to activate tamoxifen. Results from previous research have however been conflicting, so CYP2D6 testing is not currently recommended in patients treated with tamoxifen. Adherence to tamoxifen has also been suggested to be poorer in women with a high activation capacity of tamoxifen. More research is needed to get a better understanding of these matters.

In the first study in this thesis we collected blood samples from 118 patients who were taking tamoxifen at the current standard dose and who were still menstruating when they were diagnosed with breast cancer, to measure their amounts of tamoxifen's potent estrogen blocking substances. We focused on the most potent estrogen blocking substance endoxifen. Side effects were more common in patients with higher levels of endoxifen. We found low amounts of endoxifen not only in patients lacking an ability to activate tamoxifen, but also in those with partly reduced activation. This is important knowledge for future studies testing personalized dosing of tamoxifen. A third of the patients had levels of endoxifen below a suggested threshold for the treatment to be effective. This highlights the importance of further research to clearly define a possible target level for endoxifen in patients treated with tamoxifen.

Results from previous research indicate that decreasing mammographic density (the part that appears "white" in a mammogram) under treatment with tamoxifen might signal that the treatment is effective and that the risk of relapse is lower. In the second project, we studied how mammographic breast density changed under treatment with tamoxifen and if this change was affected by variation in the CYP2D6 gene as well as possible additional treatment with chemotherapy and other antihormonal treatment. As expected, mammographic density did decrease during follow up. We did not find that other treatment added to the mammographic density decrease. Neither did we see that variation in the CYP2D6 gene affected the density reduction. More research is needed to determine whether mammographic density change may be used as a marker of a desired effect of tamoxifen treatment after breast cancer surgery.

Poor adherence to tamoxifen is an important issue. In our third study we compared information on adherence to tamoxifen and other possible anti-hormonal treatment after tamoxifen was stopped, as recorded in patient notes, with information on how many prescriptions the patients had filled. The agreement between the two sources of information was good. Adherence to the anti-hormonal treatment was reasonable. Patients took their medication as prescribed in around 80% of the recommended period. Adherence to the anti-hormonal treatment was not found to be affected by whether the patients had a higher or lower risks for relapse or whether they had gone through menopause or not. Adherence to tamoxifen was unexplainably lower in patients with poor ability to activate tamoxifen, despite previous research indicating that this group might have fewer side effects.

In the fourth study we investigated a larger group of around 1100 women operated due to breast cancer between 2006 and 2014 who were treated with tamoxifen. Compared with our previous investigation, fewer, 12%, of the patients had a relapse and only 4% died from breast cancer during the follow up period of 11 years. No obvious effect of poor function of the CYP2D6 gene on the patients´ prognosis was found. Breast cancer treatment has steadily improved over time. A possible negative consequence of a poor ability to activate tamoxifen is therefore likely marginal in a setting with access to combination treatment for breast cancer. Our present results do not support using CYP2D6 testing for patients with tamoxifen in a modern clinical setting. We can't exclude that CYP2D6 testing might still be of value in selected groups, such as in a low resource setting, where for many patients, including those with a high risk of relapse, tamoxifen is their only treatment. Testing to make sure that patients treated with tamoxifen reach sufficient levels of active estrogen-blocking substances for effectiveness but also to avoid unnecessarily high levels that might be associated with severe side effects, might still be relevant in the future.

In conclusion, more research is needed to find better markers predicting the benefit of tamoxifen as well as for early evaluation of the effectiveness of the treatment. Improving side effects to tamoxifen and optimizing adherence is also important. We therefore plan to initiate a study with individualized dosing of tamoxifen to see whether quality of life and adherence to the treatment can be improved.

# Populärvetenskaplig sammanfattning av avhandlingen

Cancerläkemedlet tamoxifen blockerar effekten av kvinnligt könshormon, östrogen, och motverkar på så sätt tillväxten hos hormonberoende cancerceller. Tamoxifen har länge använts för att förebygga återfall hos kvinnor som opererats på grund av östrogenkänslig bröstcancer. Effekten av tamoxifen varierar. Flera patienter får återfall trots behandlingen. Det finns idag inga tidiga markörer för att upptäcka en dålig effekt av tamoxifen. Det föreligger således ett behov av att förbättra behandlingen. Tamoxifendosen som används idag, 20 mg dagligen under fem till tio år, kan ge besvärande biverkningar.

Det övergripande målet med avhandlingen har varit att undersöka olika aspekter av behandling med tamoxifen för att bidra till ökad kunskap och en mer skräddarsydd antihormonell behandling för patienter som opererats på grund av bröstcancer. Vårt mål är att kunna erbjuda individanpassad dosering av tamoxifen för att förbättra livskvalitet och följsamhet till behandlingen.

Kroppen använder sig av ett enzym i levern som kallas Cytochrom P4502D6 (CYP2D6) för att aktivera tamoxifen till mer kraftfulla östrogenblockerande ämnen. CYP2D6-enzymet bildas av en gen som heter CYP2D6. På grund av skillnader i arvsmassan för CYP2D6-genen bildar olika individer olika mängder östrogenblockerande ämnen av tamoxifen. Tamoxifens skyddseffekt har misstänkts vara sämre hos kvinnor där CYP2D6 inte fungerar som det ska, vilket ger en sämre aktivering av tamoxifen. Vi har tidigare gjort en mindre studie med kvinnor som opererades på grund av bröstcancer för över 20 år sedan och som behandlades med tamoxifen. Vi såg att kvinnor som fortfarande menstruerade och som hade låg aktivering av tamoxifen hade en sämre prognos jämfört med dem som hade normal eller hög aktivering. Tidigare forskningsresultat är motsägelsefulla, så testning av CYP2D6 genen rekommenderas i nuläget inte hos dem som behandlas med tamoxifen. Patienter med hög aktiveringsgrad tamoxifen har beskrivits ha sämre följsamhet till behandlingen. Mer forskning behövs för att vi ska få en bättre förståelse för dessa frågor.

I avhandlingens första studie samlade vi in blod för analys av halter av östrogenblockerande ämnen från 118 patienter som fortfarande menstruerade när de fick sin bröstcancerdiagnos och som behandlades med standarddosen för tamoxifen. Vi fokuserade på det kraftfullaste östrogenblockerande ämnet endoxifen. Vi såg att biverkningar var vanligare hos patienter med höga nivåer av endoxifen. Vi fann låga halter av endoxifen inte bara hos dem med utsläckt aktiveringsförmåga av tamoxifen utan även hos patienter med partiellt nedsatt aktiveringsförmåga, vilket är viktig kunskap för framtida studier med individanpassad tamoxifendosering. Hos en tredjedel av patienterna låg endoxifennivåerna under ett föreslaget tröskelvärde för behandlingseffekt. Detta understryker vikten av mer forskning för att säkerställa ett eventuellt målvärde för endoxifen hos patienter som behandlas med tamoxifen.

Tidigare forskning tyder på att minskande brösttäthet (dvs hur mycket som är ”vitt” på mammografibilden) under behandling med tamoxifen kan tyda på att tamoxifen fungerar

och att man har en minskad risk för återfall. I avhandlingens andra studie undersökte vi hur patienters brösttäthet förändras under behandlingen med tamoxifen och om detta påverkas av skillnader i arvsmassan för CYP2D6-genen eller ytterligare förebyggande behandling som cytostatika och annan antihormonell behandling efter tamoxifen. Som förväntat såg vi att patienternas brösttäthet minskade under uppföljningen. Vi fann inte att ytterligare behandling ledde till en ökad sänkning av brösttätheten hos patienter som behandlas med tamoxifen. Vi såg inte heller något samband mellan genetisk variation i CYP2D6-genen och täthetsförändring. Mer forskning behövs för att säkerställa om mätning av mammografisk täthetsförändring kan användas som utvärdering av tamoxifeneffekt hos patienter med kombinationsbehandling efter bröstcanceroperation.

Följsamhet till hormonell behandling efter operation av bröstcancer är en viktig klinisk fråga. När vi i vår tredje studie jämförde information om patienternas följsamhet till tamoxifen och eventuell annan antihormonell behandling efter tamoxifen, utifrån patienternas journaler med information om deras receptuthämtningar av medicinerna, såg vi att överensstämmelsen mellan de två källorna var god. Följsamheten till den antihormonella behandling var rimlig. Patienterna tog sin antihormonella behandling enligt rekommendation kring 80% av tiden. Följsamheten till behandlingen påverkades inte av risken för återfall eller om man fortfarande menstruerade. Patienterna med utsläckt aktiveringsförmåga av tamoxifen hade oförklarligt nog sämre följsamhet, trots att tidigare forskning tyder på färre biverkningar i denna grupp.

I avhandlingens sista arbete undersökte vi en större grupp med ca 1100 tamoxifen-behandlade bröstcancerpatienter som opererats mellan 2006 och 2014. Jämfört med vår tidigare studie fick en betydligt lägre andel, 12%, av patienterna återfall och bara 4% dog på grund av bröstcancer under uppföljningstiden på 11 år. Vi kunde inte bekräfta något samband mellan låg aktiveringsförmåga i CYP2D6 och en sämre prognos. Behandlingen av bröstcancer har blivit mer effektiv. En möjlig negativ konsekvens av låg aktiveringsförmåga av tamoxifen är därför sannolikt marginell idag när många patienter också får annan behandling. Även om våra nuvarande resultat inte stöder att CYP2D6-testa patienter med tamoxifen i en modern situation där många patienter får en kombination av behandlingar, kan vi inte utesluta att CYP2D6-testning kan vara av värde i utvalda grupper med enbart tamoxifenbehandling, exempelvis i länder med färre resurser, där många patienter, även de med en hög återfallsrisk, behandlas med enbart tamoxifen. Provtagning för att säkerställa att patienter som behandlas med tamoxifen bildar tillräckligt med östrogenblockerande ämnen för att behandlingen ska vara effektiv, men också för att undvika onödigt höga halter som kan leda till svåra biverkningar, kan vara relevant i framtiden.

Ytterligare forskning är viktig för att hitta bättre markörer för att förutspå en god nytta av tamoxifenbehandling och för att tidigt kunna utvärdera behandlingens effekt. Att minska biverkningar samt förbättra följsamheten till behandlingen är också viktigt. Vi planerar därför en studie med individanpassad tamoxifendosering för att se om detta kan leda till färre biverkningar och bättre livskvalitet under behandlingen.

# Abstract

Adjuvant tamoxifen at the standard dose of 20 mg daily for five to ten years reduces the risk for relapse and mortality in hormone sensitive breast cancer. The effect however varies and no early marker of poor response is yet available. Varying activation of tamoxifen due to polymorphism in the CYP2D6 gene has been suggested to influence the effect of the treatment, but data are inconsistent. Our previous study in a smaller cohort of tamoxifen treated early breast cancer diagnosed 1998-2000 indicated a poorer prognosis in premenopausal patients with reduced CYP2D6 activity.

The overall aim of this thesis was to investigate various aspects of tamoxifen treatment to facilitate improved personalized endocrine treatment strategies in early breast cancer, with individualized tamoxifen dosing, to improve quality of life, adherence and prognosis.

In study I we investigated the correlation between CYP2D6 genotype and tamoxifen metabolite levels in plasma, focusing on reduced function CYP2D6 variants (n=118). We also explored the relationship between endoxifen levels and adverse effects to tamoxifen. The degree of side effects to tamoxifen appeared to be dependent on endoxifen concentration. We found a distinct correlation between CYP2D6 activity and plasma concentrations of endoxifen. The effect of reduced function variants, in particular CYP2D6*41, on endoxifen formation was greater than anticipated. Markedly reduced endoxifen concentrations were seen in all homozygous carriers of CYP2D6 no function variants and in those with two reduced activity alleles. The fraction of patients with poor tamoxifen activation might thus be larger than expected. This may be important information for future genotype-based tamoxifen dosing. Although the clinical relevance of the proposed target level of endoxifen at around 5.9 ng/mL needs to be evaluated, it is concerning that a third of our study patients had endoxifen concentrations below this level with tamoxifen at the current standard dose. This underlines the importance of further work to define a target concentration of endoxifen for clinical benefit.

In study II we investigated the effect of CYP2D6 activity and other systemic adjuvant therapy on mammographic density (MD) change (n=699) in tamoxifen treated patients. As expected, MD declined during follow up, with a more prominent decrease in the premenopausal subgroup. Other systemic adjuvant treatment did not further extend density decline in this tamoxifen treated cohort. Density reduction appeared to persist after tamoxifen was stopped. Importantly, the previously proposed correlation between CYP2D6 activity and density change in patients with adjuvant tamoxifen could not be confirmed in this cohort with modern complex systemic adjuvant treatment. More data is needed to ascertain whether mammographic density change may be used as a marker of the desired effect of adjuvant tamoxifen.

In study III we compared information from patient records to data from the National Prescribed Drug Register in Sweden on adherence to adjuvant endocrine treatment (n=1235). We also investigated the association between CYP2D6-activity, menopausal status, the patients' risk for relapse and adherence. Consistency, i.e. agreement, between

the two sources of adherence data was good, 86%, when including medication with an aromatase inhibitor (AI) after tamoxifen. In those with at least 4.5 years follow up, adherence to adjuvant tamoxifen was reasonable, 72% and increased to 82%, when including subsequent AIs, based on prescription refill data. Adherence was not found to vary by menopausal status or recurrence risk. Unexpectedly, adherence to tamoxifen was lower in CYP2D6 poor metabolizers, despite data proposing a reduced risk of adverse effects in this group.

In study IV we aimed to validate our previous findings in a larger material in a more modern setting (n=1105), with tamoxifen treated patients operated between 2006-2014, who could also be subject to improved multimodal adjuvant therapy compared to the patients in our older study and to determine if the effect of CYP2D6 genotype is affected by menopausal status. Compared with our previous study, fewer patients, 12%, had a relapse and only 4% died from breast cancer under the 11-year follow-up. In summary, no obvious correlation between poor CYP2D6 activity and a worse prognosis was found in this material, accounting for adherence to tamoxifen and CYP2D6 inhibitors. A correlation between low CYP2D6 activity and a poorer prognosis in premenopausal tamoxifen treated early breast cancer was thus not confirmed. Breast cancer treatment has steadily improved over time. A possible negative effect of poor CYP2D6 activity on clinical outcome in tamoxifen treated patients is therefore likely marginal in a clinical setting with access to multimodal postoperative breast cancer treatment. Although our results do not support CYP2D6 testing for patients with adjuvant tamoxifen in a multimodal clinical setting, we cannot exclude that CYP2D6 genotyping might still be of value in selected groups, such as in in a low resource setting, where many patients, including those at higher risk of relapse, receive tamoxifen monotherapy. Therapeutic drug monitoring of tamoxifen to secure sufficient plasma levels of endoxifen for clinical efficacy and to avoid excess drug exposure associated with severe side effects might also be relevant in the future.

In conclusion, this thesis contributes to the knowledge on CYP2D6 polymorphism and the effect of postoperative tamoxifen in a multimodal setting, the correlation between CYP2D6 genotype and tamoxifen metabolites, which is important for future dose titration studies of tamoxifen, the effect of systemic adjuvant treatment on density change in tamoxifen treated patients as well as adherence to adjuvant endocrine treatment, with focus on tamoxifen.

There is a need for improved management of side effects to tamoxifen treatment, to optimize quality of life and adherence. Therapeutic drug monitoring of tamoxifen might be an approach. More work on predictive markers and early evaluation of response to tamoxifen is warranted.

# List of scientific papers

I. Thorén L, Lindh JD, Ackehed G, Kringen MK, Hall P, Bergh J, Molden E, Margolin S, Eliasson E.

*Impairment of endoxifen formation in tamoxifen-treated premenopausal breast cancer patients carrying reduced-function CYP2D6 alleles*.

Br J Clin Pharmacol. 2021 Mar;87(3):1243-1252. doi: 10.1111/bcp.14500. Epub 2020 Aug 9. PMID: 32713032; PMCID: PMC9328423

II. Thorén L, Eriksson M, Lindh JD, Czene K, Bergh J, Eliasson E, Hall P, Margolin S.

*Impact of systemic adjuvant therapy and CYP2D6 activity on mammographic density in a cohort of tamoxifen-treated breast cancer patients*.

Breast Cancer Res Treat. 2021 Dec;190(3):451-462. doi: 10.1007/s10549-021-06386-2. Epub 2021 Sep 27. PMID: 34570302; PMCID: PMC8558195.

III. Thorén L, Margolin S, Eliasson E, Bergh J, Lindh JD.

*Adherence to endocrine therapy in early breast cancer in relation to Cytochrome P450 2D6 genotype: a comparison between pharmacy dispensation data and medical records*.

Breast Cancer Res Treat. 2023 Mar 1. doi: 10.1007/s10549-023-06887-2. PMID: 36856936.

IV. Thorén L, Lindh JD, Molden E, Kristiansen Kringen M, Bergh J, Eliasson E, Margolin S.

*CYP2D6 genotype and outcome in tamoxifen treated early breast cancer*.

Manuscript

Scientific paper not included in the thesis:

Margolin S, Lindh JD, Thorén L, Xie H, Koukel L, Dahl ML, Eliasson E.

CYP2D6 and adjuvant tamoxifen: possible differences of outcome in pre- and post-menopausal patients.

Pharmacogenomics. 2013 Apr;14(6):613-22. doi: 10.2217/pgs.13.47. PMID: 23570465.

# Contents

# List of abbreviations

| | |
|---|---|
| AI | Aromatase inhibitor |
| AET | Adjuvant Endocrine Therapy |
| AS | Activity Score |
| ATM | Ataxia-Telangiesctasia Mutated gene |
| BARD1 | BRCA1 Associated Ring Domain 1 gene |
| BC | Breast cancer |
| BMI | Body Mass Index |
| BIRADS | Breast Imaging Reporting and Data System |
| BRCA1, BRCA2 | Breast Cancer Gene 1, 2 |
| CHEK2 | Checkpoint kinase 2 gene |
| CI | Confidence Interval |
| $Cm^2$ | Square centimeter |
| CNA, CN | Copy Number Analysis, Copy Number |
| CPIC | The Clinical Pharmacogenetics Implementation Consortium |
| CYP2B6 | Cytochrome P4502B6 |
| CYP2C9 | Cytochrome P4502C9 |
| CYP2D6 | Cytochrome P4502D6 |
| CYP2C19 | Cytochrome P4502C19 |
| CYP3A4/5 | Cytochrome P4503A4/5 |
| DA | Mammographic Dense Area |
| DDD | Defined daily doses |
| DM-tamoxifen | N-desmethyl-tamoxifen |
| DFS | Disease free survival |
| DNA | Deoxyribonucleic acid |
| Dnr | Reference number in public archives, 'Diarienummer´ |
| DPWG | Dutch Pharmacogenetics Working Group |
| EBCTCG | The Early Breast Cancer Trialists' Collaborative Group |
| EM | CYP2D6 Extensive Metabolizer |
| ER | Estrogen Receptor |
| ERB2 | Erb-B2 Receptor Tyrosine Kinase 2 Gene |
| GnRH | Gonadotropin Releasing Hormoney |
| HR | Hazard Ratio |
| i.e. | id est, "that is" |
| IHC | Immunohistochemistry |
| IM | CYP2D6 Intermediate Metabolizer |
| IQR | Inter Quartile Range |
| Her2 | Human Epidermal growth factor Receptor 2 |
| $Ki_{67}$ | Nuclear protein associated with cellular proliferation |
| LOESS | Local Polynomial Regression Fitting |
| NF1B | Nuclear Factor 1B |
| NKCB | National Quality Registry for Breast Cancer |
| MD | Mammographic density |

| | |
|---|---|
| Mg | Milligram |
| ML | Milliliter |
| MLO | Mediolateral Oblique breast view |
| MPR | Medication possession ratio |
| n/N | number |
| NF1B | Nuclear Factor I B |
| NM | CYP2D6 Normal Metabolizer |
| 4-OH- tamoxifen | 4-hydroxytamoxifen |
| p | The probability to obtain a result at least as extreme as the observed result, if the null hypothesis is correct |
| PALB2 | Partner And Localizer Of BRCA2 gene |
| PARP1 | Poly ADP ribose polymerase 1 |
| PCR | Polymerase Chain Reaction |
| PM | CYP2D6 Poor Metabolizer |
| PMD | Percent Mammographic Density |
| PR | Progesterone Receptor |
| P53 | Tumor protein p53, tumor suppressor gene |
| RAD51C, RAD51D | RAD51 Paralog C/D gene(s) |
| SD | Standard Deviation |
| SERM | Selective estrogen receptor modulator |
| S phase | Synthesis Phase, the cell cycle phase when DNA is replicated |
| SÖS | Södersjukhuset |
| Tam | Tamoxifen |
| TGF | Transforming growth factor |
| UM | CYP2D6 Ultrarapid Metabolizer |

# Introduction

Adjuvant tamoxifen at a standard dose of 20 mg per day, reduces the risk of relapse and breast cancer related death in hormone receptor positive breast cancer (1). The effect varies (2). No early marker of poor response is currently available.

The overall aim of this thesis is to study different aspects of tamoxifen treatment to contribute to further personalized endocrine treatment strategies in early breast cancer, with future individualized tamoxifen dosing, to improve adherence and quality of life under treatment as well as prognosis.

Tamoxifen is activated by CYP2D6 in to more potent metabolites, in particular endoxifen (3). Varying activation of tamoxifen due to variation in the CYP2D6 gene has been suggested to influence the effect of the treatment (4–7). Previous data on how CYP2D6 genotypes influence the effect of postoperative tamoxifen treatment have been conflicting, so genotyping of CYP2D6 in order to predict effectiveness for tamoxifen is generally not implemented (8). Data from our previous study in a smaller group of breast cancer patients diagnosed 1998-2000 who were treated with adjuvant tamoxifen, indicated a poorer prognosis in premenopausal patients with reduced CYP2D6-activity (4). In this thesis we aimed to validate our previous findings in a larger cohort in a multimodal adjuvant setting, accounting for adherence to treatment, and to determine if the effect of CYP2D6 genotype is affected by menopausal status.

The association between CYP2D6 activity and the levels of metabolites is critical for future dose titration studies with tamoxifen. Endoxifen levels vary between individuals with normal and poor CYP2D6 activity. More knowledge is needed on the influence of reduced function CYP2D6 variants on endoxifen formation (9–12). We therefore studied the correlation between CYP2D6 genotypes and tamoxifen metabolite levels, with focus on reduced function CYP2D6 alleles. We also explored the relationship between endoxifen levels and adverse effects to tamoxifen.

An association between a reduction in mammographic density (MD) under tamoxifen treatment and a reduced risk for recurrence has been reported (13). A correlation between CYP2D6 activity and decline in MD during tamoxifen therapy has also been suggested (14). Change in MD has therefore been proposed as a surrogate marker for tamoxifen response (13). Previous studies have mostly been performed in patients receiving no other systemic treatment other than tamoxifen. Hence, we investigated the effect of CYP2D6 polymorphism and other systemic adjuvant treatments on change in MD in early breast cancer patients with postoperative tamoxifen.

Poor adherence to tamoxifen is an important issue. Data suggest that patients with active CYP2D6 variants have a higher risk of discontinuing tamoxifen (15). In this thesis we assessed adherence to postoperative endocrine therapy, focusing on tamoxifen. We compared information on adherence from patient records to data from the Swedish Prescribed Drug Register. We also investigated if there is a correlation between CYP2D6 activity, menopausal status, the patients' risk for relapse and adherence.

# 1 Literature review

### 1.1 Breast cancer epidemiology

Breast cancer is the most frequent female cancer worldwide. 2.3 million new cases were diagnosed in 2020 (16). Although the relative incidence is highest in developed countries, the larger populations in less developed countries have led to that over 50 % of the cases are now diagnosed in low- and middle-income regions (17). Breast cancer is the major cause of cancer related death in women. Nearly 700 000 women died due to breast cancer globally in 2020 (17).

Breast cancer accounts for around 26 % of all female cancer in Sweden (18). Male breast cancer accounts for around 1% of all Swedish breast cancer cases (19). In 2022, 9373 breast cancer cases were reported in Sweden. Around 2200 were diagnosed in the Stockholm-Gotland region (20). The incidence for breast cancer in Sweden has almost doubled since the 1960s, likely due to a longer life expectancy, the introduction of mammography screening, improved imaging techniques and lifestyle factors such as women having fewer children, being older when having their first child and increasing obesity in menopause (17). Furthermore, more than one tumor may have been reported per patient during the last two decades (21). The estimated risk of being diagnosed with breast cancer before the age of 75 years is currently roughly 9% in Sweden (18). The median age for diagnosis of breast cancer in Sweden is 65 years. Around 4% affect women younger than 40 years (20). Almost 50 % of the cases in Sweden were detected by mammography screening. Despite an increasing incidence, the mortality rate in Sweden has remained stable at around 1300 deaths from breast cancer per year, indicating a mortality decrease. The relative 5-year survival rate has increased from 75% in 1980 to 92% in 2021, likely due to earlier diagnosis and improved treatment (22).

### 1.2 Risk factors for breast cancer

Breast cancer etiology is complex and the disease is likely caused by a combination of hereditary factors, hormonal impact on the mammary glands as well as lifestyle factors (23). Apart from being female, aging is a major risk factor of breast cancer. Other risk factors reflecting the accumulative exposure of the epithelium in the breast to estrogen are early age of menarche and older age at menopause, not experiencing a full-term pregnancy or having the first pregnancy after the age of 25, not breast feeding, postmenopausal obesity, being physically inactive and exogenous hormonal elements such as oral contraceptives and hormone replacement therapy. Additional risk factors include alcohol consumption, radiation therapy to the chest or breasts before the age 30 and previous history of neoplastic disease or hyperplasia in the breast. The risk of developing breast cancer has been found to be four to six-fold higher in those with the highest mammographic density compared to those with the lowest density (24, 25).

Hereditary factors account for around 10% of breast cancer cases. A woman's risk is higher if she has family members who have been diagnosed with breast- or ovarian cancer (23, 26). Women carrying germline pathogenic variants in BRCA1, BRCA2, P53 and PALB2 have a high risk of breast cancer while women carrying variants in ATM, BARD1, CHEK2, RAD51C, and RAD51D are at moderate risk (27). A combination of relatively common low risk single nucleotide polymorphisms may also convey an increased risk (28).

### 1.3 Prognostic and predictive factors for early breast cancer

Prognostic factors help to assess a patient's prognosis. Predictive markers indicate an anticipated benefit of a specific systemic treatment (29). Prognostic factors, routinely analyzed by immunohistochemistry (IHC), include expression of estrogen receptors (ER), progesterone receptors (PR), human epidermal growth factor receptor 2 (HER2) and proliferation measured as levels of Ki-$_{67}$ protein. Tumor size, histological grade, involvement of regional lymph nodes and age are likewise important prognostic variables (30).

#### 1.3.1 The estrogen receptor (ER)

The estrogen receptors are ligand-activated transcription factors. The ERα regulates proliferation in normal as well as cancerous breast tissue (31). In the classical signaling pathway, estrogen binds to the ER in the cell's cytoplasm and the estrogen-ER-complex moves to the cell nucleus. This process activates transcription and signaling, which stimulates proliferation of mammary cells (32). Around 75%–85% of all breast cancers are defined as ER-positive (33, 34). For these, estradiol is the main growth stimulus. ERα expression is one of the major predictive markers in breast cancer as it predicts sensitivity to endocrine treatment (35). The importance of ERβ expression needs to be clarified (31, 36). ER expression is also a prognostic factor. Patients with ER positive disease have compared with those without ER expression a more favorable prognosis during the first 5 years. For ER positive disease a long-term risk of relapse after 10 years remains (2, 37).

Ligand binding assays were initially used to determine ER status. 10 fentomole/mg was generally accepted as the clinical threshold, based on data on response to endocrine therapy. IHC assays, in which monoclonal antibodies recognizes the ER, were introduced in the 1990s (38),(35). According to current Swedish guidelines, breast cancer is defined as ER positive when at least 10% of the tumor cells stain positive for ER by IHC (39). In patients with "ER-poor disease" (ER below 10%), some benefit of tamoxifen appeared in the trials of one to two years treatment, but not in the investigations of 5 years therapy. The suggested benefit in ER poor disease has thus been questioned (1). The American Society of Clinical Oncology guidelines and the European Society for Medical Oncology

however recommend that ER status should be considered positive if one percent or more of the tumor cells stain positive by IHC (30, 38). In a meta-analysis on 20 trials with adjuvant tamoxifen by the Early Breast Cancer Trialists' Collaborative Group (EBCTCG), no apparent benefit of tamoxifen was seen when ER was below 10 fentomole/mg (40).

### 1.3.2 The progesterone Receptor (PR)

Around 60% of malignant breast lesions are PR-positive. ER and PR are most commonly expressed together in early breast cancer (33). Although there are data suggesting that PR expression may be a predictor of tamoxifen response (41), a meta-analysis by the EBCTCG observed tamoxifen efficacy in ER-positive patients to be independent of PR status (40). PR expression is considered mainly a prognostic factor. Data indicate that PR-negative tumors have a poorer prognosis (1).

### 1.3.3 Human epidermal growth factor receptor 2 (HER2)

Around 10-15 % of all breast cancer cases are defined as HER2-positive (34, 42). These tumors have more copies, i.e. amplification and / or overexpression of the HER2 gene (also known as ERBB2) which leads to increased cell proliferation and a poorer prognosis, without targeted HER2 therapy. HER2 positivity predicts the benefit of anti HER2 targeted treatment (43).

### 1.3.4 $Ki_{67}$

$Ki_{67}$, a protein found in cell nuclei in dividing cells, is used as a marker of cell proliferation. $Ki_{67}$ is of prognostic importance in ER positive, HER2 negative tumors aiding the decision if a patient will benefit from chemotherapy (44),(30). Previously other methods have been used, such as measuring the S phase fraction (i.e. the fraction of tumor cells involved in chromosomal DNA synthesis) (45). The cut offs for high proliferation have varied over time. According to current Swedish guidelines, a low proliferation rate is determined as $Ki_{67}$ below 6%, intermediate proliferation as $Ki_{67}$ 6-29% and a high proliferation rate of $Ki_{67}$ of at least 30% (39).

### 1.3.5 Histological grade

Histological grade classifies breast cancer tumors according to their degree of differentiation and reflects how well the tumor cells resemble normal cells when viewed digitally or under a microscope. Histologic grade is strongly correlated with prognosis. Grade III tumors are poorly differentiated and tend to be more aggressive (46, 47). Histological grade is mainly assessed using the Nottingham Histological Grade system,

in which the grade is determined by a compilation of the fraction of tubule-forming cancer cells, nuclear pleomorphism and the mitotic rate. Each of these characteristics is assigned a score of one to three, where the highest score represents the most abnormal feature. The sum is used to grade the tumor. A sum of 3-5 represents Grade 1, a sum of 6-7 defines Grade II and a sum of 8-9 represents Grade III (39, 47). Around half of all breast cancers are currently assigned to the intermediate group, Grade II. The clinical value of this group is uncertain (39).

### 1.3.6 Stage

Tumor stage is an important prognostic factor. In the TNM staging system, "T" describes the largest size of the primary breast tumor, "N" stands for the number of affected regional lymph nodes, and "M" describes if the patients has distant metastasis (48). Metastasis to regional lymph nodes is a major prognostic factor in early breast cancer (46). The latest ($8_{th}$) edition of the TNM classification has also added other factors, such as tumor grade, proliferation rate, ER-, PR- and HER2-status as well as results from genomic panels (48).

### 1.3.7 Age

Breast cancer in younger patients (under the age of around 40) often exhibits more aggressive features. The tumors are more often ER negative, of higher grade and stage at diagnosis. Even after adjusting for other prognostic variables and treatment in multivariable analyses, young age has been shown to be a prognostic factor. Younger patients generally have a poorer prognosis and the likelihood of a genetic predisposition for breast cancer is higher compared to older women (49).

### 1.3.8 BRCA1 and 2

The presence of germline mutations in BRCA1 and BRCA2 predicts a possible benefit of adjuvant PARP inhibitors (50).

### 1.3.9 Intrinsic molecular subtypes

Currently breast cancer is categorized into intrinsic molecular subtypes based on gene expression analyses such as MammaPrint, Oncotype DX and Prosigna (42, 51). The major intrinsic breast cancer subtypes are luminal A, luminal B, HER2 enriched and basal like (42, 52). Corresponding to, although not fully overlapping, "surrogate definitions " of the molecular subtypes may also be obtained by IHC analysis of ER, PR, $Ki_{67}$ and HER2 status. To discern between luminal A- and Luminal B-like tumors, histological grade, PR

expression and levels of $Ki_{67}$ are used (42, 53). The Intrinsic and corresponding clinico-pathological defined subtypes are summarized in Table 1.

Table 1. Intrinsic and corresponding clinico-pathological definition of breast cancer subtypes.

| **Intrinsic Subtype** | **Clinico-pathological surrogate definition of subtypes** |
|---|---|
| **Luminal A**<br><br>Low risk molecular signature | **Luminal A like** tumors are ER positive and have high PR expression, are HER2 negative, have low proliferation and low histological grade. |
| **Luminal B**<br><br>High risk molecular signature | **Luminal B like HER2 negative** tumors are ER positive with high proliferation and /or grade. PR is low or negative. This group has a higher risk of recurrence and a higher benefit of chemotherapy in addition to endocrine therapy.<br><br>**Luminal B like HER2 positive** tumors are HER2- and ER positive, can have any $Ki_{67}$ levels and positive or negative PR. This group benefits from targeted anti HER 2 therapy in addition to chemotherapy and endocrine therapy. |
| **HER2 enriched** | **HER2 non** luminal tumors are HER2 positive and ER- and PR negative. This group benefits from targeted anti HER2 treatment and chemotherapy. |
| **Basal like** | **Triple negative** breast cancer is ER-, PR- and HER2 negative and is generally recommended chemotherapy. |

In 2022 10% of all breast malignancies in Sweden were reported to be triple negative, 12 % were HER2 positive and 79% were defined as luminal (20).

Commercial gene expression analyses are currently used to obtain further prognostic data in ER-positive, HER2 negative breast cancer, with intermediate histological grade and proliferation, where the tumor´s categorization and risk profile is uncertain. These tests help to determine the patient's risk of recurrence and predict the general benefit of chemotherapy (30). Most gene expression panels have been validated for postmenopausal patients (54). Data on premenopausal women are emerging (55).

### 1.4 Treatment of early breast cancer

Modern therapies used for early breast cancer involves combinations of local modalities including surgery and radiotherapy as well as systemic treatments, such as

chemotherapy, endocrine treatment and other targeted therapies, in various sequences and combinations (30).

The choice of treatment is based on the patient's prognosis, which depends both on tumor biology and tumor burden, the predicted sensitivity to and benefit from the treatment, presence of high risk germline mutations, but also the risk of side effects, the general condition and biological age of the patient, menopausal status, comorbidity, and the patient's preferences (30).

### 1.4.1 Loco regional treatment

#### 1.4.1.1 Breast cancer surgery

Breast cancer surgery has a major role in the treatment of breast cancer and is for most patients the first step of their multimodal treatment. Almost 50% of early breast cancer patients do not have a relapse after primary surgery alone, or after surgery followed by radio therapy (56).

Over the last decades there has been a trend towards breast conserving methods and less extensive axillary staging (20, 30). Breast-conserving surgery is currently the preferred choice for most patients. A sentinel node biopsy is currently the standard method for axillary staging in clinically node negative patients. Axillary dissection is generally performed in those with known node positive disease and when the sentinel node biopsy has revealed more than two sentinel nodes with macro metastasis (30). In 2022, more than 70% of the diagnosed cancers in Sweden were amendable to breast conservation, at diagnosis or after down staging treatment and in 86% a sentinel node biopsy only was performed (20). Mastectomy may be performed for example due to tumor size in relation to breast size, in inflammatory breast cancer, tumor multicentricity, in patients with a relapse after previous breast conserving surgery and irradiation, to achieve negative surgical margins after previous non radical resection, when there are contraindications for radiotherapy, in patients carrying germ-line mutations in high-risk genes and according to the patient's preferences. The postoperative pathological assessment of the surgical specimens of the breast and axillary node(s) yields important prognostic and predictive information which aids the decision on post operative treatment (30).

#### 1.4.1.2 Radiotherapy

Postoperative radiotherapy reduces not only the risk of a local relapse but also breast cancer related mortality after both breast conserving surgery and mastectomy (56). Hypo fractionated radiation treatment has emerged during recent years (57).

Radiotherapy after breast conserving surgery is standard of care and almost halves the relative 10-year risk of any relapse and the absolute risk by 16%. The absolute risk of breast cancer specific mortality is reduced by almost 4% after 15 years (58). The absolute gain is higher in patients with positive lymph nodes, where an absolute risk reduction of recurrence at 10-years of 21% and mortality at 15 years by 9% has been reported. Extra boost radio therapy yields a further risk reduction and is recommended in patients up to 50 years of age (30). Data show that survival after breast conserving surgery followed by radiotherapy is similar to mastectomy (59), or even slightly better according to recent Swedish data (60). Radiotherapy towards the chest wall after mastectomy is recommended for patients operated due to large tumors, tumors engaging the skin or chest wall and in inflammatory breast cancer. Locoregional radiation is generally recommended to those with lymph node macro metastasis (58), (30). This treatment reduces the ten-year absolute risk of relapse by 10% and the risk of breast cancer related mortality after 20 years by 8% (30).

### 1.4.2 Systemic treatment

#### 1.4.2.1 Preoperative systemic treatment

Neoadjuvant (i.e. preoperative) treatment is increasingly used in early breast cancer. Preoperative treatment is used to downstage the tumors to reduce the surgical extent, but also yields information on the response to therapy (61). Preoperative treatment is always recommended for locally advanced and inoperable tumors. The neoadjuvant approach is at present also preferred in patients with tumors larger than two cm, with risk factors indicating a benefit of chemotherapy, i.e., in triple negative, HER2-positive, grade III or node positive disease. Four to eight cycles of anthracyclines and taxanes have been the standard for many years. Recent data show that adding the checkpoint inhibitor pembrolizumab to a chemotherapy regimen including anthracyclines, taxanes and carboplatin, improves the rate of pathological complete response and may also reduce the risk of relapse in triple negative breast cancer (57, 62). Dual anti HER2 treatment (pertuzumab plus trastuzumab) in addition to chemotherapy is currently recommended for HER2 positive patients (30). Neoadjuvant endocrine therapy, preferably with an aromatase inhibitor, may be considered in elderly patients with ER positive locally advanced tumors (30).

Patients achieving pathological complete response have a more favorable prognosis, especially in those with triple negative, HER2 positive or ER negative disease (63). The risk of recurrence increases with the extent of residual cancer burden (64).

#### 1.4.2.2 Adjuvant treatment

The purpose of adjuvant treatment is to eradicate microscopic foci of cancer cells that might remain after breast cancer surgery, or micro-metastases that have escaped

beyond the breast and regional lymph nodes, to reduce the risk of loco regional and distant relapse and breast cancer related mortality.

Treatment includes chemotherapy, anti HER2 targeted treatment, radiation therapy, endocrine treatment and intravenous bisphosphonates. The decision on which postoperative treatment is recommended is based on the patient's risk of relapse and as described previously, the predicted benefit contra risk of side effects from the treatment (30). For patients who have received neoadjuvant therapy, the response to treatment and the extent of remaining cancer cells adds additional prognostic information (30).

#### 1.4.2.2.1 Chemotherapy

Adjuvant chemotherapy is recommended in most triple negative, HER2-positive and luminal-B like tumors. The most common current regimens include anthracyclines and/ or a taxane. Initial data from adjuvant taxane trials emerged around 2005 (65). Swedish national guidelines recommended adjuvant taxanes from 2009 (66). Postoperative chemotherapy with anthracyclines and taxanes reduces the relative risk of breast cancer mortality by roughly a third during the first 10 years, compared to no chemotherapy. The benefit depends on the absolute risks without chemotherapy, so the absolute gain is lower in patients at a low risk of recurrence. The addition of a taxane to anthracyclines has been found to yield a relative reduction of breast cancer specific mortality by around 13% after 8 years (and a nearly 3 % absolute gain) (67). Recent data suggest that the addition of carboplatin and the taxane paclitaxel to anthracyclines is an effective alternative in triple negative disease (68). Adjuvant capecitabine improves disease free survival (DFS) and yields an absolute gain of around 4 % in overall survival (OS) in patients with remaining cancer cells after neoadjuvant therapy with anthracyclines and taxanes. The highest benefit is found in triple negative tumors (69).

#### 1.4.2.2.2 Checkpoint inhibitors

For patients operated due to triple negative breast cancer, who have received neoadjuvant pembrolizumab, the treatment continues postoperatively (62).

#### 1.4.2.2.3 Anti HER2 targeted therapy

Adjuvant treatment with trastuzumab was introduced in routine care in 2005, when the efficacy of the treatment in early breast cancer was reported. Adjuvant trastuzumab in addition to chemotherapy reduces the relative risk for relapse and breast cancer death by approximately one third during the first decade in HER2 positive patients. The absolute reduction of mortality is around 6% (43, 70).

Adjuvant trastuzumab emtansine is currently recommended for patients with residual cancer after neoadjuvant chemotherapy plus targeted HER2 treatment with trastuzumab and pertuzumab. In those with remaining invasive disease after neoadjuvant therapy containing a taxane and / or an anthracycline and trastuzumab, trastuzumab emtansine is found to decrease the relative risk of relapse or death by around 50%, compared to those who received postoperative trastuzumab only. The absolute OS gain at five years is around 7% (71).

Oral neratinib may be considered in selected high-risk, ER positive HER2 positive patients, where a 2.5% improvement in 5-year DFS had been reported. The benefit in those previously treated with neoadjuvant anti HER2 dual blockade is not known (30).

#### 1.4.2.2.4 Bisphosphonates

Adjuvant use of bisphosphonates in postmenopausal women reduces the absolute risk of bone metastasis by around 2% and yields an absolute increased breast cancer specific survival of around 3%. The relative risk of breast cancer mortality at 10 years is reduced by 18%. The risk of fractures is also reduced in those with a risk of treatment related bone loss (72).

#### 1.4.2.2.5 PARP inhibitors

Individuals with pathogenic germline mutations in BRCA1 or BRCA2 have a deficiency in DNA repair by homologous recombination. PARP inhibitors inhibit and trap PARP1, an enzyme thar repairs DNA. BRCA associated cancer cells are sensitive to inhibition of PARP1, as the other repair mechanism needed for cell survival, homologous recombination, is deficient. Recent data show that one year adjuvant treatment with the PARP inhibitor Olaparib yields an absolute OS gain by around 3% in BRCA-positive patients operated due to high risk HER2 negative breast cancer (50).

#### 1.4.2.2.6 CDK4/6 inhibitors

In hormone sensitive HER2 negative high risk patients, adjuvant treatment with the CDK 4/6 inhibitor abemaciclib for two years in addition to endocrine treatment has recently been shown to give a 25% reduction in the relative risk of an invasive relapse and an absolute improvement of nearly 4% in 2-year invasive DFS, compared with postoperative endocrine treatment only (73).

#### 1.4.2.2.7 Endocrine treatment

Five years standard adjuvant antihormonal treatment in ER positive breast cancer reduces the risk of relapse and improves survival for this group substantially. The relative breast cancer mortality at 15 years is reduced by roughly 30-40% (1, 74, 75). Postoperative antihormonal treatment with either tamoxifen, or an aromatase inhibitor (and ovarian suppression in premenopausal patients), is therefore usually recommended for luminal-like cancers (30). The preferred choice and duration of endocrine treatment is based on the patient's risk of recurrence, age, menopausal status, comorbidity and potential side effects. Extended treatment for up to 10 years is recommended for patients with a higher risk of recurrence (66, 76).

##### 1.4.2.2.7.1 Aromatase inhibitors

In postmenopausal women androgens produced in other organs and tissues than the ovaries such as adipose tissue and adrenal glands, are converted to estrogens by the enzyme aromatase. Aromatase inhibitors (AI) decrease the amount estrogens produced by inhibiting this enzyme. Letrozole and anastrozole are reversible non-steroidal inhibitors. Exemestane is an irreversible steroidal inhibitor. Premenopausal women cannot use AIs alone, as AIs do not block the estrogen synthesis in the ovaries (77).

Five years of an AI has been shown to reduce 10-year breast cancer mortality rates by around 40% compared with no antihormonal therapy (75). In a meta-analysis by the EBCTCG comparing treatment with an AI versus tamoxifen in postmenopausal women, five years of an AI was found to reduce the relative risk of relapse by 30% and the absolute risk by around 4%, compared with five years tamoxifen during the periods when the therapies differed, however not thereafter. The relative risk of breast cancer death after 10 years was reduced by around 15% and the absolute risk by roughly 2%, compared with tamoxifen. Two or three years of tamoxifen followed by an AI up to five years reduced the absolute risk of mortality by roughly 1% after 10 years compared to five years tamoxifen. When comparing five years of an AI with an AI for two-three years followed by switch to tamoxifen, no apparent additional benefit was seen (75).

Common side effects to AIs include menopausal symptoms, vaginal dryness, muscle and joint pain, stiffness, a moderately increased risk of osteoporosis and an absolute risk of around 3% for fractures (75, 78).

AIs could according to Swedish national guidelines in 2002 be discussed for selected patients, mainly those with severe side effects to tamoxifen or a contraindication for tamoxifen ( i.e increased risk of thrombosis). Since 2009 postmenopausal patients, especially those with a higher risk of relapse, are in general recommended an AI upfront.

AI may also be used sequentially with tamoxifen (66, 79). A meta-analysis showed that extended AI treatment improves DFS (HR 0.75; 95% CI, 0.66-0.86) and reduces the risk of contralateral breast cancer. No significant difference regarding overall survival was however found (80). High-risk patients may be recommended extended treatment with an AI up to around 8 years, or an AI after 5 years of tamoxifen. Those experiencing severe side effects to AIs are recommended changing their treatment to tamoxifen (30).

#### 1.4.2.2.7.2 Ovarian suppression

In premenopausal women estrogen is mainly produced by the ovaries. Gonadotropin releasing hormonal agonists (GnRH) down regulates GnRH receptors in the pituitary gland, which suppresses synthesis of luteinizing hormone as well as follicle-stimulating hormone, which in turn inhibits the ovaries from producing estrogen, causing temporary menopause (81).

Ovarian suppression in conjunction with tamoxifen compared to tamoxifen alone improves survival (HR 0.78; 95% CI, 0.60 - 1.01), with an absolute improvement of around 2% (82). The clinical benefit of ovarian suppression is most meaningful for patients at a higher risk of relapse. This combination has therefore been recommended high risk premenopausal patients according to Swedish national guidelines since 2009 (66). A meta-analysis by EBCTCG in 2022 showed that treatment with an AI for 3-5 years instead of tamoxifen in premenopausal patients also receiving ovarian suppression reduced the absolute 5-year recurrence risk with 3.2%, but not OS, likely due to too short follow up (74). Other recent data have found an absolute risk reduction in mortality of roughly 3% in patients treated with an AI in conjunction with ovarian suppression compared with tamoxifen plus ovarian suppression (83). As the combination of an AI with ovarian suppression has substantial side effects, this regimen is generally recommended patients with a higher recurrence risk (74, 82, 83).

#### 1.4.2.2.7.3 Tamoxifen

Tamoxifen, a so called selective estrogen receptor modulator (SERM), hinders estrogen from stimulating cell growth in ER positive breast cancer cells by competitively binding to the ER (84). This blocks or alters expression of estrogen dependent genes, resulting in inhibition of the estrogen-dependent growth-signaling pathway (85). Tamoxifen has been shown to increase oxidative stress in breast cancer cells, inducing apoptosis (86) and has been suggested to affect the regulation of growth factors in cancer cells, reducing levels of the stimulatory insulin-like growth factor and transforming growth factor alpha and increasing concentrations of sex hormone binding globulin in postmenopausal women, so

that levels of free estradiol in serum are lowered. The inhibitory factor TGF beta is induced, which may suppress tumor growth (87).

Walpole and colleagues discovered Tamoxifen in 1962. The initial plan to use tamoxifen as a post coital contraceptive failed. Instead, tamoxifen became the first targeted treatment for breast cancer. Tamoxifen was approved for patients with advanced breast cancer in the early 1970s. Later the indication was extended to adjuvant treatment for postmenopausal and finally in the 1990s for premenopausal patients. Tamoxifen has also been approved for use in male breast cancer and in the preventive setting (84). Several trials with tamoxifen in the preventive setting have been conducted. Tamoxifen as breast cancer prevention is however to date not in clinical use in Sweden (88) (89).

The positive effect of adjuvant tamoxifen has been established in many randomized trials, summarized in meta-analyses by the EBCTCG. The relative benefit of adjuvant tamoxifen has been found to be irrespective of age, lymph node involvement and whether the patient has received chemotherapy or not (40, 90).

In the initial trials of adjuvant tamoxifen one or two years of tamoxifen was used (90). Later data show that five years of adjuvant treatment was clearly superior to shorter duration and this treatment duration was the standard for a long period of time (1, 40, 90). Five years adjuvant tamoxifen has been shown not only to substantially reduce the risk for relapse both locally and distant during treatment, but also throughout the first decade after diagnosis. The relative risk of recurrence is roughly halved, with an absolute reduction of 13% during treatment and by around 30% the following five years. The relative risk for contralateral breast cancer is reduced by around 40%. Moreover, breast cancer mortality is reduced about a third, with an absolute reduction of 9 %, throughout the first 15 years (40). Data from the ATLAS study comparing five versus 10 years of adjuvant tamoxifen, showed an additional absolute reduction of recurrence by around 3% and breast cancer mortality by around 2% from 10 years adjuvant tamoxifen, after 15 years (76). Similar results from the aTTom study also indicate a benefit from 10 years tamoxifen treatment (91). Patients at high risk of relapse are therefore recommended 10 years of adjuvant tamoxifen.

A recent Swedish study investigated the long-term effect of two years postoperative tamoxifen in premenopausal women with three decades of follow-up. The relative risks of breast cancer related events were reduced by almost 40% and distant relapse by almost 30%, as compared with no tamoxifen treatment. A lower risk of relapse was seen after 15 years, suggesting a long-term benefit of the treatment (92).

Although no major trial performed a randomized comparison between different doses of tamoxifen, a review of the early clinical trials of adjuvant tamoxifen revealed that the efficacy of tamoxifen at 20 mg daily appeared equivalent to 30 or 40 mg/ day (90), establishing 20 mg daily as the standard dose.

Tamoxifen is effective in both pre- and postmenopausal patients (1). For premenopausal patients with a lower risk for relapse, tamoxifen is still the standard treatment. In younger patients with a higher risk, tamoxifen may be used in combination with ovarian suppression. For postmenopausal patients AIs are an alternative (75, 83).

In a meta-analysis of ER positive early breast cancer patients with T1 tumors ( less than 2.0 cm) or T2 disease (tumor diameter over 2.0 cm and up to 5.0 cm) and less than 10 positive nodes, who were recommended 5 years endocrine treatment, the risk of distant relapse within 20 years was small, 13%, in T1, node negative patients, but the risk of relapse increased in larger tumors with many involved lymph nodes, to 40%, in those with at least 4 positive nodes. Roughly 60% received tamoxifen. This indicates a wide variability in the efficacy of the treatment (2). To date ER expression is the only predictive marker for tamoxifen efficacy (40).

1.4.2.2.7.4 Side effects to tamoxifen

Estrogen receptors are expressed not only in breast tissue, but also in the uterus, ovaries, the musculoskeletal-, cardiovascular-, central nervous- and the immune systems (93). Side effects may occur due to binding of tamoxifen to ER in other organs besides the breast. In breast tissue tamoxifen acts as an antagonist. In contrast, tamoxifen has agonistic effects in the endometrium and bone tissue, resulting in endometrial hyperplasia as well as increased bone mineralization (32).

The most common side effects are menopausal symptoms, such as hot flashes and sweats, affecting up to 80% of the patients (94). Other common adverse effects include sleep disturbances, mood swings, lowered libido, vaginal dryness, joint pain and weight gain (78, 94). Serious, although rare, side effects include a slightly increased risk of endometrial cancer mainly for postmenopausal women, who have a cumulative risk at around 3% with extended treatment, as well as a small risk of venous thrombosis and pulmonary embolism (HR 1.87, CI 1.13–3.07). No significant increased mortality due to other causes than breast cancer has however been reported (40, 76). Tamoxifen lowers cholesterol levels, decreases the risk of coronary heart disease and improves bone health (95).

## 1.5 Cytochrome P4502D6 (CYP2D6)

The cytochrome P450 enzymes constitute the main hepatic metabolizing system for lipids, hormones, toxins, and drugs. Cytochrome P4502D6 (CYP2D6) protein is highly expressed in the liver, but is also found in the brain, intestines, and lymphoid cells. CYP2D6 has several endogenous substrates, such as tyramine in the brain (96, 97).

Although CYP2D6 only accounts for up to 5% of the hepatic cytochrome amount (98), CYP2D6 is a major drug-metabolizing enzyme, metabolizing around 25% of all drugs in clinical use, including tricyclic antidepressants, serotonin selective reuptake inhibitors, opioids such as codeine and tramadol, beta blockers, antihistamines, antiemetics such as ondansetron, antiarrhythmics, antiviral agents and tamoxifen (96, 99-101).

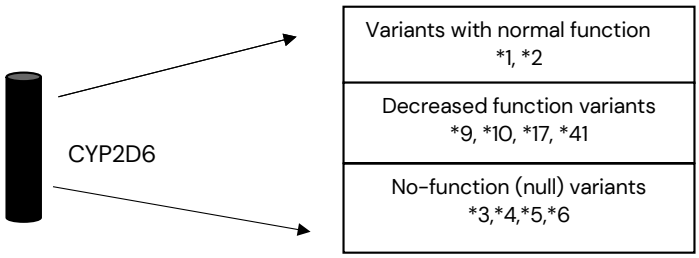
In the 1960s large variations in plasma levels of the same dose of the antidepressant nortriptyline was shown (102). In the 1970s, variation in metabolism and side effects to the antihypertensive drug debrisoquine and the antiarrhythmic drug spartein was found. In 1985 an extremely high metabolic activity in a patient requiring remarkably high doses of the antidepressant nortriptyline to obtain therapeutic plasma levels of the drug was reported. In 1989 the CYP2D6 locus on chromosome 22q13.2 was described (96, 101).

The CYP2D6 gene is very polymorphic. More than 170 allelic variants are documented (103). Variants within CYP2D6 genes are designated a star (*) number. Most of these variants are a result of single nucleotide variants ( i.e. a DNA base in the CYP2D6 gene is changed compared with the normally occurring base) or small insertion or deletions (104). The CYP2D6 gene can be duplicated or multiplicated. As many as 13 copies of a functional allele has been described (102). CYP2D6 can also harbor deletions. The metabolic activity of the CYP2D6 enzyme ranges from no function to increased functionality due to the interindividual variation in the CYP2D6 gene (9).

### 1.5.1 CYP2D6 alleles and genotype

An allele is a version of a DNA sequence at a certain location (locus). An individual has two CYP2D6 alleles, one on each chromosome (one allele inherited from the mother and one from the father). This allele combination defines her / his genotype (105). The most common CYP2D6 alleles are presented below (Figure 1) (9).

Figure 1. Summary of the most important CYP2D6 alleles (9).



Reduced CYP2D6 activity may be due genetic variations rendering the enzyme unstable (CYP2D6*10) or with reduced affinity (CYP2D6*17). Abolished CYP2D6 activity can be explained by gene deletions (CYP2D6*5), single nucleotide variants causing altered splicing, deleterious amino-acid changes, introduction of stop codons, causing no functional protein and thus no functional enzyme, whereas duplications of active CYP2D6 alleles lead to increased enzymatic activity. Functional copies are most common (96, 102, 104, 106). The metabolic activity of the different combination of variants ranges from no to increased function (9).

### 1.5.2 CYP2D6 Phenotypes

The individual CYP2D6 genotype is translated into a predicted phenotype, i.e. CYP2D6 activity. CYP2D6 activity groups are defined as follows: poor metabolizers (PM), who are not able to metabolize or activate drugs via CYP2D6, intermediate metabolizers (IM), who exhibit decreased CYP2D6 activity compared with individuals with a normal phenotype, (NM), with a normal enzyme activity (9). The term extensive metabolizers (EM) has also been used for this group (106). Ultra rapid metabolizers (UM) exhibit an increased CYP2D6 activity (9).

Initially, the different CYP2D6 activity groups were defined using so called probe substrates for CYP2D6. CYP2D6 genotyping has subsequently become the most common method to predict the activity in CYP2D6 (107). Individuals with two nonfunctional alleles have been classified as PM, individuals with a combination of one functional variant or two reduced function alleles as IM, carriers of two functional alleles (e.g. CYP2D6 *1/*1) as NM, while UM carry multiple functional CYP2D6 alleles (101).

More recently, an alternative system for translating CYP2D6 genotypes into predicted phenotypes, using an activity score (AS), has evolved. In this system each CYP2D6 allele is assigned an AS of 0 to 1, based on its function. The sum of the scores assigned to each variant is used to predict an individual´s CYP2D6 activity. No function alleles have been given the value 0, whereas decreased function alleles traditionally have been assigned an AS of 0.5, (i.e an estimated 50% reduced activity compared to fully functional variants) and normal function alleles the value 1. If an individual has multiple copies of a functional CYP2D6 allele, the additional copy or copies are also counted when assessing the total AS (9). The metabolic activity in individuals with an extra copy of a functional allele, i.e. three instead of two functional copies, corresponds to 150% compared to those with two functional copies. Previous investigations have differed whether individuals with an AS of 1 were defined as NM or IM (11). The need for reevaluation of the AS assigned decreased function alleles has been discussed (108). Downgrading the predicted AS of the reduced function variant CYP2D6*10 from 0.5 to 0.25 has recently been suggested (108). According to the latest consensus guidelines by the Clinical Pharmacogenetics Implementation Consortium (CPIC) and the Dutch Pharmacogenetics Working Group (DPWG), individuals with an AS sum of 0 are defined as PM, those with a sum over 0, but

less than 1.25 as IM, while those with an AS over 1.25 but less than 2.25 are considered NM, and an AS score exceeding 2.25 defines UM (Table 2) (108).

Table 2 summary of classification of CYP2D6 phenotypes, i.e CYP2D6 activity groups (9, 101),(108)

| **CYP2D6 activity groups** | **Poor metabolizers (CYP2D6 PM)** | **Intermediate metabolizers (CYP2D6 IM)** | **Normal metabolizers (CYP2D6 NM)** | **Ultrarapid metabolizers (CYP2D6 UM)** |
|---|---|---|---|---|
| CYP2D6 genotype | 2 non-functional alleles | 1 functional variant or 2 alleles with reduced function | 2 functional alleles | >2 fully functional alleles |
| AS according to current CPIC/DPWG guidelines (108) | AS = 0 | 0 > AS < 1.25 | 1.25 > AS <2.25 | AS > 2.25 |
| **CYP2D6 metabolic activity** | No activity | Reduced activity | Normal activity | Increased activity |

1.5.3 Frequency of CYP2D6 alleles

The frequency of CYP2D6 variants varies between different populations (99, 100). The CYP2D6*2 allele is the most common variant globally. CYP2D6*10 is most frequently found in African and Asian populations, while CYP2D6*3 and CYP2D6*6 are distinct for individuals of European ancestry (99). CYP2D6*4 is the most frequent null variant in Caucasians. CYP2D6*4 has been found in around 21 %, CYP2D6*5 in around 4% and CYP2D6*41 in 7% of the Swedish population (109),(110).

Reports on the prevalence of CYP2D6 phenotype groups, in particular IM and NM, vary not only between different populations, but also based on varying definitions in previous studies. Roughly 60-90% of the population word wide is predicted to be NM (100). Around 5-9 % are suggested to be PM and UM range between 1% and roughly 20% (100, 111). CYP2D6 UM are more frequent in certain African populations (99, 102, 111). The reported frequencies of PM and UM in Sweden are approximately 7% and 1-2% respectively, while up to 35-40% have been estimated to be IM (102, 110, 112).

The impact of CYP2D6 activity depends on how much of the drug is activated or eliminated by CYP2D6 in comparison to other pathways. CYP2D6 polymorphism is of clinical significance for many CYP2D6-metabolized drugs. The individual's CYP2D6 activity may not only affect the efficacy but also the safety of the drug. CYP2D6 PM may have several-fold increased exposure to drugs primarily metabolized by CYP2D6, such as

risperidone and metoprolol, and thereby an increased risk of side effects without relevant dose adjustments (113). In contrast, CYP2D6 PM have an insufficient effect of codein at standard doses, while UM have an increased risk of side effects. CYP2D6 UM may also need higher doses of antidepressants metabolized via CYP2D6 to achieve a therapeutic effect. Pharmacogenomic guidelines with therapeutic recommendations for more than 20 drugs involving CYP2D6 have been developed (96) (101).

### 1.5.4 Methods for detection and interpretation of CYP2D6 genotype

Several methods may be used to genotype and phenotype CYP2D6 (114). Direct phenotyping, measuring the real-time activity in CYP2D6 has been used since the late 1970s. A "probe drug" metabolized by CYP2D6, such as dextromethorphan, is administered, followed by measurement of levels of the unchanged drug its metabolites in urine or plasma to determine the urinary metabolic ratio (115). This method is generally considered the golden standard method for measuring CYP2D6 activity, as it reflects the combined effects of the genotype, environmental and endogenous factors on the individual metabolic activity (115). The major disadvantage of this method is the cumbersome procedure and the duration for results to be finalized (113).

In 1990, the first test for detecting variation in CYP2D6*3 and *4 was reported. Development of the polymerase chain reaction (PCR) and Sanger sequencing enabled detection of additional variants (105). These methods have subsequently generally been replaced by newer techniques (114). To date there are no consensus on which alleles should be tested (105).

Real time TaqMan PCR is commonly used to genotype CYP2D6. In the first step allele-specific PCR primers labeled with fluorescent probes are included. If the specific target DNA sequence is present, the probe is cleaved by Taq DNA polymerase. During the following real time PCR-process, fluorescent dye is released if the probe recognizes and binds to the specific gene sequence. The fluorescent signals are detected as they accumulate during the PCR cycles. Reactions are characterized by when amplification of a specific target is initially detected. The signals are quantified and various CYP2D6 alleles can thereby be discriminated (96, 116). This method has a high sensitivity and specificity in identifying known CYP2D6 alleles, there are several commercial assays, the cost is relatively low and a venous blood sample from the patient to secure DNA can be used. Small nucleotide changes, insertion and deletion of alleles can by this manner be identified (96, 101). A disadvantage is that most routine panels do not include rare variants with abolished CYP2D6 activity and that assays used in different laboratories may differ in the variants they detect. Different probes are needed for detection of the different alleles. Contamination of DNA can happen, resulting in false signals (113, 117).

CYP2D6 copy number analysis (CNA) is generally also performed by real time PCR. The detected signal is used to quantify the number of gene copies. The normal copy number

(CN) is two. A CN less than two indicates a gene deletion. This is interpreted as the person being either a homozygous carrier (CN=0) or heterozygous carrier (CN=1) of CYP2D6*5 (96, 101).

Microarray assays detecting single nucleotide polymorphisms in CYP2D6 are also used. Some assays do not include analysis of deletions or duplications and structure variants, while more specialized products can detect copy number variations (96).

Next-generation sequencing of the entire CYP2D6 gene is an emerging method (96, 117). Challenges of this method include accurate determination of CYP2D6 alleles contra interfering pseudogenes, characterization of structural variants, and the interpretation of new or rare alleles (117).

### 1.5.5 Bioactivation of tamoxifen by CYP2D6

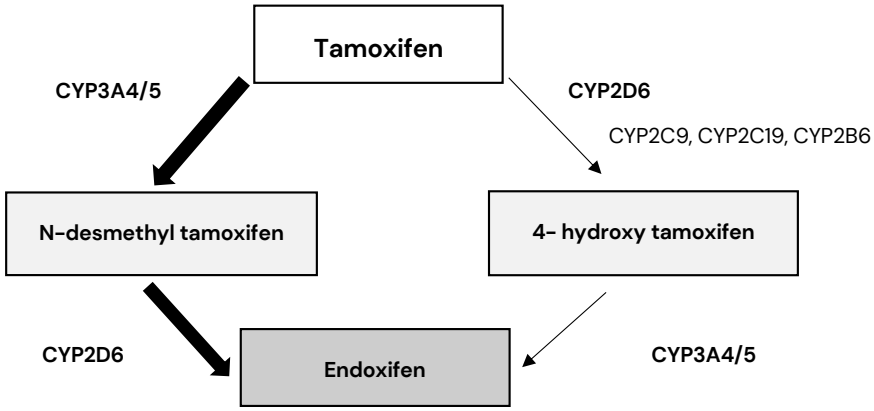



Figure 2. Tamoxifen metabolism. Tamoxifen is activated to the more potent metabolite endoxifen in a metabolic pathway largely dependent on CYP2D6. Simplistic figure based on Jin et al (118).

Tamoxifen is a prodrug with a weak antiestrogenic effect. After ingestion some metabolism takes place in the small intestine, but the main metabolism takes place in the liver (119). Bio-activation by hepatic CYP2D6 enzyme converts tamoxifen to more potent antiestrogenic metabolites, in particular endoxifen (Figure 2) (3). In the predominant metabolic pathway, responsible for more than 90% of tamoxifen's metabolism, tamoxifen is demethylated primarily by Cytochrome P4503A4/5 (CYP3A4/5) into N-desmethyl tamoxifen, which is converted by CYP2D6 into endoxifen.

In the other metabolic, pathway CYP2D6 hydroxylates tamoxifen into 4-hydroxytamoxifen (4-OH-tamoxifen), with a small contribution by Cytochrome P4502C9 (CYP2C9), Cytochrome P4502C19 (CYP2C19) and Cytochrome P4502B6 (CYP2B6). 4-OH tamoxifen is converted by CYP3A4/5 to endoxifen (3, 9).

Tamoxifen is absorbed quickly and maximal serum concentration is reached in 4-7 hours. Steady state is achieved in around 4 weeks. Tamoxifen´s half-life is around 5-7 days. Endoxifen's half-life is 49-68 hours (120). Tamoxifen´s metabolites are further inactivated by hepatic uridine glucuronosyltransferases and sulfotransferases, so they can be excreted via bile or urine (119).

### 1.5.6 CYP2D6 activity and outcome in tamoxifen treated early breast cancer

Varying tamoxifen activation due to genetic variation in CYP2D6 has been proposed to affect the effect of the treatment and that patients with poor CYP2D6 activity might benefit less from the treatment (4-7). Many studies have reported on the influence of varying CYP2D6 activity on the effect of postoperative tamoxifen since the study by Goetz and colleagues in 2005, where CYP2D6 PM were found to have a poorer outcome (121). Results have however been contradictory (8, 122-124). CYP2D6-genotyping to predict tamoxifen efficacy is therefore currently not generally implemented in clinical routine (8).

Explanations for the varying results have been debated. Methodological issues may be important. For instance, two large studies, ATAC and BIG 1-98, that did not find an association between CYP2D6 genotype and prognosis in tamoxifen treated patients genotyped tumor DNA (122, 123). Loss of heterozygosity due to chromosomal instability is common in breast cancer. Tumors may not correctly reflect germline CYP2D6 and can result in misclassified CYP2D6 activity. CYP2D6 genotyping is therefore recommended on DNA from blood or from saliva (9). The CYP2D6 alleles which were analyzed in previous studies have varied. Extensive testing of the most important CYP2D6 variants is important (8, 124). Patient groups in most previous studies have been either mixed or included only postmenopausal patients (8, 122-124). Most studies have not accounted for adherence or concomitant potent CYP2D6 inhibitors (118, 125). The duration of tamoxifen treatment has varied, the study design may have been retrospective or prospective, endpoints and follow up time have also differed (120).

In our previous study in almost 400 Swedish early breast cancer patients, diagnosed 1998-2000, who were recommended 5 years of adjuvant tamoxifen, we studied the influence of CYP2D6 activity on prognosis. When focusing on patients medicating with tamoxifen for at least a year (313 patients), we found a correlation between reduced CYP2D6-activity (i.e. ≤50% of normal) and relapse and also breast cancer specific mortality ($p = 0.025$ and $p = 0.034$ respectively). In a subgroup analysis, the effect of

CYP2D6 activity on recurrence and breast cancer specific mortality remained only in the premenopausal group consisting of 70 patients (p = 0.014 and p = 0.043 respectively) (4). This represented a new finding. Later data from Saladores and colleagues also suggest that CYP2D6 activity predicts prognosis in premenopausal tamoxifen treated early breast cancer (126). More data is needed to determine the value of CYP2D6 testing for patients recommended adjuvant tamoxifen.

### 1.6 Endoxifen – tamoxifen's principal active metabolite

Initially, 4-hydroxytamoxifen (4 OH-tamoxifen) was thought to be tamoxifen's primary active metabolite, as 4 OH-tamoxifen was found to be an up to 100-fold more active antiestrogen than tamoxifen (127). Later endoxifen, with the same antiestrogenic activity, was reported to reach up to 10 times higher plasma concentrations than 4 OH-tamoxifen, indicating endoxifen as tamoxifen's major active metabolite (128). Moreover, endoxifen, in contrast with 4 OH-tamoxifen, targets the ER directly, inducing proteasomal degradation of the receptor (119, 125) blocking ER- mediated cancer cell growth (120). Data from an in vitro report indicates that this anti proliferative effect of endoxifen is highly concentration dependent (119).

Increasing CYP2D6 activity is correlated with increasing concentrations of endoxifen in plasma. Low endoxifen levels are seen in poor metabolizers (129-131). CYP2D6 PM and IM achieve around 75% and 60% lower levels of endoxifen concentrations, respectively, compared to CYP2D6 NM (120). The impact of the various allele combinations in CYP2D6 IM on endoxifen formation is not extensively investigated (11, 12, 132, 133).

There is currently no settled target range for plasma endoxifen (107, 126, 134-137). Two previous investigations have proposed a threshold at 5.9 ng/mL (134) and 5.2 ng/mL respectively (126). The first, a retrospective study by Madlensky et al. in pre- and postmenopausal tamoxifen treated early breast cancer, divided endoxifen concentrations into five levels. Those in the lowest level ( i.e. 20% of the patients) had a 26% lower DFS rate than the other quintiles (134). Hence, the putative threshold for endoxifen at 5.9 ng/mL is a statistical cut-off from this study. In the second, smaller retrospective study by Saladores et al. on premenopausal patients, study participants were divided into four groups according to endoxifen levels. Endoxifen levels in the lowest quartile, below 5.2 ng/mL, were associated with a higher risk of metastasis compared with endoxifen concentrations in the highest quartile, above 12.9 ng/mL (126). Data from a third retrospective study, by Helland et al., indicate that individuals with endoxifen levels below 3.3 ng/mL have a poorer outcome compared to those with higher concentrations (138). One prospective study did not find a correlation between CYP2D6 genotype, endoxifen levels and outcome (139), possibly due to short follow up. Another prospective investigation found a correlation between

endoxifen levels roughly corresponding to the suggested thresholds of around 5 ng/mL and event free survival, but only when dichotomizing endoxifen concentrations (131). A very small study in 48 patients observed a higher risk of relapse in patients with endoxifen concentrations above 70 ng/mL (140). This upper threshold has not been confirmed in other investigations.

### 1.6.1 Endoxifen levels and side effects

Although endoxifen levels were not found to be correlated with severity of hot flashes in tamoxifen treated patients in a few studies (141, 142), other reports suggest that patients with higher endoxifen concentrations are more prone to experience side effects (143-145). CYP2D6 UM, who generally have higher endoxifen levels (130), seem to have more side effects at the standard dose of tamoxifen (146).

### 1.6.2 Tamoxifen dose escalation and endoxifen levels

Studies on CYP2D6 PM and IM treated with increased tamoxifen doses have shown higher concentrations of endoxifen - without increased short term severe side effects. The effect of dose escalation in PM however seems to be more limited (147-156) (table 3). Further data on potential long-term benefits and adverse effects of dose escalation are needed.

Table 3. Summary of studies in early breast cancer on tamoxifen dose escalation.

| **Study** | **N** | **Study design** | **Results** |
|---|---|---|---|
| Maggadani et al., 2021 (156) | 151 | CYP2D6 IM (n=26): Tamoxifen 40 mg daily.<br>NM: 20 mg. | -Endoxifen levels as in NM on 20 mg.<br>-Increased dyspareunia and lowered libido after dose increase, other side effects similar. |
| Braal et al., 2021 (149) | 145 | Tamoxifen escalated to max 40 mg/ day, in patients with endoxifen< 16 nM. | -Endoxifen ≥ 16 nM in 89% of patients but only in 1/3 of PM.<br>-Side effects not increased. |
| Khalaj et al., 2019 (150) | 134 | CYP2D6NM: tamoxifen 20 mg.<br>CYP2D6IM: 30 mg/day.<br>CYP2D6IM/PM (AS 0.5, n=2): 40 mg/day. | - Endoxifen levels in IM, IM/PM after dose escalation as in NM on 20 mg.<br>-Hot flashes, severe side effects not worse. |
| Fox et al., 2016 (147) | 122 | Tamoxifen dose increased up to 60 mg in patients with endoxifen < 30 nM. | -Endoxifen > 15 nM in IM/EM/UM, 60% of PM.<br>-No association of endoxifen levels and hot flashes. |
| Welzen et al., 2015 (151) | 42 | CYP2D6 IM/PM (n=16): Tamoxifen dose increased to 40 mg /day.<br>EM/UM: 20 mg. | -Endoxifen levels increased.<br>-Dose escalation in PM insufficient to increase levels comparable to EM.<br>-Side effects not deterred. |
| Martinez de Dueñas et al., 2015, 2023 (154, 157) | 249 | CYP2D6PM (n=11): tamoxifen dose increased to 40 and 60 mg, 4 months each, then 20 mg daily up to 5 years.<br>IM/EM/UM: 20 mg. | -Endoxifen levels in PM as in EM at 20 mg after both levels of dose escalation, without increased side effects.<br>-No effect of dose escalation on clinical outcome. |
| Hertz et al., 2016 (152) | 353 | CYP2D6PM and IM: tamoxifen increased to 40 mg/day.<br>CYP2D6 EM/UM: tamoxifen 20 mg/day | - IM: endoxifen levels comparable to EM/UM at 20 mg. Levels in PM significantly lower<br>-Side effects not deterred. |
| Dezentjé et al., 2015 (148) | 24 (12 IM, 12 PM) | Doses of tamoxifen increased up to 120 mg/day. | - IM escalated to in mean 45 mg, PM to 90 mg. Endoxifen levels in IM and 80% of PMs then as in NM. All patients > 5.97 ng/ml.<br>-Side effects not deterred. |
| Kiyotani et al., 2012 (153) | 98 | IM: tamoxifen dose increased from 20 to 30 and 40 mg/day. | -Endoxifen levels in IM after dose escalation comparable to levels in NM at standard dose.<br>-No difference in side effects. |
| Irvin et al., 2011 (155) | 119 | EM: tamoxifen 20 mg / day<br>IM/PM: 40 mg daily. | -Endoxifen levels higher after dose escalation than in PM, IM at standard dose. Levels in PM significantly lower.<br>-No difference in side effects. |

### 1.6.3 Influence of other metabolizing enzymes on endoxifen levels

The current definitions of CYP2D6 activity only explain around 35-50 % of the variability in endoxifen concentrations (9). The background for this is not completely understood. Genetic variation in other minor enzymes involved in the activation of tamoxifen, such as CYP2C19, CYP2C9, CYP2B6, CYP3A4/5 as well as sulfotransferases and UDP-glucuronosyltransferases, involved in the inactivation and elimination of tamoxifen, endoxifen and 4 OH tamoxifen, might also to some extent contribute to the varying concentrations of endoxifen and the other metabolites (3, 8, 9, 129).

Tamoxifen as well as the other metabolites do to some extent have inhibitory effects at the ER (158). Apart from 4 OH tamoxifen, their concentrations in vivo have in general been too low to show a significant antagonistic effect (119). In the study by Helland et al. a threshold for 4 OH tamoxifen at roughly 3 nM was suggested (138).

#### 1.6.3.1 CYP2C19

The CYP2C19 gene is also involved in the tamoxifen pathway, converting tamoxifen to 4-hydroxytamoxifen (Figure 2) and is also involved in the metabolism of estrogen and progesterone (159). CYP2C19 is highly polymorphic. The most common allele with no enzyme activity is CYP2C19*2. CYP2C19*17 results in ultrarapid metabolism (109). The frequency of CYP2C19*2 and CYP2C19*17 in Sweden is around 14% and 19% respectively (109). Data indicate that individuals with increased CYP2C19 activity generate higher concentrations of active tamoxifen metabolites (119). Data on the effect of CYP2C19-polymorphism and survival in patients with postoperative tamoxifen therapy are contradictory, so the clinical value of CYP2C19 testing for tamoxifen treatment is to date unclear (8, 160).

#### 1.6.3.2 Nuclear factor 1B (NFIB)

Currently unknown genetic variants, epigenetic regulation and transcriptional regulators may also account for some of the variability in CYP2D6 activity (96). The polymorphic nuclear factor 1b (NFIB) has been reported to be involved in tumor growth and in the regulation of pharmacogenes, including CYP2D6. NF1B inhibits CYP2D6 expression. One study reported that individuals carrying a NF1C allele had a higher activity in CYP2D6 than those with a NFIB TT genotype (161).

### 1.6.4 Non genetic factors that may affect levels of endoxifen

Apart from the dose of tamoxifen, adherence, concomitant medication with CYP2D6 inhibitors and circadian rhythm are non-genetic factors that might contribute to the variability in endoxifen levels. Some studies have reported higher endoxifen levels with

increasing age, while lower levels have been observed with increasing body mass index (BMI) and during the winter season (120, 125, 162).

#### 1.6.4.1 CYP2D6 inhibitors

Several drugs can inhibit the activity in CYP2D6. Co-medication with CYP2D6 inhibiting drugs may result in an individual having a less active CYP2D6 phenotype than predicted by their genotype (120, 129). The antidepressants bupropion, fluoxetine and paroxetine are known strong inhibitors of CYP2D6, duloxetine and the anti-fungal terbinafine are examples of moderate inhibitors. The antidepressant citalopram exerts weak inhibition of CYP2D6 (163).

Although co-mediation with potent CYP2D6-inhibiting drugs, such as paroxetine or fluoxetine has been found to reduce endoxifen concentrations, data on the influence of CYP2D6 inhibitors on outcome in tamoxifen treated patients are inconclusive. A recent review did not find a negative effect on prognosis in patients with concurrent tamoxifen and antidepressant treatment (164). Studies have varied in the potency of included CYP2D6 inhibitors, definitions of overlapping treatment and only a few have specified the patients' CYP2D6 activity (125, 164). The effect of herbal remedies on CYP2D6 is insufficiently studied. Bush mint has been found to inhibit CYP2D6 (11). Data suggest that curcumin, a component in turmeric, sesamin and the herb goldenseal, have a CYP2D6 inhibiting effect. To date there are no recognized CYP2D6 inducing drugs (96).

### 1.6.5 Measuring endoxifen in plasma

Liquid chromatography–tandem mass spectrometry is the main method used to measure plasma concentrations of endoxifen (165). This method separates, identifies and quantifies components in a mixture. Sample components separate as they flow through a column into a detector where target analytes are detected based on an electrical signal generated by specific properties. The detected analyte signal is translated into a chromatogram and the metabolite can thus be quantified (166).

## 1.7 Mechanisms for non-metabolic endocrine resistance

Tamoxifen resistance may also affect the response to endocrine therapy. Tamoxifen resistance is complex and can arise when the ER is no longer expressed or when mutations affecting the ER gene, such as ESR1, occur, causing uncontrolled ER signaling independent of estrogen. Changes in transcription activating factors that interact with the ER, overexpression of cell cycle regulators such as cyclin D and minichromosome maintenance proteins are other proposed mechanisms. Hormones, cytokines,

epigenetics, and growth factors also play a role in the development of endocrine resistance in breast cancer cells (167-170).

### 1.8 Adherence to tamoxifen

#### 1.8.1 Definition of adherence

Adherence, persistence and compliance are terms used to describe how patients follow treatment recommendations. According to The International Society for Medication Adherence, adherence defines the "the process by which patients take their medications as prescribed" (171). Compliance is generally regarded to be synonymous with adherence but is also thought to reflect the patient´s obedience in following the prescribing physician´s recommendation (172, 173). Persistence defines the duration of treatment. Non persistence equals premature discontinuation (173, 174).

#### 1.8.2 Methods for assessing adherence to adjuvant endocrine treatment (AET)

While most studies have used retrospective information from prescription and other medical databases, some have obtained retrospective information from medical records. Interviews and patient self-reports have also been used. Data has also been collected from prospective trials, where adherence may not have been the primary outcome (175) (172, 176). A few reports have included measurements of tamoxifen or endoxifen levels as surrogate markers of tamoxifen adherence (126, 162, 177).

The definitions of adherence and discontinuation vary. Medication possession ratio (MPR), estimating the proportion of prescribed days' supply of the medication under a specified period, is frequently used to assess adherence to antihormonal treatment. The lower limit of 80%, i.e., at least 80% days covered by the medication, is commonly used to define adherence to AET (172, 175, 176, 178). Discontinuation is often defined as a treatment lapse longer than a specified period, which has ranged from around 45 to 180 days in previous reports (172). In Sweden 3 months' supply of a drug can be dispensed at a time. A gap of 180 days indicates that two dispenses were missed, which may result in a shortage of the drug.

All methods have limitations. Patient reports and questionnaires are easy and inexpensive but are susceptible to recall bias. Adherence might be insufficiently documented in medical records. Pill counts can be distorted (173). All these methods tend to overestimate adherence. Adherence rates are generally higher in clinical trials, due to the selection of patients and the attention study patients receive. Measurements of tamoxifen and its metabolites to address adherence needs to be further studied. Dispensing information is usually considered the most objective method of exposure to

a certain medication. Large cohorts with long follow up are facilitated. Dispensing databases do however not contain clinical information and prescription refills do not guarantee that the patient has actually taken the medication (178) (173).

### 1.8.3 Adherence rates to adjuvant endocrine treatment

Previous investigations have described reduced adherence within the first year, decreasing over time, with rates of adherence varying from around 30 to 90% at 5 years (171, 172, 176). The wide span of adherence to AET may partly be explained by the varying methods and definitions of adherence and discontinuation. Comparing data is difficult. Few studies have used several methods comparing adherence to AET in the same cohort (171, 179, 180).

In several trials with AET adherence rates have been relatively high. In the National Surgical Adjuvant Breast and Bowel Project B-14 trial, where patients received adjuvant tamoxifen or placebo, adherence rates were nearly 80% in both study arms at 5 years. In another similar study, 5-year adherence to tamoxifen was around 70% (181). Rather surprisingly considering the side effect profile, adherence to AIs has been found to be comparable or slightly better compared with tamoxifen in some studies (181).

In a previous Swedish register study, a third of the patients were non-adherent to their adjuvant endocrine treatment (AET) after three years (182), in two other around half of the patients completed the recommended treatment (183, 184). In other Swedish reports, around 80-90 % of the patients were defined as adherent at 5 years (185-187) (Table 4).

Table 4. Summary of Swedish studies on adherence to adjuvant endocrine treatment (AET).

| Author, year | N | Year of BC diagnosis, region, age at diagnosis | AET type | Adherence, Non adherence definition | Follow up, years | Study design, data sources | Adherence rates |
|---|---|---|---|---|---|---|---|
| Markkula 2012 (188) | 417 | 2002-2010, Southern Sweden, 25-99 | Tam, AI, switch OK | Non adherence: AET stopped at 1 or 2-year follow up / pause in AET > 20% of study period | 2 | Prospective study<br><br>Patient notes, questionnaire | 91% at 1-year, 90% at year 2 |
| Wigertz 2012 (182) | 1741 | 2005, Uppsala, Örebro, Stockholm, Gotland <40 - > 80 | Tam, AI, switch OK | Adherence: MPR ≥ 80% Nonadherence: > 180 days between refills | 3 | Retrospective study<br><br>Registry data | 69% at 3 years |
| He 2015 (183) | 3395 | 2005-2008, Stockholm, Gotland, <40 - > 65 | Tam, AI, switch OK | Non adherence/ discontinuation > 180 days between 2 dispenses | 5 | Retrospective study<br><br>Registry data, questionnaire | 46% at 5 years |
| Lundgren 2018 (186) | 634 | 2009-2012 Jönköping region, <40 - > 80 | Tam, AI, switch OK | Adherence: MPR ≥ 80% | 5 | Retrospective study<br><br>Registry data | 91% at year 3. 92% after 5 years |
| Wulaningsih 2018 (187) | 4645 | 2006-2009 Stockholm, Gotland, Uppsala, Örebro, Northern region, <50 - > 65 | Tam, AI, switch OK | Adherence: MPR > 80% | 5 | Retrospective study<br><br>Registry data | 79% at 5-year follow up |
| Andersson 2019 (185) | 21016 | 2008-2010 Nationwide 41-74 | Tam, AI, switch OK | Adherence: MPR ≥80% | 5 | Retrospective study<br><br>Registry data | 88% at 3 years, 83% after 5 years |
| He 2019 (184) | 5098 | 2001-2008 Stockholm, Gotland 40-69 | Tam, AI, switch OK | Non adherence: >180 days since last filled prescription | 5 | Retrospective study<br><br>Registry data | 51% at 5 years |

### 1.8.4 Factors affecting adherence to AET

Side effects are the major reason for discontinuing AET (176, 183, 189). Other factors associated with poorer adherence include older or younger age, increasing costs for the patient, not having follow up with an oncologist, switching from one form of endocrine treatment to another, lower perceived necessity of the treatment or perception of a suboptimal role in treatment decisions as well as low social support (176). Being unmarried, having a greater comorbidity or a high educational level, use of analgesics, hormone replacement therapy, hypnotics or sedatives are other factors that have been reported predicting discontinuation of AET (183) (187). Adherence has also been found to vary between urban and rural areas in Sweden (185). A correlation between higher CYPD26 activity and early discontinuation of tamoxifen has been reported (176) (190). Data suggest that CYP2D6 UM are more likely than NM to stop their treatment with tamoxifen at an early stage (146).

### 1.8.5 Adherence to AET and outcome

Poor adherence has in several studies been associated with poorer outcome (191-195). Suboptimal adherence is therefore an important clinical problem. Compared to patients who discontinued endocrine treatment, patients who restarted AET are reported to have a better outcome (196).

## 1.9 Mammographic density

Mammographic density (MD), defined as the radiolucent, i.e."white" area of a mammogram, consists of glandular and connective tissue (24). Women with higher MD have a larger amount of stromal and epithelial cells and less adipose tissue in their breasts (197).

Several factors have been shown to influence MD. Premenopausal women have higher MD than postmenopausal women. MD declines with increasing age. The decline is most distinct during the menopausal transition, in average around 6 $cm_2$ over three years (198) (199). The average yearly decline in density in premenopausal women is around 1% and 0.5 % in post-menopausal women (200). MD decreases with increasing BMI. Genetic predisposition affects MD, as does race. Asian women have the highest MD. There is a positive correlation between higher MD and hormone replacement therapy as well as with consuming a "western diet" and alcohol. In contrast, giving birth at a young age, multi-parity and breastfeeding are associated with lower MD (24, 25, 29, 201, 202).

Mammographically dense areas of the breast have been shown to reflect increased amounts of collagen, immune cells (197) and expression of COX-2 (203), indicating that

these areas may represent an inflammatory environment. ERα has been shown to be more frequently expressed in the mammary stroma in patients with a higher MD (204).

MD is a risk factor for breast cancer (24, 25). Women with the highest density, i.e. at least 75% dense area, have four to six times increased risk of getting breast cancer compared to those with the lowest density, i.e. less than 5% dense area (25). Furthermore, the sensitivity of mammograms decreases with increasing MD, making it harder to identify malignant lesions (205). In one report, the sensitivity of mammogram was only 48% in women having the highest breast density, corresponding to Breast Imaging Reporting and Data system (BIRADS) D (206).

In the US around 7% of women aged 40–74 years have been found to have extremely dense breasts (207). An European study reported that 6% of the women were classified as BIRADS D (208). Currently, MD is not reported systematically in Sweden.

Traditionally, MD has been measured by visual assessment by radiologists using classification systems such as Wolfe, Tabar and the widely used BIRADS density categorization (24). Cumulus, a half-automated technique has also been used (209).

Most previous investigations on MD change have been based on analogue mammograms. The visual assessment methods are highly dependent on reader skill, cannot account for dissimilar breast proportions in the images and are labor intense (210). As digital images have been introduced, commercial programs such as Volpara, Quantra and STRATUS have emerged, providing digital automated assessment (211)(176)(212). STRATUS is an example of an automated program that measures and aligns images to minimize measurement variability (210).

Percent Mammographic Density (PMD) defines the dense area as a proportion of the total breast area on a mammogram (24). Absolute Dense Area (DA) measures the absolute dense and non-dense tissue. PMD is highly, inversely associated with BMI. In contrast, Dense Area (DA) is weakly correlated with BMI (213, 214).

### 1.9.1 Change in MD under tamoxifen treatment

Previous data, including a preventive trial (89) have indicated promising data for reduction in MD as an early indicator of response to tamoxifen. Data indicate that women whose MD decline after initiation of tamoxifen therapy have better outcomes. A reduction of MD between 10% to 20% has indicated a reduced risk of recurrence and mortality (13, 24, 29, 215-220). In one report a decrease in mammographic density of 20% or more reduced breast cancer specific mortality by 50% - an effect that persisted for more than 15 years (219).

MD decline under tamoxifen therapy has been shown to be more marked in patients with higher baselined density and in premenopausal patients (221),(13). Most investigations have used mammograms collected around a year to a year and a half after cancer diagnosis (216). Nyante and colleagues reported that most of the density reduction took place within around a year after the start of tamoxifen treatment (215).

Tamoxifen suppresses the development of alveoli, proliferation of epithelial cells and extracellular matrix turnover in mammary tissue (222). The mechanism of the reduction of MD under tamoxifen treatment is not yet completely explained. CYP2D6 genotype has been found to affect MD decline in one previous report in postmenopausal tamoxifen treated patients (14).

Few investigations have accounted for adherence to tamoxifen when assessing MD change and results are conflicting (215),(218, 223). Most investigations on MD change have also largely been performed in patients with tamoxifen alone either as adjuvant treatment or in breast cancer prevention. Chemotherapy may induce menopause and may be an important explanation for MD decline in younger patients. A few studies have noted a more pronounced reduction of MD following chemotherapy in younger women (217, 223-225). Most studies have not detected an effect of AIs on density change (13, 223, 226, 227). Data on the correlation between adjuvant ovarian suppression and MD change is limited (228). No association between change in mammographic density and radiation therapy is evident (223, 225, 229).

# 2 Research aims

The overall aim of this thesis is to investigate different aspects of tamoxifen treatment to contribute to further personalized endocrine treatment strategies for hormone sensitive early breast cancer, including individualized tamoxifen dosing, to improve adherence and quality of life under treatment, as well as prognosis.

The specific aims of the four studies were as follows:

Study I:

- To explore the association between CYP2D6 genotypes and levels of tamoxifen metabolites in premenopausal breast cancer patients with ongoing postoperative tamoxifen therapy. Our focus was mainly on CYP2D6 variants with reduced function, especially CYP2D6*41, the most frequent reduced function variant in the Swedish population.
- To investigate the association between endoxifen concentrations and reported adverse effects to tamoxifen.

Study II:

- To study the influence of CYP2D6 activity on MD change, in pre- and post-menopausal breast cancer patients with postoperative tamoxifen treatment.
- To investigate the effect of additional systemic adjuvant treatmentent on MD change.

Study III:

- To study the agreement between information from pharmacy refills and medical notes on adherence to adjuvant endocrine therapy.
- To investigate the agreement between pharmacy refill data and medical notes on potential medication with CYP2D6 inhibiting medication.
- To investigate the correlation between menopausal status, CYP2D6 genotypes, risk for relapse and adherence to postoperative endocrine treatment.

Study IV:

- To validate findings from our previous study, where an association between CYP2D6-activity and outcome mainly in premenopausal patients was found, in a larger cohort, subject to improved complex systemic treatment, adjusting for adherence to treatment.
- To determine if the effect of CYP2D6 genotype is affected by menopausal status.

# 3 Materials and methods

## 3.1 Overview of material and methods in this thesis

Table 5. Overview of material and methods used in the four studies in this thesis.

| Study | I | II | III | IV |
|---|---|---|---|---|
| Full study cohort<br>1256 BC patients operated 2006 – 2014 who initiated adjuvant tamoxifen at the Oncology Departments at the Karolinska University hospital/ SÖS, with biobanked DNA | | | | |
| CYP2D6 genotyped, CYP2D6 activity predicted | | | | |
| Study patients | n=118<br>Premenopausal at BC diagnosis, ongoing tamoxifen | n=699<br>> 3 months tamoxifen, digital baseline- and ≥ 1 digital follow-up mammogram | n=1235<br>Initiated tamoxifen as their first AET | n=1105<br>Initiated adjuvant tamoxifen, AIs or GnRH after tamoxifen less than 1 year |
| Specific analyses | Levels of tamoxifen, endoxifen, DM-tamoxifen, 4-OH tamoxifen<br><br>Predicted vs. observed CYP2D6 activity<br><br>Side effects to tamoxifen in relation to endoxifen levels | DA at baseline across age<br><br>Relative DA change under follow up by menopausal status<br><br>Relative DA change at year 1, 2, 5 in relation to treatments and CYP2D6 activity | Adherence to AET: MPR ≥ 80% over 4.5 - 5 years, based on medical notes / dispensing data (n=899)<br><br>Concistency between the 2 sources of information on adherence to AET: Dispensed doses of AET / AET intake in medical records | Association between CYP2D6 activity and clinical outcome |
| Statistical analysis | Descriptive statistics<br><br>Kruskal Wallis<br><br>Linear regression<br><br>Fisher's exact test | Descriptive statistics<br><br>Student's t test<br><br>Fisher's exact test<br><br>Wilcoxon test<br><br>LOESS<br><br>Non-linear b-spline regression<br><br>p-trends | Descriptive statistics<br><br>Fisher's exact test<br><br>Proportion with adequate (80–125%) or poor consistency (<80% or >125%)<br><br>Subgroup analyses based on CYP2D6 activity, menopausal status, recurrence risk<br><br>n, % of patients with CYP2D6 inhibitor by prescription refills and medical notes | Descriptive statistics<br><br>Multivariable Cox proportional hazard models<br><br>Subgroup analyses based on menopausal status, tamoxifen ≥ 1 year, HER2 status, tamoxifen as only endocrine treatment<br><br>Kaplan–Meier analysis |

### 3.2 Study population

Germ-line DNA from blood-samples from newly diagnosed breast cancer patients at Södersjukhuset and the Karolinska University Hospital, Stockholm, Sweden, has been bio-banked for future research since 2006.

The National Quality Registry for Breast Cancer (NKCB), including data on diagnosed breast cancer cases and pre- as well as postoperative treatment was fully established in 2008. Hospitals in the Stockholm Gotland region began reporting to the NKCB in 2007. Before this, corresponding data were registered in the Historic regional breast cancer registry (230). Using the NKCB and the Historic regional breast cancer registry, we identified around 4800 patients operated due to breast cancer in Stockholm between January 2006 and January 2014, who were registered as planned to receive adjuvant tamoxifen treatment. Of these, 1514 had bio-banked DNA. According to an initial medical record review, 258 of the patients received other endocrine treatment, while 1256 initiated adjuvant tamoxifen therapy.

Initially, we included 1249 patients with treatment of more than one month tamoxifen. Later we decided to also include the 7 patients with shorter tamoxifen duration. The full study cohort used in this thesis thus consists of 1256 patients undergoing primary breast cancer surgery between January 2006 – January 2014, with available bio banked DNA and who initiated postoperative tamoxifen treatment at the Departments of Oncology at Södersjukhuset or at the Karolinska University Hospital. All the patients have been genotyped for CYP2D6, as described below.

#### 3.2.1 Study I

511 of the patients in the full study cohort were according to medical records premenopausal at breast cancer diagnosis. 196 of these were still medicating with tamoxifen in January 2017. For practical reasons, only the 190 patients residing in the Stockholm area were sent a written invitation to participate in the study by providing a blood sample for measurement of tamoxifen metabolites. 118 patients were included.

#### 3.2.2 Study II

Patients from the full study cohort who according to medical records had at least three months of upfront tamoxifen treatment alone, or in combination with ovarian suppression, a digital baseline mammogram (i.e. the latest available screening mammogram prior breast cancer diagnosis) and at least one digital follow-up mammogram were included. The follow-up mammogram could be at the earliest 3 months after diagnosis as a

significant decrease in density has been reported after 3 months of tamoxifen treatment (231).

Patients with tamoxifen as second line endocrine treatment (n=14), or who had discontinued tamoxifen before 3 months (n=45), where digital baseline- and / or follow up mammograms were unavailable (n=410), for whom data of BMI was missing (n=45), or CYP2D6 activity could not be defined (n=4), were excluded. As MD measurements were performed on the breast unaffected by breast cancer, patients with a history of contralateral breast cancer or had bilateral breast cancer at baseline (n=39) were also excluded. 699 patients were included.

### 3.2.3 Study III

The full study cohort was used in this study. An updated review of medical records revealed yet one patient who had not initiated tamoxifen treatment. The study cohort thus consisted of 1255 patients who initiated adjuvant tamoxifen therapy. Patients were excluded if they initiated their AET with an AI or ovarian suppression alone (n=20) and not tamoxifen as their first AET, or if their CYP2D6 status was inconclusive (n=5). 1235 patients were included.

### 3.2.4 Study IV

The full study cohort, consisting of the 1255 patients who initiated adjuvant tamoxifen treatment, was used in this study. Patients were excluded if they initiated treatment with an AI or ovarian suppression alone rather than tamoxifen as their first adjuvant endocrine therapy (n=20), or if their CYP2D6 genotype was undecided (n=4). To minimize the compensating effect of other endocrine treatments on outcome, patients receiving AIs and / or ovarian suppression, without tamoxifen, for more than one year during the first 5 years of follow up (n= 126) were excluded. 1105 patients were included.

### 3.3. Methods used in all studies in this thesis

#### 3.3.1 Clinico- pathological data collection from medical records

Data on tumor characteristics, menopausal status and BMI at diagnosis, breast cancer treatment, concomitant relevant CYP2D6 inhibitors, side effects and adherence to endocrine therapy, recurrence and deaths in the full study cohort have retrospectively been collected from patient records at the Oncological Departments at Södersjukhuset and at the Karolinska University Hospital into a database designed for the projects in this thesis. Information from this database has been used in all four studies.

Fluoxetine, paroxetine, haloperidol, duloxetine, levomepromazine, zuclopenthixol, thioridazine, diphenhydramine, amiodarone, quinidine, terbinafine, cinacalcet and bupropion were defined as clinically important CYP2D6 inhibitors. Sertraline, a moderate CYP2D6 inhibitor, was included as local guidelines at the time when data was collected discouraged concomitant medication with Sertraline and tamoxifen.

#### 3.3.2 CYP2D6 genotyping

All patients in the full study cohort were CYP2D6 genotyped on DNA from the bio-banked blood samples. The CYP2D6 genotyping procedure was primarily performed at Diakonhjemmet Hospital in Oslo, Norway by using allele specific validated TaqMan real-time PCR reaction assays. The CYP2D6 genotyping panel is described below (table 6).

Table 6. CYP2D6 genotyping panel at Diakonhjemmet

| **Allele variants** | **Activity** |
|---|---|
| CYP2D6*3, *4,*6 | No function |
| CYP2D6*9, *10, *41 | Reduced function |
| CNA | Gene deletions/ duplications, multiplications |

If none of the described variants were detected, the genotype was defined as CYP2D6*1/*1.

A copy number analysis (CNA) was performed by real-time PCR to detect CYP2D6 gene deletions (i.e. heterozygous (CN=1) or homozygous (CN=0) for CYP2D6*5, or increased-function variants due to extra gene copies (n=3 or 4) of CYP2D6*1. The initial analysis could distinguish 0–4 allele copies, but did not discern whether CYP2D6*1, *4 or *41 was duplicated, leading to a simplified interpretation that the extra allele was fully active. As the genotyping method had certain limitations regarding 3 or more copies, a

supplementary CNA analysis at the Pharmacological Department at the Karolinska Hospital in Huddinge, Sweden, was performed for patients with CN of 3 or more. In study I and II the patients carrying CYP2D6*1/*4, CYP2D6*1/*41, or CYP2D6*4/*41, in combination with CN 3 or 4, were defined as having an inconclusive genotype. As the laboratory in Norway further developed their genotyping method, more conclusive, although not yet validated, information on duplicated alleles apart from CYP2D6*1 was obtained.

For the 7 patients with tamoxifen treatment of less than one month, CYP2D6 genotyping was performed at the laboratory in Huddinge, with a similar gene panel, including CYP2D6*1, *3, *4, *10, *41, *17 and a CNA.

### 3.3.3 Determining the predicted CYP2D6 phenotypes

Each CYP2D6 allele was designated an activity score (AS) according to CPIC guidelines. In summary, no function alleles were assigned the activity value 0, decreased function alleles 0.5 and normal function alleles the value 1. The total activity of the normal genotype CYP2D6*1/*1 with 2 fully functional alleles was set to 2.0 (9). In study III and IV the activity score for CYP2D6*10 was downgraded to 0.25 according to the latest CPIC recommendations (108). The sum of the AS values for each allele was used to classify the patients into predicted CYP2D6 activity groups. In study I, II and IV the latest recommendations from the CPIC and DPWG were used (108): CYP2D6 PM: AS = 0, CYP2D6 IM: AS = 0.25 or 1.0, CYP2D6 NM: AS = 1.5–2.25 and CYP2D6 UM: AS > 2.25. In study II, which was actually the first study we initiated, CYP2D6 PMs were defined as having an AS of 0, CYP2D6 IMs scores of 0.5 / 1.0, CYP2D6 EM/NMs 1.5–2.0, while those with an AS of more than 2.0 were defined as CYP2D6 UM (9).

Based on the findings in study I, a wider definition of the CYP2D6 PM group, where patients with the no function variants CYP2D6 *3/*4/*5/*6 in combination with a reduced function allele, CYP2D6 *9/*10/*41, were categorized as CYP2D6 PM was also used in study II-III. Although CYP2D6*41 is designated an AS of 0.5 in current guidelines, results from recent studies indicate a lower range (232),(233). In study IV, we therefore also used an alternative AS of 0.15 for CYP2D6 *41.

### 3.3.4 Statistical Analysis

Descriptive statistics were used to summarize and characterize the study patients. P-values (2-sided) < 0.05 were in all tests considered statistically significant. 95% Confidence intervals (CI) were also used (apart from study I).

### 3.4 Specific methods for the four studies in this thesis

#### 3.4.1 Study I

3.4.1.1 Measurements of tamoxifen and tamoxifen's metabolites endoxifen, N-desmethyl-tamoxifen (DM tamoxifen) and 4-hydroxy-tamoxifen (4-OH- tamoxifen)

The collected blood samples were centrifuged and the plasma was frozen until analysis. Plasma concentrations of tamoxifen, endoxifen, 4-OH-tamoxifen, and DM tamoxifen were measured by ultra performance liquid chromatography–tandem mass spectrometry (TSQ Quantiva with Dionex ltimate 3000 system, Thermo Scientific, Waltham, MA, USA).

3.4.1.2 Predicted vs observed CYP2D6 activities

The predicted CYP2D6 activity was estimated based on designated AS according to current guidelines for CYP2D6 genotypes with two no function variants, the combination of a no function allele and the reduced function variant CYP2D6*41, two copies of CYP2D6*41 alleles, the combination of the fully functioning allele CYP2D6*1 with a no function variant or CYP2D6*41, 2 copies of the normal function CYP2D6*1 alleles and duplications or multiplications of CYP2D6*1 (108). The AS of 2 copies of CYP2D6*1 was set to 2.0.

The observed CYP2D6 activity was defined as the ratio between plasma levels of endoxifen and DM-tamoxifen. Hereby, interindividual variation in CYP2D6 activity was identified, as CYP2D6 is the only enzyme responsible for metabolizing DM-tamoxifen into endoxifen (figure 2).

3.4.1.3 Side effects to tamoxifen in relation to plasma levels of endoxifen

Data on reported adverse effects to tamoxifen were retrospectively retrieved from medical notes and were graded as follows;

No side effects: no notes of side effects, mild: records stating mild side effects, severe: periods of tamoxifen discontinuation due to adverse effects and / or symptom relieving treatment (except vaginal estrogen) and / or sick leave due to side effects, moderate: notes of moderate side effects.

The four levels of side effects were compared to five endoxifen levels, corresponding to the strata in the study by Madlensky and colleagues.

3.4.1.4 Statistical analysis

Boxplots were used to illustrate the distribution of endoxifen levels for the predicted CYP2D6 genotype groups. The proportion of patients with endoxifen levels below the suggested threshold of < 5.9ng/mL was determined. The nonparametric Kruskal–Wallis test was used to test differences in median endoxifen concentrations between the CYP2D6 genotype groups; homozygous carriers of null alleles versus homozygous carriers of reduced function alleles or those carrying a reduced function allele and a null allele.

A linear regression model was used to analyze the linear relationship between the observed CYP2D6 activity (the dependent variable) and the predicted CYP2D6 activity (the explanatory variable). To focus on CYP2D6*41, the regression line was based on cases who did not carry any reduced function alleles apart from *41. The results were depicted in a scatterplot with the predicted CYP2D6 activity on the x axis and observed CYP2D6 activity on the y axis. The proportion of CYP2D6*41 carriers with a lower activity than predicted by current guidelines, i.e., with an observed CYP2D6 activity below the regression line, was calculated. The test of given proportions was used to compare the proportion of CYP2D6*41 carriers below the regression line to 0.5, i.e to test a deviation from symmetrically distributed activities.

Fisher's exact test was used to compare levels of side effects between the levels of endoxifen concentrations.

Statistical analyses were conducted using R 3.6.1.

### 3.4.2 Study II

#### 3.4.2.1 Mammographic density measurement

MD of the breast unaffected by cancer was assessed at baseline and during follow up. The STRATUS system was used to align images prior measuring and comparing the average MD (210). We chose to assess MD by Dense Area (DA) as we only had knowledge of BMI at breast cancer diagnosis. DA is weakly correlated to BMI (213, 214). DA was calculated by dividing the dense area in the breast by the total breast area, in $cm^2$. We used the mediolateral oblique views, as other views are not routinely used for screening mammography (i.e. the baseline mammography). Mammograms up till January 2018 were used.

#### 3.4.2.2 Statistical analysis

Differences in important characteristics of the included and not included patients from the full study cohort were assessed using Student's t test on continuous variables, Fisher's exact test for the categorical variables and Wilcoxon test for non-parametric data such as CYP2D6 activity.

Local Polynomial Regression (LOESS Curve Fitting), a method for fitting a smooth curve between two variables, was used to describe the average MD at baseline across age (234). The curve was compared to MD across age in women without breast cancer from the KARMA cohort (210). LOESS was also used to map out the average DA decline during tamoxifen treatment by menopausal status during follow up. The study patients contributed with assessment of MD change until the end of follow up or until they discontinued their endocrine treatment. Patients with a later contralateral breast cancer or who performed a contralateral-/ bilateral prophylactic mastectomy after study baseline, were censored at the date of diagnosis or surgery.

Relative density change (DA at follow up minus baseline DA divided by baseline DA) was calculated. As we observed that the density decrease was more pronounced in the early phase of the treatment, we analyzed the mean relative DA decrease during tamoxifen treatment at year 1, 2 and 5 using non-linear b-spline regression. 95% CI were calculated using 1000 bootstrappings. Analyses were stratified by systemic adjuvant treatments (chemotherapy, goserelin, and AIs) and CYP2D6 activity. Non-linear b-spline regression was used to calculate average density change between mammograms 5 years prior to tamoxifen discontinuation until 5 years after. As we observed what appeared to be a trend of density reduction with higher CYP2D6 activity at year 5 in postmenopausal patients with tamoxifen monotherapy, p-trends for CYP2D6 activities were estimated by linear regression in postmenopausal women at year 5.

The statistical analyses were performed using SAS 9.4.

### 3.4.3 Study III

#### 3.4.3.1 Information from the National Prescribed Drug Register in Sweden

The National Prescribed Drug Register in Sweden, linked with personal identity numbers, was established in 2005. The register includes information on all prescribed drugs dispensed at pharmacies in Sweden (235).

Data from the National Prescribed Drug Resister on prescription refills between January 2006 and January 2018 on AET (tamoxifen, AIs and GnRH analogues) and clinically relevant CYP2D6 inhibitors were acquired before the initiation of study III. Information included the Anatomical Therapeutic Chemical Classification System drug classification, product name and strength, pack size, the number of prescribed packages, the date of prescription, the date of dispersion and the Defined Daily Doses (DDD) for each prescription.

#### 3.4.3.2 Definition of adherence

Adherence to AET was defined as a Medication Possession Ratio (MPR) of at least 80% over a follow-up period of 4.5 to 5 years. As it was challenging to determine whether ovarian suppression had been dispensed at the same time or sequentially to tamoxifen, we chose to focus on adherence to tamoxifen and AIs.

#### 3.4.3.3 Definition of consistency

The term consistency was used to describe the agreement between AET exposure based on medical records and the National Prescribed Drug Register. Consistency was defined as the DDD of dispensed of AET / the DDD of AET intake documented in medical notes. Adequate consistency was defined to be within the range of 80 to 125%, inspired by margins used in bioequivalence studies (236) and non inferiority margins, e.g. when comparing duration of anti HER2 treatment (43).

#### 3.4.3.4 Statistical analysis

The proportion of patients with adequate consistency (80–125%) and with poor consistency (<80% or >125%) was calculated and depicted in a scatter plot. Subgroup analyses were performed based on CYP2D6 activity, menopausal status and the estimated recurrence risk. Patients were defined at high risk of relapse if they had positive lymph nodes and /or tumors with high proliferation rate ($Ki_{67}$ >20 / S phase >10%) and / or high grade (III) tumors and / or HER2 positive tumors and / or had received chemotherapy

(30, 237). Fisher's exact test was used to compare frequencies of adherence between groups. The number and proportion of patients medicating with a CYP2D6 inhibitor at least once during follow-up was compared between prescription refills and medical notes.

Statistical analyses were as in study I performed using R 3.6.1.

### 3.4.4 Study IV

#### 3.4.4.1 Information from the National Prescribed Drug Register in Sweden

Data from prescription renewals between January 2006- January 2018 on AET and clinically relevant CYP2D6 inhibitors, were retrieved from the National Prescribed Drug Register in Sweden .

#### 3.4.4.2 Statistical analysis

The Cox proportional hazards model is commonly used for survival analyses. The model estimates the effect of an exposure on time-to-event variables. Hazards ratios (HR), of the outcomes are calculated. Multivariable Cox proportional hazard models were used to investigate the correlation between CYP2D6 activity and relapse or breast cancer specific mortality, with the predicted CYP2D6 activity as a continuous variable. The estimated HR thus referred to a 1-unit increase in CYP2D6 activity, for example from 0 (PM) to 1 (IM). In the model we controlled for the following potential confounding factors: age and menopausal status at breast cancer diagnosis, CYP2D6 inhibiting medication during the first five years, having a high estimated risk of relapse and adherence to tamoxifen. The risk of recurrence was estimated using the prognostic factors described in study III. Adherence was defined as the MPR for tamoxifen and was calculated as the duration of follow-up in days / by the number of dispensed tamoxifen doses during the follow-up period, disregarding doses beyond the end of follow-up. A subgroup analysis, where the main analyses were repeated separately for pre- and postmenopausal patients was conducted. Subgroup analyses were also conducted based on tamoxifen as the only endocrine treatment and on HER2-status, to exclude a possible effect of trastuzumab in our current investigation, as no patients in our previous report had received anti HER2 treatment. Finally, as in our study from 2103 (4), we also did a separate analysis of all patients and for pre- and postmenopausal patients separately, with at least one year's initial tamoxifen treatment, without consideration of adherence thereafter.

The Kaplan–Meier method was used to estimate survival. Patients were as in our previous report (4), divided into two groups according to predicted CYP2D6 activity, i.e. $\leq$ 50% enzyme activity versus > 50% activity, compared with the "normal" activity of CYP2D6*1/*1, and for the four groups of estimated CYP2D6 activity (PM, IM, NM and UM). Time at risk was calculated from the date of tamoxifen initiation. In the analysis of time to relapse, data from patients without a recurrence was censored at the date of their last follow up. In the analysis of time to breast cancer specific death, data was censored at the date of death or on the last date in 2022 when the patients' vital status was determined by review of medical records.

All statistical analyses were performed by using R 3.6.1.

# 4 Results and discussion

**4.1 Study I**

4.1.1 Main findings

114 patients were available for analysis. The median age at breast at study inclusion was 52.5 years. In mean the patients had medicated with tamoxifen for 4.7 years at the time for blood sampling.

CYP2D6*41 was as expected in this predominantly Caucasian cohort the most frequent variant with reduced activity (82%). 2% of the study patients were classified as CYP2D6 ultra rapid metabolizers, 47% as extensive metabolizers, 44% as intermediate metabolizers and 7% as poor metabolizers, using current guidelines (108).

An obvious correlation between CYP2D6 genotype and endoxifen concentrations in plasma was seen (figure 3)

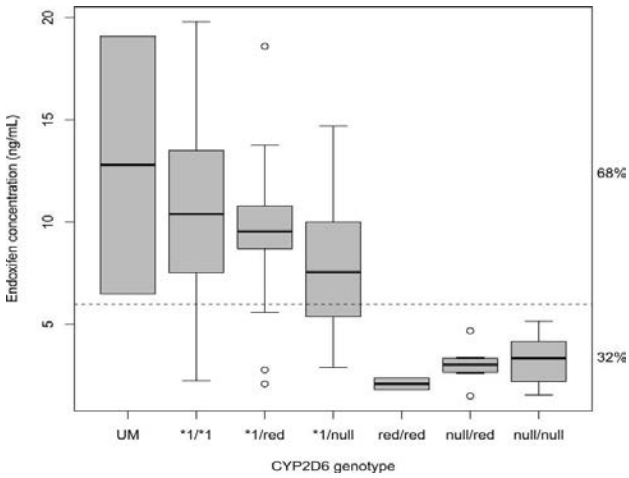



Figure 3. Endoxifen levels based on CYP2D6 genotype groups. The dashed lines depicts the proposed therapeutic threshold at 5.9 ng/mL. Reprinted with permission from the British Journal of Clinical Pharmacology (232).

As depicted in figure 3, in a third of the included women endoxifen concentrations were lower than the putative threshold for tamoxifen effect at 5.9 ng/mL. Endoxifen levels were below this level in all CYP2D6 PM ( carrying 2 null variants) and also in those currently defined as IM ( 2 alleles with reduced activity or one allele with no function plus a reduced-function variant).

81% of the patients carrying CYP2D6*41-alleles had a significantly lower activity in CYP2D6 than predicted. In fact, we did not find a difference between endoxifen levels in patients with 2 CYP2D6*41 copies compared to levels in PM (p = 0.338).

Women whose endoxifen levels were below 5.9 ng/mL, had either no or only mild adverse effects to tamoxifen, while those with higher levels had reports of moderate to severe adverse effects.

### 4.1.2 Discussion

Low endoxifen levels were as expected observed in patients defined as CYP2D6 PM but also in those carrying reduced function CYP2D6 variants, currently defined as IM (108). Specifically, carriers of CYP2D6*41 displayed a lower metabolic capacity than predicted by the current AS of 0.5 (108). Endoxifen levels in homozygous *41, carriers were comparable to levels observed in PM. This observation is supported by recent data on several CYP2D6 metabolized drugs, including tamoxifen, indicating a lower AS ranging from 0.05–0.15 (10) (238). Further corrections of the AS, especially for CYP2D6*41, might thus be relevant. Importantly, the proportion of patients with poor bio-activation of tamoxifen might be larger than currently expected.

A target range for plasma endoxifen is not established (134),(126),(138),(239), so endoxifen testing is currently not recommended in clinical practice. A recent small investigation found a correlation between endoxifen concentrations below 15 nmol/L, roughly corresponding to the suggested thresholds at 5.9 ng/mL (134) and 5.2 ng/mL (126) and a poorer prognosis. No such association was seen using the putative cut off at 3.3 ng/ mL (138). A caveat is that no association was observed when using concentrations of endoxifen on a continuous scale (131). Although the clinical relevance of the proposed target level of endoxifen at 5.9 ng/mL needs to be validated, it is still concerning that a third of the patients in our study had concentrations below this level. This underlines the importance of further prospective studies to define a target concentration of endoxifen and the other tamoxifen metabolites for clinical efficacy.

Patients with a reduced CYP2D6 activity might benefit from a higher dose of tamoxifen. As previous reports have shown that the effect of doubling the dose in PM appears limited (147-156), our results indicate that the number of patients for whom dose escalation would likely not be the best choice, might be larger than expected.

The current definitions of CYP2D6 genotype may explain only around 50 % of the variability in endoxifen formation (9). The background for the substantial variability of endoxifen concentrations observed in the group carrying the normal function allele CYP2D6*1 cannot yet be fully explained but is in keeping with other data (133, 134, 139, 240). Although results from a recent genome wide association study indicate that CYP2D6 is the major genetic regulatory factor for endoxifen levels (158), rare CYP2D6 variants might not have been detected by our genotyping method and genetic variation

in other enzymes involved in the metabolism of tamoxifen may also have contributed to the variability. Further research might identify to date unknown variants or regulating factors affecting CYP2D6 expression (158). Non genetic factors, for example BMI, might also be of importance (120, 125, 162). Data on BMI was in this study collected only from medical notes. Future algorithms integrating clinical and genetic factors to predict endoxifen concentrations might be valuable (119).

Although data on the relationship between side effects and endoxifen levels are inconsistent (15, 141, 142, 144, 241, 242), our observation of increasing side effects with higher endoxifen levels is in keeping with findings from several reports (143, 144),(15, 241, 242). Further prospective studies are needed to evaluate the correlation between levels of tamoxifen metabolites and adverse effects.

An important limitation is the study size. Our results require validation in a larger material. Our focus was on the patients who were premenopausal at diagnosis, as our previous report suggested that CYP2D6 activity seemed to be important for outcome in premenopausal tamoxifen treated patients (4) and there is some evidence that endoxifen levels might increase with age (119, 125). Some of the patients in this study were, however, likely postmenopausal at the time of inclusion. Information on adverse effects were retrospectively collected without a validated measure and information on CYP2D6 inhibitors was extracted from medical records only.

Although therapeutic drug monitoring of tamoxifen and endoxifen testing is to date controversial, individualized tamoxifen dosing based on CYP2D6 genotype and / or endoxifen levels might not only detect patients who might benefit of a higher tamoxifen dose, or another endocrine regimen, but might also be of value for those where high endoxifen levels and severe side effects might motivate a lower dose to improve adherence and quality of life during treatment.

### 4.2 Study II

4.2.1 Main findings

699 patients were included. Tamoxifen was the only endocrine therapy for most of the patients (82%). Twelve percent of the patients were treated with an AI after tamoxifen, 6% of the premenopausal patients received ovarian suppression together with tamoxifen and a very limited portion (1%) of the younger patients had switched to an AI in combination with ovarian suppression. Around a quarter of the patients, mainly premenopausal, received chemotherapy. The duration between the date of breast cancer diagnosis and the last mammogram was in mean 4.9 years. 7% of the study patients were defined as CYP2D6 PM, 36% as CYP2D6 IM, 54% as CYP2D6 EM, while 3% were CYP2D6 UM.

Mean DA in the baseline mammogram was higher in younger patients compared to older. Density at baseline was also higher in the study patients compared to women not operated due to breast cancer. Mean relative DA declined during follow up (Figure 4). The density decrease was most pronounced during the first year and during this period density reduction was more marked in the premenopausal group.

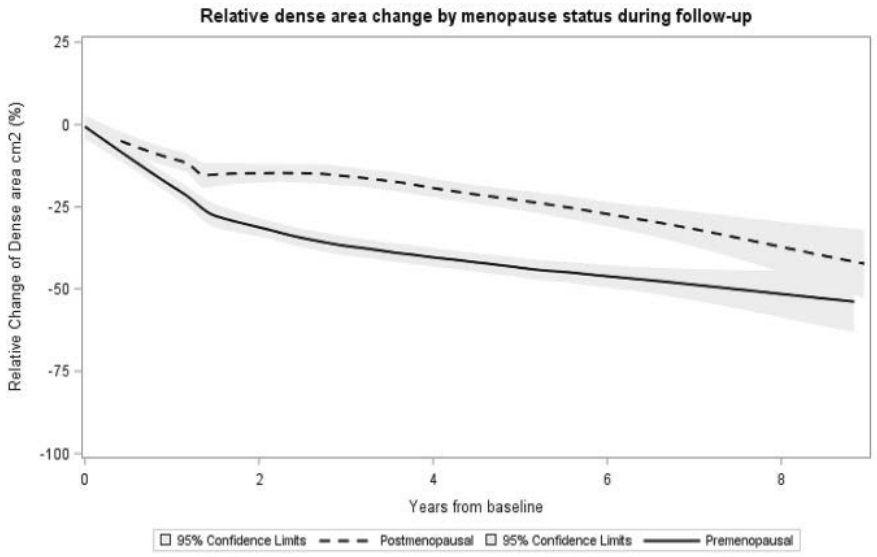



Figure 4. Relative DA change under follow up, according to menopausal status. Reprinted with permission from Breast Cancer Research and Treatment (243).

No significant effect of other systemic treatment, i.e. chemotherapy, AIs, or ovarian suppression on density change was seen. Nor could an impact of CYP2D6 activity on DA change be verified.

No obvious increase in MD after stopping tamoxifen treatment was seen.

4.2.2 Discussion

Having a high breast density is a well-known risk factor for breast cancer (24, 25) and as expected baseline density was higher in our study patients compared to the external non cancer cohort. As expected, we also observed a clear reduction of mammographic density during follow up in this tamoxifen treated cohort. The observed density decline in this study was similar to previous data in tamoxifen treated patients (219). Our findings were also consistent with other reports suggesting that density decline during tamoxifen treatment is larger in premenopausal patients (221) (13) and that MD decline is more distinct during the menopausal transition (198, 199), likely due to a hormonal factor.

Previous studies assessing density change under tamoxifen treatment have mainly included patients with tamoxifen as their only systemic treatment (13, 29), a rare situation in the clinical setting today. The patients in our study could also have received adjuvant chemotherapy, anti HER2 treatment and may have switched endocrine treatment. Previous studies are also heterogenous as for cohort sizes, mammogram modality, MD measures, cut offs for density change, characteristics of study patients and follow up (13, 29, 216). Comparing data can thus be challenging.

No additional effect of other systemic treatment on density decline was apparent. No effect of chemotherapy on density change was seen in the whole study cohort. Only a few reports have adjusted for the effect of chemotherapy on MD change and while most indicate a larger density decrease following chemotherapy in premenopausal women (217, 223-225), these investigations were not designed to study the added effect of chemotherapy on density change in patients also treated with tamoxifen. Moreover, chemotherapy regimens have differed over time. In contrast to another report in a somewhat younger cohort, ovarian suppression did not further reduce MD in our limited goserelin treated material (228). As most studies, we did not detect an effect of AIs on density change (13, 223, 226, 227). We chose not to present data from the subgroup of patients who in addition to chemotherapy received anti HER2 treatment, given the small size and unreliable results.

The earlier indicated correlation between CYP2D6 activity and density change under adjuvant tamoxifen treatment was not confirmed. Our findings suggest that CYP2D6 genotype has no significant modifying impact on MD change in patients with complex systemic adjuvant therapy. A study with tamoxifen monotherapy for six months in the preventive setting showed a correlation between CYP2D6 activity and MD decline in premenopausal women, but not in the postmenopausal group (244). More knowledge is needed to better understand the background of tamoxifen´s effect on MD change. One theory is that a threshold of endoxifen may be needed for density to decrease. It is

possible that lower tamoxifen metabolite levels may be needed for effect in the preventive versus the adjuvant setting (245).

Data on adherence to tamoxifen and MD change are limited (215, 218). The pattern of density decline in our study was not apparently affected by tamoxifen discontinuation. The number of patients with available digital mammograms after tamoxifen discontinuation was however limited. Further studies are needed to investigate whether younger patients experience a change in MD after stopping tamoxifen.

The major limitation is the limited study size. More than 40% of the patients from the full cohort were excluded due to digital mammogram availability. In this study outcome data were not available, so we could not evaluate the effect of density change on prognosis.

Further studies, with consideration of adherence, are needed to ascertain whether mammographic density change may be used as a marker of the desired effect of adjuvant tamoxifen. It is possible that evaluating MD change might be of value in the preventive setting. Further investigations are also needed to assess the association between endoxifen levels and MD decline and whether patients without a MD decrease under tamoxifen might benefit from another endocrine regimen.

### 4.3 Study III

4.3.1 Major findings

1235 patients were included. Most of the patients only had tamoxifen as their AET. 14 % switched from tamoxifen to an AI. 41% were premenopausal and 40% were defined at high risk of relapse. 7% percent were CYP2D6 PM, 90% were NM/IM and 3% were UM.

Agreement between the data sources on adherence was within the acceptance- interval, i.e. 80–125%, in 84% of the patients for tamoxifen and in 86% when including switch to an AI.

Poor consistency below 80% (i.e. fewer dispensed doses of adjuvant endocrine treatment compared to the recorded use in medical notes) was observed in 9% of the patients. This was most frequently seen in the premenopausal- and high-risk groups, as well as in CYP2DPM. Poor consistency > 125%, (i.e. a larger amount of dispensed doses of endocrine treatment compared to the use according to medical notes ) was seen in 5% of the patients, most frequently in the postmenopausal-/low risk groups, but again also in CYP2D6 PMs.

In patients with a minimum of 4.5 years follow up (n= 899), 77% were adherent to tamoxifen based on medical notes and 72% according to prescription refill data. When including patients treated with an AI after tamoxifen, adherence was 88% and 82% respectively. Adherence was not found to vary by menopausal status or recurrence risk ( Figure 5).

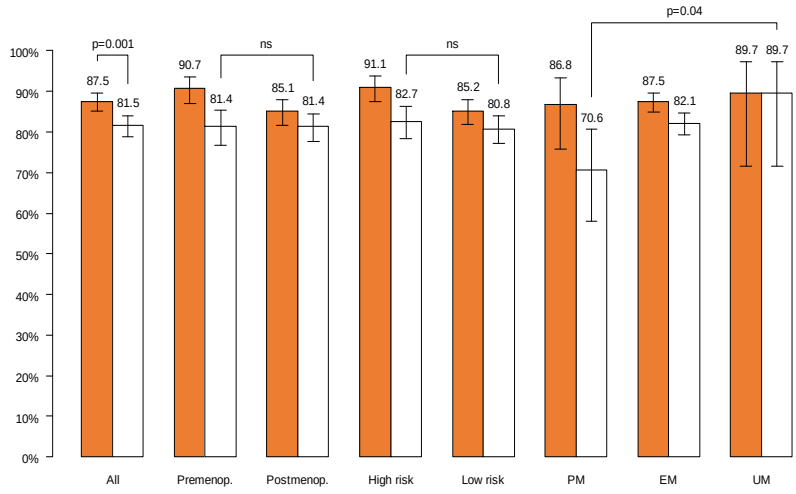



Figure 5. Proportion of patients adherent to adjuvant endocrine treatment based om medical notes and pharmacy refill data. Reprinted with permission from Breast Cacer Research and Treatment (246).

Adherence to tamoxifen was markedly lower in CYP2D6 PM (54%) compared to those defined as having a normal or high CYP2D6 activity (73% and 82% ) according to pharmacy refill data. CYP2D6 PM were also found to have a poorer adherence when including switch to AIs (71%) compared to the other two CYP2D6 activity groups (82% and 90%, respectively) based on pharmacy refill data.

52 patients had treatment with CYP2D6 inhibiting drugs according to medical notes. 73 additional patients were dispensed CYP2D6 inhibitors.

### 4.3.2 Discussion

Despite the positive effect of AET (1, 74-76), poor adherence is common. Patients may discontinue their treatment within months and adherence decreases over time (171, 172, 176). Poor adherence is associated with a poorer prognosis (181, 188, 191-195). In the study by Font et al. nonadherence to endocrine therapy (defined as MPR< 80% at 5 years) was correlated with a 2-fold risk of breast cancer death at roughly 6 years of follow up (195). In the prospective Swedish study by Markkula and colleagues, nonadherence to AET at year one was correlated with a nearly threefold increased risk of breast cancer events (247). Suboptimal adherence not only to tamoxifen, but all oral AET is therefore an important clinical issue.

Patients' reports on adherence are susceptible to recall bias, so medical notes tend to overestimate adherence (173, 178, 179). Even though there is a risk of possible overlap of prescribed medication, prescription databases are not susceptible to reporting bias and represent prescriptions in clinical practice. They are therefore commonly regarded as the most objective source of adherence information (178) (173). Although adherence to AET according to medical notes was, as expected, better than dispensing data revealed, the agreement between the data sources was in this study good. Few studies have compared multiple adherence measures to AET in the same material (171, 179, 180). Differences between medical records and dispensing data in our study were not as prominent compared to another European investigation (179).

In this study adherence to tamoxifen at 4.5 to 5 years was acceptable, 72 %, and 82% when including subsequent AIs, based om prescription refill data. In two previous Swedish studies, defining discontinuation of AET as more than 180 days between two consecutive dispenses, only half of the patients completed the recommended five-year treatment (183, 184). A longer gap than normal between prescription refills does not always equal non adherence as a treatment pause may be based on recommendation from the care giver (178). Our results are in keeping with data from other Swedish reports, using a comparable measure of adherence (185-187). Future research would benefit from using a standardized definition and measure of adherence to AET, to facilitate comparisons and easier identification of where actions are most necessary (248).

The unexpected finding of CYP2D6 PM having noticeably lower adherence to tamoxifen compared to those with a normal or high metabolic capacity in this study is in contrast to previous data indicating that CYP2D6 PM have fewer side effects to tamoxifen and a higher likelihood of better adherence (190). There is a probability that bias regarding other factors affecting adherence, for example comorbidity, marital status, social support, educational level and symptom relieving treatment (176) that we have no data on in this material, might have affected the results. Furthermore, the potentially low side effect profile in PM might have led to a lower perceived necessity of the treatment. Consistency was also poorer in the CYP2D6 PM group. CYP2D6 is expressed in brain tissue and is involved in the transformation of endogenous neuroactive substances, which might contribute to varying personality traits (249, 250). Whether this might affect adherence is speculative but might be an approach to pursue in future research. More knowledge is also needed on the association between levels of tamoxifen metabolites and side effects and how this in turn affects adherence.

Follow up routines at the oncological departments have varied in different time periods. High risk patients have had more frequent visits with their oncologist, while low risk patients have had less scheduled contact. In the later years of the study low risk patients had one initial visit with their oncologist, telephone contact with their nurse at year one of follow up ,and instead of yearly telephone contact had yearly letters reminding them of the importance of their endocrine treatment. We do not have data on whether the varying follow up routines may have impacted on adherence. Despite a higher benefit of treatment, more intensified follow up and previous data indicating better adherence to endocrine treatment for patients with a higher risk of relapse (187), adherence to AET was in our material however not better for high risk individuals compared to those with a more favorable prognosis. Moreover, in the premenopausal group, containing most of the high risk individuals, fewer doses were dispensed compared to the recorded intake in medical records, indicating that adherence was poorer than treating physicians or nurses were aware of. Intensified actions, including better communication, educational interventions on the benefits of AET, as well as information and management of side effects are essential for improving adherence, especially in young patients at a higher risk of relapse.

An important limitation in this study is the retrospective compilation of adherence data from medical notes, without access to all caregivers. Although adherence might be enquired and documented in varying degrees by different care givers, the sum of documented adverse effects is however likely average as many health care providers took part in the study patients´ follow up. Uniform assessment and documentation of adherence would facilitate evaluation and comparisons of adherence.

Future research to clarify whether therapeutic drug monitoring of tamoxifen with individualized dosing might improve adherence to endocrine treatment in early breast cancer is also warranted.

### 4.4 Study IV

4.4.1 Major findings

1105 patients were included in this study. 86% of the patients received no other adjuvant endocrine treatment besides tamoxifen. 42% were premenopausal at breast cancer diagnosis. 40% were defined as having a high estimated risk of relapse. 7 % of the study patients were classified as CYP2D6 poor metabolizers, 37% as intermediate metabolizers, 53% as normal metabolizers and 3% as ultra rapid metabolizers. Follow up was in median 11 years. During this time, 12% patients had a relapse and 4% died due to breast cancer.

We did not find a significant effect of CYP2D6 activity on breast cancer relapse (HR 1.18, CI 0.92; 1.52) or breast cancer mortality (HR 1.41, CI 0.93; 2.13) in the whole study cohort. No impact of CYP2D6 activit,y on clinical outcome was seen in the premenopausal subgroup (HR 0.85, CI 0.45; 1.61), nor in postmenopausal patients as for relapse (HR 1.43, CI 0.99; 2.01). We did observe a correlation between increasing CYP2D6 activity and an increased risk of breast cancer mortality in the postmenopausal subgroup (HR 1.90, CI 1.02: 3.55, p=0.043).

Similarly, the subgroup analysis in the patients with at least an initial year of tamoxifen treatment (n=1019), failed to show a correlation between CYP2D6 genotype and clinical outcome (HR 1.23, CI 0.86; 1.47 and HR 1.36, CI 0.89; 2.10). Neither stratifying for HER2 status, nor using the alternative AS for CYP2D6*41 changed the results (data not shown). Focusing on the patients receiving no other endocrine treatment besides tamoxifen did not reveal an effect of CYP2D6 activity on outcome (data not shown).

The Kaplan-Meier analyses did not indicate any differences in relapse or breast cancer mortality between the two groups with high versus low CYP2D6 activity (Figure 6 and 7), nor when analyzing the CYP2D6 phenotype groups , PM/ IM/ NM/ UM (data not shown).

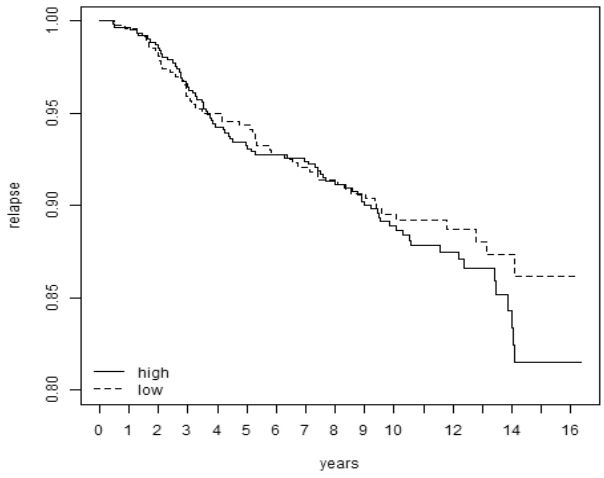



Figure 6. CYP2D6 activity and breast cancer recurrence

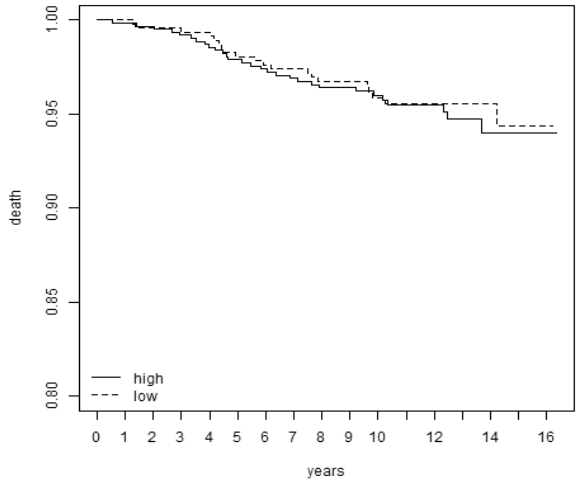



Figure 7. CYP2D6 activity and breast cancer related mortality

4.4.2 Discussion

In conclusion, no correlation between CYP2D6 activity and clinical outcome was shown in this cohort of tamoxifen treated early breast cancer patients, who could have received multimodal adjuvant therapy. The previously indicated correlation between CYP2D6 genotype and prognosis in premenopausal patients with postoperative tamoxifen was thus not confirmed in this material.

The possible risk of poorer prognosis with increasing CYP2D6 activity that was seen in some subgroup analyses in postmenopausal women cannot currently be explained. As the CIs are rather narrow, we can exclude that a large risk increase with increasing CYP2D6 activity exists. There is a likelihood that this contradictive observation is caused by unknown confounders. For example, we do not have information on non metabolic factors for tamoxifen resistance, for example mutations in the ER or loss of the ER (167-170) that might have influenced our results.

A major difference between our current and previous report (4) is the lower event rate in our present material. Breast cancer specific mortality was only 4 %, compared with 11% in the older study. Survival rates have steadily increased over time, not only in Sweden (22), but in most EU countries, likely mainly due to earlier detection and advancements in oncological treatment (251), (252). Survival in our material was even better than average for hormone sensitive breast cancer patients diagnosed roughly during a similar time frame (20). The large postmenopausal group, largely overlapping with those with tamoxifen monotherapy were in this investigation defined as having a lower risk of relapse, which likely explains the low breast cancer event rate.

Systemic breast cancer treatment has steadily improved over the last decades. Other systemic postoperative therapy not metabolized by CYP2D6 might compensate for a reduced activation of tamoxifen in individuals with poor CYP2D6 activity. The patients who received chemotherapy were largely premenopausal in both the previous and in the current cohort. The fraction of patients who received chemotherapy was however larger in our present investigation. It is possible that the more effective chemotherapy, and HER2 therapy for those who were HER2 positive, reduced the risk of relapse in the premenopausal high risk patients to such an extent that they were not as dependent on fully functional CYP2D6 activity when medicating with tamoxifen, as in our previous study.

Data on CYP2D6 polymorphism and the efficacy of adjuvant tamoxifen in early breast cancer are conflicting (8, 122, 123, 129, 139, 253, 254). Our current findings are in line with a prospective investigation in a similar clinical setting (139). A recent study where no association between CYP2D6 activity and prognosis was shown, found that endoxifen levels above 15 nmol/L correlated with a better outcome (131). It is possible that lower concentrations of active tamoxifen metabolites might still be needed for clinical effectivity in patients with modern combination treatment. Further prospective studies on endoxifen concentrations and outcome are warranted to identify a possible target range in patients with modern systemic adjuvant therapy in combination with tamoxifen.

The variability of endoxifen levels between individuals with the same genotype, especially in carriers of CYP2D6*1/*1, as described previously (133, 134, 139, 240, 246), may reduce the predictive power of CYP2D6 genotype on prognosis. Genetic variation in other enzymes involved in the metabolism of tamoxifen, for example CYP2C19, and the nuclear factor NF1B, which has been found to influence the regulation of CYP2D6, might possibly also affect the response to tamoxifen (255), (125), (159), (161). Importantly, most previous studies investigating the impact of CYP2D6 genotype in tamoxifen treated patients have not accounted for adherence, which might have affected the results (125). Information on adherence was in our older cohort only obtained from medical notes and there is a possibility that poor adherence in CYP2D6 PM, as observed in study III in this thesis, might have influenced our previous data, so that the effect of CYP2D6 activity was clearer.

A limitation in the current study is incomplete prescription refill data on CYP2D6 inhibitors and adherence to tamoxifen after January 2018. Data from 2018- 2022 are pending. A supplementary analysis is planned when data are complete. We do not yet have data on how adherence in our cohort affected outcome. This will be analyzed in another paper.

In conclusion, our present result suggests that a possible effect of CYP2D6 activity on clinical outcome in patients with adjuvant tamoxifen is likely minor in a modern clinical reality with access to complex treatment, as other parts of multimodal adjuvant breast cancer therapy have advanced. The importance of varying CYP2D6 activity might be different in a limited resource setting, where more high risk patients are treated only with tamoxifen (256). Hence, CYP2D6 genotyping to predict tamoxifen efficacy in a current multimodal setting is not supported by the present results. Therapeutic drug monitoring might still be of value, to secure a critical concentration of endoxifen.

### 4.5 Methodological considerations

Internal validity of a study depends on the design of the study, how rigorously the included parameters are measured and how accurately the findings reflect the group that was investigated. A high internal validity depends on few systematic errors, such as confounding and bias.

The external validity refers to how well the observations from an investigation can be expected to apply to other settings, in our case, how well our results are generalizable to similar breast cancer populations (257).

Strengths of the studies in this thesis include the genotyping of CYP2D6 from blood with allele coverage relevant for our mainly Caucasian study population, the relatively large prospectively collected study base of unselected early breast cancer patients with multimodal therapy reflecting standard of care, detailed clinical information including adherence to tamoxifen and relevant CYP2D6 inhibiting medication and relatively long follow up.

The used methods for CYP2D6 genotyping are rigorously validated, have a high sensitivity and specificity in identifying the tested CYP2D6 alleles. Misclassification of the individual CYP2D6 genotype due to loss of heterozygosity was not a problem as CYP2D6 was analyzed on germline DNA rather than tumor tissue. Rare CYP2D6 variants might however not have been detected by our genotyping method and might have been falsely classified as CYP2D6*1/*1. The method used for analyzing tamoxifen and its major metabolites is likewise rigorously validated and has a very high specificity and sensitivity. Information on adverse effects in study I was however retrospectively collected without a validated measure, increasing the risk of misclassification bias. Using digital images and the automated STRATUS system (177), reduced the variability in our measurements and the risk of misclassifying MD decline. Several automated systems can assess MD change today (211)(176)(212). Recent data indicate that density measurements by STRATUS might be more accurate than another automated system, Volpara (258). Comparisons with the normal age-related MD decline during follow up in study II could not be performed as we did not have a control group with breast cancer patients without tamoxifen and other systemic adjuvant treatment. Such a control group might be difficult to acquire in a modern clinical reality.

Selection bias refers to a systematic difference between individuals who are included in a study and those who are not included. This may affect both the external and internal validity. A likely selection bias of patients with high tolerability for tamoxifen therapy exists in study I, as the included patients had been taking tamoxifen for around 5 years. Consequently, patients with serious side effects are likely to be underrepresented. The side effect profile in relation to different ranges of endoxifen might thus be different in a clinical setting. In study II, where roughly 40% of the patients from the full cohort were excluded mainly because of unavailable digital mammograms, the excluded patients from the full study cohort were compared with the included patients to identify a

possible selection bias in the study cohort. As no significant differences were observed between the groups as for the most relevant factors for MD change, the degree of selection bias however appears to be low.

Patients' reports on adherence are susceptible to recall bias. Dispensing data is not susceptible to this, but there is a risk of overlapping medication (173, 178, 179). Comparing two methods to assess adherence in study III reduced biases inherent to both methods, which increased the validity of our results. In the same study there is however a probability that there was a bias regarding other factors affecting adherence (176) that we did not have data on and thus could not control for, between the groups of CYP2D6 activity. There is a possibility that the varying follow up routines may have introduced a bias in the reporting on adherence in medical records. Although is reasonable to assume that our results are relevant in similar settings, our findings may not be generalizable to patients in other countries or health care systems with other systems for prescription refills and follow up of patients.

The effect of the studied exposure on a certain outcome may be mixed with the effects of a confounding factor, so that the true relationship is distorted. Investigations without randomization, such as the studies used in this thesis carry a risk of confounding. Regression models are commonly used to adjust for confounding in retrospective studies. In study IV we therefore adjusted for confounding factors when analyzing the correlation between CYP2D6 activity and breast cancer relapse or breast cancer related death in the multivariable Cox proportional hazard models, increasing the validity of our results. Confounding may also occur due to unknown factors. It is likely that the risk of poorer outcome with increasing CYP2D6 activity observed in study IV is due to unknown confounding factors that we were not able to adjust for in our analysis.

Statistical power refers to the likelihood of not making a type II error, i.e., accepting the null hypothesis when it should have been rejected. Statistical power depends on both the study size and the size of the effect. In study IV, the low frequency of breast cancer events might have rendered our investigation underpowered to be able to show a true effect of CYP2D6 activity on clinical outcome. The rather narrow 95% CIs of the HRs for relapse and breast cancer mortality however indicate that this is unlikely. For example, the lower limit of the interval for relapse represented a risk increase of no more than 19% in CYP2D6 PM compared to CYP2D6 NM. It is unlikely that a true risk increase greater than that would not have been included in the interval due to a type II error.

### 4.6 Ethical considerations

Ethical approval was granted for each study in this thesis by the ethical review board at Karolinska Institutet, Sweden. Written informed consent was provided from all newly diagnosed breast cancer patients agreeing to biobank DNA for future breast cancer research (Ethical permit ref. number 02-061). In accordance with our ethical approvals (amendment Dnr 2014/427-31, 016/1184-31, 2016/1698–32 and Dnr. 2018/2644-32), we did

not approach the patients with bio-banked DNA for CYP2D6-genotyping (study I-IV), assessment of MD change (study II), or for collecting data from the National Prescribed Drug Register in Sweden, as the results would not change the patients' treatment or follow up and informing them might cause unnecessary concern.

In accordance with the ethical permit for study I (2016/1184–31), invited patients received written study information and were offered telephone contact with the study responsible doctors to ensure all questions were answered before signing informed consent. All patients provided written informed consent before inclusion. The study patients also agreed to publication of results in a scientific paper.

All data in this thesis have been presented at group level and information on individual patients cannot be recognized. None of the results in this thesis will change the study patients' treatment or follow up and they will therefore not ,be informed of their results. The included patients will personally not gain from contributing. Instead, we hope that the results from this thesis might be of value for future breast cancer patients.

# 5 Conclusions

- The effect of reduced function CYP2D6 variants, in particular CYP2D6*41, on endoxifen formation appears to be greater than current guidelines anticipate.

- The group of patients with poor activation of tamoxifen might be larger than currently expected. This may be of importance for future genotype based considerations of tamoxifen dosing.

- In breast cancer patients with adjuvant tamoxifen therapy, other systemic adjuvant treatment does not seem to provide additional MD decline.

- The proposed correlation between CYP2D6 activity and MD change could not be attested in early breast cancer with modern complex systemic therapy.

- Density decline appeared to persist after tamoxifen was stopped.

- Agreement between medical notes and pharmacy refill data on adherence to adjuvant endocrine therapy was good.

- Adherence to postoperative endocrine therapy was reasonable, especially when including patients changing their treatment from tamoxifen to an AI.

- Poor Metabolizers had poor adherence to tamoxifen, in spite of previous data implying a lower risk of adverse effects.

- No obvious correlation between CYP2D6 activity and prognosis was confirmed in this material of early breast cancer with multimodal modern adjuvant therapy, including tamoxifen.

- A possible impact of CYP2D6 activity on prognosis in patients with adjuvant tamoxifen is likely minor in a current multimodal clinical setting. Our present results do not support CYP2D6 genotyping to predict tamoxifen efficacy in a setting with access to complex postoperative breast cancer treatment.

- The effect of CYP2D6 polymorhism might be different in a clinical setting with more limited resources, where tamoxifen may be the only systemic treatment.

# 6 Points of perspective

There is a growing need for personalized medicine and an increasing interest in using genetic testing to aid the selection of specific treatments for breast cancer patients. As there is a substantial variability in the effect of postoperative tamoxifen treatment (2), the search for markers for better treatment prediction and early evaluation of response needs to continue.

The influence of other enzymes and factors involved in the bioactivation of tamoxifen on clinical outcome in early breast cancer is uncertain (8, 159-161, 255). Genotyping of CYP2C19 and NF1B has been performed for all patients in the full study cohort. Data have however not yet been analyzed. We plan to investigate whether NF1B - and CYP2C19 status might affect clinical outcome in our cohort of CYP2D6 genotyped tamoxifen treated patients.

In a future paper, we also plan to investigate how adherence to adjuvant endocrine treatment and its relation to CYP2D6 genotype affected outcome in our breast cancer cohort. It would also be of interest to investigate whether the different CYP2D6 genotypes, and the possible varying personality traits (249, 250), might be of importance for the varying adherence not only to tamoxifen, but also to other drugs, such as antidepressants.

Strategies to increase adherence to AET may include use of automated refill reminders and other electronic tools (181). Improved management of side effects to adjuvant endocrine treatment to optimize adherence and ultimately outcome in early hormone sensitive breast cancer is also essential.

Further prospective studies on the association between concentrations of tamoxifen metabolites, in particular endoxifen, and clinical outcome in tamoxifen treated early breast cancer are warranted, especially in younger patients with multimodal adjuvant treatment, so that a possible target range in patients with modern systemic therapy in combination with tamoxifen may be identified.

Although a certain threshold for endoxifen in plasma for tamoxifen efficacy is currently highly unclear and also might vary depending on whether the patient receives other effective systemic treatment, therapeutic drug monitoring of tamoxifen might still be an approach to improve adherence to the treatment by avoiding unneeded exposure correlated with intolerability. This might especially be of value for patients with very high endoxifen levels and severe side effects, where a lower dose might improve adherence, side effects and quality of life during treatment. We therefore plan to initiate a randomized prospective dose titration study with individualized tamoxifen dosing, to better recognize which levels of endoxifen in plasma are associated with severe side effects and whether side effects and adherence to treatment may be improved by individualized tamoxifen dosing.

Further investigations, with consideration of adherence, are as mentioned previously warranted to ascertain whether mammographic density change may be used as a marker of the desired effect of postoperative tamoxifen treatment in a modern clinical setting. In a subsequent paper, we plan to analyze the impact on MD decline under tamoxifen treatment and outcome in the cohort of study II. In the limited group of patients with available data on tamoxifen metabolites and density change, we also plan to do an exploratory analysis of whether a certain level of endoxifen may be needed for density change to occur and if there is a correlation between endoxifen levels, density change and clinical outcome. A new risk assessment tool using the whole mammogram to analyze change in several different mammographic features with malignancy potential is currently studied (259). I hope to also incorporate this model in future research, to further evaluate its' potential as a marker for early response to endocrine treatment.

CYP2D6 genotypes vary between ethnic groups (99, 100, 110). Most research on the impact of CYP2D6 polymorphism on tamoxifen efficacy has been conducted in Caucasian and Asian populations, with limited work in for example African populations (256). Further work in low resource settings might be helpful to get a better understanding of the clinical value of CYP2D6 genotyping in selected tamoxifen treated populations with predominantly tamoxifen monotherapy.

Solanidine, an alkaloid present in potatoes is found in serum of individuals who ingest potatoes, with a long half-life. Recent data show that measuring solanidine metabolism from serum samples by liquid chromatography mass spectrometry analyses, may be a promising method for predicting the CYP2D6 PM phenotype (113). Further studies evaluating this method would be interesting.

Several studies are trying to determine whether oral endoxifen is an effective treatment in breast cancer, both in the metastatic or neoadjuvant setting. Endoxifen might be a possible option in the future for patients with poor activation or who cannot tolerate tamoxifen. So far, phase I and II studies have shown promising antitumor activity, with acceptable side effects (260, 261).

Molecular profiling of breast cancers, with next generation sequencing of targeted DNA panels will likely play an increasingly important role in clinical practice. Genomic confirmation of hormone sensitive breast cancers with a low risk of relapse might also allow for future de-escalation of endocrine therapy to improve quality of life and risk of serious side effects (262).

The analyses of future early breast cancer will most likely be more complex, but also more informative, to facilitate treatment recommendations.

I hope that future, more individualized treatment strategies for hormone sensitive early breast cancer will help to improve adherence and quality of life under treatment as well as prognosis.

# 7 Acknowledgements

This thesis would not have been possible without the support and encouragement from colleagues, friends, and family. I would like to express my deepest gratitude to each one of you!

I would especially like to express my appreciation to:

**Sara Margolin**, my brilliant and inspiring main supervisor and dear colleague; for believing in me, for your unconditional support and immense patience, for helping me to calm down and lift my spirits as needed. Thank you for so generously sharing your profound knowledge on oncology and on research, for shedding new light on problems and suggesting creative and wise solutions not only in our research projects and our many manuscript writing sessions, but equally in our daily clinical practice. You have shown me that research is interesting and fun! Thank you for always making time for questions, for so many rewarding and fun discussions on research, oncology, tamoxifen, life in general and for many good laughs. I would not have done this without you.

**Erik Eliasson**, my co-supervisor; for your continuous support and enthusiasm and for inspiring me to learn more about pharmacogenetics and therapeutic drug monitoring. Thank you for always making me feel welcome at your department at Huddinge. Thank you for sharing your endless and indispensable expertise on CYP2D6, tamoxifen metabolites, research in general and for all your good advice. Thank you also for the many creative and inspiring discussions and manuscript writing sessions - always with constructive and prompt feedback, throughout these years.

**Jonatan Lindh**, my co-supervisor; for indispensable work with statistical analyses and for your razor-sharp answers to statistical questions. Thank you for the many fun hours in Huddinge when you guided me through our analyses and gently kept me from going astray. Thank you for all our inspiring and fun discussions, for all your valuable comments and support, and last but not least - for so generously explaining the perplexity of statistics in such an educational manner.

**Mikael Eriksson**, my co-supervisor; for invaluable work on our project with mammographic density and for good company during the many hours we spent working on the analyses at MEB. Thank you for sharing your knowledge on mammographic density change and boot-strappings, for helpfully answering my questions and for our interesting discussions on markers for treatment response.

All other co-authors and research partners for the papers in this thesis; for your cooperation, for sharing your expertise, for invaluable comments and advice. Your contributions have added substantial value to the papers in this thesis. I would especially like to thank **Espen Molden** and **Mariann Kristiansen Kringen**, for your invaluable work

with genotyping and for introducing me to the mysteries of how it is done at your laboratory in Oslo, **Jonas Bergh**, for sharing your expertise on breast cancer and it's treatment, and for many animated discussions, **Per Hall** for sharing your expertise on mammographic density, for your enthusiasm for breast cancer prevention and for letting me be a part of the KARISMA trial, **Kamila Czene** for interesting discussions on research and mammographic density and **Gerd Ackehed**, for invaluable help with the analyses of tamoxifen metabolites.

**Per Tornvall**, Head of the Department of Clinical Science and Education at Södersjukhuset; for accepting me as a PhD candidate.

**Anna Nopp Scherman**, Study Director at the Department of Clinical Science and Education at Södersjukhuset; for guidance and administrative help during my doctoral education.

**Malin Holm Blomqvist** at the Department of Clinical Science and Education at Södersjukhuset; for excellent and swift help with administrative issues.

**Georg Engel**, head of the Department of Oncology at SÖS; for your enthusiasm for oncology, for your interest in research and for support to finalize my doctoral education.

I would also like to thank the former heads of the Department of Oncology at SÖS and the former Department of Oncology at Karolinska/ SÖS who have supported my doctoral education.

**Ellinor Elinder**, head over the doctors at the breast cancer unit, but also a longtime dear colleague; for all your support over the years and for your effort to provide me with time for research.

All dedicated colleagues and dear friends at the breast cancer unit at SÖS –**Tommy Fornander, Gerard Winblad, Anna von Wachenfeldt, Jenny Lundin, Camilla Wendt, Anna Larsson Wråke, Elin Barnekow, Ellinor Elinder, Anders Lideståhl, Emmy Ericsson, Sara Hardell, Kathrin Wode and Ulla Blom Goldman**. Thank you all for being the best colleagues one can imagine, for your support, always interesting discussions, so many good laughs and for covering for me during all these years. I would also like to thank all my former colleagues and friends at the breast cancer unit that I have had the pleasure to work with over the years.

**Ingveldur Björnsdóttir**, dear colleague and friend; for your support over the years.

All present and former **nurses and assistant nurses** at the breast cancer unit and the hereditary unit at the Department of Oncology at SÖS; for invaluable teamwork.

**Administrative personnel** at the Department of Oncology at SÖS; for valuable technical support in times of need.

**All other present and former colleagues**, co-workers and friends at the Department of Oncology at SÖS; for being such a great team and for all the good times over the years.

The National research school in Oncology (**NatiOn**); for inspiring lectures, scientific knowledge, many friends and study buddies.

My fellow "PhD student group" at the Department of Oncology at SÖS**; Elin Barnekow, Anders Lideståhl, Shabane Barot, Jenny Lundin, Tove Ekdahl Hjelm and David Kudren**; for moral support, pep-talks, valuable advice on how to facilitate life as a PhD student and informative journalclubs.

**Roxanna Hellgren**, head of the mammography unit at SÖS; for your friendship, encouragement, creative discussions and for invaluable practical advice in times of need as a doctoral student.

**Susanne Agartz**; for valuable help preparing the DNA samples for analysis and transport to Norway.

**Irma Fredriksson** and **Christina Linder Stragliotto**, for interesting discussions during my half-time seminar.

**All my dear friends**, new and old; for being there for me and for all the good times. I hope to have much more fun with you in the foreseeable future.

**My mother Jane, father Lennart and my sister Marie-Louise**; for your love and support. For always being there for me. I will always be there for you.

**My extended family**; for your support.

**All the breast cancer patients** who so generously participated in the studies in this thesis and the patients and families I meet in my daily practice; for motivating me to engage in research.

Above all, my husband **Sven and my children Rebecka and Elliot** – thank you for everything! I love you endlessly.

*This thesis was supported by grants from The Cancer Society in Stockholm, Percy Falk's Foundation for research on Prostate Cancer and Breast Cancer, The Swedish Breast Cancer Association, grants from the Stockholm County Council (FoUI- 953166 and FoUI-961013), CIMED (FoUI-963062), and by grants from Jonas Bergh´s research group which is supported by grants from the Swedish Cancer Society (19 0292 PJ 01 H and 19 0189 US01 H), The Research Funds at Radiumhemmet (164003), Karolinska Institutet, Stockholm County Council (ALF2017-1341), FOU (Karolinska University Hospital account number 907515).*

# 8 References


1. Early Breast Cancer Trialists' Collaborative Group (EBCTCG). Effects of chemotherapy and hormonal therapy for early breast cancer on recurrence and 15-year survival: an overview of the randomised trials. Lancet. 2005 May 14-20;365(9472):1687-717.
2. Pan H, Gray R, Braybrooke J, et al. 20-Year Risks of Breast-Cancer Recurrence after Stopping Endocrine Therapy at 5 Years. N Engl J Med. 2017;377(19):1836-46.
3. Desta Z. Comprehensive Evaluation of Tamoxifen Sequential Biotransformation by the Human Cytochrome P450 System in Vitro: Prominent Roles for CYP3A and CYP2D6. Journal of Pharmacology and Experimental Therapeutics. 2004;310(3):1062-75.
4. Margolin S, Lindh JD, Thoren L, et al. CYP2D6 and adjuvant tamoxifen: possible differences of outcome in pre- and post-menopausal patients. Pharmacogenomics. 2013;14(6):613-22.
5. Goetz MP, Suman VJ, Hoskin TL, et al. CYP2D6 metabolism and patient outcome in the Austrian Breast and Colorectal Cancer Study Group trial (ABCSG) 8. Clin Cancer Res. 2013;19(2):500-7.
6. Kiyotani K, Mushiroda T, Imamura CK, et al. Significant effect of polymorphisms in CYP2D6 and ABCC2 on clinical outcomes of adjuvant tamoxifen therapy for breast cancer patients. J Clin Oncol. 2010;28(8):1287-93.
7. Schroth W GM, Hamann U, et al. Association between CYP2D6 polymorphisms and outcomes among women with early stage breast cancer treated with tamoxifen. JAMA. 2009; 2009;302(13):1429–1436.
8. Chan CWH, Li C, Xiao EJ, et al. Association between genetic polymorphisms in cytochrome P450 enzymes and survivals in women with breast cancer receiving adjuvant endocrine therapy: a systematic review and meta-analysis. Expert Reviews in Molecular Medicine. 2022;24.
9. Goetz MP, Sangkuhl K, Guchelaar H-J, et al. Clinical Pharmacogenetics Implementation Consortium (CPIC) Guideline for CYP2D6 and Tamoxifen Therapy. Clinical Pharmacology & Therapeutics. 2018;103(5):770-7.
10. Haslemo T, Eliasson E, Jukic MM, et al. Significantly lower CYP2D6 metabolism measured as the O/N-desmethylvenlafaxine metabolic ratio in carriers of CYP2D6*41 versus CYP2D6*9 or CYP2D6*10: a study on therapeutic drug monitoring data from 1003 genotyped Scandinavian patients. Br J Clin Pharmacol. 2019;85(1):194-201.
11. Gaedigk A, Dinh JC, Jeong H, et al. Ten Years' Experience with the CYP2D6 Activity Score: A Perspective on Future Investigations to Improve Clinical Predictions for Precision Therapeutics. J Pers Med. 2018;8(2).
12. Hertz DL, Snavely AC, McLeod HL, et al. In vivoassessment of the metabolic activity of CYP2D6 diplotypes and alleles. British Journal of Clinical Pharmacology. 2015;80(5):1122-30.
13. Shawky MS, Martin H, Hugo HJ, et al. Mammographic density: a potential monitoring biomarker for adjuvant and preventative breast cancer endocrine therapies. Oncotarget. 2017;8(3):5578-559.
14. Li J, Czene K, Brauch H, et al. Association of CYP2D6 metabolizer status with mammographic density change in response to tamoxifen treatment. Breast Cancer Res. 2013;15(5):R93.
15. He W GF, Eriksson M, Eliasson E, et al. CYP2D6 Genotype Predicts Tamoxifen Discontinuation and Prognosis in Patients With Breast Cancer. J Clin Oncol. 2020 Feb 20;38(6):548-557.
16. 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.
17. Wilkinson L GT. Understanding breast cancer as a global health concern. Br J Radiol. 2022 Feb 1;95(1130):20211033.
18. NORDCAN: Cancer Incidence, Mortality, Prevalence and Survival in the Nordic Countries, Version 9.2 (23.06.2022). Association of the Nordic Cancer Registries. Cancer Registry of Norway.2022. Available from: https://nordcan.iarc.fr/. Accessed on 16/05/2023.
19. Statistikdatabas för cancer, 2022. Available from: https://sdb.socialstyrelsen.se/if_can/val.aspx. Accessed on 16/05/23.

20. Nationellt kvalitetsregister för bröstcancer (NKCB). Available from: https://statistik.incanet.se/brostcancer/. Accessed on 16/05/23.
21. CANCER I SVERIGE - REGISTERDATA ÖVER FÖREKOMST OCH DÖDLIGHET 1970-2017. Available from: https://cancercentrum.se/globalassets/vara-uppdrag/statistik/cancer_i_sverige_registerdata_forekomst_dodlighet_1970-2017.pdf. Accessed on 16/05/23.
22. Statistik Bröstcancer: Cancerfonden; 2021. Available from: https://www.cancerfonden.se/om-cancer/statistik/brostcancer. Accessed on 16/05/23.
23. Sun YS ZZ, Yang ZN, Xu F, et al. Risk Factors and Preventions of Breast Cancer. Int J Biol Sci. 2017 Nov 1;13(11):1387-1397.
24. Huo CW, Chew GL, Britt KL, et al. Mammographic density-a review on the current understanding of its association with breast cancer. Breast Cancer Res Treat. 2014;144(3):479-502.
25. McCormack VA, dos Santos Silva I. Breast density and parenchymal patterns as markers of breast cancer risk: a meta-analysis. Cancer Epidemiol Biomarkers Prev. 2006;15(6):1159-69.
26. Singletary SE. Rating the risk factors for breast cancer. Ann Surg. 2003 Apr;237(4):474-82.
27. Breast Cancer Association Consortium, Dorling L, Carvalho S, Allen J, González-Neira A, Luccarini C, et al. Breast cancer risk genes - association analysis in more than 113,000 women. N Engl J Med. 2021;384(5):428–39.
28. Foulkes WD. Inherited susceptibility to common cancers. N Engl J Med. 2008 Nov 13;359(20):2143-53.
29. Atakpa EC TM, Cuzick J, Brentnall AR. Mammographic density, endocrine therapy and breast cancer risk: a prognostic and predictive biomarker review. Cochrane Database Syst Rev. 2021 Oct 26;10(10):CD013091.
30. Cardoso F, Kyriakides S, Ohno S, et al. Early breast cancer: ESMO Clinical Practice Guidelines for diagnosis, treatment and follow-updagger. Ann Oncol. 2019;30(8):1194-220.
31. Thomas C, Gustafsson JA. The different roles of ER subtypes in cancer biology and therapy. Nat Rev Cancer. 2011;11(8):597-608.
32. Deroo BJ, Korach KS. Estrogen receptors and human disease. J Clin Invest. 2006;116(3):561-70.
33. Rhodes A. Frequency of oestrogen and progesterone receptor positivity by immunohistochemical analysis in 7016 breast carcinomas: correlation with patient age, assay sensitivity, threshold value, and mammographic screening. Journal of Clinical Pathology 2000;53:688-696. 2000.
34. Nationellt kvalitetsregister för Bröstcancer (NKBC): Årsrapport 2021. Available from: https://statistik.incanet.se/brostcancer/ Accessed on 16/05/23.
35. Allred DC, Brown P, Medina D. The origins of estrogen receptor alpha-positive and estrogen receptor alpha-negative human breast cancer. Breast Cancer Res. 2004;6(6):240-5.
36 Jonsson P, Katchy A, Williams C. Support of a bi-faceted role of estrogen receptor beta (ERbeta) in ERalpha-positive breast cancer cells. Endocr Relat Cancer. 2014;21(2):143-60.
37. Colleoni M SZ, Price KN, Karlsson P, et al. Annual Hazard Rates of Recurrence for Breast Cancer During 24 Years of Follow-Up: Results From the International Breast Cancer Study Group Trials I to V. J Clin Oncol. 2016 Mar 20;34(9):927-35.
38. Hammond ME HD, Dowsett M, Allred DC, et al. American Society of Clinical Oncology/College Of American Pathologists guideline recommendations for immunohistochemical testing of estrogen and progesterone receptors in breast cancer. J Clin Oncol. 2010 Jun 1;28(16):2784-95.
39. Pathology SSo. KVAST-dokument brostpatologi. Available from: https://svfp.se/kvast/brostpatologi/kvast-dokument/. Accessed on 16/05/23.
40. Early Breast Cancer Trialists' Collaborative Group (EBCTCG); Davies C, Godwin J, Gray R, 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 Aug 27;378(9793):771-84.

41. Stendahl M RL, Nordenskjöld B, Jönsson PE, et al. High progesterone receptor expression correlates to the effect of adjuvant tamoxifen in premenopausal breast cancer patients. Clin Cancer Res. 2006 Aug 1;12(15):4614-8.
42. Dai X, Li T, Bai Z, Yang Y, et al. B. Breast cancer intrinsic subtype classification, clinical use and future trends. Am J Cancer Res. 2015 Sep 15;5(10):2929-43.
43. Early Breast Cancer Trialists' Collaborative group (EBCTCG). Trastuzumab for early-stage, HER2-positive breast cancer: a meta-analysis of 13 864 women in seven randomised trials. Lancet Oncol. 2021 Aug;22(8):1139-1150.
44. Davey MG, Hynes SO, Kerin MJ, et al. Ki-67 as a Prognostic Biomarker in Invasive Breast Cancer. Cancers (Basel). 2021 Sep 3;13(17):4455.
45. Beresford MJ, Wilson GD, Makris A. Measuring proliferation in breast cancer: practicalities and applications. Breast Cancer Res. 2006;8(6):216.
46. Fitzgibbons PL PD, Weaver D, Thor AD, et al. Prognostic factors in breast cancer. College of American Pathologists Consensus Statement 1999. Arch Pathol Lab Med. 2000 Jul;124(7):966-78.
47. Elston CW, Ellis, I.O., 1991. Pathological prognostic factors in breast cancer. I. The value of histological grade in breast cancer: experience from a large study with long-term follow-up. Histopathology 19, 403–410.
48. Giuliano AE, Connolly, J.L., Edge, S.B., et al., 2017. Breast Cancer-Major changes in the American Joint Committee on Cancer eighth edition cancer staging manual. CA: A Cancer Journal for Clinicians 67, 290–303.
49. Azim HA Jr, Partridge AH. Biology of breast cancer in young women. Breast Cancer Res. 2014 Aug 27;16(4):427.
50. Geyer CE, Garber, J.E., Gelber, R.D., et al. 2022. Overall survival in the OlympiA phase III trial of adjuvant olaparib in patients with germline pathogenic variants in BRCA1/2 and high-risk, early breast cancer Annals of Oncology 33, 1250–1268.
51. Kjällquist U, Acs B, Margolin S, et al. J. Real World Evaluation of the Prosigna/PAM50 Test in a Node-Negative Postmenopausal Swedish Population: A Multicenter Study. Cancers (Basel). 2022 May 25;14(11):2615.
52. Network CGA. Comprehensive molecular portraits of human breast tumours. Nature. 2012 Oct 4;490(7418):61-70.
53. Goldhirsch A WE, Coates AS, Gelber RD, et al. Personalizing the treatment of women with early breast cancer: highlights of the St Gallen International Expert Consensus on the Primary Therapy of Early Breast Cancer 2013. Ann Oncol. 2013 Sep;24(9):2206-23.
54. Andre F, Ismaila, N., Allison, K.H., et al., 2022. Biomarkers for Adjuvant Endocrine and Chemotherapy in Early-Stage Breast Cancer: ASCO Guideline Update. Journal of Clinical Oncology 40, 1816–1837.
55. Lundgren C, Bendahl PO, Church SE, et al. PAM50 subtyping and ROR score add long-term prognostic information in premenopausal breast cancer patients. NPJ Breast Cancer. 2022 May 9;8(1):61.
56. Clarke M, Collins R, Darby S, et al.; Early Breast Cancer Trialists' Collaborative Group (EBCTCG). Effects of radiotherapy and of differences in the extent of surgery for early breast cancer on local recurrence and 15-year survival: an overview of the randomised trials. Lancet. 2005 Dec 17;366(9503):2087-106.
57. Burstein HJ CG, Thürlimann B, Weber WP, et al. Panelists of the St Gallen Consensus Conference. Customizing local and systemic therapies for women with early breast cancer: the St. Gallen International Consensus Guidelines for treatment of early breast cancer 2021. Ann Oncol. 2021 Oct;32(10):1216-1235.
58. Early Breast Cancer Trialists' Collaborative Group (EBCTCG); Darby S MP, Correa C, Taylor C, et al. Effect of radiotherapy after breast-conserving surgery on 10-year recurrence and 15-year breast cancer death: meta-analysis of individual patient data for 10,801 women in 17 randomised trials. Lancet. 2011 Nov 12;378(9804):1707-16.
59. Litière S WG, Fentiman IS, Rutgers E, et al. Breast conserving therapy versus mastectomy for stage I-II breast cancer: 20 year follow-up of the EORTC 10801 phase 3 randomised trial. Lancet Oncol. 2012 Apr;13(4):412-9.

60. de Boniface J, Szulkin R, Johansson ALV. Survival After Breast Conservation vs Mastectomy Adjusted for Comorbidity and Socioeconomic Status: A Swedish National 6-Year Follow-up of 48 986 Women. JAMA Surg. 2021 Jul 1;156(7):628-637.
61. Mieog JS vdHJ, van de Velde CJ. Preoperative chemotherapy for women with operable breast cancer. Cochrane Database Syst Rev. 2007 Apr 18;2007(2) : CD005002.
62. Schmid P CJ, Pusztai L, McArthur H, et al. KEYNOTE-522 Investigators. Pembrolizumab for Early Triple-Negative Breast Cancer. N Engl J Med. 2020 Feb 27;382(9):810-821.
63. Cortazar P ZL, Untch M, Mehta K, et al. Pathological complete response and long-term clinical benefit in breast cancer: the CTNeoBC pooled analysis. Lancet. 2014 Jul 12;384(9938):164-72. Epub 2014 Feb 14. Erratum in: Lancet. 2019 Mar 9;393 10175) :986.
64. Yau C, Osdoit, M., Van Der Noordaa, et al., 2022. Residual cancer burden after neoadjuvant chemotherapy and long-term survival outcomes in breast cancer: a multicentre pooled analysis of 5161 patients. The Lancet Oncology 23, 149–160.
65. Early Breast Cancer Trialists' Collaborative Group (EBCTCG); Peto R DC, Godwin J, Gray R, et al. Comparisons between different polychemotherapy regimens for early breast cancer: meta-analyses of long-term outcome among 100,000 women in 123 randomised trials. Lancet. 2012 Feb 4;379(9814):432-44.
66. Nationellt vårdprogram för bröstcancer 2009. Available from: http://www.swebcg.se/wp-content/uploads/2016/09/Nationellt-vardprogram-Brostcancer-2009.pdf. Accessed on 16/07/23.
67. Early Breast Cancer Trialists' Collaborative Group (EBCTCG). Electronic address: bc.overview@ctsu.ox.ac.uk; Early Breast Cancer Trialists' Collaborative Group (EBCTCG). Anthracycline-containing and taxane-containing chemotherapy for early-stage operable breast cancer: a patient-level meta-analysis of 100 000 women from 86 randomised trials. Lancet. 2023 Apr 15;401(10384):1277-1292.
68. Yu KD YF, He M, Fan L, et al. Effect of Adjuvant Paclitaxel and Carboplatin on Survival in Women With Triple-Negative Breast Cancer: A Phase 3 Randomized Clinical Trial. JAMA Oncol. 2020 Sep 1;6(9):1390-1396.
69. Joensuu H, Kellokumpu-Lehtinen, P.-L., Huovinen, et al. (2022). Adjuvant Capecitabine for Early Breast Cancer: 15-Year Overall Survival Results From a Randomized Trial. Journal of Clinical Oncology : Official Journal of the American Society of Clinical Oncology., 40(10), 1051–1058.
70. Cameron D P-GM, Gelber RD, Procter M, et al. Herceptin Adjuvant (HERA) Trial Study Team. 11 years' follow-up of trastuzumab after adjuvant chemotherapy in HER2-positive early breast cancer: final analysis of the HERceptin Adjuvant (HERA) trial. Lancet. 2017 Mar 25;389(10075):1195-1205.
71. von Minckwitz G, Huang CS, Mano MS, et al.; KATHERINE Investigators. Trastuzumab Emtansine for Residual Invasive HER2-Positive Breast Cancer. N Engl J Med. 2019 Feb 14;380(7):617-628.
72. Early Breast Cancer Trialists' Collaborative Group (EBCTCG). Adjuvant bisphosphonate treatment in early breast cancer: meta-analyses of individual patient data from randomised trials. Lancet. 2015 Oct 3;386(10001):1353-1361.
73. Johnston SRD HN, Hegg R, Toi M, et al.; monarchE Committee Members and Investigators. Abemaciclib Combined With Endocrine Therapy for the Adjuvant Treatment of HR+, HER2-, Node-Positive, High-Risk, Early Breast Cancer (monarchE). J Clin Oncol. 2020 Dec 1;38(34):3987-3998.
74. Early Breast Cancer Trialists' Collaborative Group (EBCTCG). Aromatase inhibitors versus tamoxifen in premenopausal women with oestrogen receptor-positive early-stage breast cancer treated with ovarian suppression: a patient-level meta-analysis of 7030 women from four randomised trials. Lancet Oncol. 2022 Mar;23(3):382-392.
75. Early Breast Cancer Trialists' Collaborative Group (EBCTCG). Aromatase inhibitors versus tamoxifen in early breast cancer: patient-level meta-analysis of the randomised trials. Lancet. 2015 Oct 3;386(10001):1341-1352.

76. 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. The Lancet. 2013;381(9869):805-16.
77. Miller W. Aromatase inhibitors: mechanism of action and role in the treatment of breast cancer. Semin Oncol. 2003 Aug;30(4 Suppl 14):3-11.
78. Condorelli R V-LI. Managing side effects in adjuvant endocrine therapy for breast cancer. Expert Rev Anticancer Ther. 2018 Nov;18(11):1101-1112.
79. Nationellt vårdprogram för bröstcancer år 2000. Available from Nat vardpr brost 2000.doc (swebcg.se). Accessed on 16/07/23.
80. Xu L ZZ, Xiang Q, Liu Q, et al. Extended Adjuvant Therapy With Aromatase Inhibitors for Early Breast Cancer: A Meta-analysis of Randomized Controlled Trials. Clin Breast Cancer. 2019 Oct;19(5):e578-e588.
81. Huerta-Reyes M, Maya-Núñez G, Pérez-Solis MA, et al. Treatment of Breast Cancer With Gonadotropin-Releasing Hormone Analogs. Front Oncol. 2019 Oct 1;9:943.
82. Francis PA, Fleming GF, Láng I, et al.; SOFT Investigators and the International Breast Cancer Study Group (a division of ETOP IBCSG Partners Foundation). Adjuvant Endocrine Therapy in Premenopausal Breast Cancer: 12-Year Results From SOFT. J Clin Oncol. 2023 Mar 1;41(7):1370-1375.
83. Pagani O WB, Fleming GF, Colleoni M, et al. SOFT and TEXT Investigators and the International Breast Cancer Study Group (a division of ETOP IBCSG Partners Foundation). Adjuvant Exemestane With Ovarian Suppression in Premenopausal Breast Cancer: Long-Term Follow-Up of the Combined TEXT and SOFT Trials. J Clin Oncol. 2023 Mar 1;41(7):1376-1382.
84. Jordan VC. Tamoxifen: a most unlikely pioneering medicine. Nat Rev Drug Discov. 2003;2(3):205-13.
85. Jordan VC, 1994 Molecular mechanisms of antiestrogen action in breast cancer. Breast Cancer Research and Treatment 31, 41–52.
86. Yang G, Nowsheen S, Aziz K, Georgakilas AG. Toxicity and adverse effects of Tamoxifen and other anti-estrogen drugs. Pharmacol Ther. 2013;139(3):392-404.
87. Jordan VC. Fourteenth Gaddum Memorial Lecture. A current view of tamoxifen for the treatment and prevention of breast cancer. Br J Pharmacol. 1993 Oct;110(2):507-17.
88. Cuzick J SI, Bonanni B, Costantino JP, et al. SERM Chemoprevention of Breast Cancer Overview Group. Selective oestrogen receptor modulators in prevention of breast cancer: an updated meta-analysis of individual participant data. Lancet. 2013 May 25;381(9880):1827-34.
89. Eriksson M EM, Borgquist S, Hellgren R, et al. Low dose tamoxifen for mammographic density reduction – a randomized controlled trial. J Clin Oncol. 2021 Jun 10;39(17):1899-1908.
90. Early Breast Cancer Trialists' Collaborative Group.Tamoxifen for early breast cancer: an overview of the randomised trials. Lancet. 1998 May 16;351(9114):1451-67.
91. Rea D, Handley K, Bowden SJ, et al. aTTom: Long-term effects of continuing adjuvant tamoxifen to 10 years versus stopping at 5 years in 6,953 women with early breast cancer. Journal of clinical oncology : official journal of the American Society of Clinical Oncology. 2013;31 18_suppl:5.
92. Ekholm M, Bendahl, P.O., Fernö, M., et al., 2019. Effects of adjuvant tamoxifen over three decades on breast cancer–free and distant recurrence–free interval among premenopausal women with oestrogen receptor–positive breast cancer randomised in the Swedish SBII:2pre trial. European Journal of Cancer 110, 53–61.
93. Gustafsson J-Å. What pharmacologists can learn from recent advances in estrogen signalling. Trends in Pharmacological Sciences. 2003;24(9):479-85.
94. Moon Z, Hunter MS, Moss-Morris R, et al. Factors related to the experience of menopausal symptoms in women prescribed tamoxifen. J Psychosom Obstet Gynaecol. 2017;38(3):226-35.
95. Love RR, Baum M, Love SM, et al. Clinical practice to address tamoxifen nonadherence. Breast Cancer Res Treat. 2020 Dec;184(3):675-682.:

96. Taylor C, Crosby I, Yip V, Maguire P, Pirmohamed M, Turner RM. A Review of the Important Role of *CYP2D6* in Pharmacogenomics. Genes (Basel). 2020 Oct 30;11(11):1295. doi: 10.3390/genes11111295. PMID: 33143137; PMCID: PMC7692531
97. Kane M. CYP2D6 Overview: Allele and Phenotype Frequencies. 2021 Oct 15. In: Pratt VM, Scott SA, Pirmohamed M, et al., editors. Medical Genetics Summaries [Internet]. Bethesda (MD): National Center for Biotechnology Information (US); 2012-. Available from: https://www.ncbi.nlm.nih.gov/books/NBK574601/
98. Tornio A, Backman JT. Cytochrome P450 in Pharmacogenetics: An Update. Adv Pharmacol. 2018;83:3-32.
99. Zhou Y, Ingelman-Sundberg M, Lauschke VM. Worldwide Distribution of Cytochrome P450 Alleles: A Meta-analysis of Population-scale Sequencing Projects. Clin Pharmacol Ther. 2017;102(4):688-700.
100. Gaedigk A, Sangkuhl K, Whirl-Carrillo M, et al. Prediction of CYP2D6 phenotype from genotype across world populations. Genet Med. 2017;19(1):69-76.
101. Zanger UM, Raimundo S, Eichelbaum M. Cytochrome P450 2D6: overview and update on pharmacology, genetics, biochemistry. Naunyn Schmiedebergs Arch Pharmacol. 2004;369(1):23-37.
102. Bertilsson L, Dahl, M.-L., Dalén, P., et al.., 2002. Molecular genetics of CYP2D6: Clinical relevance with focus on psychotropic drugs. British Journal of Clinical Pharmacology 53, 111–122.
103. Pharmacogene Variation Consortium (PharmVar), CYP2D6. Last uppdated May 21, 2023. Available from: Pharmacogene Variation Consortium (PharmVar), CYP2D6. Accessed on 16/07/23.
104. Sim SC, Ingelman-Sundberg M. Update on allele nomenclature for human cytochromes P450 and the Human Cytochrome P450 Allele (CYP-allele) Nomenclature Database. Methods Mol Biol. 2013;987:251-9.
105. Nofziger C TA, Sangkuhl K, Whirl-Carrillo M, et al. A. PharmVar GeneFocus: CYP2D6. Clin Pharmacol Ther. 2020 Jan;107(1):154-170.
106. Ingelman-Sundberg M, Sim SC, Gomez A, et al. Influence of cytochrome P450 polymorphisms on drug therapies: pharmacogenetic, pharmacoepigenetic and clinical aspects. Pharmacol Ther. 2007;116(3):496-526.
107. Schroth W, Winter S, Murdter T, et al. Improved Prediction of Endoxifen Metabolism by CYP2D6 Genotype in Breast Cancer Patients Treated with Tamoxifen. Front Pharmacol. 2017;8:582.
108. Caudle KE, Sangkuhl K, Whirl-Carrillo M, et al. Standardizing CYP2D6 Genotype to Phenotype Translation: Consensus Recommendations from the Clinical Pharmacogenetics Implementation Consortium and Dutch Pharmacogenetics Working Group. Clin Transl Sci. 2020 Jan;13(1):116-124.
109. Petrović J, Pešić, V., Lauschke, V.M., 2020. Frequencies of clinically important CYP2C19 and CYP2D6 alleles are graded across Europe. European Journal of Human Genetics 28, 88–94.
110. Zhou Y LV. Population pharmacogenomics: an update on ethnogeographic differences and opportunities for precision public health. Hum Genet. 2022 Jun;141(6):1113-1136.
111. Llerena A, Naranjo MEG, Rodrigues-Soares F, et al. Interethnic variability of CYP2D6alleles and of predicted and measured metabolic phenotypes across world populations. Expert Opinion on Drug Metabolism & Toxicology. 2014;10(11):1569-83.
112. Dahl ML JI, Bertilsson L, Ingelman-Sundberg M, et al. Ultrarapid hydroxylation of debrisoquine in a Swedish population. Analysis of the molecular genetic basis. J Pharmacol Exp Ther. 1995 Jul;274(1):516-20.
113. Wollmann BM SE, Kringen MK, Molden E, et al. Prediction of CYP2D6 poor metabolizers by measurements of solanidine and metabolites-a study in 839 patients with known CYP2D6 genotype. Eur J Clin Pharmacol. 2023 Apr;79(4):523-531.
114. Saladores PH, Precht, J.C., Schroth, et al., 2013. Impact of metabolizing enzymes on drug response of endocrine therapy in breast cancer. Expert Review of Molecular Diagnostics 13, 349–365.

115. Streetman DS BJJ, Nafziger AN. Phenotyping of drug-metabolizing enzymes in adults: a review of in-vivo cytochrome P450 phenotyping probes. Pharmacogenetics. 2000 Apr;10(3):187-216.
116. Thermo Fischer Scientific. Real -time PCR-basics. Abailable from: https://www.thermofisher.com/se/en/home/life-science/pcr/real-time-pcr/real-time-pcr-learning-center/real-time-pcr-basics/essentials-real-time-pcr.html. Accessed on 16/05/23.
117. Pratt VM CL, Del Tredici AL, Gaedigk A, et al. Recommendations for Clinical CYP2D6 Genotyping Allele Selection: A Joint Consensus Recommendation of the Association for Molecular Pathology, College of American Pathologists, Dutch Pharmacogenetics Working Group of the Royal Dutch Pharmacists Association, and the European Society for Pharmacogenomics and Personalized Therapy. J Mol Diagn. 2021 Sep;23(9):1047-1064.
118. Jin Y, Desta Z, Stearns V, et al. CYP2D6 genotype, antidepressant use, and tamoxifen metabolism during adjuvant breast cancer treatment. J Natl Cancer Inst. 2005;97(1):30-9.
119. Helland T, Alsomairy S, Lin C, et al. Generating a Precision Endoxifen Prediction Algorithm to Advance Personalized Tamoxifen Treatment in Patients with Breast Cancer. Journal of Personalized Medicine. 2021;11(3).
120. Sanchez-Spitman AB, Swen JJ, Dezentje VO, et al. Clinical pharmacokinetics and pharmacogenetics of tamoxifen and endoxifen. Expert Rev Clin Pharmacol. 2019;12(6):523-36.
121. Goetz MP RJ, Suman VJ, Safgren SL, et al. Pharmacogenetics of tamoxifen biotransformation is associated with clinical outcomes of efficacy and hot flashes. J Clin Oncol. 2005 Dec 20;23(36):9312-8.
122. Regan MM, Leyland-Jones B, Bouzyk M, et al. CYP2D6 genotype and tamoxifen response in postmenopausal women with endocrine-responsive breast cancer: the breast international group 1-98 trial. J Natl Cancer Inst. 2012;104(6):441-51.
123. Rae JM, Drury S, Hayes DF, et al. CYP2D6 and UGT2B7 genotype and risk of recurrence in tamoxifen-treated breast cancer patients. J Natl Cancer Inst. 2012;104(6):452-60.
124. Province MA, Goetz MP, Brauch H, et al. CYP2D6 genotype and adjuvant tamoxifen: meta-analysis of heterogeneous study populations. Clin Pharmacol Ther. 2014;95(2):216-27.
125. Binkhorst L, Mathijssen RH, Jager A, et al. Individualization of tamoxifen therapy: much more than just CYP2D6 genotyping. Cancer Treat Rev. 2015;41(3):289-99.
126. 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.
127. Lien EA SE, Lea OA, Lundgren S, et al. Distribution of 4-hydroxy-N-desmethyltamoxifen and other tamoxifen metabolites in human biological fluids during tamoxifen treatment. Cancer Res 1989;49:2175–83.
128. 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.
129. Mulder TAM, de With M, del Re M,et al. Clinical CYP2D6 Genotyping to Personalize Adjuvant Tamoxifen Treatment in ER-Positive Breast Cancer Patients: Current Status of a Controversy. Cancers. 2021;13(4).
130. 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.
131. Almeida T SW, Nardin J, Mürdter TE, et al. (Z)-Endoxifen and Early Recurrence of Breast Cancer: An Explorative Analysis in a Prospective Brazilian Study. J Pers Med. 2022 Mar 22;12(4):511.
132. Haslemo T, Eliasson E, Jukić MM, et al. Significantly lower CYP2D6 metabolism measured as the O/N-desmethylvenlafaxine metabolic ratio in carriers of CYP2D6*41 versus CYP2D6*9 or CYP2D6*10: a study on therapeutic drug monitoring data from 1003 genotyped Scandinavian patients. Br J Clin Pharmacol. 2019 Jan;85(1):194-201.

133. Puszkiel A, Arellano C, Vachoux C, et al. Factors Affecting Tamoxifen Metabolism in Patients With Breast Cancer: Preliminary Results of the French PHACS Study. Clin Pharmacol Ther. 2019 Sep;106(3):585-595.
134. 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.
135. Hwang GS, Bhat R, Crutchley RD, et al. Impact of CYP2D6 polymorphisms on endoxifen concentrations and breast cancer outcomes. Pharmacogenomics Journal. 2018;18(2):201-8.
136. Neven P, Jongen L, Lintermans A, et al. Tamoxifen Metabolism and Efficacy in Breast Cancer: A Prospective Multicenter Trial. Clin Cancer Res. 2018;24(10):2312-8.
137. Sanchez-Spitman AB, Dezentje VO, Swen JJ, et al. A prospective study on the effect of endoxifen concentration and CYP2D6 phenotypes on clinical outcome in early stage breast cancer patients receiving adjuvant tamoxifen. Journal of Clinical Oncology. 2018;36(15).
138. 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 Res. 2017;19(1):125.
139. Sanchez-Spitman A DV, Swen J, Moes DJAR, et al. Tamoxifen Pharmacogenetics and Metabolism: Results From the Prospective CYPTAM Study. J Clin Oncol. 2019;2019 Mar 10;37(8):636-646
140. Love RR, Desta, Z., Flockhart, D., et al., 2013 CYP2D6 genotypes, endoxifen levels, and disease recurrence in 224 Filipino and Vietnamese women receiving adjuvant tamoxifen for operable breast cancer. Springerplus. 2013 Dec;2(1):52.
141. Jager NG1 KR, Vincent AD, van Schaik RH, et al. Hot flashes are not predictive for serum concentrations of tamoxifen and its metabolites. BMC Cancer 2013 Dec 28;13:612.
142. Jansen LE, Teft WA, Rose RV, et al. CYP2D6 genotype and endoxifen plasma concentration do not predict hot flash severity during tamoxifen therapy. Breast Cancer Res Treat. 2018;171(3):701-8.
143. Lorizio W, Wu AHB, Beattie MS, et al. Clinical and biomarker predictors of side effects from tamoxifen. Breast Cancer Research and Treatment. 2011;132(3):1107-18.
144. Rolla R VM, Meola S, et al: Side effects associated with ultrarapid cytochrome P450 2D6 genotype among women with early stage breast cancer treated with tamoxifen. Clin Lab 58:1211-8, 2012.
145. 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.
146. He W, Grassmann F, Eriksson M, et al. CYP2D6 Genotype Predicts Tamoxifen Discontinuation and Prognosis in Patients With Breast Cancer. J Clin Oncol. 2020 Feb 20;38(6):548-557.
147. 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. Clin Cancer Res. 2016;22(13):3164-71.
148. Dezentje 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.
149. Braal CL JA, Hoop EO, Westenberg JD, et al. Therapeutic Drug Monitoring of Endoxifen for Tamoxifen Precision Dosing: Feasible in Patients with Hormone-Sensitive Breast Cancer. Clin Pharmacokinet. 2022 Apr;61(4):527-537.
150. Khalaj Z, Baratieh Z, Nikpour P, et al. Clinical Trial: *CYP2D6* Related Dose Escalation of Tamoxifen in Breast Cancer Patients With Iranian Ethnic Background Resulted in Increased Concentrations of Tamoxifen and Its Metabolites. Front Pharmacol. 2019 May 24;10:530.
151. Welzen MB, Dezentjé, V. O. , van Schaik, et al. (2015). The Effect of Tamoxifen Dose Increment in Patients With Impaired CYP2D6 Activity. Therapeutic Drug Monitoring, 37 (4), 501-507.
152. Hertz DL, Deal A, Ibrahim JG, Walko CM, et al. Tamoxifen Dose Escalation in Patients With Diminished CYP2D6 Activity Normalizes Endoxifen Concentrations Without Increasing Toxicity. Oncologist. 2016;21(7):795-803.

153. Kiyotani K, Mushiroda T, Imamura CK, et al. Dose-adjustment study of tamoxifen based on CYP2D6 genotypes in Japanese breast cancer patients. Breast Cancer Research and Treatment. 2012;131(1):137-45.
154. Martinez de Dueñas E OAE, Blancas Lopez-Barajas I, Ferrer Magdalena T, et al. Adjusting the dose of tamoxifen in patients with early breast cancer and CYP2D6 poor metabolizer phenotype. Breast. 2014 Aug;23(4):400-6.
155. Irvin WJ Jr WC, Weck KE, Ibrahim JG, et al. Genotype-guided tamoxifen dosing increases active metabolite exposure in women with reduced CYP2D6 metabolism: a multicenter study. J Clin Oncol. 2011 Aug 20;29(24):3232-9.
156. Maggadani BP, Junusmin KI, Sani LL, et al. CYP2D6 Genotyping for Personalized Therapy of Tamoxifen in Indonesian Women with ER+ Breast Cancer. 2021 Jun 30. (medRxiv).
157. Blancas I L-RM, Martínez de Dueñas E, Herrero-Vicent C, et al. Early increase in tamoxifen dose in CYP2D6 poor metaboliser breast cancer patients and survival: A propensity score matching analysis. Breast. 2023 Mar 23;69:342-348.
158. Khor CC WS, Sutiman N, Mürdter TE, et al. Cross-Ancestry Genome-Wide Association Study Defines the Extended CYP2D6 Locus as the Principal Genetic Determinant of Endoxifen Plasma Concentrations. Clin Pharmacol Ther. 2023 Mar;113(3):712-723.
159. Sim S, Lovrot J, Lindh JD, et al. Effect of CYP2C19 and CYP2D6 genotype on tamoxifen treatment outcome indicates endogenous and exogenous interplay. Pharmacogenomics. 2018;19(13):1027-37.
160. Sanchez-Spitman AB, Swen JJ, Dezentjé VO, et al. Effect of CYP2C19 genotypes on tamoxifen metabolism and early-breast cancer relapse. Scientific Reports. 2021;11(1).
161. Lenk HÇ, Klöditz K, Johansson I, et al. The Polymorphic Nuclear Factor NFIB Regulates Hepatic CYP2D6 Expression and Influences Risperidone Metabolism in Psychiatric Patients. Clinical Pharmacology & Therapeutics. 2022;111(5):1165-74.
162. Nardin JM, Schroth W, Almeida TA, et al. The Influences of Adherence to Tamoxifen and CYP2D6 Pharmacogenetics on Plasma Concentrations of the Active Metabolite (Z)-Endoxifen in Breast Cancer. Clin Transl Sci. 2020 Mar;13(2):284-292.
163. Flockhart DA, Thacker, D., McDonald, C. et al. The Flockhart Cytochrome P450 Drug-Drug Interaction Table. Division of Clinical Pharmacology, Indiana University School of Medicine. (Updated 2021). [cited march 2023]. Available from: https://drug-interactions.medicine.iu.edu.
164. Bradbury H, B., Beltran-Bless, A.-A., Alzahrani, M., et al. (2022). Time to Update Evidence-Based Guideline Recommendations About Concurrent Tamoxifen and Antidepressant Use? A Systematic Review. Clinical Breast Cancer., 22(3), e362–e373.
165. Jager NGL, Rosing, H., Linn, S. C., et al. (2012). Importance of highly selective LC-MS/MS analysis for the accurate quantification of tamoxifen and its metabolites: focus on endoxifen and 4-hydroxytamoxifen. Breast Cancer Research and Treatment., 133(2), 793–798.
166. Thermo Fischer Scientific. Liquid-chromatography-information. Available from: www.thermofisher.com/se/en/home/industrial/chromatography/ chromatography-learning-center/liquid-chromatography-information.html. Accessed on 16/05/23.
167. Goetz MP. The development of endoxifen for breast cancer. Clin Adv Hematol Oncol. 2018 Feb;16(2):102-105.
168. Rani A SJ, Giamas G, Murphy J. Endocrine Resistance in Hormone Receptor Positive Breast Cancer-From Mechanism to Therapy. Front Endocrinol (Lausanne). 2019 May 24;10:245.
169. Løkkegaard S ED, Alves CL, Bennetzen MV, et al. MCM3 upregulation confers endocrine resistance in breast cancer and is a predictive marker of diminished tamoxifen benefit. NPJ Breast Cancer. 2021 Jan 4;7(1):2.
170. Dahlgren M GA, Brueffer C, Gladchuk S, et al. Preexisting Somatic Mutations of Estrogen Receptor Alpha (ESR1) in Early-Stage Primary Breast Cancer. JNCI Cancer Spectr. 2021 Apr 22;5(2):pkab028.
171. Yussof I, Mohd Tahir NA, Hatah E, et al. Factors influencing five-year adherence to adjuvant endocrine therapy in breast cancer patients: A systematic review. The Breast. 2022;62:22-35.

172. Ayres LR, Baldoni Ade O, Borges AP, et al. Adherence and discontinuation of oral hormonal therapy in patients with hormone receptor positive breast cancer. Int J Clin Pharm. 2014;36(1):45-54.
173. Osterberg L, Blaschke T. Adherence to medication. N Engl J Med. 2005;353(5):487-97.
174. Raebel MA SJ, Karter AJ, Konieczny JL, et al. Standardizing terminology and definitions of medication adherence and persistence in research employing electronic databases. Med Care. 2013 Aug;51(8 Suppl 3):S11-21.
175. 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. J Clin Oncol. 2016;34(21):2452-9.
176. Murphy CC, Bartholomew LK, Carpentier MY, et al. Adherence to adjuvant hormonal therapy among breast cancer survivors in clinical practice: a systematic review. Breast Cancer Res Treat. 2012;134(2):459-78.
177. Pistilli B PA, Ferreira AR, Di Meglio A, et al. Serum Detection of Nonadherence to Adjuvant Tamoxifen and Breast Cancer Recurrence Risk. J Clin Oncol. 2020 Aug 20;38(24):2762-2772.
178. Schneeweiss S, Avorn J. A review of uses of health care utilization databases for epidemiologic research on therapeutics. J Clin Epidemiol. 2005;58(4):323-37.
179. Font R, Espinas JA, Gil-Gil M, et al. Prescription refill, patient self-report and physician report in assessing adherence to oral endocrine therapy in early breast cancer patients: a retrospective cohort study in Catalonia, Spain. Br J Cancer. 2012;107(8):1249-56.
180. Hagen KB, Aas T, Kvaløy JT, et al. Adherence to adjuvant endocrine therapy in postmenopausal breast cancer patients: A 5-year prospective study. The Breast. 2019;44:52-8.
181. Chlebowski RT, Kim J, Haque R. Adherence to endocrine therapy in breast cancer adjuvant and prevention settings. Cancer Prev Res (Phila). 2014;7(4):378-87.
182. Wigertz A, Ahlgren J, Holmqvist M, et al. Adherence and discontinuation of adjuvant hormonal therapy in breast cancer patients: a population-based study. Breast Cancer Research and Treatment. 2012;133(1):367-73.
183. He W, Fang F, Varnum C, et al. Predictors of Discontinuation of Adjuvant Hormone Therapy in Patients With Breast Cancer. J Clin Oncol. 2015;33(20):2262-9.
184. He W EL, Törnberg S, Strand F, et al. Discontinuation of adjuvant hormone therapy among breast cancer patients not previously attending mammography screening. BMC Med. 2019 Jan 31;17(1):24.
185. Andersson A, Väppling AVW, Jong AD, et al. Adherence to adjuvant endocrine therapy after breast cancer in Sweden 2008-2010: A nationwide survey. Journal of Clinical Oncology. 2019;37(15_suppl):523-.
186. Lundgren C, Lindman H, Rolander B, et al. Good adherence to adjuvant endocrine therapy in early breast cancer - a population-based study based on the Swedish Prescribed Drug Register. Acta Oncol. 2018;57(7):935-40.
187. Wulaningsih W, Garmo H, Ahlgren J, et al. Determinants of non-adherence to adjuvant endocrine treatment in women with breast cancer: the role of comorbidity. Breast Cancer Res Treat. 2018;172(1):167-77.
188. Markkula A, Hietala M, Henningson M, et al. Clinical profiles predict early nonadherence to adjuvant endocrine treatment in a prospective breast cancer cohort. Cancer Prev Res (Phila). 2012;5(5):735-45.
189. Paranjpe R, John G, Trivedi M, et al. Identifying adherence barriers to oral endocrine therapy among breast cancer survivors. Breast Cancer Res Treat. 2018.
190. Rae JM, Sikora MJ, Henry NL, et al. Cytochrome P450 2D6 activity predicts discontinuation of tamoxifen therapy in breast cancer patients. Pharmacogenomics J. 2009;9(4):258-64.
191. McCowan C, Shearer J, Donnan PT, et al. Cohort study examining tamoxifen adherence and its relationship to mortality in women with breast cancer. Br J Cancer. 2008;99(11):1763-8.
192. Makubate B, Donnan PT, Dewar JA, et al. Cohort study of adherence to adjuvant endocrine therapy, breast cancer recurrence and mortality. Br J Cancer. 2013;108(7):1515-24.

193. Hershman DL, Shao T, Kushi LH, et al. Early discontinuation and non-adherence to adjuvant hormonal therapy are associated with increased mortality in women with breast cancer. Breast Cancer Res Treat. 2011;126(2):529-37.
194. McCowan C, Wang S, Thompson AM, et al. The value of high adherence to tamoxifen in women with breast cancer: a community-based cohort study. Br J Cancer. 2013;109(5):1172-80.
195. Font R, Espinas JA, Barnadas A, et al. Influence of adherence to adjuvant endocrine therapy on disease-free and overall survival: a population-based study in Catalonia, Spain. Breast Cancer Res Treat. 2019.
196. He W, Smedby KE, Fang F, et al. Treatment Restarting After Discontinuation of Adjuvant Hormone Therapy in Breast Cancer Patients. J Natl Cancer Inst. 2017;109(10).
197. Huo CW, Chew G, Hill P, et al. High mammographic density is associated with an increase in stromal collagen and immune cells within the mammary epithelium. Breast Cancer Res. 2015;17:79.
198. Engmann NJ, Scott C, Jensen MR, et al. Longitudinal Changes in Volumetric Breast Density in Healthy Women across the Menopausal Transition. Cancer Epidemiology, Biomarkers & Prevention. 2019;28(8):1324-30.
199. 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.
200. Azam S, Sjölander A, Eriksson M, et al. Determinants of Mammographic Density Change. JNCI Cancer Spectr. 2019 Feb 4;3(1):pkz004.
201. Eriksson M, Czene K, Pawitan Y, et al. A clinical model for identifying the short-term risk of breast cancer. Breast Cancer Res. 2017;19(1):29.
202. Nazari SS, Mukherjee P. An overview of mammographic density and its association with breast cancer. Breast Cancer. 2018;25(3):259-67.
203. Chew GL, Huo CW, Huang D, et al. Increased COX-2 expression in epithelial and stromal cells of high mammographic density tissues and in a xenograft model of mammographic density. Breast Cancer Res Treat. 2015;153(1):89-99.
204. Pang JM, Byrne DJ, Takano EA, et al. Breast Tissue Composition and Immunophenotype and Its Relationship with Mammographic Density in Women at High Risk of Breast Cancer. PLoS One. 2015;10(6):e0128861.
205. Wanders JOP, Holland K, Karssemeijer N,et al. The effect of volumetric breast density on the risk of screen-detected and interval breast cancers: a cohort study. Breast Cancer Res. 2017;19(1):67.
206. Kolb TM, Lichy J, Newhouse JH. Comparison of the performance of screening mammography, physical examination, and breast US and evaluation of factors that influence them: an analysis of 27,825 patient evaluations. Radiology. 2002 Oct;225(1):165-75.
207. Sprague BL, Gangnon RE, Burt V, et al. Prevalence of mammographically dense breasts in the United States. J Natl Cancer Inst. 2014;106(10).
208. Ciatto S, Bernardi D, Calabrese M, et al. A first evaluation of breast radiological density assessment by QUANTRA software as compared to visual classification. Breast. 2012;21(4):503-6.
209. Byng JW, Yaffe MJ, Jong RA, et al. Analysis of mammographic density and breast cancer risk from digitized mammograms. Radiographics. 1998 Nov-Dec;18(6):1587-98. doi: 10.1148/radiographics.18.6.9821201.
210. Eriksson M, Li J, Leifland K, et al. A comprehensive tool for measuring mammographic density changes over time. Breast Cancer Res Treat. 2018 Jun;169(2):371-379.
211. Astley SM HE, Sergeant JC, Warwick J, et al. A comparison of five methods of measuring mammographic density: a case-control study. Breast Cancer Res. 2018 Feb 5;20(1):10.
212. Lee HN SY, Han KH. Comparison of mammographic density estimation by Volpara software with radiologists' visual assessment: analysis of clinical-radiologic factors affecting discrepancy between them. Acta Radiol. 2015 Sep;56(9):1061-8.
213. Haars G vNP, van Gils CH, Grobbee DE, et al. Measurements of breast density: no ratio for a ratio. Cancer Epidemiol Biomarkers Prev. 2005 Nov;14:2634-40.

214. Boyd L, G. A., Byng, J. W., Little, L. E., et al. (1998). The relationship of anthropometric measures to radiological features of the breast in premenopausal women. British Journal of Cancer., 78(9), 1233–1238.
215. Nyante SJ, Sherman ME, Pfeiffer RM, et al. Longitudinal Change in Mammographic Density among ER-Positive Breast Cancer Patients Using Tamoxifen. Cancer Epidemiol Biomarkers Prev. 2016;25(1):212-6.
216. Mullooly M, Pfeiffer RM, Nyante SJ, et al. Mammographic Density as a Biosensor of Tamoxifen Effectiveness in Adjuvant Endocrine Treatment of Breast Cancer: Opportunities and Implications. J Clin Oncol. 2016;34(18):2093-7.
217. Kim J, Han W, Moon HG, et al. Breast density change as a predictive surrogate for response to adjuvant endocrine therapy in hormone receptor positive breast cancer. Breast Cancer Res. 2012;14(4):R102.
218. Kim WH, Cho N, Kim YS, et al. Mammographic density changes following discontinuation of tamoxifen in premenopausal women with oestrogen receptor-positive breast cancer. Eur Radiol. 2018 Aug;28(8):3176-3184.
219. Li J, Humphreys K, Eriksson L, et al. Mammographic density reduction is a prognostic marker of response to adjuvant tamoxifen therapy in postmenopausal patients with breast cancer. J Clin Oncol. 2013;31(18):2249-56.
220. Mullooly M, Nyante SJ, Pfeiffer RM, et al. Involution of Breast Lobules, Mammographic Breast Density and Prognosis Among Tamoxifen-Treated Estrogen Receptor-Positive Breast Cancer Patients. J Clin Med. 2019;8(11).
221. Engmann NJ, Scott CG, Jensen MR, et al. Longitudinal Changes in Volumetric Breast Density with Tamoxifen and Aromatase Inhibitors. Cancer Epidemiol Biomarkers Prev. 2017;26(6):930-7.
222. Hattar R, Maller O, McDaniel S, et al. Tamoxifen induces pleiotrophic changes in mammary stroma resulting in extracellular matrix that suppresses transformed phenotypes. Breast Cancer Res. 2009;11(1):R5.
223. Eriksson L, He W, Eriksson M, et al. Adjuvant Therapy and Mammographic Density Changes in Women With Breast Cancer. JNCI Cancer Spectr. 2018;2(4):pky071.
224. Sandberg ME, Li J, Hall P, et al. Change of mammographic density predicts the risk of contralateral breast cancer--a case-control study. Breast Cancer Res. 2013;15(4):R57.
225. Knight JA, Blackmore KM, Fan J, et al. The association of mammographic density with risk of contralateral breast cancer and change in density with treatment in the WECARE study. Breast Cancer Res. 2018;20(1):23.
226. Vachon CM, Suman VJ, Brandt KR, et al. Mammographic breast density response to aromatase inhibition. Clin Cancer Res. 2013;19(8):2144-53.
227. Prowell TM, Blackford AL, Byrne C, et al. Changes in breast density and circulating estrogens in postmenopausal women receiving adjuvant anastrozole. Cancer Prev Res (Phila). 2011;4(12):1993-2001.
228. Yang H, Zong X, Yu Y, et al. Combined effects of goserelin and tamoxifen on estradiol level, breast density, and endometrial thickness in premenopausal and perimenopausal women with early-stage hormone receptor-positive breast cancer: a randomised controlled clinical trial. Br J Cancer. 2013;109(3):582-8.
229. Bagnera S, Milanesio L, Brachet Cota PB, et al. Does accelerated hypofractionated adjuvant whole-breast radiotherapy increase mammographic density or change mammographic features? Br J Radiol. 2015;88(1055):20150312.
230. Manual Nationellt Kvalitetsregister för Bröstcancer. 2021. Available from: https://cancercentrum.se/globalassets/cancerdiagnoser/brost/kvalitetsregister/ manual-nationellt-kvalitetsregister-for-brostcancer.pdf. Accessed on 16/05/23.
231. Bäcklund M EM, Hammarström M, Thoren L, et al. Time to Mammographic Density Decrease After Exposure to Tamoxifen. Oncologist. 2022 Jul 5;27(7):e601-e603.
232. Thorén L, Lindh JD, Ackehed G, et al. Impairment of endoxifen formation in tamoxifen-treated premenopausal breast cancer patients carrying reduced-function CYP2D6 alleles. British Journal of Clinical Pharmacology. 2021;87(3):1243-52.

233. Molden E, Jukic MM. CYP2D6 Reduced Function Variants and Genotype/Phenotype Translations of CYP2D6 Intermediate Metabolizers: Implications for Personalized Drug Dosing in Psychiatry. Front Pharmacol. 2021;12:650750.
234. Cleveland W S. Robust Locally Weighted Regression and Smoothing Scatterplot. Journal of the American Statistical Association, Vol. 74, No. 368. (Dec., 1979), pp. 829-836.
235. National Prescribed Drug Register. 2023. Available from: https://www.socialstyrelsen.se/en/statistics-and-data/registers/national-prescribed-drug-register/. Accessed on 16/05/23.
236. EMA guidelines for investigation of bioequvivalence [Internet]. Available from: https://www.ema.europa.eu/en/documents/scientifc-guideline/guideline. Accessed on 16/05/23.
237. Coates AS, Winer, E.P., Goldhirsch, A., et al., 2015. Tailoring therapies—improving the management of early breast cancer: St Gallen International Expert Consensus on the Primary Therapy of Early Breast Cancer 2015. Annals of Oncology 26, 1533–1546.
238. Frederiksen T, Areberg, J., Schmidt, E., et.al., 2021. Cytochrome P450 2D6 genotype–phenotype characterization through population pharmacokinetic modeling of tedatioxetine. CPT: Pharmacometrics & Systems Pharmacology 10, 983–993.
239. Sanchez-Spitman AB, Moes, D.-J.A.R., Swen, J.J., et al. 2020. Exposure–response analysis of endoxifen serum concentrations in early-breast cancer. Cancer Chemotherapy and Pharmacology 85, 1141–1152.
240. Schroth W, Winter S, Mürdter T, et al. Improved Prediction of Endoxifen Metabolism by CYP2D6 Genotype in Breast Cancer Patients Treated with Tamoxifen. Front Pharmacol. 2017 Aug 24;8:582.
241. Lee CI FP, Balakrishnar B, Balleine RL, et al. Tamoxifen-induced severe hot flashes and endoxifen levels: is dose reduction a safe and effective strategy? Breast. 2019 Aug;46:52-57. doi: 10.1016/j.breast.2019.05.009. Epub 2019 May 6.
242. El Desoky ES TA, Mousa HS, Ibrahim A, et al. Value of therapeutic drug monitoring of endoxifen in Egyptian premenopausal patients with breast cancer given tamoxifen adjuvant therapy: A pilot study. J Oncol Pharm Pract. 2022 Dec 25:10781552221146531.
243. Thorén L, Eriksson M, Lindh JD, et al. Impact of systemic adjuvant therapy and CYP2D6 activity on mammographic density in a cohort of tamoxifen-treated breast cancer patients. Breast Cancer Res Treat. 2021 Dec;190(3):451-462.
244. He W, Eriksson M, Eliasson E, et al. CYP2D6 genotype predicts tamoxifen discontinuation and drug response: a secondary analysis of the KARISMA trial. Annals of Oncology. 2021;32(10):1286-93.
245. DeCensi A JH, Helland T, Puntoni M, et al. Association of CYP2D6 genotype and tamoxifen metabolites with breast cancer recurrence in a low-dose trial. NPJ Breast Cancer. 2021 Mar 25;7(1):34.
246. Thoren L, Margolin S, Eliasson E, Bergh J, Lindh JD. Adherence to endocrine therapy in early breast cancer in relation to Cytochrome P450 2D6 genotype: a comparison between pharmacy dispensation data and medical records. Breast Cancer Res Treat. 2023.
247. Markkula A HM, Henningson M, Ingvar C, et al. Clinical profiles predict early nonadherence to adjuvant endocrine treatment in a prospective breast cancer cohort. Cancer Prev Res (Phila). 2012 May;5(5):735-45.
248. Kardas P, Aguilar-Palacio I, Almada M, et al. The Need to Develop Standard Measures of Patient Adherence for Big Data: Viewpoint. J Med Internet Res. 2020 Aug 27;22(8):e18150.
249. Ingelman-Sundberg M PA, Jukic MM. Polymorphic expression of CYP2C19 and CYP2D6 in the developing and adult human brain causing variability in cognition, risk for depression and suicide: the search for the endogenous substrates. Pharmacogenomics. 2014;15(15):1841-4.
250. Peñas-Lledó EM LA. CYP2D6 variation, behaviour and psychopathology: implications for pharmacogenomics-guided clinical trials. Br J Clin Pharmacol. 2014 Apr;77(4):673-83.
251. Jani C SI, Rupal A, Al Omari O, et al. Trends in Breast Cancer Mortality Between 2001 and 2017: An Observational Study in the European Union and the United Kingdom. JCO Glob Oncol. 2021 Dec;7:1682-1693. Erratum in: JCO Glob Oncol. 2022 Jan;8:e2200015.

252. Taylor C MP, Probert J, Broggio J, et al. Breast cancer mortality in 500 000 women with early invasive breast cancer in England, 1993-2015: population based observational cohort study. BMJ. 2023 Jun 13;381:e074684.
253. Province MA, Goetz MP, Brauch H,et al. CYP2D6 genotype and adjuvant tamoxifen: meta-analysis of heterogeneous study populations. Clin Pharmacol Ther. 2014;95(2):216-27.
254. Wang H, Ma X, Zhang B, et al. Chinese breast cancer patients with CYP2D6*10 mutant genotypes have a better prognosis with toremifene than with tamoxifen. Asia Pac J Clin Oncol. 2022 Apr;18(2):e148-e153.
255. Beelen K OM, Severson TM, Koornstra RH, et al. CYP2C19 2 predicts substantial tamoxifen benefit in postmenopausal breast cancer patients randomized between adjuvant tamoxifen and no systemic treatment. Breast Cancer Res Treat. 2013 Jun;139(3):649-55.
256. Nthontho KC, Ndlovu, A.K., Sharma, et al. 2022. Pharmacogenetics of Breast Cancer Treatments: A Sub-Saharan Africa Perspective. Pharmacogenomics and Personalized Medicine Volume 15, 613–652.
257. Ramirez-Santana M. Limitations and Biases in Cohort Studies. Cohort Studies in Health Sciences. InTech; 2018. Available from: http://dx.doi.org/10.5772/intechopen.74324.
258. Lorenzo Ficorella ME, Kamila Czene, Goska Leslie, et al. Incorporating continuous mammographic density into the BOADICEA breast cancer risk prediction model. [abstract]. In: Proceedings of the American Association for Cancer Research Annual Meeting 2023; Part 1 (Regular and Invited Abstracts); 2023 Apr 14-19; Orlando, FL. Philadelphia (PA): AACR; Cancer Res 2023;83(7_Suppl):Abstract nr 4174.
259. Eriksson M, Czene, K., Strand, F., et al., 2020. Identification of Women at High Risk of Breast Cancer Who Need Supplemental Screening. Radiology 297, 327–333.
260. Goetz MP, Suman VJ, Reid JM, Northfelt DW, Mahr MA, Ralya AT, et al. First-in-Human Phase I Study of the Tamoxifen Metabolite Z-Endoxifen in Women With Endocrine-Refractory Metastatic Breast Cancer. J Clin Oncol. 2017;35(30):3391-400.
261. Jayaraman S RJ, Hawse JR, Goetz MP. Endoxifen, an Estrogen Receptor Targeted Therapy: From Bench to Bedside. Endocrinology. 2021 Dec 1;162(12):bqab191. doi: 10.1210/endocr/bqab191. Erratum in: Endocrinology. 2021 Dec 1;162(12):
262. Opdam M, Van Der Noort, V., Kleijn, M., et al., 2022. Limiting systemic endocrine overtreatment in postmenopausal breast cancer patients with an ultralow classification of the 70-gene signature. Breast Cancer Research and Treatment 194, 265–278.