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STUDIES OF SIDE EFFECTS RELATED TO ADJUVANT BREAST CANCER REGIMENS WITH FOCUS ON CHEMOTHERAPY

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«Ἓν μόνον ἀγαθὸν εἶναι, τὴν ἐπιστήμην,
καὶ ἓν μόνον κακόν, τὴν ἀμαθίαν»

“There is only one good, knowledge, and one evil, ignorance”

Sokrates 470 – 399 BC

To the people that left a mark on my life

ABSTRACT

Radiotherapy, endocrine therapy, chemotherapy and targeted therapy adjunct to surgery have a critical role in the management of early breast cancer for outcome improvement. Anthracycline containing chemotherapy reduces 10-year incidence of breast cancer mortality by an absolute 6.5% compared to no adjuvant chemotherapy and addition of taxanes to anthracycline regimes leads to additional gain of 2.8% in 8-years breast cancer mortality. Dose dense chemotherapy improves breast cancer mortality by an estimated relative reduction of 40%. Trastuzumab, the first anti-HER2 agent among plenty, administered for one year, led to a paradigm shift in the management of HER2 positive breast cancer and significantly altered prognosis. Adjuvant trastuzumab improves disease free survival by a relative risk reduction of 40%. Despite beneficial effect of chemotherapy and anti-HER2 targeted therapy, acute or late toxicity can be dose limiting and even influence patients' quality of life during treatment and long time after it is completed. Given the good prognosis of breast cancer and the millions of breast cancer survivors, therapy related toxicity affects a considerable number of women. Efforts to escalate or de-escalate treatment are ongoing and potential trade-offs in safety are monitored, resulting at best to improved benefit-risk balance.

The aim of the current thesis was to examine heart failure outcomes and management after breast cancer diagnosis compared to a population of women with heart failure and toxicity outcomes related to tailored dose dense chemotherapy namely, cardiotoxicity after combination with trastuzumab, neutropenia related events and premature ovarian insufficiency.

The Swedish Registry for heart failure (SwedeHF) and the national health care registries were utilised for the purposes of **Paper I**. Patients enrolled in the SwedeHF registry between 2008 and 2013 were included and followed for a median period of two years. Patients with breast cancer history, identified through the National Cancer Registry, and age-matched controls (1:5) were investigated. Heart failure related baseline characteristics and outcomes did not differ amid presence of breast cancer history among women registered in the SwedeHF registry with incident heart failure. Differences in the history of myocardial infarction, administration of aspirin and device therapy were observed among women with prevalent heart failure, depending on previous breast cancer history. Breast cancer history did not alter heart failure outcomes but time from heart failure diagnosis did; women with prevalent heart failure had worse survival than those with incident heart failure.

Papers II-IV investigated different toxicity aspects related to the population enrolled and treated in the PANTHER phase III study comparing tailored (protocol predefined dose escalation or de-escalation) and dose dense (every two weeks) chemotherapy to standard dose three weekly chemotherapy. **Paper II**, investigated if tailored dose dense chemotherapy can further improve trastuzumab efficacy, compared to combination with standard chemotherapy and whether this combination would jeopardise cardiac safety; both parts of the study were predefined. The trastuzumab and tailored dose dense group demonstrated a 32% relative

improvement in risk for breast cancer relapse but the results did not reach formal statistical significance. Despite small reductions of left ventricular ejection fraction at four- and six-years follow-up, no clinically meaningful difference in the risk for cardiotoxicity was demonstrated between tailored dose dense chemotherapy and standard chemotherapy compared as administration per HER2 treatment or as per chemotherapy group.

Compliance to the planned chemotherapy schedule is related to better breast cancer outcomes, underscoring the value of prophylactic granulocyte-colony stimulating factor (G-CSF). Efficacy of G-CSF in preventing neutropenic events and delays in the delivery of the planned chemotherapy dose were examined in a secondary analysis in **Paper III**. Administration of G-CSF reduced the risk for neutropenic events defined as febrile neutropenia or infection with low absolute neutrophil count. Although a comparison between the two treatment groups was not possible, within group investigation in the standard chemotherapy group revealed improved adherence to planned schedule by fewer dose delays when G-CSF was administered.

Chemotherapy-induced amenorrhea is a therapy-related adverse event but it also improves breast cancer survival. Efficacy of dose dense chemotherapy has been assumed to derive from increased incidence of chemotherapy-induced amenorrhea. Thus, the exploratory, post hoc studies in **Paper IV** investigated the incidence of chemotherapy-induced amenorrhea and impact on treatment outcomes, excluding patients receiving gonadotropin releasing hormone agonists. Even though the delivered mean chemotherapy doses in the experimental treatment group were higher than in the standard chemotherapy group, amenorrhea incidence at two years of follow-up between the two treatment groups did not differ. Hence, benefit of dose dense chemotherapy is deemed to stem from chemotherapy effect per se and not aggravated gonadal toxicity. Breast cancer relapse events were too few to make any inference on the impact of amenorrhea on breast cancer relapse outcomes and longer follow-up is required. Menstruation status did not impact efficacy of allocated treatment although a non-significant benefit from tailored dose dense chemotherapy was demonstrated and persisted in most breast cancer subgroups. Sub-group analysis of the different breast cancer subtypes did not reveal any influence of the baseline menopause status on the efficacy of given chemotherapy schedule, except in triple negative breast cancer. There is no known biological plausibility to explain this interaction and the possibility of a chance finding cannot be excluded.

In summary, at median follow-up of two years, having previously been diagnosed with breast cancer did not alter heart failure outcomes, compared to controls, although some limited discrepancies in existing comorbidities and heart failure treatment were observed. Moreover, increasing dose intensity did not relate to excess cardiotoxicity when combined with trastuzumab, did not increase the risk for neutropenic events, provided G-CSF prophylaxis is administered, and did not affect risk for premature ovarian insufficiency. Conclusively, patients with early breast cancer should be offered efficient breast cancer therapy related to their risk level, with proper supportive therapy and assessment of potential comorbidities such as cardiovascular risk factors.

LIST OF SCIENTIFIC PAPERS

I. Hedayati E, **Papakonstantinou A**, Gernaat SAM, Altena R, Brand JS, Alfredsson J, Bhoo-Pathy N, Herrmann J, Linde C, Dahlstrom U, Bergh J, Hubbert L. *Outcome and Presentation of Heart Failure in Breast Cancer Patients; Findings from a Swedish register-based study.* Eur Heart J Qual Care Clin Outcomes 2019

II. **Papakonstantinou A**, Matikas A, Bengtsson N-O, Malmström P, Hedayati E, Steger G, Untch M, Hübbert L, Johansson H, Hellström M, Gnant M, Loibl S, Greil R, Moebus V, Foukakis T, Bergh J. *Efficacy and safety of adjuvant tailored dose dense chemotherapy and trastuzumab for resected HER2-positive breast cancer: Results from the phase 3 PANTHER trial.* Cancer 2019

III. **Papakonstantinou A**, Hedayati E, Hellström M, Johansson H, Gnant M, Steger G, Greil R, Untch M, Moebus V, Loibl S, Foukakis T, Bergh J, Matikas A. *Neutropenic complications in the PANTHER phase III study of adjuvant tailored dose-dense chemotherapy in early breast cancer.* Acta Oncol 2019

IV. Matikas A, **Papakonstantinou A**, Hellström M, Hemming J, Steger G, Greil R, Loibl S, Gnant M, Moebus V, Untch M, Foukakis T, Bergh J. *Incidence of amenorrhea and impact on breast cancer outcomes during tailored dose dense chemotherapy for high-risk early breast cancer.* (Manuscript)

LIST OF ADDITIONAL RELEVANT PAPERS

I. **Papakonstantinou A,** Foukakis T, Rodriquez-Wallberg K.A, Bergh J. *Is estradiol monitoring necessary in women receiving ovarian suppression for breast cancer?* J Clin Onc 2016

CONTENTS

LIST OF ABBREVIATIONS

ABCSG-12	Austrian Breast and Colorectal Cancer Study Group -12
ANC	Absolute Neutrophil Count
ATLAS	Adjuvant Tamoxifen: Longer against Shorter
ATM	Ataxia Telangiectasia Mutated gene
aTTom	Adjuvant Tamoxifen- To Offer More?
b.i.d.	Twice a day (bis in die)
BRCA	Breast Cancer gene
BCRFS	Breast Cancer Relapse-Free Survival
CHEK2	Checkpoint Kinase 2 gene
CHF	Congestive Heart Failure
CI	Confidence Interval
DFS	Disease-Free Survival
EBCTCG	Early Breast Cancer Trialists' Collaborative Group
EC	Epirubicin, Cyclophosphamide
ECG	Electrocardiogram
ECHO	Echocardiography
ECOG PS	Eastern Cooperative Oncology Group Performance Status score
EFS	Event-Free Survival
ER	Oestrogen Receptor
FEC	5-fluorouracil, Epirubicin, Cyclophosphamide
FN	Febrile Neutropenia
G-CSF	Granulocyte-Colony Stimulating Factor
GnRHa	Gonadotropin Releasing Hormone agonist
HER2	Human Epidermal Growth Factor Receptor -2
HR	Hazard Ratio
IHC	Immunohistochemistry
IQR	Interquartile Range
LVEF	Left Ventricular Ejection Fraction
MRI	Magnetic Resonance Imaging
MUGA	Multigated acquisition scan

NYHA	New York Heart Association
OS	Overall Survival
PALB2	Partner And Localiser of BRCA2
PR	Progesterone Receptor
PTEN	Phosphatase and TENsine homolog deleted in chromosome 10 gene
ROS	Reactive Oxygen Species
RR	Risk Ratio
SOFT	Suppression of Ovarian Function Trial
TEXT	Tamoxifen and Exemestane Trial
TNBC	Triple Negative Breast Cancer
TNM	Tumour, Nodes, Metastasis classification
TP53	Tumour Protein 53 gene
WHO	World Health Organisation

BACKGROUND

1.1 Epidemiology of breast cancer

Breast cancer is the fifth leading cause of death among women in high-income countries and the leading cancer site among women in Europe in 2018, according to the World Health Organization (WHO) reports (1, 2). Worldwide breast cancer incidence and 5-year prevalence in 2018 was estimated at about 2.1 and 6.9 million respectively; about one in four women diagnosed with cancer (1). Figures 1 and 2 present global cancer epidemiological data among women reported in 2018; incidence (according to diagnoses, not individuals) and 5-year prevalence, respectively (3). Notably, male breast cancer is rare and comprises only approximately 1% of all breast cancer diagnoses (4).

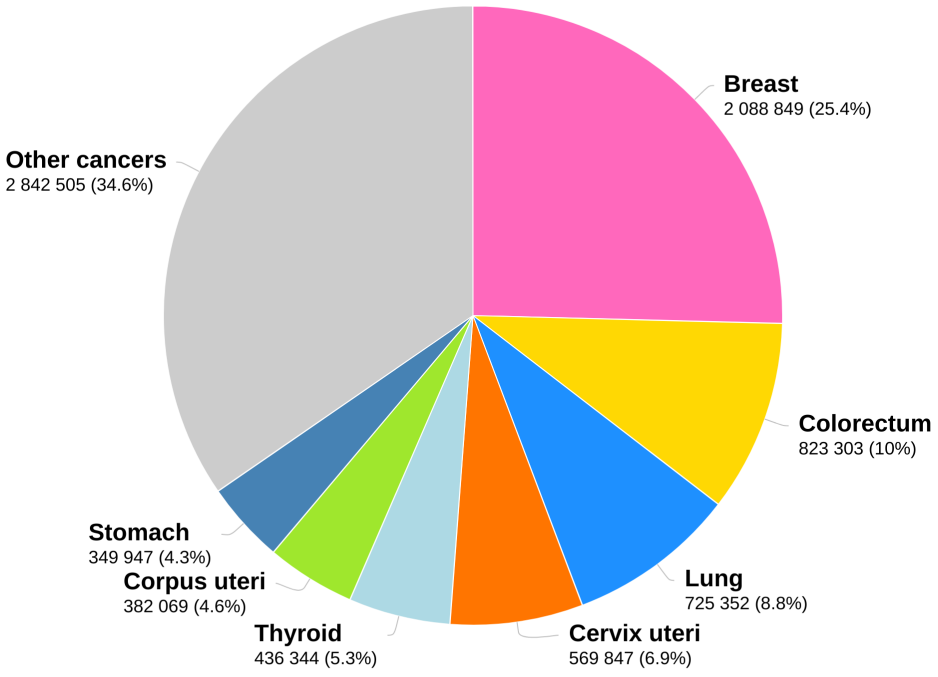

Figure 1. *Global estimated cancer incidence (total number of diagnoses) among women of all ages in 2018. (Globocan 2018, IARC. Non-melanoma skin cancer excluded).*

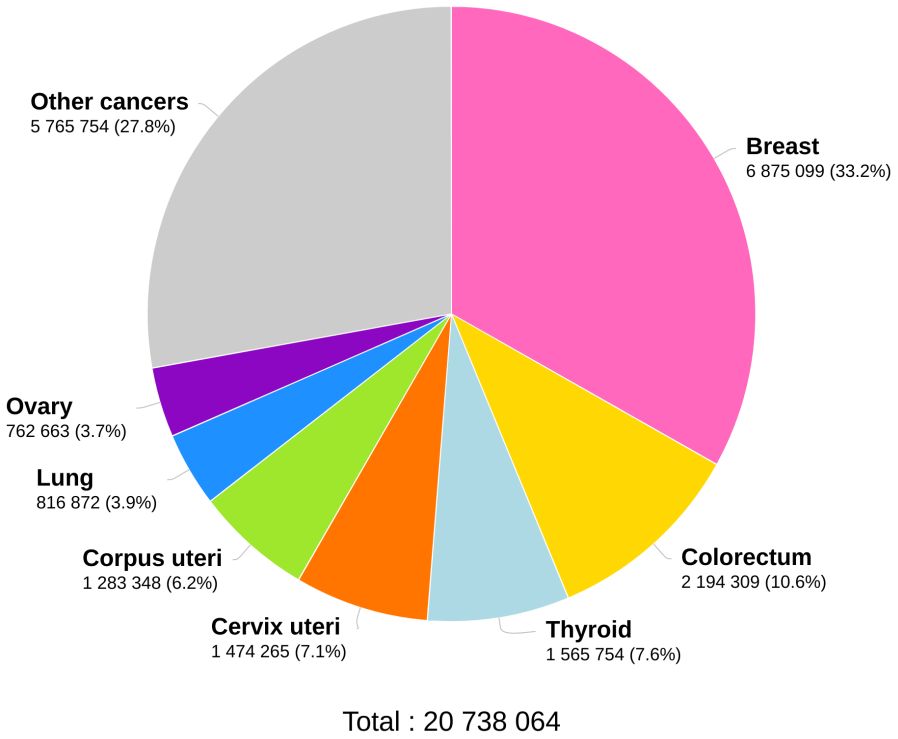

Figure 2. *Global estimated 5-year cancer prevalence among women of all ages in 2018. (Globocan 2018, IARC. Non-melanoma skin cancer excluded).*

Incidence of breast cancer has increased over time due to the introduction of mammography screening and ageing of the world's population (5, 6). Forouzanfar et al reported a three-fold increase of breast cancer incidence between 1980 and 2010, with a 3.1% annual rate of increase (7). At the same time, survival rates have been greatly improving, largely because of early detection due to screening, advances in the systemic therapy of breast cancer and improved comprehension of the disease biology and the importance of lifestyle factors (8). Breast cancer survival is continuously improving over time but in Europe there are still persisting differences between countries, with reported worse survival in eastern and parts of southern Europe (9, 10). Global differences between breast cancer incidence and mortality in Sweden and Europe are depicted in figure 3. In Sweden, 7 558 women were diagnosed with breast cancer in 2016 and age-standardized incidence in 2018 was estimated to 89.8 individuals per 100 000 inhabitants (11, 12). Mean age of diagnosis is 66 years and 86.1% of the affected women are alive and breast cancer free 10 years after diagnosis (12). In line with other western countries, breast cancer mortality rate in Sweden remains low despite increased incidence over time, as demonstrated in figure 4 (12).

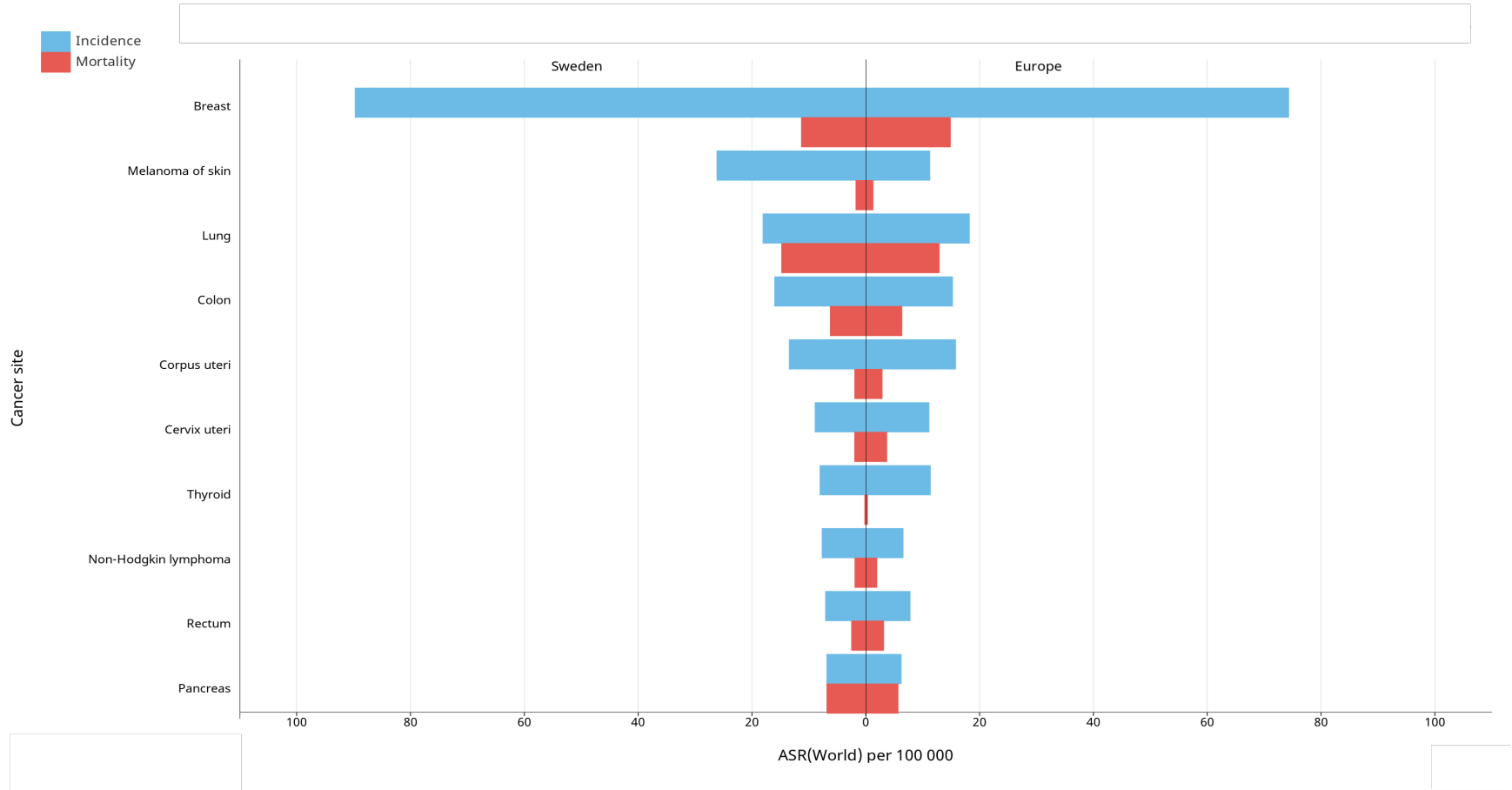

Figure 3. *Age-standardized cancer incidence and mortality among the five most common malignancies in women, Sweden versus Europe (Globocan 2018, IARC).*

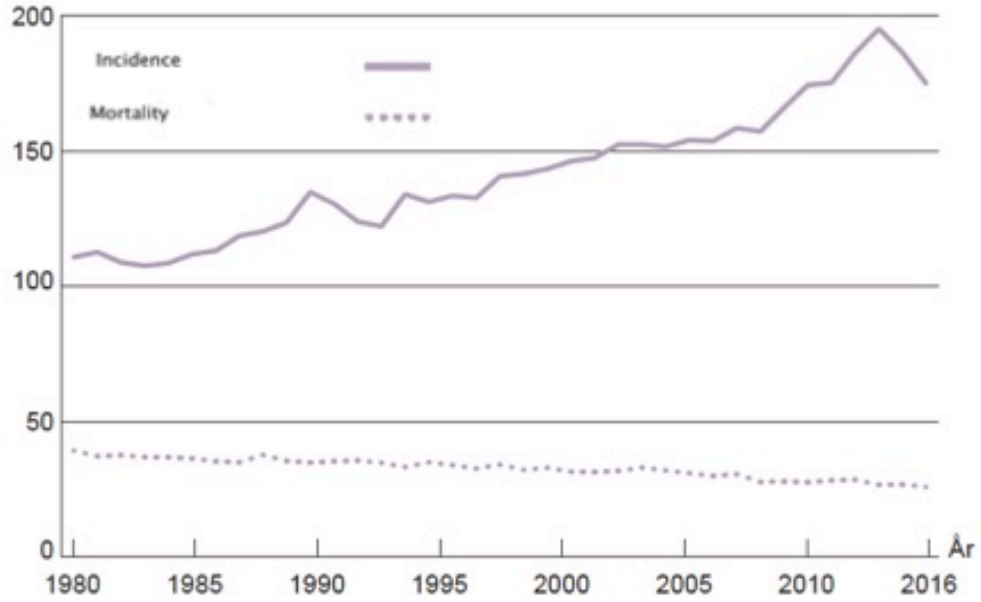

Figure 4. *Breast cancer incidence and mortality trend over time in Sweden. (Cancer i siffror 2018, Socialstyrelsen och Cancerfonden i samarbete).*

1.2 Risk factors and aetiology

Further understanding of breast cancer aetiology is of outmost importance for the development of intervention and prevention strategies. Exposure to endogenous and exogenous oestrogens, increased age, ionising radiation, high mammographic breast density, previous history of breast lesions and genetic predisposition increase the risk for developing breast cancer (13). Exposure to oestrogens refers to both intrinsic factors, such as early menarche and late menopause, and extrinsic factors such as low- or nulliparity, delayed first pregnancy, and menopausal replacement therapy (13-15). Despite some difference in the risk levels, all types of systemic menopausal replacement therapy have been associated to excess breast cancer incidence and increased breast cancer mortality, with a risk upsurge with longer duration of treatment (15, 16). Additionally, modifiable lifestyle factors such as postmenopausal obesity, lack of physical exercise, alcohol and tobacco use have also been identified to increase breast cancer risk (17).

About 5-10% of all female breast cancer is attributed to genetic predisposition (18). Women with triple negative breast cancer have a higher probability of having a hereditary breast cancer and are reported to be almost 6 times more likely to have a BRCA1 mutation compared to women with other subtypes of breast cancer (18, 19). Germline mutations in BRCA1 and BRCA2 account for about 30% of hereditary breast cancer but other less frequent gene mutations have also been described, such as TP53, PTEN, CHEK2, PALB2 and ATM (18, 20-23). Even in men, BRCA1 or BRCA2 germline mutations increase the lifetime risk for breast cancer up to 10% (24). The affected individuals usually present with a strong family history and are younger at diagnosis, mandating penetration of family history among both men and women diagnosed with breast cancer (18).

1.3 Breast cancer screening and diagnosis

Even though early detection is often cited to improve breast cancer outcomes, the impact of mammography screening on breast cancer mortality is debated (25). Comparisons of breast cancer mortality before and after mammography screening adaptation show little benefit on mortality solely on screening whereas a UK-based independent panel estimated that available published data imply a 20% survival improvement among women that are offered mammography screening between the age of 50 and 70 years. Controversial survival benefit is often juxtaposed to potential harms such as overdiagnosis and overtreatment, but the possibility of early diagnosis of asymptomatic women with low tumour burden that can potentially lead to de-escalation of cancer therapy should not be neglected (26, 27).

Many countries have adapted mammography screening, but participation, age intervals and frequency of mammograms vary widely. In Sweden, the National Board of Health and Welfare recommends population-based screening of women between 40 and 74 years old every 18-24 months, but regional variations exist. Internationally, there is currently a large interest towards development of risk-adapted screening aiming to allow for personalised screening and intervention methods (28). However, no meaningful benefit on survival has yet been reported and formal recommendations are lacking, except for women with increased breast cancer lifetime risk (above 20%), where magnetic resonance imaging (MRI) screening is recommended (29, 30).

Lege artis investigational inquiries of breast symptoms or a lump in the breast include combination of three modalities, so called triple diagnostics; physical examination of the breast and regional lymph nodes, imaging with mammography and ultrasound and fine needle aspiration or core biopsy (6). MRI of the breast can also be utilised in selected cases, for example pre-treatment staging in women with tumour difficult to define or in selected cases of lobular breast carcinoma (31).

1.4 Clinical and genomic assessment

Complete classification, staging and characterisation of the tumour is mandatory and constitutes the basis for treatment recommendations. The assessment includes both prognostic (distinguish patients more likely to have better or worse outcome, regardless of treatment) and predictive (predict benefit, or lack thereof, from specific intervention) biomarkers. Some of the available biomarkers are dual; for example amplification of the human epidermal factor receptor 2 (HER2) predisposes to worse breast cancer outcomes but simultaneously predicts benefit from treatment with anti-HER2 agents.

A robust pathology report should include (32):

a) *Histological type* according to the WHO classification system. The most common among the 19 different major subtypes of breast carcinomas, are invasive carcinoma of no special type (up to 75%) and lobular carcinomas (5-15%) (figure 5) (32).

b) *Histological grade* according to the Elston-Ellis grading system based on mitotic count, nuclear polymorphism and proportion of tubule formation (33). As described

in table 1, each parameter is scored from one to three and grading is thereafter classified according to the sum of the three variables to: grade I well-differentiated (sum equals 3-5 points), grade II moderately-differentiated (6-7 points) and grade III poorly differentiated (8-9) points (33, 34). Mitotic count is counted in hot spots and per 10 fields but discrepancies between different microscopes should be taken into consideration.

c) Existence of tumour emboli in small vessels surrounding the tumour; described as *lymphovascular invasion* (32).

d) Stage of the tumour according to the Union for International Cancer Control (UICC) *TNM* system describing tumour size (T), nodal status (N) and the existence or absence of metastatic lesions (M) (32). Staging is preoperatively estimated clinically and radiologically by assessment of the tumour size, the existence (or not) of palpatory or pathologically enlarged axillary or supraclavicular lymph nodes and known macro-metastatic lesions. The pathological TNM stage is subsequently described according to measurements of the resected tumour specimen and lymph nodes.

e) *Surgical margins*, described as mm. In invasive breast cancer, positive margin is defined by "ink on tumour" (35).

f) *Axillary node* status (32).

g) Testing for expression of o*estrogen (ER) and progesterone (PR) receptor and HER2 amplification* is imperative. The testing is performed primarily on protein lever through immunohistochemistry (IHC). However, for HER2 the IHC assessment is accompanied by in-situ hybridisation if immunohistochemistry shows ambiguous results (reported as 2+).

h) The *Ki67* score; a tumour proliferation and growth biomarker. The score should be reported according to international and institutional guidelines. There is a substantial inter-observer variability and Ki67 can also be affected by other factors such as long time before tissue sections, long time in room temperature and use of fixation solutions other than neutral formalin (36). Due to disparities between laboratories, local cut-off values apply. In samples with hot spots, these should be evaluated and reported. Digital pathology is expected to further improve reliability and reproducibility of Ki67 scoring (36, 37).

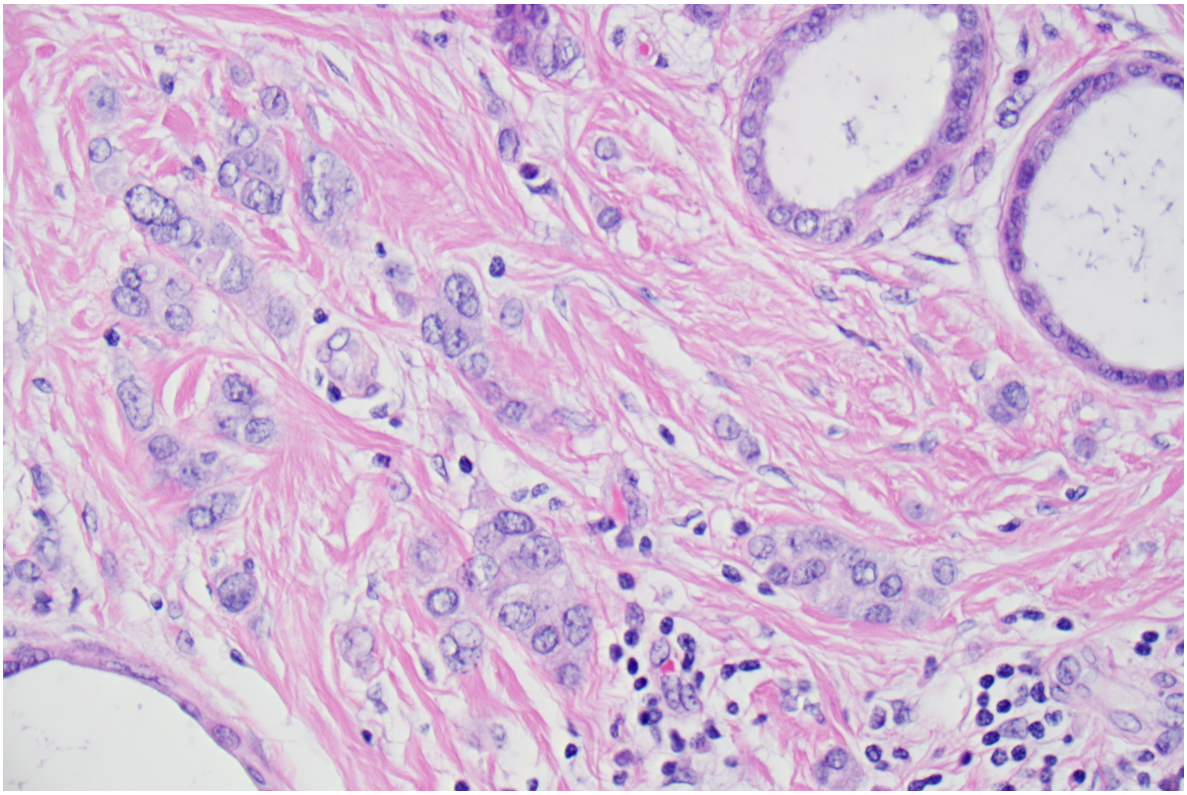

Figure 5. *Ductal breast carcinoma of no special type. Courtesy of Dr Felix Haglund*

Parameter			**Score**
Tubule formation	**Nuclear pleomorphism**	**Mitotic counts**	
Majority of tumour	Regular uniform cells	<8	1
Moderate	Moderate increase in size and variability	8-16	2
Little or none	Marked variation	>16	3

Table 1. *Histological grade according to the Elston Ellis system.*

1.5 Intrinsic molecular breast cancer subtypes

Perou et al described distinct molecular patterns in breast cancer samples and paved the way for a more tailored, tumour biology-guided classification and management of both early and metastatic breast cancer (38). The different gene expression patterns led to 4 clusters: basal-like, HER2+, normal-breast-like and luminal/epithelial (38). Given the global heterogeneity of access to gene expression analysis, IHC is currently used as a surrogate test to distinguish the different molecular subtypes and it identifies five distinct intrinsic subtypes (39-41):

Luminal A: Represent the most common molecular subtype of breast cancer (60-70%) and is characterised by low proliferation measured by Ki67, strong ER and PR expression and are usually HER2 negative.

Luminal B-like HER2-: Account for about 10-20% of breast cancers and is characterised by ER expression, PR expression lower than luminal A, high proliferation according to Ki67 and lack of HER2 expression.

Luminal B-like HER2+: Characterised by the same biomarkers as above but with additional expression of HER2.

HER2-enriched (non luminal): Present with high proliferation, HER2-expression but lack ER and PR expression. All HER2-positive cancers, regardless ER/PR status, account for about 15% breast cancers.

Triple-negative (TNBC): Lack both endocrine receptors (ER and PR) and HER2 expression and is commonly accompanied by high proliferation.

Notably, the histopathologically identified subtypes do not entirely overlap with the molecular subtypes (42). For example, only 71% of TNBC are classified as basal-like and not all HER2 positive tumours identified by IHC are HER2 enriched (43). Furthermore, subsequent studies have revealed intratumoural heterogeneity with significant impact on survival (44). More importantly, discrepancies of ER, PR and HER2 expression between the primary tumour and relapse shed light on tumour-evolutionary aspects with major bearing on breast cancer care (45). A retrospective analysis including patients with breast cancer relapse, revealed up to one third (32.4%) of the patients had altered ER expression at relapse, directly affecting chances of survival (46). Additionally, PR and HER2 expression was altered in 40.7% and 14.5% respectively (46). A similar study reported significant deterioration of overall survival among 14.2%, respective, 39.6% of patients that lost ER or PR expression after breast cancer relapse (47).

1.6 Genomic risk assessment

The mentioned clinical markers provide valuable information to accommodate distinction of patients at increased risk for breast cancer recurrence and death and therefore candidates for postoperative (adjuvant) treatment, such as radiotherapy, chemotherapy, endocrine and targeted therapy. Complementary gene-expression signatures, such as OncotypeDx,

MammaPrint and Prosigna, can predict breast cancer outcomes and aid selection of patients at low genomic risk that are probably not benefited by adjuvant chemotherapy (48-52). The gene expression profiles could fill the gap in the unmet need to limit overtreatment with adjuvant chemotherapy. The profiles could aid to better identify patients with intermediate clinical risk that will not benefit from adjuvant chemotherapy but their value as prognostic factors and their clinical usefulness is still undetermined.

Gene expression profiles can be utilised today for treatment recommendations to avoid adjuvant chemotherapy in node negative patients at such low absolute risk that treating them would not provide meaningful benefit (53, 54). Of note, access to gene expression analysis possibilities around the globe varies gradely. Available data for patients with node positive breast cancer are still immature and cannot be largely applied yet.

1.7 Treatment modalities for early breast cancer

1.7.1 Surgery

Surgery upfront versus neoadjuvant chemotherapy

Multimodal approach is nowadays the pillar for breast cancer management. Surgical excision of the primary tumour, by breast conserving surgery or mastectomy prior to application of any other treatment modality has long been considered the standard approach to early breast cancer. However, the advancement of systemic therapy prior to surgery (neoadjuvant) has been practice changing, especially among women with triple negative or HER2 positive breast cancer. The choice of whether to recommend neoadjuvant therapy depends on the tumour's molecular subtype, size, lymph node status, comorbidity and the patient's preference. Same chemotherapy regiments administered prior to or after surgery result in similar risk for distant recurrence, breast cancer specific and overall survival (55). Downsizing the tumour can facilitate surgery and increase the probability to avoid mastectomy in larger tumours. Neoadjuvant therapy further provides the possibility of prospective in-vivo assessment of treatment effect enabling identification of non-responders and potentially tailoring treatment accordingly (56). Eradication of tumour from the breast and the lymph nodes, defined as pathological complete response (pCR), is a clinically relevant outcome and considered by many to provide a surrogate estimation of better outcomes, mainly in HER2 positive and triple negative breast cancer (57). The importance of the latter is supported by the reported survival improvement among patients that achieved pCR after neoadjuvant therapy, in a pooled analysis (57). Thus, administration of systemic breast cancer therapy prior to surgery, allows for selection of the patients that did not respond with pCR and will benefit from salvage adjuvant therapy, an assessment that is not be possible after primary surgery. The use of neoadjuvant therapy for tumours larger than 2 cm in triple negative or HER2 positive breast cancer is currently recommended (34, 58).

Identifying poor responders and adding capecitabine as salvage adjuvant approach for patients with HER2-negative residual tumour after neoadjuvant chemotherapy improved 5-

year recurrence free survival (absolute gain 6.5%, hazard ratio [HR] 0.70, 95% Confidence Interval [CI] 0.53 – 0.92; p=0.01) and 5-year overall survival (absolute gain 5.6%, HR 0.59, 95% CI 0.39 – 0.90; p=0.01) in a study published by Masuda et al in 2017 (59). Similarly, adjuvant treatment with the antibody-drug conjugate trastuzumab-emtansine for patients with residual HER2 positive tumour after neoadjuvant trastuzumab and taxane-containing chemotherapy, halved the risk for recurrence and led to an absolute risk reduction of 11% (60). Additional surrogate methods for identification of patients at increased risk for relapse after neoadjuvant therapy such as residual cancer burden and post-neoadjuvant therapy staging are under investigation (34, 61).

Hormone-receptor positive tumours lacking HER2 amplification have thus far not achieved as high pCR rates as HER2 positive and triple negative tumours but there are currently on-going studies examining addition of CDK4/6 inhibitors to endocrine therapy as neoadjuvant approach in high-risk luminal B breast cancer. The incidence of objective responses has, thus far, not been encouraging but reported trends of improved gene-signature based risk estimations hint on potential place of neoadjuvant therapy also in high-risk luminal B breast cancer and the issue should therefore be considered unresolved (62-64).

Surgical methods

Appropriate surgical approach is decided upon tumour-related factors (size) and patient-related factors (volume of the breast, patient's preference, pregnancy). Up to 90% of breast cancer recurrences are local recurrences after breast conserving surgery but postoperative radiotherapy has been shown to reduce the risk of local or distant recurrence by half and provide a relative reduction of breast cancer mortality by 18% (Risk Ratio [RR] 0.82, 95% CI 0.75 – 0.90; p<0.001) (65, 66). Recent retrospective studies support superior breast cancer specific and overall survival after breast conserving surgery and radiotherapy than mastectomy alone, in small, node negative tumours (67, 68). However, intrinsic limitations of retrospective studies and obvious bias by selection of patients with more aggressive tumours to undergo mastectomy, call for caution in the interpretation of the results.

Regional axillary lymph nodes are usually treated by sentinel node biopsy and/or axillary dissection. Sentinel node biopsy has negligible side effects and is used as a prognostic marker to de-escalate axillary surgery in patients with negative sentinel node biopsy. Addition of axillary dissection in sentinel node negative breast cancer does not improve survival and it is hence not recommended (69). On the contrary, it can aggravate morbidity, such as lymphedema (70). Moreover, axillary dissection has so far been the standard praxis in sentinel node positive disease, however recent studies demonstrate that, for selected patients, sentinel node biopsy alone is not inferior to complementary axillary surgery. It is, thus, considered safe to refrain axillary dissection among patients with lymph node metastasis up to 2 cm (69, 71, 72). The timing of sentinel node biopsy and extend of axillary surgery in the context of neoadjuvant therapy is under investigation.

Breast reconstruction with implant or autologous tissue after mastectomy is considered safe regarding oncological outcomes and can be performed immediately or delayed depending of the tumour characteristics and the patient's preference (73-77). Direct reconstruction after inflammatory breast cancer is though discouraged and delayed reconstruction should also be considered in the case of locally advanced breast cancer with skin or thoracic wall involvement.

1.7.2 Radiotherapy

Since breast conserving surgery became the surgical method of choice for small breast tumours, there was a concern on the risk of local recurrence. However, administration of adjuvant radiotherapy on the remaining breast evens out increased risk of recurrence after breast conserving surgery (65, 66). In absolute numbers, adjuvant radiotherapy after breast conserving surgery reduced 10-year risk of recurrence by 15.7% and 15-year risk for breast cancer death by 3.8% according to the Early Breast Cancer Trialists' Collaborate Group (EBCTCG) report in 2011 (65). Efficacy of radiotherapy is even more prominent among women with lymph node tumour burden, associated to an absolute reduction of 10-year risk for recurrence by 21.2% (65). Nevertheless, according to randomised and non-randomised data, omitting radiotherapy in women older than 70 years with small, hormone receptor positive breast cancer does not lead to worse overall or disease-free survival and can be a safe option, provided adjuvant endocrine therapy is administered (78-80).

Addition of boost to the tumour bed in the radiotherapy plan, following breast conserving surgery, does not improve breast cancer survival but improves local control. Administration of boost is correlated to an absolute reduction of the 20-year cumulative incidence of ipsilateral breast cancer by 4.4% (16.4% versus 12.0% in no boost group) (81). The absolute risk reduction is more prominent among younger patients (absolute gain 11.6% for those under 40 years and 5.9% for those 41-50 years) and administration of boost on the tumour bed is thus deemed clinically relevant to be provided to women younger than age 50. Importantly, boost significantly increases severe fibrosis in corresponding levels; 1.8% in no boost group compared to 5.2% in the boost group ($p<0.0001$) (81).

Another important aspect is the place of adjuvant radiotherapy after mastectomy. A large meta-analysis from the EBCTCG in 2014 did not demonstrate significant improvement of the risk for loco-regional recurrence after 10 years (RR 1.06, 95% CI 0.76 – 1.48; $p>0.1$) or 20-year risk for breast cancer mortality (RR 1.18, 95% CI 0.89 – 1.55; $p>0.1$) after adjuvant radiotherapy of the chest wall, amid women that had undergone mastectomy and axillary dissection. On the contrary, adjuvant radiotherapy in this setting increased the risk for overall mortality (RR 1.23, 95% CI 1.02 – 1.49; 2p=0.03) (82). However, women with lymph node positive disease had a significant benefit from adjuvant radiotherapy after mastectomy, both in terms of 10-year breast cancer recurrence and 20-year breast cancer specific survival; absolute gain of 10.6% and 8.1% respectively (82).

Dose per fraction and number of fractions in adjuvant breast cancer radiotherapy has been changing over time. Hypofractionated radiotherapy (nowadays 2.65 Gy divided in 15

fractions) has been shown of equal biological efficacy as conventional fractionation with 50 Gy divided by 2 Gy fractions (83). In addition, shorter treatment duration has been demonstrated to be beneficial in terms of lower risk for skin toxicity and telangiectasia but impact on the risk for long-term cardiotoxicity and lung-fibrosis is unknown (83).

1.7.3 Chemotherapy

As abovementioned, chemotherapy in early breast cancer, can be delivered preoperatively (neoadjuvant) or as additional treatment after primary surgery (adjuvant chemotherapy). Historically, adjuvant chemotherapy was offered to patients with node positive breast cancer. However, the development of different predictive and prognostic biomarkers has led to a more complex assessment of relapse risk and chemo-/endocrine-sensitivity prior to treatment decisions on individual level. A large meta-analysis of the EBCTCG on individual patient data from 100 000 women revealed reduction of breast cancer mortality by one third after adjuvant chemotherapy (84).

Initially the combination of cyclophosphamide, methotrexate and 5-fluorouracil (CMF) reduced breast cancer mortality at 20 years by one fourth, compared to no adjuvant chemotherapy (85). Addition of anthracyclines compared to no adjuvant chemotherapy decreased relative risk for breast cancer mortality by 21% (absolute risk reduction at 10 years 6.5%) (84). The benefit of anthracyclines persisted regardless age, nodal involvement, ER status, tumour size and differentiation (84). Moreover, a higher cumulative dose of anthracycline-containing regimens was shown to be superior to CMF by reducing the relative risk of 10-year breast cancer mortality by one fifth (absolute risk reduction 4.1%) (84).

Further on, addition of taxanes improved relative risk for breast cancer mortality by 13%. Anthracyclines and taxanes given in sequence were superior in terms of breast cancer recurrence compared to concomitant administration, with an absolute reduction of recurrence by 3.2% (RR 0.87, 95% CI 0.80 – 0.94; $p<0{\cdot}001$) (84).

There is a strong biological rationale for intensification of chemotherapy regimens. Increasing dose or shortening the treatment interval (dose-dense chemotherapy), can potentiate treatment effect and produce better cancer-related outcomes. It is generally hypothesised that intensifying chemotherapy dose would increase the number of tumour cells killed and shortening of the treatment interval would enhance treatment effect by providing less time for the recovery of the tumour-cells. This is supported by different hypotheses with the Norton-Simon hypothesis having a central role. Simon and Norton suggested that ‘chemotherapy results in a rate of regression of tumour volume that is proportional to the rate of growth for an unperturbed tumour of that size' (86). In addition, Norton suggests that reduction of the tumour volume by chemotherapy can lead to faster tumour re-growth between cycles, since small tumours are considered to grow faster than larger tumours (87). Another hypothesis supports that drug-resistance develops spontaneously after administration of chemotherapy and as such, early introduction of intensive therapy and considering alternating regimens (such as anthracyclines and taxanes in sequence) can further improve efficacy of administered therapy (88).

In accordance with these hypotheses, the individual patient data based meta-analysis of EBCTCG published in 2019 reported a benefit in breast cancer mortality with dose-intense anthracycline and taxanes adjuvant chemotherapy compared to standard chemotherapy (89). In particular, when the same agents were administered but with shorter interval (two weeks versus three weeks), 10-year recurrence of any type was reduced in absolute numbers by 4.3% (24% versus 28.3%) and breast cancer survival by 2.8% (16.8% versus 19.6%). This was translated in a relative risk reduction for recurrence by 17% (RR 0.83, 95% CI 0.76 – 0.91) and for breast cancer mortality by 14% (RR 0.86, 95% CI 0.77 – 0.96). Non-breast cancer mortality did not increase with intensive chemotherapy and combined with the overall survival improvement, suggests that the survival benefit is solely due to reduced breast cancer mortality (89).

Despite the clear benefit from increasing dose intensity of anthracyclines and taxanes, increasing cyclophosphamide dose intensity and/or cumulative dose in the adjuvant setting in the NSABP B22 and B25 trials, did not correspond to improved disease-free or overall survival (90, 91). The phase III PANTHER trial, further discussed in detail in the current thesis, compared standard dose 3-weekly chemotherapy with tailored dose dense chemotherapy (92). A fundamental difference between the PANTHER and the B22 and B25 trials is the protocol pre-defined dose escalation according to toxicity of the anthracycline, taxanes and cyclophosphamide doses. Thus, cyclophosphamide escalation was not performed to all participants of the PANTHER trial but it was reserved for those probably undertreated according to standard therapy.

Notably, the relative benefit from increased dose intensity in the EBCTCG meta-analysis persisted regardless patient and tumour characteristics; age, ER status, nodal status, HER2 status, tumour size, Ki67 and grade (89). The proportional outcomes in ER positive and ER negative disease imply long-term survival in ER positive disease is not uniquely related to adjuvant endocrine therapy but is also an aftereffect of adjuvant chemotherapy. Importantly, increased dose-intensity was not compromised by increased toxicity; deaths from cardiovascular disease, acute myeloid leukaemia or other malignancy did not differ from standard chemotherapy schedules.

Relative dose intensity (RDI) of chemotherapy defined as the ratio of delivered dose intensity to the standard dose intensity is directly related to cancer-specific outcomes in both early and metastatic setting. In particular, delivery of RDI $\geq$ 85% in early breast cancer has been related to improved breast-cancer specific and overall survival (93, 94). Even in the metastatic setting, increased RDI is related to improved objective response (95). Current practice usually relies dosing on the body surface area, not taking into account inter-patient variations in pharmacogenomics and pharmacodynamics (96). However, a recent publication suggests that excess adiposity can lead to lower RDI and worse breast cancer survival (97). In addition, individually tailoring chemotherapy dose based on haematological toxicity improved 5-year event free survival (RR 0.79, 95% CI 0.63 – 0.99; p=0.04) even though the benefit in overall survival did not reach significance (92). Such observations strengthen the need for development of improved methods for individualised dose calculation based on the intrapersonal unique pharmacodynamic and pharmacokinetic characteristics.

1.7.4 Anti-HER2 therapy

The discovery of the HER2/neu as oncogene related to breast cancer in 1985 has revolutionised care and outcomes for this particular subgroup (98). Shortly after, in 1987, Slamon et al reported correlation of HER2/neu amplification and worse breast cancer prognosis with the contemporary treatment approach (99). The subsequent development of the humanized monoclonal antibody trastuzumab, and later on other anti-HER2 agents, led to a cascade-like evolution of the management of HER2 amplified breast cancer and constitutes a brilliant example of translational research directly impacting clinical praxis and benefiting the patients. In the first reported study, trastuzumab added to chemotherapy improved objective response and time to progression and, overwhelmingly, improved survival with a 20% reduction in risk of death among heavily pre-treated women with metastatic, HER2 amplified, breast cancer (100). Trastuzumab efficacy is potentiated by concomitant chemotherapy treatment, as already demonstrated in early trastuzumab trials in metastatic breast cancer, with reported response rate of trastuzumab in combination with cisplatin superior to trastuzumab alone (101). Synergistic effect was also present in combination with taxanes, anthracyclines and cyclophosphamide and currently there is a recommendation for administration of anti-HER2 therapy in combination with chemotherapy or at least to initiate with concomitant therapy (100, 102).

Following the unequivocal success of trastuzumab, several other anti-HER2 agents were developed. The antibody-drug conjugate T-DM1, that combines trastuzumab with the chemotherapeutic agent emtansine, the recombinant, humanised, monoclonal antibody pertuzumab and the tyrosine kinase inhibitors lapatinib and neratinib are the ones currently available after approval by the competent authorities.

In the context of neoadjuvant therapy for HER2 positive breast cancer, addition of pertuzumab to neoadjuvant chemotherapy and trastuzumab (with trastuzumab continuing even postoperatively for total treatment duration one year) in the NeoSphere trial, improved complete pathological response and five-year survival (103). Even though difference in event-free (EFS) and overall survival (OS) was not statistically significant (HR EFS 0.81, 95% CI 0.52 – 1.26 and HR OS 0.72, 95% CI 0.41 – 1.27), neoadjuvant administration of lapatinib in combination with trastuzumab in the NeoALTTO trial led to numerically less events at six years follow-up compared to trastuzumab alone (85% versus 79% respectively) and demonstrated the survival impact of pathologic complete response after neoadjuvant therapy in HER2 positive breast cancer (104). Another neoadjuvant trial (KRISTINE) demonstrated substantial benefit from dual blockade but it also underscored the value of combination of anti-HER2 treatments with conventional chemotherapy (105). The TRAIN-2 study provided support for the use of non-anthracycline regimens during neoadjuvant therapy for HER2 positive breast cancer; an essential step to reduce risk for cardiotoxicity (105, 106). Hence, dual HER2 blockage (pertuzumab and trastuzumab) with backbone chemotherapy is presently standard of care in the neoadjuvant setting for HER2 amplified breast cancer.

In the adjuvant setting, 5099 individuals were enrolled in the Herceptin Adjuvant (HERA) trial investigating one and two years' treatment duration that demonstrated superiority of one-year trastuzumab compared to control with relatively equal effect for ER positive and ER negative breast cancer. Disease free survival was improved by 24% (HR 0.76, 95% CI 0.68 –

0.86), the risk of death was reduced by 26% (HR 0.74, 95% CI 0.64 – 0.86) and 63% were breast cancer free at 10 years (107). The value of adjuvant trastuzumab was further investigated and a Cochrane review including eight studies revealed improved disease free survival by 40% with the administration of trastuzumab in HER2 positive breast cancer (108). Despite the initial hypothesis that lapatinib would have better efficacy than trastuzumab, the head to head comparison of the two as adjuvant therapies in the ALTTO trial demonstrated 34% worse DFS with lapatinib compared to trastuzumab as single anti-HER2 therapies, at the updated 4.5 years median follow-up (HR 1.34, 95% CI 1.13 – 1.60) (109). Adding pertuzumab for the whole duration of adjuvant trastuzumab (1 year) did not benefit the 3-year invasive-disease free survival for node negative tumours but was superior among patients with positive lymph nodes (HR 0.77, 95% CI 0.62 – 0.96; p=0.64) (110).

The considerably arbitrary one-year treatment duration of adjuvant trastuzumab has been challenged in both directions. The HERA trial failed to show any survival benefit from increasing treatment duration to two years (107). Shorter duration of adjuvant trastuzumab, such as 9 weeks or 6 months, has also been tested due to trends of higher incidence of cardiac events with longer trastuzumab duration. Most of the shorter duration studies have a non-inferiority design and thus far only the PERSEPHONE trial (6 months versus one-year trastuzumab) has fulfilled non-inferiority criteria with an absolute benefit in disease-free survival (DFS) in the longer duration of 0.4% (111-113). Four other trials investigating shorter intervals (SOLD, Short-HER, PHARE and HORG) have shown gains in DFS with one-year trastuzumab in the range of 2-3% (114-117). To conclude, one-year trastuzumab treatment, including a chemotherapy part with taxane-including regimens, remains the standard of care, despite concerns for trade-offs in cardiac events (111-116, 118).

Moreover, as aforementioned, addition of T-DM1 as adjuvant therapy halved the risk of death from invasive disease for patients not achieving complete pathological response after neoadjuvant therapy, corresponding to an absolute benefit of 11% (HR 0.50, 95% CI 0.39 – 0.64) (60). As a result, adjuvant salvage therapy with T-DM1 is currently recommended for patients with considerable tumour burden at surgery following neoadjuvant therapy for HER2 amplified breast cancer.

Even in the metastatic setting, dual blockade with pertuzumab and trastuzumab in combination with docetaxel, substantially improved prognosis by improving overall survival with 15.7 months and median progression-free survival by half a year (119). Backbone chemotherapy in combination with pertuzumab and trastuzumab is today the treatment of choice for first line therapy in HER2 positive metastatic breast cancer and the rest of the abovementioned anti-HER2 agents further enrich our armamentarium and prolong survival. Development in the care of metastatic HER2 positive breast cancer is rapid and continuous, with more drugs such as tucatinib and deruxtecan in the pipeline (120-122).

1.7.5 Endocrine therapy

ER and/or PR expression is both a prognostic marker of improved breast cancer survival and predictive of benefit from endocrine therapy with reduction of breast cancer relapse and survival (123). Tamoxifen is a selective oestrogen receptor modulator (SERM) and can act as oestrogen antagonist in breast tissue but as oestrogen agonist in, for example, uterus. After menopause, oestrogen production is limited to peripheral tissues through aromatisation of androstendione to estradiol. The aromatase inhibitors (AI) letrozole, anastrozole and exemestane inhibit this process leading to reduction of estradiol levels.

Five years adjuvant tamoxifen among patients with ER positive early breast cancer improved 10-year overall survival by 6% compared to two years of tamoxifen; 80% versus 74% respectively and reduced breast cancer mortality by 30% during the first 15 years following breast cancer diagnosis (RR 0.70, 95% CI 0.64 – 0.75; p<0.001) (124) (125).

The effect of ovarian ablation alone (by gonadotropin-releasing hormone agonists [GnRHa], surgery or radiation) is less prominent among premenopausal women that have received chemotherapy, probably due to early ovarian function suppression secondary to chemotherapy-induced amenorrhea (CIA) but addition of GnRHa to tamoxifen demonstrates a relative risk reduction for breast cancer death by 15% (123, 126, 127). Benefit in overall survival from addition of GnRHa to tamoxifen is mostly for women with high-risk breast cancer that remain premenopausal after adjuvant chemotherapy, and this combination is recommended for the specific population (128). A jointed analysis of the Suppression of Ovarian Function Trial (SOFT) and the Tamoxifen and Exemestane Trial (TEXT) revealed improved disease-free survival by an absolute difference of 4% with ovarian suppression and AI compared to ovarian suppression and tamoxifen (HR 0.77; 95% CI, 0.67 – 0.9; p<0.001) but no survival benefit (128). On the other hand, the ABCSG-12 trial reported lack of benefit from AI and GnRHa compared to tamoxifen and GnRHa and increased risk of death from any cause (HR 1.63, 95% CI 1.05 – 1.45) (129, 130). The lack of survival benefit in the SOFT/TEXT trials, the reported worse survival from GnRHa and AI versus GnRHa and tamoxifen in the ABCSG-12 trial together with reports on hormonal escape during GnRHa treatment advise for monitoring of estradiol levels to ensure complete ovarian suppression when AI in combination with GnRHa is offered to premenopausal women (131). Sufficient ovarian suppression during concurrent therapy with AI is of paramount importance and has been debated in a dedicated article (131).

In postmenopausal women with early breast cancer, 5 years of AI further improved breast cancer mortality (RR=0·86, 95% CI 0.80 – 0.94; p=0·0005) even thought the benefit on breast cancer recurrence was only present during the treatment period (132, 133). Even switching to AI after 2-3 years tamoxifen, with total endocrine therapy duration for 5 years showed slightly increased breast cancer outcomes compared to 5 years tamoxifen alone (132).

A minimum of five years adjuvant endocrine therapy has been the standard recommendation for a few decades. However, almost half of the recurrences happen after the first 5 years of follow-up and the risk of breast cancer distant-recurrence among women with hormone receptor positive early breast cancer can persist up to 20 years, depending on tumour burden and grade (134, 135). Extending tamoxifen treatment to 10 years in the Adjuvant Tamoxifen

Longer Against Shorter (ATLAS) and adjuvant Tamoxifen To offer more (aTTom) trials demonstrated further improvement of the risk for breast cancer recurrence and breast cancer specific mortality (136, 137). In the ATLAS trial, the benefit of 10 years tamoxifen became more obvious after year 10 for both risk for recurrence (RR 0.75, 95% CI 0.62 – 0.90) and breast cancer mortality (RR 0.71, 95% CI 0.58 – 0.88) (136). In line with these results, longer tamoxifen duration in the aTTom trial had a significant impact on breast cancer recurrence after treatment year 7 (RR during years 7-9: 0.84, 95% CI 0.73 – 0.95) and breast cancer mortality after year 10 (RR 0.77, 95% CI 0.64 – 0.92) (137).

Similarly, in the MA.17 trial, extended endocrine therapy with the aromatase inhibitor letrozole after 5 years of tamoxifen improved risk for recurrence by 42% and among women with lymph node positive breast cancer it also demonstrated benefit in overall survival (HR 0.61, 95% CI 0.38 – 0.98; p=0.04) (138). Most valuable effect of prolonged AI appears to be after 5 years tamoxifen. Extended treatment with additional five years of AI for patients already treated with AI for five years demonstrated reduced risk for breast cancer recurrence but this was not translated to better breast cancer specific or overall survival (139-141). However, there is a significant heterogeneity among patients identified as high-risk and therefore careful selection of the candidates for prolonged endocrine therapy is warranted (51). In summary, based on the presented results, prolonged endocrine therapy for 10 years is currently standard of care for high-risk breast cancer.

1.7.6 Bisphosphonates

Despite indications of benefit from adjuvant bisphosphonates in premenopausal women in the ABCSG-12 study, the large meta-analysis of EBCTCG on the efficacy of 2-5 years treatment with bisphosphonates in 2015, reported benefit in overall breast cancer related outcomes only in postmenopausal women (129, 142). The specific group benefited by a relative risk reduction of distant recurrence and breast cancer death by 18% (RR 0.82, 95% CI 0.74 – 0.92 and 95% CI 0.73 – 0.93, respectively) and improvement of the risk for recurrence in the bone by 28% (RR 0.72, 95% CI 0.60 – 0.86) (142). Type of administered bisphosphonate, treatment schedule, administered chemotherapy, and tumour burden did not affect efficacy (142). A subsequent Cochrane meta-analysis added further data on survival benefit of bisphosphonates administration in postmenopausal women (HR 0.77, 95% CI 0.66 – 0.90; p=0.001) and, so far, lack of such in premenopausal women (143).

Denosumab, a fully human monoclonal antibody that inhibits osteoclast activity, is widely used for the treatment of osteoporosis. In terms of breast cancer efficacy, adjuvant administration of denosumab has been shown superior to bisphosphonates in terms of skeletal-related events (RR 0.78, 0.72 – 0.85; p< 0.001) (143). Denosumab led to significant gain in disease-free survival compared to placebo in the ABCSG-18 study whereas the, also placebo controlled, study D-CARE reported no benefit in disease-free survival or risk for bone metastasis from denosumab (144, 145). In the context of osteoporosis, concerns have been raised on optimal treatment duration and management after treatment discontinuation due to evidence reporting harmful rebound effect and increased bone fractures following

denosumab cessation (146). To stipulate the impact of denosumab discontinuation on breast cancer related outcomes is far from simple but the available data should raise awareness. Adjuvant bisphosphonates are considered by many as a way to partly counterpart increased osteoporosis risk related to long-term aromatase inhibitors administration. It is though unclear whether a potential rebound effect after denosumab discontinuation can aggravate bone health among these patients.

1.7.7 Immunotherapy

Immune checkpoint inhibitors have transformed management and survival of several solid tumours such as lung cancer, malignant melanoma and renal cancer. In breast cancer, the leap has not been equally impressive. Expression of programmed death ligand 1 (PD-L1) on the immune cells infiltrating TNBC tumours impedes the immune response against the tumour and therefore constitutes a reasonable therapeutic target. Despite that, efficacy of immune checkpoint has been, thus far, shown mostly in metastatic TNBC.

Neoadjuvant pembrolizumab, a monoclonal antibody that inhibits interaction of programmed death-1 (PD-1) receptor with PD-L1 or PD-L2, in combination with chemotherapy led to a higher rate of pathologic complete response rate by 13% compared to chemotherapy alone, a statistically significant improvement (147). Pembrolizumab treatment effect was consistent, regardless PD-L1 receptor status.

Atezolizumab, a selective PD-L1 antagonist, in combination with nab-paclitaxel did not improve overall survival among patients with metastatic or unresectable TNBC compared to nab-paclitaxel alone in the intention-to-treat analysis (HR 0.86, 95% CI 0.72 – 1.02) (148). Although, stratified exploratory analysis revealed overall survival benefit of atezolizumab, compared with placebo, in the PD-L1 positive group (HR 0.71, 95% CI 0.54 – 0.94) (148). Early phase trials also suggest pembrolizumab efficacy in the metastatic setting as monotherapy or in combination with other therapies; both for TNBC but also for other breast cancer subtypes suggesting there still may be place for immunotherapy in both early and metastatic breast cancer (149-151).

1.8 Common toxicity of breast cancer therapies

1.8.1 Surgery

Development in breast cancer surgery has been towards minimising surgical intervention and shortening hospitalisation time. A Danish study reported, in a health care system similar to the Swedish, that 72% of all breast cancer operations were performed as outpatient with a rate of wound-related re-admission within 30 days following surgery as low as 2% (152). Up to 10% of the patients can experience any type of short-term complication across different surgical approaches (153). The patients should be informed of potential complications such as

hematoma or seroma requiring drainage, infection and complications related to the flap or expander used in reconstructions.

Another known complication of breast cancer surgery is lymphedema. Lymphedema can augment after axillary lymph node dissection and/or radiotherapy and can present shortly or long-time after surgery. For example, long-term follow-up of the IBCSG 23-01 trial revealed that 13% of the patients that underwent axillary lymph node dissection (n=447) developed lymphedema of any grade, about one in five developed sensory neuropathy (19%) and almost one in ten (9%) motor neuropathy (154). Similarly, McLaughlin et al reported incidence of lymphedema in 16% of patients with axillary surgery compared to 5% of those with sentinel node biopsy (155). These results are in concordance with the AMAROS study where twice as many patients developed lymphedema, defined as increase of arm circumference 10% or above, five years after axillary surgery compared to axillary radiotherapy alone (13% versus 6%, $p<0.001$) (72).

Beyond physical complications, breast cancer surgery can have psychological downsides such as those related to alteration of the person's perceived body image. The cosmetic result is often considered the major advantage of breast conserving surgery and one would expect it reduced psychological impact emanating from altered body image perception. Data is however inconsistent and body image perception appears to be similar between patients undergoing breast conserving surgery and radiotherapy or mastectomy with reconstruction at two years following surgery (156). Interestingly, at the same follow-up, patients that underwent mastectomy alone report better body image compared to patients with mastectomy and direct reconstruction (156).

1.8.2 Radiotherapy

Side effects of radiotherapy are generally considered to be limited to the irradiated area and the surrounding tissues, although some data suggest that it could also increase the risk for marrow neoplasms (157). Lungs and heart are organs of particular interest in the adjuvant radiotherapy of breast cancer. Most common short-term toxicity of radiotherapy of the breast, with or without regional lymph nodes, is skin toxicity in the irradiated area and it can vary from erythema to moist desquamation (158). Radiation pneumonitis is less common and can occur as acute side effect or as long-term toxicity in the form of lung fibrosis (159, 160). Ionizing radiation can injure all the anatomical structures of the heart and the large vessels through inflammation and fibrosis liable for a range of long-term conditions such as pericarditis, valvular dysfunction, cardiomyopathy and coronary artery disease. As expected, in the case of breast cancer, risk for long-term cardiovascular side effects is increased in left-sided breast cancer and history of cardiac disease. There is a clear association between distribution of stenosis in coronary arteries and the radiotherapy field (161).

Administration of radiotherapy has been related to increased risk of secondary cancer, such as lung cancer, and non-breast cancer mortality secondary to cardiac disease (162). A recent publication reported cumulative incidence of radiation-associated breast angiosarcoma after radiotherapy for breast cancer to 0.1% with increased risk for older patients and those receiving radiotherapy after breast conserving surgery (163). Long-term smoking is related to increased risk for lung cancer (4% versus 0.3% for non-smokers) and cardiovascular mortality (1% versus 0.3 %), according to a meta-analysis based on individual data from breast cancer patients treated with modern radiotherapy techniques (162).

The evolution of the radiotherapy techniques for treatment planning and delivery have led to sharp reduction in the incidence of acute pericarditis, however chronic pericarditis still develops up to 12% at 30 years after chest irradiation (164, 165).

Early radiotherapy trials using older techniques showed excess mortality related to reasons other than breast cancer, leading to the conclusion that radiotherapy-related side effects can even out benefit on breast cancer mortality (166). On the other hand, a meta-analysis by the EBCTC,G including 13 500 women, reported no increase in 10-year non-breast cancer mortality in more recent (1989 – 2003) radiotherapy versus no radiotherapy trials; 5% versus 4.8% respectively (167). One can hypothesise, that newer technology, such as, for example, gating and coordination with breathing variation will further reduce radiation to the heart and lungs during breast and chest wall radiotherapy, with or without node irradiation. Nonetheless, potential confounding from radiotherapy needs to be taken into consideration when assessing potential cardiotoxic effects of systemic therapies, particularly in left-sided breast cancer.

1.8.3 Chemotherapy

Chemotherapy administered as adjuvant, neoadjuvant or palliative therapy in breast cancer can cause short-term and long-term toxicity. The frequency, character and severity of the related toxicity usually depend primarily on the type of regimens used and the dosing schedules.

Adjuvant breast cancer chemotherapy-related short-term toxicity develops during chemotherapy and particularly anthracycline-based regimens tend to cause more short-term toxicity than non-anthracycline ones (168). The most commonly described symptoms are fatigue, nausea and emesis, alopecia, myelosuppression and mucositis. The reported frequency of nausea has been varying but, nowadays, it is expected to be lower due to the wide adaptation of primary prophylaxis with serotonin antagonists (such as ondansetron and palonosetron) and neurokinin-1 receptor antagonist (aprepitant) (169). Specific chemotherapy regimens such as taxanes can also cause peripheral neuropathy up to 22% and myalgia (168, 170).

Long-term toxicity can occur during the course of chemotherapy and become persistent, but it can also develop long time after chemotherapy completion. Common late toxicities can include cardiovascular disease, cardiomyopathy with or without heart failure, secondary malignancies and chemotherapy-induced amenorrhea and subsequent transient or permanent premature ovarian insufficiency and infertility (168). The latter has also been associated with long-term increased risk for osteoporosis and cardiovascular disease (171). Early studies have also reported varying frequency of weight gain. Up to 83% of women receiving adjuvant chemotherapy gain weight, especially premenopausal women in general or premenopausal women that develop chemotherapy-related ovarian suppression (168, 172).

Cognitive dysfunction has been observed both during and after adjuvant chemotherapy for breast cancer. Objective and subjective memory impairment have been reported and even though the severity of daily memory lapses may not differ from healthy individuals, they indeed have a bearing on the quality of life of the individual (173, 174). A common parameter between many reported longitudinal studies is the presence of subjective cognitive impairment and lack of objective findings, questioning the mere existence of a direct neurophysiological damage from chemotherapy. However, the nature of the cognitive changes, other concomitant treatment and methodological hurdles, have caused large heterogeneity between the studies and hamper conclusions. Neuroimaging studies have reported structural and functional differences between breast cancer patients and controls suggesting increased effort and overcompensation being the source of lack of objective findings despite the patients' experience of failing cognitive function (175). Although existence of cognitive dysfunction during and after chemotherapy is reported, it is more likely the result of other psychoneurological conditions such as fatigue and endocrine alterations due to endocrine therapy, rather than direct toxic effect of chemotherapy (176-178).

Secondary haematological malignancies such as acute myeloid leukaemia/myelodysplastic syndrome (AML/MDS) have also been reported as unwanted events after adjuvant breast cancer chemotherapy. However, an analysis by Praga et al, reported that patients treated with commonly used anthracycline and cyclophosphamide doses (epirubicin $\leq$ 720 mg/m^2 and cyclophosphamide $\leq$ 6300 mg/m^2) had reported cumulative probability of AML/MDS after 8 years of follow-up as low as 0.37% (95% CI 0.13 – 0.61%) (179). On the contrary, high cumulative epirubicin and cyclophosphamide dose significantly increased cumulative probability of AML/MDS at 8 years to 4.97% (95% CI 2.06 – 7.87) (179). Cumulative epirubicin dose of more that 720 mg/m^2 was related to an almost 7-fold increased risk, compared to lower cumulative doses (HR 6.80, 95% CI 2.86 – 16.13) (179). Similarly, the EBCTCG also reported incidence of less then 1% in their large meta-analysis on polychemotherapy benefit in early breast cancer (84). The mentioned meta-analysis based on individual patient data from 100 000 women that participated in >100 clinical trials, actually revealed that adjuvant chemotherapy did not lead to excess mortality. On the contrary, overall mortality was in accordance with breast cancer specific mortality (84).

Tailored, dose-dense chemotherapy results in higher chemotherapy doses and, as expected, higher acute toxicity but does not exaggerate long-term cardiotoxicity or haematological toxicity (92, 96). In the EBCTCG meta-analysis from 2019, rates of death secondary to AML/MDS were not affected by increased chemotherapy dose intensity; 33 events with AML/MDS were reported in the dose-intensive group (n=18623) and 17 in the standard schedules (n=18750) (89). In the PANTHER phase III trial patients received not only shorter chemotherapy intervals (2 weeks) but also individualised dose escalation (92). Incidence of AML/MDS was consistently low, with three patients in the tailored and dose dense treatment group (n=1001) compared to two patients in the standard chemotherapy group (n=999) (92).

Cardiotoxicity, neutropenia and chemotherapy-induced amenorrhea are related to the current thesis and will be discussed separately.

1.8.4 Endocrine therapy

Commonly reported side effects of endocrine therapy include hot flashes (30-40%), fatigue, mood disturbances, sexual dysfunction and musculoskeletal pain, although tamoxifen and AIs can exhibit differential side effects (180). Tamoxifen has been related to slight increase of risk for endometrial cancer (1.2% versus 0.4% in the aromatase inhibitor group), ischemic cerebrovascular events and thromboembolic episodes, whereas AIs increase the risk for osteoporosis, bone fractures (absolute excess 2.7%) and arthralgia (132, 181).

Interestingly, placebo controlled trials in the adjuvant setting report similar incidence of symptoms among patients receiving endocrine therapy and placebo. In the NSABP B-42 trial investigating prolonged letrozole treatment versus placebo after 5 years endocrine therapy in postmenopausal women, incidence of arthralgia and back pain did not differ between the placebo and the letrozole group; both adverse events were reported around 2% in both groups (182). Even other endocrine-therapy related side effects such as hot flashes, myalgia and depression were equally rare (182). Similarly, the MA-12 trial comparing 5 years tamoxifen versus placebo among premenopausal women reported indifferent frequency of hot flashes (82% vs. 81%), arthralgia (23% vs. 29%), myalgia (17% vs. 19%), headache (12% vs. 15%), and vaginitis/vaginal fibrosis (27% vs. 20%) (183). Notwithstanding different trials and drugs administered, the incidence of side effects in the premenopausal women of the MA-12 trial is clearly higher than the postmenopausal women enrolled in the NSABP-12. It is therefore highly relevant to question whether the reported side effects can be genuinely ascribed solely to adjuvant endocrine therapy or could be related to other reasons, considering the concordance in the reported incidences between both patients treated with active agents and placebo.

Furthermore, concerns have been raised on potential cardiovascular negative effects of AI with two recent reviews reporting contradicting results (184, 185). It is speculated that excess cardiac events in the aromatase inhibitor group could depend on positive effect of tamoxifen

and not real harmful effect of AI. Figure 6 summarises common toxicity in the different treatment modalities.

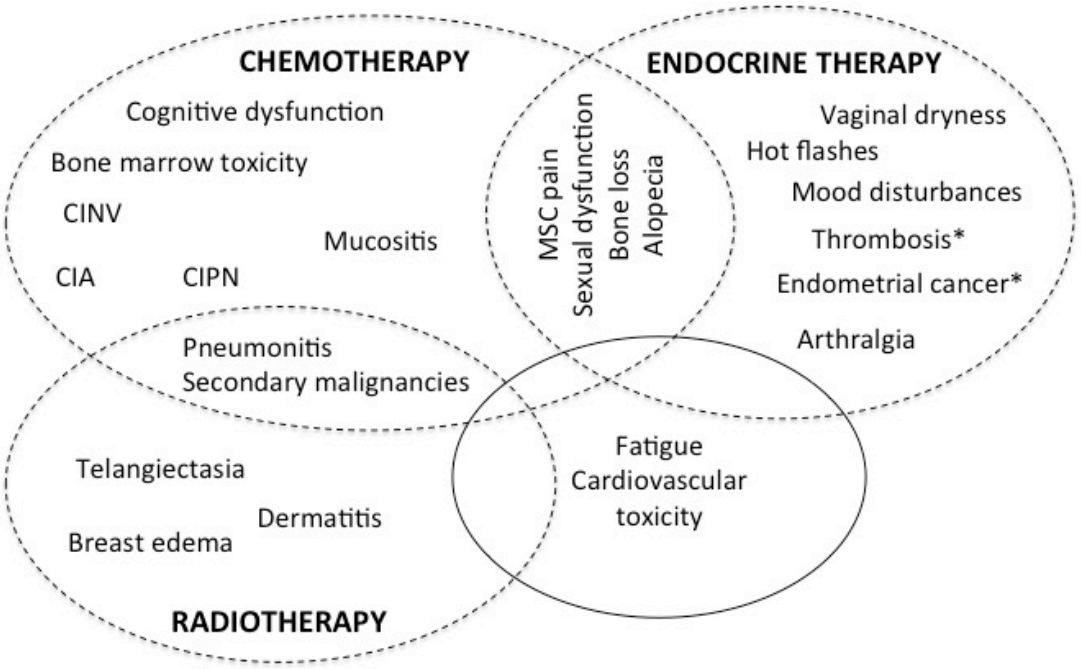

Figure 6. *Common toxicities related to the different breast cancer treatment modalities. *Related to tamoxifen. CIA: Chemotherapy-induced amenorrhea, CINV: Chemotherapy-induced nausea and vomiting, CIPN: Chemotherapy-induced peripheral neuropathy, MSC: musculoskeletal*

1.9 Breast cancer chemotherapy-related cardiotoxicity

There is a perplex relationship between breast cancer and heart disease with shared risk factors such as lack of physical exercise, smoking, obesity and old age, consisting a common denominator. Additionally, it was early established that breast cancer therapy could have potentially detrimental effects on the cardiovascular system. With an expected 5-year breast cancer survival of up to 90% (with regional variations), there is an increased prevalence of breast cancer survivors at risk for developing cardiovascular disease or heart failure after treatment for early breast cancer. Table 2, summarises risk factors and onset of cardiotoxicity for the most commonly used chemotherapeutic and targeted agents in early breast cancer treatment.

Compared to age-matched controls, women with breast cancer history have lower risk of coronary revascularisation but similar risk for cardiovascular death (186). Importantly, the presence of cardiovascular risk factors is the drive to greater risk for cardiovascular disease and mortality, regardless breast cancer history (186). The 10-year-risk of cardiovascular disease is equal or higher than breast cancer relapse risk among 80% of women with hormone receptor positive breast cancer (187). In a large retrospective analysis, women diagnosed with

early breast cancer had increased 10-year incidence of ischemic heart disease, arrhythmias and cerebrovascular disease rather than heart failure, compared to controls (188). Despite younger age and presence of fewer risk factors among those diagnosed with breast cancer and treated with anthracyclines and/or trastuzumab, the risk for cardiovascular disease was increased compared to age-adjusted population (188).

A unanimous definition of chemotherapy-related cardiotoxicity is difficult, but it is generally considered to be related to either direct damaging effect of the cancer therapy on the heart function and structures or, among patients with cardiovascular risk factors, the acceleration of the development of cardiovascular disease (189). In an effort to structure this complex and heterogeneous field, Ewer and Lippman reported a classification system based on injury type, reversibility and presence of structural abnormalities (190, 191). They characterised *Type I* chemotherapy-induced cardiotoxicity as cardiomyocyte death, with dose-depended and irreversible damage, and *Type II* as cardiomyocyte dysfunction with largely dose-independent and reversible damage (190, 192-195). Anthracyclines have long been considered the prototype of type I chemotherapy-induced cardiotoxicity whereas trastuzumab-related toxicity is considered primarily of reversible type II toxicity. However, the system was not absolute and combined damages were soon described, such as for example scar formation among patients with presumably type II cardiotoxicity (196). Therefore, Perez et al recommended the addition of three more categories; type III coronary disease related (e.g. due to radiotherapy or 5-FU), type IV miscellaneous including myocarditis or Takotsubo's cardiomyopathy and type V indirect cardiotoxicity that is related to conduction abnormalities, arrhythmia and hypertension (197).

In a harmonisation effort, the European Society of Cardiology (ESC) proposed a diagnostic algorithm for cardiotoxicity, depending on the modality used and divided cardiovascular complications of cancer therapy in nine different categories. The recommended cut-off value for echocardiography, suggestive of cardiotoxicity is *"reduction of left ventricular ejection fraction (LVEF >10%) to a value below the lower normal limit"(189).* Other modalities recommended for screening of cardiotoxicity include cardiac MRI and biomarkers such as Troponin I, high-sensitivity troponin I and the natriuretic peptides B-type natriuretic peptide (BNP) and N-terminal fragment B-type natriuretic peptide (NT-proBNP) (189). LVEF is defined as the percentage of the total volume of blood in the left ventricle ejected at each heart contraction. Heart failure is a syndrome related to the existence of typical symptoms or signs; symptoms such as shortness of breath, ankle oedema and fatigue, and signs such as peripheral oedema and elevated jugular venous pressure may also accompany it (198). According to the ESC guidelines heart failure is currently divided into three categories depending on LVEF levels (198). Heart failure with reduced EF (HFrEF) is characterised by EF <40% whereas heart failure with preserved EF (HFpEF) is characterised by LVEF >50% accompanied by symptoms and/or signs, elevated natriuretic peptides and either structural changes in the heart or diastolic dysfunction (198). Similar clinical criteria but LVEF values

between 40-49% characterise the third category, namely heart failure with mid-range EF (HFmrEF) (198).

Risk factors and onset of cancer therapy-related cardiotoxicity of adjuvant breast cancer therapy drugs			
Anthracyclines	**Cyclophosphamide**	**Taxanes**	**Anti-HER2 therapy**
Risk factors			
Infusion time (bolus administration increases risk for cardiotoxicity)	Total bolus dose (seen mostly in doses >140 mg/kg)	Cardiotoxicity risk unclear due to lack of data and interaction with other agents such as anthracyclines.	Short time between anthracyclines and anti-HER2 treatment
Lifetime cumulative dose		Arrhythmias related to taxanes have been described; bradycardia, atrioventricular block, conduction disturbances, atrial fibrillation, subraventricular and ventricular tachycardias.	
Other concomitant chemotherapy, immunotherapy or targeted therapies	Other concomitant chemotherapy		Concomitant or previous use of anthracyclines
Mediastinal irradiation	Mediastinal irradiation		Mediastinal irradiation
Pre-existing heart conditions Hypertension			Pre-existing heart condition/ low LVEF Hypertension
Age (>65 years old or paediatric population)	Older age		Older age
Renal failure Female gender			High BMI
Onset			
Acute (<1%) Early (within 1 year) Late (several years, median 7 years)	Acute		During treatment

Table 2. *Risk factors and onset of cancer therapy-related cardiotoxicity of commonly used adjuvant breast cancer therapy drugs; anthracyclines, cyclophosphamide, taxanes and anti-HER2 treatment. BMI: Body Mass Index, HER2: Human epidermal growth factor receptor-2, LVEF: Left ventricular ejection fraction.*

The recommended by ESC categories of cardiovascular complications are (189):

1. Myocardial dysfunction and heart failure
2. Coronary artery disease
3. Valvular disease
4. Arrhythmias, especially those induced by QT-prolonging drugs
5. Arterial hypertension
6. Thromboembolic disease
7. Peripheral vascular disease and stroke
8. Pulmonary hypertension and
9. Pericardial complications.

Several efforts are made also by other organisations to provide a common definition of cancer therapy-related cardiotoxicity. For example, the Food and Drug Administration (FDA) defines it *"as >20% decrease in LVEF when baseline LVEF is normal, or >10% decrease when baseline LVEF is not normal"* (199). On the other hand, the recently published ESMO consensus guidelines consider LVEF decline by 10%, and especially if below the absolute value of 50%, qualifying as cardiotoxicity requiring further assessment and management (200). Despite reduction of LVEF by 10 points being the historically available cut-off value, recent studies, such as the SHORT-Her study adapted LVEF decline from baseline by 15 points as cardiotoxicity indicator (115, 200).

Observed indications of chronic myocardial damage expressed as elevated troponin measured at one of the follow-up visits in the Atherosclerosis risk in communities project (ARIC), in cancer survivors even after adjustment for "traditional" cardiovascular risk factors, imply distinct pathophysiology among cancer survivors than population (201). These findings support publications challenging the theory of distinction between acute and late-onset cardiotoxicity, meaning there is probably one process that is identified at different stages. Cardinale et al, reported development of asymptomatic anthracycline-induced cardiomyopathy among patients with normal baseline LVEF and no severe comorbidity, after mean follow-up of 5 years in about 9% of the patients; 98% of which had occurred within one year but were subclinical (202). An earlier publication by the same group reported that shorter time to treatment start of cardiac medication was related to good recovery after early diagnosis and immediate initiation of indicated cardiac medication for patients with LVEF 45% or less (203).

Another highly debated aspect is the stand on cardioprotection and up to date there is no conclusive data. The OVERCOME randomized study (n=90) reported improved outcomes

with prophylactic enalapril and carvedilol in intensively treated patients with haematological malignancies (incidence of death, heart failure or final LVEF<45%; 6.7% vs. 24.4% in control arm, p = 0.02) but the MANTICORE 101-Breast (n=99) did not show benefit of primary cardioprotection during adjuvant therapy for early breast cancer (204, 205).

Cardiotoxicity related to adjuvant therapy after breast cancer is an important issue, not only for a healthy survivor, but also in the case of relapse and/or metastatic disease and primarily, but not exclusively, for patients with HER2 amplified breast cancer. Taking into account the increased risk of cardiovascular disease in older age, it is highly probable that patients with primary or metastatic breast cancer present with cardiovascular disease, a risk factor for cardiotoxicity per se. At the same time, when diagnosed with cancer, a life-threating disease, the patients should not be deprived of curating treatment due to the fear of cardiac complications. Thus, optimal monitoring, management and potential benefit from early interventions or even cardioprotection in selected patients in high risk for cardiotoxicity remains an unmet need.

Anthracycline-associated cardiotoxicity

Anthracyclines were extracted from the Streptomyces bacterium and doxorubicin and epirubicin are two members of the anthracycline family administered in early and metastatic breast cancer. These two drugs are an integral part of adjuvant breast cancer regimens such as 5-fluorouracil, doxorubicin and cyclophosphamide (FAC), 5-fluorouracil, epirubicin and cyclophosphamide (FEC), doxorubicin and cyclophosphamide (AC) and docetaxel, doxorubicin and cyclophosphamide (TAC). Their harmful effect to the cardiomyocytes, in form of cardiotoxicity, is related primarily to cumulative dose, age, pre-existing heart conditions and way of administration (injection) (192, 206-208). Administration of doxorubicin over 48 or 90 hours prevented cardiotoxicity in contrast to a short infusion of 15-30 minutes (209).

A retrospective, registry-based follow-up of a randomised study (n=961) with a median follow-up of almost 17 years, demonstrated a 3-fold increase in cumulative risk for heart failure for patients treated with epirubicin (23/446) based polychemotherapy compared to CMF (9/515), even at mean epirubicin dose about 450 mg/m^2 (210). On the other hand, the large meta-analysis by EBCTCG in 2011 supports breast cancer survival benefit transcends over cardiotoxicity and subsequent cardiac mortality from anthracyclines (84). Thus, appropriate assessment of the cardiotoxicity risk versus breast-cancer survival benefit is of major value for the individual patient and risks versus benefit should be properly communicated to the patient.

Redox recycling through generation of reactive oxygen species (ROS) was initially believed to be the major mediator of anthracycline-related cardiotoxicity (211, 212). In recent years, alternative pathways have been proposed, such as myofibrillar disarray, mitochondrial

apoptosis and disruption of sarcomeres (211, 213-215). The different pathways involved in anthracycline-related cardiotoxicity are described in figure 7 (216). Anthracyclines are considered to cause cytotoxic effects through inhibition of topoisomerase II and differential action on tumour cells and cardiomyocytes has been proposed (211, 216, 217). Topoisomerase II is an enzyme involved in regulating DNA tangles by cutting both the DNA strands of a DNA helix simultaneously and after the repair is complete, the pairs are brought together again. Inhibition of topoisomerase II does not allow for the repair of the DNA leading to cell death. Topoisomerase II is classified in two categories, IIa and IIβ, and current data support that anthracycline anti-cancer effect is mediated by inhibition of topoisomerase IIα whereas detrimental effects on cardiomyocytes are caused through inhibition of topoisomerase IIβ and lethal DNA double strand breaks (211, 216).

Morphological changes in the cardiomyocytes have been demonstrated even in doxorubicin doses as low as 200 mg/m^2. Nowadays, doxorubicin is administered up to a maximum cumulative dose of 400-450 mg/m^2 since the risk for cardiotoxicity increases exponentially in higher doses (218). Other derivatives such as epirubicin and pegylated doxorubicin are less cardiotoxic at equivalent myelosuppressing doses, thus allowing for higher cumulative doses. A ratio of 0.5:1 epirubicin-to-doxorubicin has been identified for equivalent cardiotoxic potential and 0.75:1 for equivalent myelotoxicity (219).

Even though initially no maximum cumulative dose was established for epirubicin, Nielsen et al reported in 1990 severe cardiotoxicity from epirubicin cumulative doses exceeding 1000 mg/m^2 (220, 221). Later on, it was shown that cumulative risk of cardiotoxicity increased exponentially up to 15% at 1 000 mg/m^2 in contrast to 4% and 1.4% for doses 900 mg/m^2 and <600 mg/m^2 respectively (221-223). As a result, maximum epirubicin cumulative dose is currently at 900 mg/m^2 (189). However, a study of Danish population with metastatic breast cancer revealed that a 5% risk for cardiotoxicity translated to varying maximum tolerable cumulative epirubicin dose depending on the patient's age, comorbidity, hormonal therapy and chest irradiation (220). Thus, the maximum cumulative dose and risk for cardiotoxicity for the individual should be considered in relation to the presence of confounding factors. Figure 8 graphically presents reported cumulative cardiotoxicity incidence at different cumulative dose levels of doxorubicin and epirubicin from historical and more recent publications (189, 206, 221, 223, 224).

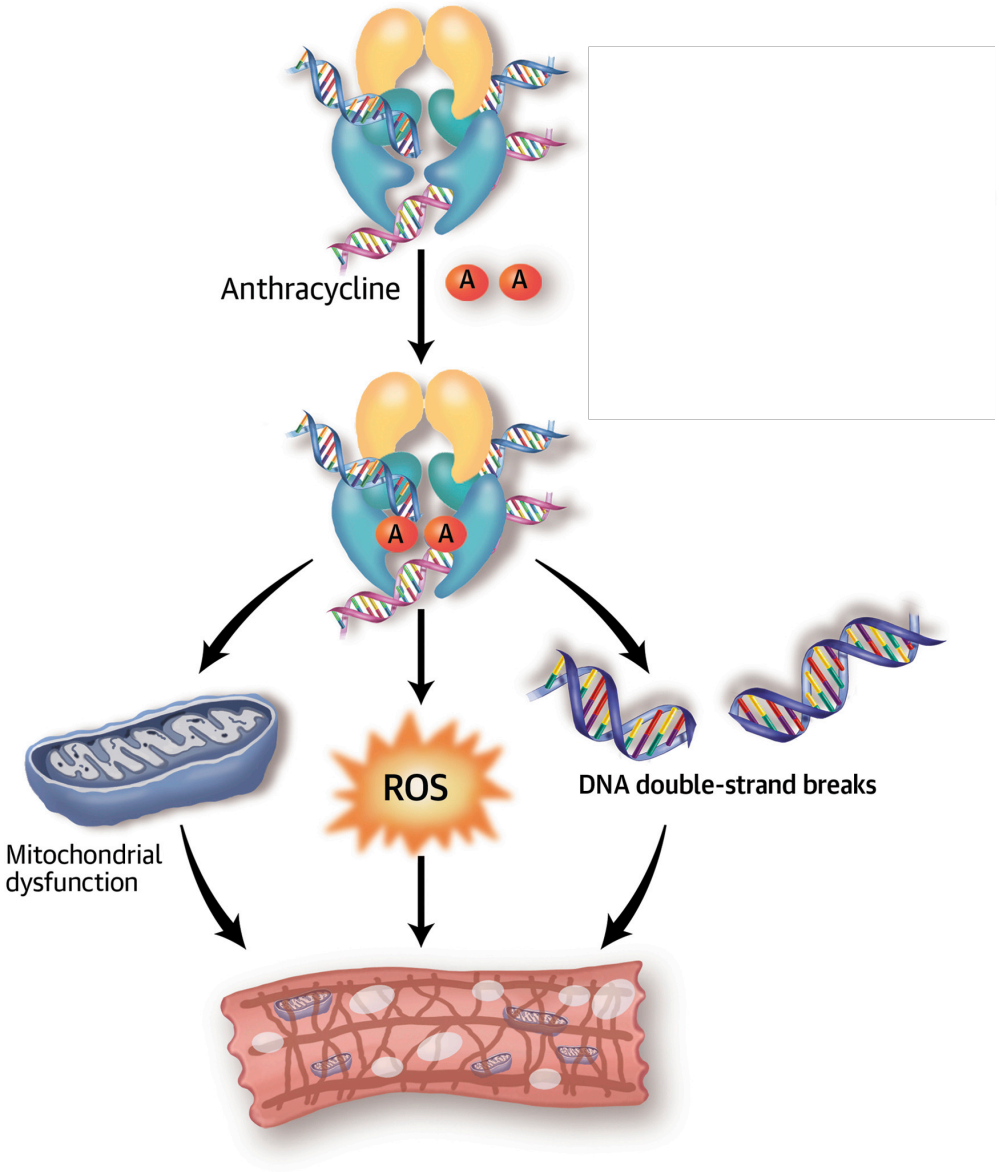

Figure 7. *Pathways involved in anthracycline-associated cardiotoxicity. ROS: Reactive oxygen species (Adapted and reprinted from J Am Coll Card, Vejngposa and Yeh, 2014:64:938-945, ©2014 with permission from Elsevier)*

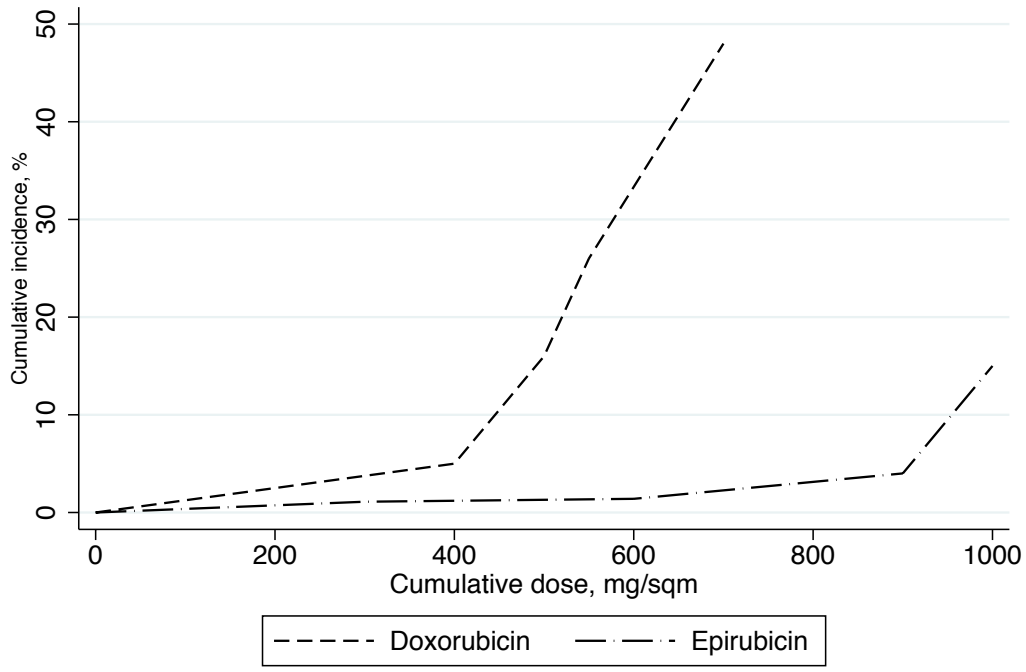

Figure 8. *A compilation of reported cumulative incidence of doxorubicin and epirubicin-associated cardiotoxicity.*

Trastuzumab-associated cardiotoxicity

HER2 is expressed in the cardiomyocytes and in the presence of neuregulin has an important role in to myocardial stress adaptation and survival (225). The effect of HER2 is mediated by regulation of apoptosis, cell growth, cell adhesion and angiogenesis, through activation of signalling pathways like extracellular signal-regulated kinase (ERK), 1/2-mitogen activated protein kinase (MAPK) and phosphoinositide-3-kinase-Akt (PI3K-Akt) (figure 9) (226). In animal studies, cardiac –restricted HER2 knockout mice exhibit abnormal cardiac development and dilating cardiomyopathy features (227). In contrast to anthracyclines, the structural and functional changes on the cardiomyocytes and inhibition of contractile elements caused by trastuzumab do not lead to cell death and therefore this kind of toxicity is considered reversible (189, 190, 228). Compromised cell recovery and repair by trastuzumab after anthracycline-related cell damage is considered to underlie increased incidence of cardiotoxicity when trastuzumab and anthracyclines are given concomitantly or in sequence but within very short interval (226).

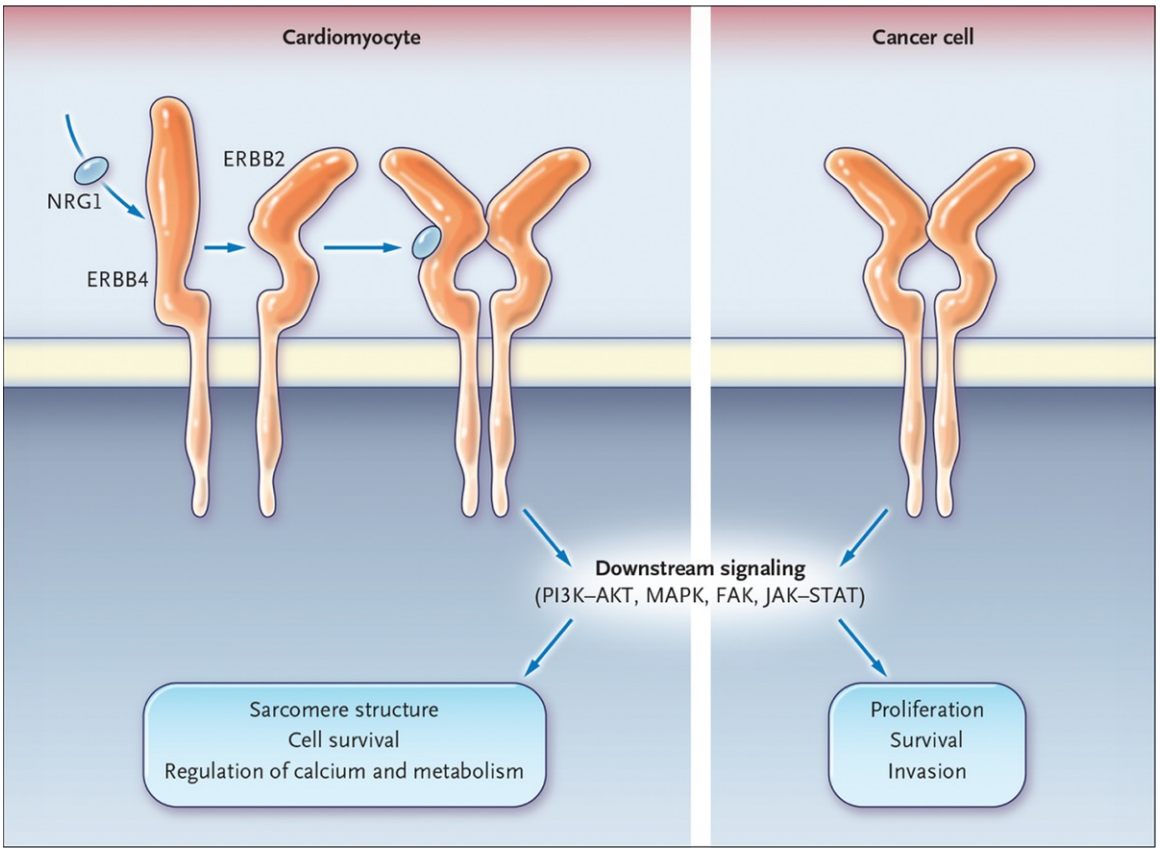

Figure 9. *HER2 signalling pathway in the cardiomyocyte and in cancer cells. ERBB2, also known as HER2-neu: Epidermal growth factor 2, ERBB4, also known as HER4: Epidermal growth factor 4, FAK: Focal adhesion kinase, JAK-STAT: Janus kinases- signal transducer and activator of transcription proteins, MAPK: mitogen-activated protein kinase, NRG1: Neuregulin 1, PI3-AKT: phosphatidylinositol 3-kinase–AKT (Reproduced with permission from Cote el al, N Engl J Med 2012;367:2150-2153, © Massachusetts Medical Society)*

The true incidence of trastuzumab-induced cardiotoxicity, the ideal window between anthracyclines and trastuzumab administration and the status of prophylactic cardiac medication are still disputed. The initial studies performed by Slamon, investigating efficacy of trastuzumab in patients with metastatic HER2 positive breast cancer, surprisingly revealed 27% heart failure of New York Heart Association functional class (NYHA) III or IV among patients treated with concomitant anthracyclines and trastuzumab compared to 8% of those not receiving addition of trastuzumab (100, 229). An independent Cardiac Review and Evaluation Committee (CREC) was founded with mission to supervise trastuzumab trials. The CREC established some criteria and described trastuzumab-cardiotoxicity syndrome *"as decline in left ventricle ejection fraction (LVEF) of at least 5% to less than 55% with accompanying signs or symptoms of congestive heart failure (CHF), or a decline in LVEF of at least 10% to below 55% without accompanying signs or symptoms"* (229). Reflecting the impact of previous delivered chemotherapy, an assessment of the early trastuzumab trials by the CREC reported cardiac dysfunction, according to published predefined criteria, up to 3% when single trastuzumab was administered as first line treatment and 5% when administered

as second or third line (229). Subsequent adjuvant trials have reported markedly lower cardiotoxicity incidence; as high as 7.3% (107, 230). Notably, younger, healthy individuals with normal LVEF and no pre-existing cardiac conditions were enrolled in these trials, not always reflecting the population treated in real life conditions.

The HERA trial reported asymptomatic or mildly symptomatic heart failure with significant LVEF drop by 10% below baseline and below 50% among 4.4% of patients treated with trastuzumab for 1 year but almost the double after two years trastuzumab (7.3%) (107). Even if trastuzumab-induced cardiotoxicity is considered to be dose-independent most studies investigating shorter trastuzumab duration report increased cardiotoxicity with longer treatment duration (114, 115, 118, 231). A recently published meta-analysis of six trials reported compared 1-year trastuzumab with shorter duration. One-year trastuzumab increased pooled risk for cardiac dysfunction (defined as heart failure or significant LVEF decline) compared to shorter treatment intervals (8.2% vs. 4.8%) but the overall incidence remained low and less than 10% (232).

The prospective observational OHERA study included non-selected patients (n=3 938) that fulfilled the indication for adjuvant trastuzumab (233). Cardiac assessments were performed per local guidelines and therefore introduce heterogeneity in the results. Interestingly 49 patients (1.3%) had known history of heart failure at baseline and approximately 42% had more than one known risk factor for cardiovascular disease. Only a small percentage of the patients developed heart failure (n=160, 2.8%) and median time to diagnosis was 5.7 months (233). Unsurprisingly, five of six patients suffering cardiovascular death had pre-existing cardiovascular risk factors (233). The study confirmed previously reported risk factors for trastuzumab-related cardiotoxicity such as pre-existing cardiac conditions, hypertension, obesity and age over 65 years and, additionally, identified normal range but low baseline LVEF (≤55%) as independent risk factor (233). Table 3 presents incidence and definition of cardiac events reported in some of the adjuvant trastuzumab trials with varying trastuzumab duration (107, 114, 115, 230, 233-235). Difference in the definition of cardiac events, assessment and duration of follow-up should be taken in consideration.

Early phase studies of (neo)adj T-DM1 with treatment duration 1 year demonstrate reportedly low incidence of asymptomatic LVEF decline (2.7%, n=4) but no cardiovascular events or heart failure (236). A recent individual patient data pooled meta-analysis of T-DM1 trials including 1961 patients showed low rates of cardiotoxicity with heart failure/significant LVEF drop as low as 0.71% and cardiac ischemia 0.1% and a total rate for cardiotoxicity at 3.37% (237). Age over 65 years, low baseline LVEF (below 55%) and the combination of T-DM1 with pertuzumab were identified as risk factors for T-DM1 related cardiotoxicity (237).

CARDIAC SAFETY ENDPOINTS IN SELECTED ADJUVANT TRASTUZUMAB TRIALS				
Study	**Number of patients**	**Trastuzumab duration**	**Primary cardiac safety endpoints**	**Outcome**
SHORT-HER (Conte el al, 2018)	N=1254	1 year vs. 9 weeks	- LVEF decline >15% or LVEF decline >10% & absolute value <50% - Heart failure - Other cardiac events of grade ≥2	9 weeks 4.3% 1 year 13.1% [c]
SOLD (Joensuu et al, 2018)	N=2174	1 year vs. 9 weeks	Heart failure, myocardial infarction or cardiac/coronary surgery	9 weeks 2%[c] 1 year 4%
PERSEPHONE (Earl et al, 2019)	N=4089	1 year vs. 6 months	- Symptoms of cardiac disease and/or signs of HF and/or new/altered cardiac medication within 12 months from trastuzumab start -LVEF absolute value <50% or reported as low without quantification	6 months 8%[c] 1 year 11% 6 months 9% 1 year 11%
HERA[a] (Cameron et al, 2017)	N=5102	2 years vs. 1 year	- NYHA III-IV - LVEF decline >10% & absolute value <50% - Cardiac death	1 year 1% 2 years 1%
BCIRG06[a] (Slamon et al, 2011)	N=3222	1 year trastuzumab and: anthracycline chemotherapy (AC-TH) vs. non-anthracycline chemotherapy (TCH)	- Heart failure - Sustained asymptomatic LVEF decline >10%	AC-TH 2%[c] TCH 0.4% AC-TH 18.6%[c] TCH 9.4%
OHERA[b] (Lidbrink et al, 2019)	N=3938	Included patients fulfilling recommendation for 1 year trastuzumab	- Symptomatic HF (NYHA II-IV)	2.8% (NYHA III-IV 1%)

Table 3. *Reported incidence of cardiac adverse events in selected adjuvant trastuzumab trials with varying trastuzumab treatment duration. AC-TH: Doxorubicin/Cyclophosphamide (AC) followed by docetaxel/trastuzumab (TH) with trastuzumab duration for 1 year, HF: heart failure, LVEF: Left ventricular ejection fraction, NYHA: New York Heart Association functional class, TCH: Docetaxel/carboplatin/trastuzumab with trastuzumab duration one year*
[a]The group that was treated with trastuzumab is not reported here, [b]Non-randomised observational study, [c]p ≤0.01.

Other anti-HER2 agents have also shown low cardiotoxicity rates and combinations have not increased cardiotoxicity rates. Dual HER2 blockage with the addition of pertuzumab to trastuzumab did not exacerbate cardiac safety (110, 119, 238-241). In fact, in the CLEOPATRA trial, there were numerically less left ventricle dysfunction events in the combination of docetaxel, trastuzumab and pertuzumab (6.6%) than in the docetaxel and trastuzumab group (8.6%) (119). It is unclear whether this is a chance finding or if there could a plausible biological explanation related to the distinct way the two drugs block HER2 activation. Likewise, a pooled analysis of the combination of pertuzumab with non-anthracycline chemotherapy, trastuzumab or erlotinib revealed no added cardiotoxicity (242). Moreover, lapatinib and trastuzumab alone, in combination or in sequence, reported cardiotoxicity between 2-3% (109, 243). A meta-analysis of different lapatinib studies also reported cardiac adverse events as low as 3% (244). Finally, neratinib in sequence or in combination with trastuzumab does not either demonstrate significant cardiotoxicity (245-247).

In the majority of the patients that develop trastuzumab-related heart failure, LVEF recovers on average 6-9 months after treatment discontinuation (233, 248). Patients with 6 months trastuzumab recovered more rapidly than the ones that received 12 months treatment in the PERSEPHONE trial (112). Nonetheless, continuing trastuzumab even after LVEF decline below 50% seems to be relatively tolerable (249). In a retrospective analysis including 60 patients by Houssain et al, the majority of the patients (61%) that continued trastuzumab besides reduced LVEF could continue without cardiac event and only 13 % developed a cardiac event (249). As expected, almost all patients (91%) received cardiological care with introduction or titration of cardiac medication and after median follow-up of 633 days there was no difference in the final LVEF between patients that interrupted trastuzumab (and eventually restarted afterwards) and the ones that continued with trastuzumab (54% vs. 56%, p=0.29) (249).

In similar manner, initiation of anti-HER2 treatment in patients with baseline LVEF less than 50%, even if asymptomatic, has been met with reservation. The results from the SAFE-HEaRT study though reported that up to 90% (27 out of 30) of asymptomatic patients with LVEF 40-49% at baseline could complete planned anti-HER2 treatment with proper cardiac medication and cardiac monitoring (250).

In summary, anti-HER2 agents are integral part of HER2 positive breast cancer management with undisputable impact on survival. Interpretation of the results from various publications calls for caution since different definitions of cardiotoxicity are used and varying chemotherapy regiments in combination or in sequence with trastuzumab have been administered in different studies.

Cyclophosphamide and cardiotoxicity

Cyclophosphamide is an alkylating agent belonging to the nitrogen mustard family and is administered in combination with anthracyclines during treatment for early or metastatic

breast cancer. Sulphur mustard was a milestone in the development of chemotherapy and it was the first chemotherapeutic to be tested in a clinical trial.

Cardiotoxicity with acute onset related to cyclophosphamide has been described and it is related to high delivered dose at one occasion (> 150mg/kg) rather than cumulative dose (251, 252). In doses over 150 mg/kg acute heart failure up to 33% is reported with onset within days or few weeks and dose per administration (calculated on weight or body surface area) is directly related to the risk of cardiotoxicity (252, 253). It is suggested that the cyclophosphamide metabolites 4-hydroxy-cyclophosphamide and acrolein, mediate cardiotoxicity through ROS generation and suppression of the activity of aldehyde dehydrogenase leading to complex pathways (254). Partly, the mitochondria are damaged by ROS and impediment of the Krebs cycle by increased mitochondrial membrane permeability (255). Furthermore, ROS generation and subsequent oxidative stress can activate nuclear factor kB (NF-kB) leading to cytokine release (256).

When administered in immunomodulating aim such as in rheumatological conditions or to prevent graft rejection, cyclophosphamide doses are low and the risk for cardiac adverse events is expected to be insignificant. In breast cancer, cyclophosphamide is usually given in combination with anthracyclines in doses 500-600 mg/m^2. A person with a mean body surface area of 1.73 m^2 would then be expected to receive 865 – 1038 mg; doses below those expected to increase the risk for cardiotoxicity. However, in protocols such as the PANTHER trial that will be discussed in the current thesis, cyclophosphamide doses can escalate up to 1200 mg/m^2. Even though, the risk for cyclophosphamide-related acute cardiotoxicity is not entirely negligible, one would not expect cyclophosphamide to impose major impact on the risk and incidence of long-term cardiotoxicity.

Taxanes and cardiotoxicity

These microtubuli inhibitors belong to a drug class called plant alkaloids and as the name implies they are derived from plants; specifically from the Pacific Yew tree. Docetaxel and Paclitaxel are the ones commonly used in the management of breast cancer. In early breast cancer, taxanes are most often used in sequence to anthracycline regimens, although taxane-only schedules in combination with trastuzumab are now also an option for HER2 positive early breast cancer. Taxanes are considered to disturb the conduction system of the heart and potentially cause arrhythmias. They have not shown the same cardiotoxic potential as anthracyclines and are regarded as a safe alternative, even though the cumulative risk is unclear. Data from trials investigating anthracycline-free regimens demonstrate low rates and indifferent cardiotoxicity incidence between anthracycline/cyclophosphamide and docetaxel/cyclophosphamide combinations but long-term data are scarce (257-259).

Fluorouracil and cardiotoxicity

Fluorouracil (5-FU) and its oral pro-drug capecitabine are synthetic drugs and are widely used across different solid tumours as single therapy, in chemotherapeutic combinations and as radiosensitisers during radiotherapy.

Cardiotoxic potential is mediated mainly due to endothelial injury and vasospasm-induced ischemia during the treatment period and presented as angina, myocardial infarction and ventricular arrhythmia (260). However, not all symptomatic patients have findings in line with vasospasm or endothelial damage and transient changes similar to Takutsubo's cardiomyopathy has also been suggested as underlying mechanism (261). Reported data provide varying but low incidence (<10%) of fluorouracil-related ischemia but, once manifested, can be fatal to 8% of the patients (262). Efforts to re-challenge should be accompanied with caution due to 90% risk for recurrence of symptoms and mortality up to 13% (261).

Administration as infusion causes toxicity in 2-18% and pharmacodynamics during administration as oral capecitabine are comparable to bolus or short infusion (261). The risk for cardiotoxicity (3-9%) was similar to infusion administration implying that both treatment schedule and drug-specific pharmacokinetics impact the risk for cardiotoxicity in this case (261).

1.10 Neutropenia and G-CSF prophylaxis

Haematological toxicity in the form of anaemia, leukopenia/neutropenia and thrombocytopenia is common dose-limiting toxicity following chemotherapy administration. Neutropenia can be complicated with fever (febrile neutropenia, FN) or opportunistic infections. FN is a potentially life-threatening condition, can require hospitalization, antibiotics administration and impose considerable health economic costs on both the patient and the health care system. The common terminology criteria for adverse events (CTC AE) version 3.0, describes FN as fever over 38.5 ^{o}C and absolute neutrophil count [ANC] <1.0 x10^{9}/L whereas the more recent version 5.0 requires fever over 38.3 ^{o}C as single measure or at least 38 ^{o}C persisting for more than one hour.

About 8 patients per 1000 receiving contemporary standard-dose chemotherapeutics develop FN (263). Median length of hospitalization due to FN for breast cancer patients is reported to 4 days and mean cost per hospitalization in Western countries is estimated at approximately 13 500 € (263, 264). Development of neutropenia and FN can have an impact on dose intensity during chemotherapy, which in turn can negatively affect cancer related outcomes (89, 95, 265). A meta-analysis including 10 trials performed by Kuderer et al, reported increase in mean relative dose intensity with granulocyte-colony stimulating factor (G-CSF) support from 86.7% to 95.1% (mean difference 8.4%, p=0.001) (266).

The use of exogenous G-CSF has shortened the length of neutropenia, length of hospitalization and severity of febrile neutropenia but potential impact on mortality is debated (266, 267). G-CSF is a main regulator of granulopoiesis and the survival, proliferation and differentiation of granulocytes (neutrophils, basophils and eosinophils); the body's critical

soldiers against microbial infections and inflammatory processes. Initially it has a synergistic action with "early acting cytokines" and promotes the proliferation of hematopoietic stem cells, which in turn will engage in myeloid differentiation for the production of neutrophils (268, 269). Subsequently, G-CSF acts as "late acting cytokine" and influences granulocyte maturation (268). Expression of the G-CSF receptor is encountered in both immature myelocytes and mature neutrophils and it seems that G-CSF levels are regulated by the latter through a negative-feedback loop (270). Exogenous administration of G-CSF has been shown to expand the mitotic pool of progenitor granulocytes (myeloblasts, promyelocytes, myelocytes) and mobilise the postmitotic pool (metamyelocytes to neutrophils) (268). Finally, G-CSF administration can also promote peripheral neutrophil activation (271).

Use of G-CSF has also permitted for higher relative dose intensity without excess myelotoxicity (272). G-CSF primary prophylaxis is recommended by international guidelines for chemotherapy regimens with risk for FN 20% or above (263, 273, 274). Risk assessment should include: i) tumour factors such as cancer type and stage, ii) planned chemotherapy regimen and iii) patient-related factors such as age, comorbidities, poor performance, low baseline blood cell counts, tolerance of previous chemotherapy or previous episode of neutropenic fever (263, 274).

Over time there has been a steady decline in mortality related to neutropenic complications from 10% to 5.4% (275). Breast cancer patients have had low FN in-hospital mortality compared to FN in other malignancies and it has been accordingly declining (276). FN in-hospital mortality was reported at 3.6% in 2006 by Kuderer et al but Pathak et al in 2015 reported incidence of in-hospital mortality related to FN reduced to 2.6% (276, 277). The reduction of FN related mortality has been attributed to the use of less toxic chemotherapeutic agents, more effective treatment of FN and awareness for early detection of sepsis (275).

The available G-CSF agents are divided into two major categories: the short-acting filgrastim, and the long-acting pegfilgrastim. Filgrastim is cleared by the kidneys and requires administration several days in a row until the nadir phase is passed as depicted in figure 10. Pegfilgrastim on the other hand, has a sustained-released formulation that requires only one administration after each chemotherapy cycle and is cleared through circulating neutrophils.

Despite the well-documented beneficial effect of G-CSF, adherence to guidelines has not been optimal. Occasionally, patients do not receive G-CSF prophylaxis in spite of adequate risk level, or G-CSF prophylaxis is discontinued early during the chemotherapy course without coverage for all cycles (278). Based on observations that the risk for FN is highest during the first two cycles but then declines, a phase III study, reported by Aarts et al, compared administration of G-CSF for only the first two cycles of adjuvant breast cancer chemotherapy or all subsequent cycles (279). The study had to close prematurely after an interim analysis revealed 5 times higher risk of FN (HR 5.4, 95% CI 2.3 – 12.6) when G-CSF administration was limited to the first two cycles (38%) compared to prophylaxis at every chemotherapy cycle (10%) (279). These results, together with the fact that 24% of the patients who discontinued G-CSF after two cycles developed FN after the third cycle, further underline the importance and benefit of G-CSF prophylaxis.

FEC followed by docetaxel (D) is widely used for adjuvant or neoadjuvant treatment of breast cancer. This schedule was in the PACS01 trial compared to six cycles of FEC and

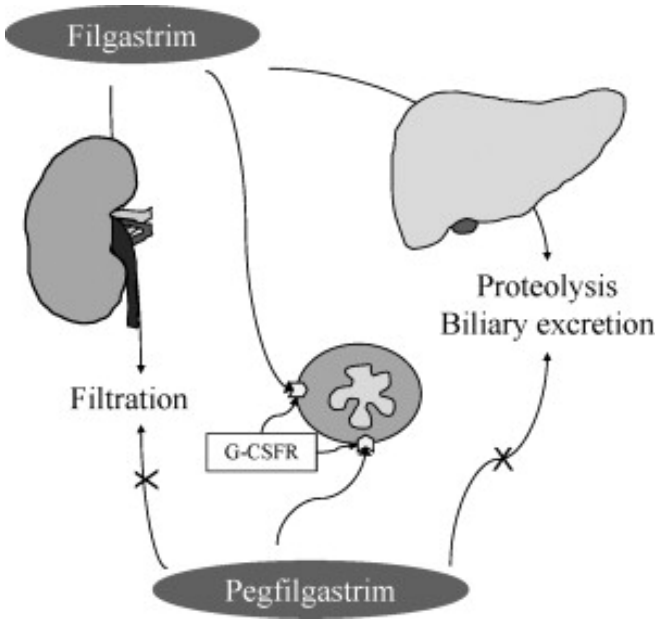

Figure 10. *Elimination of filgrastim and pegfilgrastim. G-CSFR: Granulocyte colony stimulating factor receptor. (Reprinted from Crit Rev Onco Hematol, 72(1):21-44, Crea et al, © 2009, with permission from Elsevier).*

grade 3-4 FN was reported among 11.2% of the patients treated with the FEC/D sequential schedule, compared to 8.4% of patients receiving only FEC (280). It is worth mentioning that only secondary G-CSF prophylaxis was allowed in the protocol and if G-CSF was administered during FEC, it was discontinued upon docetaxel initiation (280). A retrospective study by Miguel et al, reported even higher FN incidence of 21% during docetaxel and 7% during EC (281). Additional retrospective data report 26% FN among 168 patients that were treated with FEC-D and about half of the events occurred during FEC and half during D (282). The same study reported also three-fold higher incidence of FN among patients that did not receive G-CSF versus those who did; 31% versus 10%. Interestingly, patients that received trastuzumab in combination with chemotherapy reportedly had higher risk for developing FN. Febrile neutropenia grade 4 despite pegfilgrastim prophylaxis was reported in 14% of patients that received anthracycline and cyclophosphamide after previous taxane and carboplatin in a recent neoadjuvant Belgian study (283).

Whether any of the two G-CSF formulations is superior than the other is debated. A recent meta-analysis showed little difference between the two, and supports that potential superiority of pegfilgrastim could be related to suboptimal dosing of short-acting filgrastim (284). Conversely, early reports from 2009 and 2013 claim higher cost effectiveness with pegfilgrastim and also underline the patient-perspective of only receiving one injection (285, 286).

The initial approval of filgrastim in 1991 recommended initiation of treatment already 24 hours after chemotherapy completion until absolute neutrophil count (ANC) was over 10 x 10^9/L and duration could be up to two weeks. Mean reported duration in early clinical trials was 10-11 days (287). However, in real life setting and subsequent clinical trials, delayed start and shorter treatment duration demonstrated equivalent effect and shorter duration is

most usually used today (288-290). Nowadays, ESMO guidelines recommend administration of filgrastim at 5 μg/kg/day s.c. within 24-72 hours after chemotherapy and until the ANC nadir is over, usually at ANC levels of 2-3 x 10^9/L (263). Pegfilgrastim is recommended at a single fixed dose of 6 mg the day after chemotherapy (263). There is data implying administration of pegfilgrastim 72 hours after chemotherapy could be more beneficial by causing less leucocytosis while maintaining adequate prophylaxis towards neutropenia (291). However, it is early data from a small non-randomised study and should be assessed accordingly.

The most commonly reported side effect during administration of G-CSF is bone and/or musculoskeletal pain and it was reported up to almost 20% of patients in a review including 17 randomised trials (266). Pathogenesis of G-CSF related bone pain is not restrictively due to bone marrow quantitative and qualitative expansion; quantitative due to expansion of the granulocyte progenitors and qualitative due to stimulation of mature cells and secretion of cytokines (292). Other suggested mechanisms are through G-CSF action on pathways involved in pain modulation but also through involvement in sensitisation of peripheral receptors to nociceptive stimuli by modulating inflammatory response (290, 293, 294).

Administration of primary G-CSF prophylaxis during adjuvant breast cancer treatment is feasible and commonly used. On the contrary, the benefit of adjunctive G-CSF after manifested FN is ambiguous. G-CSF administration in combination with antibiotics appears to shorten the length of hospitalisation but impact on FN-related mortality is not clear (267). Thus, blind administration of G-CSF after manifested neutropenia is not recommended. It should, however, be considered in patients with high risk for infection-related complications or co-morbidities that predispose to poor outcome in case of prolonged infection and/or hospitalization.

1.11 Chemotherapy-induced gonadal toxicity

Globally, 31% (n=644 753) of women diagnosed with breast cancer in 2018 were younger than 50 years old (3). Ovarian ablation by surgery or irradiation or suppression by GnRHa improves breast cancer outcomes among women with breast cancer with ER positive or unknown status (123). In absolute numbers, 15-year risk for recurrence is improved by 4.3% ($p<0.001$) and risk for breast cancer death by 3.2% (123). Moreover, ovarian suppression with luteinising hormone releasing hormone agonists (LHRHa) in addition to tamoxifen, chemotherapy, or both, in ER positive breast cancer improved breast cancer recurrence and death (126).

Nevertheless, even in the absence of ablation, ovarian function among young women can be impaired due to chemotherapy, leading to amenorrhea and premature menopause (chemotherapy-induced amenorrhea, CIA). This becomes more relevant when considering

that average age at spontaneous, natural menopause is 51 years (295). The incidence of CIA varies greatly from 10% to 97% (296-298).

Even though loss of primordial follicles is often cited as the cause of CIA, several pathways are reported to be involved. Oocyte death through chemotherapy-induced damage to somatic cells (necessary for the survival of the oocytes) and "burn out of follicle reserve" in an effort to replace apoptosis of developing follicles are alternative pathways (299). Moreover, acute vascular damage, reduction of the blood flow in the ovary and fibrosis of the cortical stroma have been reported following doxorubicin administration (295).

The heterogeneity of the reported incidence results depends on a plethora of reasons such as type of chemotherapy agents administered and duration of treatment, duration of follow-up, age at breast cancer diagnosis and subsequent long-term endocrine therapy. The risk of CIA seems to be increased when chemotherapy is administered during the follicular phase of the menstrual cycle (300). Mean time to induction of CIA is reportedly shorter in older women (2-4 vs. 6-16 months) (301). Addition of tamoxifen leads to a two-fold increase of amenorrhea incidence after chemotherapy with or without trastuzumab (OR, 2.12; 95% CI, 1.13 - 4.00); probably through hypothalamus-ovarian feedback loop (297, 302-305).

A meta-analysis by Zavos et al, reported increasing CIA incidence with increasing age at diagnosis; 26% for women under 35 years old and 77% for women above 40 years old (297). A recent combined analysis of the PACS04 and PACS05 studies demonstrated menstruation recovery in 63% of women younger than 35 years, 56% amid those aged 35-39 years and a drastic reduction to 20% among women older than 40 years (306). Increased risk for premature ovarian failure among older women is likely a result of chemotherapy-induced destruction of follicles among women with a de facto low primordial follicles reserve (307). Even breast cancer survivors who remained, per definition, premenopausal after adjuvant chemotherapy have diminished follicle pool and reduced levels of anti-Müllerian hormone (AMH) compared to age- and pregnancy-matched controls (302). Administration of tamoxifen was related to lower follicle count, AMH and inhibin B and higher estradiol levels (302).

Notably, amenorrhea in direct conjunction to the completion of adjuvant chemotherapy is not a sign of permanent premature ovarian failure. Recovery of ovarian function and resumption of menstruation has been seen within 24 months after end of chemotherapy. As expected, younger women (<35 years) are more likely to resume menstruation than older women and up to 85% of the former can recommence menstruation (308). Most available data are due to older chemotherapy trials and, nowadays, prolonged duration of endocrine therapy is recommended for high-risk breast cancer. Hence, potential impact and confounding of adjuvant endocrine therapy and concomitant use of GnRHa should be considered when interpreting results and advising a patient. Relation of menstruation recovery and time from completion of chemotherapy is depicted in figure 11 (306).

Infertility is the most commonly associated detrimental effect related to CIA, although not the only one. Fertility preservation methods such as oocyte or embryo cryopreservation have been developed with success and the field is further developing; for example, with

transplantation of ovarian tissue or uterus. Recent reports support GnRHa administration during chemotherapy reduces the risk of CIA and premature ovarian insufficiency (309). An individual patient data meta-analysis of five major trials by Lambertini et al reported lower premature menopause incidence (14.1% versus 39.9%; adjusted OR 0.38, 95% CI, 0.26 – 0.57) and increased rate of pregnancies (10.3% versus 5.5%) after chemotherapy in combination with GnRHa versus chemotherapy alone, without impact on disease-free or overall survival (310). A multivariate analysis revealed administration of GnRHa and young age at diagnosis as having a prophylactic effect, further supported by the fact that all pregnancies presented among women younger than 40 years old at the time of diagnosis (310). Thus, GnRHa probably have a place in the prevention of ovarian function and fertility without compromising breast cancer outcomes.

Apart from infertility, ovarian insufficiency in young age can also trigger other long-term effects related to low oestrogen levels and can deteriorate quality of life. Such examples are menopause-related vasomotor symptoms, osteoporosis and increased risk for cardiovascular disease and related mortality due to premature menopause (172, 311). Iatrogenic ovarian failure secondary to chemotherapy was found to be related to decreased sexual interest but did not independently affect sexual function among women treated for breast cancer (312). Hormonal substitution in this population is categorically contraindicated and thus other methods should be employed for relief of these patients.

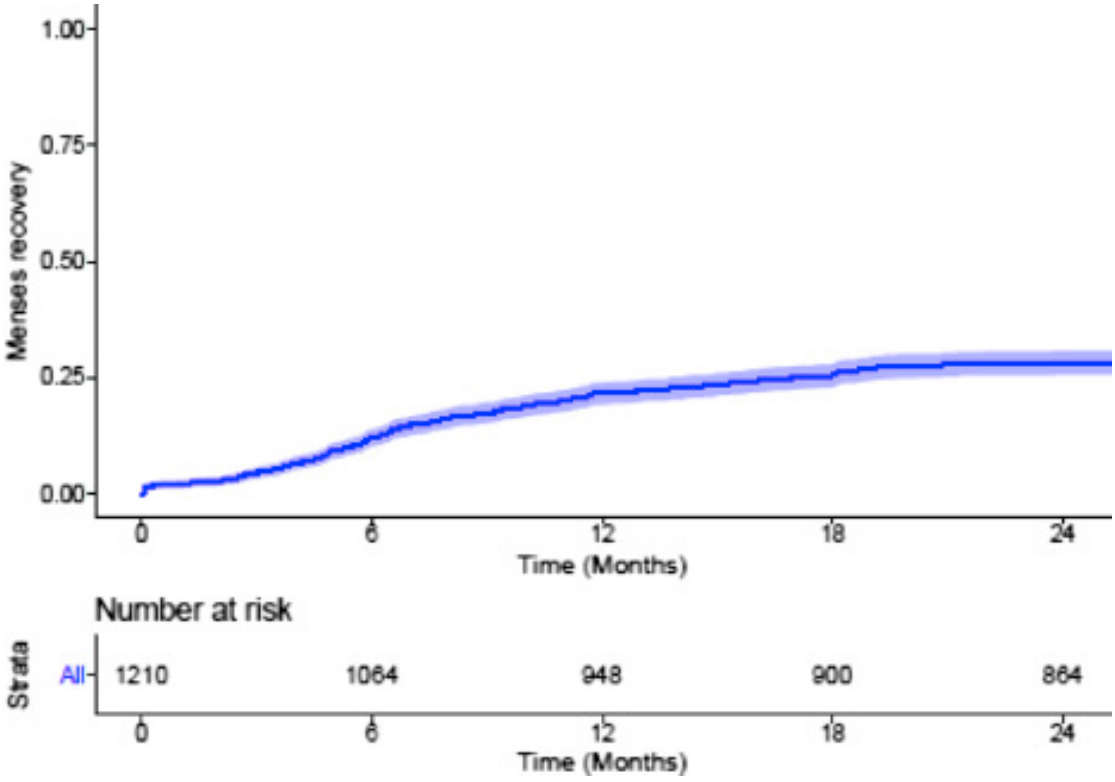

Figure 11. *Resumption of menstruation up to 24 months after completion of chemotherapy for early breast cancer. (Reprinted from Clin Breast Cancer, 19(1):63-70, Pistilli et al, ©2019, with permission from Elsevier)*

In spite of the notable side effects, the significant survival benefit of this specific chemotherapy-related toxicity should be emphasised, not least considering the persistence of the CIA-associated improvement of breast cancer outcomes regardless oestrogen receptor status (296). In a prospective randomised trial investigating doxorubicin-cyclophosphamide (AC) and docetaxel (D) in sequence (AC-T), concomitantly (TAC) or only AC, women with premenopausal status at diagnosis and subsequent development of CIA reported improved overall survival and disease free survival; RR0.76 and 0.70 respectively ($p=0.04$ and $p<0.001$) (296). The favourable effect of CIA in terms of disease-free and overall survival is further supported by a meta-analysis from 2014 (RR OS 0.60 and RR DFS 0.67) and a retrospective analysis of the MA.5 trial (HR OS 0.40, 95% CI 0.22 – 0.72 and HR relapse-free survival 0.51, 95% CI 0.32 – 0.82) (313, 314).

Increased chemotherapy-dose intensity is cited to elope greater risk for subsequent CIA. Dose intensive chemotherapy in the IBCSG Trial 15-95 resulted in amenorrhea 7-9 months after randomisation in 93%, versus 78% after standard chemotherapy (315). Amenorrhea was more likely to become permanent after dose intensive therapy and the difference in transient and permanent amenorrhea between the two treatment schedules was more prominent among women younger than 40 years; 61% versus 24% for the latter (315). Maintaining same cumulative dose but increasing dose-density has not led to increased CIA incidence (305, 316, 317).

Even if cumbersome, assessment of menopausal status is of paramount importance for women with hormone receptor positive breast cancer in order to decide upon appropriate endocrine therapy. Even though AIs are superior to tamoxifen, establishing a patient is postmenopausal prior to treatment initiation is a prerequisite. Thus, efficacy of AIs in women with ovarian function, even if not at adequate levels to cause menstruation, is impeded by simultaneous estradiol production in the ovaries. Moreover, AIs in seemingly postmenopausal young women can stimulate the hypothalamus/pituitary pathway and subsequently increase estradiol levels by FSH release (318). Figure 12 presents hormonal regulation of oestrogen production through the hypothalamus/pituitary-ovaries pathway in premenopausal women.

Despite addition of GnRHa to AI when offered to young premenopausal patients, complete ovarian function inhibition needs to be ensured due to potential survival concerns otherwise (129). Since ovarian function may spontaneously recover several months after the end of chemotherapy, hormone-levels shortly after chemotherapy administration should not be considered reliable. Thus, a patient's age, menopausal status, gynaecological history and given chemotherapy should be taken into consideration when assessing probability of premature ovarian failure and endocrine therapy.

Gonadal toxicity and agents used in adjuvant breast cancer chemotherapy regimens

The type of chemotherapeutic agent used and achieved cumulative dose have a direct impact on the risk of CIA (297). Alkylating agents, of which cyclophosphamide is of interest in the

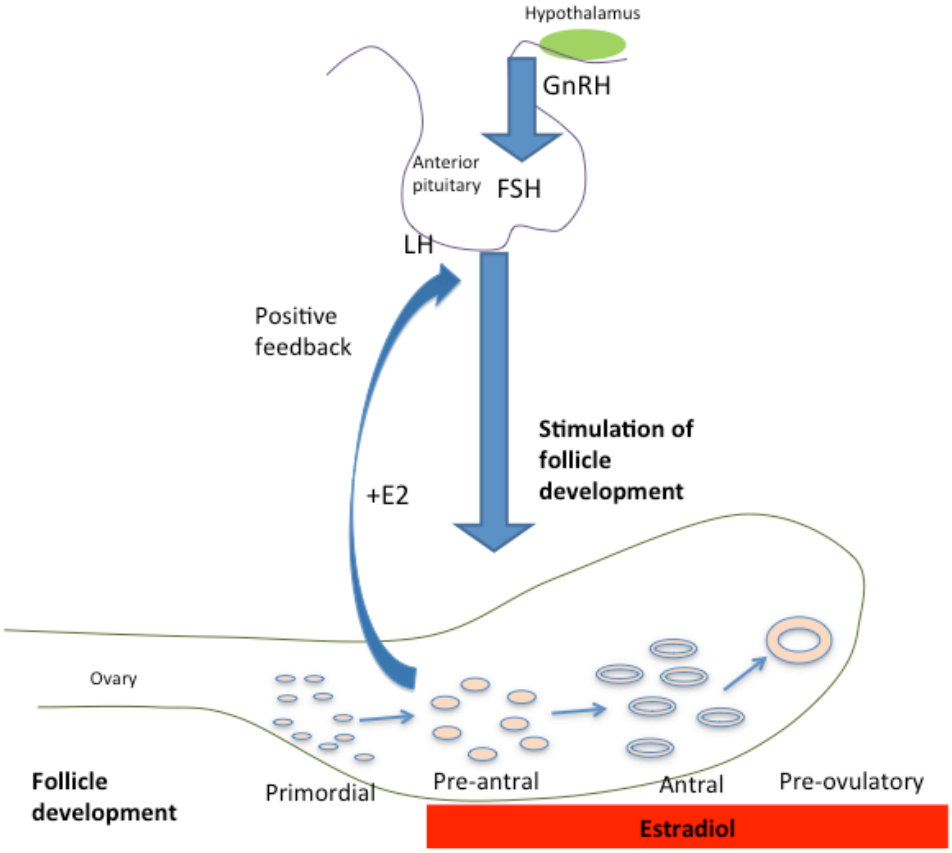

Figure 12. *Regulation of oestrogen production in the ovaries and follicle development in premenopausal women. (Modified from Papakonstantinou A et al: J Clin Oncol 34: 1573-1579, 2016, Reprinted with permission. © 2016 American Society of Clinical Oncology. All rights reserved.)*

treatment of early and metastatic breast cancer, are considered highly toxic to the ovaries damaging both resting and growing follicles (319). Cyclophosphamide is reportedly related to permanent CIA in up to 97% of women older than 40 years old (298). The lack of cell-cycle specificity is considered to be responsible for the 4-9.3 fold increased risk of amenorrhea among patients treated with cyclophosphamide compared to controls (319). On the contrary, cell-cycle specific drugs such as 5-fluorouracil do not seem to impact follicle number (303).

An early trial published in 1986, reported amenorrhea in 80% of premenopausal women treated with doxorubicin-containing regimens (320). The data regarding potency of anthracycline-regimens versus CMF in terms of CIA is inconsistent pointing at similar CIA incidence after anthracyclines and CMF or increased CIA after CMF, probably due to higher cyclophosphamide doses (304, 308, 314). A retrospective analysis of the PACS04 and PACS05 trials singled out non-alkylating chemotherapy to increase likelihood of menstruation resumption (306).

The gonadal toxicity related to taxanes is largely unknown. Administered most often in combination or in sequence with anthracycline and cyclophosphamide containing regimens, it is indeed difficult to investigate actual impact from taxanes. Available studies report conflicting results, from reduced CIA incidence to equal and further to increased (295, 301, 308). Animal studies have demonstrated blockage of ovulation without impact on fertility

supporting a transient toxicity (321). In addition, in rat experiments apoptosis was mainly seen in mature follicles following paclitaxel administration and fertility was affected directly after the infusion but recovered at 24 days (322). Accordingly, potential gonadotoxic effect of taxanes is probably mild, transient and diluted in the amenorrhea caused by accompanying chemotherapeutics. Fluorouracil does not seem to increase risk for amenorrhea and is also no longer used as widely as before in the management of early breast cancer (295).

AIM OF THE THESIS

The overall aim of the thesis was to further examine specific chemotherapy-induced toxicities, namely cardiotoxicity, neutropenia prevention and chemotherapy-induced premature ovarian failure, and the impact of intensifying chemotherapy intervals and individual dose tailoring.

In particular, specific aims of the thesis were:

1. To investigate how heart failure after breast cancer diagnosis differs from heart failure in the general population in terms of clinical features, aetiology and treatment (**Paper I**).

2. To investigate exposure, efficacy and cardiac safety of trastuzumab in combination with tailored and dose-dense chemotherapy compared to combination with standard 3-weekly regimen in the PANTHER phase III trial (**Paper II**).

3. To investigate incidence of neutropenic events with and without G-CSF and the impact of G-CSF on neutropenia related treatment delays (**Paper III**).

4. To assess whether tailored and dose-dense chemotherapy can impact CIA incidence and breast cancer specific outcomes and whether menopause status at breast cancer diagnosis can influence the effect of the allocated chemotherapy regimen (**Paper IV**).

PATIENTS AND METHODS

3.1 Data source

Registries

The Swedish National Registries were utilised for Paper I. In Sweden, the existence of a unique twelve-digit personal number issued at birth or when migrating to Sweden, enables follow-up of patients through life and cross-reference between different national registries. All registries but the Swedish Heart Failure Registry are maintained and managed by the National Board of Health and Welfare (323). Extraction of data from the registries was performed after approval from relevant regional ethics committee in Linköping and procedures complied with Swedish legislation valid at the time.

The *Swedish National Hospital Discharge Register* (NHDR) was established in 1964 and has had complete national coverage since 1987 (324). It contains health care related information from in-patient (in-hospital) care and outpatient specialist care but no information from primary health care visits is registered. Since 2015, reporting is mandatory on monthly basis. The positive predictive value (PPV) of the registry varies between diagnoses but validity is generally high and between 85 and 95% (324).

The *Swedish Cancer Registry* was founded in 1958 and has national coverage. Reporting of all new diagnosed cancer is mandatory by law and the registry is the source of official cancer statistics for Sweden. With about 99% of the diagnoses morphologically verified, the registry is considered of good quality and a completeness survey by Barlow et al published in 2009 reported good validity of the registry (325). Underreporting was less than 4% and related to site. For example, breast cancer had very low underreporting and probably insignificant impact on epidemiological studies (325).

The *Swedish Cause of Death Register* (COD), also with complete national coverage, is updated annually and includes information from 1961 when computers were introduced and a historic registry between 1952-1961. It includes information on deaths of all persons registered in Sweden at the time of death; regardless if the place of death is Sweden or abroad. However, registration of cause of death for persons not registered in Sweden is available only from 2012 and onward. The registry does not include stillborns (326).

The *Swedish Prescribed Drugs Register* (PD) has been around in years but it was expanded to include the patient's identification in the middle of 2005 (327). The registry uses the Anatomical Therapeutic Chemical Classification System (ATC) and contains data on all dispensed prescription drugs for each patient throughout the country (327).

The *Swedish Heart Failure registry (SwedeHF) or RiksSvikt* was founded in 2000, has nationwide coverage and is managed by Uppsala Clinical Research Centre. The registry is implemented in the whole of Sweden and registers clinically diagnosed heart failure from 70 out of 80 hospitals and 100 out of 1000 primary care facilities (328). The clinically diagnosed term refers to the fact that it only requires a physician's assessment as heart failure to be added in the heart failure registry and ECHO or other investigations are not mandatory.

Reporting in the registry is voluntary and ejection fraction is recorded for about 90% of the cases (328). It is estimated that the registry covers 54% of prevalent inpatient heart failure cases but coverage for incident heart failure is only 9.5% (both in- and out-patient) (328, 329).

The PANTHER trial

Data source for Papers II-IV was the Pan-European Tailored Chemotherapy (PANTHER) trial. This academic international, multicentre, open-label, randomised phase III trial enrolled patients in 86 centres in Sweden, Germany and Austria between 2007 and 2011 (92). In summary, women between 18 and 65 years old, in good physical condition (ECOG PS 0-1), with radically resected high-risk early breast cancer; lymph node positive or high-risk node-negative (defined as younger than 35 years old or tumour larger than 2 cm with histological grade 3 and hormone receptor negativity). Patients with distant metastases or prior history of major cardiovascular disease were not eligible for inclusion in the trial. Random allocation 1:1 to the experimental or the standard treatment schedule was performed (figure 13). In total 1006 women were allocated to receive tailored dose-dense chemotherapy and 1011 to receive standard treatment (n=2017).

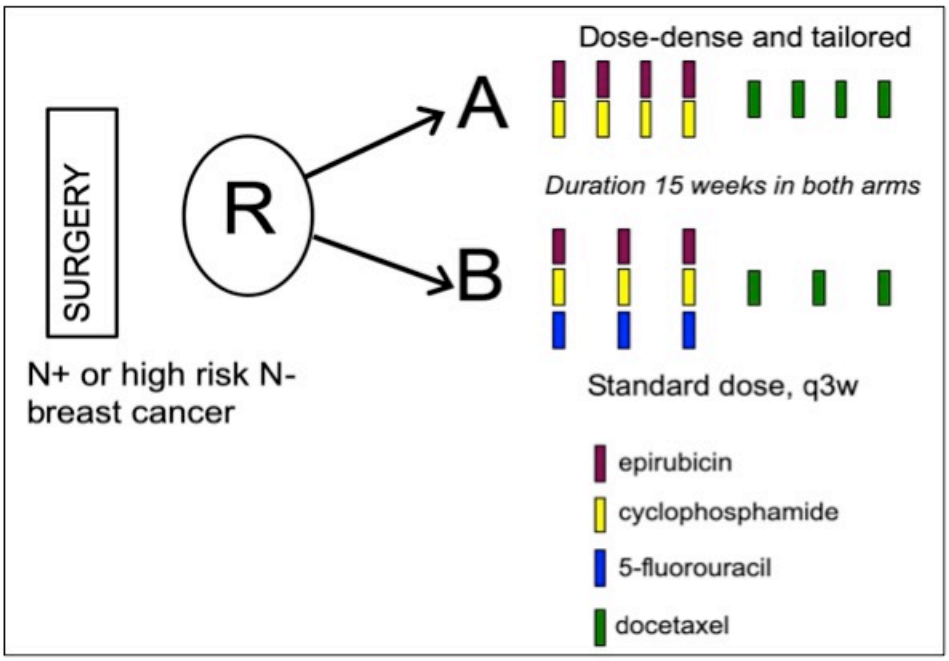

Figure 13. *Description of the design of the PANTHER trial. Adjuvant trastuzumab for one year was administered after completion of chemotherapy or concomitant with docetaxel. N= node, q3w= every three weeks, R= randomisation. Courtesy Dr Theodoros Foukakis*

The standard treatment group received three cycles of standard FEC administered every three weeks (F 500 mg/m^2, E 100 mg/m^2, C 500 mg/m^2) followed by three cycles of docetaxel 100 mg/m^2 every three weeks. The experimental treatment group received four cycles of tailored and dose dense chemotherapy epirubicin (38-120 mg/m^2, starting at 90 mg/m^2) and cyclophosphamide (450-1200 mg/m^2, starting at 600 mg/m^2) every two weeks (EC), followed by four cycles of tailored and dose dense docetaxel, (75-100 mg/m^2, starting at 75 mg/m^2) every two weeks. In the case of HER2 positive breast cancer, adjuvant trastuzumab was administered for one year, according to international guidelines. Trastuzumab started initially after accomplishment of all adjuvant chemotherapy and after October 2007, trastuzumab was recommended to begin in conjunction with docetaxel. Chemotherapy dose in the experimental group was tailored according to protocol-predefined guidelines according to haematological toxicity on day 8, 11/12 and 14/15, based on retrospective data indicating survival benefit for patients that developed severe haematological toxicity (330, 331). In addition, the protocol also predefined possibility for dose modification in case of severe non-haematological toxicity; namely diarrhoea, stomatitis, fatigue, neuro- and liver toxicity. Efficacy results after a median follow-up of 5.3 years (interquartile range [IQR] 4.5 – 6.1 years) were published in 2016 (92).

The PANTHER phase III trial was approved by ethics committees in the different regions and relevant competent authorities in Sweden, Germany and Austria and procedures relevant to the projects of the thesis are in concordance with current laws of Sweden and the Helsinki declaration. All patients included in the PANTHER trial provided written informed consent. Information recorded on the case-report forms of the trial and specific study-related echocardiograms were collected for the purposes of the current thesis.

Cardiac safety sub-study

A subset of patients from the PANTHER trial enrolled at the Swedish sites of Stockholm, Lund/Malmö and Umeå were asked to participate in the sub-study investigating safety of trastuzumab when tailored dose-dense chemotherapy is administered. Patients with HER2 positive breast cancer and equal number of control patients with HER2 negative breast cancer, matched for age, treatment group and institution were included. All patients provided separate informed consent for the sub-study. ECHO or MUGA, related biomarkers, cardiac medication and NYHA classification are registered at baseline, 4-, 6- and 10-years follow-up. The study obtained approval by the regional ethics committee in Stockholm and all study-related procedures followed current Swedish legislation and the Helsinki declaration.

3.2 Study population

Paper I

For the purpose of paper I, the SwedeHF registry was scrutinised to identify women with heart failure that were reported in the registry between 2008 and 2013. Of the 17 540 women identified, 2539 were excluded due to previous cancer diagnosis other than breast cancer, based on information from the Swedish Cancer Registry. Finally, 14 998 women were

deemed eligible for further investigation. Of these, 632 had been previously diagnosed with breast cancer. For the formation of the study cohort a selection was made on a 1:5 matching: for every person with heart failure and known breast cancer history five controls matched for age were selected. Finally, n= 3792 women with heart failure were included in the analysis of which n=632 also had prior history of breast cancer and n=3 160 did not.

Demographic characteristics, clinical signs and symptoms related to heart failure, NYHA classification and potential comorbidities, heart failure treatment, history of cancer and cause of death were collected from the registries. In addition, available results from ECG, ECHO and biochemical tests related to heart failure were registered. The data was additionally cross-referenced to the Cause-of-Death registry for the purpose of cause-specific survival analysis.

Paper II

Papers II-IV investigated different subpopulations of the women enrolled in the PANTHER phase III trial described earlier. For the intention-to-treat efficacy analysis of adjuvant trastuzumab in combination to tailored dose-dense chemotherapy versus trastuzumab and standard chemotherapy, population of interest was all patients with HER2 positive breast cancer (N=342).

The prospective, observational cardiac safety sub-study enrolled patients in the PANTHER trial treated in Sweden (n=157). In total 78 women with HER2 positive and 79 women with HER2 negative breast cancer were included.

Paper III

All patients participating in the PANTHER phase III trial that received at least one cycle of chemotherapy were included (n=2000); 1001 patients received tailored dose dense chemotherapy and 999 received standard chemotherapy. The PANTHER trial database and original case report forms (CRFs) were the source of information on the use of G-CSF, neutropenia and infection-related complications, treatment delays and dose reductions before and after each cycle.

For the patients treated with tailored and dose-dense chemotherapy, primary prophylaxis with G-CSF was mandatory. The type of G-CSF to be used was not pre-specified and both pegfilgrastim and filgrastim (or biosimilars) were administered. The study protocol mandated pegfilgrastim to be administered as single dose on day 2. Filgrastim was administered on days 4-11 during the first part of the adjuvant treatment with tailored dose-dense epirubicin and cyclophosphamide and on days 4-10 during docetaxel. Importantly, the tailored dose-dense chemotherapy group also received primary prophylaxis with antibiotics (ciprofloxacin 500 mg b.i.d on days 5-12) during EC. During docetaxel, ciprofloxacin was recommended only as secondary prophylaxis.

Initially, the protocol did not include guidelines for primary G-CSF prophylaxis for patients treated in the standard chemotherapy group and secondary prophylaxis was recommended in case of absolute neutrophil count less than 1.5 x 10^9/L or febrile neutropenia. However, in the summer of 2010, after interaction with the Independent Data Safety and Monitoring

Committee following a pre-planned safety analysis this policy changed. The protocol recommended then primary prophylaxis with G-CSF even for patients treated with standard chemotherapy schedule, but it was still not mandatory. The decision was communicated to the investigators on June 18th 2010.

Paper IV

The case report forms of the PANTHER trial were examined to identify menopausal status of trial participants at baseline. In total 1913 participants with available menopause status at baseline were identified and included in the current paper; n=956 from the tailored dose-dense chemotherapy group and n=957 from the standard chemotherapy group. Further data from the trial database were retrieved regarding menopausal status at two years follow-up, administered endocrine therapy, use of GnRHa and breast cancer outcome data.

3.3 The common terminology criteria for adverse events (CTC AE)

"An adverse event is any unfavourable sign, symptom or disease temporally associated with the use of medical treatment that may or may not be related to the medical treatment or procedure" (332). In the controlled environment of clinical trials, registration of adverse events (AEs) is a prerequisite to ensure safety of the trial participants as well as to gain knowledge on safety issues of the drugs under investigation. Severity of the reported adverse events is graded on a scale from 1 to 5, with 5 related to AEs with fatal outcome. Grade 1 refers to mild AEs, usually asymptomatic and not requiring intervention and grade 2 AEs are of moderate severity and can require low scale interventions. More severe AEs are graded as grade 3 if they are severe, require interventions, limit possibility for self-care activities but are not considered life-threatening and grade 4 are severe, life-threatening conditions that require urgent action. CTC AE version 3.0 was applied for registration of AEs in the PANTHER trial and has been utilised for the purpose of paper III (table 4). Neutropenic events in paper III included FN according to CTC AE version 3.0 and infection with neutropenia (ANC < 1.0 $x10^9$/L).

3.4 Echocardiography and MUGA

Reported LVEF in papers I and II are based on estimations from echocardiography (ECHO) or multigated acquisition scan (MUGA), depending on the centre. The normal ranges of LVEF vary slightly between genders but in general, LVEF 50% or above is considered as normal and this has also been the cut-off used for inclusion in the PANTHER trial.

The ECHO is a non-invasive test and therefore participants do not risk any harm but is subjected to inter-operator variations. There are various methods of visualisation and calculation of LVEF with the most recommended one today being the modified Simpson's biplane method, a two-dimensional method but in praxis both the latter and visual estimations are applied. The modified Simpson's method uses both four-chamber and two-chamber views to trace endocardial border in end-systole and end-diastole.

BLOOD/BOOD MARROW						Page 1 of 1
		Grade				
Adverse Event	Short Name	1	2	3	4	5
Bone marrow cellularity	Bone marrow cellularity	Mildly hypocellular or ≤25% reduction from normal cellularity for age	Moderately hypocellular or >25 – ≤50% reduction from normal cellularity for age	Severely hypocellular or >50 – ≤75% reduction cellularity from normal for age	—	Death
CD4 count	CD4 count	<LLN – 500/mm^3 <LLN – 0.5 x 10^9 /L	<500 – 200/mm^3 <0.5 – 0.2 x 10^9 /L	<200 – 50/mm^3 <0.2 x 0.05 – 10^9 /L	<50/mm^3 <0.05 x 10^9 /L	Death
Haptoglobin	Haptoglobin	<LLN	—	Absent	—	Death
Hemoglobin	Hemoglobin	<LLN – 10.0 g/dL <LLN – 6.2 mmol/L <LLN – 100 g/L	<10.0 – 8.0 g/dL <6.2 – 4.9 mmol/L <100 – 80g/L	<8.0 – 6.5 g/dL <4.9 – 4.0 mmol/L <80 – 65 g/L	<6.5 g/dL <4.0 mmol/L <65 g/L	Death
Hemolysis (e.g., immune hemolytic anemia, drug-related hemolysis)	Hemolysis	Laboratory evidence of hemolysis only (e.g., direct antiglobulin test [DAT, Coombs'] schistocytes)	Evidence of red cell destruction and ≥2 gm decrease in hemoglobin, no transfusion	Transfusion or medical intervention (e.g., steroids) indicated	Catastrophic consequences of hemolysis (e.g., renal failure, hypotension, bronchospasm, emergency splenectomy)	Death
ALSO CONSIDER: Haptoglobin; Hemoglobin.						
Iron overload	Iron overload	—	Asymptomatic iron overload, intervention not indicated	Iron overload, intervention indicated	Organ impairment (e.g., endocrinopathy, cardiopathy)	Death
Leukocytes (total WBC)	Leukocytes	<LLN – 3000/mm^3 <LLN – 3.0 x 10^9 /L	<3000 – 2000/mm^3 <3.0 – 2.0 x 10^9 /L	<2000 – 1000/mm^3 <2.0 – 1.0 x 10^9 /L	<1000/mm^3 <1.0 x 10^9 /L	Death
Lymphopenia	Lymphopenia	<LLN – 800/mm^3 <LLN x 0.8 – 10^9 /L	<800 – 500/mm^3 <0.8 – 0.5 x 10^9 /L	<500 – 200 mm^3 <0.5 – 0.2 x 10^9 /L	<200/mm^3 <0.2 x 10^9 /L	Death
Myelodysplasia	Myelodysplasia	—	—	Abnormal marrow cytogenetics (marrow blasts ≤5%)	RAEB or RAEB-T (marrow blasts >5%)	Death
Neutrophils/granulocytes (ANC/AGC)	Neutrophils	<LLN – 1500/mm^3 <LLN – 1.5 x 10^9 /L	<1500 – 1000/mm^3 <1.5 – 1.0 x 10^9 /L	<1000 – 500/mm^3 <1.0 – 0.5 x 10^9 /L	<500/mm^3 <0.5 x 10^9 /L	Death
Platelets	Platelets	<LLN – 75,000/mm^3 <LLN – 75.0 x 10^9 /L	<75,000 – 50,000/mm^3 <75.0 – 50.0 x 10^9 /L	<50,000 – 25,000/mm^3 <50.0 – 25.0 x 10^9 /L	<25,000/mm^3 <25.0 x 10^9 /L	Death

Table 4. *Grade of selected adverse events related to bone marrow toxicity, according to the Common Terminology Criteria for Adverse Events (CTC AE) version 3.0 (published August 2003).*

MUGA is also non-invasive, however small amount of radioisotope is injected intravenously, which may cause pain and discomfort to the patient. This method also exposes the patient to non-ionising radiation, which is less appealing for repeated measurements and is currently not widely performed. MUGA is considered to be less subjective to inter-observer variations but different computer processing programmes and different centres can still impact heterogeneity in the measurements (333). Additionally, since MUGA is based on imaging projection, it can be subject to systematic errors.

Ejection fraction actually represents the fraction of the volume of blood in the left ventricle ejected in systole (stroke volume, SV) in relation to the volume of the same chamber at the end of diastole (end-diastolic volume, EDV). Stroke volume is estimated by subtraction of end-systolic volume (ESV) of the left ventricle from the end-diastolic volume (EDV-ESV). Thus, LVEF can be calculated from:

$$LVEF = [(EDV-ESV)/EDV] \times 100 \text{ or } LVEF=[SV/EDV] \times 100$$

3.5 Statistical analysis

To better characterise study population in **Paper I** and to facilitate statistical comparisons, the Swedish National Hospital Discharge Register was examined to identify date of heart failure diagnosis. The date of inclusion in the heart failure registry was considered as index date. If a patient had been registered in the SwedeHF registry within 1 month from the registered date of heart failure diagnosis, she was classified as "incident heart failure" whereas enrolment after more than 1 month from heart failure diagnosis was classified as "prevalent heart

failure". The distinction between incident and prevalent heart failure was thereafter utilised for group comparisons.

In Papers II and IV, time for event-free patients was calculated from date of randomisation (start date) until the date of last clinical visit (end date).

The primary endpoint of **Paper II** was breast cancer relapse-free survival (BCRFS) defined as time from randomisation to breast cancer relapse or breast-cancer specific death. Secondary outcomes of overall survival (OS), event-free survival (EFS) and distant disease-free survival (DFS) were defined as follows: i) OS: time to death by any cause, ii) EFS: defined as time to breast cancer relapse, contralateral breast cancer, any other malignancy or death, regardless cause, iii) DFS: time to distant metastases or death due to breast cancer.

In **Paper III**, no direct comparison of neutropenia incidence between the two treatment schedules was performed upon two main reasons. Firstly, because almost all patients treated with tailored dose-dense chemotherapy received primary prophylaxis with G-CSF, in adherence to the study protocol. Secondly, because in the specific group more chemotherapy cycles were delivered (8 versus 6 in the standard chemotherapy group) leading to a baseline increased risk of neutropenia and thereby not directly comparable to the standard treatment group. Although primary prophylaxis even in the standard group was communicated as per June 16th 2010, the 1st of August 2010 was defined as dichotomous date, permitting adequate time for the information to reach the investigators and be incorporated at the study centres.

The primary objective of **Paper IV** was BCRFS, as defined above, per menopause status subgroups analysis per breast cancer subtype. The secondary endpoint was to examine incidence of CIA, defined as cessation of menstruation after chemotherapy and up to two years of follow-up, among patients that were premenopausal at breast cancer diagnosis and did not receive GnRHa.

Baseline characteristics of the population of each paper were described as absolute numbers and percentages for categorical variables. Means were instead presented for continuous variables and age was presented as median. T-test was applied for comparison between groups of continuous variables and Fisher's exact test or χ^2 test were performed for comparisons of categorical variables. No corrections for multiple testing were performed. A 5% significance level was chosen for two-sided tests.

Results from randomised trials can suffer from missing data or protocol violations. Two approaches to address the latter is to analyse data by extracting data from patients that did not comply to the protocol (per protocol analysis, PP) or include all patients and analyse data according to the group the patients were randomised in (intention to treat analysis, ITT). The ITT analysis is more conservative, aiming to minimise introduction of potential bias by excluding patients from the analysis. On the other hand, PP analysis is more prone to give the efficacy measurements in ideal conditions, reducing bias by false treatment and protocol violations. ITT is a more complete analysis and data from every patient included in the trial is analysed, with the exception of patients that no data is available or they did not receive any treatment at all. An ITT approach has been applied for the purposes of paper II-IV.

Survival analysis investigates the time from a specified start point until the occurrence of an event of interest. The time from start to the event is referred to as survival time. Even though

death is usually the event of interest, the analysis can be used for various types of events that can be defined with certainty. Such examples in oncology can be time to disease progression and type to cancer relapse (local or metastatic).

The Kaplan-Meier (KM) curve is a non-parametric method commonly used to visualise survival and to estimate probability of survival over time. Individuals that prematurely finish their follow-up for reasons other than the study endpoints (e.g. due to loss to follow-up), are being censored but the time until the last known follow-up visit can be accounted in the analysis. However, some basic assumptions are required for the use of KM; that the censored patients have the same probability of survival as those still in the study, that survival prospects are the same regardless time of inclusion and that the expected event happens within a specific time period. In paper I the KM-curve was applied to visualise and calculate cumulative incidences for death due to any cause, death due to cardiovascular disease and due to heart failure in four different groups; incident heart failure +/- breast cancer history and prevalent heart failure +/- breast cancer history. In papers II and IV reverse KM-curve (1-KM) was utilised to estimate median follow-up time. Median follow-up time is defined as the follow-up time where the KM-function is less than or equal to 0.5.

Another method utilised in survival analysis context is the Cox regression (or proportional hazards regression) for survival analysis that estimates the effect of different variables on the time-to event. Despite being regarded as non-parametric, this method assumes that variables affecting survival are constant over time. The Cox regression survival analysis was applied for estimation of time to failure, hazard ratios and 95% confidence intervals.

Even though the Kaplan Meier curve is widely applied in cancer research, in the presence of competing risk for other events, this method can overestimate the cumulative probability of an event (cause-specific failure). Competing risks are events that can alter the probability of the occurrence of the event of interest. To provide some examples in the context of breast cancer research, development of second primary cancer or death due to causes other than breast cancer can be considered as competing risks, if the endpoint of interest is breast cancer relapse or death. Therefore, for the purposes of the survival analysis in paper II, cumulative incidence curves were applied for estimation of breast cancer outcomes. This function takes into consideration the fundamental contribution of other causes (competing risks) in the overall outcome.

RESULTS

Paper I – Outcome and Presentation of Heart Failure in Breast Cancer patients: Findings from a Swedish Register-based Study.

This registry-based study investigated differences in clinical presentation of heart failure, received heart failure care and survival in women with heart failure with and without previous breast cancer history.

In total, 1764 women with incident heart failure (iHF) (of which 294 with breast cancer history) and 2028 with prevalent heart failure (pHF) (338 with previous breast cancer) were included in the analysis with a median follow-up time of 2 years. Median time from breast cancer (BC) diagnosis to heart failure diagnosis was 6.2 years (IQR 3.3 – 8.4 years). Table 5, describes baseline characteristics of these individuals.

Cardiovascular risk factors did not differ significantly between the four groups (iFH, iHF + BC, pHF, pHF + BC). Interestingly, all groups reported mean BMI that falls in the overweight range, around 27 kg/m^2. In addition, a little more than half of the patients in each group had hypertension, without any significant interaction with breast cancer diagnosis or time from heart failure diagnosis. No differences were present regarding smoking habits and presence of diabetes mellitus.

Overall, no statistically significant variations were seen in cardiovascular risk factors, pre-existing comorbidities, clinical features and heart failure care among patients with and without breast cancer, in the group classified as iHF. However, some small deviations were noticed in the management of patients with pHF. As presented in table 6, incidence of history of myocardial infarction among patients with pHF was significantly lower among patients with breast cancer history; 21.6% vs. 28.6%, $p<0.01$. Fewer patients with pHF and breast cancer history had undergone coronary revascularization (11.8% vs. 16.2%, $p<0.01$) or were prescribed aspirin (47.6% vs. 55.1%, $p=0.01$) compared to those without previous breast cancer. Although device therapy was not commonly applied in any of the groups, patients with pHF and BC history were significantly less frequently treated with implantable cardioverter-defibrillators and cardiac resynchronisation therapy than patients with only pHF; 0.6% vs. 1.6 % and 0.9% vs. 3% respectively.

Within group comparisons did not show any impact on the cumulative incidence for all-cause mortality, cardiac mortality and mortality due to heart failure at 1-, 2- and 5-year follow-up in any of the groups (figure 14). On the other hand, inter-group comparison revealed increased mortality on every endpoint in the pHF group. In both groups, mortality rates increased with time, for example 1-year all-cause mortality for iHF with breast cancer was 15.3% compared to 43.2% 5-year all-cause mortality for the same group.

In conclusion, history of breast cancer diagnosis did no significantly impact aetiology and survival of heart failure nor overall survival among patients with established heart failure. In the group of women with longer duration of heart failure (pHF) some differences were seen

in regards of myocardial infarction incidence and heart failure management depending on breast cancer history status.

Characteristics	Incident HF			Prevalent HF		
	Without BC (*N* = 1470)	With BC (*N* = 294)	*P*-value	Without BC (*N* = 1690)	With BC (*N* = 338)	*P*-value
Age (years), median (IQR)	77 (68–85)	77 (68–85)		79 (73–86)	79 (73–86)	
HF diagnosis, *n* (%)			<0.01			<0.01
Outpatient clinic	467 (31.8)	117 (39.8)		781 (46.2)	162 (47.9)	
Inpatient department	1003 (68.2)	177 (60.2)		909 (53.8)	176 (52.1)	
Time between HF diagnosis and SwedeHF enrolment (years), median (IQR)	0.0 (0.0–0.0)	0.0 (0.0–0.0)	0.36	2.1 (0.4–5.5)	1.8 (0.4–5.0)?	0.12
Year of SwedeHF enrolment, *n* (%)			0.20			0.20
2008	297 (20.2)	65 (22.1)		396 (23.4)	89 (26.3)	
2009	224 (15.2)	52 (17.7)		298 (17.6)	52 (15.4)	
2010	251 (17.1)	39 (13.3)		248 (14.7)	44 (13.0)	
2011	273 (18.6)	50 (17.0)		294 (17.4)	63 (18.6)	
2012	243 (16.5)	41 (13.9)		198 (11.7)	43 (12.7)	
2013	182 (12.4)	47 (16.0)		256 (15.1)	47 (14.0)	
Specialty enrolling, *n* (%)			0.62			0.63
Cardiology	674 (50.8)	145 (53.1)		812 (53.7)	156 (51.8)	
Internal medicine	641 (48.3)	127 (46.5)		684 (53.7)	142 (47.2)	
Geriatrics	12 (0.9)	1 (0.4)		15 (1.0)	3 (1.0)	
Follow-up time (years), median (IQR)	2.2 (0.9–2.8)	2.0 (0.8–4.0)		1.8 (0.7–3.4)	2.1 (0.7–3.5)	
Mean	2.0	2.0		2.0	2.0	
Maximum	6.0	6.0		6.0	6.0	
Time between BC and HF (years), median (IQR)	NA	6.2 (3.3–8.4)	NA	NA	6.2 (2.8–8.4)	NA
<1 year, *n* (%)	NA	25 (8.5)		NA	24 (7.1)	
1–5 years, *n* (%)	NA	95 (32.3)		NA	114 (33.7)	
>5 years, *n* (%)	NA	174 (59.2)		NA	200 (59.2)	
New BC or metastatic disease after HF, *n* (%)			<0.01			<0.01
BC	12 (0.8)	55 (19)	<0.01	10 (0.6)	72 (21.3)	<0.01
Metastases	24 (1.6)	13 (4.4)	<0.01	17 (1.0)	15 (4.4)	<0.01

Table 5. *Baseline characteristics of women registered in the SwedeHF registry according to time from heart failure diagnosis to inclusion in SwedeHF and presence or not of breast cancer history. BC: breast cancer, HF: heart failure, IQR: interquartile range, NA: not available*

	Incident HF			Prevalent HF		
	Without BC (*N* = 1470)	With BC (*N* = 294)	*P*-value	Without BC (*N* = 1690)	With BC (*N* = 338)	*P*-value
Pre-existing comorbidities, *n* (%)						
Myocardial infarction	268 (18.2)	43 (14.6)	0.15	484 (28.6)	73 (21.6)	<0.01
Unstable angina	83 (5.6)	9 (3.1)	0.08	141 (8.3)	28 (8.3)	1.00
Stable angina	188 (12.8)	31 (10.5)	0.33	446 (26.4)	88 (26.0)	0.95
Atrial fibrillation/flutter	492 (33.5)	93 (31.6)	0.59	964 (57.0)	186 (55.0)	0.51
Stroke	199 (13.5)	37 (12.6)	0.71	281 (16.6)	52 (15.4)	0.63
Treatment						
Beta-blocker, *n* (%)	921 (62.7)	184 (62.6)	1.00	1462 (86.5)	298 (88.2)	0.48
Agents acting on the renin–angiotensin system, *n* (%)	871 (59.3)	184 (62.6)	0.30	1393 (82.4)	290 (85.8)	0.15
Angiotensin-converting enzyme inhibitor	615 (41.8)	126 (42.9)	0.75	987 (58.4)	199 (58.9)	0.90
Angiotensin receptor blocker	328 (22.3)	70 (23.8)	0.59	561 (33.2)	122 (36.1)	0.31
Mineral corticoid receptor antagonist, *n* (%)	166 (11.3)	36 (12.2)	0.62	623 (36.9)	118 (34.9)	0.54
Calcium channel blockers, *n* (%)	374 (25.4)	77 (26.2)	0.83	438 (25.9)	81 (24.0)	0.50
Diuretics, *n* (%)	877 (59.7)	176 (59.9)	>0.99	1506 (89.1)	301 (89.1)	>0.99
Statins, *n* (%)	539 (36.7)	96 (32.7)	0.21	765 (45.3)	149 (44.1)	0.72
Aspirin, *n* (%)	636 (43.3)	120 (40.8)	0.48	932 (55.1)	161 (47.6)	0.01
Coronary revascularization, *n* (%)	166 (11.3)	24 (8.2)	0.12	274 (16.2)	40 (11.8)	<0.01
Device therapy, *n* (%)			>0.99			0.03
Implantable cardioverter-defibrillators	5 (0.3)	2 (0.7)		27 (1.6)	2 (0.6)	
Cardiac resynchronization therapy/+implantable cardioverter-defibrillators	4 (0.3)	1 (0.3)		50 (3.0)	3 (0.9)	

Table 6. *Pre-existing comorbidities and treatment for the women with heart failure included in the study. BC: Breast cancer, HF: heart failure*

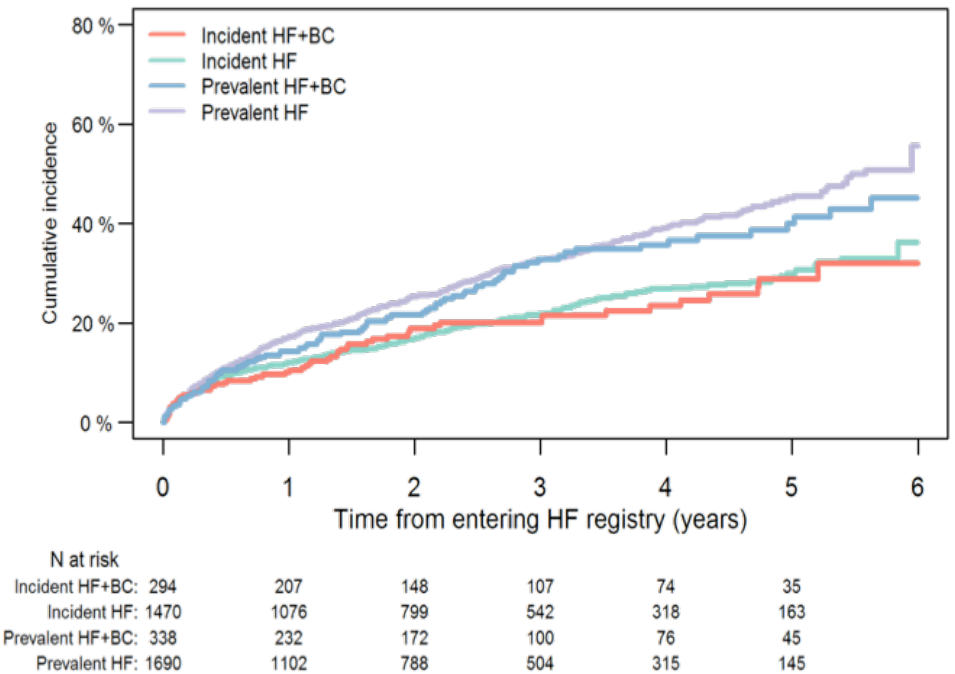

Figure 14. *Cumulative incidence of heart failure specific mortality among patients included in the study. BC: breast cancer, HF: heart failure.*

Paper II – Efficacy and Safety of Tailored and Dose-Dense Adjuvant Chemotherapy and Trastuzumab for Resected HER2-Positive Breast Cancer: Results From the Phase 3 PANTHER Trial.

The aim of this study was to investigate efficacy and safety of trastuzumab in combination with tailored and dose-dense adjuvant chemotherapy.

Trastuzumab exposure and efficacy analysis

There were 342 HER2-positive patients enrolled in the PANTHER phase III trial of which 182 were treated with standard and 160 with tailored dose-dense chemotherapy. Median follow-up for the analysis was 5.3 years (IQR 4.4 – 6.3 years). Three hundred thirty-five patients received at least one dose of trastuzumab. Three patients withdrew consent, two were lost to follow-up and two more did not receive any trastuzumab due to other reasons, with balanced numbers between the treatment groups. Median age and tumour-related baseline characteristics were balanced between the standard therapy and tailored dose-dense chemotherapy and are presented in table 7.

	Standard chemotherapy N=182 (%)		**Tailored dose-dense chemotherapy** N=160 (%)	
Median age, years (range)	48.3 (21.4-66.4)		51.5 (28.0-69.5)	
Tumor size, mm				
0-20	73	(40.1)	61	(38.1)
21-50	92	(51.5)	87	(54.4)
>50	17	(9.3)	12	(7.5)
Positive nodes				
0	6	(3.3)	6	(3.8)
1-3	99	(54.4)	89	(55.6)
4-9	53	(29.1)	43	(26.9)
>9	24	(13.2)	22	(13.8)
Tumor grade				
I	0	(0.0)	3	(1.9)
II	64	(35.2)	46	(28.7)
III	118	(64.8)	110	(68.8)
Missing	0	(0.0)	1	(0.6)
Hormone receptors				
ER and/or PR (+)	109	(59.9)	105	(65.6)
ER and PR (-)	73	(40.1)	55	(34.4)
Ki-67 %				
≤20	33	(18.1)	27	(16.8)
>20	96	(52.7)	85	(53.1)
Missing	53	(29.1)	48	(30.0)
Trastuzumab				
Administered	175	(96.2)	155	(96.9)
Not administered	7	(3.8)	5	(3.1)

Table 7. *Clinical baseline characteristics of patients with HER2 positive breast cancer in the PANTHER phase III trial. ER: oestrogen receptor, PR: progesterone receptor*

In total 15 out of 29 patients discontinued trastuzumab prematurely due to cardiotoxicity; 10 in tailored dose-dense group and 5 in standard group. One patient had persistently low LVEF after 3 years, whereas three patients did not perform other evaluations and two died due to metastatic disease. Of the remaining nine, two patients reported recovered LVEF at one year follow-up, three at two years, two at three years and two at five years. Other reasons for trastuzumab discontinuation were disease progression (n=4), and other reasons (n=10).

The combination of dose-dense chemotherapy and trastuzumab led to a non-significant relative reduction of the risk for breast cancer relapse by 32% compared to standard treatment (HR=0.68, 95% CI 0.37 – 1.27, p = 0.231), as presented in figure 15. In absolute numbers, 16 versus 26 breast cancer relapse events were reported in the tailored dose-dense and standard chemotherapy group respectively, in an intention to treat analysis. The 5-year breast cancer relapse-free survival was 89.1% and 85.3% respectively with an absolute gain of 3.8% (95% CI 3.7% – 11%). No significant interaction between treatment efficacy and hormone receptor status was demonstrated and similar trends were noted for all secondary endpoints.

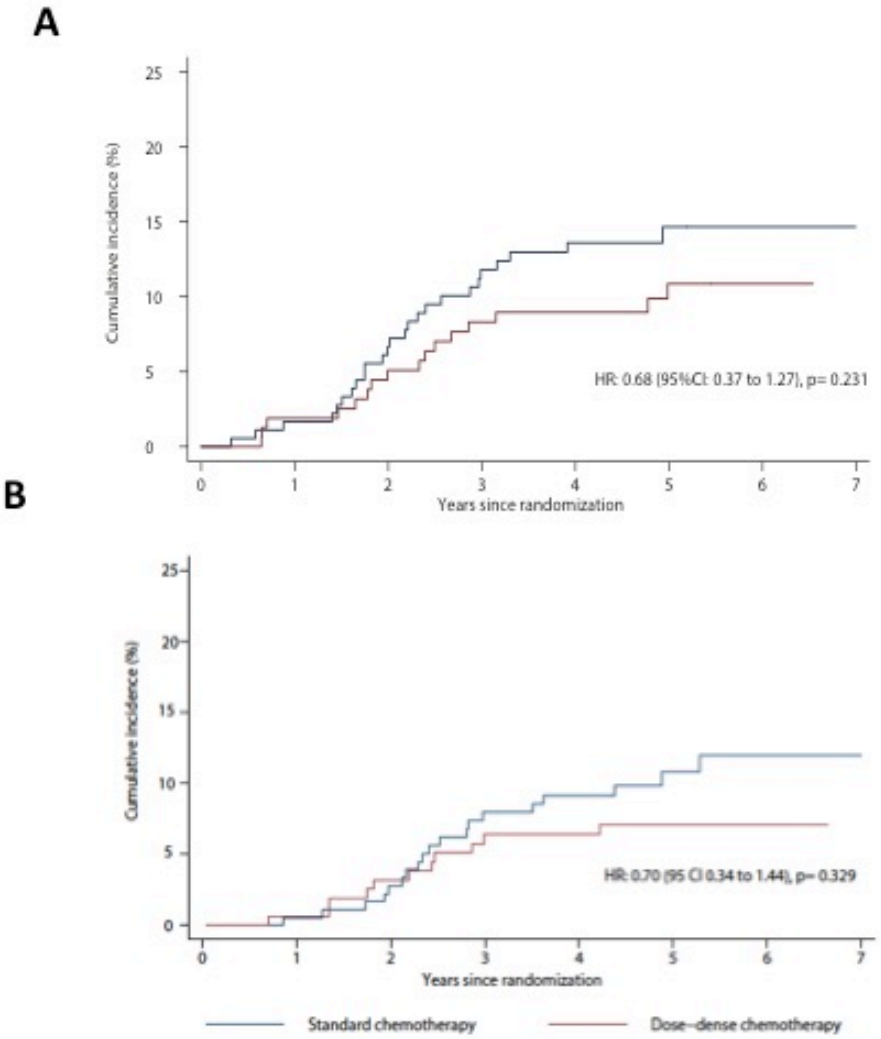

Figure 15. *Breast cancer relapse-free survival (A) and overall survival (B) for patients with HER2 positive breast cancer treated with standard or tailored dose-dense chemotherapy in the PANTHER phase III trial.*

Cardiac outcomes HER2 positive versus HER2 negative

Among the patients enrolled in the PANTHER trial in Sweden (n=780), 176 were assessed eligible to be included in the cardiac safety sub-study and finally 157 consented to participate. Figure 16, depicts the Consolidated Standards of Reporting Trials (CONSORT) diagram of the PANTHER HER2 cardiac safety sub-study. Baseline characteristics between the HER2 positive group and the matching control group were not entirely balanced. Median age, tumour sidedness and delivered chemotherapy were in balance but the groups differed in regards of hormone receptor status and received radiotherapy (table 8). The numbers were balanced in regards of allocated chemotherapy treatment and the mean epirubicin doses were similar; 346.1 mg/m^2 in the HER2 positive group and 344.4 mg/m^2 in the control group.

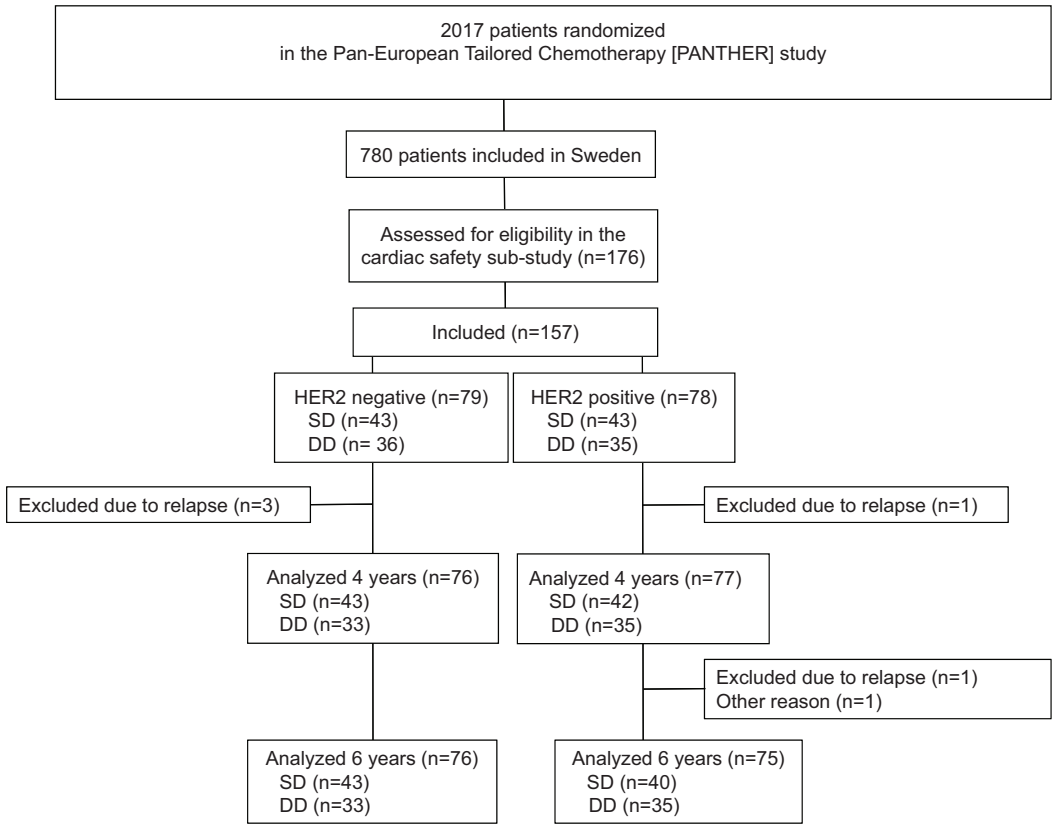

Figure 16. *Consolidated Standard of Reporting Trials diagram of the PANTHER HER2 cardiac safety sub-study. DD: Tailored dose-dense chemotherapy. SD: Standard chemotherapy.*

Normal cardiac function at inclusion, according to ECHO or MUGA, was required to fulfil eligibility criteria for the PANTHER phase III trial. In the sub-study, mean baseline LVEF was similar in the two groups; 61.2% for the HER2 positive group and 62.5% for the HER2 negative group. At four years follow-up, four patients were excluded due to breast cancer relapse. Mean LVEF did not differ between groups for the remaining n=153; 58.7% HER2 positive and 59.9% HER2 negative. Accordingly, at 6-years follow-up, n=151 patients were investigated. Mean LVEF and difference from baseline was similar between the groups and even though it had declined from baseline, mean LVEF remained within normal range; 58.8% among patients treated with trastuzumab and 59% among those who did not. Both at 4- and 6-years follow-up, LVEF drop of 10% or higher, was comparable between the groups (table 9). Additionally, reported electrocardiographic abnormalities, NYHA classification, prescription of cardiac medication, frequency of hypertension or diabetes mellitus and levels of cardiac biomarkers were similar between the treatment groups and not clinically or statistically significant.

	HER2 positive N=78 (%)		**HER2 negative** N=79 (%)		***P* value**
Median age, years (range)	51.5 (28.4 – 64.7)		50.6 (29.1 – 64.9)		0.58
Center					0.92
Umeå	32	(41.0)	30	(38.0)	
Lund	9	(11.5)	9	(11.4)	
Stockholm	38	(47.4)	40	(50.6)	
Side					0.75
Right	34	(43.6)	37	(46.8)	
Left	44	(56.4)	42	(53.2)	
Chemotherapy					1.0
Tailored dose-dense	35	(44.9)	36	(45.6)	
Standard	43	(55.1)	43	(54.4)	
ER					0.02
Positive	50	(64.1)	64	(81)	
Negative	28	(35.9)	15	(19)	
PR					<0.001
Positive	37	(47.4)	62	(78.5)	
Negative	41	(52.6)	17	(21.5)	
ECG					0.49
Normal	73	(93.6)	74	(93.7)	
Abnormal	5	(6.4)	3	(3.8)	
Not done	0	(0.0)	2	(2.5)	
Radiotherapy					0.03
Local	16	(20.5)	25	(31.7)	
Loco-regional	48	(61.5)	52	(65.8)	
Not given	14	(18)	2	(2.5)	
MUGA/ECHO					0.18
Mean LVEF	61.19 %		62.47 %		
Missing	3	(3.8)	2	(2.5)	

Table 8. *Baseline characteristics of HER2 positive and HER2 negative groups in the PANTHER cardiac safety sub-study. ECG: Electrocardiogram; ECHO: Echocardiography; ER: Oestrogen receptor; LVEF: Left ventricular ejection fracture; MUGA: multigated acquisition; PR: Progesterone receptor.*

	Trastuzumab whole cohort	Trastuzumab FEC/D	Trastuzumab tDD EC/D		No trastuzumab Whole cohort	No Trastuzumab FEC/D	No Trastuzumab tDD EC/D
	N=78	N=43	N=35		N=79	N=43	N=36
Baseline							
Mean LVEF	61.2 %	61.7 %	60.6 %		62.5 %	62.6 %	62.4 %
4-years follow up							
Mean LVEF	58.7 %	58.3 %	59.2 %		59.9 %	61.2 %	58.1 %
LVEF value drop ≥ 10%	11 (14.3 %)	7	4		10 (13.2 %)	4	6
Mean difference from baseline	2.3 %	0.6 %	3.5%		2.6 %	1.6 %	4.1 %
6-years follow up							
Mean LVEF	58.8 %	59.5 %	57.8 %		59 %	61.8 %	55.8 %
LVEF value drop ≥ 10%	12 (16 %)	7	5		11 (16.6%)	5	6
Mean difference from baseline	2.4 %	2.2 %	2.6 %		3.4 %	0.7 %	6.6 %

Table 9. *Left ventricular ejection fraction changes at baseline, 4- and 6-years follow-up in the PANTHER cardiac safety sub-study according to HER2 status and delivered chemotherapy. FEC/D: standard chemotherapy 5-fluorouracil/epirubicin/cyclophosphamide and docetaxel, HER2: Human Epidermal growth factor Receptor 2, LVEF: Left Ventricular Ejection Fraction, tDD EC/D: tailored and dose dense epirubicin/cyclophosphamide and docetaxel*

Cardiac outcomes tailored dose-dense versus standard chemotherapy

A predefined comparison of LVEF decline between the two treatment schedules revealed statistically lower LVEF among patients treated in the tailored dose-dense group, even though mean LVEF remained within normal range. Moreover, at 4-years follow-up there was a statistically significant LVEF decline within both treatment schedules ($p<0.01$ for both groups). Similar decline was seen at 6-years follow-up within the tailored dose-dense group ($p<0.001$). None of the observed LVEF declines responded to clinically relevant findings. Higher mean epirubicin dose in the tailored dose-dense group (400.5 mg/m^2) compared to the standard chemotherapy group (296.3 mg/m^2) did not translate to increased cardiotoxicity.

In summary, trastuzumab in combination with tailored dose-dense chemotherapy showed a trend to improved breast cancer outcomes, though not significantly. There was no direct impact on cardiotoxicity following trastuzumab administration.

Paper III – Neutropenic Complications in the PANTHER phase III study of Adjuvant Tailored Dose-Dense Chemotherapy in Early Breast Cancer.

Utility of G-CSF and neutropenic complications in the PANTHER trial were investigated in this secondary, retrospective study.

Among the 2000 patients administered at least one chemotherapy cycle, 1001 were treated according to the tailored dose-dense schedule and 999 according to the standard schedule. As per protocol recommendation, the majority of the patients in the former group received primary prophylaxis with G-CSF (98.9% during EC and 97.4% during docetaxel) whereas in the latter group about half the patients received primary prophylaxis; 49.7% during FEC and 63.8% during docetaxel (D). As noted in table 10, pegfilgrastim was the most popular G-CSF administered in the PANTHER trial, although the choice of which G-CSF to use was left on the physician. An exploratory analysis on the efficacy of pegfilgrastim versus filgrastim did not show any statistically significant difference.

Among the patients treated with standard chemotherapy schedule, 5.4% during FEC and 6.3% during D developed grade 3-4 neutropenic events respectively. In comparison, despite provision for prophylactic G-CSF, 7% during tailored dose dense EC and 1.6% during docetaxel reported such events. Rate of infection with normal ANC was similar among patients treated with anthracyclines, regardless dose intensity, and standard docetaxel, ranging between 2.5% and 3.7%. On the other hand, tailored dose dense docetaxel was related to more infections with normal ANC (7.4%), although the same group developed fewer events of infection accompanied with neutropenia (0.8%). Notably, neutropenic complications were not related to any grade 5 toxicity (death) in the study.

	Tailored dose-dense chemotherapy group			Standard interval chemotherapy group		
	During tdd EC			*During 3-weekly FEC*		
	Secondary prophylaxis *n* (%)	Primary prophylaxis *n* (%)	Total *n* (%)	Secondary prophylaxis *n* (%)	Primary prophylaxis *n* (%)	Total *n* (%)
Type of G-CSF						
Short-acting G-CSF	1 (20)	89 (8.99)	90 (8.99)	3 (5.56)	77 (15.49)	80 (8.01)
Long-acting G-CSF	4 (80)	850 (85.86)	854 (85.31)	49 (90.74)	372 (74.85)	421 (42.14)
Different G-CSF at times		51 (5.15)	51 (5.09)	2 (3.70)	48 (9.66)	48 (9.66)
Missing						22
None			6			426
Total	5	990	1001	54	497	999
	During tdd Docetaxel			*During 3-weekly Docetaxel*		
	Secondary prophylaxis *n* (%)	Primary prophylaxis *n* (%)	Total *n* (%)	Secondary prophylaxis *n* (%)	Primary prophylaxis *n* (%)	Total *n* (%)
Short-acting G-CSF	1 (50)	111 (11.91)	112	6 (13.64)	81 (12.94)	87
Long-acting G-CSF	1 (50)	768 (82.40)	769	38 (86.36)	500 (79.87)	538
Different G-CSF at times	0	53 (5.69)	53	0	45 (7.19)	45
Missing			4			17
None			9			293
Total	2	922	947	44	626	980

Table 10. *Type of G-CSF used in the PANTHER phase III trial. EC: epirubicin, cyclophosphamide, FEC: 5-fluorouracil, epirubicin, cyclophosphamide, G-CSF: granulocyte-colony stimulating factor, tdd: tailored dose-dense*

Administration of G-CSF reduced the risk of neutropenic events overall (odds ratio, OR 0.44, 95% CI 0.35 – 0.55) and more specific in the standard chemotherapy group, change of prophylaxis policy led to significantly fewer grade 3 or 4 neutropenic events (OR 0.48, 95% CI 0.31 – 0.73).

Compliance to planned treatment according to protocol and delays or dose reductions due to neutropenia were calculated per chemotherapy cycle. A total of 13 343 chemotherapy cycles were delivered in the study, of which 10 391 with G-CSF support and 2950 without. The risk for febrile neutropenia was significantly increased in the absence of G-CSF support whereas the overall risk for infection was not affected by G-CSF administration despite a significant disparity on the risk of infection depending on ANC levels. The risk for grade 3-4 infection with low ANC was significantly higher among patients that did not receive G-CSF support compared to those who did but the opposite was observed for infection with normal ANC, as described in table 11.

Overall, 5.6% (n=753) of planned chemotherapy cycles were delayed of which more than half was due to administrative or other reasons. Delays related to leukopenia and/or infections were distributed as follows: 131 were delayed due to infection, 114 due to neutropenia (n=114), 51 due to leukopenia and 16 due to neutropenic fever. Chemotherapy cycles among patients treated in the standard chemotherapy group were more likely to be delayed (3.3%) compared to the tailored dose-dense regimen (2.6%) even though a formal statistical comparison was not performed. Administration of G-CSF support significantly reduced risk of delay for the subsequent chemotherapy cycle due to neutropenia (OR 0.098, 95% CI 0.06 – 0.15) or leukopenia (OR 0.32, 95% CI 0.18 – 0.58). This became more obvious when the policy change for G-CSF support in the standard chemotherapy group resulted in reduction of the risk for chemotherapy delay due to neutropenia (OR 0.52, 95% CI 0.32 – 0.84). Dose reductions overall in the standard chemotherapy group were very low; 165 out of a total of 5705 cycles were reduced in dose due to neutropenic event, infection with normal ANC and neutropenia or leukopenia. Dose reductions in the tailored dose dense therapy group were protocol predefined and were therefore not considered for this analysis.

		With G-CSF		Without G-CSF		Unknown	Total	Odds Ratio
		Total cycles 10,391	%	Total cycles 2950	%	93	13,434	
Febrile neutropenia	Grade 1–2	19	0.18%	6	0.20%	0	25	
	Grade 3–4	126	1.21%	87	2.95%	5	218	OR 0.40* 95% C.I. 0.30–0.54
Infection with ANC < 1.0	Grade 1–2	167	1.61%	82	2.78%	6	255	OR 0.52* 95% C.I. 0.35–0.79
	Grade 3–4	74	0.71%	40	1.36%	2	116	
Infection with normal ANC	Grade 1–2	525	5.05%	102	3.46%	1	628	
	Grade 3–4	158	1.52%	24	0.81%	0	182	OR 1.88* 95% C.I. 1.22–3.03

Table 11. *Neutropenic events (febrile neutropenia and infection with low ANC) and infection with normal ANC with and without G-CSF. ANC: Absolute neutrophil count, G-CSF: granulocyte-colony stimulating factor, OR: odds ratio, CI: Confidence interval. Grade reported according to CTC AE v 3.0.*

In conclusion, tailored dose-dense chemotherapy with G-CSF support did not increase haematological toxicity. Administration of G-CSF reduced neutropenic events and allowed for better compliance on the planned treatment schedule.

Paper IV – Incidence of Amenorrhea and Impact on Breast Cancer Outcomes During Tailored Dose Dense Chemotherapy for High-Risk Early Breast Cancer.

The aim of this post-hoc study was to examine whether gonadal toxicity related to tailored and dose dense chemotherapy can impact breast cancer related outcomes and if menopause status at breast cancer diagnosis can impact chemotherapy efficacy on high-risk early breast cancer outcomes.

This retrospective, exploratory analysis included 1913 participants in the PANTHER trial with available menopause status at baseline; 1036 were premenopausal and 877 were postmenopausal at inclusion. Figure 17 presents distribution of patients in the treatment groups based on baseline menopausal status and allocated treatment. Treatment allocation was balanced between the two different menopause status groups.

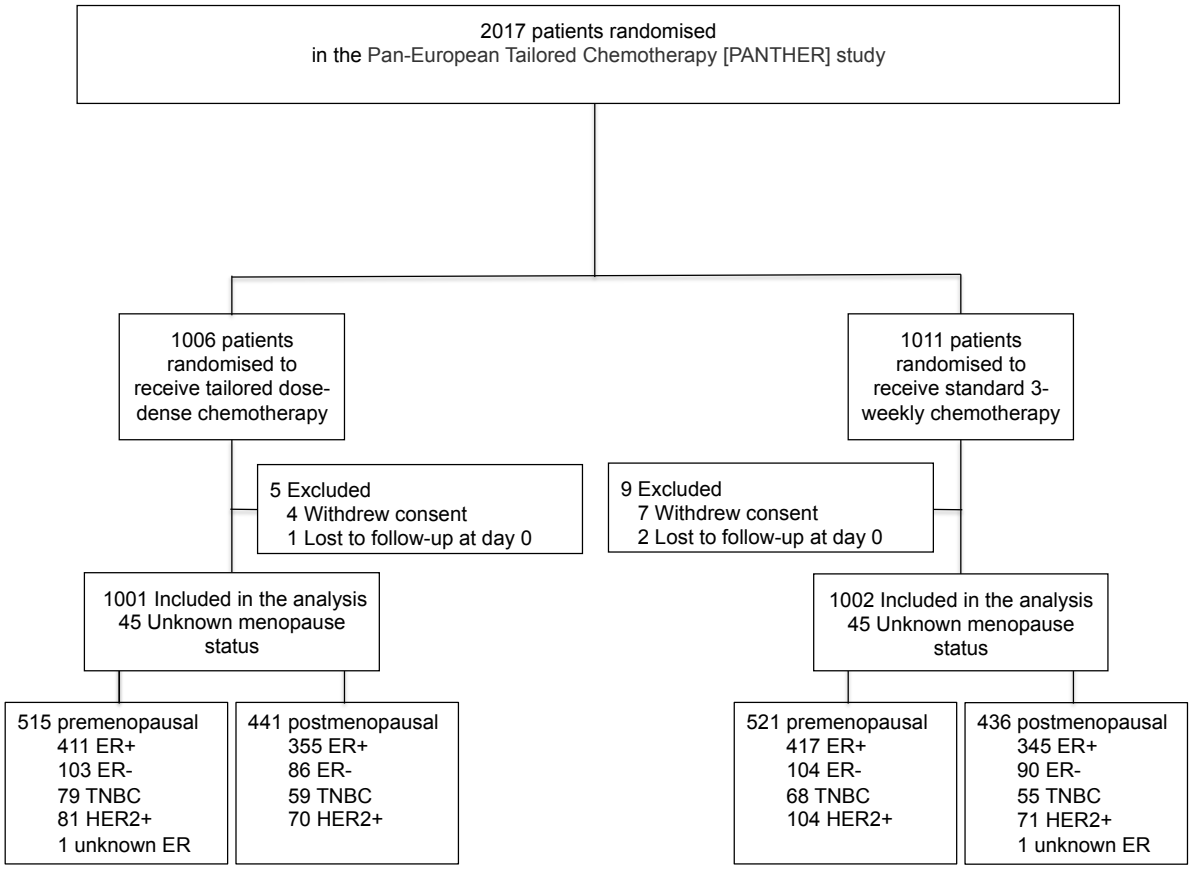

Figure 17. *Consolidated Standard of Reporting Trials diagram of the PANTHER HER2 according to baseline menopause status and allocated treatment. DD: Tailored dose-dense chemotherapy. ER: Oestrogen Receptor, HER2: human epidermal growth factor 2, SD: Standard chemotherapy, TNBC: Triple negative breast cancer.*

Baseline characteristics are presented in table 12 and were balanced in terms of tumour size, nodal status, hormonal receptor and HER2 status. Imbalance was present regarding tumour grade, Ki67 and type of performed surgery. Premenopausal women had higher grade and Ki67 tumours and underwent more mastectomies.

In total, 251women experienced breast cancer relapse event; 136 premenopausal and 115 postmenopausal. Overall, tailored and dose dense chemotherapy improved BCRFS regardless menopause status but did not reach statistical significance. Compared to standard chemotherapy, women treated with tailored dose dense chemotherapy were 17% less likely to experience BCRFS event if they were premenopausal (HR 0.83, 95% CI 0.59 – 1.16) and

	Premenopausal patients N=1036 (%)		**Postmenopausal patients** N=877 (%)		**P value**
Median age, years (range)	45.2 (21.4 – 65.3)		58.6 (26.4 – 69.5)		<0.001
Tumor size, mm					
0-20	424	(40.9)	354	(50.5)	
21-50	538	(51.9)	457	(52.1)	0.76
>50	69	(6.7)	66	(7.5)	
Missing	5	(0.5)	0	(0.0)	
Positive nodes					
0	32	(3.1)	28	(3.2)	
1-3	604	(58.3)	488	(55.6)	0.48
4-9	284	(27.4)	244	(27.8)	
>9	116	(11.2)	117	(13.3)	
Tumor grade					
I	64	(6.2)	44	(5.0)	
II	480	(46.3)	463	(52.8)	0.003
III	492	(47.5)	364	(41.5)	
Missing	0	(0.0)	4	(0.5)	
Hormone receptors					
ER and/or PR (+)	828	(80.0)	700	(79.8)	
ER/PR (-)	207	(20.0)	176	(20.1)	0.96
Missing	1	(0.1)	1	(0.1)	
HER2 status					
Positive	185	(17.9)	141	(16.1)	0.30
Negative	851	(82.1)	736	(83.9)	
Ki-67 %					
≤20	288	(27.8)	267	(30.4)	
>20	373	(36.0)	270	(30.8)	0.03
Missing	375	(36.2)	340	(38.7)	
Type of operation					
Breast conserving	518	(50.0)	502	(57.2)	0.002
Mastectomy	518	(50.0)	375	(42.8)	

Table 12. *Baseline characteristics of patients enrolled in the PANTHER trial according to menopausal status at baseline. ER: Oestrogen receptor; FEC/D: 5-fluorouracil/epirubicin/cyclophosphamide and docetaxel; HER2: human epidermal growth factor receptor 2; PR: progesterone receptor; tdd EC/D: tailored dose dense epirubicin/ cyclophosphamide and docetaxel*

26% less likely if they were postmenopausal (HR 0.74, 95% 0.51 – 1.06) at the time of breast cancer diagnosis. In absolute numbers, experimental treatment improved 5-year BCRFS in premenopausal women by 2.5% (-1.8 – 6.8%, p=0.254) and postmenopausal women by 4.6% (0.1 – 9.2%, p=0.049). There was no significant interaction between baseline menopausal status and allocated treatment.

At the predefined, for this analysis, two years' time point, about one third (n=128) of the premenopausal women in each group (31.3% in the standard and 32.2% in the experimental therapy group) experienced gonadal toxicity and cessation of menstruation (OR=1.04; 95% CI 0.77 – 1.39). There was an absolute reduction of BCRFS events among patients that experienced chemotherapy-related premature ovarian insufficiency. Forty-eight (8.11%) events among women with CIA and seven (12.28%) among those who still menstruated, numbers too small to provide power to detect potential benefit.

Further subgroup analysis per breast cancer subtype revealed statistically non-significant reduction of BCRFS events by the experimental treatment among the 1528 patients with hormone responsive breast cancer, with or without HER2 amplification. This effect remained regardless menopausal status and no formal interaction between efficacy of received chemotherapy regimen and menopausal status in hormone receptor positive patients was present. Interestingly, among the 261 patients with triple negative breast cancer, a significant interaction between treatment and menopausal status was demonstrated (test of interaction p=0.043). Tailored and dose dense adjuvant chemotherapy improved BCRFS in postmenopausal women (HR=0.44, 95% CI 0.19 – 1.03; p=0.06) but had the opposite effect among premenopausal women (premenopausal, HR=1.29, 95% CI 0.69 – 2.40; p=0.426) (figure 18). Interaction was present exclusively in the TNBC group and the test of interaction was not significant when the entire hormone-receptor negative patients with also HER2 positive tumours was considered (p=0.155).

In summary, increased dose intensity did not augment gonadal toxicity. Efficacy of allocated treatment was not influenced by the patient's menopausal status at the time of breast cancer diagnosis with the exception of TNBC. A finding that warrants caution and should be further investigated, considering the exploratory, not-predefined nature of the analysis.

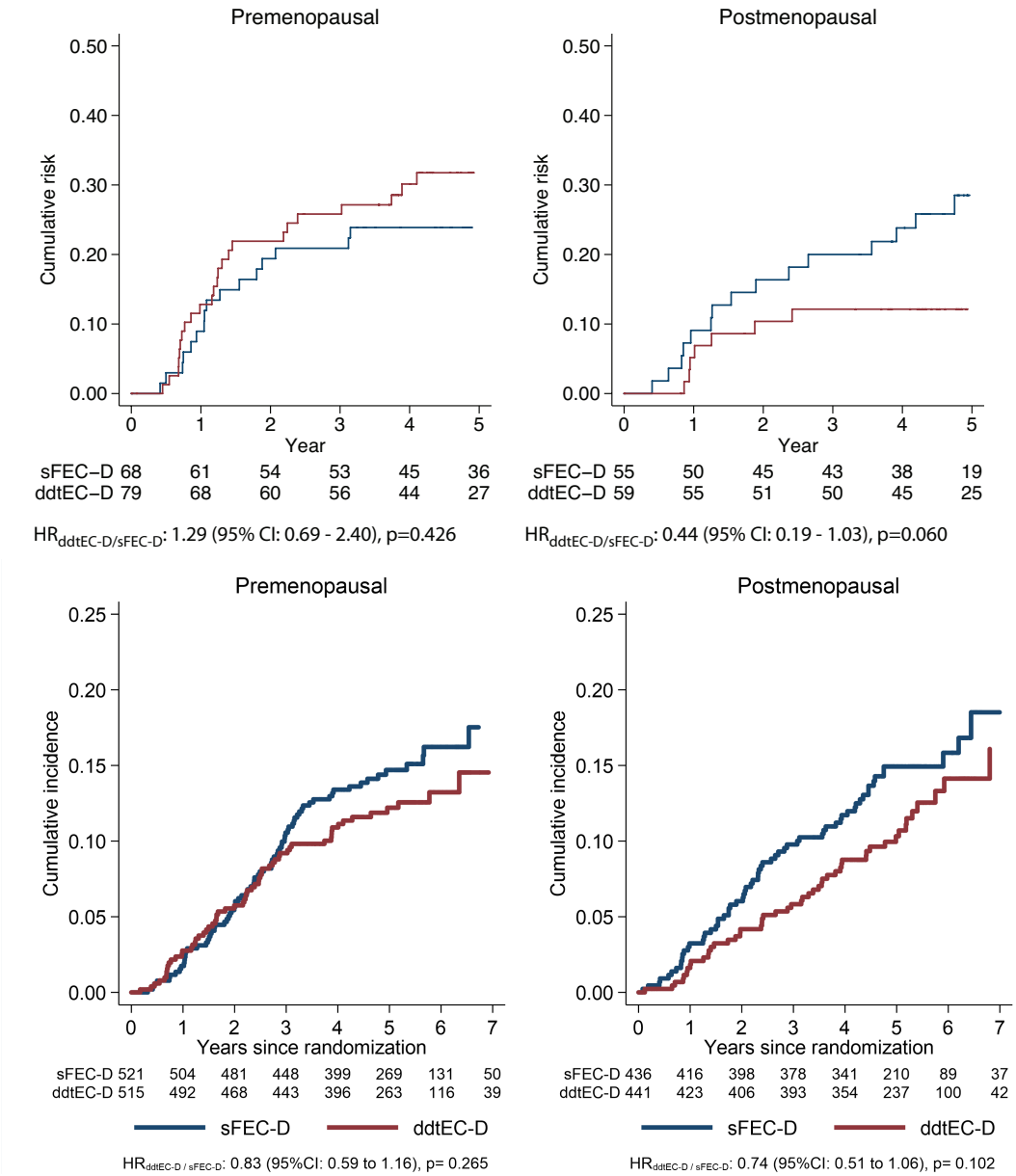

Figure 18. *Cumulative incidence for breast cancer relapse-free survival premenopausal versus postmenopausal, study population (top) and triple negative breast cancer population (lower) in the PANTHER trial. sFEC-D: standard interval 5-fluorouracil/epirubicin/cyclophosphamide and docetaxel; ddtEC-D: tailored and dose dense epirubicin/cyclophosphamide and docetaxel; HR: hazard ratio; CI: confidence interval*

DISCUSSION

Breast cancer is a disease affecting a significant number of women around the globe with major bearing on individual, societal and health-economic level. The rapid development in breast cancer management and advancements in further understanding disease biology have improved survival and increased prevalence of long-term survivors. Despite systemic adjuvant anticancer therapies for breast cancer being life-saving, the impact on the person's quality of life and, potentially, survival through serious side effects is not negligible.

In the current thesis both acute toxicities; neutropenia, infections and acute cardiotoxicity during trastuzumab and long-term toxicities such as long-term cardiotoxicity leading to heart failure and chemotherapy induced gonadal toxicity were studied.

Paper I

Management of heart failure nowadays is multifaceted and is directly related to the stage of the disease and the symptoms. Paper I utilised the Swedish national registries to compare women with heart failure and a breast cancer history to an age-matched female population with cardiac insufficiency. No differences were noted between breast cancer survivors and general population with reference to heart failure in the iHF group. Despite similarities in symptoms and heart failure aetiology, differences in the reported comorbidities and heart failure therapies were demonstrated in the pHF group. History of acute myocardial infarction (AMI) was more common among women with pHF and no history of breast cancer. A finding also consistent with the more frequent utilisation of aspirin or revascularisation that was observed in this group.

Despite imbalance in the history of AMI, women with BC history were as likely to have heart failure due to ischemic heart disease as those without. This discrepancy could be either due to diagnosis of ischemic heart disease at an early stage before leading to established myocardial infarction or most likely due to distinct aetiology of coronary artery disease and ischemia among breast cancer survivors. Whether the former stems from increased awareness after breast cancer diagnosis or the latter is related to the anticipated distinct ischemic cardiomyopathy pathophysiology due to previous chemotherapy and/or radiotherapy cannot be assessed in the present paper. Nonetheless, potential bias in expediting investigations and monitoring in favour of women with breast cancer history is of importance and merits further exploration.

Age is an important confounder in the risk of both breast cancer and heart failure. The cohort was therefore controlled with age-matched population, to adjust for the higher risk of cardiovascular disease with increased age. This introduced balance between the groups but may have masked potential differences regarding age of onset of heart failure or rate of progression between the two groups.

Chronic heart failure is a successfully deteriorating condition and thus early detection and intervention is essential, not least to prevent sudden worsening leading to acute heart failure and decompensation. In line with the progressive course of chronic heart failure, higher mortality rates were demonstrated among women with prevalent heart failure (pHF) compared to the ones with shorter interval since heart failure diagnosis (iHF), irrespective breast cancer history. On the other hand, the median follow-up time of two years might be considered short in order to assess potentially distinct heart failure mortality among women with breast cancer history and pHF.

Patients with cancer other than breast cancer were excluded from the analysis, and thus any cardiovascular aftereffect is uniquely related to breast cancer therapy. Information on the potential breast cancer therapy women in the cohort received or were still receiving after heart failure diagnosis would have facilitated evaluation of the results but this data is not available in the heart failure registry. Chemotherapy-induced cardiomyopathy and subsequent heart failure is often cited to be associated with worse prognosis and a more aggressive course than cardiomyopathy of other aetiology such as idiopathic or due to ischemic heart disease (334). However, this has been challenged in the recent years due to increased awareness and improved heart failure outcomes. Intra-group comparison in the iHP and pHF did not reveal any difference in the overall survival or mortality due to cardiovascular disease or heart failure between women that had been diagnosed with breast cancer and not. Thus, heart failure after breast cancer diagnosis and treatment can likely have the same outcomes as heart failure of other causes, if properly and timely addressed.

Paper II

Increased chemotherapy dose intensity improves breast cancer outcomes (89). Calculation of chemotherapy dose regardless inter- and intrapatient pharmacodynamics and pharmacogenetic variations faces the risk of over- and undertreatment (96). Individually tailoring chemotherapy dose according to levels of maximal bone marrow tolerance, as in the PANTHER trial, enables individually adjusted dose levels. Thus, an accepted assumption could be that it may also lead to increased cardiotoxicity.

As previously stated, current guidelines recommend neoadjuvant approach for HER2 positive breast cancer and the results from the KATHERINE study, demonstrating meaningful improvement of breast cancer outcomes can be considered practice changing (58, 60). In consequence, the relevance of optimisation of adjuvant therapy in HER2 amplified breast cancer can be questioned. However, implementation of neoadjuvant therapy as such is significantly low across countries, even with different economic and health care background (335-338). Thus, adjuvant trastuzumab is still extensively exploited and the continuous quest for appropriate escalation or de-escalation is justified.

Adding chemotherapy, with shorter intervals and individually adjusted doses, to trastuzumab in HER2 positive breast cancer led to a non-significant improvement of breast cancer relapse by approximately one third, compared to standard backbone chemotherapy. It remains to be seen if longer follow-up can lead to significant improvement of breast cancer relapse and survival. The subgroup analysis of HER2 positive breast cancer patients treated with adjuvant trastuzumab in the GIM2 trial (n=132) did not show statistically significant benefit of adjuvant trastuzumab delivered after dose dense chemotherapy over trastuzumab in sequence with standard interval chemotherapy (339). On the contrary, hazard ratio for both DFS and OS were close to 1 and with wide confidence intervals. However, this was a post hoc analysis of a small portion of a subgroup of the study and without stratification for trastuzumab treatment between the two chemotherapy schedules. Similarly, the large meta-analysis by EBCTCG showed that trastuzumab did not impact treatment efficacy, however, the use of trastuzumab was not standard in half of the included trials (89).

Molecularly identified HER2 enriched breast cancer by PAM50, could predict better effect in terms of higher probability of pCR, after anti-HER2 neoadjuvant therapy (340). On one hand, failure to report benefit from dose dense chemotherapy and trastuzumab, could therefore derive from dilution of the results by HER2-enriched tumours that respond to anti-HER2 treatment without particular benefit from intensifying chemotherapy. On the other hand, exploration of escalation of chemotherapy would be a logical step forward to improve breast cancer outcomes for HER2 non-enriched tumours.

Trastuzumab in combination to tailored dose dense chemotherapy was safe despite increased mean epirubicin dose delivered in the experimental group. That being said, a reduction of mean LVEF through time was indeed observed despite lack of clinical relevance. This observation suggests a negative long-term undesirable effect of chemotherapy rather than trastuzumab. Importantly, there was a significant reduction of LVEF from baseline at six years follow-up in the tailored dose dense. This further suggests the observed LVEF decline more likely results from the higher mean epirubicin dose administered in the tailored dose dense group and underscores the need to investigate whether cardiac function will further decline at the planned 10-years follow-up.

Paper III

The benefit from escalating dose intensity is directly associated with preventing dose reductions or delays due to haematological toxicity. Paper III confirmed the undisputable prophylactic effect of G-CSF in terms of neutropenia and neutropenic events. G-CSF allowed for dose escalation in the experimental group without excess haematological toxicity. On the contrary, it must be emphasised that despite higher mean chemotherapy doses delivered in the experimental arm, no grade 5 toxicity related to haematological side effects was reported in the PANTHER trial. The lack of comparison between the two treatment groups can be considered a limitation of the study. This becomes though more understandable if each

chemotherapy cycle is considered as potential source of neutropenia. Thus, the eight cycles delivered in the experimental group were de facto more prone to cause neutropenic events compared to the six cycles in the standard group. The former received also G-CSF and antibiotics per protocol requirements, making potential comparison meaningless and methodologically unsound.

The safety of G-CSF administration has been questioned after recognition of expression of myeloid growth factor receptors in hematopoietic cells (341). G-CSF support has been reported to slightly increase the risk for secondary malignancies such AML/MDS (RR=1.85, 95% CI 1.19 – 2.88), while simultaneously improving overall survival (RR=0.92, 95% CI 0.90 – 0.95) (342). The risk of overtreatment with G-CSF needs to be considered and adherence to international guidelines is crucial. In the investigated chemotherapy schedules in paper III, supportive treatment with G-CSF was beneficial and outweighed potential long-term side effects, not least considering the low incidence of AML/MDS reported in the study (92).

Although may be contra-intuitive, dose dense treatment schedules are reported to cause less leukopenia, mainly due to the practically mandatory and selective administration of G-CSF during these schedules compared to the standard regimens (89). G-CSF support resulted in fewer dose reductions and delays due to neutropenia even in the standard group. This becomes more obvious after comparing compliance to planned treatment among participants treated with standard chemotherapy, before and after the policy change. Increased use of G-CSF improved adherence to the study treatment, in regards to delays and dose reductions due to neutropenic events. The latter highlights the benefit of G-CSF even in standard chemotherapy regimens in breast cancer, considering the detrimental effect that a reduction in relative dose intensity can have on breast cancer outcomes (95, 265). Increased incidence of infections with normal ANC when G-CSF was administered, probably reflect infections that would still appear, but the G-CSF treated patients were able to hold a normal neutrophil count.

Paper IV

Moving forward, paper IV investigated if tailored dose dense chemotherapy would also increase gonadal toxicity and thus affect breast cancer outcomes. Risk for chemotherapy-induced amenorrhea, at two years after randomisation, among women that did not receive GnRHa was equal in both treatment groups. One explanation for this could be the tailoring part of trial design. Chemotherapy dose was escalated for patients who did not experience severe toxicity but it was also de-escalated for patients that did. Thus, patients that in the standard group might had been overtreated, in the experimental group they received a dose adapted to their individual. Similarly, patients that required dose escalation would have probably been undertreated in the standard group. It would therefore be rational to assume

that iatrogenic premature ovarian suppression was equal in the two groups due to the dose adjustments depending on toxicity in the experimental group.

At the same time, other confounding factors could have influenced the results. Patients that received GnRHa were excluded from the amenorrhea analysis since amenorrhea due to chemotherapy was the endpoint of the analysis. However, GnRHa was based on the treating physician's recommendation and not on random allocation, and may thus mask CIA incidence. Moreover, GnRHa administration during chemotherapy seems to have a protective effect on ovarian function and can potentially alter the results but such information is lacking and this practice was not widely applied during the conduct of the PANTHER trial (310).

Chemotherapy-induced amenorrhea is associated with improved breast cancer outcomes (296, 313, 314). It has been speculated by many that the driver of dose dense chemotherapy effect is no other than higher CIA incidence due to increased cumulative doses. This is however contradicted by the similar amenorrhea rate between the two treatment groups in the PANTHER trial and by analogous results from other trials comparing dose dense and standard chemotherapy regimens (305, 316). Thus, treatment efficacy of dose dense chemotherapy appears to be related to genuine cytotoxic effect rather than consequence of higher rate of premature ovarian insufficiency. Comparison of BCRFS between premenopausal women that suffer cessation of menstruation and those who did not was not feasible due to the limited number of premenopausal women that experienced breast cancer relapse events.

In the overall population, regardless breast cancer histological subtype, tailored dose dense chemotherapy led to a non-significant improvement in BCRFS in both premenopausal and postmenopausal women. However, no formal interaction was demonstrated, implying that the beneficial effect of experimental treatment, even if not statistically significant, was due to increased dose intensity and density, regardless menstruation status at time of chemotherapy delivery.

Subgroup analysis by tumour subtype revealed a positive interaction between menopausal status and allocated treatment for women with triple negative breast cancer. There was a non-statistically significant trend to worse BCRFS among premenopausal women treated with tailored dose dense chemotherapy. On the contrary, improved BCRFS was observed among postmenopausal women in the same treatment group. The aforementioned menopause status-allocated treatment interaction did not persist when only hormone receptor negative patients were included, probably due to the improved outcomes of the HER2 positive patients due to trastuzumab masking other effects. If this finding is an aftereffect of the patients' younger age in the premenopausal group rather than menstruation status itself, then similar findings should be present in the overall analysis and the other subgroups analysis. Additionally, based on the report of the main results of the PANTHER trial and the EBCTCG meta-analysis, dose dense chemotherapy effect is independent of age and ER status (89, 92). Thus, same effect of the tailored dose dense chemotherapy would be expected in both hormone-receptor positive and negative women, facts suggesting our findings are most probably a chance finding.

While acknowledging the risk for false positive findings, potentially yet unknown biological factors leading to worse prognosis in young, premenopausal women with tumours lacking receptors for treatment other than chemotherapy, should not be disregarded. Randomisation in clinical trials is expected to balance known and unknown baseline factors but an exploratory sub-group analysis is more prone to biases. Thus, while considering pitfalls in the study design, these results should raise caution and be further investigated.

FUTURE PERSPECTIVES

In addition to the valuable information retrieved from the studies, some reflections warrant further investigation and could consist future research activities:

1. What oncological treatment did the patients in paper I receive and did results of similar heart failure outcomes persist after adjusting for chemotherapy and left-sided radiotherapy?

2. The reasons leading to significantly lower administration of device therapy as measure to treat heart failure among women with breast cancer history merits further investigation. Considering the usual chronic nature of heart failure, can longer follow-up reveal significant differences in heart failure outcomes?

3. We, partly, move towards more anthracycline-free regimens with anthracycline-free neoadjuvant therapy and salvage with T-DM1 and there is also an increasing trend in adjuvant paclitaxel in combination with trastuzumab for early HER2 positive breast cancer. Would an anthracycline-free regimen lead to fewer discontinuations of trastuzumab due to cardiotoxicity? Would the long-term cardiotoxicity risk after trastuzumab be positively affected and can then the overall benefit of trastuzumab subsequently increase without the trade-off of anthracycline cardiotoxicity?

4. As described, there are compelling data on the usefulness of increased chemotherapy intensity in high-risk breast cancer. Tailored dose-dense chemotherapy in the PANTHER trial significantly improved event-free survival and although improvement in overall survival did not reach significance it clearly indicates a trend to better survival. Would there be a place for a randomised controlled trial between dose dense and tailored dose dense chemotherapy?

5. G-CSF is indicated when expected neutropenic fever rates exceed 20%. Could G-CSF also be useful in the treatment of frail patients with lower estimated neutropenic fever risk? Would it allow for more patients to receive chemotherapy; both (neo)adjuvant and in metastatic disease? Would it be clinically relevant and/or cost-effective?

6. Further follow-up is justified to investigate long-term survival effect of CIA among the premenopausal PANTHER patients due to the long natural history of ER-positive disease.

7. Can we gather individual patient data from other dose-dense chemotherapy studies to investigate interaction of dose dense chemotherapy and menopausal status in a larger triple negative breast cancer population?

CONCLUSION

Breast cancer is the most common cancer among women in most countries. The constantly improving prognosis of breast cancer, almost 8 out of 10 women diagnosed with breast cancer are alive after 10 years, has led to increasing number of long-term survivors. Management of early breast cancer is multiprofessional involving various disciplines and treatment approaches. In addition, the median age of diagnosis (around 60-65 years in Europe) coincides with an age when also the risk for cardiovascular disease and other comorbidities increases. Thus, breast cancer therapy is further perplexed with an important ethical dilemma; provide enough care to survive a potentially life-threatening disease as cancer and concurrently keep unwanted short and long-term effects of surgery, chemotherapy, targeted therapy and radiotherapy at the minimum. Or as Hippocrates said, to: *"follow that system of regimen which, according to my ability and judgment, I consider for the benefit of my patients, and abstain from whatever is deleterious and mischievous"*. The *"primum non nocere"*. As such, our studies aimed to better characterise heart failure after breast cancer diagnosis and to investigate trastuzumab-related cardiotoxicity, neutropenic events and premature ovarian insufficiency related to intensified breast cancer chemotherapy regimens.

In conclusion:

- History of breast cancer did not predict a worse heart failure prognosis, despite observed differences in the management of heart failure. The differences demonstrated in the frequency of ischemic heart disease and administration of aspirin and, more importantly, device therapy highlight the need for development of heart failure management guidelines adapted to the need of the cancer patient and cancer survivors. Longer follow-up is warranted and will shed more light in the impact of differences in heart failure management among women with prevalent heart failure with and without breast cancer. Collaboration between cardiologists and oncologists is essential for the assessment and management of patients at risk for cardiotoxicity and those who develop cardiac adverse events related to cancer therapy.

- The combination of dose-dense chemotherapy and trastuzumab decreased the relative risk for relapse by 32%, compared to standard treatment, a statistically non-significant difference. The combination was feasible and did not lead to increased cardiotoxicity, until six years of follow-up. Longer follow-up is warranted to assess long-term cardiotoxicity and efficacy.

- Prophylactic G-CSG allowed for dose escalation without excess neutropenia related adverse events and, significantly, no neutropenia-related deaths were observed in the study. In line with existing data, G-CSF support reduced neutropenic events and dose reductions, allowing for increased relative dose intensity, an important factor for improved survival outcomes. These results come to add in the significance of

appropriate use of G-CSF prophylaxis in both standard and dose dense breast cancer chemotherapy regimens.

- Tailored and dose-dense adjuvant chemotherapy did, thus far, not increase risk for CIA and was associated with statistically non-significant improvements in outcomes compared with standard therapy, regardless of menopausal status and amenorrhea. It is uncertain whether there is a plausible biological explanation for the observed negative effect of tailored and dose-dense chemotherapy among premenopausal patients with triple negative breast cancer or it is merely due to a chance finding. Nonetheless, these results further support the efficacy of dense chemotherapy independent of CIA.

ACKNOWLEDGEMENTS

Conducting this work has been a long and rewarding experience, with challenges on both personal and professional levels. The eventful journey to my Ithaca would not have reached its completion without all the wonderful people around me to whom I am profoundly thankful.

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