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Integration of Tumor and Host Factors – Implications for Breast Cancer Prognosis

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LUND
UNIVERSITY

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Abstract

Breast cancer is the most common cancer among women worldwide, although the mortality rates have decreased in the last decades. Breast cancer is a heterogeneous disease, and treatment resistance is a significant clinical problem in spite of the overall high survival rates. Improved screening programs, novel surgical techniques, and adjuvant treatments have contributed to the improved survival, but more extensive detection of breast cancer and subsequent treatment also imply higher rates of overtreatment. Therefore, it is vitally important to identify new tumor markers or host factors for patients who are at risk of recurrence, as well as patients who would benefit from less treatment. This approach would lead to more personalized breast cancer treatment. The aim of this thesis is to elucidate whether combining host factors, including genetic constitution and lifestyle factors, with tumor characteristics could yield a more comprehensive understanding than either factor alone for the prognosis of breast cancer.

In **paper I**, moderate to high coffee consumption was associated with higher frequency of ER-negative tumors but a lower risk of early breast cancer events among tamoxifen-treated patients with ER-positive tumors. Furthermore, the combinations of low coffee consumption with the germline *CYP1A2* rs762551 C-allele and *CYP2C8*3*, respectively, were associated with a significantly increased risk of early breast cancer events, indicating that integrating genotype and lifestyle factors may impact the prognosis of breast cancer.

In **paper II**, any preoperative or postoperative alcohol consumption was weakly associated with a lower risk of early breast cancer events. This association was modified by axillary lymph node status, in that any alcohol consumption was associated with a significantly lower risk of early breast cancer events among patients with axillary lymph node involvement but not in patients without axillary lymph node involvement. The results do not support recommending that all breast cancer patients abstain from low to moderate alcohol consumption.

In **paper III**, the *CYP1A2* rs762551 C-allele was strongly associated with a higher risk of breast cancer events among aromatase inhibitor-treated patients, and the main impact was found within the first five years. In addition, the impact of the *CYP1A2* rs762551 C-allele was modified by genotypes of *AhR* Arg554Lys and *CYP19A1* rs4646, and the combined genotypes could further improve the prediction of aromatase inhibitor response. If validated, these genotypes could be used as predictive markers for aromatase inhibitor response.

In **paper IV**, tumor-specific COX-2 expression was associated with significantly less aggressive tumor characteristics and was independently associated with a lower risk of early breast cancer events. The association was modified by a history of oral contraceptive (OC) use, preoperative non-steroidal anti-inflammatory drug (NSAID) use, and tumor size. If the findings were validated in an independent prospective cohort or within a randomized trial, history of OC use and tumor size might need to be considered when designing or evaluating clinical outcomes in a randomized controlled trial of adjuvant NSAIDs or COX-2 selective inhibitors for breast cancer patients.

In conclusion, a more comprehensive view of tumor characteristics combined with host factors could be beneficial when assessing breast cancer prognosis and may provide a method for more personalized medicine in the treatment of breast cancer patients.

Key words: Breast cancer, prognosis, CYP1A2, CYP2C8, coffee consumption, alcohol consumption, cyclooxygenase-2

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To my beloved family

To Gunilla and all breast cancer patients
– you are the heroes of our time

Content

List of Original Papers

This thesis is based on the following papers, which are referred to in the text by their Roman numerals:

I. Coffee prevents early events in tamoxifen-treated breast cancer patients and modulates hormone receptor status
Maria Simonsson, Viktoria Söderlind, Maria Henningson, Maria Hjertberg, Carsten Rose, Christian Ingvar, Helena Jernström
Cancer Causes Control. 2013 May;24(5):929-40.

II. Pre- and postoperative alcohol consumption in breast cancer patients: impact on early events
Maria Simonsson, Andrea Markkula, Pär-Ola Bendahl, Carsten Rose, Christian Ingvar, Helena Jernström
SpringerPlus. 2014 May 22;3:261.

III. *CYP1A2* – a novel genetic marker for aromatase inhibitor response in the treatment of breast cancer patients
Maria Simonsson, Srinivas Veerla, Andrea Markkula, Carsten Rose, Christian Ingvar, Helena Jernström
BMC Cancer. 2016 Mar 31;16(1):256.

IV. The prognostic impact of COX-2 expression in breast cancer depends on oral contraceptive history, preoperative NSAID use, and tumor size
Maria Simonsson, Sofie Björner, Andrea Markkula, Björn Nodin, Karin Jirström, Carsten Rose, Signe Borgquist, Christian Ingvar, Helena Jernström
Manuscript submitted

Abbreviations

AACR – American Association for Cancer Research

ADH – Alcohol Dehydrogenase

ADME – Absorption, Distribution, Metabolism, and Elimination

AhR – Aryl Hydrocarbon Receptor

AIs – Aromatase Inhibitors

ALDH – ALdehyde-DeHydrogenase

ATAC trial – Arimidex, Tamoxifen, Alone, or in Combination trial

ATLAS trial – Adjuvant Tamoxifen: Longer Against Shorter trial

AUDIT – Alcohol Use Disorders Identification Test

BC-Blood Study – Breast Cancer and Blood Study

BCS – Breast Conserving Surgery

BIG 1-98 trial – Breast International Group 1-98 trial

BMI – Body Mass Index

CIS – Carcinoma *In Situ*

CLS-B – Crown-Like Structures of the Breast

COX-1/2 – CycloOXygenase 1/2

CRP – C-Reactive Protein

DCIS – Ductal Carcinoma *In Situ*

DMET™ chip – Drug Metabolizing Enzymes and Transporters chip

DNA – DeoxyriboNucleic Acid

EBCTCG – Early Breast Cancer Trialists' Collaborative Group

EPIC study – European Prospective Investigation into Cancer and nutrition study

ER – Estrogen Receptor α

FNA – Fine Needle Aspiration

GnRH agonist – Gonadotropin-Releasing Hormone agonist

GWAS – Genome-Wide Association Studies

HER-2 – Human Epidermal growth factor Receptor 2

HR – Hazard Ratio

HRT – Hormone Replacement Therapy

IGF-1 – Insulin-like Growth Factor 1

ISH – *In Situ* Hybridization

LCIS – Lobular Carcinoma *In Situ*

LD – Linkage Disequilibrium

MHT – Menopausal Hormone Therapy

MRM – Modified Radical Mastectomy

NFκB – Nuclear Factor-κB

OCs – Oral Contraceptives

PCR – Polymerase Chain Reaction

PGE2 – ProstaGlandin E2

PgR – Progesterone Receptor

PTGS1 gene – Prostaglandin-endoperoxide Synthase 1 gene

RFS(5) – Five year Recurrence-Free Survival

ROS – Reactive Oxygen Species

SERMs – Selective Estrogen Receptor Modifiers

SNPs – Single Nucleotide Polymorphisms

STAT-3 – Signal Transducer and Activator of Transcription 3

TMA – Tissue Microarray

TNF-α – Tumor Necrosis Factor α

TNM system – Tumor Node Metastasis system

WHR – Waist-to-Hip Ratio

2-OHE – 2-HydrOxyEstrogens

16α-OHE1 – 16α-HydrOxyEstrone

Introduction

Breast cancer is the most common cancer among women worldwide, accounting for over 1.6 million new cases in 2012 (Torre *et al*, 2015). Globally, breast cancer is also the leading cause of cancer death in women (Torre *et al*, 2015). In high-income countries such as Sweden, lung cancer has taken over as the leading cause of cancer death in the last decade (Engholm *et al*, 2010; NORDCAN; Torre *et al*, 2015). Breast cancer incidence in Sweden is still increasing, and over 8,800 female patients were diagnosed with breast cancer in 2014. One in nine women are expected to be diagnosed before the age of 75 years (Socialstyrelsen, 2013; Socialstyrelsen, 2015). Nevertheless, the prognosis of breast cancer has significantly improved in the last decades, and the 10-year survival in Sweden is now over 80% (Socialstyrelsen, 2013). This improved survival rate has been attributed to earlier detection through screening programs, improved tumor profiling, and adjuvant therapies (Autier *et al*, 2011; Broeders *et al*, 2012; Gelmon *et al*, 2012). See Figure 1 for incidence and mortality rates in Sweden over time.

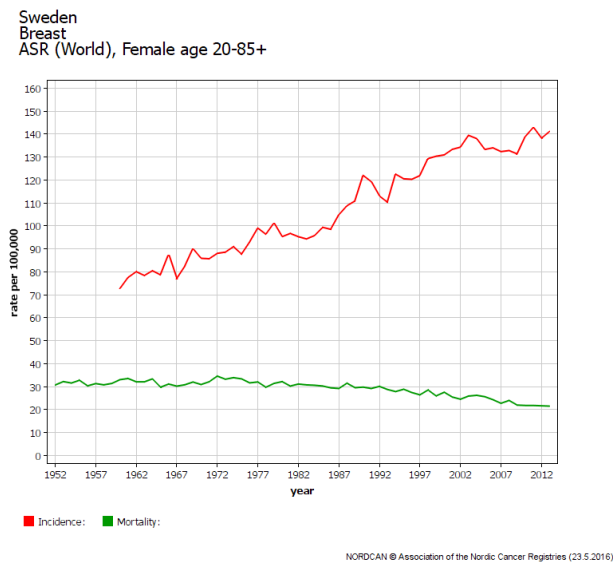


Figure 1. Incidence and mortality of breast cancer in Sweden
In the last decades, the incidence of breast cancer has increased while the mortality has declined. Graph from Nordcan (Engholm *et al*, 2010; Engholm *et al*)

Risk factors for breast cancer can be divided into non-modifiable factors, including age, gender, height, genetic constitution, and endogenous hormones, as well as modifiable factors, including exogenous hormones, weight, breast density, and lifestyle factors (Maas *et al*, 2016), which may also influence epigenetic constitution (Ambrosone *et al*, 2015; Bell & Beck, 2010). The strongest risk factor for breast cancer is female sex. Very few men are diagnosed with breast cancer, with only 44 being diagnosed in Sweden in 2014 (Socialstyrelsen, 2015). Age is also an important risk factor, and the highest incidence of breast cancer is now among women aged 65 to 74 years in Sweden (Socialstyrelsen, 2015). Hereditary breast cancer accounts for 5–10% of all cases in Sweden, and the high risk genes include *BRCA1/2* (Foulkes, 2008). In addition to the high-risk genes, several other mutations or deoxyribonucleic acid (DNA) alterations have been identified. These mutations confer a lower risk increase compared to the high-risk genes but have a higher prevalence (Foulkes, 2008).

The endogenous hormone profile of the patient is also an important risk factor and depends on reproductive factors, such as age at menarche and menopause, age at first child, parity, and duration of breast feeding (Collaborative Group on Hormonal Factors in Breast, 2002; Collaborative Group on Hormonal Factors in Breast, 2012; Dartois *et al*, 2016; Eshre Capri Workshop Group, 2004). Additionally, exogenous hormones such as oral contraceptives (OCs) and menopausal hormone therapy (MHT) may influence the risk, which is dependent on the type, dosage, age, and duration of MHT use (Chlebowski *et al*, 2015a; Collaborative Group on Hormonal Factors in Breast, 1996; Jernström *et al*, 2003a; Jernström *et al*, 2005; Olsson *et al*, 2003). High socioeconomic status has been associated with a higher risk of breast cancer (Larsen *et al*, 2011; Lundqvist *et al*, 2016). This association may in part be mediated by differences in reproductive factors, alcohol consumption, and MHT use (Larsen *et al*, 2011). In the last decade, other risk factors have been identified, with increased attention being devoted to diet, alcohol consumption, lack of physical activity, and obesity (Buckland *et al*, 2013; Carmichael, 2006; Chlebowski, 2013; Maas *et al*, 2016; Shield *et al*, 2016). These factors are modifiable and thus important in the choice of lifestyle for the individual woman. Additionally, gene-environment interactions may further improve the identification of high-risk women, but data is lacking and more research is needed (Rudolph *et al*, 2016).

The risk factors for breast cancer overlap to a large extent with prognostic markers for breast cancer survival. However, it is still not clear if and how many of these factors are related to the prognosis (Barnett *et al*, 2008; Carmichael, 2006; Ewertz *et al*, 1991; Goldhirsch *et al*, 2013; Goodwin *et al*, 2015). Breast cancer is a heterogeneous disease, and treatment resistance is a significant clinical problem in spite of the overall high survival rate (Ambrosone *et al*, 2015; Miller & Larionov, 2012; Nandy *et al*, 2014; Osborne & Schiff, 2011; Ziauddin *et al*, 2014). Additionally, many patients are overtreated and needlessly suffer from side effects (Miller & Larionov, 2012). According to one meta-analysis, over 60% of the patients who did not receive

chemotherapy in randomized trials survived without this treatment for 10 years. This indicates that approximately 60% of the patients who received chemotherapy based on the recommendations from these trials could have survived 10 years without this treatment and were thus considered overtreated (Early Breast Cancer Trialists' Collaborative *et al*, 2012; Scharl *et al*, 2015). Hence, it is vitally important to find prognostic markers for patients at risk of recurrence and contralateral breast cancer, as well as for patients who would benefit from less treatment. This approach would lead to more personalized breast cancer treatment (Scharl *et al*, 2015).

This thesis focuses on tumor and host factors in relation to female breast cancer recurrence and survival. The aim is to elucidate whether combining host factors, comprising genetic and lifestyle factors, with tumor characteristics could yield a more comprehensive view than either factor alone for the prognosis of breast cancer.

The Normal Breast and Breast Cancer Development

The female breast is composed of mammary ductal cells, milk-producing epithelial cells, and fat cells (McGee *et al*, 2006). The alveolar epithelium of the lobules consists of two layers, which contain the milk-producing apical luminal cells and basal myoepithelial cells. The myoepithelial cells contract during lactation and deliver milk into the ducts and out to the nipple (Sherratt *et al*, 2016). Together, these cells form the ducts, lobes, and lobules of the breast and a continuous basement membrane surrounds the breast epithelium. The mammary gland architecture thus forms a structure similar to a tree, with the ducts, lobes, and lobules surrounded by adipose tissue and stroma that is rich in connective tissue (Sherratt *et al*, 2016), see Figure 2.

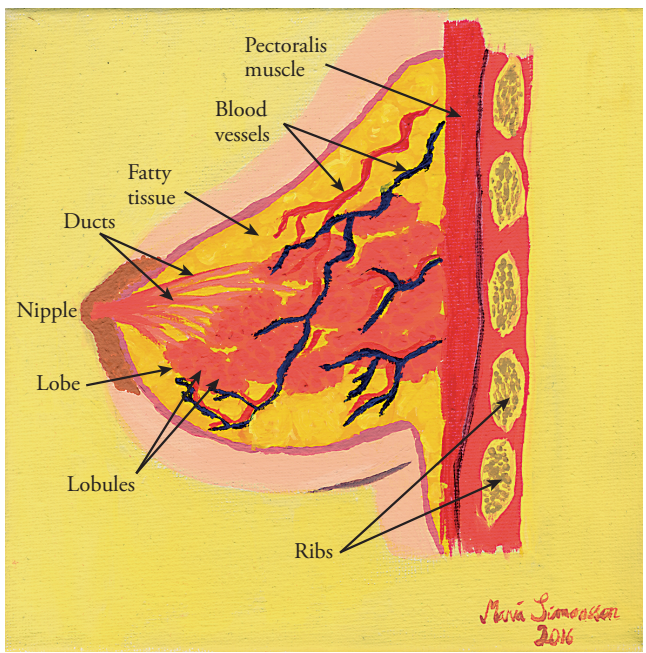


Figure 2. Anatomy of the breast
Maria Simonsson, 2016

Unlike other organs, the majority of the breast development occurs after birth. An undeveloped breast is present at birth, with further epithelial growth depending on hormones and beginning at puberty. During puberty, the immature gland develops highly proliferating terminal-duct lobular units (TDLUs), which branch through the fat to form the mature epithelial tree (Lanigan *et al*, 2007; Russo & Russo, 1994; Russo *et al*, 1982). The development of the mammary gland continues throughout life, and the epithelium undergoes many cycles of proliferation, remodeling, and cell death, which are most pronounced at puberty, pregnancy, lactation, and involution after pregnancy (Lanigan *et al*, 2007), but also during each menstrual cycle (Longacre & Bartow, 1986). The development is dependent on systemically released steroid and peptide hormones, which induce local paracrine signals to control the correct development of the gland (Lanigan *et al*, 2007). The stroma and the tumor microenvironment have received increased attention for involvement in breast carcinogenesis and more recently in metastasis and recurrence (Lanigan *et al*, 2007).

There are many identical mechanisms for normal breast development, carcinogenesis, and the transition from carcinoma *in situ* (CIS) to invasive cancers, including recruitment of fibroblasts, immune cells, and other stromal cells (Lanigan *et al*, 2007). However, breast cancer is more disorganized compared to the normal breast constitution and has escaped the control mechanisms of the immune system. The mechanisms behind the transition from a normal cell to an infiltrative tumor are known today as the hallmarks of cancer.

Hanahan and Weinberg published an extensive review in 2000 on what was known at the time about tumorigenesis. They proposed that most or even all human tumors share six common characteristics, which they called the hallmarks of cancer (Hanahan & Weinberg, 2000). The hallmarks are shown in figure 3, among which growth, invasion, survival, and induction of angiogenesis are vitally important. Since this publication in 2000, these hallmarks have played a crucial role in cancer research and education around the world. An update was published a decade later, and four new hallmarks were added: avoiding immune destruction, tumor-promoting inflammation, genome instability and mutation, and deregulating cellular energetics (Hanahan & Weinberg, 2011). Although the hallmarks were constructed with carcinogenesis in mind, targeted therapies against tumor markers of these hallmarks may be valuable for the treatment of cancer and therefore also for prognosis, see Figure 3.

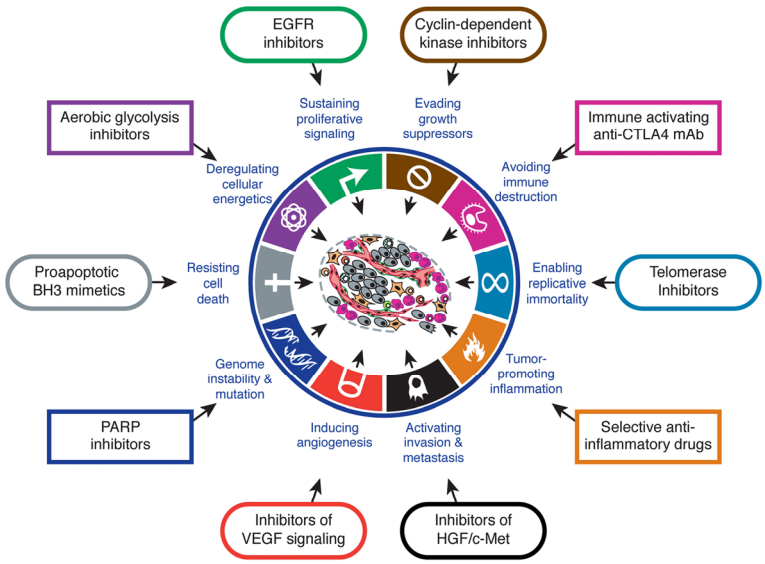


Figure 3. Hallmarks of cancer
Reprinted from The Cell, volume 144, issue 5, Hanahan D, Weinberg RA, Hallmarks of Cancer: The Next Generation, 646-674. Copyright (2011), with permission from Elsevier (Hanahan & Weinberg, 2011).

A breast tumor often develops over a long period of time, and several premalignant lesions are believed to be important for the transition of normal breast epithelium to breast cancer. Atypical hyperplasia and CIS are examples of premalignant lesions that are histologically classified as between TDLUs and invasive cancer. These premalignant lesions are characterized by increased proliferation with no metastatic potential per se, but they provide increased risks of 5 and 10% for developing invasive breast cancer, respectively (Allred *et al*, 2001). Carcinoma *in situ* can be derived from epithelial cells of the duct (ductal CIS (DCIS)) or the lobe (lobular CIS (LCIS)). Carcinoma *in situ* is a determined precursor of invasive carcinoma, and it is distinguished from invasive carcinoma by an intact basal membrane. Invasive cancers have a disrupted basal membrane with cancer cells in the stroma (Lanigan *et al*, 2007).

The histological classification is also applied to invasive cancer. The majority of invasive breast cancers are ductal carcinomas (75%), and the second most common histological type of breast cancer is lobular carcinoma (15%). Other histological subtypes are mucinous, tubular, comedo, inflammatory, medullary, and papillary carcinoma. Together, these other types account for 10% of all cases of breast cancer (Li *et al*, 2005). Since 2001, there has been a transition towards classification based on molecular subtypes instead of histological classification (Sørlie *et al*, 2001). For a more thorough description, see the chapter entitled, "Tumor Prognostic and Predictive Factors."

Diagnosis

Self-detection

Most often, patients do not present with any subjective symptoms at diagnosis, largely due to increased detection through mammography. Through screening, the tumors are found at an early stage (Tabár *et al*, 1985) and are often not noticeable by the patient. However, if the tumor is self-detected, the most common symptom of breast cancer is a lump in the breast. Other symptoms are changes in the size or shape of the breast, peau d'orange, dimpling, puckering, or scaling of the breast, or retraction of the nipple. Rarer symptoms include pain or discomfort from the breast, and discharge of serous or blood-stained liquid from the mammilla (Bloom *et al*, 1962; Kurkure *et al*; Regionala cancercentrum i samverkan, 2014).

Screening

Screening with mammography was introduced in a few districts in Sweden in the early 1970s and throughout the country in the 1990s (Rosén *et al*, 2000). The Swedish National Board of Health and Welfare now recommends that all women from 40 to 74 years old participate in the screening program (Regionala cancercentrum i samverkan, 2014). For women with high risk of breast cancer such as *BRCA1/2* mutation carriers, screening with magnetic resonance tomography (MR) is recommended instead of mammography to improve the diagnostic accuracy and reduce the dose of irradiation (Regionala cancercentrum i samverkan, 2014).

Mammographic screening reduces the relative risk of breast cancer mortality by approximately 20–40% (Massat *et al*, 2016; Nyström *et al*, 2002; Tabár *et al*, 2011). However, there is ongoing discussion about whether mammographic screening is needed now when better adjuvant treatment options are available, as well as whether the screening is associated with increased overdiagnosis (Puliti *et al*, 2012). Overdiagnosis is the detection of a tumor by screening that would not have been discovered during the lifetime of a woman, and the estimate of overdiagnosis with mammography ranges from approximately 1 to 10% (Puliti *et al*, 2012). However,

according to a recent study, treatment improvements in the last two centuries were not sufficient on their own to explain the improved survival among breast cancer patients. Mammography was also independently associated with better breast cancer specific survival (Kaplan *et al*, 2015), indicating a need for continued screening programs.

Triple Diagnostic Procedure

The triple diagnostic procedure is a standardized practice for suspected breast tumors and includes: 1) clinical assessment, 2) radiographic examination, and 3) cytology or biopsy. The clinical examination includes extensive anamnesis, including family history and other risk factors for breast cancer, as well as inspection and palpation of the breast and regional lymph node sites. The radiographic examination is either performed with a clinical mammography or an ultrasound. According to the Swedish national guidelines, clinical mammography is considered the primary choice if the patient does not have any contraindications (Regionala cancercentrum i samverkan, 2014).

The risk of inducing a new cancer by the radiation from the mammography is very low, and the benefits are considered to be much higher than the small risk of introducing a new cancer (Mattsson *et al*, 2000). For patients with a suspicious finding on mammography, more extensive mammography is needed and ultrasound is performed. According to the Swedish national guidelines, ultrasound is the primary choice for women under 30 years of age, pregnant, and breast-feeding women. Ultrasound is also often used as a supplement to mammography and may be helpful as a guide during tumor biopsy (Regionala cancercentrum i samverkan, 2014). If the clinical and radiographic examinations show a high likelihood of cancer, a cytology or biopsy may further guide the therapeutic decision (Regionala cancercentrum i samverkan, 2014).

Tumor Prognostic and Predictive Factors

Breast cancer is a heterogeneous disease classified according to tumor characteristics (Weigelt *et al*, 2005). Most breast cancers present at an early stage and the whole tumor can often be removed surgically. However, micrometastases may remain undetected locally or at distant sites and can result in a relapse up to decades later if untreated. Therefore, prognostic and predictive factors are needed to guide the choice of treatment (Early Breast Cancer Trialists' Collaborative, 2005; Weigelt *et al*, 2005). A prognostic factor is associated with risk of recurrence and corresponds to the natural history of the disease, while a predictive factor is associated with the prognosis after a given treatment has been administered. In other words, prognostic factors help to determine whether a patient needs adjuvant treatment, while predictive factors help to determine which treatment to offer the patient. Since 1978 the expert panel of the St. Gallen International Breast Cancer Conference has biannually reviewed the current literature and published treatment consensus recommendations based on established prognostic and predictive markers (Coates *et al*, 2015). These recommendations constitute a significant decision basis for the Swedish national guidelines for breast cancer treatment (Regionala cancercentrum i samverkan, 2014).

Tumor Stage (TNM system)

The tumor node metastasis system (TNM system) is a common staging system used for all solid tumors and was developed by Pierre Denoix in the 1940s. The TNM system is now a worldwide staging system maintained by the Union for International Cancer Control and the American Joint Committee on Cancer (Edge & Compton, 2010; Union for International Cancer Control (UICC)). This system serves to categorize patients into four prognostic stages (0–IV) according to combinations of three prognostic markers: tumor size, axillary lymph node involvement, and distant metastasis. Tumor size is one of the most important prognostic factors for breast cancer, in which patients with larger tumors have worse prognosis compared to patients with smaller tumors (Tabár *et al*, 1992). Therefore, invasive tumor size has been divided into

four groups with increasing risk: T1: 1–20 mm; T2: 21–50 mm; T3: >50 mm; and T4: skin or muscular involvement irrespective of size. The tumor size can be macroscopically measured clinically, before surgery (cT), and microscopically by the pathologist postoperatively after the tumor has been removed (pT) (Grabau, 2014).

Axillary lymph node involvement is considered the most important prognostic factor and is critical for determining the treatment of breast cancer patients. A higher number of involved lymph nodes confers a higher risk of recurrence and lower overall survival (Cianfrocca & Goldstein, 2004). The number of axillary lymph nodes is commonly classified into three categories: no involved nodes, one to three positive nodes, and four or more positive nodes (Grabau, 2014). According to the ninth St. Gallen consensus, four or more positive lymph nodes are considered to give a high risk by themselves, while one to three nodes are considered to indicate intermediate risk (Goldhirsch *et al*, 2005). Distant metastases in breast cancer are still incurable, and the treatment regime is palliative with a focus on increasing the life expectancy and maintaining the quality of life of the patient (Regionala cancercentrum i samverkan, 2014).

Tumor Grade

In Sweden, the Elston-Ellis Nottingham histological grade is used for grading breast cancer. The grading system classifies tumors according to the similarities between tumor cells and normal breast tissue. The grading system is based on scores for tubule formation, nuclear pleomorphism, and mitotic count. This system provides a measure of the differentiation of the tumor, and the added score gives the grade of the tumor ranging from I to III, where grade I is well differentiated, grade II is moderately differentiated, and grade III is poorly differentiated and has the worst prognosis (Bloom & Richardson, 1957; Elston & Ellis, 1991). Elston and Ellis showed that patients with grade I tumors had significantly better survival compared to patients with grade II or III tumors (Elston & Ellis, 1991). However, the prognostic role of grade II is controversial. Other methods such as gene expression profiles and the proliferation marker Ki-67 may reclassify patients with grade II into groups with low and high risk, respectively (Klintman *et al*, 2010; Sotiriou *et al*, 2006).

Estrogen Receptor

The estrogen receptor (ER) is an intracellular receptor and acts primarily as a DNA-binding transcription factor. Once the ER is activated by estrogens, it is translocated into the nucleus, binds to estrogen response elements in the DNA, activates gene

expression, and stimulates proliferation. Recent studies have also shown other roles of the ER in mitochondria and the plasma membrane (Simpson & Santen, 2015). The two classes of ER are ERα and ERβ, with ERα having the most estrogenic effects (Warner *et al*, 1999). ERα was characterized in 1960 (Jensen, 1975), while ERβ was characterized as late as 1996 (Kuiper *et al*, 1996). It is well established that the estrogen effect in the cell is mediated by the ERs (Dickson & Stancel, 2000; Kuiper & Gustafsson, 1997; Warner *et al*, 1999). The prognostic significance of ERβ is not entirely clear, but it seems that ERβ may have a different impact on survival, depending on ER and PgR expression (Taneja *et al*, 2010). Hereafter, ER will be used to refer to ERα.

ER is expressed in over 80% of all breast cancers in Sweden (Karlsson *et al*, 2014; Oh *et al*, 2015; Simonsson *et al*, 2014), but the expression differs internationally (Chlebowski *et al*, 2005). Overall, ER expression is a favorable prognostic factor in breast cancer and is associated with lower histological grade, lower proliferation index, and older age at diagnosis (Thorpe *et al*, 1986). Moreover, ER expression is associated with lobular carcinomas rather than medullary or inflammatory carcinoma, and over 90% of lobular breast carcinomas are ER-positive. Ductal carcinomas (of no special type) have a lower frequency of ER expression compared to lobular carcinomas, and approximately 80% of ductal carcinomas are ER-positive (Arpino *et al*, 2004).

ER is also a predictive factor for endocrine therapy, and adjuvant treatment with endocrine therapy is now given only to patients with ER-positive tumors since endocrine therapy has little or no effect on the risk of recurrence or mortality in patients with ER-negative tumors (Early Breast Cancer Trialists' Collaborative *et al*, 2011b; Early Breast Cancer Trialists' Collaborative Group, 1998). Before the report from the Early Breast Cancer Trialists' Collaborative Group (EBCTCG) in 1998, patients were given endocrine therapy based on their menopausal status (Early Breast Cancer Trialists' Collaborative Group, 1998; van Nes *et al*, 2011). In Sweden, a tumor is considered to be ER-positive if over 10% of the nuclei express ER (Grabau, 2014; Sydsvenska bröstcancergruppen, 2016), while internationally, only 1% of the nuclei have to be stained for the tumor to be classified as ER-positive (Goldhirsch *et al*, 2009). However, according to the St. Gallen report from 2015, tumors with frequencies of ER expression between 1 and 9% are considered to have worse prognosis and should receive not only endocrine therapy but other systemic treatments as well (Coates *et al*, 2015).

The independent prognostic impact of ER has been hard to identify since ER is associated with other favorable prognostic factors. In addition, ER status is also associated with the type of treatment, which may impact the prognosis. The favorable prognostic effect of ER also seems to be lost after several years and patients with ER-positive tumors tend to relapse late (Osborne *et al*, 1980; Taneja *et al*, 2010), while the risk of recurrence among patients with ER-negative tumors is highest shortly after diagnosis (Early Breast Cancer Trialists' Collaborative *et al*, 2011b).

Progesterone Receptor

The progesterone receptor (PgR) is similar to the ER in structure and function and is also a hormone-dependent nuclear transcription factor (Taneja *et al*, 2010). In normal breast tissue and in several breast cancer cell lines, PgR expression is induced by estrogen (Taneja *et al*, 2010). Moreover, while ER is most important in the ductal elongation of the mammary gland during puberty, PgR is more important during the differentiation of the lobules during lactation (Taneja *et al*, 2010). In Sweden, the cut-off for PgR positivity is 10%, the same as for ER positivity (Grabau, 2014; Sydsvenska bröstcancergruppen, 2016). Recently, an additional cut-off of 20% was proposed to categorize tumors into low and high PgR expression (Sydsvenska bröstcancergruppen, 2016).

PgR expression is associated with less aggressive tumors, whereas ER- and PgR-negative tumors are often associated with a more aggressive phenotype (Taneja *et al*, 2010). The expression of PgR is regulated by ER, meaning the expression of PgR is indicative of a functioning ER and thus a potentially better response to tamoxifen treatment (Allred, 2010). However, in the EBCTCG meta-analysis from 2011, the effect of tamoxifen in patients with ER-positive disease was independent of PgR status (Early Breast Cancer Trialists' Collaborative *et al*, 2011b). Therefore, the exact role of PgR signaling in breast cancer and the crosstalk between PgR and ER is still unclear.

In a recent study by Mohammed *et al.* published in *Nature* in 2015, activation of PgR was shown to lead to expression of antiproliferative genes and the resulting gene signature was associated with a good prognosis among breast cancer patients (Mohammed *et al*, 2015). Interestingly, co-treatment of ER-positive breast cancer cell lines with an ER antagonist (tamoxifen) and progesterone inhibited estrogen-dependent growth, indicating a potential future benefit of PgR agonist therapy (Mohammed *et al*, 2015).

Human Epidermal Growth Factor Receptor 2

Human epidermal growth factor receptor 2 (HER-2) is a glycoprotein that belongs to the epidermal growth factor (EGFR) receptor family. The gene encoding the protein (*HER-2/neu, c-erbB2*) is recognized as an oncogene (Slamon *et al*, 1987; Taneja *et al*, 2010). HER-2 is overexpressed in 10–30% of invasive breast cancers, primarily due to gene amplification (Regionala cancercentrum i samverkan, 2014; Taneja *et al*, 2010). The determination of HER-2 in breast cancer samples can be performed using immunohistochemistry to measure the overexpression of HER-2 or by *in situ* hybridization (ISH) to measure the gene amplification. Using immunohistochemistry,

HER-2 expression is scored on a scale of 0 to 3+, depending on the fraction of positive cells and the membrane staining intensity, while the ISH results are "amplified" or "not amplified" (Grabau, 2014; Taneja *et al*, 2010). In Sweden, HER-2 is first measured by immunohistochemistry. Tumors with scores of 0 and 1+ are classified as negative, while complementary analysis with ISH is performed for tumors classified as 2+ or 3+ (Grabau, 2014).

HER-2 amplification is associated with a poor prognosis in breast cancer patients, with two-fold increased mortality compared to patients with HER-2 negative tumors (Slamon *et al*, 1987). However, HER-2 is also a predictive factor for targeted treatments with monoclonal antibodies directed at HER-2 (Taneja *et al*, 2010). Thus, for patients receiving the targeted treatment, HER-2 is not necessarily associated with a poor prognosis. Today, triple-negative tumors that express neither ER, PgR, nor HER-2 are considered to have the poorest prognosis, and new potential treatment targets must be identified for these patients (Dent *et al*, 2007; Gelmon *et al*, 2012). Two potential new targets for triple-negative tumors are the androgen receptor and the EGFR (Collignon *et al*, 2016; Elebro *et al*, 2015).

Ki-67

Ki-67 is a proliferation marker that is expressed during the S, G1, G2, and M phases of the cell cycle but is not present during cell-cycle arrest in G0 (Gerdes *et al*, 1984). In 2009, Ki-67 was included as a prognostic marker of breast cancer by both St. Gallen and the Swedish Breast Cancer Group (SweBCG) (Goldhirsch *et al*, 2009; Regionala cancercentrum i samverkan, 2014). Ki-67 has been shown to be of most importance in differentiating patients with good and poor prognosis in patients with ER-positive disease and grade II tumors (Klintman *et al*, 2010).

The evidence is clear that Ki-67 has prognostic significance, especially in the higher interval. However, it is still debated regarding how to evaluate the expression and which cut-off should be used (Coates *et al*, 2015). Some studies have proposed measuring Ki-67 in the hot spots of the tumors, where the level is highest, while others suggests measuring Ki-67 as a global value (Leung *et al*, 2016). Additionally, Ki-67 is measured as a continuous variable, which, together with the analytical disparities, hinders the definition of a cut-off (Coates *et al*, 2015; Polley *et al*, 2015). However, a threshold of 20% or higher is considered to be high (Goldhirsch *et al*, 2013). An international working group investigating Ki-67 is working towards standardization of the methodology (Leung *et al*, 2016). In 2015, St. Gallen stated that immunohistochemical Ki-67 has less analytical validity than newer but more expensive molecular testing (Coates *et al*, 2015). Nevertheless, Ki-67 is an inexpensive way to measure the

proliferation index and has been routinely analyzed in Lund, Sweden since 2009 (Regionala cancercentrum i samverkan, 2014).

Molecular Subtypes

Sørlie *et al*. developed the "intrinsic" molecular subtypes in 2001 to further understand the biology and heterogeneity of breast tumors by studying the combination of multiple genetic alterations (Sørlie *et al*, 2001). Their study resulted in five molecular subtypes with different prognoses: luminal A, luminal B, HER-2 enriched, normal breast-like, and basal-like subtype (Sørlie *et al*, 2001). Their study was the first to divide the ER-positive group into two subtypes with different prognosis, where patients with luminal B tumors had a poorer prognosis compared to patients with luminal A tumors (Sørlie, 2016; Sørlie *et al*, 2001). Since then, a smaller gene set that measures the expression of 50 genes has been built into an assay called prediction analysis of microarray (PAM50). This method can be used as a simpler alternative for determining the four major intrinsic subtypes: luminal A, luminal B, HER-2 enriched, and basal-like subtype, using reverse-transcriptase quantitative PCR (Guiu *et al*, 2012; Parker *et al*, 2009; Sørlie, 2016).

Due to the cost and complexity of gene expression analysis, surrogate immunohistochemical definitions for the intrinsic subtypes were developed and included in the 2011 St. Gallen International Consensus (Goldhirsch *et al*, 2011). In addition to histological grade, the surrogate subtyping with immunohistochemistry determines the treatment of patients today (Goldhirsch *et al*, 2013). However, according to a recent study, the traditional TNM system still seems to have prognostic importance and should not yet be discarded in favor of molecular subtypes (Orucevic *et al*, 2015). The molecular subtypes using the immunohistochemical surrogate definitions from St. Gallen are presented in table 1.

Other genetic tests have been developed and are being tested in clinical trials, such as OncotypeDX and MammaPrint. The Sweden Cancerome Analysis Network - Breast (SCAN-B) consortium was initiated in 2010 as a prospective, multicenter approach to identify new prognostic genetic markers through whole transcriptome RNA-sequencing. The final aim of the study is to reduce the time to discovery, validation, and clinical implementation of novel predictive tests for breast cancer prognosis and treatment response (Saal *et al*, 2015).

Table 1. Molecular subtypes and immunohistochemical surrogate definitions

Definitions from the Swedish national guidelines (Regionala cancercentrum i samverkan, 2014), which are adapted and marginally modified from the 2013 St. Gallen Consensus (Goldhirsch *et al*, 2013).

	Luminal A	**Luminal B (HER-2 negative)**	**Luminal B (HER-2 positive)**	**HER-2 positive**	**Triple negative**
ER status	ER-positive (>10%) &	ER-positive (>10%) &	ER-positive (>10%) &	ER-negative (≤ 10%) &	ER-negative (≤ 10%) &
HER-2 status	HER-2 negative (0-1+ or not amplified) &	HER-2 negative (0-1+ or not amplified) & one of the following:	HER-2 positive (amplified or 3+) independent of other factors	HER-2 amplified or 3+ &	HER-2 negative (0-1+ or not amplified) &
PgR status	PgR-positive (>10%) &	PgR-negative (≤ 10%) /low (≤ 20%) or		PgR-negative (≤ 10%)	PgR-negative (≤ 10%)
Ki-67 index	Ki67 low (≤ 20%) &	Ki67 high (> 20%) or			
Tumor grade	Grade I or II	Grade III			

Treatment

Surgery is the primary treatment for invasive breast cancer but is often complemented with adjuvant systemic treatment and radiotherapy. Adjuvant treatment aims to control or eradicate any remaining cancer cells, and the treatment choice for each patient is based on the prognostic and predictive factors described. According to the present national Swedish guidelines, neoadjuvant therapy should be considered for patients with locally advanced or primary unresectable tumors—i.e., large tumors in relation to the breast size—as well as for patients with lymph node involvement or triple-negative disease (Regionala cancercentrum i samverkan, 2014). Neoadjuvant treatment offers an opportunity to shrink the tumor, increase the chances of a breast-conserving surgery (BCS), and monitor the treatment response prior to surgery. The choice of neoadjuvant treatment is based on the prognostic and predictive factors and includes chemotherapy, targeted therapies including anti-HER-2 treatment, and endocrine therapy (Regionala cancercentrum i samverkan, 2014).

For patients not receiving neoadjuvant therapy, adjuvant treatment with chemotherapy, targeted treatments, radiotherapy, and/or endocrine treatment may follow after surgery. The treatment choice is based on the individual patient's tumor characteristics and overall health status (Regionala cancercentrum i samverkan, 2014). Patients who present with a metastatic disease follow a different scheme, and the treatment is more individualized. The aim in this setting is to prolong life, maximize quality of life, and ease symptoms from the breast cancer. After a breast cancer diagnosis, each patient and the treatment options are discussed in a multidisciplinary conference by breast cancer surgeons, pathologists, and/or cytologists, oncologists, radiologists, and contact nurses. The multidisciplinary conference should discuss each patient before and after surgery for correct assessment and to choose the best possible treatment for the patient. The multidisciplinary conference was established 25 years ago in Sweden and have been associated with increased survival, although with weak and limited evidence (Houssami & Sainsbury, 2006; Patkar *et al*, 2011).

Surgery

The technique of breast cancer surgery has evolved in the last century, starting with Halsted's radical mastectomies (Halsted, 1894) and developing into modified radical mastectomies (MRM), where the pectoralis muscle and lymph nodes are spared, and then to BCS, where the lump is removed and the rest of the breast is spared (Aspegren *et al*, 1988; Blichert-Toft *et al*, 1992). Modern breast surgery also comprises oncoplastic surgery with replacement of the breast substance in order to improve the cosmetic result (Al-Ghazal *et al*, 2000; Rosenqvist *et al*, 1996). BCS is considered the primary choice of surgery when possible—i.e., when the tumor can be radically removed with a good cosmetic result (Regionala cancercentrum i samverkan, 2014; Veronesi *et al*, 2002). An MRM is chosen if the tumor is too large or multifocal to be radically removed with a good cosmetic result using BCS. An MRM is also recommended when the ratio between the tumor size and the total breast volume is high (Regionala cancercentrum i samverkan, 2014). Several studies have shown that BCS followed by radiotherapy decreases the risk of local recurrence, breast-cancer specific mortality, and all-cause mortality (Clarke *et al*, 2005; Early Breast Cancer Trialists' Collaborative *et al*, 2011a). Moreover, BCS followed by radiotherapy has a comparable overall survival to MRM (Veronesi *et al*, 2002).

After excision, the tumor is painted with ink on different sides to guide the pathologist. Currently, there is no international consensus on the surgical margin (Morrow, 2009). In Sweden, the tumor is considered radically removed by the pathologist if there is no microscopically visible tumor on the ink of the removed tissue (Regionala cancercentrum i samverkan, 2014).

Sentinel node assessment for evaluation of the involved axillary lymph nodes is now standard before neoadjuvant therapy or in surgery, when neoadjuvant treatment is not recommended. Lymph nodes that are suspicious clinically or on ultrasound should always be biopsied pre-operatively or before neoadjuvant treatment. If the sentinel node biopsy is positive and identifies a macro- or micrometastasis, an axillary lymph node dissection is recommended. However, if the sentinel node biopsy is negative, such surgery is not needed and the patients may thus be spared the side effects associated with resection of the axillary lymph nodes, such as lymphedema (Regionala cancercentrum i samverkan, 2014).

Chemotherapy

Chemotherapy lowers the risk of micrometastases. According to the latest EBCTCG meta-analysis, a high-dose anthracycline-based regime confers a relative reduction in breast cancer mortality by 36% and an absolute risk reduction of 6.5% in 10 years compared to no adjuvant chemotherapy (Early Breast Cancer Trialists' Collaborative *et al*, 2012). Large studies have shown that chemotherapy is most effective in combination regimens, and the best possible regimens with anthracyclines and taxane confer a 13% absolute risk reduction of breast cancer mortality (Early Breast Cancer Trialists' Collaborative *et al*, 2012).

According to the Swedish national guidelines, the primary choice for adjuvant or neoadjuvant chemotherapy is three cycles of a high-dose anthracycline-based regime and then three cycles of the taxane Docetaxel (Regionala cancercentrum i samverkan, 2014). Adjuvant chemotherapy is recommended for patients with triple negative disease who have a tumor larger than 5 mm or lymph node involvement, as well as to patients with ER-positive, HER-2 negative tumors larger than 10 mm, or those with lymph node involvement if one of the following criteria is fulfilled: age under 35 years at diagnosis, a luminal B tumor irrespective of age, age under 50 years with a luminal A tumor and at least one involved lymph node, or a luminal A tumor and at least four positive lymph nodes irrespective of age. For patients with HER-2 positive tumors, chemotherapy in combination with targeted treatment is offered if the tumor is larger than 5 mm or there are any positive lymph nodes (Regionala cancercentrum i samverkan, 2014). However, chemotherapy is associated with side effects such as neutropenia and infections, as well as severe side effects such as neurotoxicity, leukemia, and cardiotoxicity (Azim *et al*, 2011; Early Breast Cancer Trialists' Collaborative *et al*, 2012). New results from the MINDACT study presented at the American Association for Cancer Research (AACR) annual meeting in 2016 indicated that the gene assay MammaPrint was able to identify a subset of patients who could be spared from adjuvant chemotherapy (Poh, 2016).

Trastuzumab

Therapies that target HER-2 are another possibility for the treatment of breast cancer. In the 1990s, Baselga *et al*. showed that a monoclonal antibody caused regression of metastatic tumors (Baselga *et al*, 1996). Since then, several anti-HER-2 drugs have been approved for clinical use. One of the first approved drugs was trastuzumab, which significantly improved both overall survival and disease-free survival in a Cochrane meta-analysis on patients with HER-2 positive early breast cancer (Moja *et al*, 2012).

The results also indicated that trastuzumab should be administered concurrently with chemotherapy and for a duration of one year (Moja *et al*, 2012). Side effects of trastuzumab include an elevated risk of congestive heart failure. Therefore, before and during treatment, the heart function of the patients is carefully monitored with echocardiography (Moja *et al*, 2012).

In the neoadjuvant or adjuvant setting, the Swedish national guidelines recommend one year of trastuzumab administered concurrently with chemotherapy for patients with HER-2 amplified (or IHC 3+) tumors that are either larger than 5 mm or have positive lymph nodes (Regionala cancercentrum i samverkan, 2014; Sydsvenska bröstcancergruppen, 2016). There are also newer HER-2 inhibitors such as Pertuzumab and Lapatinib, which have received approval for clinical use in Sweden. However, the use is still restricted to patients with metastatic disease or locally advanced recurrences (Regionala cancercentrum i samverkan, 2014).

Radiotherapy

Radiotherapy reduces the risk of locoregional recurrence and distant metastases as well as breast cancer mortality after both BCS and mastectomy. However, the absolute risk reduction depends on the patients' risk of recurrence as determined by prognostic factors (Early Breast Cancer Trialists' Collaborative *et al*, 2014; Early Breast Cancer Trialists' Collaborative *et al*, 2011a). According to international and Swedish national guidelines, radiotherapy should be recommended to patients with a 10-year recurrence risk of over 20%, thus including patients who have received a BCS, irrespective of other factors, as well as patients who received an MRM and presented with four or more positive axillary lymph nodes (Kurtz & Party, 2002; Regionala cancercentrum i samverkan, 2014). Furthermore, after a mastectomy, radiotherapy against the thoracic wall is recommended for patients with large or multifocal tumors. For mastectomized patients with T4 tumors or lymph node involvement, locoregional radiotherapy directed against the thoracic wall and locoregional lymph node stations is recommended (Regionala cancercentrum i samverkan, 2014). The most common side effect after radiotherapy is inflammation of the skin, while more rare side effects include pneumonitis, cardiovascular events, and lung cancer (Darby *et al*, 2013).

Endocrine Treatment

The two main types of endocrine treatments are selective estrogen receptor modifiers (SERMs) and AIs, which have different mechanisms of action and different target groups. According to the national guidelines in Sweden, adjuvant endocrine treatment is currently recommended for patients with ER-positive tumors who fulfill one of the following criteria: luminal A tumors larger than 10 mm, luminal B tumors larger than 5 mm, or tumors with signs of locoregional lymph node involvement. The recommended treatment period has until recently been five years for all patients (Regionala cancercentrum i samverkan, 2014). However, the recommendations were changed when results were published from the Adjuvant Tamoxifen: Longer Against Shorter (ATLAS) trial. The ATLAS study showed that patients with ER-positive disease could benefit from another five years of tamoxifen, with absolute risk reduction of 2.8% for mortality and 3.7% for recurrence (Davies *et al*, 2013). The recommended treatment period for patients with lymph node-negative disease is still five years in Sweden, but for patients with lymph node involvement, prolonged endocrine therapy for up to 10 years should now be considered.

Tamoxifen – SERMs

Tamoxifen is a SERM that acts as a competitive ER antagonist and blocks the proliferative signaling of ER (Osborne & Schiff, 2011). However, this drug is also a partial ER agonist and stimulates ER in some organs more than others (Shang & Brown, 2002). Tamoxifen is a prodrug and has to be metabolized to its more active metabolites to exert its main antiestrogenic effects. Recent studies have shown that tamoxifen has a carryover effect after treatment ends and a risk reduction in mortality for up to 15 years (Davies *et al*, 2013; Ekholm *et al*, 2016). The relative and absolute risk reductions in breast cancer mortality are 30% and 9.2%, respectively (Early Breast Cancer Trialists' Collaborative *et al*, 2011b). The relative risk of breast cancer recurrence also decreases with 39% over 15 years, with an absolute risk reduction of 13.2%.

Both pre- and postmenopausal patients may be treated with tamoxifen. The side effects of tamoxifen are derived from the antagonistic as well as from the agnostic effect on the ER and includes menopausal symptoms such as hot flashes, increased risk of endometrial cancer, and thromboembolic events (Cronin-Fenton *et al*, 2014; Early Breast Cancer Trialists' Collaborative, 2005). However, the agonistic effect of tamoxifen on ER antagonizes the risk of developing osteoporosis (Krum *et al*, 2008). According to the Swedish national guidelines, tamoxifen is recommended for premenopausal patients for five years.

Aromatase Inhibitors

Aromatase inhibitors block the conversion of androgens to estrogens by inhibiting the catalyzing enzyme, aromatase. However, this inhibition only affects the aromatization in peripheral tissues, mostly fat, and not the aromatization in the ovaries. Thus, AIs have little or no effect in premenopausal patients with active ovarian production of estrogens (Dowsett & Haynes, 2003).

Several trials have shown that AIs more effectively reduce the risk of recurrence than tamoxifen for early breast cancer, including the "Arimidex, Tamoxifen, Alone, or in Combination" (ATAC) trial (Cuzick *et al*, 2010) and the Breast International Group (BIG) 1-98 trial (Regan *et al*, 2011). In addition, EBCTCG recently published a meta-analysis and showed that AIs decreased the absolute risk of breast cancer recurrence in 10 years by 3.6% and reduced the absolute risk of breast cancer mortality within 10 years by 2.1% (Early Breast Cancer Trialists' Collaborative *et al*, 2015b). The side effects differ between tamoxifen and AI since AIs inhibit all remaining estrogen production. AIs confer a higher risk of arthritis, muscle pain, dry mucous membranes, osteoporosis, fractures, and cardiovascular events (Chlebowski *et al*, 2015b; Cuzick *et al*, 2010). Another recent study calculated a benefit/risk index based on six health outcomes: breast cancer distant recurrence, hip fracture, endometrial cancer, pulmonary embolism, stroke, and coronary heart disease. The outcomes were assigned the same weight, and the results indicated a better benefit/risk index for AIs compared to tamoxifen among almost all patient categories. Tamoxifen showed a benefit in only the oldest patients with previous myocardial infarction (Chlebowski *et al*, 2015b).

The Swedish national guidelines now recommend AIs either alone for five years or sequentially with tamoxifen as a first line of treatment for all postmenopausal patients, as well as for premenopausal patients with a worse prognosis, such as those 35 years or younger at diagnosis or those who have regained ovarian function after chemotherapy (Regionala cancercentrum i samverkan, 2014). Premenopausal patients who receive AIs must also receive ovarian suppression, most commonly with gonadotropin-releasing hormone (GnRH) agonists (Del Mastro *et al*, 2016). When prolonged endocrine therapy for up to ten years is indicated, treatment with tamoxifen is recommended for the last five years since AIs are not recommended for more than five years (Regionala cancercentrum i samverkan, 2014).

Although both tamoxifen and AIs substantially improve the prognosis of breast cancer patients, endocrine resistance is still a major problem and afflicts many patients through intrinsic or acquired resistance (Osborne & Schiff, 2011). Most studies have focused on resistance to tamoxifen (Osborne & Schiff, 2011), which may have agonistic effects on the ER in breast cancer cells under some circumstances, leading to tamoxifen resistance (Goldhirsch *et al*, 2005). This has been shown for breast cancer cells expressing high levels of the co-activator A1B1 and HER-2 (Goldhirsch *et al*, 2005).

Moreover, mutations in *ESR1*, the gene encoding ER, have been associated with acquired resistance to tamoxifen and AIs (Fribbens *et al*, 2016; Rugo *et al*, 2016). However, most of the resistance is still unexplained. Therefore, it is very important to find new markers of both intrinsic and acquired endocrine resistance (Osborne & Schiff, 2011).

Bisphosphonates

A very recent addition to the adjuvant treatments is bisphosphonates, which was added to the South Swedish guidelines in April 2016 (Sydsvenska bröstcancergruppen, 2016). Bisphosphonates are osteoclast inhibitors and have been shown to decrease bone metastases in metastatic breast cancer. A meta-analysis from EBCTCG analyzed the results from randomized studies of adjuvant bisphosphonates and concluded that there was an absolute risk reduction of 5-year fracture risk by 1.2%. Among postmenopausal patients, bisphosphonates conferred an absolute risk reduction of 2.2% for bone metastases and 3.1% for breast cancer mortality (Early Breast Cancer Trialists' Collaborative *et al*, 2015a).

In Sweden, postmenopausal patients with lymph node involvement are now offered the bisphosphonate zoledronic acid intravenously every sixth month in addition to other adjuvant therapy over a total of three years (Regionala cancercentrum i samverkan, 2014; Sydsvenska bröstcancergruppen, 2016).

Host Prognostic Factors

In terms of cancer, much interest and research involve prognostic markers of the tumor. However, despite all measures taken in attempts to personalize treatment for each patient, many patients are still overtreated and could have survived without any adjuvant treatment. In other patients, recurrence arises in spite of the advances in treatment. Therefore, new tumor markers as well as prognostic or treatment predictive host factors are needed. Among the host factors, there are still controversies and unknown areas that need further research before implementation in a clinical setting.

Age at Diagnosis

Age at diagnosis is a known risk factor for breast cancer but is somewhat controversial as a prognostic factor. Very young and very old patients have been proposed to have a poorer prognosis, but the data has been conflicting (Barchielli & Balzi, 2000; Brandt *et al*, 2015; Fredholm *et al*, 2009; Yancik *et al*, 2001). Furthermore, there are contradictory results whether all young patients have a poor prognosis or if certain subgroups of patients have worse prognosis. However, most studies are in agreement that age less than 35 years at diagnosis is associated with a poorer prognosis (Colleoni *et al*, 2006; El Saghir *et al*, 2006; Park *et al*, 2002). A recent Swedish study with over 4,400 patients showed that patients younger than 40 years at diagnosis had the worst prognosis, especially among patients with lymph node positive disease, as well as patients over 80 years old at diagnosis (Brandt *et al*, 2015). Additionally, age impacts the choice of treatment with regard to menopausal status and general health status of the patient.

Genetic Factors

In spite of the control mechanisms in eukaryote human cells, mutations in DNA occur and are sometimes inherited. There are several kinds of mutations, such as copy number, tandem repeats, insertions, and deletions (Bertram, 2000). However, this

thesis focuses on the more common mutations, single-nucleotide polymorphisms (SNPs). SNPs are point mutations, where one nucleotide of the DNA chain has been exchanged to another. For example, some people in a population have an A allele at a particular site of the chromosome, while others have a C allele. SNPs are classified by where they are located in the gene. SNPs in an exon are called coding SNPs, while those in a non-coding region such as an intron or intergenic region are called non-coding SNPs. In addition, a coding SNP can be non-synonymous and thus not confer any change in the amino acid sequence, or synonymous when the SNP leads to a change in the amino acid sequence that is translated (Katsonis *et al*, 2014). Such a change in the amino acid sequence may lead to different phenotypes or different susceptibility to disease, or they can be neutral (Wang & Moult, 2001).

A non-coding SNP may have regulatory effects through *cis*- or *trans*-acting elements. A *trans*-acting element is usually a locus of the DNA string that contains a gene, and the effect of the element is mediated by the protein encoded in that gene. In contrast, a *cis*-acting element does not code for a protein but is located in a non-coding region and usually functions as a binding site for transcription factors. Thus, *cis*-acting elements regulate the transcription of nearby genes. Mutations within these elements could cause an altered affinity to the binding site and thereby up- or downregulate gene expression (Wittkopp & Kalay, 2012).

Among the global population, 10,000 sites or one variant per 300 bases is present in at least 1% of the population, and these common SNPs can explain 90% of the genetic variation in the population (International HapMap, 2003). These variations are inherited, and one allele is therefore linked to other alleles that were already present when the latest mutation took place. These associations between alleles lead to linkage disequilibrium (LD), which differs between ethnic groups (Chakravarti, 1999; International HapMap, 2003). Each set of SNPs that is present on a chromosome or a part of a chromosome is called a haplotype. Due to the association between the SNPs, only a few tag SNPs need to be examined to identify the majority of the genetic variation in a region (International HapMap, 2003). Since humans have chromosome pairs, alleles on the same locus on a homologous chromosome make up the genotype of a person. Similarly, a diplotype is a matched pair of haplotypes on homologous chromosomes (Zuo *et al*, 2014).

SNPs in multiple chromosomes and different genes in the germline DNA have been associated with prognosis in breast cancer. However, due to the cost, complexity, and need for validation in large cohorts or randomized clinical studies, diagnostic or predictive genetic routines have not yet been introduced. In the last decade, a new method was developed to analyze thousands to millions of tag SNPs that capture most of the genome at the same time with SNP arrays. Such studies investigating associations between SNPs and major diseases are called Genome-Wide Association Studies (GWAS). Since the first study was published in 2005 (Klein *et al*, 2005), such studies

have spread around the world and been used for multiple diseases (Welter *et al*, 2014). The attitudes towards GWAS are divided, and the causal effects cannot be estimated using only GWAS as they are non-candidate-driven studies, and a pre-specified hypothesis about a gene is not included, in contrast to candidate-gene-specific-driven studies. Furthermore, theinterpretation of the results has sometimes been difficult, and the most common SNPs identified in GWAS studies are not coding SNPs. In fact, approximately 90% of the SNPs are located in non-coding regions (Manolio, 2010; Tak & Farnham, 2015). Thus, if and how these SNPs could be causal in regard to the risk or progression of a disease is still controversial (Manolio, 2010; Tak & Farnham, 2015).

However, it would be very expensive to perform functional follow-up studies on every potential SNP. Therefore, new methods for prioritizing the SNPs have been suggested (Tak & Farnham, 2015). Other methods with a narrower approach are SNP array analyses with chips that contain a small selection of SNPs. One example is a chip that focuses on polymorphisms in genes related to absorption, distribution, metabolism, and elimination (ADME), such as polymorphisms in the cytochrome P450 (CYP450) system (Burmester *et al*, 2010). This method is more candidate-driven than GWAS. The chip was developed for pharmacogenomics with the aim to avoid toxicity and for improved individualized therapy. Such a chip was used in paper III of this thesis to find new predictive markers for AIs.

Cytochrome P450 System

The CYP450 family is a superfamily of enzymes that have evolved through repeated gene duplications. The CYP450 enzymes are membrane-bound proteins and metabolize several thousand endogenous and exogenous substrates. Many drugs are metabolized by CYP450 enzymes, and while most undergo deactivation during metabolism, others are bioactivated into their active metabolites. This capacity of CYP450 enzymes makes them essential in the phase I metabolism of drugs and are thus important for interindividual drug metabolism and interactions, which have clinical relevance (Danielson, 2002).

Genetic variation such as SNPs in the CYP450 enzymes may influence drug levels and cause interindividual responses to treatments. Many studies have investigated the prognostic and predictive impact of these variations, as summarized on the CYP-allele website (http://www.cypalleles.ki.se) (Sim & Ingelman-Sundberg, 2010). CYP19A1 (aromatase) is the key enzyme in the androgen to estrogen metabolism, catalyzing the conversion of androstenedione to estrone and testosterone to estradiol in a rate-limiting step (Simpson & Santen, 2015). In premenopausal women, this conversion is mainly localized to the ovaries, while in postmenopausal women, the estrogen synthesis in the

ovaries is very limited. Estrogen synthesis in postmenopausal women is instead dependent on the aromatization in peripheral tissues, mainly peripheral fat tissue, which is important in the choice of endocrine therapy (Simpson, 2004).

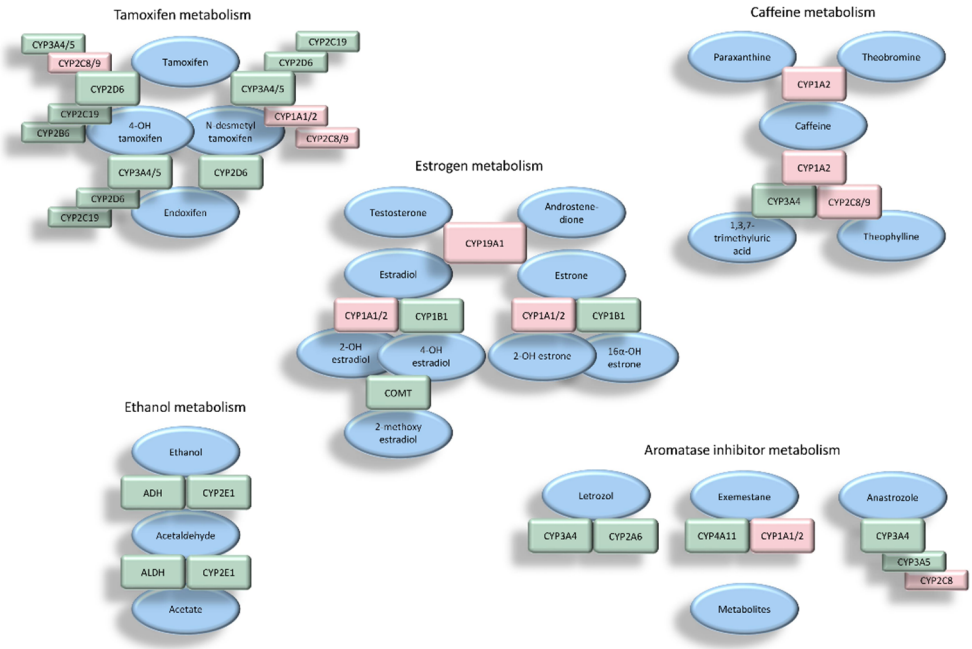


Figure 4. Metabolizing enzymes involved in the metabolism of estrogens, tamoxifen, aromatase inhibitors, caffeine, and ethanol. Pink boxes indicate enzymes investigated in this thesis. Each figure should be read from the top to the bottom.

Estrogens are metabolized by CYP1A1, CYP1A2, CYP1B1, CYP3A4, CYP3A5, and other non-CYP450 enzymes, including 17β-hydroxysteroid dehydrogenase (17β-HSD), catechol-O-methyltransferase (COMT), sulfotransferases (SULTs), UDP-glucuronosyltransferases (UGTs), and glutathione-S-transferases (GSTs) (Raftogianis *et al*, 2000; Thompson & Ambrosone, 2000). Furthermore, many breast cancer medications interact with or are metabolized by CYP enzymes (Binkhorst *et al*, 2015; Kamdem *et al*, 2010; Rodriguez-Antona & Ingelman-Sundberg, 2006). CYP2D6 is the key enzyme in the tamoxifen metabolism and metabolizes tamoxifen to its metabolites 4-hydroxytamoxifen, 4-desmethyltamoxifen, and its most active metabolite, endoxifen (Jin *et al*, 2005). However, several other CYP450 enzymes also contribute to the tamoxifen metabolism, including CYP1A2, CYP1B1, CYP2B6, CYP2C8/9, CYP2C19, CYP3A4, and CYP3A5 (Cronin-Fenton *et al*, 2014; Desta *et al*, 2004; Mürdter *et al*, 2011). Moreover, the steroidal AI exemestane has been shown to be metabolized by CYP4A11 and CYP1A1/2 *in vitro* (Kamdem *et al*, 2011). The regulation of these enzymes varies. Two of these enzymes, CYP1A1 and CYP1A2, share a promoter and are both regulated by the aryl hydrocarbon receptor (AhR) (Li *et al*,

1998). The association between polymorphisms in CYP450 enzymes and prognosis in different treatment groups were investigated in papers I and III of this thesis. For a summary of the metabolism of estrogens, tamoxifen, AIs, caffeine, and ethanol, see figure 4.

Lifestyle Factors

In the last decades, increasing evidence of lifestyle factors as prognostic markers have emerged. Lifestyle factors are modifiable factors and could thus be altered by the patient herself after diagnosis and during treatment. Some examples include smoking, body constitution, diet, coffee, and alcohol consumption.

Anthropometric Factors

Obesity is a well-known risk factor for postmenopausal breast cancer and induces cell proliferation (Pérez-Solis *et al*, 2016). Furthermore, obesity has been associated with a poorer outcome in breast cancer patients (Nechuta *et al*, 2016). Conversely, low weight and weight loss have also been associated with a worse prognosis (Ewertz *et al*, 1991). Body mass index (BMI) has been the standard measurement to characterize body composition, but the causal link between BMI and breast cancer prognosis is still unclear. A recent study found an altered gene expression within luminal A tumors among obese patients with alterations in cell cycle control and tumorigenesis. Thus, personalized targeted treatment may be beneficial for these patients (Toro *et al*, 2016).

In recent years, other measurements such as central obesity measured by waist circumference or waist-to-hip ratio (WHR) have emerged as potentially better measurements of body composition (James *et al*, 2015). Moreover, breast size has been proposed as a potential independent prognostic factor in breast cancer (Markkula *et al*, 2012a). Circulating levels of estrogens are increased among obese postmenopausal women, and obesity is now recognized as an inflammatory condition (Simpson & Santen, 2015). However, which of these measurements that constitutes the best measurement for body composition in relation to breast cancer is still debated, and further research is needed (James *et al*, 2015).

Physical activity has been associated with a lower risk of recurrence and lower mortality compared to physical inactivity (Schmid & Leitzmann, 2014). In a pooled analysis by Nelson *et al*. from 2016, physical inactivity and comorbidities among patients with ER-positive tumors attenuated the association between high BMI and increased risk of mortality seen in other studies (Nelson *et al*, 2016). This is in line with the results from another pooled analysis from 2016, where post-diagnosis physical activity was

associated with lower mortality, but it was not associated with breast cancer recurrence (Nechuta *et al*, 2016).

Socioeconomic Status

Although a high socioeconomic status is associated with increased risk of breast cancer (Larsen *et al*, 2011; Lundqvist *et al*, 2016), several studies have shown that patients with higher socioeconomic status have lower breast cancer mortality than patients with lower socioeconomic status (Eaker *et al*, 2009; Lagerlund *et al*, 2005; Larsen *et al*, 2015; Lundqvist *et al*, 2016). A European study recently showed that patients with low socioeconomic status had double the risk of late-stage breast cancer compared to patients with high socioeconomic status (Orsini *et al*, 2016). However, the lower mortality among patients with high socioeconomic status could not be explained by only less aggressive tumor characteristics or less aggressive treatment (Eaker *et al*, 2009), suggesting that other mechanisms are important.

Smoking

Tobacco smoke contains nearly 70 established carcinogens and increases the risk of many cancer types (Torre *et al*, 2015; U.S. Department of Health and Human Services, 2010). Smoking causes a chronic inflammatory state and DNA damage, it prevents apoptosis, and it may impact the hormonal profile in women (U.S. Department of Health and Human Services, 2010). Some studies have investigated the prognostic significance of smoking in breast cancer, but the results have been inconsistent. However, most studies have found an increased risk of recurrence or breast cancer-specific mortality with smoking (Bérubé *et al*, 2014; Nechuta *et al*, 2016). A recent study based on the same cohort on which this thesis is based showed that AI-treated patients who smoked had an increased risk of recurrence compared to non-smokers who received the same treatment. Smoking did not appear to impact the risk of recurrence in other treatment groups (Persson *et al*, 2016).

Hormonal Factors

The results regarding the association between oral contraceptives (OCs) and prognosis have been inconsistent (Lu *et al*, 2011; Schönborn *et al*, 1994), but a recent meta-analysis showed no association between OC use and breast cancer-specific mortality (Zhong *et al*, 2015). However, a paper published the same year as, and thus not included in the meta-analysis, showed that a history of teenage OC use was associated with an increased risk of breast cancer events among patients younger than 50 years old

at diagnosis. Additionally, ever OC use was associated with a better prognosis among patients 50 years and older who had received treatment with aromatase inhibitors (AIs). This indicates that a history of any OC use may have prognostic potential among certain subgroups but not among all patients (Huzell *et al*, 2015).

MHT or hormone replacement therapy (HRT), as it was called until recently, was introduced in the early 1940s. However, 30 years later, it was recognized that therapy with estrogen only gave rise to endometrial cancer. Thereafter, progesterone analogs were added in what is called combination MHTs for women with an intact uterus to reduce the risk of endometrial cancer. Although combination MHT has been associated with the risk of breast cancer (Chlebowski *et al*, 2015a), the association of MHTs with prognosis has yielded varying results. Some studies showed that a history of MHT conferred a better prognosis (Holm *et al*, 2014; Jernström *et al*, 1999; Rosenberg *et al*, 2008), while a recent study from the Women's Health Initiative found an association between poorer prognosis and combined MHT but a better prognosis with estrogen-only MHT (Chlebowski *et al*, 2015a).

Coffee Consumption

Coffee is a refined beverage with the highest consumption in the world, and Sweden has currently the fifth highest coffee consumption in Europe (The European Coffee Federation, 2014). Coffee consumption has received much attention and it is important for a large part of the population whether the popular beverage is harmful or not. In the last decade, the general opinion has switched from believing that coffee is generally harmful to a more positive view (Cano-Marquina *et al*, 2013).

Coffee is a mixture of several hundred bioactive ingredients, such as caffeine, polyphenols, lipids, and phytoestrogens (Gaascht *et al*, 2015). These compounds may have both carcinogenic and anti-carcinogenic effects. However, the mixture depends on several factors, such as the species and growth conditions of the plant, as well as the preparation method, such as drying, roasting, brewing, and the addition of sugar or dietary products (Gaascht *et al*, 2015). Several CYP450 enzymes are involved in the metabolism of caffeine, and CYP1A2 is a key enzyme (Butler *et al*, 1989). Other enzymes involved in the metabolism of caffeine are CYP2C8/9, CYP3A4, and CYP2E1 (Berthou *et al*, 1991; Kot & Daniel, 2008). According to a recent review by Gaascht *et al.,* different compounds in coffee affect all hallmarks of cancer and are antioxidants, anti-inflammatory, cell proliferation inhibitors, cell cycle progression inhibitors, metabolism mediators, angiogenesis inhibitors, invasion and metastasis inhibitors, pro-apoptotic, immune modulators, and cytotoxic agents (Gaascht *et al*, 2015).

The phenolic compound in coffee has been shown to have antioxidative effects by reducing reactive oxygen species (ROS) and consequently reducing DNA damage (Gaascht *et al*, 2015). Conversely, another study showed that another compound in

coffee, hydroxyl hydroquinone, may generate ROS and thereby induce apoptosis in breast cancer cells (Shashni *et al*, 2013). One study found caffeine to inhibit mitosis and induce cell differentiation (Michels *et al*, 2002), and a more recent study suggested that caffeine is associated with improved DNA repair (Nikitina *et al*, 2015). Others have found caffeic acid to influence the cell cycle through downregulation of cyclin D1 (Oleaga *et al*, 2012).

Furthermore, coffee has been shown to activate ER and trigger transcription of ER-responsive genes, such as *PGR* (Divekar *et al*, 2011). Recent results from the Nurses' Health Study showed that coffee consumption, particularly caffeinated coffee, was significantly associated with a longer telomere length of leukocytes among women (Liu *et al*, 2016). These results indicate a new mechanism by which coffee may influence health status and possibly cancer. As a follow-up to paper I in this thesis, mechanistic studies were performed and showed that caffeine, and caffeic acid to some extent, downregulated the levels of growth-promoting ER and insulin-like growth factor 1 receptor (IGF1R) in breast cancer cells, and concomitantly reduced proliferation and induced cell death (Rosendahl *et al*, 2015).

Caffeine has also been associated with an altered hormone profile in pre- and postmenopausal women (Ferrini & Barrett-Connor, 1996; Jernström *et al*, 2003b; Kotsopoulos *et al*, 2009; Sisti *et al*, 2015). A previous study from a subgroup of the cohort used for this thesis showed that the plasma ratio of 2-hydroxyestrogens (2-OHE) to 16α-hydroxyestrone (16α-OHE1) was higher among coffee drinkers than other patients. The ratio of 2-OHE to 16α-OHE1 was also increased by higher alcohol consumption and among patients who had received tamoxifen and radiotherapy (Klug *et al*, 2006). 2-OHE acts as weak estrogens or even anti-estrogens, while 16α-OHE1 is a stronger estrogen with procarcinogenic properties. A high ratio of 2-OHE to 16α-OHE1 may thus be associated with a better prognosis (Schneider *et al*, 1984), indicating that coffee consumption may affect the prognosis of breast cancer patients.

Regular coffee consumption has also been shown to decrease the expression of inflammatory genes, including interleukin-6, and analytical studies also indicated that this effect was at least in part due to chlorogenic acid and metabolites (Gaascht *et al*, 2015). Moreover, coffee has been associated with lower circulating levels of inflammatory markers (Loftfield *et al*, 2015). In breast cancer cell lines, treatment with the lipid kahweol found in coffee inhibited the transcriptional activity of STAT-3, which decreased inflammation (Gaascht *et al*, 2015). Caffeine has also been shown to decrease the secretion of cytokines (Gaascht *et al*, 2015). In summary, coffee contains many anti-inflammatory compounds that may impact the tumor microenvironment and thus potentially impact the risk and prognosis of breast cancer.

Most studies have found a decreased risk of cancer at any site with coffee consumption (Lukic *et al*, 2016). Results from studies investigating the association between coffee and breast cancer risk have been inconsistent, with some studies showing a decreased risk, especially among heavy coffee consumers (Baker *et al*, 2006; Bhoo-Pathy *et al*, 2015; Oh *et al*, 2015), while others found no association (Bøhn *et al*, 2014; Lukic *et al*, 2016). Some of these studies found a decreased risk among subgroups of patients such as postmenopausal patients (Bhoo-Pathy *et al*, 2015; Bøhn *et al*, 2014), patients with ER-positive tumors (Oh *et al*, 2015), or patients with ER-negative tumors (Li *et al*, 2013). The potential prognostic significance of coffee consumption for breast cancer patients had not been studied prior to paper 1 in this thesis.

Alcohol Consumption

Ethanol has been associated with increased risk of many cancer types, such as liver, colorectal, head and neck cancers, and breast cancer. Overall, the main alcohol-induced pathway for carcinogenesis is believed to involve ethanol and its metabolism. Ethanol is primarily metabolized in the liver by two enzymes: alcohol dehydrogenase (ADH), which metabolizes ethanol into its primary carcinogenic metabolite acetaldehyde, and aldehyde dehydrogenase (ALDH), which metabolizes acetaldehyde into acetate (Shield *et al*, 2016). However, there are wide variations in the interindividual ethanol metabolism, depending on genetic polymorphisms, which in turn have different ethnic distributions (Edenberg, 2007). The metabolism of ethanol results in the formation of ROS, which leads to DNA damage (Shield *et al*, 2016).

Ethanol is also metabolized by CYP2E1 into both acetaldehyde and acetate, which leads to the formation of ROS and promotes the conversion of procarcinogens to carcinogens (Hayashi *et al*, 1991; Shield *et al*, 2016). However, while ROS contributes to carcinogenesis, too much oxidative stress may have toxic effects on the cancer cells. Many cancer cells have higher ROS production than normal cells. Therefore, further ROS from exogenous agents may have therapeutic potential (Trachootham *et al*, 2009). An example of an existing agent that acts through production of ROS is radiotherapy, where the production of ROS is a main mechanism behind its cytotoxicity (Salehifar & Hosseinimehr, 2016).

Alcohol consumption has been identified as a risk factor for breast cancer, and both light and moderate to heavy drinking increase the risk (Shield *et al*, 2016). Furthermore, low alcohol consumption and smoking have been associated with low socioeconomic status (Aarts *et al*, 2013; Cederfjäll *et al*, 2004). Although alcohol increases risk of breast cancer overall, the increased risk in most studies is confined to postmenopausal patients (Dartois *et al*, 2016; Strumylaite *et al*, 2015) or patients with ER-positive tumors (Shield *et al*, 2016; Wang *et al*, 2015). The exact mechanisms by

which alcohol confers an increased risk of breast cancer are not fully understood, but some mechanisms have been identified.

One of the so far identified main mechanism underlying alcohol-induced breast carcinogenesis includes an effect on the estrogen metabolism (Hartman *et al*, 2016) and increased serum estrogen levels, which may be a reason for the higher risk increase among patients with ER-positive tumors (Shield *et al*, 2016). Additionally, *in vitro* studies have shown that alcohol leads to an increased ER expression and increased proliferation, while BRCA-1 levels decreased, although the complete transcriptional mechanism remains unclear (Pérez-Solis *et al*, 2016). Moreover, alcohol consumption has been associated with increased mammographic density (Brand *et al*, 2013), which has been associated with a higher risk of breast cancer, but a recent study did not find such an association (McDonald *et al*, 2016).

The results regarding alcohol's potential prognostic significance for recurrence and mortality have been inconsistent, with some studies showing a beneficial effect of alcohol on prognosis (Barnett *et al*, 2008; Harris *et al*, 2012; Reding *et al*, 2008) and others finding a poorer prognosis with alcohol consumption (Kwan *et al*, 2013; Vrieling *et al*, 2012). A meta-analysis showed no effect on overall survival of post-diagnosis alcohol consumption but a slightly improved survival of pre-diagnostic alcohol consumption (Ali *et al*, 2014). However, a recent study within the Women's Health Initiative did not find any significant association between pre- or postdiagnostic alcohol consumption and all-cause mortality (Lowry *et al*, 2016). Few studies have investigated the association between alcohol consumption and recurrence, and the results have been heterogeneous and could not be combined in the meta-analysis (Ali *et al*, 2014). However, in a recent pooled analysis including patients recruited from the United States and Shanghai with ER-positive tumors, any alcohol intake was associated with late recurrence, but no trend for increasing alcohol intake and higher risk of recurrence was observed (Nechuta *et al*, 2016).

Different recommendations and long-term survivorship care plans are applied around the world for breast cancer patients regarding alcohol consumption. With respect to survival and quality of life, it is important to elucidate whether postoperative alcohol consumption, which is modifiable, has an impact on the risk of recurrence and survival, which was the purpose of paper II in this thesis.

Inflammation and Cancer

Inflammation and evading immune destruction are two of the new hallmarks of cancer. These hallmarks may at first seem paradoxical. The immune system inhibits tumor growth, and the presence of cytotoxic T-cells in breast tumors improves outcome (Brenner *et al*, 2016), but the exact role of the immune system in breast cancer is still unresolved. However, inflammation in the tumor microenvironment is often beneficial for tumorigenesis. The inflammation contributes to acquiring other hallmarks by supplying bioactive molecules such as growth factors, anti-apoptotic factors, proangiogenic factors, and factors that facilitate invasion and metastasis (Hanahan & Weinberg, 2011).

Nuclear factor-κB (NFκB) and signal transducer and activator of transcription 3 (STAT-3) are often mutated and thus activated or constitutively activated by tumor necrosis factor-α (TNF-α) in cancer. This activation leads to expression of growth factors such as vascular endothelial growth factor and cytokines (Gaascht *et al*, 2015). Additionally, inflammatory cells can release ROS, which are mutagenic (Hanahan & Weinberg, 2011). Inflammation is also linked to obesity, and signs of inflammation known as crown-like structures of the breast (CLS-B) have been identified in the breasts of obese patients. CLS-B are inflammatory foci with an aggregation of necrotic macrophages that surrounds adipocytes. The presence of CLS-B may be a biomarker of increased risk or poor prognosis in breast cancer (Morris *et al*, 2011).

Cyclooxygenase 2

Cyclooxygenase (COX) is an enzyme that catalyzes the conversion of arachidonic acids to prostaglandin G2 and H2, which in turn are converted to other prostaglandins, prostacyclin, and thromboxane. Prostaglandins are involved in many diverse processes, such as renin release, thromobocytic aggregation, and inflammation (Howe *et al*, 2001). COX exists as isoforms COX-1 and COX-2, which differ in function, regulation, and tissue distribution (Picot *et al*, 1994). COX-1 is constitutively expressed in most tissues, and its downstream products prostaglandins have an important function in normal cellular activities. COX-2 is constitutively expressed in kidney, bone, and brain tissues, but not in other tissues. COX-2 expression in other tissues is instead induced by growth

factors, cytokines, and oncogenes such as HER-2, and in turn mediates cell growth and inflammation (DeWitt *et al*, 1993; Howe *et al*, 2001).

COX-2 is overexpressed in many types of cancers, including colorectal and breast cancer (Howe *et al*, 2001). Subbaramaiah *et al*. showed that COX-2 expression leads to increased aromatase and PgR expression via prostaglandin E2 (PGE2) in overweight patients (Subbaramaiah *et al*, 2012). Several inflammatory parameters are increased by obesity, particularly prostaglandin E2 expression (PGE2) (Simpson & Santen, 2015). The increased aromatase expression, and thereby increased testosterone-to-estrogen conversion, are mediated by COX-2 and could thus be a mechanistic explanation of the association between obesity and breast cancer risk (Subbaramaiah *et al*, 2012). In breast cancer, COX-2 expression has been associated with a worse prognosis in most (Holmes *et al*, 2011; Ristimäki *et al*, 2002; van Nes *et al*, 2011) but not all studies (Ahn *et al*, 2015; Gunnarsson *et al*, 2006; Kelly *et al*, 2003). In other studies, COX-2 expression was associated with different prognosis, depending on other tumor factors such as ER status (Glynn *et al*, 2010; Haffty *et al*, 2008) and Ki-67 (Park *et al*, 2012).

Non-steroidal anti-inflammatory drugs (NSAIDs) are COX inhibitors and inhibit the enzymatic activity by binding to the enzyme binding site of COX. Most NSAIDs are not selective and thus inhibit both thromboxane and prostaglandin synthesis. Newer NSAIDs with COX-2 selective inhibition mainly inhibit prostaglandin synthesis, and not thromboxane synthesis, were developed and prescribed during the 1990s. However, due to an increased risk of cardiovascular events and prothrombotic effects with selective COX-2 inhibitors, two COX-2 inhibitors were withdrawn during the early 2000s, and the prescription of the remaining COX-2 inhibitors has dropped (Santilli *et al*, 2016). Later, it was proposed that polymorphisms in the gene encoding COX-1, the prostaglandin-endoperoxidase syntetase 1 (*PTGS1*) gene, and in the C-reactive protein (CRP) gene would interact with NSAIDs and may contribute to the susceptibility for cardiovascular events (St Germaine *et al*, 2010). In colorectal cancer, COX-2 inhibition is a promising target, and postdiagnostic aspirin use was associated with better overall survival but not colorectal-specific survival in a recent meta-analysis (Li *et al*, 2015).

Randomized clinical trials have examined the effect of COX-2 inhibition in the preoperative and metastatic setting of breast cancer, but the results have been inconclusive (Aristarco *et al*, 2016; Brandão *et al*, 2013; Martin *et al*, 2010). The impact of COX-2 inhibitors in the adjuvant setting of breast cancer is currently not known, but randomized trials are ongoing (ClinicalTrials.gov identifiers; NCT00502684, NCT02429427, NCT01431053, NCT01806259, and NCT02141139). Larger studies and meta-analyses are therefore needed along with randomized controlled trials to determine the prognostic significance of COX-2 and COX-2 inhibition in breast cancer. In paper IV of this thesis, the association between tumor-specific COX-2 expression and prognosis was investigated in a large patient cohort.

Interactions between Genotype, Lifestyle, and Therapies

Tumor characteristics, CYP450 enzymes, host factors, and breast cancer treatment constitute a complex web of interacting factors that may have significant clinical implications. Gene-environment interactions have been thoroughly studied in relation to cancer and are known etiologic factors for cancer risk (Simonds *et al*, 2016). Since the CYP450 enzymes are involved in the metabolism of both endogenous and exogenous substances, many interactions between host factors and treatments include the CYP450 enzymes. A clinical example is the interaction between *CYP2D6* genotypes, CYP2D6 inhibitors, and the efficacy of tamoxifen metabolite activity. Many antidepressants are CYP2D6 inhibitors and thus might interfere with the metabolism of tamoxifen into its active metabolites (Binkhorst *et al*, 2015). However, the results have been inconsistent regarding both inhibition of CYP2D6 (Binkhorst *et al*, 2015; Lash *et al*, 2010; Regan *et al*, 2012) and the genotypes of *CYP2D6* (Binkhorst *et al*, 2015; Jernström *et al*, 2009; Jin *et al*, 2005; Lash *et al*, 2011; Markkula *et al*, 2014; Wegman *et al*, 2007). It has been proposed that the serum concentration of the metabolite endoxifen may be a better predictor of tamoxifen response (Hennig *et al*, 2015).

In addition to CYP2D6, the metabolism of tamoxifen involves other CYP450 enzymes such as CYP1A2 and CYP2C8/9. Polymorphisms of *CYP1A2* and *CYP2C8* were investigated in relation to breast cancer prognosis among tamoxifen-treated patients in papers I and III. Genetic polymorphisms in *CYP1A2* account for the main interindividual variation of CYP1A2 enzyme activity, but the activity is also modified by environmental factors (Ghotbi *et al*, 2007). Estrogens, alcohol consumption, and OCs inhibit CYP1A2, while coffee and smoking induce the enzyme activity (Ghotbi *et al*, 2007; Le Marchand *et al*, 1997). Additionally, AIs have been shown to interfere with CYP450 enzymes, including CYP1A2, with some AIs being metabolized by CYP1A2 and others inhibiting the enzyme (Buzdar *et al*, 2002; Kamdem *et al*, 2011; Kamdem *et al*, 2010). CYP2C8 is involved in the metabolism of estrogens, arachidonic acids, and approximately 20% of clinically used drugs, including the chemotherapeutic agent paclitaxel (Goldstein, 2001). Since several CYP450 enzymes are involved in the metabolism of estrogens, tamoxifen, caffeine, and ethanol, interactions could be caused

by the competition of several substrates for the same enzyme as well as inhibition or induction of the enzyme.

Alcohol also modulates the innate and the adaptive immune response, which in turn may have an impact of the prognosis of cancer. However, whether this results in immunosuppression or activation is complex. For example, chronic alcohol intake activates the immune system, particularly T-cells, NK cells, and dendritic cells. Few studies have investigated the impact of alcohol consumption on the immune system in cancer patients, whose immune systems may be altered. In hepatocellular cancer and lymphoma, alcohol was associated with altered levels of regulatory T-cells, but the clinical impact of these changes is uncertain (Meadows & Zhang, 2015). A Danish prospective cohort study found an interaction of NSAIDs on the association between alcohol consumption and breast cancer risk. NSAID users with an intake of 13 g of alcohol per day had a higher risk of breast cancer than non-users of NSAIDs with a consumption of less than 3 g of alcohol per day (Kopp *et al*, 2016), indicating that decreased inflammation in patients who consume alcohol may not be beneficial. However, the exact role of alcohol in inflammation and breast cancer remains unclear. In conclusion, gene-environment, gene-drug, drug-drug, and drug-lifestyle interactions may be important in the clinical setting for breast cancer prognosis and treatment response.

Aims of the Thesis

The overall aim of this thesis is to elucidate the associations between genetic host factors, lifestyle factors, and tumor protein expression and how these factors alone or combined may be used as prognostic and/or predictive factors in breast cancer. The specific aims of the included papers are as follows:

Paper I

To investigate the impact of coffee consumption on tumor characteristics and the risk of early breast cancer events in relation to breast cancer treatment and genotypes of *CYP1A2* rs762551 and *CYP2C8*3*.

Paper II

To investigate the association between preoperative and postoperative alcohol consumption and the risk of early breast cancer events, early distant metastases, and all-cause mortality according to adjuvant treatment and tumor characteristics.

Paper III

1) To perform an exploratory analysis using a DMET™ chip to find new genetic treatment predictive markers of AI in a subset of an AI-treated extended cohort.

2) To examine the potential markers found with the DMET™ chip with a special focus on SNPs in *CYP19A1* in relation to risk of early events in an extended cohort of 201 breast cancer patients who received adjuvant AI treatment.

Paper IV

1) To investigate the association between tumor-specific COX-2 expression and tumor characteristics and to evaluate the prognostic significance of tumor-specific COX-2 expression in relation to ER-status and in different treatment groups.

2) To analyze potential effect modifications of NSAID and tumor and lifestyle factors, including body constitution on the association between tumor-specific COX-2 expression and breast cancer prognosis.

Materials, Methods, and Methodological Considerations

The Breast Cancer and Blood Study

The papers included in this thesis are based on the Breast Cancer and Blood study (BC-blood study), which is a population-based prospective cohort of female breast cancer patients diagnosed at Skåne University Hospital in Lund, Sweden, as of October 2002. Patients diagnosed with primary breast cancer who did not have any other cancer diagnosis within the last 10 years were invited to participate. The patients were asked to fill out a three-page questionnaire preoperatively and a less extensive one-page questionnaire at follow-up visits at three to six months; seven to nine months; one, two, and three years postoperatively; and biannually at home thereafter up to 11 years postoperatively. The questions regarding alcohol consumption were obtained from the validated Alcohol Use Disorders Identification Test (AUDIT) developed by the World Health Organization (Saunders *et al*, 1993). See Table 2 for included variables in this thesis.

At the preoperative visit, the patients' weight, waist and hip circumference, and breast volumes were measured by trained research nurses. The standardized measurement of breast volume was performed using plastic cups of increasing size (Ringberg *et al*, 2006). This procedure has been shown to be a reliable method for measuring breast size (Hansson *et al*, 2014). The anthropometric measures except for breast volume and height were obtained again at the postoperative visits. Blood samples were collected by research nurses preoperatively and at the postoperative visits. The blood was centrifuged, and the samples were frozen at –80° Celsius within two hours of collection.

Table 2. Variables collected from the questionnaires

Most variables were collected preoperatively and some were collected postoperatively (as indicated).

Variables collected from the questionnaires	
Age	Date of birth.
Medications	Medications used during the past week prior to filling out the questionnaire, including complementary alternative medicine use.
Coffee consumption	The preoperative and the postoperative questionnaires provided nine consumption levels ranging from 0 to 8 cups of coffee per day.
Alcohol frequency	Alcohol frequency was included in the preoperative questionnaire: never, ≤1 time/month, 2-4 times/month, 2-3 times/week, and 4+ times/week.
Alcohol intake	Number of drinks consumed during the past week was reported at the preoperative visit as well as at each follow-up visit: none, 1-3 drinks, 4-9 drinks, 10-19 drinks, and 20+ drinks.
Smoking status	Present smoking status including smoker and occasional (social) smoker.
Reproductive history	Number of pregnancies and children, age at menarche, and age at menopause.
MHT use	Current or previous use, duration and type of MHT.
OC use	Any history of OC use, starting age, duration prior to age 20, duration prior to first child, and total duration of OC use.
Previous breast surgery	Type of operation.

Data on tumor characteristics was obtained from the patient's pathology report, including tumor size, histological grade, number of involved axillary lymph nodes, ER and PgR status, HER-2 amplification, and Ki-67. The tumors were analyzed at the department of pathology at Skåne University Hospital in Lund. Analysis of HER-2 amplification was introduced to the clinical routine in November 2005 for patients younger than 70 years old. Ki-67 was routinely analyzed as of March 2009. Information regarding adjuvant treatment of the patients was retrieved from patient charts and questionnaires to obtain as accurate information as possible. Adjuvant treatment data was registered up to but not after the last follow-up or any breast cancer event.

The genotyping for the BC-blood study was carried out for patients included until October 2008. Papers I and III are therefore based on patients included between October 2002 and October 2008. Paper II is based on data from patients included between October 2002 and December 2011. Since the tissue micro arrays (TMAs) were constructed for patients included until June 2012, paper IV is based on data from patients included between October 2002 and June 2012. Flowcharts of included and excluded patients of the individual papers are presented below.

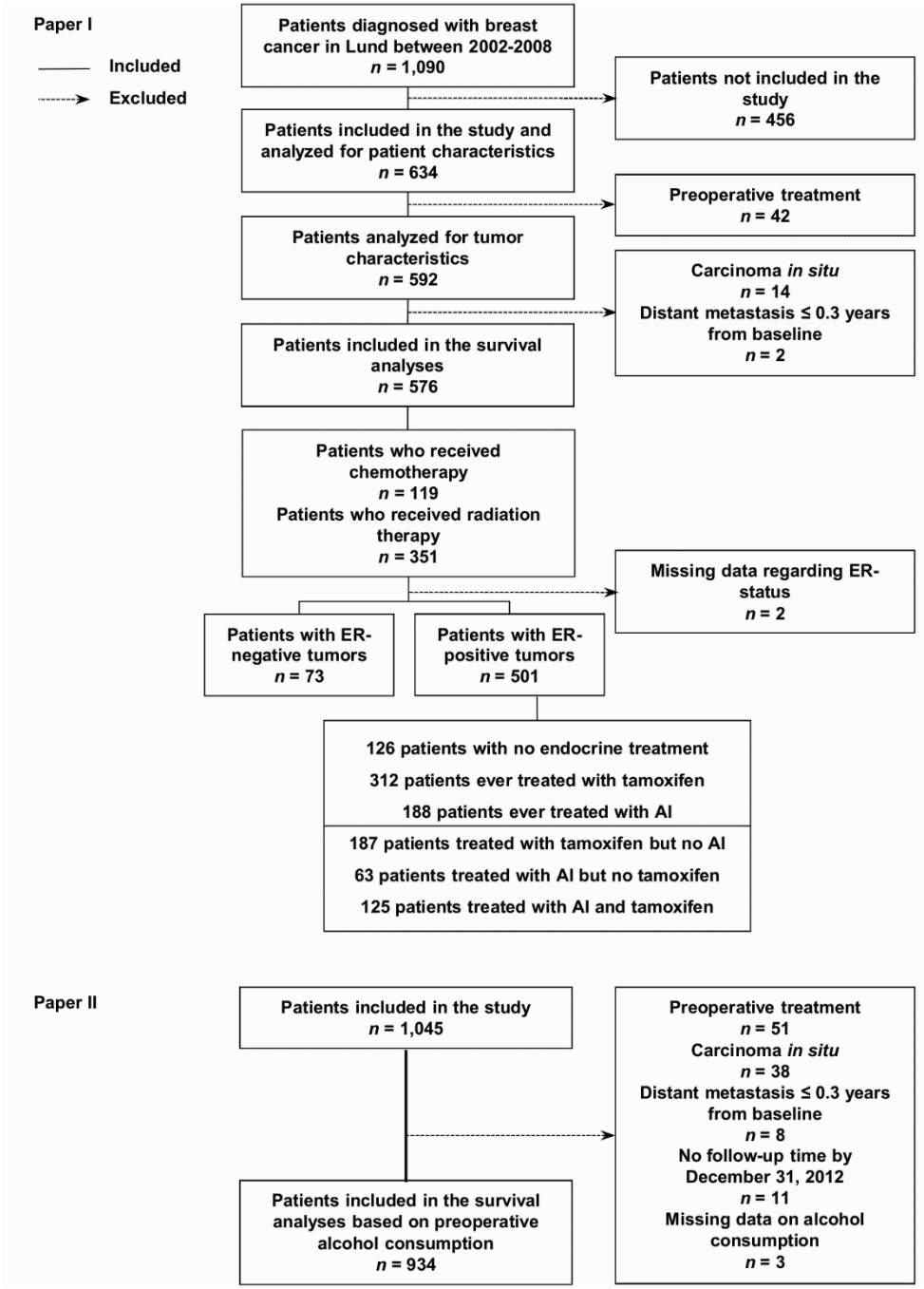


Figure 5. Flowchart of included and excluded patients in papers I and II

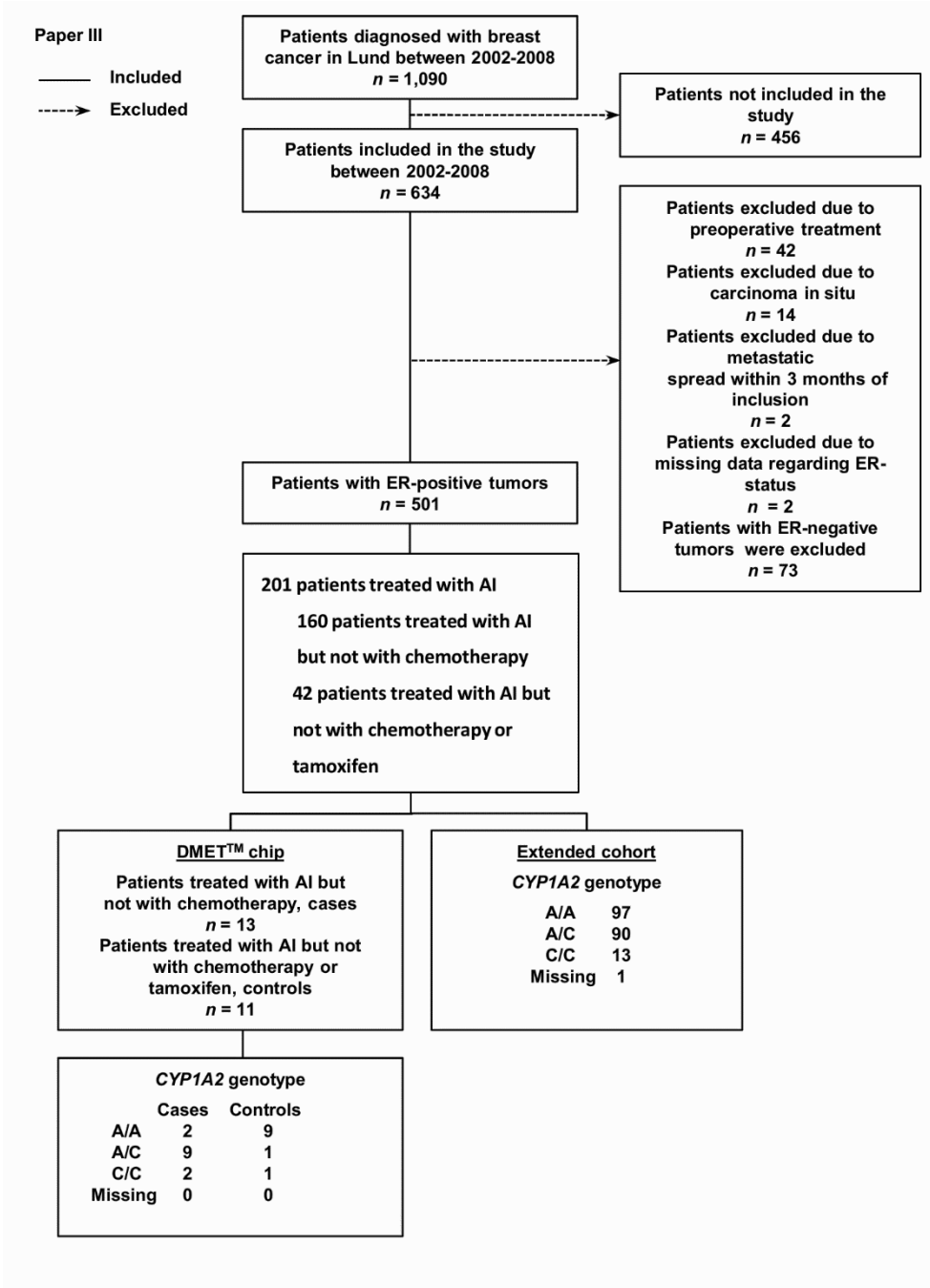


Figure 6. Flowchart of included and excluded patients in paper III

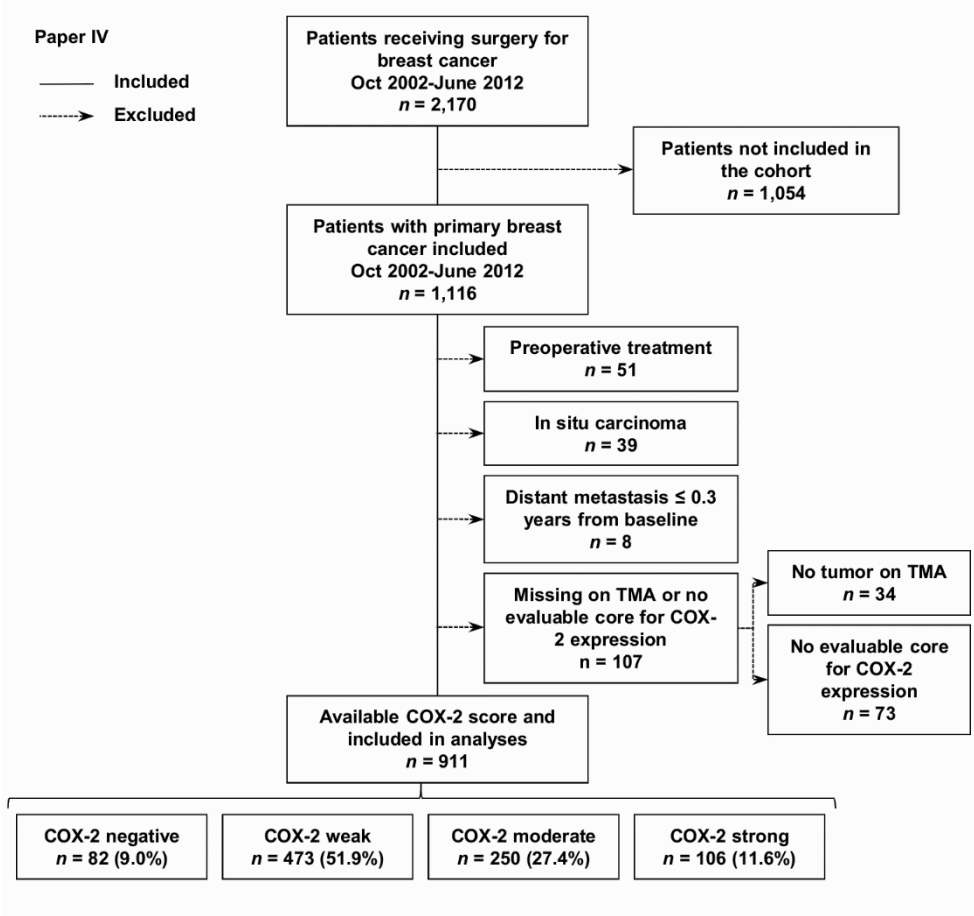


Figure 7. Flowchart of included and excluded patients in paper IV

Registries

Date of death was obtained from the Swedish Population Registry, which is virtually 100% complete since it is compulsory for the physician in charge of the patient to provide a death certificate and a certificate of the cause of death to the registry according to Swedish law (1990:1144 and 1990:1147) (Johansson & Westerling, 2000). Complementary to patient charts and pathology reports, information concerning breast cancer events was collected from the Southern Swedish Regional Tumor Registry, which is a subdivision of the Swedish Cancer Registry. Every healthcare provider in Sweden is required to report newly detected cancers to the Swedish Cancer Registry,

and the completeness of this register is very high, with 96% of all cancer diagnoses in the latest update (Barlow *et al*, 2009).

Methods and Methodological Considerations

Genetic Analyses

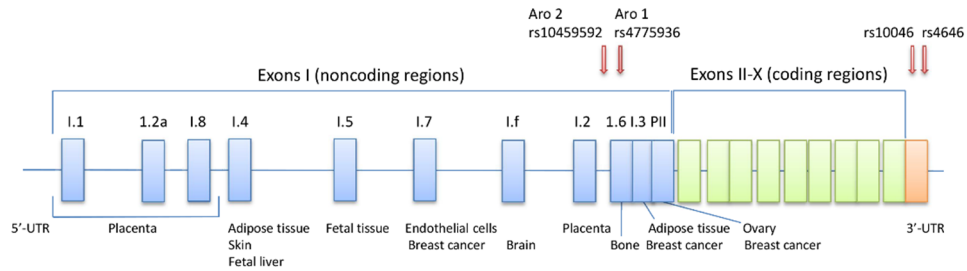
The Wizard Genomic DNA Purification Kit (Promega, Madison, USA) was used for extracting genomic DNA from the leukocyte portion of the frozen peripheral blood. For papers I and III, four methods for genotyping were used: sequencing, iPlex, TaqMan, and a microarray chip, the DMET™ (Drug Metabolizing Enzymes and Transporters) Plus Premier Pack (Affymetrix Santa Clara, CA, USA). The DMET™ chip includes 1931 SNPs in 227 ADME-related genes on a single array. In this assay, the sequence-specific information at each SNP locus is amplified with multiplex polymerase chain reaction (PCR). The PCR product then undergoes enzymatic fragmentation, end-labeling, and hybridization to an array that contains allele-specific oligonucleotides used for SNP discrimination and genotyping (Burmester *et al*, 2010). The experimental analysis of the DMET™ chip data was performed for paper III at SCIBLU Genomics at Lund University for 24 AI-treated patients (13 cases, 11 controls).

Genotyping with iPlex and TaqMan was performed at the Region Skåne Competence Centre (RSKC Malmö), Skåne University Hospital, Malmö, Sweden, for patients included until October 2008. The genotypes were used in paper I and paper III. For paper I, the *CYP2C8*3* SNPs (rs11572080 and rs10509681), were genotyped with iPlex reagents. For paper III, the *CYP19A1* SNPs rs700518, rs4646, Aro1 (rs4775936), and Aro2 (rs10459592), as well as two functional *AhR* SNPs Arg554Lys (rs 2066853) and Val570Ile (rs4986826), were genotyped with iPlex reagents. For papers I and III, *CYP1A2* rs762551 and *CYP19A1* rs10046 were genotyped with TaqMan SNP allelic discrimination assay. For *CYP1A2* rs762551, data was missing for 19 patients. Sequence data from an earlier project was available for *CYP1A2* rs762551, and genotypes for 16 patients with missing values on TaqMan could be filled in. The concordance between the two methods was 99.8%. For the genetic analyses with iPlex and TaqMan, 10% of the samples were run in duplicates as a validation set, and the concordance was 100% for all SNPs. All SNPs were in Hardy-Weinberg equilibrium, indicating a random selection of patients.

For paper III, *CYP19A1* haplotypes and diplotypes were constructed using cross-tabulation of the four *CYP19A1* SNPs among the 634 patients who were genotyped. Haplotypes or diplotypes that were present in less than 5% of the patients were

classified as rare variants and combined. The most likely combinations of SNPs were used to construct the haplotypes and diplotypes used in the paper, as shown in figure 8.

A)



SNP	Genotype	MAF
rs4646	CC AC AA	0.29
rs10046	CC CT TT	0.52
rs4775936	CC CT TT	0.49
rs10459592	TT GT TT	0.40

B)

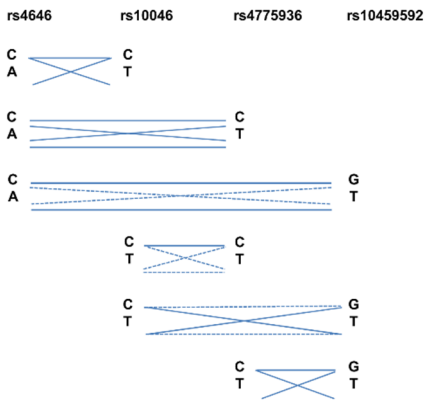


Figure 8. The *CYP19A1* gene and haplotype construction
A) Illustration of the *CYP19A1* SNPs in relation to the positions of the promoters of the *CYP19A1*. The different promoters regulate the expression of *CYP19A1* in different tissues, as indicated. B) Illustration of cross tabulation that was used to create the *CYP19A1* haplotypes. Continuous lines indicate common allele combinations and dotted lines indicate less common allele combinations.

Tissue Microarray and Immunohistochemistry

Tissue microarrays (TMAs) of invasive tumors were evaluated for paper IV. The TMAs were constructed from paraffin-embedded tissue, and two 1.0 mm cores were sampled from non-necrotic areas with invasive cancer. The two cores from each primary tumor were mounted into TMA recipient blocks. For COX-2 staining, 4-µm-thick TMA slices were deparaffinized and pretreated using the automatic PT-link system, followed by staining with COX-2 antibody (ab15191, diluted 1:750 for 30 min at pH 7; Abcam, Cambridge, UK) and an EnVision FLEX high-pH kit in an Autostainer Plus according to the manufacturer's instructions (DAKO, Glostrup, Denmark). In a previous study of the current cohort, COX-2 expression was evaluated in a subset of the patients that were included for paper IV. However, the median staining intensity was very high in the previous study, with a dilution of 1:250. To obtain a wider distribution of staining intensities, new slides of the TMA were re-stained with a more diluted antibody for paper IV.

Cytoplasmic COX-2 expression was evaluated by two independent observers who were blinded to patient information. COX-2 expression was evaluated for the fraction of positive cells, and staining intensity and a joint score for the two cores was obtained. The fraction (0–100%) was defined by preset categories (0%, 1–10%, 11–25%, 26–50%, 51–75%, and >75%). The staining intensity was defined as 0 = negative, 1 = weak, 2 = moderate, and 3 = strong. In cases of discrepancy, a re-evaluation was performed by the observers, and consensus was reached. Since COX-2 was found to be expressed in the majority of the cancer cells if the intensity was positive, the fraction of COX-2 positive cells was excluded for further analyses.

Manual scoring is relatively subjective for evaluation of the expression of biomarkers (Stålhammar *et al*, 2016). The estimation is dependent on the observers' experience and optimal light conditions in the room, and there is a problem with intra- and interobserver variability. Digital image analysis, where the scoring is digitalized, is a more objective method compared to manual scoring and may offer less variability. However, this technique also has limitations, and a source of variance is poor identification of tumor tissue. Although promising, the technique may need further validation (Stålhammar *et al*, 2016).

Statistical Methods and Considerations

The majority of the data analyses were performed with IBM SPSS versions 19.0 or 22.0. Stata version 12.1 was used for analysis of competing risks and conditional hazards in paper II. All *P*-values were two tailed, and a *P*-value of <0.05 was considered significant. Analysis of data from the DMET™ chip in paper III was performed using

the DMET™ console software. Nominal *P*-values are presented without adjustments for multiple testing, with the exception of paper III, where a *P*-value of <0.005 was considered significant in the analysis of the DMET™ data.

Survival Analyses

The log-rank test and Cox proportional hazard model were used for survival analyses in all papers included in this thesis. Kaplan-Meier graphs were used in all papers to illustrate survival probabilities stratified by the variable of interest. These three methods are the most commonly used for survival analyses in cancer journals (Clark *et al*, 2003). For the papers in this thesis, any breast cancer event was considered the primary endpoint. Any of the first occurring event of ipsilateral, contralateral, regional, or distant metastases was considered a breast cancer event (in paper I, event-free survival was incorrectly named breast cancer-free survival by mistake). Distant metastases were considered a secondary endpoint for papers I and III, while distant metastases and overall survival were considered secondary endpoints for paper II. The definition of the different endpoints changed during the time the cohort was compiled and the individual papers were written. Therefore, for paper IV, the newest definition used by the BIG 1-98 trial with breast cancer-free interval and distant metastasis-free interval was used since the events did not include death.

The Kaplan-Meier method for estimation of survival probabilities (Kaplan & Meier, 1958) is a nonparametric method where the estimated survival probability changes at the time of each event. The corresponding Kaplan-Meier survival curve is a plot of these survival probabilities against time. The number of patients at risk decreases with increasing follow-up, not only because of events of interest but also due to censoring, which leads to increased uncertainty of the estimates towards the tail of the curves (Pocock *et al*, 2002). Therefore, it has been proposed that curtailing the plots before the end of follow-up on the x-axis would be valuable—for example, when only five patients remain in one group (Altman, 1991). However, whether curtailing the plots should be performed or not is still debated (Pocock *et al*, 2002), and for the papers in this thesis, the plots were not curtailed.

The log-rank test is a non-parametric test and is the most widely used method for comparing two or more survival curves. This method compares the observed number of events to the expected number under the null hypothesis of no survival differences in each treatment group. Then, the method compares a test statistic based on these numbers to a χ^2 distribution with degrees of freedom equal to the number of groups minus one to determine the *P*-value (Clark *et al*, 2003; Peto *et al*, 1977). The Cox proportional hazards model (Cox regression) is a semi-parametric method that gives the hazard ratio (HR), which is an estimate of the hazard in one group relative to that in another group (Clark *et al*, 2003; Cox, 1972). For non-categorical variables, the

resulting estimates should be interpreted as the relative hazard increase per unit of the variable studied. The Cox regression model requires the assumption of proportionality between the hazard curves to be fulfilled—for example, the hazard curves should not cross or deviate in different directions (Bradburn *et al*, 2003). In addition, some other requirements have to be met for survival analyses, including both log-rank tests and Cox regressions, to yield valid results (Clark *et al*, 2003), see Table 3.

Table 3. Requirements for survival analyses
Adapted from Clark *et al.* (Clark *et al*, 2003).

Requirements for survival analysis	Requirements met in papers I-IV
Uniformative censoring; i.e., censored patients lost to follow-up should have the same risk of event as patients that remain in the study.	The follow-up rates were high, and few patients were therefore lost to follow-up. Furthermore, for the patients who did not answer the follow-up questionnaires, a research nurse reviewed their patient charts in order to discover an event. In addition, register-based searches were performed for all patients at regular intervals and before the last follow-up time. Patients who moved from the region were censored and lost to follow-up, but are likely to have the same risk of event as those who remained in the study.
Adequate length of follow-up to capture enough events for sufficient power.	Power calculations were performed for papers I, III, and IV. However, power calculations were not performed for paper II and for subgroup analyses in any of the studies. The power was limited for the subgroup analyses in all papers. A post hoc power calculation for paper II revealed that the power was 0.78 for the study to identify an HR of 0.69. See text for further details.
Similar completeness of follow-up between the groups.	The follow-up rates calculated for paper I were high and the follow-up is likely to be similar between groups. If the follow-up was not equal between some groups, this would not have led to a significant bias due to the high follow-up rates.
Homogeneity of prognostic factors and treatment during the follow-up period; i.e., the survival probabilities for patients included early should be the same for patients recruited later in the study.	Treatment was administered according to the standard of care at the Skåne University Hospital at the time of inclusion of the patients. However, the treatment regimens have changed according to new guidelines during the time the cohort was compiled (Regionala cancercentrum i samverkan, 2014). The change in the treatment guidelines could lead to a better survival probability for patients included later in the study. For papers III and IV, two follow-up times were used, and the risk of events over the whole study period as well as early events were calculated, which would have lowered the risk of heterogeneity of treatment.
No differences between different centers in a multicenter study.	The BC-blood study in Lund only includes patients diagnosed and treated at Skåne University Hospital in Lund and thus this requirement is not applicable.

In paper II, the association between current alcohol consumption (any vs. null) at different time points during follow-up and risk of breast cancer events was estimated using a series of Cox proportional hazards models. The first analysis examined the whole time interval from the preoperative visit (time 0 for all patients in the study) to

the last follow-up. In this analysis, alcohol use (yes vs. no) at baseline was used as a covariate in univariable and multivariable analyses. In the second analysis, time 0 was defined as the minimum number of days from the preoperative visit to the first postoperative visit observed among the patients in the cohort. In this analysis, which is conditional on survival up to this time point, the covariate alcohol use was updated to the status at the first postoperative visit for the patient with the shortest time interval from the preoperative visit to the first postoperative visit. In the third analysis, time 0 was defined as the second shortest time interval between the preoperative visit and the first postoperative visit, and so on. This way of handling covariates, which are updated during the follow-up, is often referred to as landmark analysis. In this setting, the patients' current alcohol consumption at the most recent follow-up visit before or at time 0 was used for each interval. Thus, updated HRs were calculated for a series of overlapping time intervals. The HR for each time period is conditional on being alive and event free up to the visit of the beginning of that particular time-period.

Patients with missing data on preoperative alcohol consumption (n=3) were excluded. The "last observation carried forward" method was used for patients with missing data for current alcohol consumption at the respective postoperative visits. Thus, the value of alcohol consumption at the previous visit was imputed for postoperative alcohol consumption. Performing this analysis with conditional HRs updated with up to four years of follow-up enabled the evaluation of the impact of postoperative alcohol consumption on the risk of breast cancer events. The effect of postoperative alcohol consumption is a modifiable factor, which the patients may change after diagnosis, in contrast to preoperative or prediagnostic alcohol consumption, which has already happened and cannot be altered afterwards. Therefore, it is also valuable to examine the influence of postoperative alcohol consumption on the risk of recurrence, although this approach is not as straightforward as examination of baseline characteristics and is more prone to bias. Since the analysis is conditional on the patients being alive and event free at the start of each time period, a selection bias of healthier patients may have been introduced. However, the preoperative alcohol consumption was also investigated, and the hazards were comparable to the hazards obtained for the postoperative alcohol consumption.

Competing Risks Analysis

In survival analyses, there is often a possibility that more than one type of event could occur, as competing risks. The definition of a competing risk is an event that hinders the observation of an event of interest or alters its probability (Gooley *et al*, 1999). In other words, a competing risk competes with the event of interest to remove individuals from the population at risk. For example, if the outcome is cancer recurrence, death from causes other than cancer needs to be considered as a competing risk event since it

hinders the observation of cancer recurrence. There are different opinions about whether to incorporate the competing risk in the analysis or not, as well as when and how to do it (Berry *et al*, 2010; Chappell, 2012; Dignam *et al*, 2012; Koller *et al*, 2012). In the presence of competing risks, one option is to fit a cause-specific Cox proportional hazards model, in which competing events are regarded as loss to follow-up. The corresponding hazard ratios should then be interpreted in an artificial world where all the competing risks have been eliminated. The second frequently used analysis strategy is to fit a Cox model to the subdistribution hazards, which incorporates the competing risk events (Dignam *et al*, 2012; Pintilie, 2007). The former approach could be beneficial when the aim of the study is to test whether a specific factor is biologically relevant or whether a treatment is effective. With the latter method, the observed incidence of the event of interest is directly compared between groups. However, this approach can be generalized only to other populations with similar competing risks (Pintilie, 2007).

In paper II, the association between alcohol consumption and risk of any breast cancer event was investigated. Since alcohol may also increase risk of death due to other causes, a competing risk analysis was performed using the Fine and Gray model for subhazards (Fine & Gray, 1999). This approach keeps the patients who experience a competing risk in the risk set and thus illustrates those who will never experience the event of interest (Koller *et al*, 2012). The competing risk analysis led to essentially the same results as a standard Cox regression analysis in paper II, indicating that the potential influence of death due to any cause did not significantly bias the results. Furthermore, the result of the competing risk analysis indicated that censuring for death yields similar results to a competing risk analysis for this cohort.

The Kaplan-Meier estimate is based on the assumption that the event of interest is the only possible event. Since the life expectancy of humans is limited, analyses of cause-specific mortality or recurrence introduces competing risks and a bias in the estimate downwards (Andersen *et al*, 2012). In contrast, analyses of all-cause mortality incorporate all possible events, and the estimate is thus unbiased in this sense. In analyses where competing risks are present, there is no longer a one-to-one correspondence between the survival rate and the risk estimate, the HR. Thus, the HR could not be computed based on the survival rate. Instead of using the Kaplan-Meier estimate or one minus the Kaplan-Meier estimate, a cumulative incidence based on both the event of interest and competing risk events would introduce less bias to the curves and survival estimate (Andersen *et al*, 2012; Dignam *et al*, 2012). However, both methods seem to be useful depending on the research question (Chappell, 2012; Koller *et al*, 2012). See figure 9 for comparisons between cumulative incidence and one minus Kaplan-Meier curves for COX-2 expression.

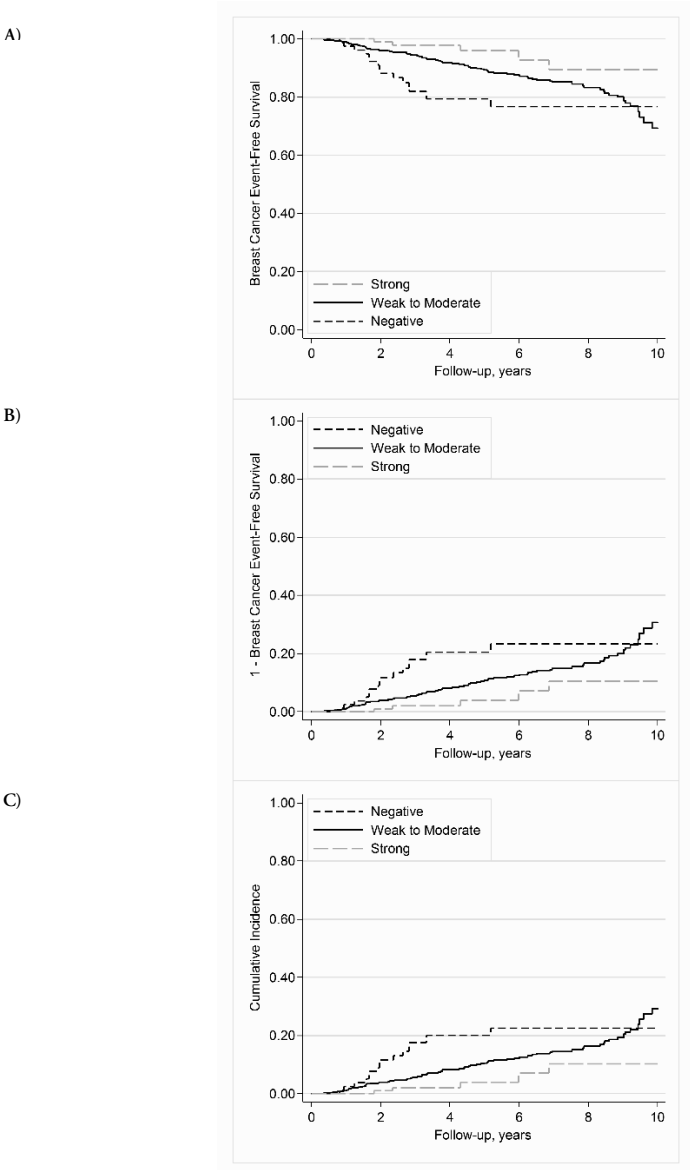


Figure 9. Comparison between different types of graphs for the association between COX-2 and risk for breast cancer events
A) Kaplan-Meier estimates of event-free survival, B) Biased estimates of cumulative event incidences defined as 1 minus the Kaplan-Meier estimates, and C) Unbiased cumulative event incidences estimated using a method that takes the competing risk i.e. death without a preceding breast cancer event, into account. In this material, with relatively few competing events per group, only minor discrepancies were seen between the 1 minus Kaplan-Meier estimates and the unbiased cumulative incidence estimates. However, all the unbiased cumulative incidence estimates were lower than the corresponding cumulative incidence estimates calculated using the Kaplan-Meier method and at 10 years, the absolute differences between the two varied from 0.4 to 1.5 percentage units. This is in accordance with the theory, which states that Kaplan-Meier estimates are always biased towards worse survival (higher cumulative incidence) if the follow-up is censored at the time of a competing event.

Type I, Type II, and Systematic Errors

When designing or interpreting results from a study, two types of errors – *random errors* and *systematic errors* – must be considered (Rothman & Greenland, 1998). *Random errors* reflect the random variability in the data that cannot be explained by selection processes or confounding factors. A confidence interval for an observed parameter such as HR is a rough measure of the uncertainty in the data, and the *P*-value is a measure that summarizes the amount of evidence against the null hypothesis. More specifically, the *P*-value is defined as the probability of observing an effect that is as strong or stronger than the effect observed in the study if the null hypothesis is true. The significance level is usually set at $\alpha = 0.05$, which corresponds to a 5% risk of falsely rejecting the null hypothesis when it is true, i.e. a type I error (Rothman & Greenland, 1998). If multiple hypothesis tests are performed at the 5% level, the joint probability of falsely rejecting at least one of the null hypotheses will be considerably larger than 5%. Corrections for multiple testing are therefore often performed. However, a correction that is too stringent would increase the number of false negative results, i.e. type II errors. Therefore, many agree that corrections for multiple testing should not be performed in exploratory analyses (Bender & Lange, 2001). To correct for the tendency of higher likelihood of type I errors, exploratory studies always need validation in confirmatory studies where corrections for multiple testing should be carried out (Bender & Lange, 2001).

The papers included in this thesis were considered exploratory, so corrections for multiple testing where not carried out. However, in paper III, where nearly 2000 SNPs were investigated, the significance level for each analysis was set to 0.005 to decrease the risk of false positive associations. The amount of genotyping in paper III did not reach the amount of SNPs genotyped in a GWAS, nor was it intended to. Instead, a more candidate-driven analysis was performed with SNPs in only ADME-related genes. Therefore, the significance level was not chosen as low as for GWAS, but it was somewhat corrected compared to the most commonly used significance level of 0.05.

The probability of type II errors is usually set at 20%, which corresponds to 80% power of detecting stipulated effects as significant. For the genetic analyses, the sample size was determined using a series of simulations with 5,000 replicates for each scenario. Survival times were simulated from two exponential distributions with intensity parameters uniquely defined by the 5-year recurrence-free survival probability in the low-risk allele, $RFS_0(5)$, and the ratio of the two hazards (HR), high versus low risk. Uniform recruitment in a 5-year interval was assumed, followed by an additional 2.5 years of observation, leading to an average follow-up of 5 years for the event-free patients (range 2.5 to 7.5 years). For each scenario and simulated dataset, the null hypothesis (equal RFS between the two groups) was evaluated using a log-rank test at the 5% significance level.

All combinations of the following scenarios were evaluated:

- Prevalence of the high-risk allele (p): 10%, 20%, and 30%
- $RFS_0(5)$: 60%, 70%, and 80%
- Sample size: 300 and 600

The fraction of simulations for which the null hypothesis is rejected is an estimate of the power for each scenario. For the low sample size, we found the power to be insufficient (<80%) for several reasonable scenarios. By doubling it to 600, HRs between 1.5 (p=0.3, $RFS_0(5)$=60%) and 3.4 (p=0.1, $RFS_0(5)$=80%) can be detected with at least 80% power. These effects were considered reasonable, so the larger sample size was chosen for the genetic analyses.

The analyses for each of the papers included in this thesis were exploratory, and specific power calculations prior to the analyses were not possible to perform because the frequency of the exposure variable was not known. Power calculations were performed after the study was initiated for papers I, III, and IV with adequate results. However, power calculation for subgroup analyses were not performed, but the power of these smaller groups is certainly lower than for all patients. No power calculation was performed for paper II, but a power calculation with the Power and Sample Size Program (Dupont & Plummer, 1998) after the study was performed, which indicated that the power was 0.78 for the study to identify a HR of 0.69 with a type I error probability of 0.05.

Systematic errors stem from systematically incorrect measurements or non-random inclusion and cannot disappear if the sample size is increased, in contrast to random errors. Systematic errors are also called bias and include three broad categories: *selection bias*, *information bias*, and *confounding* (Rothman & Greenland, 1998). *Selection bias* may occur when the association between an outcome and an exposure differs between the included patients and the non-included patients. Patients with a better health status are more likely to participate in studies, which may have conferred a selection bias of the BC-blood study. However, the non-included patients with worse health status at diagnosis may have been more likely to experience early metastasis. The patients with metastases included within three months of inclusion were excluded from the analyses, which may have reduced the risk for this kind of selection bias. Furthermore, those with higher socioeconomic status have been shown to be more likely to participate in scientific studies (Galea & Tracy, 2007). This may cause a selection bias since these patients are more likely to be diagnosed with cancer and to have a better prognosis than patients with lower socioeconomic status.

The second category of *systematic bias* is *information bias*, which may arise when information collected from or about the study participants is inaccurate. One example is recall bias, which arises when the exposure information is gathered after the event has occurred. Thus, patients who have been diagnosed with the disease are more likely to

recall exposures that occurred before diagnosis more accurately compared to healthy patients. Recall bias is a form of differential misclassification, in which the classification error is associated with the event of interest (Rothman & Greenland, 1998). This type of bias is less likely to have occurred in the BC-blood study because all patients were diagnosed before study entry. Furthermore, the information is collected from the study participants at inclusion and not after a breast cancer event has happened, which decreases the risk of information bias between patients who have not experienced a breast cancer event and patients who have recurred. Another type of information bias is nondifferential misclassification, which may arise when there is no association between misclassification and the outcome. Nondifferential misclassification of a dichotomous variable often leads to bias towards the null (Rothman & Greenland, 1998). In paper II, a self-presentational bias concerning any alcohol consumption is most likely to be present, since self-reporting of alcohol consumption has been shown to be underestimated (Embree & Whitehead, 1993). However, this self-presentational bias is likely to be the same for patients who recur and those who do not recur and may thus be classified as a nondifferential misclassification.

The third category of bias is *confounding*, which is a central issue for non-randomized studies. The definition of a confounder is that both the exposure and the outcome are associated with a third variable, i.e. a confounder. In a randomized trial, known and unknown confounders are spread between the groups. However, in non-randomized studies, there are factors that differ between patients who have not experienced an outcome and those who have. Therefore, in non-randomized studies, there is a need for adjustments for confounding factors in a multivariable model or stratification by the confounding factor (Rothman & Greenland, 1998). In all papers included in this thesis, adjustments and stratifications according to potential confounders have been performed. This procedure most likely does not account for all possible confounders within the study but is an attempt to control for factors that are likely to constitute the majority of the underlying confounding.

Causality

In 1964, the Surgeon General published a model for assessing causality of smoking with disease (Surgeon General's Advisory Committee on Smoking and Health & Public Health Service. Office of the Surgeon General, 1964; Surgeon General's Advisory Committee on Smoking and Health & Public Health Service. Office of the Surgeon General, 2004). Based on this model, Sir Austin Bradford Hill wrote a review in 1965 about association and causation and established what would later be called the Bradford Hill Criteria or Hill's criteria for causation. These criteria are an attempt to create a framework for the judgment of whether an association between an incidence and a possible consequence is a direct or an indirect effect of the incidence (Hill, 1965).

However, these criteria were never meant to be rules that must be obeyed for an association to be considered causal, but rather as a guide to recognize a factor as causal or not causal (Hill, 1965). A description of these nine criteria and a declaration of whether they were met in the papers of this thesis are presented in Table 4.

Table 4. The Bradford Hill Criteria of causation in relation to the findings in this thesis
Established by Sir Austin Bradford Hill in 1965 (Hill, 1965).

Criteria	Explanation	Criteria met in papers I-IV
Strength of the association (effect size)	The larger the effect size, the higher the likelihood that it is causal.	**Paper I**: The effect sizes were generally high, between 2.5 and 6.2. **Paper II**: The effect sizes were somewhat smaller, 0.40 to 0.66 (corresponding to 1.5 to 2.5). **Paper III**: The effect sizes varied between 2.2 and 10. **Paper IV**: The effect sizes varied between 1.7 and 4.5.
Consistency (reproducibility)	If the findings were reproduced in different places, by different people, and in different circumstances and times, the likelihood of causation is higher.	**Paper I**: The main result regarding the prognostic significance of coffee consumption in tamoxifen-treated patients was reproduced in a follow-up study of the BC-blood study with twice as many patients and longer follow-up time. **Paper II**: The result regarding the prognostic significance of alcohol consumption has been replicated by some other studies, but not all. **Paper III**: The results regarding *CYP1A2* and *AhR* have not been replicated yet, and this study was the first to find this association. **Paper IV**: The main result contrasts with the majority of published papers. However, most of these studies have been small with no adjustments for other factors. Moreover, the interactions with lifestyle factors have not been described previously.
Specificity	The more specific an association between a factor and outcome is, the higher the likelihood for causation i.e. the association is limited to specific people and specific types and sites of disease. However, this characteristic should not be overemphasized according to Bradford Hill.	The factors investigated in this thesis are all involved in some way in the pathway of estrogen conversion and metabolism and may thus be specifically involved in estrogen-dependent cancers. However, the factors investigated in this thesis have also been associated with other diseases and other types of cancer and are not entirely specific for breast cancer. The papers in this thesis are based on primary breast cancer patients with the primary endpoint of risk of any breast cancer event. This is relatively specific compared to overall survival, which includes a wider spectrum of types and sites of disease.
Temporality	The cause must proceed the effect, which may be particularly important for diseases of slow development, thus the effect must occur after the delay.	The markers of interest for all papers were selected preoperative host characteristics for **papers I–III** and tumor characteristics from the primary tumor for **paper IV**. An exception was postoperative alcohol consumption for **paper II**. However, the postoperative consumption proceeded the effect (recurrence).
Biological gradient	The probability of causality is higher if the association reveals a biological gradient, a dose-response effect between the factor and the outcome.	**Paper I**: There was no dose-response effect with increasing coffee consumption. However, there seemed to be a threshold effect, and the survival curves for moderate and high coffee consumption were completely overlapping. **Paper II**: A tendency for a dose-response effect was observed with increasing preoperative alcohol consumption, but the middle groups were overlapping. Furthermore, the groups of high and no alcohol consumption were small. However, there seemed to be a threshold effect of any alcohol consumption. **Paper III:** There were too few patients with a C/C genotype for a gradient to be observed. **Paper IV:** A dose-response effect was observed for increasing COX-2 staining intensity.
Plausibility	If the effect is biologically plausible, it would increase the likelihood of causality.	**Paper I**: The observed association could be biologically plausible with current knowledge about antitumor effects of coffee consumption. **Paper II**: The observed effect of alcohol consumption on prognosis may be plausible, and

	However, this depends on current biological knowledge.	one mechanism could be the production of reactive oxygen species during the metabolism of alcohol. However, more research is needed, and the exact mechanisms for the effect of alcohol on tumor prognosis are not known. **Paper III**: The observed prognostic effect of *CYP1A2* in combination with *AhR* or *CYP19A1* could be plausible since the expression of these genes are involved in the metabolism of estrogens and AIs, but further evaluation is needed. **Paper IV**: The exact mechanisms are not known, and current knowledge cannot entirely explain the interaction between COX-2 and lifestyle factors or tumor size.
Coherence	The cause-and-effect association should not seriously conflict with experimental findings, and coherence between epidemiological and laboratory results increase the likelihood of causality. However, lack of experimental evidence cannot nullify the epidemiological observation in humans.	**Paper I**: Experimental findings support the associations, and caffeine is involved in many antitumor processes and has been shown to reduce the expression of ER. **Paper II**: Most experimental findings suggest a role of ethanol in carcinogenesis. However, experimental studies have shown a reduced risk of distant metastases in mice. Further studies are needed to understand the role of alcohol in the prognostic setting. **Paper III**: No experimental findings are yet available for *CYP1A2* genotypes in AI-treated breast tumors. **Paper IV**: There are conflicting experimental findings, but most of them support an aggressive phenotype of COX-2 positive breast tumors, in contrast to our findings. However, the influence of adjuvant treatments or patient characteristics have not yet been investigated experimentally.
Experiment	If the frequency of the associated events is affected when the causal agent is removed, the likelihood of causality is greatly increased.	**Papers I and II**: Randomized trials after diagnosis have not been carried out, but could possibly be performed. **Paper III**: It is not currently possible to remove the agent since it is a germline SNP. However, for papers I–III, randomization or removal of the agent is possible to do *in vitro* and possibly *in vivo*. **Paper IV**: Randomized trials with selective COX-2 inhibitors are ongoing, but the results have been inconsistent.
Analogy	If the effect is similar to already known factors, weaker evidence could be accepted for causality.	Other dietary factors, genetic factors, and tumor-specific expression of inflammatory markers have all been investigated in relation to breast cancer before and may influence breast cancer cells and hormone receptors. Additionally, gene-environment interactions have been reported previously, indicating a similar effect to the findings of the present study.

External Validity

Skåne University Hospital in Lund serves a population of almost 300,000, and patients are not referred to other hospitals for surgery. Therefore, this cohort is considered population based. The inclusion criterion was primary breast cancer for which the patients received surgery at Skåne University Hospital in Lund, and the exclusion criteria were patients who did not receive breast cancer surgery and patients with a history of other cancers within the last 10 years.

The latest comparison between patients included and all those who received breast cancer surgery at Skåne University Hospital in Lund was performed for paper IV, which showed similar characteristics for included and non-included patients. At the time this cohort was compiled, 2,170 women received surgery for breast cancer and 51.4% of

these patients were included in the study. The frequencies of ER and PgR expression were similar to the frequencies observed in paper IV. Furthermore, the median age of 61 years was the same for the included patients and all patients who received surgery at the hospital during this time period. An earlier comparison performed for patients included between October 2002 and October 2008 showed a somewhat higher inclusion rate (58%) and a somewhat lower frequency of ER and PgR, with differences of approximately 1 and 2 percentage points, respectively (Lundin *et al*, 2011; Persson *et al*, 2016). The earlier comparison also showed one-year lower median age at diagnosis. However, the expression of ER and PgR and the median age were comparable for patients included within that timeframe. In the earlier comparison, a lack of available research nurses was estimated to account for the majority of the non-inclusion in the study, and only approximately 5% of the patients were missed due to unverified diagnosis at the time of surgery. Moreover, the follow-up rates of the study were high (Simonsson *et al*, 2013).

In conclusion, this cohort resembles primary breast cancer patients diagnosed at Skåne University Hospital in Lund. Therefore, we believe that the results are generalizable to the underlying population of breast cancer patients. Due to the global heterogeneity of breast cancer characteristics and treatment (Unger-Saldaña, 2014), the extent to which the results may be generalized to breast cancer patients worldwide remains to be elucidated.

Ethical Considerations

The study was approved by the ethics committee at Lund University (Diarienummer (Dnr)75-02, Dnr37-08, Dnr658-09, Dnr58-12, Dnr379-12, Dnr227-13, Dnr277-15, and Dnr458-15). A written informed consent was obtained from all participants prior to study entry. There were no severe risks identified for the patients who participated in the cohort because it was not a clinical trial but an observational study. The only invasive procedure was blood samples, which were collected preoperatively for all patients and at the postoperative visits for patients who were alive and event free at the follow-up visit. The participants were not offered any compensation for study participation. However, at the postoperative visits, the patients met with a research nurse and were thus offered more time and attention for healthcare than non-included patients.

Results and Discussion

Paper I

Results

Coffee consumption was associated with altered hormone receptor status

Higher coffee consumption was significantly associated with increasing frequency of ER-negative tumors. In addition, the odds ratio for discordant receptor status i.e. ER-positive, PgR-negative tumors, was less than half for patients with moderate to high coffee consumption (≥2 cups/day) compared to patients with low coffee consumption (0–1 cups/day).

Coffee consumption was associated with a lower risk of early breast cancer events

Coffee consumption was neither associated with risk of early breast cancer events among all patients nor among those who had received radiotherapy or AIs. Among patients who had received chemotherapy, a borderline significant decreased risk of early breast cancer events was observed with moderate to high coffee consumption compared to low coffee consumption.

Coffee consumption predicted risk for early breast cancer events among tamoxifen-treated patients

Among the tamoxifen-treated patients with ER-positive tumors, moderate to high coffee consumption was significantly associated with more than half the risk of early breast cancer events compared to low coffee consumption.

CYP1A2 and CYP2C8 genotypes in combination with coffee consumption predicted risk for early breast cancer events among tamoxifen-treated patients

CYP1A2 rs762551 was not associated with risk of early breast cancer events among the tamoxifen-treated patients. However, when combining the genotype of *CYP1A2* rs762551 and coffee consumption, patients with any C-allele and low coffee consumption had over 3-fold higher risk for early breast cancer events compared to the other tamoxifen-treated patients. Furthermore, increasing number of the *CYP2C8*3* allele was significantly associated with a 2-fold increased risk for early events among

tamoxifen-treated patients. When combining the genotype of *CYP2C8*3* and coffee consumption, patients with any *CYP2C8*3* allele and low coffee consumption had six times the risk for early events compared to the other tamoxifen-treated patients.

Discussion

This study indicated that coffee consumption may modulate the hormone receptor status of breast tumors and decrease the risk of early breast cancer events among tamoxifen-treated patients with ER-positive tumors. The median follow-up time of nearly five years is relatively short, and the long-term effects of coffee consumption could not be evaluated. This is particularly important for ER-positive tumors, which tend to relapse late (Osborne *et al*, 1980). Until recently, however, endocrine treatment was administered during the first five years, and a potential drug-lifestyle and drug-gene interaction would thus be most likely to occur during this time period. In a recent follow-up study within the BC-blood study with twice as many patients and two years longer follow-up time than the current cohort, moderate to high coffee consumption was still significantly associated with half the risk for breast cancer events. Furthermore, *in vitro* studies showed a downregulation of ER, which is in line with the findings of a lower frequency of ER-positive tumors among patients with moderate to high coffee consumption in the present study (Rosendahl *et al*, 2015).

As described thoroughly in the chapter on host prognostic factors, many of the anti-inflammatory and anti-tumor effects of coffee are attributed to caffeine. The questionnaire for the BC-blood study did not include any question about whether the coffee was caffeinated or decaffeinated. However, less than 1% of the coffee consumed in Sweden is decaffeinated (The European Coffee Federation, 2012), so decaffeinated coffee should not confer a significant bias. Additional weaknesses are that the questionnaire did not include questions regarding the size of the coffee cups and if milk or sugar were added. Furthermore, coffee consumption and other possible confounders were self-reported and could confer a self-presentational bias. However, the median coffee consumption in this study was similar to the median coffee consumption in Sweden (The European Coffee Federation, 2012), and the reported coffee consumption was stable during follow-up, indicating that the preoperative coffee consumption is a reliable variable.

The *CYP1A2* rs762551 is a noncoding SNP located in intron 1 of the *CYP1A2* gene. This SNP enhances the *CYP1A2* inducibility and the A/A genotype is highly inducible, especially by coffee consumption (Djordjevic *et al*, 2010) and smoking (Sachse *et al*, 1999). Moreover, the *CYP1A2* rs762551 C-allele has been associated with an increase in blood pressure and increased habitual coffee intake (Sim *et al*, 2013). *CYP2C8*3* may influence the CYP2C8 enzyme metabolic activity. Since CYP1A2 and CYP2C8/9 are part of the metabolism of caffeine and tamoxifen (Butler *et al*, 1989; Cronin-Fenton

et al, 2014; Desta *et al*, 2004; Jernström *et al*, 2009; Kot & Daniel, 2008), our hypothesis is that caffeinated coffee results in an increased activation of tamoxifen via these enzymes and thus confers a lower risk of breast cancer events. An additional hypothesis is that low coffee consumption would lead to higher levels of phosphorylated Akt (Hashimoto *et al*, 2004), which in turn could induce ER-ligand independent activity (Cui *et al*, 2003) and thereby lead to a higher frequency of early events. If the results of the present study are validated, integrating the genotype and coffee consumption in the clinical setting may thus lead to better therapeutic choices and evidence-based lifestyle recommendations during treatment.

Paper II

Results

Preoperative alcohol consumption was not associated with a worse prognosis but weakly associated with a better prognosis

Higher preoperative alcohol consumption was not associated with risk of early breast cancer events. Any alcohol consumption was borderline associated with lower risk for early events, distant metastases, and all-cause mortality compared to no alcohol consumption. Stratified analyses according to adjuvant treatment did not modify the results.

A competing risk analysis yielded similar results

Competing risk analyses were performed using the Fine and Gray model with breast cancer events as the event of interest and death due to any cause as the competing risk. The analyses showed similar results to the adjusted Cox regression analyses for preoperative alcohol consumption.

Axillary lymph node involvement modified the association between alcohol consumption and the risk for early breast cancer events

There was a significant interaction between axillary lymph node status and preoperative alcohol consumption on the risk for early events. Any preoperative alcohol consumption was significantly associated with lower risk for early events among patients with any axillary lymph node involvement but not among patients without lymph node involvement.

Current alcohol consumption was associated with lower risk for early events, primarily among lymph node positive patients

The conditional hazards model indicated that any preoperative alcohol consumption was weakly associated with risk for early breast cancer events, and this association leveled off with time. However, the point estimate of the HR was below 1.0 for up to three years postoperatively. For axillary lymph node negative patients, there was no association between alcohol consumption and risk for early events at any time point during follow-up. For axillary lymph node positive patients, a moderate association between any alcohol consumption was observed, and the point estimate of the HR was below 1.0 for up to four years postoperatively.

Discussion

This study indicates that preoperative as well as postoperative alcohol consumption may be associated with a lower risk of breast cancer events. Most of the studies before and after this paper was published have shown lower mortality or no association between alcohol consumption and mortality, although few studies have investigated the association between alcohol consumption and breast cancer recurrence (Ali *et al*, 2014). A recent study on alcohol use and breast cancer survival within the Women's Health Initiative concluded that alcohol consumption does not seem to have a substantial impact of the mortality in breast cancer patients (Lowry *et al*, 2016), which is partly in line with the weak overall findings of the present study. However, another recent study reported an increased risk of late events in patients with ER-positive tumors (Nechuta *et al*, 2016), indicating a need for further research regarding alcohol consumption and long-term survival.

One of the mechanisms behind the findings in this study may be that while alcohol consumption confers a higher risk of breast cancer, it gives rise to less aggressive tumors with higher frequency of ER-positive tumors, possibly due to alcohol's effect on estrogen metabolism and levels. Alcohol consumption was associated with a higher frequency of ER-positive tumors in the present study, but there was no effect modification by ER status in the survival analyses. Alcohol consumption may also lead to a higher plasma ratio of 2-OHE to 16α-OHE1 and thus a more favorable estrogenic profile (Klug *et al*, 2006). In line with the results of a lower risk of distant metastases among patients with any alcohol consumption, a mouse model showed that alcohol consumption in high and moderate doses protected against distant metastases (Vorderstrasse *et al*, 2012). However, the mechanism behind the interaction of axillary lymph node status on the association between alcohol consumption and risk for early events warrants further investigation. A potential explanation is that patients with axillary lymph node involvement tend to relapse earlier than lymph node negative patients. Such later events in the lymph node negative group may have been missed due

to the relatively short median follow-up time of three years. An alternative hypothesis is that the cancer cells that had already metastasized to the axillary lymph node at the time of diagnosis differed with respect to alcohol response from those tumor cells that had not metastasized.

The questions regarding alcohol consumption were based on AUDIT, but not all eight questions from AUDIT were included. A limitation of the current study was a lack of information regarding the type of alcoholic beverage and size of glass. However, the median reported alcohol consumption was similar to the consumption reported in another population-based Swedish cohort of 10,000 women (Cederfjäll *et al*, 2004), indicating that the alcohol variable is likely to be reliable. Moreover, the questionnaire did not include questions regarding history of alcohol consumption or co-morbidities. The patients classified as abstainers in this cohort may thus be true teetotalers, but some may also be former alcohol abusers or have other comorbidities that may have influenced both alcohol consumption and morbidity and mortality (Green & Polen, 2001). In addition, low alcohol consumption has previously been reported to be a marker of non-adherence to endocrine treatment in the present cohort (Markkula *et al*, 2012b), although it does not explain the increased risk for early events among patients with axillary lymph node involvement. Another potential confounder of the association is a socioeconomic status, which was not registered in the present study. However, alcohol consumption and co-morbidities may account for the survival differences between patients with low and high socioeconomic status. This indicates that alcohol may be a confounder in the association between socioeconomic status and survival, and not the other way around (Aarts *et al*, 2013). Nevertheless, high alcohol consumption increases the risk of developing several different types of diseases, such as head and neck cancer and liver cancer (Eriksson, 2015; Grewal & Viswanathen, 2012), and caution is advised.

In conclusion, more experimental and clinical research is warranted to investigate the mechanisms behind the effect of alcohol consumption on breast cancer with regard to metastasis and prognosis. The postoperative alcohol consumption is modifiable after a cancer diagnosis, and consequently, whether alcohol consumption interferes with the treatment or affects the prognosis is important for patients and physicians. The results should be interpreted with caution due to the relative short follow-up. However, although validation is needed, this study does not support recommending that all patients abstain from low to moderate alcohol consumption after a breast cancer diagnosis.

Paper III

Results

CYP1A2 genotype predicted risk for breast cancer events among AI-treated patients

Of the 1,931 SNPs in the analysis of the DMET™ chip, only one met the pre-specified significance level of $P<0.005$. The *CYP1A2* rs762551 C-allele was significantly associated with increased risk of breast cancer events in the 24 AI-treated patients of the DMET™ chip analysis and in the extended cohort of 201 AI-treated patients. The main prognostic impact of the SNP was found during the first five years.

AhR predicted risk for breast cancer events among AI-treated patients

The *AhR* Arg554Lys SNP was not significant in the analysis of the DMET™ chip. However, in the extended cohort, any A-allele of *AhR* Arg554Lys was significantly associated with higher risk for breast cancer events and with early events within five years.

Combined genotypes of CYP1A2 and AhR predicted risk for breast cancer events among AI-treated patients

Although there was no significant interaction between the SNPs in *CYP1A2* and *AhR*, a combined genotype yielded a multiplicative association. Patients with at least one minor allele in both genes (any *CYP1A2* C-allele and any *AhR* A-allele) had an over 8-fold increased risk for events compared to patients who were homozygous for the major alleles in both genes (*CYP1A2* A/A and *AhR* G/G). Patients with at least one minor allele in one but not both genes had an almost 3-fold risk of events.

Combined genotype of CYP1A2 and CYP19A1 predicted risk for breast cancer events among AI-treated patients

No *CYP19A1* SNP was significantly associated with risk for breast cancer events neither in the DMET™ chip analysis nor the extended analysis. However, there was a significant interaction between *CYP1A2* rs762551 and *CYP19A1* rs4646. Patients with any C-allele of *CYP1A2* rs762551 and C/C genotype of *CYP19A1* rs4646 had a 3-fold increased risk for breast cancer events compared to the rest of the AI-treated patients. The main prognostic impact was found during the first five years.

Discussion

In this study, a novel genetic prognostic marker for AI-treated patients was identified. The results indicate that patients with any C-allele of *CYP1A2* rs762551 have a

significantly worse prognosis, especially when they also have the A-allele of *AhR* or C/C genotype of *CYP19A1*. Although the *CYP1A2* rs762551 SNP has been associated with inducibility, this SNP does not seem to significantly alter the gene expression (Ingelman-Sundberg *et al*, 2007). However, the SNP may be in *cis* or *trans* with other SNPs and instead have a regulatory function. The expression of *CYP1A2* is regulated by AhR, and the functional *AhR* Arg554Lys SNP has been associated with altered expression of AhR, where the G-allele (coding to arginine) confers higher expression compared to the A-allele (coding to lysine). There is also cross talk between AhR and ER, and a rat model showed that ligand-activated AhR had antiestrogenic properties, partly due to a decrease in ER expression in ductal epithelial cells (Helle *et al*, 2016). Thus, patients with G/G genotype of *AhR* would have lower ER levels and more effective *CYP1A2* transcription and expression.

Increased expression of *CYP1A2* would lead to increased 2-hydroxylation of estrogens and thus to a less estrogenic profile of the remaining estrogen levels during AI treatment. The C-allele of the *CYP1A2* rs762551 SNP has previously been associated with a lower plasma ratio of 2OHE to 16αOHE1 both pre- and postoperatively (Klug *et al*, 2006). Additionally, an AhR antagonist was shown to rescue *BRCA1* and ER in an ER-negative human breast cancer cell line. Conversely, the AhR antagonist also antagonizes the estrogen-dependent expression of BRCA-1 without effects on ER in an ER-positive human breast cancer cell line, which indicates different function of AhR in ER-negative versus ER-positive tumors (Romagnolo *et al*, 2015).

In line with the findings of the present study, a recent review of the role of *CYP19A1* polymorphisms in response to AIs showed great discrepancies between studies and suggested that the role of *CYP19A1* polymorphisms in response to AIs is questionable (Blackburn *et al*, 2015). However, a meta-analysis concluded that rs4646 may be a predictive factor for AI response, although further research is warranted (Artigalas *et al*, 2015). One hypothesis behind the discrepancies between studies could be that there is an interaction between *CYP1A2* rs762551 and *CYP19A1* rs4646, as described in the present study. However, the subgroups were small, and the results should be interpreted with caution. Furthermore, the 24 AI-treated patients genotyped with the DMET™ chip was part of the extended cohort of 201 AI-treated patients. Therefore, validation studies are needed to confirm these results.

This study was one of the first to use a DMET™ chip, and the results need validation, preferably within a randomized controlled trial of AI versus placebo or tamoxifen, such as the BIG 1-98 or ATAC trial. Thereafter, a randomized controlled trial based on the genotypes could further strengthen the results. If the findings were validated, the genotypes could be determined as predictive or prognostic factors and could guide physicians in the choice of adjuvant endocrine treatment and thus more personalized medicine.

Paper IV

Results

Tumor-specific COX-2 expression was associated with a higher age at diagnosis and less aggressive tumor characteristics

Higher intensity of tumor-specific COX-2 expression was significantly associated with increasing age at diagnosis, lower histological grade, lower Ki-67, lower frequency of both HER-2 positive tumors and triple negative tumors, and higher frequency of ER-positive and PgR-positive tumors. COX-2 negativity was associated with lower frequency of smaller tumors.

Tumor-specific COX-2 expression predicted risk for early breast cancer events within the first five years of follow-up

Higher COX-2 expression was independently associated with lower risk for early breast cancer events during the first five years of follow-up but not significantly thereafter in the multivariable model. Furthermore, COX-2 expression was not associated with prognosis in stratified analyses according to type of adjuvant treatment.

The association between tumor-specific COX-2 expression and prognosis was modified by OC use, NSAID use, and invasive tumor size

There were significant interactions between COX-2 expression and OC use, NSAID use, and invasive tumor size on the risk for breast cancer events. Higher COX-2 expression was associated with lower risk for breast cancer events among patients who had used OCs, NSAID users, and patients with large invasive tumor size.

Discussion

In this study, COX-2 expression was associated with less aggressive tumor characteristics and weakly associated with lower risk for early breast cancer events. In addition, OC use, NSAID use, and tumor size modified the association between COX-2 expression and risk for breast cancer events. These effect modifications have not been reported previously.

The results of COX-2 in relation to prognosis contrast with many previous studies (Holmes *et al*, 2011; Ristimäki *et al*, 2002; van Nes *et al*, 2011), but not all of them(Ahn *et al*, 2015; Gunnarsson *et al*, 2006; Kelly *et al*, 2003). However, most of the previous studies included 200 patients or fewer and did not perform multivariable analyses. When multivariable analyses were performed, they were not significant in most studies. Furthermore, the present study is one of the largest studies evaluating COX-2

expression in relation to breast cancer prognosis, and adjustments were made for tumor characteristics and age, which are known prognostic factors and thus potential confounders. The association between tumor-specific COX-2 expression in the present study was only attributable to the first five years of follow-up, when all patients were analyzed. Moreover, the association between COX-2 expression and risk for early breast cancer events was weak, and the results should be interpreted with caution. However, the interactions strengthened the association and yielded higher effect estimates.

The subgroup analysis of patients with any NSAID use was small, although larger than many published studies performed on COX-2 expression in relation to risk for breast cancer events (Kelly *et al*, 2003; O'Connor *et al*, 2004; Schmitz *et al*, 2006; Surowiak *et al*, 2005; Zhang *et al*, 2008). The immunohistochemical staining, analysis, and cut-off values for COX-2 expression have not been standardized and may account for differences between studies. In the present study, patients with COX-2 negative tumors had the worst prognosis. So as not to violate the assumption of proportional hazards for Cox regression, patients with weak and moderate COX-2 staining intensities were combined in the survival analyses. Other studies that combined tumors with low or moderate COX-2 staining with COX-2 negative tumors may have missed this group of patients.

Moreover, the association between tumor-specific COX-2 expression and tumor characteristics is unclear, with some studies showing associations with favorable characteristics (Dhakal *et al*, 2012; Nakopoulou *et al*, 2005) and others showing associations with more aggressive characteristics (Haffty *et al*, 2008; Ristimäki *et al*, 2002). There are several ongoing clinical trials with COX-2 inhibitors as adjuvant treatments for breast cancer. The inconsistent results between studies regarding the prognostic impact of COX-2 expression in breast cancer may be due to several factors. If the findings of the present study were validated, history of OC use, tumor size, and COX-2 expression might need to be taken into account when designing or evaluating outcomes in randomized controlled trials with NSAIDs for breast cancer patients.

Conclusions

Paper I

Moderate to high coffee consumption was associated with a lower frequency of tumors with discordant hormone receptor status. Furthermore, higher coffee consumption was associated with a decreasing frequency of ER-positive tumors. Among tamoxifen-treated patients with ER-positive tumors, moderate to high coffee consumption was significantly associated with less than half the risk for early breast cancer events compared to low coffee consumption. The poorest prognosis was found in patients with any *CYP1A2* rs762551 C-allele or *CYP2C8*3* with low coffee consumption. If validated, moderate to high coffee consumption might improve the prognosis of patients with these genotypes.

Paper II

Preoperative and postoperative alcohol consumption was weakly associated with lower risk for early breast cancer events, and the association was modified by axillary lymph node involvement. Alcohol consumption was moderately associated with lower risk for early breast cancer events among patients with axillary lymph node involvement, but not in those without axillary lymph node involvement. This study does not support that all breast cancer patients should abstain from low to moderate alcohol consumption, although the follow-up time was relatively short, and the long-term impact of alcohol consumption could not be investigated in this study.

Paper III

The *CYP1A2* rs762551 SNP was identified as a novel potential predictive marker for early AI treatment response in breast cancer patients in both the 24 patients analyzed with the DMET™ chip and in the extended cohort of 201 AI-treated patients. In addition, a combination of genotypes of *CYP1A2* rs762551 with *CYP19A1* rs4646 or

AhR Arg554Lys further improved the prediction of early AI treatment response. If confirmed in an independent cohort or within a randomized clinical trial, genotyping breast cancer patients might guide the choice of adjuvant breast cancer treatment and thus provide a path towards more personalized medicine.

Paper IV

Higher tumor-specific COX-2 expression was significantly associated with older age at diagnosis, less aggressive tumor characteristics, and lower risk for early breast cancer events within five years of inclusion. The prognostic impact of COX-2 expression did not differ by ER-status, BMI, or adjuvant treatment. However, the prognostic impact of COX-2 was modified by a history of OC use, preoperative NSAID use, and tumor size. If the findings were validated in an independent prospective cohort or within a randomized trial, then tumor size and potentially a history of OC use might need to be considered when designing or evaluating clinical outcomes in randomized controlled trials of adjuvant NSAID or COX-2 selective inhibitors for breast cancer patients.

Future Perspectives

Analyses of modifiable host factors such as coffee consumption in relation to risk or prognosis is often based on self-reported estimates, which are hard to validate. Instead of such self-reported estimates, or as a complement to them, a biological measurement may be used to validate the estimates and to study the association of interest. For example, for coffee consumption, recent results from the European Prospective Investigation into Cancer and Nutrition (EPIC) study showed a correlation between urinary excretion of polyphenols and coffee intake, suggesting a potential biomarker for coffee consumption (Zamora-Ros *et al*, 2016). Objective biological measurement of lifestyle factors instead of subjective self-reported values may provide more objective results and validate the findings of previous studies in the future.

The results from all papers included in this thesis need validation. As the next step, it would be interesting to analyze the germline SNPs in *CYP1A2*, *CYP2C8*, and *AhR*, coffee and alcohol consumption, and tumor-specific COX-2 expression within a randomized controlled trial. If such analyses were performed, the prognostic or predictive significance of the factors could be strengthened and potentially lead to future implementation. Furthermore, experimental studies where the integration of tumor factors and lifestyle factors could be analyzed would be interesting. Such studies might contribute to the mechanistic background behind the present findings and to further strengthen or reduce the likelihood of causality of the results. In the future, a more translational approach from mechanistic experiments to observational findings may be valuable when studying the association between lifestyle and breast cancer prognosis.

In recent years, genetic tests for breast tumors have been developed and may contribute to improved selection of treatment for the individual patient. However, the tumor is influenced by many factors, including the tumor genetic constitution, host genotype, and other host factors such as lifestyle, which may affect the metabolism of drugs and xenobiotics. An example of the potential usefulness of integrating tumor and host factors is the findings in paper I regarding coffee consumption and *CYP1A2* genotype in relation to tamoxifen treatment response, as well as in paper III regarding *CYP1A2* genotype in relation to AI-treatment response. Patients with any C-allele of *CYP1A2* rs762551 do not seem to respond as well to either tamoxifen or AIs. However, any C-allele carriers who receive tamoxifen may improve their prognosis with a moderate to high coffee consumption, while for AI-treated patients who are carriers of any C-allele,

coffee consumption has not been shown to improve the prognosis. The next step would be to validate these findings and to incorporate other ADME-related genes. Subsequently, a pharmacogenetic test could be developed, which could help guide the choice of adjuvant endocrine treatment. Furthermore, evidence-based lifestyle recommendations are an interesting field. With more research, guidelines for such recommendations may be developed and eventually incorporated in the clinical praxis to avoid interactions or to enhance the treatment effect of adjuvant therapies.

In the future, a more comprehensive view with an integration of tumor and host factors might be beneficial when assessing breast cancer prognosis. This approach may lead to more personalized medicine in breast cancer.

Populärvetenskaplig Sammanfattning

Bröstcancer är den vanligaste cancersjukdomen hos kvinnor i världen och antalet kvinnor som insjuknar ökar årligen. Å andra sidan har överlevnaden i bröstcancer ökat under de senaste decennierna. Bröstcancer är i Sverige sedan några år inte längre den vanligaste dödsorsaken av cancer hos kvinnor utan den näst vanligaste efter lungcancer. Den förbättrade överlevnaden tros bero på screening med mammografi av en stor del av befolkningen, och därmed tidigare upptäckt av cancern, samt förbättrade behandlingsmetoder. Förbättrad diagnostik och behandling leder tyvärr även till att en del patienter överbehandlas och får biverkningar i onödan. Trots den höga överlevnaden är behandlingsresistens ett stort problem och en del patienter får återfall i bröstcancer. Det är därför av stor vikt att upptäcka nya markörer som kan identifiera de patienter som har risk för att få återfall och de som inte kräver så stor behandlingsinsats, för att kunna individualisera behandlingen av bröstcancerpatienter.

De flesta delar av arvsmassan är likadan hos alla människor, men vissa delar varierar, där några är så kallade genetiska normalvarianter. Risken för återfall i bröstcancer beror sannolikt på många orsaker där samverkan sker mellan genetiska faktorer, livsstilsfaktorer och tumöregenskaper. Målet med den här avhandlingen är att undersöka om man bättre kan förutsäga återfallsrisken och överlevnaden för bröstcancerpatienter med en kombination av information om tumör och patient.

En livsstilsfaktor är kaffe, vilken är en av de vanligaste dryckerna i världen. Kaffe innehåller hundratals bioaktiva ämnen och många av dessa har egenskaper som skulle kunna motverka cancertillväxt. I **delarbete I** undersökte vi om kaffekonsumtion hade ett samband med överlevnad i bröstcancer samt om sambandet skiljde sig mellan olika behandlingsgrupper. En konsumtion av två eller fler koppar kaffe per dag var kopplat till en bättre överlevnad hos patienter som behandlats med antiöstrogenläkemedlet tamoxifen. Då både kaffe och tamoxifen bryts ner av enzymerna CYP2C8 och CYP1A2, studerades även om normala genvarianter i generna *CYP2C8* och *CYP1A2* i sig själva eller i kombination med kaffekonsumtion var kopplat till återfallsrisk i bröstcancer hos patienter som behandlats med tamoxifen. Den vanligaste av de normala genvarianterna i *CYP2C8* var i sig kopplad till en lägre risk för återfall. Patienter som hade den normala genvarianten i *CYP2C8,* en normal genvariant i *CYP1A2* eller som drack minst två koppar kaffe per dag hade en bättre överlevnad än övriga patienter. Resultaten visar att ämnen i kaffe, genetiska normalvarianter och behandling samverkar. Om resultaten bekräftas i oberoende studier skulle kunskap om patientens

genetiska uppsättning och kaffekonsumtion vara värdefull för valet av behandling för den individuella patienten.

En annan livsstilsfaktor för bröstcancer är alkoholkonsumtion, där även lågt till måttligt intag av alkohol har visat sig ge en ökad bröstcancerrisk. Om alkoholkonsumtion påverkar risken för återfall i bröstcancer är däremot omstritt och resultaten från tidigare studier har varierat. I **delarbete II** undersökte vi därför om alkoholkonsumtion påverkade risken för återfall i bröstcancer. I denna studie hittades inget samband mellan alkoholkonsumtion och ökad risk för återfall, utan istället ett svagt samband mellan alkoholintag och bättre överlevnad för patienter som vid diagnos hade spridning till lymfkörtlar, men inte hos övriga patienter. Senare studier har också indikerat att måttligt alkoholintag inte verkar ge en sämre överlevnad i bröstcancer. Resultaten i den här studien gällande patienter med spridning till lymfkörtlar har inte tidigare undersökts och behöver därför bekräftas i en annan oberoende studie. Sammanfattningsvis ger vår studie inga indikationer på att rekommendera alla bröstcancerpatienter att avhålla sig helt från alkohol.

Enzymer som ingår i det så kallade CYP450 systemet är viktiga för nedbrytningen av både kroppsegna ämnen och läkemedel. Förändring i förmågan att bryta ner både kroppsegna östrogener och antihormonella läkemedel skulle kunna påverka behandlingssvaret av bröstcancerbehandlingen. Genetiska normalvarianter i gener som kodar för enzymer i CYP450 systemet skulle därför kunna vara viktiga i förhållande till överlevnad och behandlingssvar för bröstcancer. I **delarbete III** användes en metod som analyserade nästan 2000 genetiska normalvarianter hos 24 patienter vilka hade behandlats med aromatashämmare, som verkar genom att hämma den kroppsegna produktionen av östrogen. Hos dessa 24 patienter samt i en utvidgad analys med över 200 patienter sågs ett samband mellan en genetisk normalvariant i *CYP1A2* och bättre överlevnad. Kombination av den genetiska normalvarianten i *CYP1A2* med andra normalvarianter i *CYP19A1* och *AhR* generna kunde ytterligare förutsäga vilka patienter som svarar bättre på behandling med aromatashämmare. Detta var den första studien som identifierade dessa genetiska markörer för svar på behandling med aromatashämmare. Om resultaten bekräftas i en annan oberoende studie skulle information om patientens genetiska uppsättning kunna leda till en mer skräddarsydd behandling för bröstcancerpatienter.

Inflammation har nyligen blivit uppmärksammad som en del av hur cancer uppkommer. COX-2 är en markör för inflammation som ofta förekommer i brösttumörer och kan hämmas av antiinflammatoriska läkemedel. Studier pågår om behandling med dessa antiinflammatoriska läkemedel skulle kunna förbättra överlevnaden i bröstcancer. Om förekomst av COX-2 i bröstcancer i sig har ett samband med överlevnad eller återfallsrisk i bröstcancer är inte helt klarlagt. I **delarbete IV** undersöktes därför om COX-2 i tumören hade ett samband med överlevnad och risk för återfall i bröstcancer samt om andra tumörfaktorer eller livsstilsfaktorer

påverkade sambandet. Förekomst av COX-2 var i denna studie kopplat till en lägre risk för återfall, särskilt hos patienter som hade stora tumörer, hos de som någon gång hade använt p-piller och hos de som hade använt antiinflammatoriska läkemedel. Resultaten visar att inflammation och livsstilsfaktorer samspelar och kan vara av betydelse för kommande studier av antiinflammatoriska läkemedel som behandling av bröstcancer.

Sammanfattningsvis har den här avhandlingen undersökt om samband mellan genetiska faktorer, livsstilsfaktorer och tumörmarkörer kan öka möjligheterna till individualiserad behandling av bröstcancer. Kombinationen av arv och miljö är välkänt för uppkomsten av sjukdomar, men samspelet är komplicerat och verkar även vara av vikt för risken för återfall och överlevnaden i bröstcancer. En tumör i bröstkörteln är inte isolerad utan det finns ett samspel mellan patient och tumör, via genetiska faktorer, livsstilsfaktorer samt via behandlingen av bröstcancern. Att se patient och tumör som en helhet är ett steg på vägen för att uppnå en bättre, mer individualiserad bröstcancerbehandling.

"We have not succeeded in answering all our problems—indeed we sometimes feel we have not completely answered any of them. The answers we have found have only served to raise a whole set of new questions. In some ways we feel that we are as confused as ever, but we think we are confused on a higher level and about more important things. So this report does not purport to give final answers, or to claim that we now "know how to do it". We see more need for revision than ever. But we are doing better than we did. And this is a progress report, rendered with humility because of the unsolved problems we see now which we could not see before."

Earl C. Kelley, Professor of Secondary Education at Wayne University
1951 in "The Workshop Way of Learning".

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Maria Simonsson MD., was born in 1989 and grew up in Stenungsund, Bohuslän. In her spare time she enjoys reading, singing, and playing golf.

This thesis aims to elucidate whether combining host factors, including genetic constitution and lifestyle factors, with tumor characteristics could yield a more comprehensive understanding than either factor alone for the prognosis of breast cancer.

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