Evaluation and Management of Side Effects of Breast Cancer Treatment

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Evaluation and Management of Side Effects of Breast Cancer Treatment

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(met een samenvatting in het Nederlands)

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De dingen die er toe doen zijn onzichtbaar

Voor mijn ouders

In liefdevolle herinnering aan Cora Klaasse Bos

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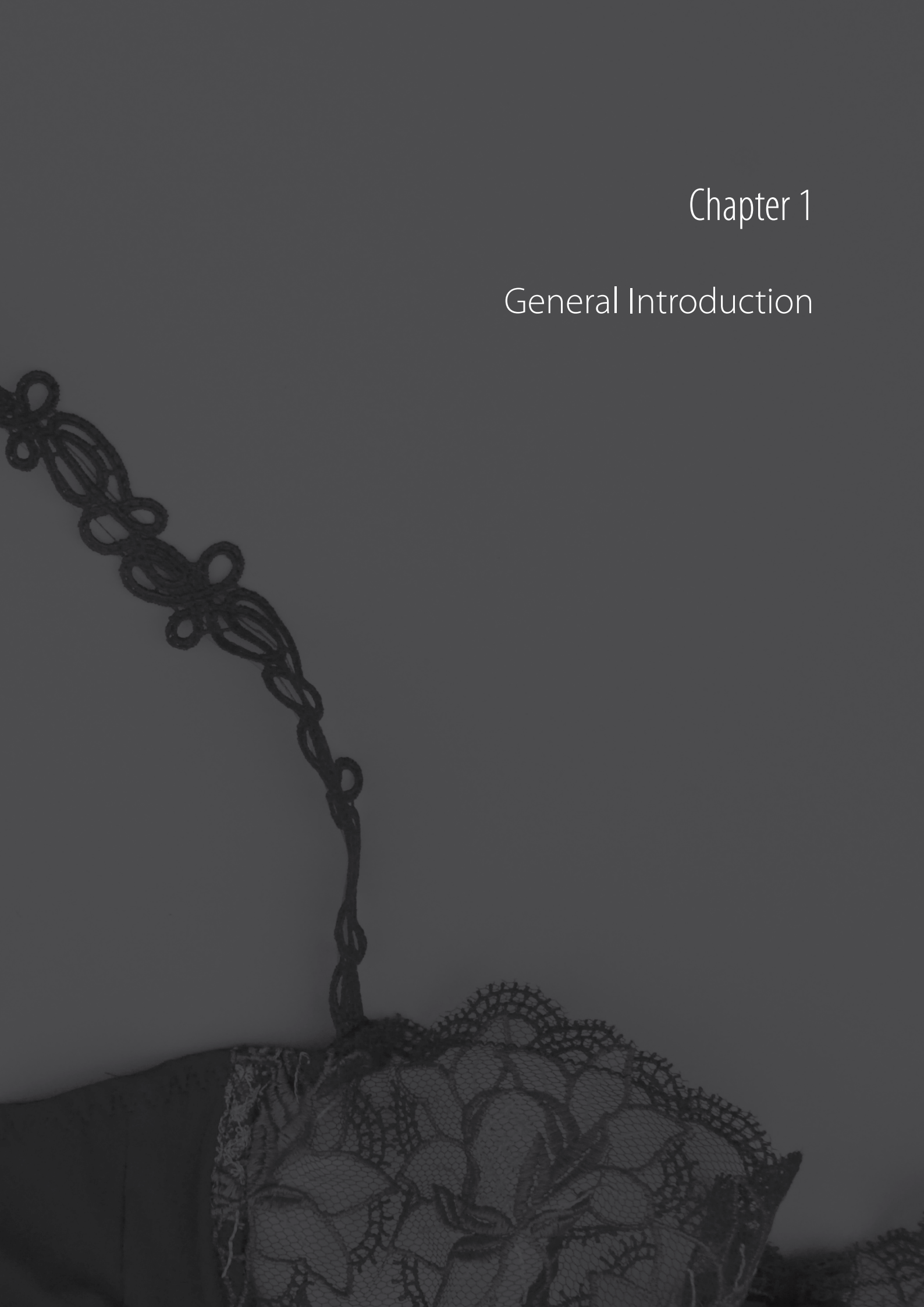
Chapter 1

General Introduction



Breast cancer

Breast cancer is one of the most frequently occurring malignant diseases. In 2008, worldwide 1.38 million new cases of breast cancer were diagnosed (23% of all malignancies) (1). The mortality rate decreased in the last decades as a result of improved early diagnosing and implementing more effective treatment regimens for early as well as for advanced breast cancer.
Surgery is the main modality for local treatment of early breast cancer. Surgery, with or without radiotherapy, results in locoregional disease control in the majority of breast cancer patients. However, microscopic disease may already have spread before locoregional therapy could be applied. If this microscopic disease remains untreated, it could develop into local recurrence and/or metastatic breast cancer. In addition to improved locoregional therapies, major steps forward have been made by implementation of adjuvant systemic hormonal therapy and/or chemotherapy in properly selected patients resulting in significant reduction in the risk of distant relapse of breast cancer and increased cure rate.
However, the downside of systemic therapy is the risk of short- and/or long-term disabling side-effects that reduce the quality of life and treatment adherence.

Menopausal symptoms

Systemic therapies for breast cancer in premenopausal women increase the risk for early menopause with the resulting loss of childbearing capacity and symptoms such as hot flashes, genitourinary atrophy and mental distress. Hot flashes are the most often occurring complaint in pre- and postmenopausal breast cancer patients. The impact of hot flashes on the quality of daily life including absence from work varies widely among affected women. When hot flashes disrupt quality of life or the quality of sleep, patients highly desire to apply effective therapies that reduce intensity and frequency of hot flashes and symptoms improving the basis for continuation of anti-cancer therapies.

Trastuzumab-related cardiac dysfunction

The human epidermal growth factor receptor HER2 is a member of the epidermal growth factor receptor (EGFR) family of transmembrane tyrosine kinases and is physiologically involved in the regulation of cell proliferation. In a subset of approximately 20% of patients with breast cancer HER2 is overexpressed. This feature is associated with shorter disease-free and overall survival compared to women with breast cancer without HER2 overexpression. Trastuzumab is a humanized monoclonal antibody against the extracellular domain of HER2 and treatment with trastuzumab, most often in combination with chemotherapy, has resulted in clinical benefit in HER2 positive advanced breast cancer and improved disease-free and overall survival in combination with adjuvant chemotherapy in HER2 positive primary breast cancer. Overall trastuzumab treatment is well tolerated, however cardiac dysfunction is one of the clinically relevant side-effects of trastuzumab treatment manifested by left ventricular

systolic dysfunction and in a small proportion of patients even in advanced congestive heart failure.

Thus, improvement in outcome achieved by systemic therapy is achieved at the cost of short- and long-term and sometimes irreversible toxicities. It is plausible that even further benefit could be attained if side-effects of therapy could be significantly reduced, thereby improving quality of life, treatment continuation and patient adherence. Thus, development and implementation of methods to avoid or ease toxicities are of major relevance. Results of research strategies to predict, recognize and improve non-adherence and to alleviate treatment related adverse events are expected to improve treatment outcome in women with early breast cancer.

This thesis

The research described in this thesis is focused on treatment of anti-cancer therapy induced menopausal symptoms and on trastuzumab-related cardiac dysfunction in women with early and advanced breast cancer. The aim is to investigate new treatment options for the management of hot flashes, and related disabling symptoms of treatment induced early menopause, and new modalities for the detection and prevention of trastuzumab-related cardiotoxicity.

Outline of this thesis

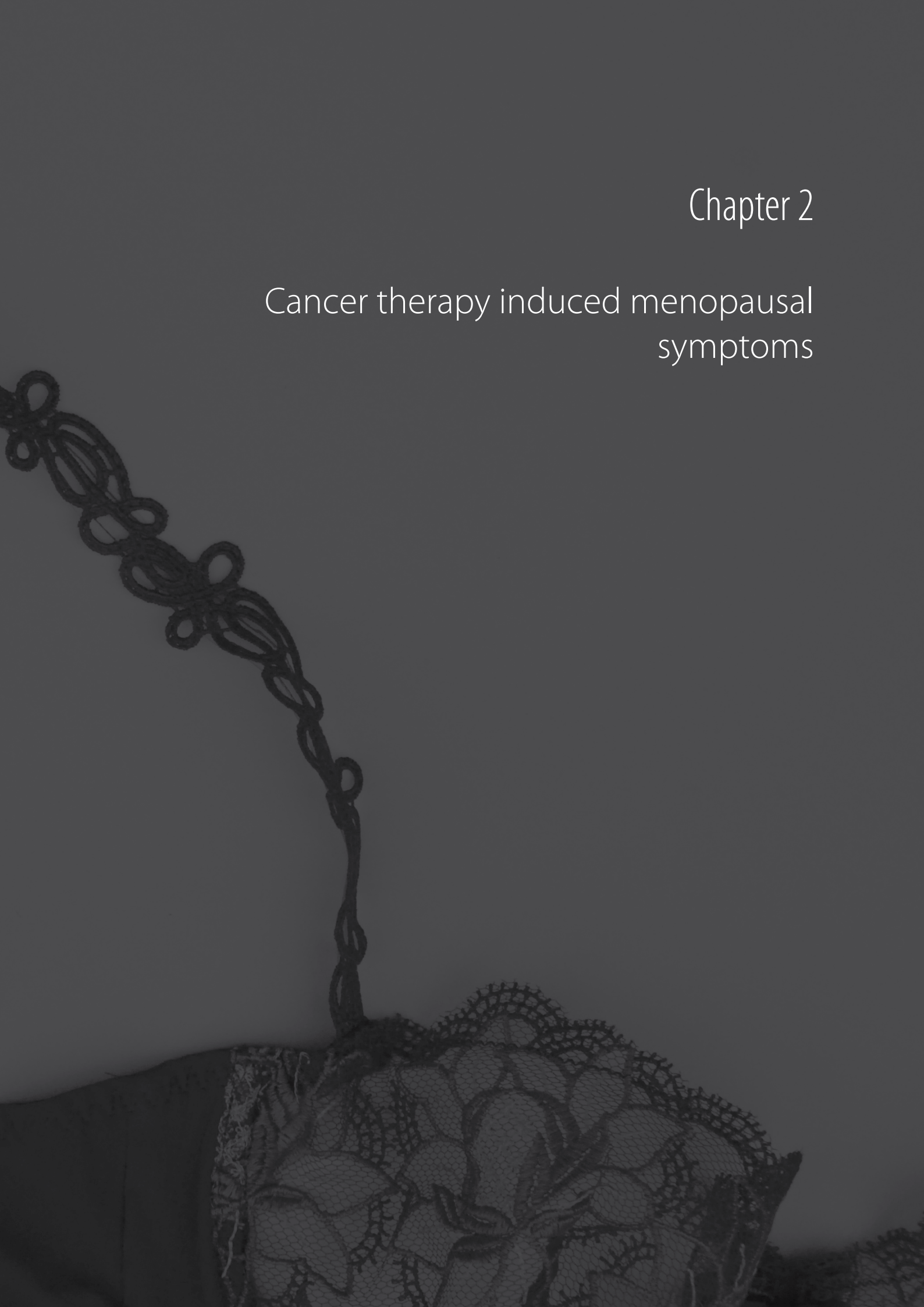
Chapter 2.1 is a review of the literature on the epidemiology and diagnosis of hot flashes and the non-pharmacological and pharmacological treatment interventions of hot flashes in breast cancer patients. Chapter 2.2 provides the results of a prospective study, a randomized placebo-controlled trial with clonidine and venlafaxine in the management of hot flashes in breast cancer patients. Both drugs are often used for this aim, but the activities to treat these symptoms and the side-effects of these drugs even in premenopausal women, have never been compared in a double blind placebo-controlled trial.
In Chapter 3.1 a concise monograph of the drug trastuzumab is presented.
Preliminary results have been published about safety, side-effects such as cardiotoxicity of trastuzumab in highly selected patient populations as part of the registration strategy. However, data are warranted about cardiotoxicity in the unselected daily-life patient population. For this aim the association between risk factors and the development and the reversibility of trastuzumab-related cardiotoxicity were investigated retrospectively of which results are outlined in Chapter 3.2.
Results of large adjuvant trastuzumab trials suggest that the trastuzumab-associated asymptomatic decline in left ventricular ejection fraction or congestive heart failure is reversible in up to as high as 86% of the patients. However, clinical trials adhere to strict eligibility criteria and patients treated in clinical trials are generally fit and often not representative of an unselected patient population. Therefore, long- term safety data of

trastuzumab treatment in primary breast cancer in an unselected patient population were explored of which results are presented in Chapter 3.3.
For safety reasons all clinical trials strictly excluded patients with low baseline LVEF values of less than 50%. Currently, guidelines derived from these trials are being followed in clinical practice, possibly resulting in exclusion of patients from receiving trastuzumab even though they may benefit from treatment, however at the cost of an uncertain risk of developing long-term cardiac side-effects. Therefore, a cohort of patients with a low baseline LVEF prior to start of trastuzumab was identified retrospectively and is outlined in Chapter 3.4.
Pharmacokinetic-pharmacodynamic (PK-PD) models are increasingly recognized as valuable tools in understanding and anticipation of drug interactions or tolerances to treatment and in aiding the development of clinical guidelines. Since little is known about the predictive factors of cardiac toxicity induced by trastuzumab exposure, a PK-PD model is developed to explore the relationship between trastuzumab exposure and changes in left ventricular ejection fractions of which results are presented in Chapter 3.5.
Chapter 3.6 presents the tentative results of a prospective, randomized, pharmacological intervention study (CANDY) at its second interim analysis. The CANDY study aims to assess prevention of trastuzumab-associated cardiotoxicity in early breast cancer patients by pharmacological intervention. This blinded interim report evaluates the safety of trastuzumab with concurrent treatment with the angiotensin II-receptor (AT1) blocker candesartan or placebo.
A summary of the results presented in this thesis is described in Chapter 4.

1. Ferlay J, Shin HR, Bray F, Forman D, Mathers C and Parkin DM. GLOBOCAN 2008, Cancer Incidence and Mortality Worldwide. International agency for Research on Cancer, 2010 (*http://globocan.iarc.fr*)

Chapter 2

Cancer therapy induced menopausal symptoms



Chapter 2.1

Symptoms and Treatment in cancer Therapy-induced Early menopause

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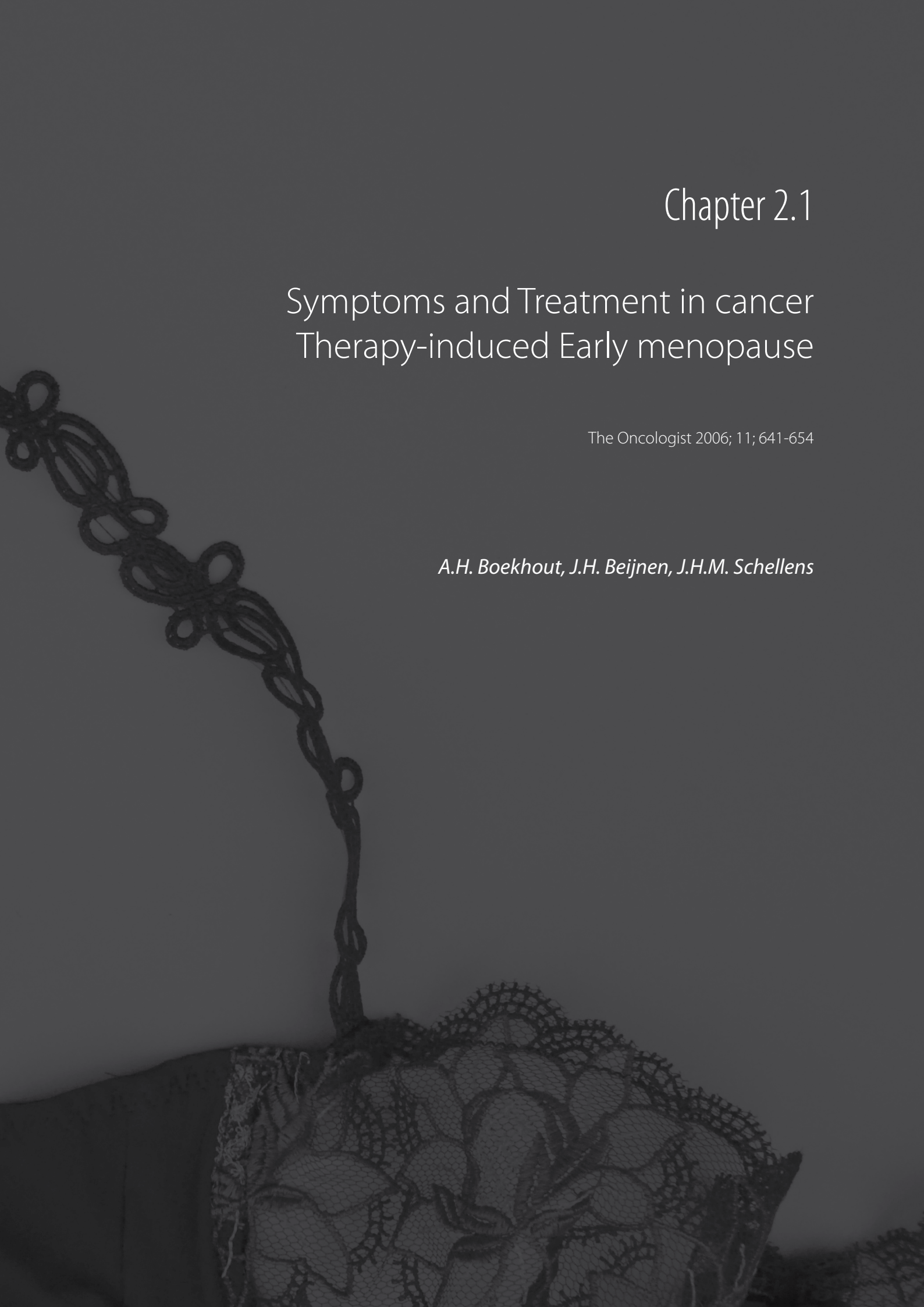

Abstract

Young women with breast cancer often experience early menopause as a result of the therapy for their malignant disease. The sudden occurrence of menopause resulting from chemotherapy, oophorectomy, radiation, or gonadal dysgenesis frequently results in hot flashes that begin at a younger age and may occur at a greater frequency and intensity than hot flashes associated with natural menopause.

Hormone therapy relieves symptoms effectively in 80%–90% of women who initiate treatment. This therapy, however, is generally contraindicated in estrogen-dependent cancers, such as breast cancer, because of the potentially increased risk for recurrence. Many agents have been investigated as potential means for alleviating hot flashes in survivors of breast cancer, such as progestagens, clonidine, gabapentin, and antidepressants. Several complementary and alternative medicines frequently used by patients have also been studied. These include black cohosh, phytoestrogens, homeopathy, vitamin E, acupuncture, and behavior strategies.

To support the use of one of more of these nonpharmacological or pharmacological options in the treatment of hot flashes in breast cancer patients, more evidence from well-controlled clinical trials is needed. In particular, soundly based scientific research with complementary and alternative medicine therapies is lacking. Pharmacological treatments appear to be more beneficial than nonpharmacological treatments.

This article reviews the current literature to assess the epidemiology and diagnosis of hot flashes and the nonpharmacological and pharmacological options for the treatment of hot flashes, in breast cancer patients in particular. When specific treatment options have not been evaluated in breast cancer patients specifically, published data on the management of hot flashes with this modality in healthy postmenopausal women are described.

Introduction

Breast cancer is the most common life-threatening cancer diagnosis in women. Although primary local control surgery is still the mainstay of treatment for early breast cancer, it often may not cure patients, as it does not eradicate micrometastases that are present in a subset of patients. In order to decrease the risk for local and distant relapse, the addition of adjuvant local radiation therapy and/or systemic hormonal and/or chemotherapy is necessary to increase the cure rate.

However, the treatment of early or advanced breast cancer, with the aim to improve survival or palliation, may induce potentially severe short- and long-term toxicities. One disabling side effect of cancer treatment is premature menopause. The risk for the development of early menopause with polyagent adjuvant chemotherapy has been reported to be in the range of 53%–89% (1). The frequency of chemotherapy-related amenorrhea varies with age, the cytotoxic agents used, and the cumulative dose (2, 3).

In premenopausal women with endocrine-responsive tumors, the additional benefit of endocrine therapies after locoregional treatment and chemotherapy has been shown in the adjuvant setting of breast cancer. Endocrine therapy results in a significant improvement in both recurrence-free survival and overall survival in patients younger than 50 years of age (4, 5). Endocrine therapies include suppression of ovarian function by irradiation, surgery, or luteinizing hormone-releasing hormone (LHRH) or gonadotropin hormone-releasing hormone (GnRH) agonists. It is an option to combine ovarian ablation with other types of endocrine therapies, including aromatase inhibitors and selective estrogen receptor downregulators (6).

However, these therapies for breast cancer in premenopausal women increase the risk for early menopause, with the resulting loss of childbearing capacity and symptoms such as hot flashes, genitourinary atrophy, and psychological distress (2, 7, 8). Hot flashes are one of the symptoms that occur with considerable frequency in premenopausal breast cancer patients. Hot flashes can interfere with quality of life and the quality or duration of sleep (9, 10).

There is a wide range of possible disruptions in day-to-day living because of the impact of hot flashes. When hot flashes disrupt quality of life or the quality of sleep in breast cancer patients, these patients should be informed about therapeutic options, and interventions should be considered in the prevention and management of hot flashes.

Pathophysiology and epidemiology of hot flashes

Definitions

A hot flash is a subjective sensation of heat that is associated with objective signs of cutaneous vasodilation and a subsequent drop in core temperature. Sweating, flushing, palpitations, anxiety, irritability, and even panic may accompany the hot flash, and women may also report night sweats. The frequency, duration, and intensity of hot flashes vary. The duration of hot flashes can last a few seconds to several minutes and vary from mild to intolerable. Some women will have hot flashes several times a month, whereas other women complain that symptoms occur every hour (11, 12).

"Hot flashes,""vasomotor symptoms,""hot flushes,""night sweats," and "climacteric symptoms" are all terms used to describe the same phenomenon. The term "hot flash" is used in this article.

Pathophysiology

The exact pathophysiological mechanisms of the occurrence of hot flashes are unknown. The theory of the cause of hot flashes is that there is a dysfunction in the central thermoregulatory set point in the hypothalamus as a result of decreased estrogen or decreased gonadal steroid levels. Studies suggest that estrogen withdrawal leads to an imbalance in plasma levels of several neurotransmitters. Norepinephrine is the primary neurotransmitter responsible for lowering the thermoregulatory set point. Plasma levels of norepinephrine metabolites are increased. The effect of higher norepinephrine and serotonin levels is to lower the thermoregulatory set point, which allows vasodilation and hot flash sensation.

The neurotransmitter serotonin might also have an important role in thermoregulation. Decreased blood serotonin levels and upregulation of serotonin receptors in the hypothalamus are associated with estrogen withdrawal. The thermoregulatory set point might be dependent on the balance of these plasma levels, and a change in the balance may trigger the hot flash sensation (11, 13, 14).

Prevalence of Hot Flashes

Hot flashes affect two thirds of postmenopausal women, and 10%–20% of all postmenopausal women find them nearly intolerable (15). Hot flashes are the most usual complaint of perimenopausal and postmenopausal women. Various entities—such as spicy foods, alcohol and drugs, systemic disease, neurological disorders, androgen deprivation, and carcinoid tumors—could lead to flashing reactions (16, 17). Hot flashes are very common in breast cancer survivors after treatment as well as because of some adjuvant treatment, such as tamoxifen. They are significantly more frequent and more severe in breast cancer patients than in women without a diagnosis of breast cancer (7, 18). The use of tamoxifen, aromatase inhibitors, or suppression of ovarian function in the adjuvant treatment of breast cancer increases the frequency and severity of hot flashes (19–21). Hot flashes were the most common adverse event in the anastrozole (18%) and in the tamoxifen (26%) group in the Immediate Preoperative Anastrozole, Tamoxifen, or Combined with Tamoxifen (IMPACT) trial and in the Arimidex(R), Tamoxifen, Alone or in Combination (ATAC) trial (22, 23).

Treatment of hot flashes

There is a diversity of treatments developed throughout the years, differing in safety, efficacy, and acceptability for alleviating hot flashes among women with a history of breast cancer. These include hormonal and nonhormonal and pharmacological and nonpharmacological treatments. Furthermore, women have also used simple strategies, such as wearing light clothes, dressing in layers, lowering the room temperature, using air conditioners, drinking cold beverages, and avoiding alcohol, spicy foods, hot drinks, and hot foods, to ameliorate their symptoms before starting complementary or pharmacological interventions (24, 25).

Placebo Effect

When reviewing published data on the effect of new therapies in the management of hot flashes, the placebo effect must be seriously considered. Several placebo-controlled trials showed a substantial placebo effect in intervention studies for hot flashes (26–31). Four weeks of placebo treatment can reduce hot flash frequency and hot flash scores by about 25%. The placebo effect on hot flash scores is, in this regard, a consistent effect (32). Therefore, when interpreting clinical data, the placebo effect must always be taken into consideration.

Complementary Interventions

Many alternative products have been used for alleviating menopausal symptoms in women with a history of breast cancer. The effect of black cohosh, phytoestrogens, homeopathy, vitamin E, acupuncture, and behavior strategies in the treatment of hot flashes are described in the next section. Clinical trials of complementary therapies are detailed in Table 1.

Black Cohosh

Black cohosh (Actaea racemosa, synonym Cimicifuga racemosa) is a plant native to eastern parts of North America. In Europe, especially in Germany, black cohosh has been used in the treatment of menopausal symptoms. The mechanism of action is uncertain. There are conflicting data on the estrogenic effect of black cohosh (33–40). The most recent data suggest that black cohosh has no estrogenic effects and is safe in the treatment of menopausal hot flashes (35, 40, 41). There are differences in the results of published clinical data on the efficacy of black cohosh in the treatment of hot flashes. In an uncontrolled trial, 80% of women reported a reduction in hot flashes after 4 weeks of treatment (42). In an open-label, randomized study of 12-months' duration, a satisfactory reduction in the frequency and severity of hot flashes in the intervention group was observed (43). A pilot study revealed that black cohosh reduced hot flashes significantly more than placebo (44). In contrast, in a randomized, double-blind, placebo-controlled trial of 60 days' duration in 69 women with a history of breast cancer, black cohosh was not significantly more effective than placebo (45). A systematic review concluded that there is no evidence for the clinical efficacy of black cohosh in the treatment of hot flashes (46).

Drug-related toxicities such as nausea, vomiting, headache, and dizziness have been reported. One case suggests a relationship between black cohosh and hepatotoxicity, but assessment of a direct correlation between the two was not possible (47).

The published data on the effect of black cohosh in the treatment of hot flashes are conflicting. That black cohosh has beneficial effects on the relief of hot flashes is not supported by evidence from methodologically sound clinical trials.

Phytoestrogens

Phytoestrogens are plant-derived, naturally occurring estrogens and have the ability to bind and activate human estrogen receptor alpha (ER-α) and human estrogen receptor beta (ER-β). Phytoestrogens have both estrogenic and antiestrogenic effects (48). Soy products and red clover are rich sources of phytoestrogens. Phytoestrogens include isoflavones, lignans, and coumestans. The effect of phytoestrogens on hot flashes has been studied in several clinical studies. Two studies showed the efficacy of soy protein in the treatment of hot

Table 1. Summary of clinical trials of complementary therapies in the treatment of hot flashes

Treatment	Design	Sample and duration	Results	Side Effects	Reference
Black cohosh					
Preparation of a standardized extract of Cimicifuga racemosa	Pilot	21 women with and without a history of breast cancer; 4 weeks	Reduction in mean daily hot flash frequency was 50%, weekly hot flash scores reduced by 56%	Joint pain	Pockaj et al. (44)
Tamoxifen with or without Menofem ®/ Klimadynon® 20mg daily (CR BNO 1055)	R, O	136 premenopausal breast cancer patients with tamoxifen-induced hot flashes; 12 months	Reduction of the severity and frequency of hot flashes (hot flashes were reported by 24.4% of patients in the intervention group and 73.9% in the usual care group)	Adverse events not related to treatment	Munoz et al. (43)
Black cohosh tablets	R, DB, PC	69 breast cancer patients; 60 days	No treatment effect on hot flashes	Adverse events not related to treatment	Jacobsen et al. (45)
Phytoestrogens					
Soy capsules; 70mg isoflavones daily	R, DB, PC	72 breast cancer patients; 12 weeks	No statistical difference in menopausal symptom scores	No significant difference in toxicity between treatment arms	MacGregor et al. (55)
Promensil (isoflavones 82mg) and Rimostil (isoflavones 57mg) daily	R, DB, PC	252 postmenopausal women; 12 weeks	Neither supplement had a clinically important effect on hot flashes	Headache	Tice et al. (53)
Soy tablets; 36mg isoflavones daily	R, DB, PC	62 postmenopausal women; 24 weeks	Soy tablets not more effective than placebo	No effect on endometrial thickness	Penotti et al.(54)
Soy beverage; 90mg isoflavones daily	R, DB, PC	123 breast cancer patients; 12 weeks	No treatment effect on hot flashes	Gastrointestinal	Van Patten et al, 2002 (52)
Soy tablets; 150mg isoflavones daily	R, DB, PC, C	177 breast cancer patients 9 weeks	No treatment effect on hot flashes	No toxicity was observed	Quella et al. (51)
Soy tablet; 50mg isoflavones daily	R, DB, PC	177 postmenopausal women 12 weeks	Hot flashes reduced by 27% versus placebo 19%	Gastrointestinal	Upmalis et al. (50)
60g soy protein, 76mg isoflavones	R, DB, PC	104 postmenopausal women; 12 weeks	Hot flashes reduced by 45% (P<0.001)	Constipation, nausea, vomiting	Albertazzi et al, 1998 (49)

C=crossover DB=double blind O=open-label P=prospective observational PC=placebo controlled R=randomized

Table 1.

Homeopathy Individualized homeopathic single remedy, a homeopathic combination medicine or a placebo	R, DB, PC	83 breast cancer patients; 1 year	No treatment effect of hot flashes	Adverse events not reported	Jacobs et al. (58)
Homeopathic approach (consultation and prescription of an individualised homeopathic remedy)	P	45 breast cancer patients;	Significant improvement in symptom scores of estrogen withdrawal	Adverse events are not reported	Thompson et al. (59)
Vitamin E Vitamin E 800IU daily	R, DB, PC, C	120 breast cancer patients; 4 weeks	Hot flashes reduced by 32% versus placebo 29%	No statistically significant difference in toxicity between the treatment arms	Barton et al. (60)
Acupuncture Electro-acupuncture, superficial needle insertion and oral estradiol	R	45 postmenopausal women; 12 weeks (6 months folluw up)	Electro-acupuncture: hot flashes reduced by 50%	No serious side effects m	Wyon et al. (62)
Electro-acupuncture, applied relaxation	R	38 postmenopausal breast; cancer patients 12 weeks	Electro-acupuncture: hot flashes reduced	No serious side effects	Nedstrand et al. (63)
Behavioural therapies Structured education and exercise program or refrain from exercising during study period		35 women 40 to 60 year old; 12 weeks	Structured education and exercise program: hot flashes reduced	Adverse events not reported	Ueda (67)
Exercise three-times weekly or oral estradiol	R	75 postmenopausal women (10 women fulfilled 12 weeks of exercise); 12 weeks treatment 24 weeks follow-up	Hot flashes reduced by 28% after 12 weeks and by 39% after 36 weeks	Adverse events not reported	Lindh-Astrand et al. (68)
Comprehensive menopausal assessment intervention program	R	76 postmenopausal breast cancer patients; 4 month	Reduction of menopausal symptoms and an improvement in sexual functioning	Adverse events are not reported	Ganz et al. (65)
Relaxation response training, reading or control group	R	33 postmenopausal women; 10 weeks	Hot flashes reduced in the relaxation response group	Adverse events are not reported	Irvin et al. (69)
Eight sessions of paced respiration, muscle relaxation or alpha-wave electroencephalographic biofeedback (placebo control)	R	33 women	Subjects undergoing paced respiration had significant reductions in hot flash frequency	Adverse events are not reported	Freedmann et al. (66)

C=crossover DB=double blind O=open-label P=prospective observational PC=placebo controlled R=randomized

flashes. In a double-blind, randomized trial of 12 weeks' duration, 40 g of protein and 76 mg of phytoestrogens per day demonstrated a significant reduction in the incidence of hot flashes compared with placebo (49). Another double-blind, controlled trial, comparing a soy isoflavone extract of 50 mg genistin and daidzin with placebo, also showed a statistically significant reduction in hot flashes (50). In contrast, a double-blind, placebo-controlled trial that evaluated soy phytoestrogens for the treatment of hot flashes in 177 breast cancer survivors failed to show a beneficial effect in the incidence and severity of hot flashes. Four weeks of treatment did not show a difference between the group taking soy tablets containing 50 mg of soy isoflavones in each tablet and the placebo group (51). In a second double-blind, placebo-controlled trial of 12 weeks of treatment in postmenopausal women who were previously treated for early-stage breast cancer, a soy beverage did not alleviate hot flashes more than placebo (52). Results of other studies also suggest that soy is not better in the reduction of hot flashes than placebo (53–55).

Several clinical studies assessed the direct relationship between an individual's dietary intake of soy products and the risk for the development of breast cancer. Few prospective data are available on the effects of phytoestrogens on breast cancer risk. Results do not show protective effects, with the exception of the consumption of phytoestrogens at young ages or the intake of high amounts (56). The effect of increased phytoestrogens in breast tissue and on the endometrium is obscure. A recent trial of red clover-derived isoflavones did not show any increased mammographic breast density in 205 women, and no effects on estradiol, gonadotropins, lymphocyte tyrosine kinase activity, or menopausal symptoms were observed (57). No randomized, controlled trials have addressed the long-term safety of phytoestrogens in patients after a diagnosis of breast cancer.

The results of the trials on the effect of phytoestrogens on hot flashes are contradictory. Comparison of all clinical studies is difficult because of the differences in products and dosages applied. It is not clear what the long-term safety of phytoestrogens is in women after a diagnosis of breast cancer. There is no evidence to support using high doses of soy products for alleviating hot flashes in breast cancer survivors.

Homeopathy

There are fewer published clinical trials to evaluate the effectiveness of homeopathy in the treatment of hot flashes. A randomized, placebo-controlled study with three arms—an individualized homeopathic single remedy (a homeopathic practitioner prescribed an individualized homeopathic medication), a homeopathic combination of three medicines (amyl nitrate 3 x (1:1,000 dilution), Sanguinaria canadensis 3 x (1:1,000 dilution), and Lachesis 12 x (1:1,000,000,000,000 dilution), and a placebo —revealed that there was no significant difference in the severity and frequency of hot flashes among the treatment arms (58). Results from a prospective observational study suggested a significant improvement in symptom scores of estrogen withdrawal after a homeopathic approach in women with breast cancer (59).

Evidently, well-controlled and good quality clinical trials assessing the efficacy and safety of homeopathic treatments are needed in the treatment of hot flashes in women after a diagnosis of breast cancer before homeopathic treatments can be advised. For now, homeopathic therapies appear not to be effective for alleviating hot flashes.

Vitamin E

A double-blind, randomized, placebo-controlled, crossover clinical trial reported a marginal statistical effect of vitamin E (800 IU/day) in the treatment of hot flashes (60). Four weeks of daily vitamin E was compared with placebo in 120 breast cancer patients. Vitamin E was associated with one less hot flash per person per day and did not induce toxicity. A crossover analysis showed that vitamin E was associated with a minimal decrease in hot flashes. At the end of the study, patients did not prefer vitamin E use over placebo.

The investigators of that study suggested that vitamin E at a dose of 800 IU daily can be used because it is inexpensive and nontoxic and it might result in a slightly better relief of hot flashes than placebo.

In other clinical trials, no statistically significant difference between treatment groups and placebo groups was found for adverse events (61). Evidence is thus limited, and before supporting vitamin E in the management of hot flashes, more clinical data are warranted. Vitamin E is not registered for this indication and should be used with caution.

Acupuncture

A randomized study of 45 postmenopausal women with vasomotor symptoms suggested that electroacupuncture decreased the number of hot flashes by 50% in 11 of 15 women studied (62). Other studies also reported a reduction in hot flashes with acupuncture (63).

A review article summarized the adverse reactions after acupuncture, such as hepatitis, subacute bacterial endocarditis, and dermatitis (64). When acupuncture is used, sterile needles are obviously necessary for the safety of patients. Especially in women who have had axillary surgery for lymph node dissection, acupuncture must be used with care to the operated arm because of the risk for the development of lymphedema.

Behavioral Therapies

A comprehensive menopausal assessment (CMA) intervention program was tested in 76 breast cancer survivors with menopausal symptoms (65). In this structured intervention program, the target symptoms were hot flashes, vaginal dryness, and stress urinary incontinence. The focus was on symptom assessment, education, counseling, and specific pharmacological and behavioral interventions. After randomization, a group of women received the usual care and another group received the interventions. Women in the usual care group were not precluded from these interventions but were not encouraged to do so. Women in the intervention group received an individualized plan of care including pharmacological and/or behavioral interventions.

The symptom assessments resulted in a reduction in menopausal symptoms and an improvement in sexual functioning in the intervention group. The comprehensive menopausal assessment included so many interventions that it is not clear which intervention was essential to relieve women from menopausal symptoms. Thus, to date, it is unknown which specific component reduced the hot flashes.

A behavioral relaxation procedure significantly reduced the frequency of hot flashes (66). The active component of this relaxation treatment was training in slow, deep breathing. Results of another study suggested that an education and exercise program alleviated climacteric symptoms (67). In a prospective study of 75 postmenopausal women with

Table 2. Summary of clinical trials of pharmacological interventions in the treatment of hot2flashes

Treatment	Design	Sample and duration	Results	Side Effects	Reference
Progestagens					
Depot medroxyprogesterone acetate 500mg on days 1, 14 and 28, or oral megestrol acetate 40mg/d	R	71 postmenopausal breast cancer patients; 6 weeks	Hot flashes reduced by 86% in the whole group of patients	Skin rashes, fluid retention, dizziness, vaginal discharge, mouth dryness	Bertelli et al. (70)
Transdermal progesterone 20mg/d	R, DB, PC	102 postmenopausal women; 48 weeks	Hot flashes reduced by 83% and by 15% in the placebo group	Vaginal bleeding	Leonettie et al. (75)
Megestrol acetate 20mg twice daily	R, DB, PC	97 breast cancer patients and men with prostate cancer; 8 weeks	Hot flashes reduced by 85% in the megestrol acetate group versus 21% placebo	Withdrawal menstrual bleeding	Loprinzi et al. (27)
Depot of medroxyprogesterone acetate 50, 100, 150mg	DB, PC	48 peri- and postmenopausal women; 12 weeks	Hot flashes reduced by 25-45% in the medroxyprogesterone acetate group	Withdrawal menstrual bleeding	Morrison et al. (73)
Medroxyprogesterone acetate 20mg/d	R, DB, PC, C	32 postmenopausal women; 12 weeks	Frequency of hot flashes reduced by 73.9% in the medroxyprogesterone acetate group	Vaginal bleeding	Schiff et al. (72)
Depot medroxyprogesterone acetate 150mg monthly	R, DB, PC	69 postmenopausal women; 24 weeks	Hot flashes reduced by 89.5% in the medroxyprogesterone acetate group versus 25% placebo	Headache, vaginal dryness	Bullock et al. (71)
Clonidine hydrochloride					
Clonidine oral 0.1mg/d	R, DB, PC	194 postmenopausal breast cancer patients with tamoxifen-induced hot flashes; 8 weeks	Frequency of hot flashes decreased with 37% (versus 20% placebo) after 4 weeks of treatment and with 38% (versus 24% placebo) after 8 weeks of treatment	Difficulty sleeping	Pandya et al. (84)
Clonidine transdermal (equivalent to a daily oral dose of 0.1mg/d)	R, DB, PC, C	116 breast cancer patients with tamoxifen-induced hot flashes; 8 weeks	Frequency of hot flashes decreased with 20% from baseline (P<.0001) and severity of hot flashes with 10% from baseline (P=.02)	Mouth dryness, constipation , itchiness under the patch, drowsiness	Goldberg et al. (26)
Clonidine transdermal (equivalent to a daily oral dose of 0.1mg/d)	R, DB, PC	30 postmenopausal women; 8 weeks	80% reported fewer hot flashes (versus 36% placebo), 73% a decrease in severity (versus 29% placebo), 67% a decrease in duration (versus 21% placebo) of hot flashes.	Transient local skin reactions (erythema and/or itching)	Nagamani et al. (79)
Clonidine oral 0.1mg, 0.2mg or 0.4mg/d	PC	10 postmenopausal women; 2 weeks	Clonidine reduced significantly the frequency of hot flashes (P<.005)	Dizziness, mouth dryness	Laufer et al. (82)
Clonidine 25 to 75 ųg twice/d	DB, PC, C	100 postmenopausal women; 4 weeks	Reduction in the frequency of hot flashes (clonidine before placebo $P \leq 0.05$); clonidine after placebo $P \leq 0.001$)	Mouth dryness	Clayden et al. (81)

C=crossover DB=double blind O=open-label P=prospective observational PC=placebo controlled R=randomized

Table 2.

Gabapentin					
Gabapentin 300mg/d or 900mg/d	R, DB, PC	420 breast cancer patients; 8 weeks	Severity of hot flash score decreased with 31% in the group gabapentin 300mg and 46% in the group gabapentin 900mg (versus 15% placebo)	Not reported	Pandya et al. (31)
Gabapentin 900mg/d	Pilot study	22 postmenopausal breast cancer patients with tamoxifen-induced hot flashes; 4 weeks	Mean decrease in hot flash duration of 73.6% (P=0.027), frequency of 44.2% (P<0.001), severity of 52.6% (P<0.001)	Nausea, rash, excessive sleepiness	Pandya et al. (84)
Gabapentin 900mg/d	R, DB, PC	59 postmenopausal women; 12 weeks	Reduction of 45% in hot flash frequency and 54% reduction in hot flash composite score (frequency and severity)	Somnolence, dizziness, rash (with or without peripheral edema)	Guttuso et al. (30)
Gabapentin 300mg to 900mg/d	Pilot	20 breast cancer patients and men with prostate cancer; 4 weeks	Hot flash score reduction was 70%	Light-headedness, dizziness.	Loprinzi et al. (83)
Venlafaxine 75mg/d	R, PC	80 postmenopausal women; 12 weeks	Reduction of hot flashes with 51% (versus 15% placebo)	Mouth dryness, sleeplessness, appetite	Evans et al. (87)
Venlafaxine 37.5mg, 75mg or 150mg/d	R, DB, PC	192 breast cancer patients; 4 weeks	Reduction of hot flashes with 37% in the venlafaxine 37.5mg group and with 61% in the venlafaxine 75mg and 150mg group (versus 27% placebo)	Mouth dryness, decreased appetite, nausea, constipation	Loprinzi et al. (29)
Venlafaxine 37.5mg, 75 mg or 150mg/d	O	Breast cancer patients; 8 weeks	Venlafaxine 37.5mg reduced hot flashes by 26% and venlafaxine 75mg, 150mg by 60%	Appetite loss, mouth dryness, dissipating nausea	Barton et al, 2002 (86) (follow up of the Loprinzi et al study (29)
Venlaxine 12.5mg oral twice/d	Pilot study	28 breast cancer patients and men with prostate cancer; 4 weeks	Reduction of hot flashes with more than 55% from baseline	Fatigue, sweating, trouble sleeping	Loprinzi et al, 1998 (28)
Fluoxetine 20mg/d	R, DB, PC	81 breast cancer patients; 4 weeks	Reduction of hot flashes with 50% versus 36% in the placebo group	Fluoxetine was well tolerated	Loprinzi et al, 2002 (89)
Paroxetine 10mg, 20mg/d	R, DB, PC, C	151 women with and without a history of breast cancer; 8 weeks	Paroxetine 10mg reduced hot flash frequency and composite score by 40.6% and 45.6% (versus 13.7% placebo). Paroxetine 20mg reduced hot flash frequency and composite score by 51.7% and 56.1% (versus 26.6% and 28.8% placebo)	Drowsiness and nausea in the paroxetine 20mg group	Stearns et al, 2005 (91)
Paroxetine 12.5mg or 25mg/d	R, DB, PC	165 postmenopausal women; 6 weeks	Paroxetine 12.5mg reduced hot flashes by 62% and paroxetine 25mg by 65% (versus 38% placebo)	Headache, nausea, insomnia	Stearns et al, 2003 (90)

C=crossover DB=double blind O=open-label P=prospective observational PC=placebo controlled R=randomized

vasomotor symptoms, women were randomized to a physical exercise program or oral estradiol therapy. Ten women fulfilled the 12-week exercise period. In five of these women, hot flashes decreased to 28% of baseline (68).
Results of a 10-week randomized trial including 33 postmenopausal women demonstrated that relaxation response training (including mental focusing, diaphragmatic breathing, and breath awareness) could significantly reduce hot flash intensity compared with that seen in a control group (69).
Behavioral interventions can be effective in the treatment of hot flashes. The drawback of behavioral trials is the impossibility of using a placebo. A substantial placebo effect must be accounted for when interpreting published behavioral therapy data in the treatment of hot flashes.

Pharmacological Interventions
Clinical trials of pharmacological therapies are outlined in Table 2.

Progestagens
Progestagens have been studied in the treatment of hot flashes. Megestrol acetate, a progestagen used in the treatment of breast cancer, decreased hot flashes by 80%, compared with a 20% decrease in hot flashes in the placebo group. The patients were women with a history of breast cancer and men with prostate cancer receiving androgen- ablation therapy. The medication was equally efficacious in men and women. One of the main side effects was menstrual withdrawal bleeding 1–2 weeks after discontinuing the megestrol acetate (27). In a randomized trial in postmenopausal breast cancer patients, one group of patients received an i.m. depot of medroxyprogesterone acetate (500 mg on days 1, 14, and 28) and another group of patients received oral megestrol acetate at a dose of 40 mg/d. Hot flashes were reduced by 86% in the entire group of patients without a significant difference between groups. The treatment was generally well tolerated. More patients in the megestrol group experienced adverse events, which, in six women, led to early discontinuation of the treatment (70). Reasons for interruption of treatment were skin rashes, dyspnea, gastric pain, and increased arterial blood pressure. More clinical studies showed that depomedroxyprogesterone acetate and medroxyprogesterone acetate are effective in the treatment of hot flashes (71–74).
Transdermal progesterone cream has also been studied, and after 4 weeks of treatment, it was associated with an 83% reduction in hot flashes in progesterone-treated patients and a 19% reduction in placebo-treated patients (75).
High-dose megestrol acetate is an effective treatment in the management of breast cancer, but the theoretical concern is that low doses of progestagens may stimulate tumor growth (76, 77). There are conflicting reports about the safety of progestagens in the treatment of hot flashes in breast cancer patients.

Neuroendocrine Agents
Clonidine hydrochloride
Clonidine hydrochloride is a centrally active α-agonist that reduces vascular reactivity and is primarily indicated in the treatment of hypertension. Several studies suggest that oral

or transdermal clonidine is effective in the treatment of hot flashes in postmenopausal women. Clonidine reduces brain norepinephrine release, raises the sweating threshold, and ameliorates hot flashes (78). Transdermal clonidine was studied in a randomized, double-blind study in postmenopausal women with hot flashes. The reduction in hot flashes was significant in patients who received transdermal clonidine. Eighty percent of patients reported fewer hot flashes, 73% reported a decrease in severity, and 67% reported a decrease in duration (79).

Another randomized, double-blind trial showed that clonidine reduced the hot flash frequency and severity, but this effect was clinically modest. Drug-related toxicities were mouth dryness, constipation, itchiness under the patch, and drowsiness (26).

A randomized, double-blind, placebo-controlled trial reported that oral clonidine (0.1 mg/d) given for at least 8 weeks is effective in the treatment of tamoxifen-induced hot flashes in women with a history of breast cancer. Hot flashes decreased by about 37% after 4 weeks of treatment compared with a 20% reduction with placebo, and after 8 weeks of treatment, a reduction in hot flashes of 38% in the clonidine group, versus 24% in the placebo group, was observed (80). The patients in the clonidine group had significantly more difficulty sleeping than patients in the placebo group.

Clinical studies demonstrated that clonidine reduced the frequency of hot flashes, but the effect of clonidine on hot flash duration and severity was marginal (81, 82).

Gabapentin

Gabapentin is a gamma-aminobutyric acid analogue that is used in the treatment of epilepsy or neurogenic pain. Two pilot studies suggested that gabapentin is effective in the treatment of hot flashes (83, 84). A 12-week, randomized, placebo-controlled trial in postmenopausal women taking gabapentin at a dose of 900 mg/d showed a reduction of 45% in hot flash frequency and a 54% reduction in hot flash composite score (frequency and severity) (30). Adverse events in the gabapentin group were somnolence, dizziness, and rash with or without peripheral edema. In some patients who took gabapentin, blood laboratory tests showed a decrease in serum albumin, total protein, total bilirubin, blood urea nitrogen, and platelets after 12 weeks of treatment, compared with baseline.

In a randomized, double-blind, placebo-controlled trial in 420 women with breast cancer, one group received a placebo, one group received gabapentin at a dose of 300 mg/d, and one group received gabapentin at a dose of 900 mg/d for 8 weeks. The percentage decreases in hot flash severity score between baseline and 8 weeks were: 31% in the 300-mg gabapentin group and 46% in the 900-mg gabapentin group. Gabapentin at a dose of 300 mg/d gave a significant reduction in hot flash frequency but not a significant reduction in the severity of hot flashes. There was a significant decrease in hot flash frequency and severity at a dose of 900 mg/d of gabapentin. A major limitation of this study is that the side effects of gabapentin have not been reported (31).

Based on these results, gabapentin can be considered effective in the management of hot flashes in postmenopausal women after a diagnosis of breast cancer. More clinical data are needed on the side effects and safety associated with the use of gabapentin in the treatment of hot flashes.

Selective serotonin reuptake inhibitors

Selective serotonin reuptake inhibitors (SSRIs) are antidepressants. Based on the modes of action of these antidepressants and the theory of the pathophysiology of hot flashes, SSRIs have been studied in the treatment of hot flashes. Specifically, the SSRIs venlafaxine, paroxetine, and fluoxetine were evaluated in the treatment of hot flashes.

Venlafaxine

Several clinical studies have been performed to evaluate the use of venlafaxine in the treatment of hot flashes. Venlafaxine affects both serotonin and norepinephrine reuptake in contrast to the SSRIs paroxetine and fluoxetine. Two pilot studies reported that hot flashes were reduced by approximately 55%–67% with venlafaxine (28, 85). Results of a double-blind, placebo-controlled trial in 192 breast cancer patients showed a significant decrease in median hot flash scores in all venlafaxine groups compared with placebo. After 4 weeks of treatment, median hot flash scores were reduced by 27% in the placebo group, by 37% in the 37.5-mg venlafaxine extended release (XR) group, and by 61% in the 75mg venlafaxine XR and 150-mg venlafaxine XR treated patients (29). At the end of 4 weeks of treatment, the investigators of the study asked the participants to participate in an 8-week open-label study. Participants received 37.5– 150 mg/day of venlafaxine XR. In the 37.5-mg group, hot flashes decreased by 26%. In the 75-mg and 150-mg group, hot flashes scores decreased by 60% (86).

In a 12-week placebo-controlled trial, postmenopausal women received 37.5 mg of venlafaxine XR for 1 week followed by 75 mg of venlafaxine XR for 11 weeks. After 4 weeks, the mean scores in the placebo and venlafaxine groups were the same. After 11 weeks of treatment, venlafaxine was associated with a 51% reduction in hot flashes, compared with a 15% reduction in the placebo group (87).

In all venlafaxine studies, venlafaxine was well tolerated. The most common adverse events included dry mouth, constipation, loss of appetite, nausea, and sleepiness. The 150-mg dose of venlafaxine XR was associated with more adverse events than the 37.5-mg and 75-mg doses of venlafaxine XR. The efficacies of 75 mg of venlafaxine XR and 150 mg of venlafaxine XR in the treatment of hot flashes were not different and were 60%. Based on these results, the advised dose of venlafaxine in the treatment of hot flashes is 75 mg/d (86).

Sexual dysfunction is a common adverse event of antidepressant treatment, especially when using SSRIs (88). In the study of Loprinzi et al. (29), the venlafaxine doses of 37.5–150 mg XR per day did not reduce libido after 4 weeks of treatment.

In conclusion, venlafaxine is effective and can be used in the treatment of hot flashes in women after a diagnosis of breast cancer. How long patients must take venlafaxine after starting this intervention and also the effectiveness of venlafaxine for durations of longer than 12 weeks are unknown. Further long-term, well-controlled clinical trials are needed to investigate the efficacy of venlafaxine in the management of hot flashes. These trials should include the side effects and, specifically, the possible interference of venlafaxine with sexual function after long-term treatment.

We are currently conducting a randomized, double-blind, placebo-controlled trial comparing venlafaxine with clonidine and placebo.

Fluoxetine

A randomized, double-blind, crossover, placebo-controlled trial in 81 breast cancer patients reported a decrease in the incidence of hot flashes by 50% using fluoxetine at a dose of 20 mg/d, versus a 36% decrease with placebo. There was no statistically significant difference in the toxicities in the two treatment arms (89). More clinical data are needed on the efficacy of fluoxetine in the management of hot flashes.

Paroxetine

Controlled-release paroxetine was evaluated in a randomized, double-blind, placebo-controlled trial in 165 postmenopausal women with hot flashes. After 6 weeks of treatment, 12.5 mg/d of paroxetine reduced hot flashes by 62% and 25 mg/d of paroxetine reduced hot flashes by 65%, whereas there was a 38% reduction in hot flashes by placebo. The most frequently reported adverse events in the controlled-release paroxetine group were headache, nausea, and insomnia. Paroxetine was well tolerated, with more adverse events in the 25-mg than in the 12.5-mg treatment group (90).

Recently published data reported that paroxetine in doses of 10–20 mg is effective in the treatment of hot flashes in women with and without a history of breast cancer. This randomized, double-blind, crossover, placebo-controlled trial demonstrated that 10 mg of paroxetine reduced hot flash frequency and the composite score by 40.6% and 45.6%, respectively, compared with 13.7% and 13.7% reductions, respectively, with placebo. Paroxetine at a dose of 20 mg reduced hot flash frequency and the composite score by 51.7% and 56.1%, respectively, versus 26.6% and 28.8% reductions, respectively, in the placebo group. The most common adverse events were drowsiness and nausea in the 20-mg paroxetine group (91). These trials demonstrated that paroxetine is effective in the treatment of hot flashes, but further clinical trials assessing the efficacy and safety of paroxetine in breast cancer survivors are needed.

Safety of SSRIs

The SSRIs are also commonly prescribed in the treatment of hot flashes in women who take tamoxifen. SSRIs are known to inhibit cytochrome P450 (CYP)2D6, an enzyme that is important for the metabolism of many drugs, such as tamoxifen, to its active metabolite endoxifen. Some studies reported an interaction of CYP2D6 polymorphisms and CYP2D6 inhibitors. Plasma concentrations of endoxifen were lower in patients who were treated with SSRIs than in patients who did not receive treatment with SSRIs in combination with tamoxifen (92). The weak inhibitor of CYP2D6, venlafaxine, had very little effect on plasma endoxifen concentrations, which is in contrast to the combination of paroxetine or sertraline with tamoxifen, which resulted in substantially lower plasma endoxifen concentrations. The variations in plasma endoxifen concentrations that are associated with CYP2D6 gene polymorphisms and CYP2D6 inhibitors can affect the antitumor efficacy or adverse events of tamoxifen (93).

Further clinical trials are needed to evaluate the safety of SSRIs in combination with tamoxifen. It is advisable to inform women after a diagnosis of breast cancer of the uncertainties about the interactions of some SSRIs and tamoxifen.

Conclusions and recommendations

Many published results of clinical trials are available in the management of hot flashes, but there are limited well-controlled trials assessing the role of pharmacologically and nonpharmacologically based treatments of hot flashes in breast cancer patients. A limitation of most trials is the duration of the treatment and follow-up of patients. The U.S. Food and Drug Administration recommended a study period of 12 weeks for trials in the treatment of hot flashes (94). Several trials in the management of hot flashes have a shorter duration than 12 weeks.

Another limitation of studies in patients with hot flashes is the absence of a placebo group. Several trials have shown a significant placebo effect in the relief of hot flashes. A placebo group as a control group is thus necessary in these studies. Therefore, when interpreting clinical data, the placebo effect should always be taken into consideration.

Only 13 trials that we found meet the criteria of a study period of 12 weeks or longer and were placebo controlled. Six studies were performed with phytoestrogens in the management of hot flashes, but the results of those studies were contradictory. One preliminary homeopathic trial showed no treatment effect on hot flashes. Four trials were performed with progestagens and all showed a reduction in the incidence and severity of hot flashes. One trial was performed with gabapentin and one trial was performed with venlafaxine in the management of hot flashes. Both showed a reduction in the incidence and severity of hot flashes in postmenopausal women. However, only two trials performed with phytoestrogens were evaluated in breast cancer patients. The other trials evaluated treatment options in the management of hot flashes in postmenopausal women without a history of breast cancer. This emphasizes the need for larger-scale and long-term prospective trials to test the efficacy and safety of selected pharmacological and nonpharmacological strategies in the management of hot flashes after the diagnosis of breast cancer and induction of early menopause. Several questions are still unanswered, including the time period that patients need pharmacological interventions after starting with the intervention in the management of hot flashes. The optimal time period of use can be based on the natural history of hot flashes, which mostly resolve within 2–3 years of menopause. Hence, one may consider tapering and discontinuation of SSRIs after about this length of time.

The first step to control hot flashes in breast cancer patients is advice and information about simple strategies in the management of hot flashes, such as wearing light clothes, dressing in layers, lowering the room temperature, using air conditioners, drinking cold beverages, and avoiding alcohol, hot drinks, or hot food to ameliorate symptoms. For pharmacological intervention of hot flashes, venlafaxine can be used. Venlafaxine is preferable to the other SSRIs for several reasons. Most clinical data are available for venlafaxine. The safety of the other SSRIs is unclear in combination with tamoxifen, and a possibly clinically relevant reduction in endoxifen, the active metabolite of tamoxifen, may take place. Another possible treatment option is clonidine, an antihypertensive agent. Gabapentin was reported to be effective in the treatment of hot flashes; however, more information on the toxicity profile of this agent is needed before prescribing gabapentin outside clinical trials.

Behavioral relaxation is preferable as a nonpharmacological treatment, as it was found to reduce hot flashes. Vitamin E and acupuncture can also be effective, but only a few clinical

data are available, and therefore, these modalities cannot be recommended outside clinical trials. Progestagens, black cohosh, and phytoestrogens should be used with caution in the treatment of hot flashes because of the unavailability of long-term safety and efficacy data and the lack of knowledge and conflicting data on possible estrogenic effects. An algorithm for the treatment of hot flashes in breast cancer patients is proposed and outlined in Figure 1.

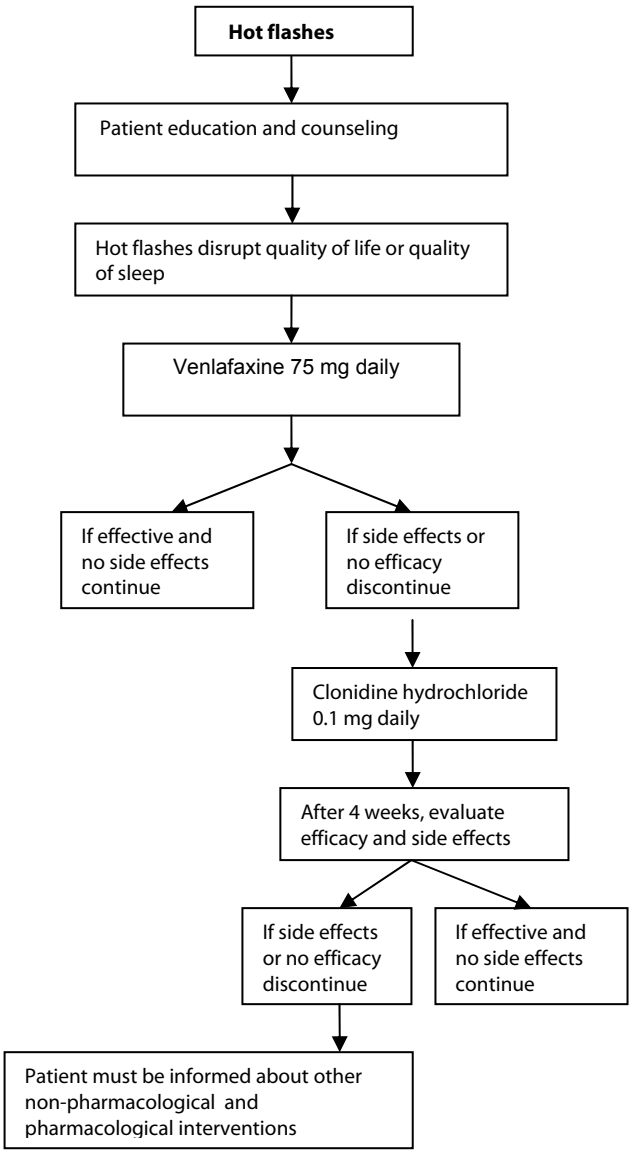

Figure 1. Proposed algorithm for the treatment of hot flashes in cancer therapy induced early menopause, based on literature review of pharmacological and non-pharmacological interventions.

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Chapter 2.2

Management of hot flashes in breast cancer patients with venlafaxine and clonidine: a randomized double-blind placebo-controlled trial

Submitted for publication

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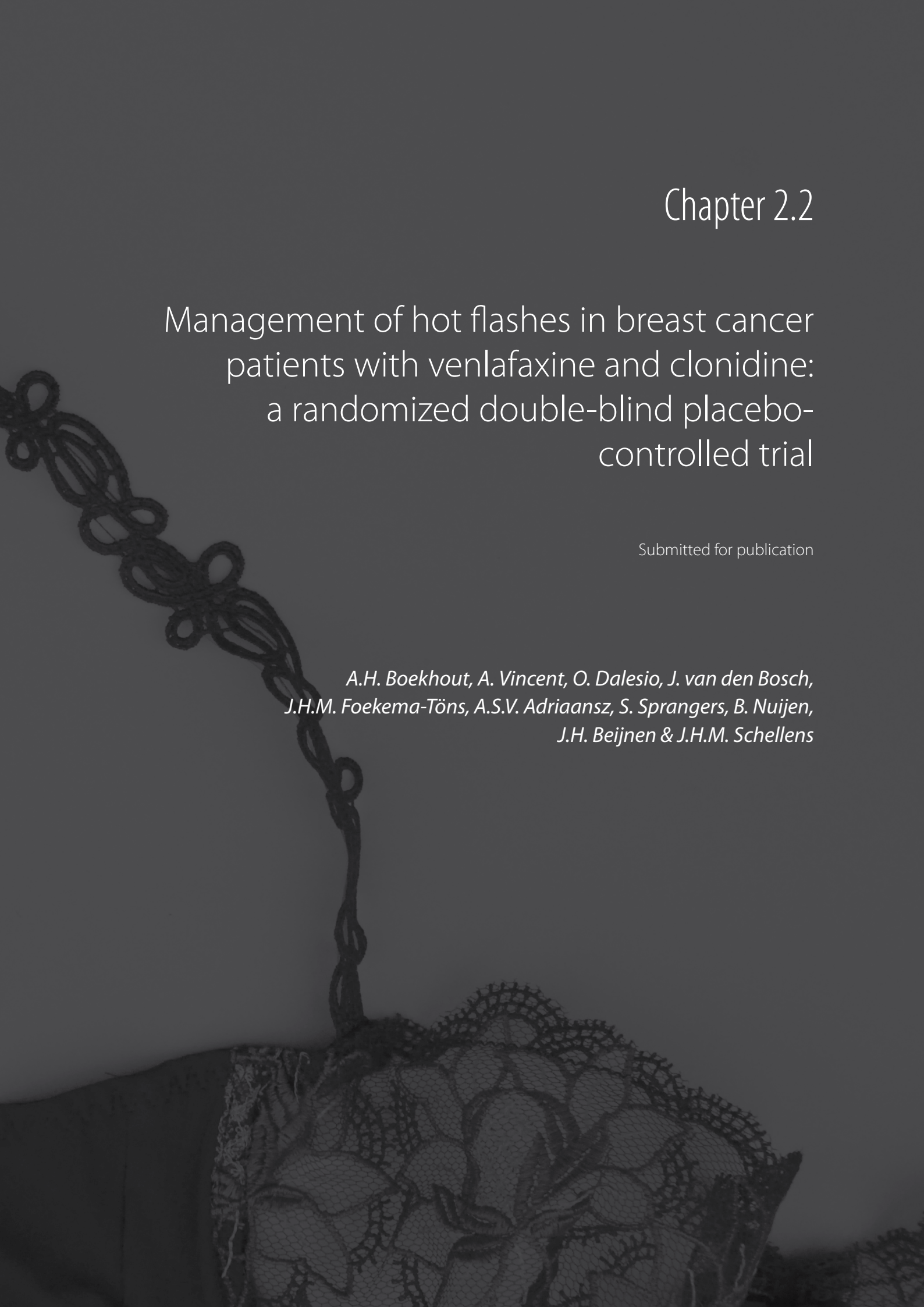

Abstract

Purpose

Therapies for breast cancer may induce hot-flashes that can affect quality of life. We undertook a double-blind, placebo controlled trial. The primary objective was to compare the average daily hot-flash scores in the twelfth week between patients treated with venlafaxine, clonidine and placebo. Additional analyses were performed of the hot-flash score over the full twelve weeks of treatment.

Patients and methods

In total 102 patients with a breast cancer history were randomized (2:2:1) to venlafaxine 75 mg, clonidine 0.1 mg or placebo daily for 12 weeks. Questionnaires at baseline and during treatment assessed daily hot-flash scores, sexual function, sleep quality, anxiety and depression.

Results

After 12 weeks a total of 80 patients were evaluable for the primary endpoint. During week 12 hot-flash scores were significantly lower in the clonidine group versus placebo ($p=0.03$), while for venlafaxine versus placebo the difference was borderline not significant ($p=0.07$). Over the course of 12 weeks the differences between both treatments and placebo were significant ($p=0.0004$ venlafaxine versus placebo, $p=0.045$ clonidine versus placebo). Sexual function and sleep quality were not significantly different between both treatment groups. Frequencies of treatment-related side-effects, nausea ($p=0.02$), constipation ($p=0.04$) and severe appetite loss were higher in the venlafaxine group.

Conclusion

Venlafaxine and clonidine are effective treatments in the management of hot-flashes in patients with breast cancer. Venlafaxine resulted in a more immediate reduction of hot-flash scores when compared with clonidine, however hot-flash scores at week 12 were lower in the clonidine group than in the venlafaxine group.

Introduction

Therapies for breast cancer in pre- and postmenopausal women, such as systemic endocrine therapy and chemotherapy, may result in symptoms such as hot-flashes (1-5). A major reason for nonadherence or early discontinuation of adjuvant breast cancer therapies are treatment-associated toxicities (6), which may affect compliance and treatment outcome (7-9). Therefore, effective treatments that moderate hot-flash toxicities may improve continuation of anti-cancer therapies.

Estrogen withdrawal results in a decrease of endorphin levels and an increase of norepinephrine and serotonin release, followed by instability of the hypothalamic thermoregulatory set-point which allows changes of the body temperature and in hot-flash sensation (10-12).

Several agents have been investigated for their ability to modify hot-flashes. The analgesics gabapentin and pergabalin and the selective serotonin re-uptake inhibitors (SSRIs) fluoxetine, citalopram and paroxetine have demonstrated a reduction in the frequency of hot-flashes (11, 13-16). However, interactions between CYP2D6 inhibitors (SSRIs) and tamoxifen are reported (17). Plasma concentrations of endoxifen were lower in patients treated with these combinations, which possibly affects the efficacy of tamoxifen (18).

In contrast the selective serotonin-norepinephrine reuptake inhibitor venlafaxine is a weak inhibitor of CYP2D6 and appears to have little effect on plasma endoxifen concentrations (17). At a dose of 75 mg daily venlafaxine demonstrated a reduction in hot-flashes by about 60% after 4 weeks of treatment (19).

At a dose of 0.1 mg daily clonidine, a centrally acting α-adrenergic agonist, demonstrated a reduction in hot-flashes of 38% after 8 weeks of treatment compared to a 24% reduction in placebo treated patients (20, 21).

Venlafaxine and clonidine are both often prescribed treatments and recommended in clinical guidelines in the management of hot-flashes (22, 23). However a three-arm trial comparing clonidine, venlafaxine and placebo in breast cancer patients has not been conducted, nor is there data concerning longer term duration of effect.

Therefore we initiated a randomized double-blind placebo-controlled multicenter trial of venlafaxine and clonidine treatment in women with a history of breast cancer. Endpoints included daily hot-flash scores at week 12 and the effect of both drugs on sexual function sleep quality, anxiety, depression and treatment side effects.

Methods

Patients

Women eligible for this trial had a history of breast cancer; were aged >18 years; had natural or chemotherapy induced menopause or were premenopausal with ovarian function suppression by surgery or luteinizing hormone-releasing hormone (LHRH); experiencing at least 2 hot-flashes per day; a performance status of 0-1; estradiol <200 pmol/L; serum creatinine <120 umol/L; thyroid stimulating hormone (TSH) between 0.5-3.9 mU/L; systolic blood pressure <160 mmHg; diastolic blood pressure <95 mmHg. Anti-estrogen

and aromatase inhibitors were allowed if they had been started at least 3 months before entering into the study. Participants were excluded if they had a history of hypersensitivity to the study medication; started treatment with antidepressants or SSRIs less than 4 weeks prior to randomization; recently used drugs that might affect study drug metabolism (*e.g.*: verapamil); a history of uncontrolled hypertension, heart disease or angina pectoris; recent myocardial infarction; planned switch in endocrine treatment during the study period; were pregnant; or were breastfeeding.

Design and procedures

Eligible participants were randomly assigned (2:2:1) to venlafaxine 75 mg, clonidine hydrochloride 0.1 mg, or placebo daily. The pharmacy of the Slotervaart Hospital prepared identical capsules containing 37.5 mg venlafaxine, 0.05 mg clonidine or placebo. Only the Data Center of the Netherlands Cancer Institute (DC-NCI) and the pharmacists had access to individual treatment assignments during the study. For verification of compliance of study treatment, patients were instructed to record the use of study treatment in a diary and to return unused tablets. Upon completion of the study, participants were given the opportunity to break the study code, and to continue or switch treatments.

Stratified randomization was performed by the DC-NCI with stratification factors: age (£35, 36-50, >51 years), duration of complaints (>or< 9 months), concurrent endocrine therapy (yes or no) and previous chemotherapy (yes or no).

During the two weeks prior to administration of study medication and during the 12 week study treatment period, participants were instructed to complete a diary. This well-validated dairy was originally developed by the North Central Cancer Treatment group, and has been used in several well-controlled trials to gather information pertaining to hot-flashes (24). Each day participants were asked to record the frequency and severity of hot-flashes (severity categories: mild, <5 minute duration; moderate 5-15 minutes; severe 15-20 minutes; or extreme >20 minutes). Further, patients were asked to record weekly adverse events including: reduced appetite, nausea, sleepiness, dizziness, fatigue, dry mouth, abnormal sweating and constipation, along with a severity grade (mild, moderate, severe or extreme) per side effect.

Levels of sleep quality, anxiety, depression and sexual function were assessed at baseline and after 4 and 12 weeks of treatment. Sleep quality was assessed by the Groningen Sleep Quality Scale (GSQ), anxiety and depression by the Hospital Anxiety and Depression Scale (HADS) and sexual function by the Sexual Activity Questionnaire (SAQ). The GSQ includes 14 questions and is not validated in breast cancer trials but has been used previously in Dutch populations to determine sleep disturbance and sleep quality (25). The HADS is a well-validated 14-item scale, including 7 questions of anxiety and 7 questions of depression, a higher score indicating more anxiety and depression (26). The SAQ is a well-validated questionnaire and used to assess the influence of hot-flashes and treatment on sexual function (27, 28). Also assessed was blood pressure and heart rate at baseline and during weeks 4 and 12; serum creatinine and TSH at screening and estrogen levels at screening and at week 12.

Participants were enrolled and monitored by nurse practitioners and made four visits to the hospital, one visit for the informed consent procedure and three visits during the study

period. During these visits each woman received study medication, the blood pressure and heart rate were assessed, occurrence of adverse events and any change in medication was recorded, and unused study medication was collected. The study was approved by the Institutional Review Board (IRB) and meets the IRB standards.

Statistical methods

The primary endpoint of the study was the daily hot-flash scores assessed during week 12. The method of analysis was decided prospectively and followed the intention-to-treat principle. The hot-flash score encompasses both severity and frequency in a single measure according to the methodology and instruments for conducting hot-flash studies (24). A severity value is allocated to each hot-flash: 1 point for mild, 2 for moderate, 3 for severe and 4 for very severe; and the daily hot-flash score is the sum of the severity values experienced that day. Given the relation with duration, the hot-flash score is approximately the number of five minute periods of hot-flashes experienced on a particular day. In several studies a substantial placebo effect has been observed indicating a reduction in frequency in hot-flashes between baseline and treatment period with a hot-flash score SD of 5 and a difference of 3.9 score units per day (24). A sample size of 40 patients in each treatment arm and 20 patients in the placebo arm would provide 80% power to detect an effect size of 0.78 when comparing each experimental arm with control at an alpha level of 0.05 in a two-tailed t-test. This sample size would give 80% power to detect an effect size of 0.63 when comparing the two experimental arms.

Hot-flash scores at week 12 were compared between the three treatment groups using a generalized linear mixed model with a Poisson error distribution. Initially the combined treated population was compared with the placebo group. If significant, then each treatment group was compared with the placebo group. As each patient provided (a maximum of) seven hot-flash scores during week 12, a random intercept was included to account for within patient correlations. The model fixed-effects were treatment, average baseline hot-flash score and the interaction of these two variables. As a sensitivity analysis, significant results were retested with adjustment for stratification factors. Two additional Poisson mixed effects models of the hot-flash scores were constructed using data from all 12 weeks. The first compared the three groups over all 12 weeks, and the other compared the three groups during weeks 1-4, 5-8 and 9-12. In both models, treatment and average baseline score were included as fixed effects, in the second model the week grouping was also included as fixed effect. Kruskal-Wallis tests were used to assess differences between the three treatment groups in baseline hot-flash scores, vital signs, and questionnaire scores. Fisher exact tests were used to assess completion of week 12 diaries, and incidence and severity (severe or extreme vs. other grades) of side-effects. The level of significance was set at 0.05 for all tests.

Results

Patients

Study participants were enrolled between October 2005 and Augustus 2009 from three Dutch hospitals, the Netherlands Cancer Institute (NKI), the Albert Schweitzer Hospital and

the Slotervaart Hospital. A total of 102 women were randomly assigned to venlafaxine, clonidine or placebo. Baseline hot-flash data was available for 95 patients (93%) and during week 12 data from 80 patients (78%) was available for analysis: 35 patients in the venlafaxine group, 28 in the clonidine group and 17 patients in the placebo (Figure 1). Early discontinuation of the study was not associated with either the venlafaxine or clonidine group (p=0.26) nor the baseline hot-flash scores (p=0.84). Baseline demographic and clinical characteristics are presented in Table 1. Baseline hot-flash scores did not differ between the three groups, nor were they different when comparing patients who completed all 12 weeks of treatment (p=0.27). Median hot-flash scores for the treatment groups are presented in Figure 2 and Table 2 and the observed hot-flash scores in Figure 3. During the twelfth week of treatment there was a reduction in hot-flash scores by roughly 45% (two treatments combined) in comparison with placebo (p=0.03), with no detectable difference between the two treatments (p=0.58). Comparing clonidine with placebo, hot-flash scores were significantly lower in the clonidine group at week 12 (p=0.03), while for venlafaxine versus placebo the difference in hot-flash scores was borderline not significant (p=0.07). Adjusting for stratification factors did not change these results.

In contrast to the week 12 assessment, the reduction compared to placebo in hot-flash scores over the entire 12 week period in the venlafaxine group was 41% (p=0.0004), while only 26% (p=0.045) in the clonidine group. In particular, hot-flash scores reduced sooner after commencement of venlafaxine treatment than during clonidine treatment. The venlafaxine group reported a decrease compared to placebo of 42% during weeks 1-4 (p=0.01), while the clonidine group reported a decrease of 22% over the same period (p=0.26). The pairwise comparisons between the venlafaxine and clonidine treatment groups during the weeks 1-4, 5-8 and 9-12 were not significant. A substantial placebo effect was found with hot-flash scores being reduced by 29% during the 12 weeks of treatment as compared to baseline ($p < 0.0001$).

The incidence of nausea (p=0.02) and constipation (p=0.04) was higher in the venlafaxine group than in the placebo group, and there was also a trend towards a higher incidence of appetite loss in the venlafaxine group (p=0.06). Regarding the occurence of severe side-effects, loss of appetite occurred more frequently in the venlafaxine group versus the clonidine group (p=0.003). Worst grade of side effects are reported in Table 3.

The occurrence of these side-effects did not result in the premature discontinuation of study treatment or an increased use of co-medication. Premature discontinuation for adverse events occurred in 2 (5%) patients in the venlafaxine group and 6 (15%) patients in the clonidine group (p=0.26, Figure 1). Sexual function and sleep quality were not significantly different between the venlafaxine and clonidine treatment groups. At week 12, the anxiety score (adjusted for baseline scores) was higher (increased anxiety) in the clonidine group than in the venlafaxine group (p=0.04) and the depression score was higher (increased depression) in the venlafaxine than in the clonidine group (p=0.03). Blood pressure and pulse values were not significantly different between the three groups.

A total of 41 patients (40%) wished to continue the study treatment after the end of the study, 14 patients (34%) in the clonidine group, 23 (56%) in the venlafaxine group and 4 (20%) in the placebo group. The comparison between the clonidine and venlafaxine treatment groups was borderline not significant (p=0.08). In 8 patients there was discrepancy between

Table 1. Patient Characteristics

Patients intent-to-treat			Ven. N= 41	Clon. N= 41	Plac. N= 20	Total N= 102
Age	median (range)		48 (28-69)	49 (32-71)	50 (34-62)	49 (28-71)
Performance Status	WHO 0		40 (98%)	41 (100%)	19 (95%)	100 (98%)
	WHO 1		1 (2%)		1 (5%)	2 (2%)
Postmenopausal after treatment of breast cancer?	no				1 (5%)	1 (1%)
	yes		41 (100%)	41 (100%)	19 (95%)	101 (99%)
Reason	age (natural)		13 (32%)	16 (39%)	3 (15%)	32 (31%)
	chemotherapy; hormonal treatment; surgery		27 (66%)	25 (61%)	15 (75%)	67 (66%)
	unknown		1 (2%)		1 (5%)	2 (2%)
	not postmenopausal				1 (5%)	1 (1%)
Number of months with hot-flashes?	median (range)		15.0 (3-72)	12 (2-93)	10.5 (3-40)	13 (2-93)
Prior treatment for hot-flashes?	no		21 (51%)	23 (56%)	13 (65%)	57 (56%)
	yes		17 (41%)	16 (39%)	7 (35%)	40 (39%)
	unknown		3 (7%)	2 (5%)		5 (5%)
Received prior radiotherapy	no		9 (22%)	8 (20%)	6 (30%)	23 (23%)
	yes		32 (78%)	33 (80%)	13 (65%)	78 (76%)
	unknown				1 (5%)	1 (1%)
Received prior chemotherapy	no		7 (17%)	7 (17%)	4 (20%)	18 (18%)
	yes		34 (83%)	34 (83%)	15 (75%)	83 (81%)
	unknown				1 (5%)	1 (1%)
Received endocrine treatment	tamoxifen	No	19 (46%)	20 (49%)	10 (50%)	49 (48%)
		Yes	22 (54%)	21 (51%)	10 (50%)	53 (52%)
	anastrozole	No	35 (85%)	34 (83%)	18 (90%)	87 (85%)
		Yes	6 (15%)	7 (17%)	2 (10%)	15 (15%)
	Gosereline	No	33 (80%)	34 (83%)	17 (85%)	84 (82%)
		Yes	8 (20%)	7 (17%)	3 (15%)	18 (18%)
	Other	No	32 (78%)	36 (88%)	15 (75%)	83 (81%)
		Yes	9 (22%)	5 (12%)	5 (25%)	19 (19%)

Abbreviations: clon, clonidine; N, number; plac, placebo; ven, venlafaxine; WHO, World Health Organization. Blank fields indicate 0%.

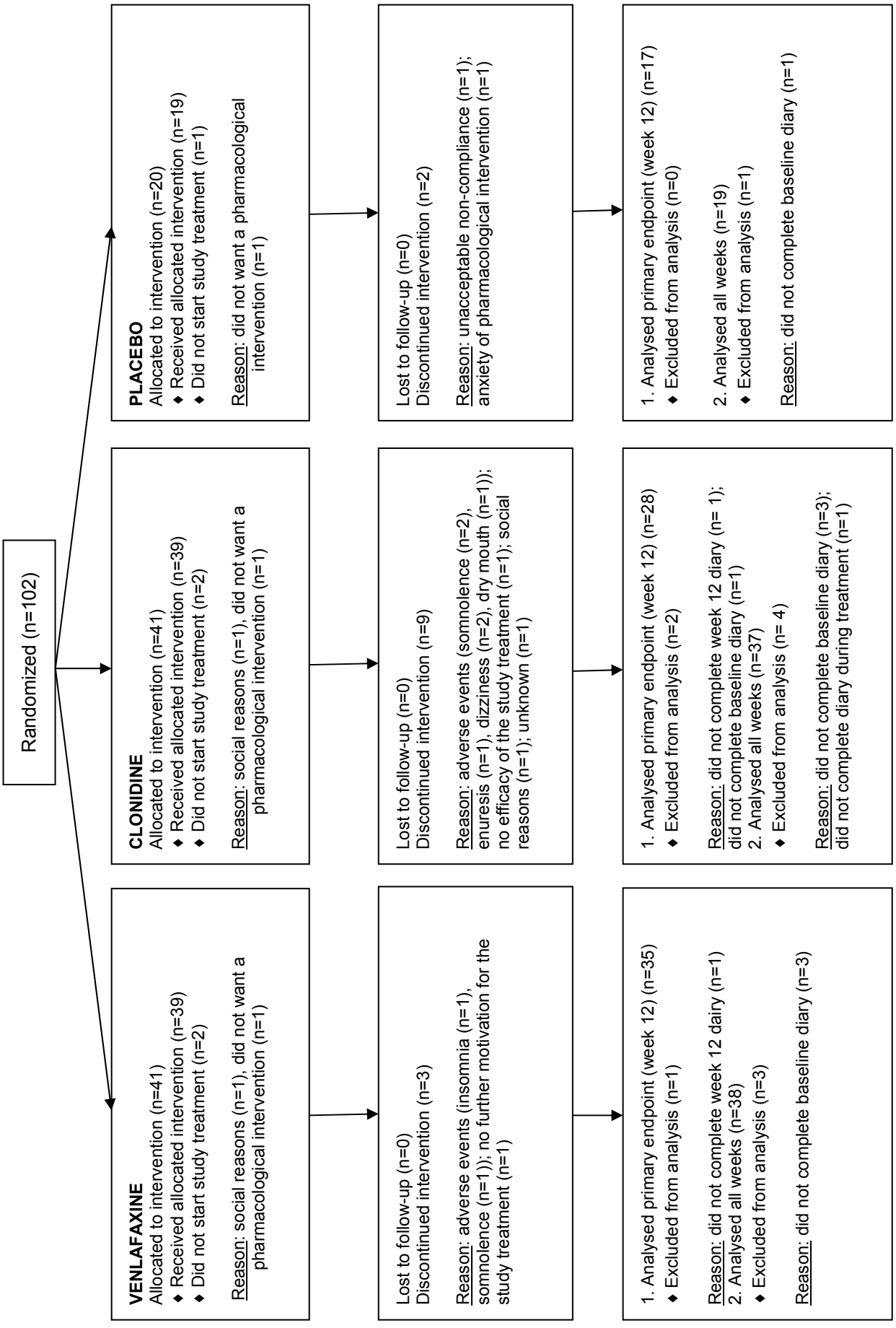

Figure 1. Trial Profile

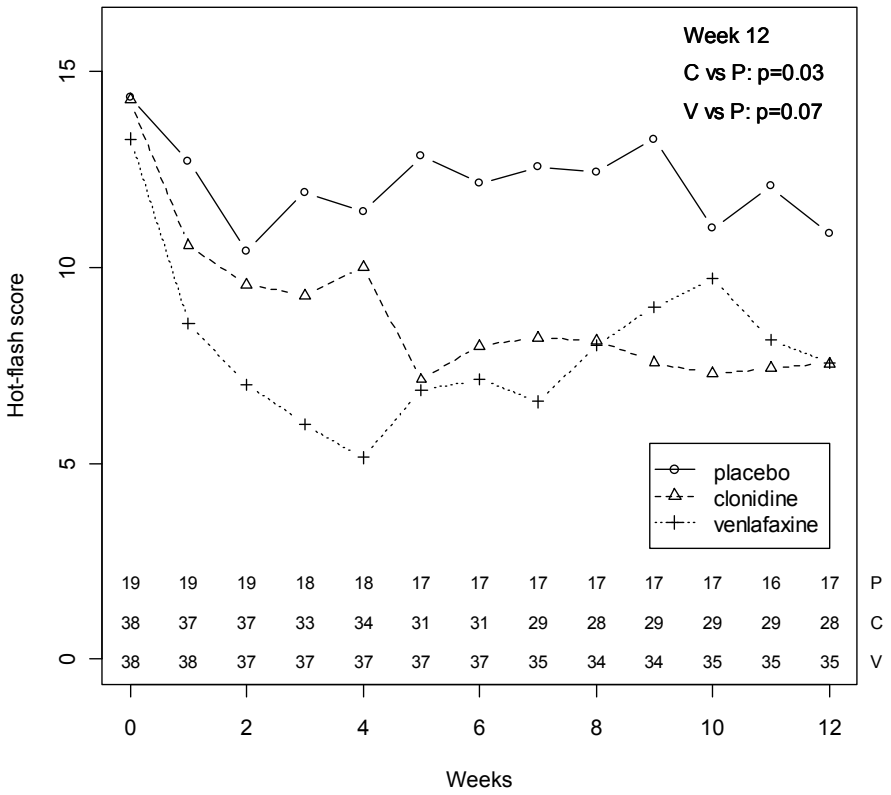

Figure 2. Median hot-flash scores during 12 weeks of treatment (c, clonidine; p, placebo; v, venlafaxine). The p-values are from the pairwise comparisons of the treatments with placebo during week 12 using the generalized linear mixed-effects models.

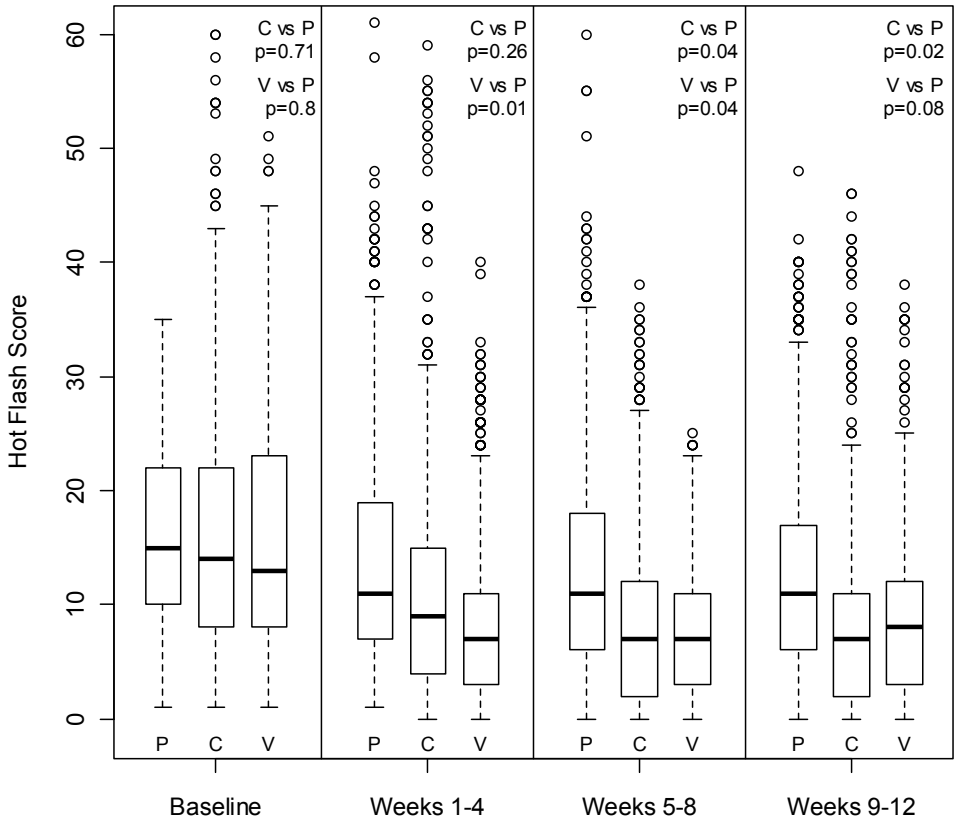

Figure 3. Hot-flash scores by month during 12 weeks of treatment (c, clonidine; p, placebo; v, venlafaxine). The p-values are from the pairwise comparisons between the treatments and placebo over the four periods.

Table 2. Median (IQR) of the observed within-patient average of the daily hot-flash scores. The p-values are the pairwise comparisons at each time-interval between the treatments and placebo using generalized linear mixed-effects models.

	placebo	clonidine	p-value	venlafaxine	p-value
Baseline	14.4 (10.3-21.8)	14.3 (9.1-22.8)	0.71	13.3 (9.0-23.0)	0.80
Weeks 1-4	12.1 (7.1-16.5)	10.3 (4.6-15.0)	0.26	6.6 (3.2-10.6)	0.01
Weeks 5-8	12.4 (6.1-17.0)	8.0 (2.2-13.1)	0.04	7.1 (3.3-10.9)	0.04
Weeks 9-12	12.0 (6.2-17.0)	7.4 (1.9-10.3)	0.02	8.1 (3.5-11.8)	0.08
Week 12	10.9 (7.4-15.8)	7.5 (2.0-10.8)	0.03	7.6 (4.0-11.4)	0.07

Abbreviation: IQR, interquartile range

Table 3. Worst grade of side effects reported during study period

Patients intent-to-treat		Ven. N= 41	Clon. N= 41	Plac. N= 20	Total N= 102
Unassessed	Did not start	2 (5%)	2 (5%)	1 (5%)	5 (8%)
	Non-completion of daybooks	1 (2%)	2 (5%)	0 (0%)	3 (8%)
Loss of appetite	None	12 (29%)	20 (49%)	11 (55%)	43 (42%)
	Mild/Moderate	13 (32%)	15 (37%)	5 (25%)	33 (32%)
	Severe/Extreme	13 (32%)	2 (5%)	3 (15%)	18 (18%)
Nausea	None	10 (24%)	20 (49%)	8 (40%)	38 (37%)
	Mild/Moderate	20 (49%)	11 (27%)	7 (35%)	38 (37%)
	Severe/Extreme	8 (20%)	6 (15%)	4 (20%)	18 (18%)
Sleepy	None	10 (24%)	12 (29%)	8 (40%)	30 (29%)
	Mild/Moderate	13 (32%)	10 (24%)	5 (25%)	28 (27%)
	Severe/Extreme	15 (37%)	15 (37%)	5 (25%)	35 (34%)
Dizziness	None	16 (39%)	17 (41%)	10 (50%)	43 (42%)
	Mild/Moderate	15 (37%)	16 (39%)	6 (30%)	37 (36%)
	Severe/Extreme	7 (17%)	4 (10%)	3 (15%)	14 (14%)
Fatigue	None	10 (24%)	10 (24%)	7 (35%)	27 (26%)
	Mild/Moderate	9 (22%)	9 (22%)	2 (10%)	20 (20%)
	Severe/Extreme	19 (46%)	18 (44%)	10 (50%)	47 (46%)
Sweating	None	19 (46%)	19 (46%)	6 (30%)	44 (43%)
	Mild/Moderate	5 (12%)	6 (15%)	4 (20%)	15 (15%)
	Severe/Extreme	14 (34%)	12 (29%)	9 (45%)	35 (34%)
Dry mouth	None	9 (22%)	14 (34%)	7 (35%)	30 (29%)
	Mild/Moderate	15 (37%)	15 (37%)	5 (25%)	35 (34%)
	Severe/Extreme	14 (34%)	8 (20%)	7 (35%)	29 (28%)
Constipation	None	12 (29%)	21 (51%)	11 (55%)	44 (43%)
	Mild/Moderate	14 (34%)	11 (27%)	6 (30%)	31 (30%)
	Severe/Extreme	12 (29%)	5 (12%)	1 (5%)	18 (18%)

Ven. = venlafaxine / Clon. = clonidine / Plac. = placebo

the self-reported compliance and the returned study medication reported in the medical file. Seven patients reported not taking medication, however study medication was not returned, while 1 patient reported compliance however returned more study medication than was possible.

Discussion

Venlafaxine and clonidine are effective treatments in the management of hot-flashes in breast cancer patients. The results of this trial accord with earlier trials in which both drugs, venlafaxine and clonidine, have been studied (19-21). However for the first time, venlafaxine and clonidine were compared with placebo in breast cancer patients over a period of 12 weeks of treatment. This duration is based on guidance of the Food and Drug Administration (FDA) for development of hormone products for menopausal symptoms (29). A meta-analysis of 5 placebo controlled trials with the studied drugs of gabapentin, sertraline and paroxetine, suggests that hot-flash treatment efficacy remains stable between week 4 and week 12 (30). However, as shown in Table 2 the efficacy of the two treatments in this study changed dramatically during of the 5 to 12 week period. Based on the currently presented data, the optimal duration of hot-flash studies, with the two active drugs venlafaxine and clonidine versus placebo should be continued for at least 12 weeks. However, the effectiveness of both treatments beyond 12 weeks cannot be determined from this study.

Two double-blind crossover trials compared venlafaxine treatment with clonidine treatment in breast cancer patients. In the study of Loibl et al. (31) venlafaxine was superior to clonidine in the reduction of frequency ($p=0.025$) and severity ($p=0.043$) of hot-flashes. In contrast, the study of Buijs et al. (32) found no superiority of venlafaxine over clonidine in efficacy, defined as ≥50% reduction in hot-flash scores. In our study, venlafaxine resulted in a more immediate reduction of hot-flash scores when compared with clonidine however at week 12 hot-flash scores were lower in the clonidine group than in the venlafaxine group. A more rapid reduction of hot-flashes suggests that venlafaxine is to be preferred above clonidine. Limitations in comparing these results include differences in dosages of clonidine, in primary endpoints, and in duration. Moreover, we compared both treatments with placebo over a longer period of 12 weeks. In our study, 12 weeks of placebo treatment reduced hot-flash scores by 29%, which was described earlier (24). Therefore a placebo group as control is required in hot-flash trials for interpreting the effects of the medications.

In this study, treatment compliance was assessed by comparing patient-reported use with the number of tablets returned. Not all patients reported medication usage and in 8 patients (8.5%) there was discrepancy between the reports of the patients and the returned tablets. In published data of clinical studies concerning the management of hot-flashes, compliance to the study treatment is often not reported.

Sexual dysfunction is a common adverse event of antidepressant therapy, especially when using SSRIs, and sleep difficulties were considered side-effect of clonidine (21, 33). In our study sexual function and sleep quality did not differ between the two treatment groups. Comparing both treatments, more symptoms of anxiety in the clonidine group and more symptoms of depression were found in the venlafaxine group. Study periods may be too

short or the efficacy of the study treatment may be to moderate to accurately assess side-effects such as sleep quality, anxiety, depression and sexual function of the study treatment (34).
The occurrence of more side-effects such as nausea in the venlafaxine group may have been related to the dose of 75 mg daily. Previous trial results suggest that venlafaxine treatment should start with a daily dose of 37.5 mg which can then be increased to 75 mg if greater efficacy is desired. A gradual increase in venlafaxine dose may be associated with fewer adverse events, and some patients may be satisfied with the efficacy of 37.5 mg daily (19).
The discontinuation rate observed in our study did not differ from that reported in previous studies (19). Although the occurrences of nausea, constipation and severe appetite loss were more frequent in the venlafaxine group, premature discontinuation for adverse events occurred more often in the clonidine group. The cause of the increased premature discontinuation in the clonidine group is unclear but possibly related with lower efficacy of the study treatment. Patients should weigh the benefits and treatment effects for continuation of the study drugs.
One limitation of this study is the relatively limited number of participants analyzed at week 12, roughly 80% of the sample size that for which this study was powered. Another is the primary outcome is self-reported, and as such is hard to guarantee the quality of the data, nor the objectivity of the patients responses.
In conclusion, hot-flash scores were significantly lower in the clonidine group than placebo at week 12, while for venlafaxine versus placebo the difference in hot-flash scores was borderline not significant. Venlafaxine resulted in a more immediate reduction of hot-flash scores and was more effective in the management of hot-flashes over 12 weeks of treatment. It is advisable to treat patients in the management of hot-flashes with venlafaxine 37.5 mg daily in the first week and an increase in the venlafaxine dose to 75 mg if greater efficacy is desired. Hot-flash trials with two active drugs and placebo should be continued longer than 4 weeks of treatment due to changes in efficacy over longer periods, and in agreement with the FDA guideline.

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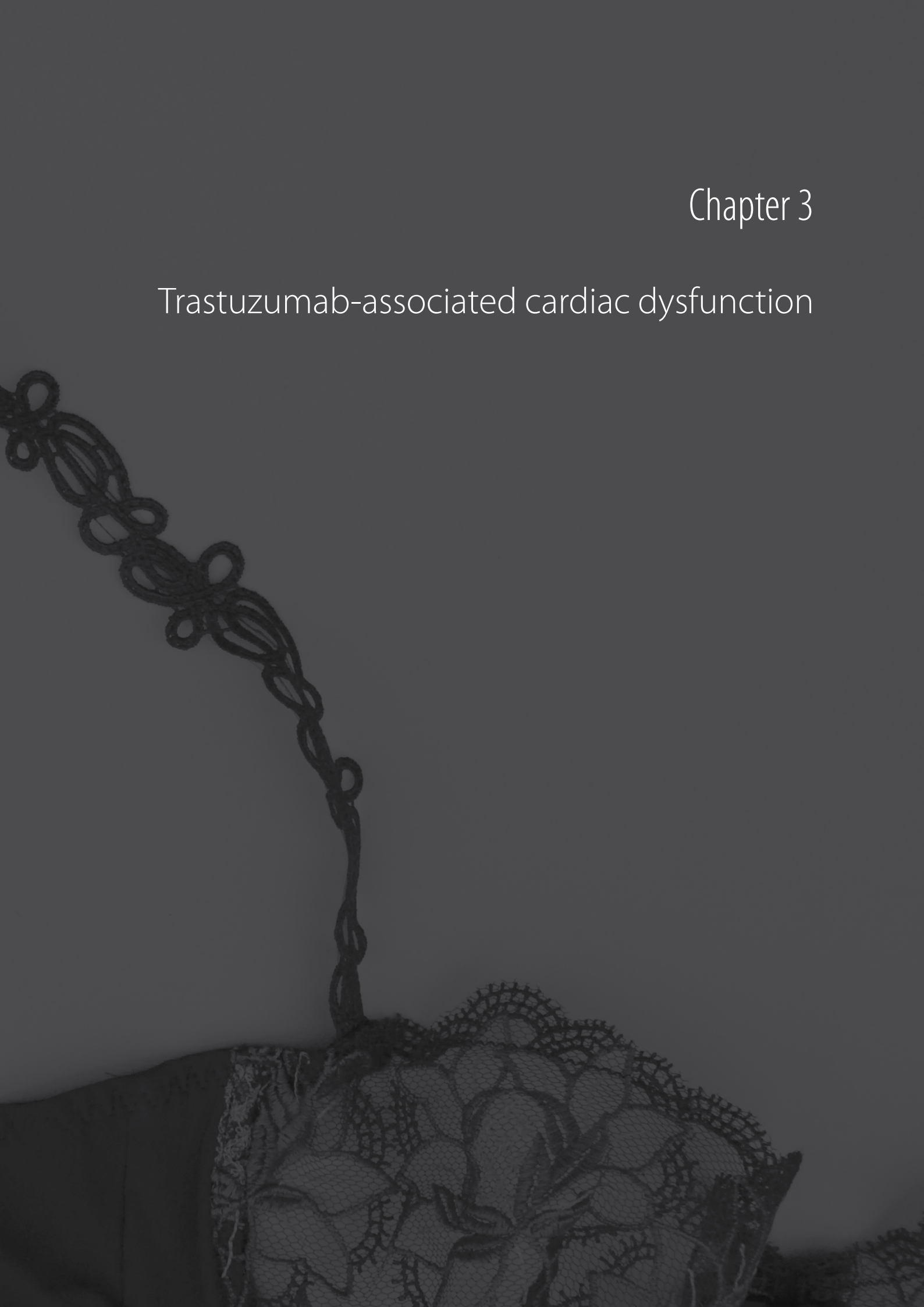
Chapter 3

Trastuzumab-associated cardiac dysfunction

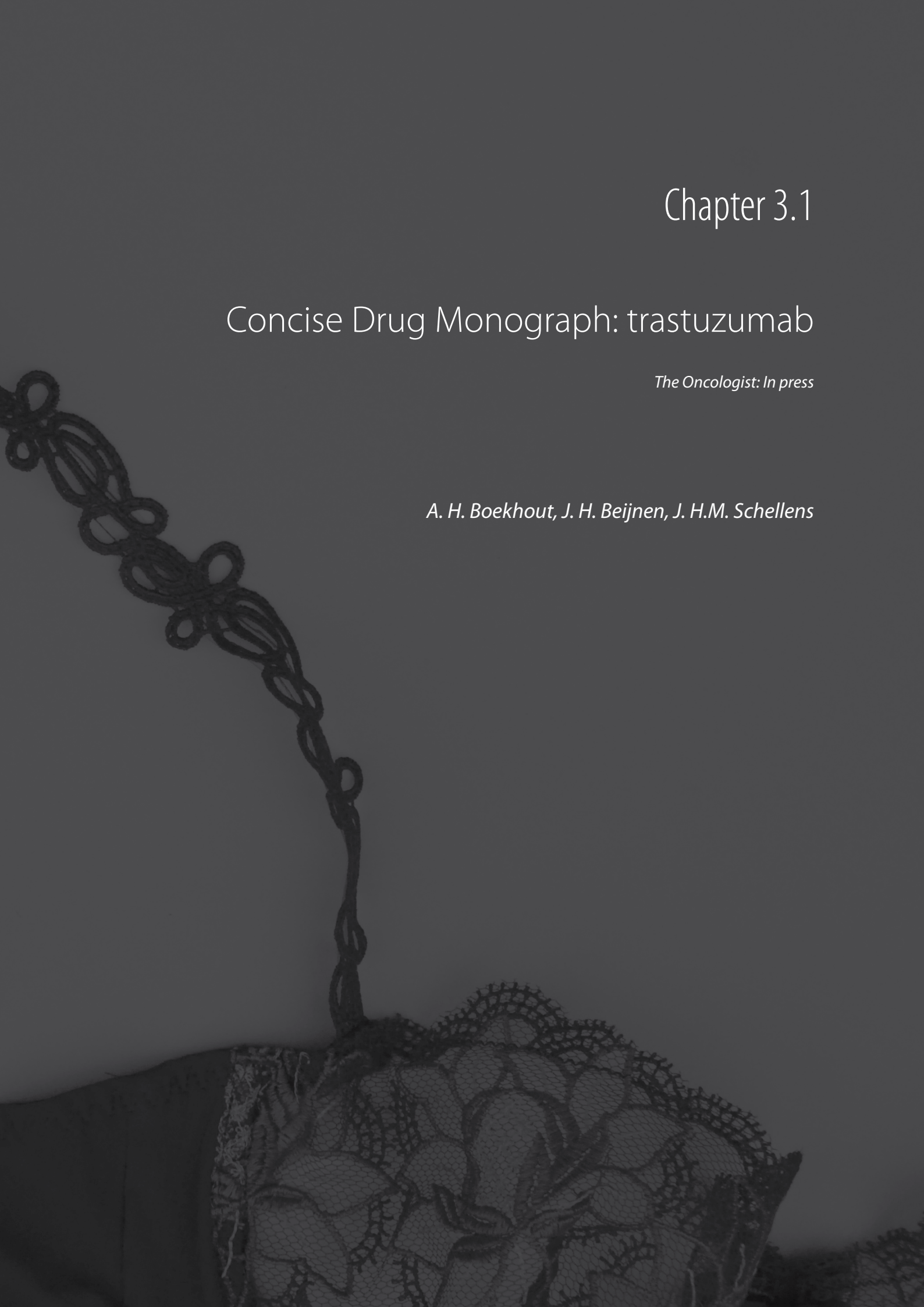


Chapter 3.1

Concise Drug Monograph: trastuzumab

The Oncologist: In press

A. H. Boekhout, J. H. Beijnen, J. H.M. Schellens



Introduction

Trastuzumab (Herceptin ®) is registered for the treatment of HER2-positive metastatic breast cancer, for adjuvant treatment of localized HER2-positive breast cancer and for HER2-positive metastatic adenocarcinoma of the stomach or gastro-esophageal junction. In the United States (US) and in Europe (EU) trastuzumab is indicated for breast cancer patients with a proven amplification of the HER2 oncogene or overexpression of the HER2 protein in the tumor. Overexpression of HER2 or amplification of the HER2 gene is associated with adverse disease prognosis, and a shorter overall and disease-free survival (1, 2). Trastuzumab is indicated in metastatic HER2-positive breast cancer patients: 1) as monotherapy after at least one or more chemotherapy regimens, 2) in combination with paclitaxel (US, EU), 3) in combination with docetaxel, and 4) in combination with an aromatase inhibitor in postmenopausal women with endocrine-responsive breast cancer, not previously treated with trastuzumab (EU). Patients with endocrine-responsive breast cancer must have failed hormonal therapy before trastuzumab is indicated (EU) (3-6). Trastuzumab is indicated in HER2-positive early breast cancer: 1) as adjuvant treatment (US, EU), and 2) as neoadjuvant treatment (EU). Trastuzumab is also indicated in HER2-positive metastatic adenocarcinoma of the stomach or gastro-esophageal junction: 1) in combination with capecitabine or 5-fluorouracil and cisplatin in patients who have not received prior anti-cancer therapy for their metastatic disease (US, EU).
Side-effects of trastuzumab treatment are often mild and mostly manageable. The major side-effect of trastuzumab treatment is reduction in left ventricular ejection fraction (LVEF), in a small proportion of patients even leading to advanced congestive heart failure (CHF), which appears to be at least partly reversible (7).

Clinical benefit of trastuzumab treatment
Trastuzumab has been shown to benefit patients with HER2-positive metastatic breast cancer when applied as monotherapy or used in combination with chemotherapy. In phase II studies, trastuzumab treatment was effective and well tolerated. An overview of the phase II studies in which trastuzumab was tested in advanced breast cancer is presented in a recent review (8). In phase III studies, the addition of trastuzumab to standard chemotherapy was associated with a longer time to disease progression (7.4 versus 4.6 months), longer duration of response (9.1 versus 6.1 months) and a longer overall survival (25.1 versus 20.3 months) (Table 1).
The addition of trastuzumab in the (neo)adjuvant setting resulted in an absolute reduction in the risk of recurrence or death. Three large randomized trials, evaluating the use of trastuzumab after adjuvant standard chemotherapy, showed beneficial effects of the addition of trastuzumab to standard adjuvant treatment. Combined analysis of the North Central Cancer Treatment Group Trial N9831 (NCCTG) and the National Surgical Adjuvant Breast and Bowel Project trial B-31 (NSABP-B31) (n=3,351), showed beneficial effects in terms of disease-free survival (87% versus 75%) and overall survival (94% versus 92%), after a median follow-up of 3 years (9). A large European study (HERA) showed that patients treated with trastuzumab had an absolute disease-free survival benefit of 6.3% (80.6% versus 74.3%) at 3 years (10). A fourth adjuvant trastuzumab trial, the BCIRG 006 study, has also shown a

Table 1 Trastuzumab treatment in metastatic breast cancer (Phase III studies)

Line of treatment	Treatment plan	No. Patients	Primary endpoint	Median OS	Incidence of CHF
First [2]	Anthracycline-based chemotherapy (A-E) + C 600 mg/m^2 or P 175 mg/m^2 (every 3 weeks) versus A-E + C followed by T or P followed by T	469	Median TTP, Chemotherapy: 4.6 months Chemotherapy + T: 7.4 months P<0.001	Chemotherapy: 20.3 months Chemotherapy + T: 25.1 months P<0.046	Class III or IV CHF: A-E + C: 8% A-E + C +T: 27% P: 1% P + T: 13%
Previous hemotherapy or endocrine therapy was permitted [51]	Anastrozol monotherapy versus anastrozol plus T (TAnDEM study) in postmenopausal women with ER/ HER positive breast cancer.	207	PFS, HR = 0.63 P = 0.0016 Anastrozol: 2.4 months Anastrozol + T: 4.8 months	Anastrozol: 23.9 months Anastrozol + T: 28.5 months P = 0.325	Class II CHF: Anastrozol + T: 1%
First [52]	P (175 mg/m^2) + T or P (175 mg/m^2) + Carboplatin (AUC = 6) + T	196	ORR, P + T: 36% P + Carboplatin + T: 52% P = 0.04	P + T: 32.2 months P + Carboplatin + T: 35.7 months P = 0.76	P + T: 2% P + Carboplatin + T: 0%

Abbreviations: A-E, anthracycline derivate: doxorubicine 60 mg/m^2 or epirubicin 75 mg/m^2; AI, aromatase inhibitor; C, cyclophosphamide; CHF, congestive heart failure; DFS, disease-free survival; D, docetaxel; E, epirubicine; FEC, fluorouracil, epirubicin, cyclophosphamide; HR, hazard ratio; ORR, objective response rate; OS, overall survival; P, paclitaxel; PFS, progression-free survival; T, trastuzumab; TTP, time to progression

disease-free survival benefit for trastuzumab when combined with standard chemotherapy. Table 2 presents an overview of the published adjuvant trastuzumab trials.

Recently, clinical benefit has been demonstrated in other malignancies with HER2 overexpression, especially in gastric cancer. The international phase III Trastuzumab for Gastric Cancer trial (ToGA), showed an overall survival of 13.5 months in the treatment group, compared with 11 months in the control group (HR 0.74; 95% CI 0.60, 091 p=0.0048)(11). This clinical improvement was considered convincing enough to halt the trial and to obtain Food and Drug Administration (FDA) and European Medicines Agency (EMA) registration for the first-line treatment of HER2-positive gastric cancer.

Clinical Use

Trastuzumab is administrated by intravenous infusion and is applied in a weekly or a 3-weekly schedule. The weekly schedule is initiated for monotherapy or in combination with chemotherapy. The weekly dose of trastuzumab is 2 mg/kg starting one week after the loading dose of trastuzumab of 4 mg/kg. The 3-weekly schedule of trastuzumab starts with a loading dose of trastuzumab of 8 mg/kg, followed by trastuzumab 6 mg/kg every 21 days. Trastuzumab in doses of 2 mg/kg can be administered as a 30-min infusion, but the higher doses of 4 mg/kg or 6 mg/kg in approximately 90 min.

Mechanism of action

Trastuzumab is a recombinant humanised IgG1 monoclonal antibody, against the extracellular domain of the HER2 receptor (Er*b*B2). The HER2 receptor consists of an extracellular ligand-binding domain, a transmembrane region and an intracellular or cytoplasmic tyrosine kinase domain. Trastuzumab binds to the extracellular domain of HER2, prevents cleavage of the extracellular domain of HER2 and thereby activation of the receptor, blocks the dimerization of HER2, mediates activation of antibody-dependent cell-mediated cytotoxicity resulting in tumor cell lysis, and promotes HER2 receptor internalization. Trastuzumab treatment is only effective in patients with an amplified HER2 oncogene or overexpression of the HER2 protein (12).

Molecular pathologic diagnosis

The accuracy of HER2 assays is essential for testing the HER2 status in breast and gastric cancer; however, the outcome of assays to determine the HER2 status varies substantially among laboratories. Validated methods for HER2 testing need to be used for resolving discrepancies in HER2 testing. The HER2 status is mostly tested by immunohistochemistry (IHC, HercepTest™), in some cases by fluorescent in situ hybridisation (FISH) or by chromogenic in situ hybridisation (CISH) (12). In current practice the use of the HercepTest is considered insufficient in patients with, for example, HER2 2 + outcome. Equivocal IHC samples (2+) must be retested for HER2 gene amplification by FISH or CISH (13). Equivocal FISH or CISH results must be confirmed by counting additional cells or repeating the test with a different method. The pathologist scores the test as 0, 1+, 2 + or 3 + and only 3 + and/or showing HER2 gene amplification by FISH or CISH identifies patients for trastuzumab treatment. In the case of gastric cancer 3 + IHC combined with positive FISH is considered necessary for trastuzumab treatment (13).

Bioanalysis of trastuzumab

Trastuzumab can be quantified in human serum by an enzyme-linked immunosorbent assay (ELISA). A precipitate-enhanced immunoassay (PEIA) has also been developed and results demonstrate a good correlation between the ELISA and PEIA method (14). It is presently not clear whether variations in plasma concentrations are predictive for toxicity and/or treatment failure.

Pharmacokinetics

The relevance of trastuzumab pharmacokinetics is unclear in relation to response or toxicity.

Elimination

Trastuzumab is metabolized to peptides and amino acids. The elimination is a complex of processes in humans but is specifically mediated by epithelial cells. Trastuzumab binds to the HER2 protein and is metabolized intracellularly. The consequence of the intracellular binding explains a dose-dependent (non-linear) elimination. The elimination of antibodies from the plasma is complex and dependent on factors such as genetics and clinical status of the patient. The half-life of trastuzumab is approximately 28 days. The wash-out period is up

Table 2 Trastuzumab treatment in primary breast cancer, the adjuvant setting

Study	Treatment plan	No. of patients	Median follow-up time in months	DFS and OS endpoint	Asymptomatic cardiotoxicity in trastuzumab treated patients	Incidence of class III or IV CHF in the trastuzumab group
NSABP B-31 trial and NCCTG N9831 [9;23]	A 60 mg/m^2 + C 600 mg/m^2 (4x every 3 weeks) followed by P 175 mg/m^2 (4x every 3 weeks) or P 80 mg/m^2 (12x weekly) versus AC followed by P plus T for 1 year	3351	36	DFS, HR = 0.48 P<0.0001 (absolute difference in DFS is 12%) OS, HR = 0.67; P=0.015	NSABP B-31 trial: 34% NCCTG N9831: 1.9%	NSABP B-31 trial: 4.1% NCCTG N9831: 1.9%
	Long-term follow-up of cardiac events	173	24	Not reported	Not reported	2%
BCIRG 006	A 60 mg/m^2 + C 600 mg/m^2 (4x every 3 weeks) followed by D 100 mg/m^2 (4x every 3 weeks) versus AC followed by D (4x every 3 weeks) + T for 1 year versus D + Carboplatin (6 x every 3 weeks) + T for 1 year	32222	36	DFS, HR = 0.61 P<0.0003 OS, HR = 0.59; P=0.004	AC-D group: 0.6% AC-DH group: 2.4% DCH: 0.4%	AC-D group: 1.2% versus AC-DH group: 2.3% AC-D group versus DCH group: 1.2%
FinHer [25]	D 100mg/m^2 followed by FEC (E 60 mg/m^2) or V 25 mg/m^2 (weekly) followed by FEC (E 60 mg/m^2) versus D followed by FEC + T or V followed by FEC + T for 9 weeks	232	36	DFS, HR = 0.42 P = 0.01 OS, HR = 0.41; P = 0.07	3.5%	3%
HERA [10;24]	anthracycline-based chemotherapy followed by T (every 3 weeks) for 1 or 2 years versus observation	Observation group n=1698 1 year T group n=1703	23.5	DFS, HR = 0.64 P < 0.0001 OS HR = 0.66; P = 0.0115	7%	Severe CHF: 0.5% Symptomatic CHF: 2%
	Long-term follow-up of cardiac events	Observation group n=1698 1 year T group n=1703	42	Not reported	9.8%	Severe CHF: 0.8% Symptomatic CHF: 1.9%

Abbreviations: CHF, congestive heart failure; A, doxorubicin; C, cyclophosphamide; DFS, disease-free survival; D, docetaxel; E, epirubicin; FEC, 5-fluorouracil, epirubicin, cyclophosphamide; HR, hazard ratio; OS, overall survival; P, paclitaxel; T, trastuzumab; V, vinorelbine

to 24 weeks after cessation of trastuzumab treatment. The renal excretion of trastuzumab is very low.

Contra-indication of trastuzumab treatment
Contra-indications for trastuzumab treatment include a history of hypersensitivity to trastuzumab treatment or murine proteins, severe dyspnea at rest due to complications of advanced malignancy, or requiring supplementary oxygen therapy.

Pregnancy and milk lactation
Data on the effect of trastuzumab treatment on the development of the human fetus are limited (15). Some cases of oligohydramnios during the second and third trimester and reversible fatal renal failure are reported in pregnant women receiving trastuzumab treatment (16). Trastuzumab treatment is indicated if the potential benefit for the mother outweighs the potential risk of the fetus, but the fetus must be strictly monitored. Lactation should be avoided during trastuzumab treatment and for 6 months after the last administration of trastuzumab.

Drug interaction and complementary and alternative medicine interaction
No controlled clinical data are available about drug interactions or complementary and alternative medicine interactions.

Alterations of patient characteristics
Data suggest that the disposition of trastuzumab is not influenced by age or renal function.

Pharmacogenetics
The aim of pharmacogenetics and trastuzumab treatment is to determine if there is a correlation between genetic polymorphism, such as in the HER2 gene, and the response to trastuzumab treatment or the development of trastuzumab-associated toxicity such as cardiotoxicity. Most of the reported polymorphisms affecting the efficacy of anticancer treatment are single nucleotide polymorphisms (SNPs) (17). Several SNPs in the extracellular, transmembrane and intracellular region of HER2 have been studied; however, their reported influence on trastuzumab efficacy remains controversial (18, 19). Currently, there are no arguments to determine the pharmacogenetic status of the HER2 gene to individualize trastuzumab treatment.

Pharmacodynamics
Recommended trastuzumab dosages are for monotherapy and for trastuzumab in combination with chemotherapy, used in the (neo-)adjuvant or metastatic setting. Higher doses and longer dosage intervals show no significant benefit over the standard dose schedules. There are no algorithms for dose reduction of trastuzumab in case of development of significant toxicity.

Special precautions

Caution should be exercised in patients who are experiencing dyspnea at rest due to complications of advanced malignancy and co-morbidities, patients with chronic heart failure, hypertension, and coronary artery disease and in patients with prior anthracycline-based chemotherapy.

Cardiac dysfunction

Short-term side-effects of trastuzumab are generally mild and manageable. However, cardiac dysfunction is an important side-effect of trastuzumab treatment. Cardiac dysfunction has been reported in patients who received trastuzumab as single agent or in combination with chemotherapy for metastatic disease or in primary breast cancer (20, 21). An indirect comparison of cardiac events between clinical studies is hampered by differences in the applied treatments, in inclusion and exclusion criteria, in the time interval between anthracycline-based chemotherapy and trastuzumab treatment, and in the definition of cardiotoxicity. A meta-analysis of randomized clinical trials in patients treated with sequential anthracycline-based chemotherapy and trastuzumab in the adjuvant setting, reported a significantly increased risk of 1.4% and 5.6% in grade III-IV CHF and asymptomatic cardiotoxicity, respectively (22).

However, the highest incidence of cardiac dysfunction is reported in metastatic breast cancer patients who were treated concurrently with anthracycline-based chemotherapy and trastuzumab (2). Based on this observation strict cardiac monitoring was incorporated in the adjuvant trastuzumab trials.

Recently, long-term cardiac safety data from three large randomized adjuvant trastuzumab trials (NCCTG N983; NSABP-B31; HERA) were presented. Patients with CHF in the NSABP-B31 and NCCTG N9831 trial were reviewed by an independent Adjuvant Cardiac Review and Evaluation Committee (ACREC) (23). The definition of CHF was defined as clinical symptoms, objective physical findings, and an LVEF drop of 10%, or an LVEF drop to an absolute LVEF below 50%. Based on previously documented cardiotoxicity data, 173 patients were evaluated: 40 patients who were treated with chemotherapy alone, 133 trastuzumab treated patients. The ACREC confirmed CHF in 8 patients (0.45%) who received chemotherapy alone and in 36 trastuzumab treated patients (2%), after a median follow-up of 2 years. A higher rate of CHF was associated with an age older than 50 years and lower LVEF values at the start of trastuzumab treatment.

A long-term follow-up study of the HERA trial evaluated the incidence of asymptomatic cardiotoxicity and CHF after a median follow-up of 3.6 years (24). A significant LVEF decrease (asymptomatic cardiotoxicity) was defined as an absolute decline of at least 10 percentage points from baseline LVEF, and to below 50%. Severe CHF was defined as NYHA class III or IV, confirmed by a cardiologist, and a significant LVEF decrease. Symptomatic CHF was defined as symptomatic CHF confirmed by a cardiologist and a significant LVEF decrease. A total of 164 trastuzumab treated patients (9.8%) and 49 patients (2.9%) who received chemotherapy alone experienced asymptomatic cardiotoxicity. Thirteen trastuzumab treated patients (0.8%) developed severe CHF versus zero patients with chemotherapy alone. Thirty-two trastuzumab patients (1.9%) developed symptomatic CHF versus 2 (0.1%) patients in the chemotherapy alone group.

In a subset of patients, trastuzumab treatment was discontinued because of cardiac disorders. In the NSABP-B31 and the NCCTG N9831 trials 16.4% of the patients discontinued trastuzumab treatment because of a confirmed asymptomatic decline in LVEF and 4.7% of the patients because of symptoms of CHF (9). In the analysis of the HERA study 5.1% of the patients discontinued trastuzumab treatment before completion of the treatment plan because of cardiac dysfunction. Premature discontinuation of trastuzumab treatment might limit trastuzumab-associated treatment benefit in the adjuvant setting. However, the optimal duration of adjuvant trastuzumab treatment is not yet determined. The efficacy of 9 weeks trastuzumab in one small adjuvant trastuzumab study (the FinHer study) was similar to the large adjuvant trials (25). This raises the question whether discontinuation of trastuzumab treatment is associated with a worse prognosis. Currently, the standard duration of adjuvant trastuzumab treatment is one year. However, the optimal duration of trastuzumab treatment still has to be determined.

Results from the NCCTG N9831 trial, NSABP-B31 trial and HERA trial, suggest that trastuzumab-associated cardiac dysfunction has a high rate of reversibility. Complete or partial recovery was observed in 86.1% of the trastuzumab treated patients with CHF in the combined analysis of the NSABP-B31 and NCCTG N9831 trial. In the HERA trial, 81% of the patients reached acute recovery of a cardiac event. An acute recovery was defined as two or more sequential LVEF assessments of ≥ 50% after the date of the cardiac event.

In their editorial Morris and Hudis criticized these reports on the point of data collection (26). Data of the long-term follow-up studies were not selected on original study data but were based on previously documented cardiotoxicity data. The retrospective design of these studies can lead to an underestimation of the incidence and reversibility of cardiotoxicity. Incomplete follow-up of the patient and under-diagnoses of other cardiac diseases can influence the accuracy of trastuzumab-associated cardiotoxicity.

All adjuvant trials had strict exclusion criteria concerning pre-existent cardiovascular morbidity. This makes the outcome of these trials difficult to translate to an unselected patient population or daily-life patient population. It is unclear whether or not classical cardiac risk factors are predisposing factors for trastuzumab-associated cardiotoxicity. The incidence of cardiotoxicity may well be higher in daily-life populations than reported in clinical trials in selected patients. Pre-existing hypertension, a smoking history, and a family history of coronary artery disease were supposed to be risk factors to develop CHF in a retrospective trial in a Canadian trastuzumab-treated patient population (27). Another retrospective trial suggested no relationship between these factors and trastuzumab-associated cardiotoxicity but there was a significant relationship between baseline LVEF and the risk of cardiotoxicity ($p=0.001$) (28). Therefore, more studies are needed in unselected patient populations to investigate the cardiac safety of trastuzumab treatment in a general population.

In conclusion, trastuzumab treatment is associated with cardiac dysfunction, is mostly medically manageable with CHF medication, and is partly reversible when trastuzumab is discontinued. Trastuzumab-associated cardiotoxicity is different from anthracycline-associated cardiotoxicity which is dose-dependent, not reversible and results in ultrastructural abnormalities as observed in myocardial biopsies. Based on current data, HER2-positive breast cancer patients can be safely treated with trastuzumab. However, we need longer follow-up of the adjuvant studies, further research to establish the incidence of trastuzumab-

induced cardiotoxicity in daily-life patient populations, and research on screening methods and cardioprotective drugs in trastuzumab-treated patients.
It is important to decrease the morbidity and mortality of trastuzumab treatment in breast cancer patients. Currently, a prospective, randomized, double-blind placebo-controlled trial is ongoing (CANDY study) in the Netherlands, to evaluate the efficacy of the angiotensin II-receptor (AT1) blocker candesartan in the prevention of trastuzumab-associated cardiotoxicity.

Mechanisms of trastuzumab-associated cardiotoxicity
In embryonic wild-type mice, HER2 is immunohistochemically present in myocardial and endocardial cells (29, 30). Cardiomyocyte HER2 expression is mostly restricted to the T-tubular network, indicating a non-random cardiac distribution pattern (31). It is therefore likely that HER2 regulates circumscriptive processes in cardiac physiology. Evidence of HER2 involvement in the physiology and pathophysiology of the heart is demonstrated in conditional mutant mice with a cardiac-restricted HER2 gene deletion. These mice showed no abnormalities at birth, but shortly after birth they developed a dilated cardiomyopathy (29, 31-33).
HER2 appears to play an important role in compensatory cardiac hypertrophy. Hypertrophic growth can serve as a compensatory mechanism for different mechanical, hemodynamic, hormonal and pathologic stimuli. Aortic banding in conditionally mutated mice with a cardiac-restricted HER2 deficiency did not result in a hypertrophic growth response.
The precise role of HER2 in human cardiac physiology and disease is still unknown. Myocardial HER2 mRNA expression was studied in left ventricle biopsies from 36 patients with severe CHF due to ischemic or non-ischemic cardiomyopathy, undergoing left ventricular assist device implantation. HER2 was upregulated after implantation of the device, whereas HER2 prior to implantation was comparable to healthy controls (34). Recently, in 6 out of 60 severe CHF patients, immunohistochemical expression of HER2 (and HER4) was shown in myocardial biopsies (35).

Clinical Monitoring
Monitoring of cardiac function
For identification of trastuzumab-related cardiotoxicity all trastuzumab-treated patients should undergo a complete medical history, physical examination, electrocardiogram and measurement of LVEF at baseline of trastuzumab treatment. Furthermore, it is recommended to monitor cardiac function by LVEF every 3 months during trastuzumab treatment.
Preliminary data suggest that plasma NT-proBNP and troponine I may be parameters to detect or predict trastuzumab induced cardiotoxicity. In a study by Perik et al., pre-treatment plasma NT-proBNP levels were higher in patients with heart failure during trastuzumab treatment (n=3) than in patients without heart failure (n=12) (36). A recently published trial revealed a significant relationship between troponin I, a well-established specific and sensitive marker of myocardial injury, and trastuzumab-associated cardiotoxicity. Patients with elevated troponine I levels, were at risk for trastuzumab-associated cardiotoxicity and recovery of trastuzumab-associated cardiotoxicity was unlikely (37). These findings suggest that NT-proBNP and troponine I may be useful parameters for identifying patients at risk

for trastuzumab induced cardiotoxicity. However, more evidence is needed before these parameters can be applied as biomarkers to identify patients at risk for development of trastuzumab-associated cardiotoxicity during trastuzumab treatment.

Infusion-related reactions of trastuzumab
Trastuzumab may cause infusion-related reactions. Most trastuzumab-related reactions occur during the first infusion or within 24 hours after the infusion. These are generally mild and occur infrequently with subsequent trastuzumab infusions. The overall incidence of severe infusion-related reactions is rare and is less than 1%. These infusion-related reactions include fever, chills, dyspnea, hypotension, bronchospasm, reduced oxygen saturation and respiratory distress. Fatal reactions, however, have been reported. In patients with severe trastuzumab-related hypersensitivity the safety of rechallenge is unknown. In 33 (85%) of 39 patients with a previous severe hypersensitivity reaction, rechallenge of trastuzumab treatment was safe with supportive therapy. (38)

Patient instructions and recommendations for supportive care
Trastuzumab should be administrated by intravenous infusion. Patients should be observed for hypersensitivity reactions during the trastuzumab administration, especially during the first infusion. It is advised to monitor the cardiac function before start of trastuzumab treatment, every 3 months during trastuzumab treatment, and at least 6 months after discontinuation of trastuzumab treatment.

Biomarkers of trastuzumab resistance
Not all HER2-positive breast cancer patients respond to trastuzumab treatment. Several mechanisms have been proposed that might explain intrinsic trastuzumab resistance. Deficiency of phosphatase tensin homologue (PTEN) and activation of PI3K results in increased activity of the AKt/mTOR signal transduction pathway and have been shown to be important biomarkers of trastuzumab resistance (39, 40). Also the overexpression of other surface receptors, such as insulin-like growth factor (IGFR) provides alternative growth-factor signalling and is related to decreased trastuzumab sensitivity (41). In vitro studies have shown that increased expression of mucin MUC4 results in increased retention of Her2 and Her3 at the cell surface. As a result, growth factor receptors cannot be degraded with increased signalling potential and decreased trastuzumab sensitivity as a result (42).

Trastuzumab treatment beyond progression
The optimal therapeutic strategy beyond progression during trastuzumab treatment is not well known. A prospective trial (prematurely closed) described the clinical outcome of 156 patients after progressive disease during treatment with trastuzumab. Patients received capecitabine alone or in combination with trastuzumab. The addition of trastuzumab to capecitabine was associated with longer time to disease progression (8.2 versus 5.6 months), longer overall survival rates (25.5 versus 20.4 months) and higher overall response rates (48.1% versus 27%) (43). Although prospective data are limited, on the basis of retrospective analysis there is consensus that trastuzumab should be continued until tumor progression. Trastuzumab is also applied beyond progression, whereby the accompanying chemotherapy

is switched. For example, trastuzumab-paclitaxel is changed into trastuzumab-capecitabine, or trastuzumab-vinorelbine. Phase II studies to support this strategy are lacking, as are studies to compare this strategy with replacement of trastuzumab by lapatinib. At present, we should focus on well-designed clinical trials to establish the optimal strategy in this setting for patients.

Novel anti-ErbB2 therapies

Patients with HER2-positive breast cancer will eventually experience relapse or progression on trastuzumab treatment. Trastuzumab binds to the extracellular domain of HER2, but an inhibition of one signal transduction pathway may not be enough, since it does not control all HER2-positive breast cancer tumors. Therefore, there is a need for novel effective anti-ErbB2 therapies. Several studies in patients with HER2-positive metastatic breast cancer have been initiated to develop multiple lines of anti-ErbB2 therapy. Small molecule thyrosine kinase inhibitors (TKIs) may add therapeutic benefit to the established anti-body based treatment. TKIs bind to the intracellular domain of HER2, usually the ATP-binding domain, thereby blocking the HER2 dimerization step by kinase inhibition and interrupting downstream pathways. Lapatinib, an orally available small-molecule, is the only TKI registered for the treatment of HER2-positive metastatic breast cancer (US, EU). The most reported side-effects of lapatinib therapy are diarrhea, skin rash and asymptomatic cardiotoxicity. Lapatinib is established as effective treatment in advanced breast cancer including cancers progressing on trastuzumab-based therapy (44). In a phase III study, 321 HER2-positive metastatic breast cancer patients were randomized to receive lapatinib plus capecitabine or capecitabine alone (previously progressive on trastuzumab treatment). The median time to progression was 8.4 months in the combination therapy group, compared with 4.4 months with the capecitabine monotherapy group ($P < 0.001$) (45, 46). Lapatinib in combination with endocrine therapy provided clinical benefit in untreated ER/HER-positive post-menopausal patients. Patients received lapatinib plus letrozol versus letrozol plus placebo. The median progression-free survival (PFS) was significantly higher in the combination group of lapatinib with letrozol compared with letrozol plus placebo; 8.2 versus 3 months, respectively. A combination of the two agents trastuzumab and lapatenib significantly improved the median PFS; 12 weeks in the lapatinib plus trastuzumab group versus 8.1 weeks in the lapatinib alone group. The overall tumor response rate was not significantly different between the two treatment arms (47).

Neratinib, a pan-ErbB receptor tyrosine kinase inhibitor, showed to be clinically active and was well tolerated in previously trastuzumab-treated patients (48). Patients with advanced HER2-positive breast cancer received neratinib 240 mg once daily. Sixty-six patients had received prior trastuzumab treatment and 70 patients were trastuzumab naive. The median PFS was 22.3 weeks for patients with prior trastuzumab and 39.6 weeks for patients with no prior trastuzumab treatment. Diarrhea was the most frequently reported side-effect (48). Phase I and II studies of trastuzumab-DM1 (T-DM1), an antibody-drug conjugate, have shown activity and was well-tolerated in patients with prior trastuzumab treatment (49). The combination of trastuzumab and pertuzumab, a recombinant humanized monoclonal antibody preventing HER2 dimerization with HER1, HER3 or HER4, was evaluated in a phase II study. The combination of trastuzumab and pertuzumab showed activity with a median

PFS of 5.5 months and was well tolerated in patients who had progression during prior trastuzumab treatment (50). Ongoing trials are investigating the efficacy of various new TKIs of HER2. Moreover, other studies are aiming to define subsets of patients with specific characteristics of the Erb gene that will most likely benefit from these new strategies.

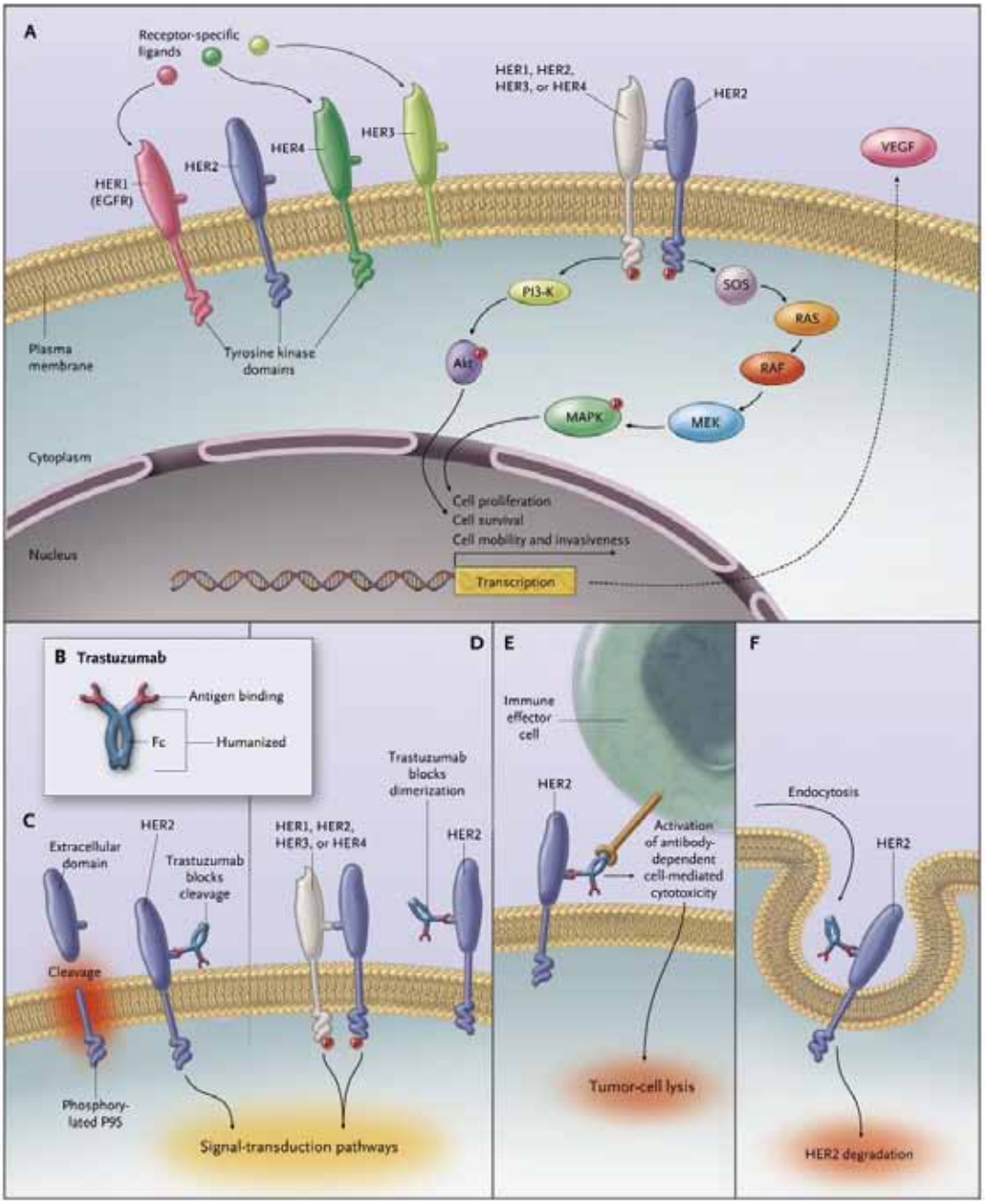


Figure 1. Signal Transduction by the HER family and potential mechanisms of action of trastuzumab (reproduced with permission from reference 23).

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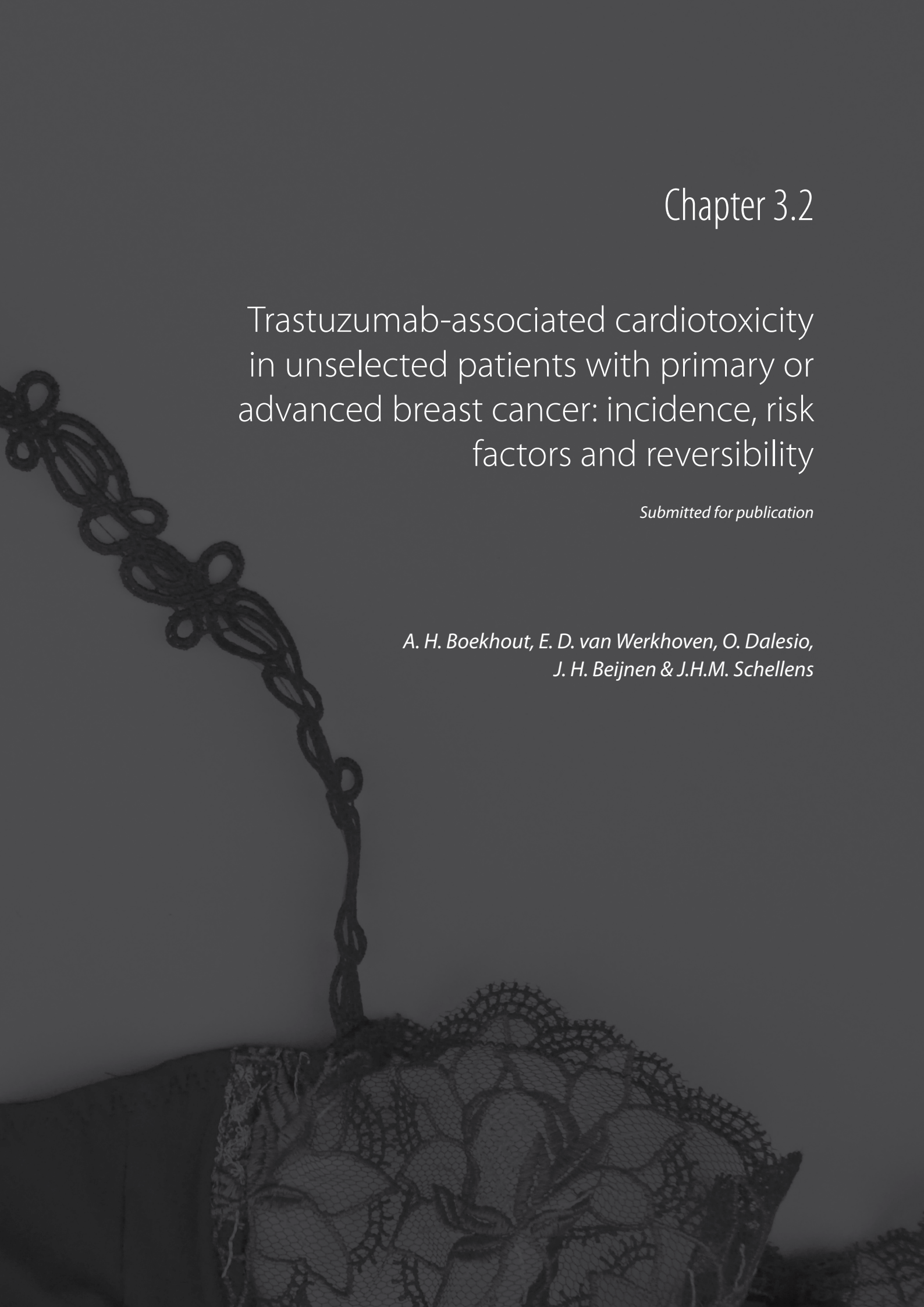
SUMMARY TABLE	
Generic name	Trastuzumab
Commercial name	Herceptin ®
Synonym	Anti HER2
Average Molecular weight	approximately 150 kDa
Mechanism of action (Fig. 1)	Trastuzumab binds to the extracellular domain of HER2, prevents cleavage of the extracellular domain of HER2 and thereby activation of the receptor, blocks dimerization of HER2, mediates activation of antibody-dependent cell-mediated cytotoxicity resulting in tumor cell lysis and promotes HER2 receptor internalization
Route of administration	Intravenous
Elimination	Elimination predominantly intracellularly in epithelial cells. Renal elimination is very low.
Terminal half-life	28 days
Main toxicities	Infusion-related toxicities such as fever, chills, dyspnea, hypotension, bronchospasm, reduced oxygen saturation and respiratory distress. Cardiotoxicity, asymptomatic decrease in left ventricular ejection fraction or congestive heart failure
Pharmacogenetics	No pharmacogenetic status to individualize trastuzumab treatment
Resistance	Several mechanisms are responsible for trastuzumab resistance; loss of phosphatase and tensin homologue deleted on chromosome ten (PTEN), activation of the PI3K pathway, overexpression of other surface receptors (IGFR) may play a role.
Unique features	Side-effects are generally mild and manageable
Main drug and CAM interactions	No controlled clinical data are available
Dose adaptations	No controlled clinical data are available.

Abbreviations: PTEN = phosphatase tensin; IGFR = insulin-like growth factor



Chapter 3.2

Trastuzumab-associated cardiotoxicity in unselected patients with primary or advanced breast cancer: incidence, risk factors and reversibility

Submitted for publication

A. H. Boekhout, E. D. van Werkhoven, O. Dalesio,
J. H. Beijnen & J.H.M. Schellens

Abstract

Background

The addition of trastuzumab to standard adjuvant chemotherapy in HER2-positive breast cancer markedly improves treatment outcome. The aim of this study was to investigate retrospectively the association between risk factors and the development of trastuzumab-associated cardiotoxicity and to identify the reversibility of trastuzumab-associated cardiotoxicity in a standard treated HER2 positive patient population.

Patients and methods

Patients were treated between January 2005 and January 2007 in the Netherlands Cancer Institute. Asymptomatic cardiotoxicity was defined as: decrease of 15 percentage points in LVEF compared with baseline and an absolute value below 50%.

Results

In total 122 patients were included in this study: 77 patients were treated for HER2 positive primary breast cancer and 45 patients for HER2 positive advanced breast cancer. A high incidence of asymptomatic cardiotoxicity of 18% and 9% in patients with and without anthracycline-based chemotherapy, respectively, was observed. In one patient (0.8%) there was evidence of symptomatic cardiotoxicity.

Conclusions

Trastuzumab treatment is associated with significant and only partly reversible cardiotoxicity in an unselected HER2 positive patient population. The combination of anthracycline-based chemotherapy and trastuzumab induces the incidence of asymptomatic cardiotoxicity. In our study there was no statistically significant association between described risk factors as age, high BMI, use of antihypertensive drug or co-morbidity. The baseline LVEF value can be an important factor in the incidence of cardiac events. Longer follow-up studies will be needed to evaluate long-term cardiac safety after trastuzumab treatment.

Introduction

Breast cancer is the most frequently reported malignancy and life-threatening cancer diagnosis in women in northern Europe and the United States. In a subset of approximately 25% of patients with breast cancer the human epidermal growth factor receptor 2 (HER2) is overexpressed. This feature is associated with shorter disease-free and overall survival compared with women with HER2 negative breast cancer (1, 2). Trastuzumab is a humanized monoclonal antibody against the extracellular domain of HER2 and has resulted in clinical benefit for women with HER2 positive metastatic breast cancer and improved disease-free and overall survival in HER2 positive primary breast cancer (3-5).
Although treatment with trastuzumab is generally well tolerated it is associated with the development of clinically manageable left ventricular systolic dysfunction (asymptomatic cardiotoxicity) and occasionally advanced Congestive Heart Failure (symptomatic cardiotoxicity, CHF) in a small proportion of patients (6-8). The incidence of cardiac events in the highly selected patient population in clinical trials was up to 10% (7-9). However, in view of the strict in- and exclusion criteria of these trials these patients may not be fully representative of the daily-life patient population that may suffer from significant co-morbidity and thus may be using more co-medication. The exact mechanism of trastuzumab-induced cardiotoxicity is still unclear and there are many other questions regarding this toxicity (10-12). Little is known about the incidence, the risk factors to induce trastuzumab related cardiotoxicity and the reversibility of this toxicity in the daily-life patient population. Therefore we explored the incidence of trastuzumab-associated cardiotoxicity in a standard treated HER2 positive patient population, possible risk factors in relation to trastuzumab treatment with prior or concomitant chemotherapy or endocrine therapy, and the reversibility of cardiotoxicity. Although the results of the left ventricular ejection fraction values (LVEF) are sensitive for identifying asymptomatic cardiotoxicity, the retrospective design of this study can lead to an under- or overestimation of the changes in LVEF values. Incomplete follow-up information on the patient, an unknown cardiac disease and the use of anthracyclines can influence the cardiotoxicity determination. Even though this study is representative for estimating the incidence of trastuzumab-associated cardiotoxicity in clinical practice and presents one of the largest European standard HER2 positive trastuzumab treated patient population.

Patients and methods

Study population

Patients treated with trastuzumab for HER2 positive primary or advanced breast cancer at the Netherlands Cancer Institute and treated between 2005 and 2007 were included in this study. Criteria for inclusion were: pre- and postmenopausal patients with histologically confirmed HER2 positive breast cancer treated with trastuzumab-based therapy for at least one month or longer and available left ventricular ejection fraction (LVEF) values, one before and at any time at least one LVEF value during trastuzumab treatment. Patients with a LVEF measurement before anthracycline-based chemotherapy, but without LVEF measurement

at the end of anthracycline-based chemotherapy and before start of trastuzumab administration and patients with a baseline LVEF value below 50% were excluded for the study. All patients fulfilling the criteria were included in the analysis.

Treatment

Trastuzumab is given according to standard practice weekly, two-weekly or tri-weekly. Trastuzumab is commonly used in combination with or after upfront chemotherapy and in patients with an ER and/or PR positive breast tumor in combination with endocrine therapy.

Cardiac evaluation

Cardiac evaluation included a LVEF value assessed by multiple-gated acquisition scanning (MUGA) and an evaluation of symptomatic cardiotoxicity. The severity of cardiotoxicity was graded according to the National Cancer Institute Common Toxicity Criteria for Adverse Events, version 3.0. Cardiac events were defined as follows: decrease of 15 percentage points in LVEF compared with baseline and an absolute value below 50%. A cardiac event was defined on the first simultaneous occurrence of; a decrease in LVEF compared with baseline and an absolute LVEF value below 50%. In this study symptomatic CHF was defined as symptomatic CHF confirmed by a cardiologist.

Statistical analysis

The following parameters were considered as risk factors for the development of cardiotoxicity: age; body mass index (BMI); history of hypertension; cardiac disease; pulmonary disease (COPD, asthma); history of hyperlipidemia; type 1 or type 2 diabetes mellitus; smoking (current or previous: time-period of discontinuation); use of co-medication at baseline or during trastuzumab treatment as angiotensin-converting enzyme (ACE) inhibitors; angiotensin blockers; diuretics; calcium antagonists and ß-blockers. The following data were evaluated as treatment factors: cumulative time, dose and schedule of trastuzumab treatment; type and (cumulative) dose of chemotherapeutic treatment; type, dose and time of endocrine therapy; dose, side (left, right or both) and field (breast- and/or chest wall irradiation) of radiotherapy and the time-period between anthracycline-based chemotherapy and the first administration of trastuzumab treatment.

Data were analysed in the total study population and in two different treatment groups: patients with anthracycline-based chemotherapy and patients without anthracycline-based chemotherapy prior to or concomitant with trastuzumab treatment.

Patient characteristics at baseline and treatment differences between the two groups were compared using the Krusskall Wallis -, the chi-square - and Fisher's exact test, where appropriate.

The incidence of a LVEF failure was defined as the time-period from the start of trastuzumab treatment to the time-point of the first occurrence of both: a decrease of 15 percentage points in LVEF from baseline and an absolute value below 50%. Patients without such a failure were censored at the time of the last LVEF measurement. Incidence-curves were made using the Kaplan-Meier method with the log-rank test assessing equality of distributions. The follow-up was calculated as the time-period between the first trastuzumab administration to the

time-point of the last LVEF measurement.
Associations between treatment characteristics and baseline risk factors with time-period to LVEF failure were tested by univariate Cox proportional hazards models. Age at the first trastuzumab administration was calculated.
An interruption of trastuzumab treatment was defined as an interruption for more than two subsequent planned trastuzumab administrations; i.e. in patients with a weekly schedule an interruption of at least two weeks. To assess the reversibility of the LVEF, the last LVEF before the interruption was compared with the next LVEF during the interruption, using Wilcoxon's signed-rank test.
To assess the effect of co-medication intended to reverse the decrease of the value of the LVEF, the last LVEF value before start of co-medication was compared with the first LVEF value after the start of co-medication treatment, using Wilcoxon's signed-rank test. All analyses were performed using SAS version 9.1 and R version 2.9.0 (13).

Results

Patient characteristics
In total one hundred fifty-seven female patients were identified that received trastuzumab in the selected time-period of January 2005 and January 2007. Out of these: twenty-four patients were not eligible because of a LVEF measurement before anthracycline-based chemotherapy but without LVEF measurement after anthracycline-based chemotherapy, before start of trastuzumab administration. Eleven patients were excluded because of a baseline LVEF value below 50%. This leaves one hundred twenty-two patients evaluable for this study: eighty-nine patients received anthracycline-based chemotherapy prior to or concomitant with trastuzumab treatment and thirty-three patients received trastuzumab treatment without anthracycline-based chemotherapy. In total seventy-seven patients were treated for HER2 positive primary breast cancer and forty-five were treated for HER2 positive advanced breast cancer. Median age of the total evaluable population at start of trastuzumab treatment was 48 years; range 29 – 73 years. The median follow-up was 12.5 months in the total study population. A low percentage of patients had baseline risk factors of inducing cardiotoxicity. Information about smoking history was available of one hundred six (87%) of the patients; median time-period between stop-point of smoking and start-point of trastuzumab treatment was 9 years.
Patient characteristics are presented in Table 1.

Treatments
Treatment characteristics are presented in Table 2. Anthracyclines were most frequently prescribed before trastuzumab administration (93%) although in six (6.7%) patients concomitant with trastuzumab treatment. These six patients were treated in a neoadjuvant study with two treatment arms for patients with HER2 positive primary breast cancer; the combination paclitaxel (70 mg/m^2), trastuzumab (4 mg/kg loading dose, then 2 mg/kg) and carboplatin (AUC=3 mg min/mL) or fluorouracil (500 mg/m^2), epirubicin (75 mg/m^2), cyclophosphamide (500 mg/m^2) and trastuzumab (FE75C-T), as described by Buzdar et

Table 1. Patient characteristics of patients with anthracycline-based chemotherapy and of patients without anthracycline-based chemotherapy		**No N= 33**	**Yes N= 89**	**Total N= 122**
Age at start herceptin	Median (range)	52 (32-73)	46 (29-69)	48 (29-73)
Age	<50	14 (42%)	53 (60%)	67 (55%)
	50-59	12 (36%)	30 (34%)	42 (34%)
	>=60	7 (21%)	6 (7%)	13 (11%)
LVEF at baseline	Median (range)	0.65 (0.53-0.83)	0.61 (0.50-0.78)	0.62 (0.50-0.83)
Body Mass Index	Median (range)	24.7 (19.6-36.1)	24.0 (17.6-52.1)	24.1 (17.6-52.1)
	<20	1 (3%)	9 (10%)	10 (8%)
	20-25	18 (55%)	50 (56%)	68 (56%)
	>25	14 (42%)	30 (34%)	44 (36%)
Smoker	Non-smoker	17 (52%)	46 (52%)	63 (52%)
	Smoker	7 (21%)	12 (13%)	19 (16%)
	Former smoker	5 (15%)	19 (21%)	24 (20%)
	Missing	4 (12%)	12 (13%)	16 (13%)
Cardiac symptoms at baseline	Total	0 (0.0%)	0 (0.0%)	0 (0.0%)
Hyperlipidemia	Total	1 (3.0%)	2 (2.2%)	3 (2.5%)
Blood pressures - systolic	Median (range)	125 (95-190)	120 (95-195)	120 (95-195)
Blood pressures - diastolic	Median (range)	81 (60-115)	80 (60-100)	80 (60-115)
History of cardiac disease	Total	0 (0.0%)	5 (5.6%)	5 (4.1%)
Diabetes Mellitus	Total	2	3	5
Diabetes Mellitus type 1	Total	1	2	3
Diabetes Mellitus type 2	Total	1	1	2
Chronic Obstructive Pulmonary Disease	Total	1 (3.0%)	2 (2.2%)	3 (2.5%)
Astma	Total	0 (0.0%)	3 (3.4%)	3 (2.5%)
Baseline co-medication for cardiac event	Total	1 (3.0%)	10 (11.2%)	11 (9.0%)
ACE inhibitors	Total	0 (0.0%)	3 (3.4%)	3 (2.5%)
Angiotensin blockers	Total	0 (0.0%)	2 (2.2%)	2 (1.6%)
Diuretics	Total	0 (0.0%)	4 (4.5%)	4 (3.3%)
Calcium antagonists	Total	0 (0.0%)	1 (1.1%)	1 (0.8%)
Beta-blockers	Total	1 (3.0%)	3 (3.4%)	4 (3.3%)

Abbreviation: LVEF, left ventricular ejection fraction

Table 2. Treatment characteristics in patient with anthracycline-based chemotherapy and in patients without anthracycline-based chemotherapy		**No** N= 33	**Yes** N= 89	**Total** N= 122
trastuzumab- weeks of treatment	Median (range)	44.0 (16.3-159.6)	42.9 (7.3-217.9)	43.4 (7.3-217.9)
trastuzumab- total dose (milligram)	Median (range)	7875 (2660-29952)	7004 (1400-31160)	7337 (1400-31160)
trastuzumab- stopped within 3 months reason	Total	2	6	8
trastuzumab- stopped reason	LVEF decrease	3 (100%)	7 (41%)	10 (50%)
	Symptomatic cardiac toxicity	0 (0.0%)	1 (6%)	1 (5%)
	LVEF decrease + other	0 (0.0%)	1 (6%)	1 (5%)
	LVEF decrease + symptomatic cardiac toxicity	0 (0.0%)	4 (24%)	4 (20%)
Comedication during trastuzumab	Total	4 (12.1%)	19 (21.3%)	23 (18.9%)
Comedication during trastuzumab- ACE inhibitors	Total	1 (3.0%)	v9 (10.1%)	10 (8.2%)
Comedication during trastuzumab- Angiotensin blockers	Total	0 (0.0%)	3 (3.4%)	3 (2.5%)
Comedication during trastuzumab- Diuretics	Total	2 (6.1%)	7 (7.9%)	9 (7.4%)
Comedication during trastuzumab- Calcium antagonists	Total	0 (0.0%)	2 (2.2%)	2 (1.6%)
Comedication during trastuzumab Beta-blockers	Total	3 (9.1%)	9 (10.1%)	12 (9.8%)
Endocrine treatment- Aromatase inhibitor	Total	15 (45.5%)	51 (57.3%)	66 (54.1%)
Endocrine treatment - Estrogen receptor downregulators	Total	5 (15.2%)	19 (21.3%)	24 (19.7%)
Endocrine treatment- LHRH or GnRH agonists	Total	6 (18.2%)	17 (19.1%)	23 (18.9%)
Radiotherapy	Total	25 (75.8%)	72 (80.9%)	97 (79.5%)
Radiotherapy- before trastzumab	Total	10 (30.3%)	61 (68.5%)	71 (58.2%)
Radiotherapy- during trastzumab	Total	13 (39.4%)	13 (14.6%)	26 (21.3%)
Radiotherapy- after trastzumab	Total	2 (6.1%)	1 (1.1%)	3 (2.5%)
Radiotherapy- breast thoracic wall	Total	9 (27.3%)	23 (25.8%)	32 (26.2%)
Radiotherapy site	No RT	8 (24%)	17 (19%)	25 (20%)
	Left side	11 (33%)	35 (39%)	46 (38%)
	Right side	13 (39%)	32 (36%)	45 (37%)
	Left and right side	1 (3%)	5 (6%)	6 (5%)
Radiotherapy- total dose RT units	Median (range)	95 (32.58-250)	100 (24-152.04)	100 (24-250)
	N	25	70	95
with epirubicin	Total	0 (0.0%)	v30(33.7%)	30 (24.6%)
with doxorubicin	Total	0 (0.0%)	59 (66.3%)	59 (48.4%)
Total dose doxorubicin in milligram	Median (range)	NA	477.5 (204-960)	477.5 (204-960)
	N	0	58	58
Total dose epirubicin in milligram	Median (range)	NA	624 (427.5-1026)	624 (427.5-1026)
	N	0	20	20

Abbreviations: CHF, congestive heart failure; LVEF, left ventricular ejection fraction; N, number; RT, radiotherapy; NA, not applicable

al (14). In our study no change in the incidence of cardiac events was seen excluding the six patients who received epirubicin-based chemotherapy concomitant with trastuzumab treatment.

Cardiac events and risk factors

Several potential risk factors were analysed. The baseline risk factors were not statistically significantly associated with the incidence of cardiotoxicity. In this study, data suggest that the baseline LVEF value was associated with the incidence of cardiac events.

Cardiac events and treatment

The total trastuzumab dose, the dose, side and timing of radiotherapy were not significantly associated with the incidence of cardiotoxicity. The median time interval between anthracycline-based chemotherapy and the start of trastuzumab treatment is longer (11 months) in the HER2 positive advanced breast cancer group compared with the primary breast cancer group (1.5 months), P=<0001. Median time-period between the last doxorubicin administration and start of trastuzumab treatment was 2.5 months and 4.5 months between the last epirubicin and trastuzumab treatment.

The severity of cardiac events was statistically significantly associated with prior or concomitant anthracycline-based chemotherapy, P=0.0004. The administration of AIs was statistically significantly associated with the incidence of cardiotoxicity, P=0.0226.

Incidence and severity (according the CTC scale) of cardiac events

In one hundred sixteen (95%) patients the treatment had been completed at the time of the analysis. The incidence rate of cardiac events observed in this study was 13% per year of treatment. Sixteen (18%) patients of the anthracycline-based treatment group and three (9.1%) patients of the group without anthracycline-based chemotherapy experienced a decrease in LVEF of at least 15% compared to baseline and to an absolute value to below 50%. The number and severity of cardiac events by treatment group is shown in Figure 1 and 2 and in Table 3 and 4.

Table 3. Cardiac events in patient with anthracycline-based chemotherapy and in patients without anthracycline-based chemotherapy

		No N = 33	**Yes N = 89**	**Total N = 122**
LVEF grade	Grade 0: 60% or higher	9 (27%)	9 (10%)	18 (15%)
	Grade I: LVEF < 60 - 50%	20 (61%)	41 (46%)	61 (50%)
	Grade II: LVEF < 50 - 40%	4 (12%)	33 (37%)	37 (30%)
	Grade III: LVEF: < 40 - 20%		6 (7%)	6 (5%)
Decrease in LVEF: lower than 50 percent absolute	Total	4 (12.1%)	39 (43.8%)	43 (35.2%)
Decrease in LVEF percent absolute AND decrease in LVEF percent relative	Total	3 (9.1%)	16 (18.0%)	19 (15.6%)

Abbreviation: LVEF, left ventricular ejection fraction

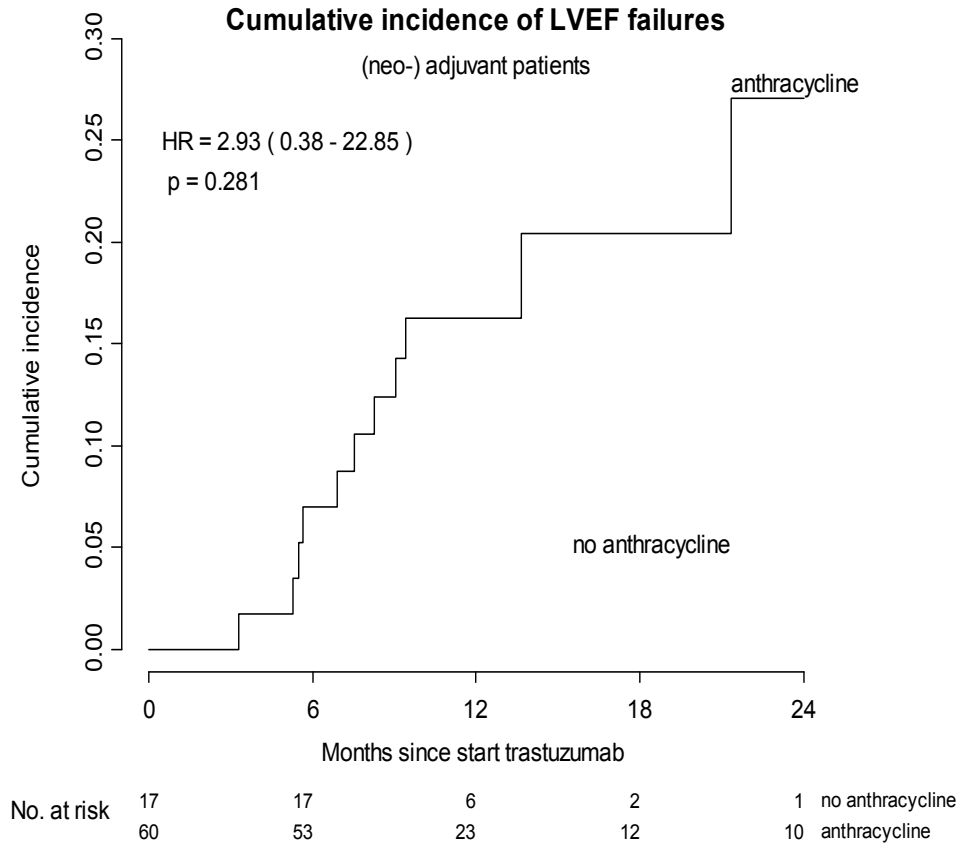


Figure 1. Incidence of LVEF failures in patients treated in the (neo) adjuvant setting of breast cancer

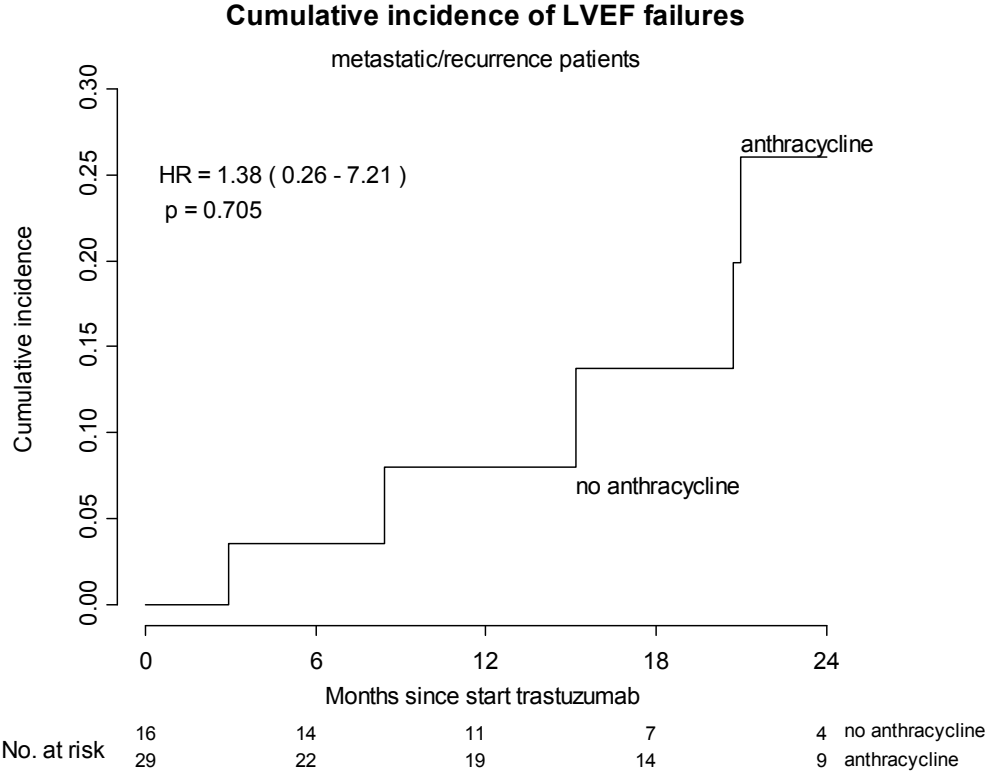


Figure 2. Incidence of LVEF failures in patients treated in the metastatic setting of breast cancer

Table 4. Cardiac events in the (neo)adjuvant treatment setting and in the metastatic treatment setting

		(neo) adjuvant N = 77	Metastatic recurrence N = 45	Total N = 122
LVEF grade	Grade 0: 60% or higher	9 (12%)	9 (20%)	18 (15%)
	Grade I: LVEF < 60 - 50%	38 (49%)	23 (51%)	61 (50%)
	Grade II: LVEF < 50 - 40%	26 (34%)	11 (24%)	37 (30%)
	Grade III: LVEF: < 40 - 20%	4 (5%)	2 (4%)	6 (5%)
Decrease in LVEF: lower than 50 percent absolute	Total	30 (39.0%)	13 (28.9%)	43(35.2%)
Decrease in LVEF percent absolute AND decrease in LVEF percent relative	Total	12 (15.6%)	7 (15.6%)	19 (15.6%)

Abbreviation: LVEF, left ventricular ejection fraction

Symptomatic cardiotoxicity

One patient, a thirty-two years old woman with primary breast cancer, experienced CHF confirmed by a cardiologist, after six cycles of FE90C and eleven cycles of trastuzumab. The LVEF value was 31% at the time of the presentation of symptoms of CHF. Two (1.6%) patients experienced non-cancer treatment related CHF. Both patients experienced atrial fibrillation, in one patient induced by hyperthyroidism. Two patients (1.6%) experienced symptomatic cardiotoxicity likely related to diastolic dysfunction, with normal systolic left ventricular function, confirmed by a cardiologist. One patient, experienced symptoms probably related but not evidently related to cardiotoxicity. In summary in one patient (0.8%) there was evidence for symptomatic cardiotoxicity induced by the sequence of anthracycline-based chemotherapy and trastuzumab treatment. This patient started with an ACE inhibitor and diuretics at the time of the presentation of signs and symptoms of CHF. After start of this medication and discontinuation of trastuzumab treatment the symptoms of CHF disappeared and the LVEF value increased.

Discontinuation of trastuzumab treatment

Thirteen (14.6%) patients, in the anthracycline-based treatment group and three (9%) patients in the group without anthracycline-based chemotherapy stopped with trastuzumab because of a cardiac event. Fourteen (18%) patients, in the (neo)adjuvant treatment group, stopped with trastuzumab permanently because of a cardiac event.

Effect of CHF medication in LVEF value

During trastuzumab treatment, fifteen patients started with standard CHF medication because of a decrease in LVEF. No statistically significant difference was seen in the reversibility of cardiac events after start of standard CHF medication.

Reversibility of LVEF values by stopping trastuzumab temporarily or permanently

In, in total twelve (9.8%) patients trastuzumab therapy was discontinued because of a decrease in LVEF. In nine (75%) patients, LVEF values during an interruption of trastuzumab

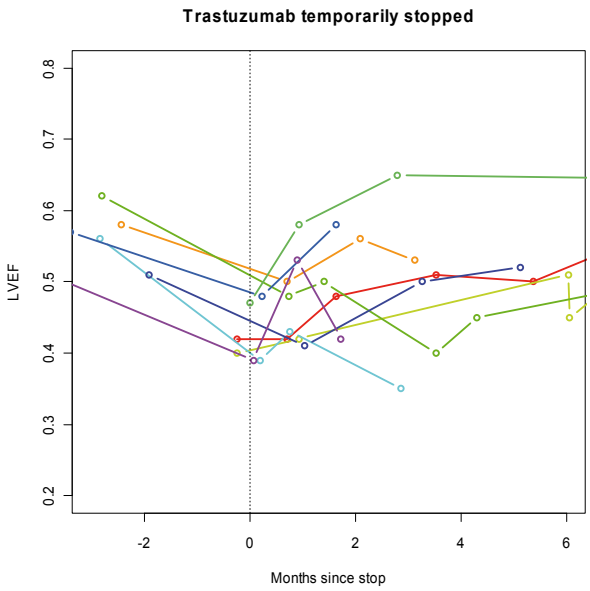


Figure 3. Reversibility of LVEF values by stopping trastuzumab treatment temporarily

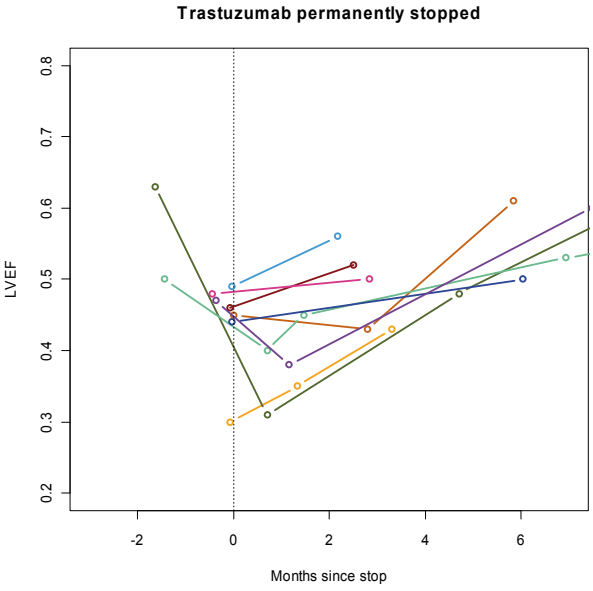


Figure 4. Reversibility of LVEF values by stopping trastuzumab treatment permanently

treatment were evaluable, Figure 3. The mean LVEF was 0.43 (SD 0.04) before interruption and 0.50 (SD 0.07) after interruption.
Sixteen (13%) patients stopped with trastuzumab permanently because of a cardiac event. In nine (56%) patients, LVEF values after discontinuation of trastuzumab treatment were evaluable, Figure 4. The mean LVEF was 0.42 (SD 0.07) before stopping and 0.46 (SD 0.07) after stopping of trastuzumab treatment. In two patients (22%) the LVEF value further decreased after discontinuation of trastuzumab treatment for 2.5 months. In both groups an association was seen in the reversibility of LVEF values after discontinuation of trastuzumab.

Discussion

Data were retrospectively collected to evaluate cardiotoxicity in patients treated with trastuzumab for HER2 positive primary or advanced breast cancer in a daily care patient population. In this analysis patients were pooled independent of the treatment setting, primary or advanced breast cancer. Additional analyses were conducted to describe possible differences in the (neo)adjuvant - and in the metastatic setting.

After a median follow up of 12.5 months, 18% of the patients in the anthracycline-based treatment group experienced asymptomatic cardiotoxicity versus 9.1% of the patients without prior or concomitant anthracycline-based chemotherapy. These data are based on the first measured LVEF drop during trastuzumab treatment. In analyzing the data of the current study we must conclude that the combination of anthracycline-based chemotherapy and trastuzumab induces a higher incidence of cardiac events than previously reported in the large trials. We also found that the severity of cardiotoxicity was significantly higher in the group with prior or concomitant anthracycline-based chemotherapy. In this respect

these results do not differ greatly from earlier reports (4, 15, 16). In a retrospective analysis of 152 trastuzumab treated patients with primary breast cancer, 24% of the patients developed trastuzumab induced cardiotoxicity. In this Canadian study all patients were treated with prior anthracycline-based chemotherapy. In our study, we analysed and compared trastuzumab-associated cardiotoxicity in patients treated with and without prior anthracycline-based chemotherapy in a European standard patient population.

Ewer proposed the use of the terms type I and type II chemotherapy-related cardiotoxicity. Type I is associated with the use of anthracyclines and defined as the destruction of myocytes whereas type II is associated with a loss of contractability (11). Our and other studies reveal that trastuzumab exacerbates the anthracycline induced cardiotoxicity.

The observed 18 percent of asymptomatic cardiotoxicity was lower than the 27 percent of cardiac events reported in the phase III trial in patients with metastatic breast cancer when trastuzumab was given concurrently with anthracyclines (5). However, it substantially exceeded the incidence of cardiotoxicity observed in the HERA and N9831 adjuvant trastuzumab trials (5, 8, 17). However, the indirect comparison of cardiac events between patients treated with trastuzumab in our study and in cited clinical studies is hampered by differences in the applied treatments, in inclusion and exclusion criteria and in the definition of cardiotoxicity. All adjuvant trials strictly excluded patients with baseline LVEF values lower than 50% (NSABP B-31 and N9831 trial) (7-10) or lower than 55% (HERA trial) (8). Almost half of the number of the cardiac events observed in the N9831 and B31 trials occurred in patients whose post-chemotherapy LVEF value was 50%-54%. A higher percentage of cardiotoxicity in our study versus the HERA trial has been attributed to the lower baseline LVEF values at the start of trastuzumab treatment. The baseline LVEF value can be a major determinant of the incidence of cardiac events according to the applied definition of cardiotoxicity. Another factor that might explain the observed differences in the incidence of cardiotoxicity is the definition of asymptomatic cardiotoxicity. Trastuzumab trials defined the number of cardiac events based on at least one significant LVEF drop or on repeated LVEF values and a significant drop in LVEF of 5, 10 or 15 percentage points from baseline or to below 50% or 55% (8, 9).

Comparing the incidence of symptomatic cardiotoxicity in our study and in cited clinical studies, the incidence in our study (0.8%) is lower than in the HERA trial (2.1%), the N9831 trial (3.3%) and in the NSABP B-31 (4.1%). In our study, the symptoms had to be confirmed by a cardiologist, which could result in an underestimation of symptomatic cardiotoxicity.

In our study known risk factors of inducing cardiotoxicity were not statistically significantly associated with the risk of a cardiac event. However, we currently further study such relationships in a cohort of patients who have been followed long-term after discontinuation of trastuzumab to substantiate these findings.

Another factor is the type of anthracycline used, epirubicin or doxorubicin. There are suggestions that epirubicin reduces the risk and severity of cardiotoxicity but based on the currently available data, no conclusion can be drawn (18). In this study data suggest that the type of anthracycline derivatives was associated with the incidence and severity of

cardiac events. However, the association between the type of anthracycline analogue and the incidence of cardiac events might also be the result of the difference in time-period between the last doxorubicin administration versus the last epirubicin administration and start of trastuzumab treatment.

The time interval between the end of the administration of anthracyclines and the start of trastuzumab treatment may influence the incidence and severity of cardiotoxicity. The median time interval between the last administration of anthracycline-based chemotherapy and the start of trastuzumab treatment is 21 days in the NSABP B-31 trial, 89 days in the HERA trial and 45 days in the (neo) adjuvant treatment group in our study. The shorter time-interval in the primary breast cancer group between anthracyclines and trastuzumab treatment may influence the higher incidence of cardiotoxicity in our study compared to the HERA trial.

Another potential risk factor associated with the development of therapy induced cardiotoxicity is the time of radiotherapy in relation to trastuzumab treatment. In our study no relation is seen in the incidence of cardiotoxicity and the use of trastuzumab concurrently with radiotherapy. At present it is not clear if there is an association between these treatments (19). More reported information about the cardiotoxicity profile induced by concurrent trastuzumab and radiotherapy is needed before drawing definitive conclusions about the safety of this approach.

In our study population an association was seen in the reversibility of the LVEF values after discontinuation of trastuzumab treatment. Data suggest that trastuzumab induced cardiotoxicity is reversible when trastuzumab is discontinued or CHF is treated (6, 20, 21).

However, 2 or 22% of patients show a reduced level of the LVEF after discontinuation of trastuzumab and our ongoing long-term follow study may unravel the clinical implications of that observation.

Premature discontinuation of trastuzumab treatment for a cardiac event occurred in 18% of the patients in the (neo) adjuvant treatment group. In contrast with the HERA study were 5.1% of the study patients discontinued trastuzumab treatment because of cardiac disorders (22). In clinical trials, in a fraction of the patients trastuzumab treatment was stopped before completion of the study treatment.

However, the percentage in an unselected patient population substantially exceeded the percentage of premature discontinuation in clinical trials.

In conclusion, high incidence of cardiac events during and after trastuzumab treatment, especially, in the combination of anthracycline-based chemotherapy and trastuzumab treatment was observed in our daily-life patient population. Reversibility was not complete in 22% of patients after discontinuation of trastuzumab for 2.5 months. Although this retrospective study can lead to an overestimation of the incidence of cardiotoxicity, these data presents the implications of trastuzumab treatment in a daily-life patient population that may suffer from significant co-morbidity and using co-medication. Based on our study results, trastuzumab induced cardiotoxicity must be weighed as a relevant clinical problem, with greater importance than would be anticipated based on data of clinical trials. Longer follow-up of large cohorts and more prospective data will be needed to extend the evaluation

of cardiotoxicity. A decrease in the morbidity and mortality of trastuzumab treatment will be needed to enable optimal therapy, without premature discontinuation in HER2 positive breast cancer patients.

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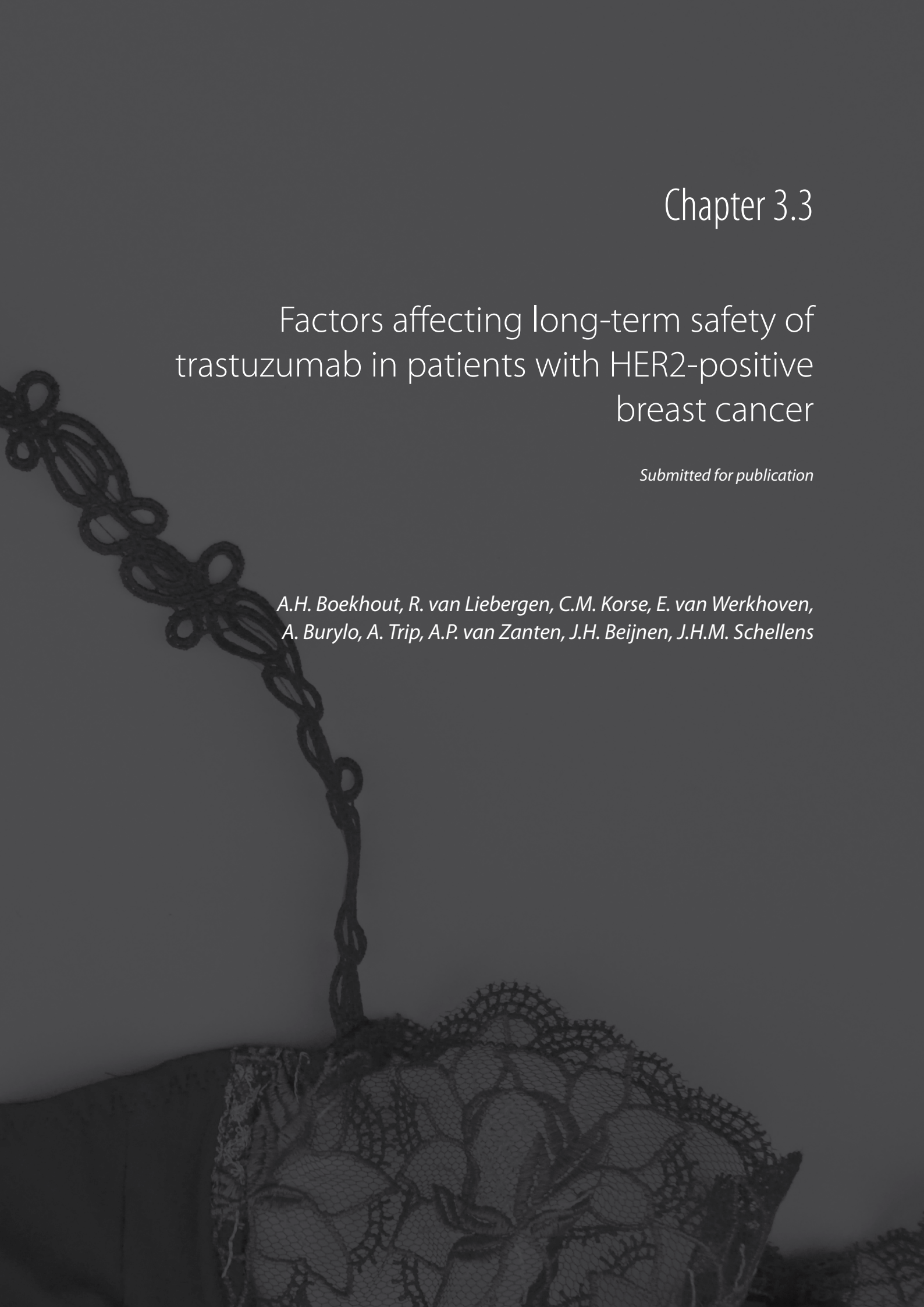

Chapter 3.3

Factors affecting long-term safety of trastuzumab in patients with HER2-positive breast cancer

Submitted for publication

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Abstract

Purpose

Trastuzumab treatment is associated with cardiac dysfunction, which usually is manifested by an asymptomatic decline in left ventricular ejection fraction (LVEF), and sometimes even in symptomatic congestive heart failure (CHF). We evaluated cardiac safety during and long-term after trastuzumab treatment in an unselected HER2 positive primary breast cancer population.

Methods

The study consisted of a retrospective review of the time-period of trastuzumab treatment and prospective data collection long-term after the end of trastuzumab treatment.
Cardiac events were defined as a decrease of 10 percentage points in LVEF compared with baseline and to an absolute LVEF of below 50%.

Results

In total 105 patients were included in this study. Eight patients developed a cardiac event. All patients with a cardiac event were pre-treated with anthracyclines and represented 12% of all anthracyclin pretreated patients. Of these eight patients, the LVEF value did not recover in one patient and recovered partially or completely in seven patients. Patients who recovered were re-treated with trastuzumab. After re-treatment the LVEF values were below 50% or lower than baseline LVEF in two patients long-term after trastuzumab treatment.
In this study population, changes in LVEF values were statistically significantly associated with changes in B-type natriuretic peptide (NT-proBNP) values (p=0.011). No statistically significant association was observed with single nucleotide polymorphisms (SNPs) in the *HER2* gene and the risk of a cardiac event.

Conclusion

Trastuzumab treatment in combination with anthracyclines is associated with significant and only partially reversible cardiotoxicity. Longer follow-up studies will be needed to evaluate long-term cardiac safety and whether cardiac markers and SNPs might be useful parameters in the prediction or detection of trastuzumab-related cardiotoxicity.

Introduction

Trastuzumab (Herceptin®) is a humanized monoclonal antibody that targets the extracellular domain of the human epidermal growth factor HER2 receptor protein and has resulted in clinical benefit in HER2 positive advanced breast cancer and improved disease-free and overall survival in HER2 positive primary breast cancer (1-3). Trastuzumab is generally well tolerated and not associated with adverse events that are commonly seen with chemotherapy. However, cardiac dysfunction is an important side-effect that can result in asymptomatic decline in left ventricular ejection fraction (LVEF) and, in a small proportion of patients, even in symptomatic congestive heart failure (CHF) (4, 5).

Trastuzumab-associated cardiac dysfunction is likely related to the HER2 receptor involvement in the physiology and pathophysiology of heart muscle contractility (6, 7). A long-term cardiac tolerability study of 173 HER2 positive advanced breast cancer patients, treated with trastuzumab as monotherapy or in combination with chemotherapy, presented an overall incidence of cardiac dysfunction of 28% after a median follow-up of 2.8 years demonstrated by reduction in the LVEF of ≥ 20 percentage points compared with baseline, decrease of LVEF to < 50%, or signs and symptoms of CHF (8). Symptoms of CHF and asymptomatic declines in LVEF values were reversible in the majority of the patients upon discontinuation and/or treatment with ACE inhibitors or other supporting medication.

Recently, long-term cardiac safety data of three large adjuvant trastuzumab trials, employing symptomatic CHF events, cardiac death or decline in LVEF value as study endpoints, were presented with a median follow-up of up to 3.6 years after discontinuation of trastuzumab (9, 10). Results of these trials suggest that trastuzumab-associated cardiac dysfunction has a low incidence rate, especially symptomatic heart failure, and a high rate of reversibility with appropriate treatment, usually consisting of ACE-inhibitors, beta-blockers or diuretics and/or discontinuation of trastuzumab treatment. However, clinical trials adhere to strict eligibility criteria and patients treated in clinical trials are fit and often not representative of an unselected patient population.

Preliminary data suggest that troponin I might be a parameter to predict trastuzumab-induced cardiotoxicity by identifying patients who are at risk of development of cardiac dysfunction and who will recovery from asymptomatic cardiotoxicity (11). However, more evidence is needed before this parameter can be applied as routine screening measurement for trastuzumab-related cardiotoxicity. It is of interest to explore relationships between troponin I, but also other markers of cardiac function, especially NT-proBNP, and trastuzumab-induced cardiotoxicity in the daily-life patient population treated with trastuzumab.

Several single nucleotide polymorphisms (SNPs) in the extracellular, transmembrane and intracellular region of HER2, have been studied to examine the impact of these polymorphisms on disease outcome and on trastuzumab-related toxicity (12-15). In a prospective clinical study of 61 advanced breast cancer patients, the presence of the Val655Ile polymorphism was found to be a risk factor for trastuzumab-related cardiotoxicity (16). However, confirmation in larger studies is needed.

In this study we recruited long-term breast cancer survivors after standard one-year adjuvant trastuzumab treatment, to determine long-term tolerantly, cardiac safety, and long-term effects of treatment of CHF and, parameters to detect or predict trastuzumab-

induced cardiotoxicity. We also analyzed the variability in the *HER2* gene for identification of potential genetic factors predisposing patients to the development of trastuzumab-related cardiotoxicity.

Patients and methods

Patients

Women with strongly HER2-positive breast cancer who had received (neo) adjuvant trastuzumab treatment were eligible. HER2-positive breast cancer was defined as an immunohistochemistry score of 3+ in the HercepTest ®and/or gene amplification by fluorescence in situ hybridization (FISH), or chromogenic in situ hybridization (CISH). Other criteria for inclusion were: age 18 years or older; available LVEF values at baseline (i.e. before trastuzumab treatment); and at least one LVEF value during the trastuzumab treatment period and written informed consent to participate in the study. Participants were excluded if they had advanced breast cancer; or were pregnant or breast feeding.

Trial design and procedures

This study explored the tolerability and safety during and long-term after trastuzumab treatment. Patients were recruited at the Netherlands Cancer Institute – Antoni van Leeuwenhoek Hospital (NKI-AVL). From May 2005 all trastuzumab treated patients who had received and finished trastuzumab as (neo) adjuvant treatment were identified through medical records and were informed of this study by letter. The study was approved by the medical ethics committee of the NKI-AVL. After patients had given written informed consent, they were registered at the Trial Office of the Data Center of the Netherlands Cancer Institute (DC-NKI). This study included a retrospective part, the chemotherapy – and trastuzumab treatment period and a prospective part, i.e. the period of data collection long-term after the end of trastuzumab treatment. Medical records were reviewed for all demographic and treatment related data including type, dose and duration of chemotherapy and endocrine therapy, side, dose and field of radiotherapy, dose, schedule and duration of trastuzumab treatment, reason of temporary or definitive trastuzumab discontinuation and the development of signs and symptoms of CHF. The prospective part of this study consisted of data collection on two predefined time-points. The first was immediately after informed consent procedure and the second was one year later. During the clinical visits the following data were recorded: medical history, including risk factors for cardiac disease, such as hypertension, diabetes type I and II, family history of hypercholesterolemia, other familiar predisposition for cardiac disease, New York Heart Association classification (NYHA), smoking status, current use of medication, complementary alternative medicine (CAM)-use, history of co-medication, as oral contraceptives and hormone replacement therapy. Physical examination was performed and vital signs (blood pressure, heart rate), performance status, body weight and height were determined at these occasions. An evaluation of cardiac abnormalities was performed by a 12-lead electrocardiogram (ECG) and an identification of the left ventricular ejection fraction by MUGA scan, or echocardiography. For MUGA scans, 400 MBq Tc-99m labelled autologous red blood cells were injected and acquisition was done

in 6 min with a large-field-of-view gamma camera with a low energy all-purpose parallel-hole collimator. An independent cardiologist reviewed the ECGs of all eligible patients. The ECGs were reviewed for heart rate, QRS time, QT time and QTc. The QT time was corrected for heart rate (QTc) according to the method of Basett. A QTc time of more than 440 msec was considered prolonged.

Laboratory measurements

Laboratory tests at the laboratory of Clinical chemistry of the NKI were performed and included hemoglobin, hematocrit, white blood cell count, platelet count, serum creatinine, serum sodium, potassium, calcium, thyroid stimulating hormone, glucose, cholesterol, low-density lipoprotein cholesterol (LDL-C), high-density lipoprotein cholesterol (HDL-C), triglycerides, bilirubin, alkaline phosphatase, ASAT, ALAT, LDH, albumin, troponin T (cTnT), troponin T high sensitivity (cTnThs) and the B-type natriuretic peptide (NT-proBNP). NT-proBNP values were measured in samples taken at baseline and long-term after trastuzumab treatment. cTnT was measured long-term after trastuzumab treatment. Troponin I (cTnI) and cTnThs and levels were measured in serum samples taken at baseline, i.e. prior to anthraycline-based chemotherapy and trastuzumab treatment. cTnI levels were analyzed at the Department of Clinical chemistry of the Slotervaart Hospital. We analyzed the cardiac markers, cTnT and cTnI because they are interchangeable in clinical practice (17). We analyzed cTnT and cTnThs because troponin T was detectable in 92% of the patients with CHF with a high- sensitivity assay, compared with a standard assay were troponin T was detectable in 10% of the patients with CHF (18).

Genotyping

One blood sample was taken for genotype analysis of the *HER2* gene. *HER2* genotyping was performed at the Department of Experimental therapy of the NKI. In this study we determined genetic variability in the extracellular domain; FcγRIIIa-158 valine (V)/phenylalanine (F), FcγRIIIa-131 histidine (H)/arginine (R) and FcγRIIIa-232 isoleucine (I)threonine (T), in the transmembrane domain; Val654Ile, Val655Ile and in the intracellular domain; P1170A. Genomic DNA was isolated from whole blood samples using QIAamp® DNA Mini Kit (Qiagen Benelux B.V., The Netherlands) and amplified by PCR reaction in a PTC-200 thermocycler (MJ Research, Inc., Waltham, MA, USA). DNA sequencing was carried out for HER2 I654V, I655V, FcγR2B I232T, and FcγR3A F158V. First, amplified DNA samples were purified using ExoSAP-IT® (USB, Germany). Secondly, purified DNA sample, DNA Sequencing Kit Big Dye TerminatorTM Cycle sequencing mix v3.1 (Applied Biosystem, Foster City, CA, USA), and a specific primer (forward or reverse, same as PCR primer) were mixed in a final volume of 20 µl, the reaction was performed in a PTC-200 thermocycler. Analysis of the DNA sequence reaction product was carried out on an Applied Biosystem 3730/3730xl DNA analyzer (Applied Biosystem, Foster City, CA, USA).

The allelic discrimination analysis was performed for HER2 A1170P and FcγR2A H166A according to the manufacturer's protocol (Applied Biosystems, Foster City, USA). The assay contained Taqman® Universal PCR Fast Master Mix No AmpErase® UNG (2x), genotyping assay mix (40x) (SNP specific unlabeled PCR primers and TaqMan® MGB probes [FAM™ and VIC® dye-labeled]), and a sample DNA.

Haplotype analysis was performed for HER2 I654V, I655V and A1170P SNPs.
The used DNA primers, PCR conditions and genotyping assay details are listed in Table 1.

Table 1. PCR, sequencing and genotyping assay

SNP	Primer Probe	PCR and sequencing primers, 5'→3' Taqman® SNP genotyping primers and probes, 5'→3'	PCR conditions
I654V (rs1801201) I655V (rs1801200)	Fwd	CCTTTCCGAATGCCAAACA	95°C 9min, 95°C 1min, 63°C 1min, 72°C 1min, 39 cycles, 72°C 10min
	Rev	CGCCTCACCTCCGTTTCCT	
I232T (rs1050501)	Fwd	CTAAGAGGAGCCCTTCCCTATGT	95°C 5min, 94°C 30sec, 54°C 45sec, 72°C 1min, 35 cycles, 72°C 7min
	Rev	AATACGGGCCTAGATCTGAATGTG	
F158V (rs396991)	Fwd	AGCTGGAAGAACACTGCTCTGCA	95°C 5min, 94°C 30sec, 55.7°C 30sec, 72°C 30sec, 45 cycles, 72°C 7min
	Rev	AACTCAACTTCCCAGTGTGAT	
A1170P (rs1058808)	Fwd	CCTGCTGGTGCCACTCT	Manufacturer's Fast RT-PCR settings, Applied Biosystems
	Rev	CGTCTTTGACGACCCCATTCTT	
	Probe	AGTCTTGG**G**CCTTTC (VIC)(mutant)	
	Probe	AGTCTTGG**C**CCTTTC (FAM)(wild type)	
H166A (rs1801274)	Fwd	CTGGTCAAGGTCACATTCTTCCA	Manufacturer's Fast RT-PCR settings, Applied Biosystems
	Rev	GCTTGTGGGATGGAGAAGGT	
	Probe	CAGAAATTCTCCC**A**TTTGGA (VIC)(wild type)	
	probe	AGAAATTCTCCC**G**TTTGGA (FAM) (mutant)	

Abbreviation: Polymerase chain reaction (PCR)

Biochemical Measurements of Cardiac markers

A volume of 10 ml of whole blood was drawn and collected in EDTA tubes according of standard practice. Blood samples were analyzed for cTnT, cTnThs, cTnI and NT-proBNP. cTnT levels were assessed by the CARDIAC T immunoassay on the Cobas h232 instrument with an Upper Limit of Normal (ULN) of 0.05 µg/l and limit of detection (LoD) of 0.03 µg/L. Serum cTnThs levels were assessed by the Troponin T electrochemiluminescence immunoassay (ECLIA) on the Cobas 601 analyzer with an ULN of 0.05 µg/L and LoD of 0.003 µg/L (Roche Diagnostics GmbH, Mannheim, Germany). Serum cTnI levels were assessed by the ADVIA Centaur using the chemiluminometric technology with an ULN of 0.04 µg/L and LoD of 0.006 µg/L (Siemens Healthcare Diagnostics, Tarrytown, NY, USA). ULN of the normal healthy population for the troponin assays were set at the 99th percentile (19, 20). Serum NT-proBNP levels were assessed by ECLIA on the Cobas 601 analyzer with an ULN of 18 pmol/L and LoD of 6 pmol/l (Roche Diagnostics GmbH, Mannheim, Germany).

Statistical methodology

The primary endpoint of the study was the change in left ventricular ejection fraction (LVEF) value before, during and long-term after trastuzumab treatment. A sample size of 100 patients was needed to provide about 85% power to detect an effect size of 0.3 with a two tailed paired t-test at a significance level of 0.05. Baseline was the date of start of trastuzumab treatment. The following patient characteristics at baseline were considered as risk factors for the development of cardiotoxicity: age; body mass index (BMI); history of hypertension; cardiac disease; pulmonary disease (COPD, asthma); history of hyperlipidemia; type 1 or type 2 diabetes mellitus; smoking (current or previous: time-period of discontinuation); use of co-medication. The possible relationship between the following treatment characteristics and the development of cardiotoxicity were evaluated: cumulative time, dose, schedule and duration of trastuzumab treatment; type, (cumulative) dose and duration of chemotherapeutic treatment and; type, dose and duration of endocrine therapy; dose, side (left, right or both) and field of radiotherapy and the time-period between anthracycline therapy and the first administration of trastuzumab.

Data were analyzed in two different treatment groups: patients with and those without anthracycline-based chemotherapy prior to or concomitant with trastuzumab treatment. Demographic and treatment characteristics were compared between the groups with the Chi-square or Fisher's exact test for qualitative variables and the Krusskall Wallis, Mann-Whitney or t-test for continuous variables. LVEF values at baseline, during treatment and in follow-up were compared with the t-test. Incidence-curves of asymptomatic cardiac events were made using the Kaplan-Meier method. Associations with parameters were estimated with Cox proportional hazards models and logrank tests. To measure the association between NT-proBNP values, genotype analyses, troponines and changes in LVEF values Spearman coefficients were calculated. Reported P-values were two-tailed and an alpha level of 0.05 was used to assess statistical significance. Analyses were performed with the use of SPSS or SAS software, version 9.1 and R version 2.9.0 (21).

Cardiac evaluation

Cardiac evaluation included LVEF values assessed by multiple-gated acquisition scanning (MUGA), or echocardiography and an evaluation of symptomatic cardiotoxicity. An asymptomatic cardiac event was defined as follows: decrease of 10 percentage points in LVEF compared with baseline and to an absolute value of below 50%. Complete recovery of asymptomatic cardiotoxicity was defined as a LVEF value equal to the baseline LVEF value with a margin of 5 absolute points. A partial recovery of asymptomatic cardiotoxicity was defined if the patient had two or more sequential LVEF values that did not meet the definition of an asymptomatic cardiac event with an LVEF value of 50% or greater.

Symptomatic cardiotoxicity was defined as: clinical signs and symptoms of congestive heart failure. A judgement of the degree of the patient's recovery was recorded and based on the clinical findings, cardiac medication used and LVEF recovery. Complete recovery of symptomatic cardiotoxicity was defined as the disappearance of clinical findings and symptoms. A partial recovery of symptomatic cardiotoxicity was defined by continued but less severe signs and symptoms.

Results

Patient characteristics and treatments

From May 2005 170 patients had received and finished trastuzumab treatment. Forty-three patients were not eligible for this study based on the following criteria: 10 patients had developed distance metastasis, 8 patients were treated in another hospital, 3 patients were excluded as no baseline LVEF value was available, and 23 patients were on treatment in other adjuvant clinical trials. In total 127 patients were informed of this study and 108 patients (85%) responded to participate. A total of 105 patients were eligible for this study. One patient was not evaluable for analysis of the primary endpoint because there was no long-term LVEF measurement. In total 66 patients received anthracycline-based chemotherapy prior to or concomitant with trastuzumab treatment and 39 patients received trastuzumab treatment without anthracycline-based chemotherapy. The median age was 50 years; range 31-71 years. The median follow-up was 36 months; range 13-56 months. The median follow-up was longer (41 months) in the anthracycline-based treatment group compared with the group patients without anthracycline-based chemotherapy (28 months) ($p< 0.001$).

The median cumulative trastuzumab dose was 6835 mg; range 1400-11500 mg. The cumulative trastuzumab dose in patients who experienced a cardiac event was statistically significantly lower than in patients without a cardiac event, $p=0.003$, and was 3315 mg compared with 7010 mg respectively. The median time interval between the last anthracycline infusion and start of trastuzumab treatment was 21 days; range 12-1694 days. In six patients (9%) epirubicin was prescribed concomitant with trastuzumab treatment. Demographic and treatment characteristics are shown in Table 2.

Cardiac Events

The mean difference between the most recent LVEF value before trastuzumab treatment and the LVEF value measured long term after trastuzumab treatment was -1.7 points, $p=0.005$. Eight patients experienced a decrease in LVEF of at least 10 percentage points compared to baseline and to an absolute LVEF of below 50%. These patients represented 7.6% of the total cohort of 105 patients and as they all had been pre-treated with an anthracycline they represented 12.1% of the latter group. The Kaplan-Meier estimated 3-year incidence of events was 12% (95% CI, 4 - 20%).

Anthracycline-based chemotherapy was statistically significantly associated with the incidence of cardiac events ($p=0.026$). The number of cardiac events is shown in Figure 1.

Ten patients (9.5%) experienced a grade II cardiac toxicity (LVEF range, 40-50%) and another two patients (1.9%) a grade III toxicity (LVEF range, 20-40%) during trastuzumab treatment. The severity of cardiac toxicity was related with anthracycline-based chemotherapy ($p<0.003$).

One patient, a 55 year old woman, experienced a myocardial infarction two years after the adjuvant systemic therapy with anthracycline-based chemotherapy and trastuzumab treatment. In this patient the following risk factors for the development of cardiotoxicity were available: medical history of hypertension and hyperlipidemia, previous smoker (38 pack years) and a BMI >25 at start of trastuzumab treatment. The severity of cardiac events by treatment group is shown in Table 3.

Table 2. Patient Characteristics by anthracycline-based treatment group and by Cardiac Event group*

		No anthracyclines		Anthracyclines		Anthracyclines without Cardiac Event		Anthracyclines with Cardiac Event	
		N=39	%	N=66	%	N=58	%	N=8	%
Age	Median (range)	50 (32-71)		50 (31-68)		51.5 (31-68)		44.5 (38-59)	
Follow-up in months	Median (range)	28 (13-46)		41 (13-58)		41 (13-55)		35 (18-49)	
LVEF at baseline	Median (range)	67 (53-84)		64 (38-80)		64 (38-80)		60 (55-72)	
Smoker	Non-smoker	12	30.8	20	30.3	17	29.3	3	37.5
	Smoker	9	23.1	6	9.1	4	6.9	2	25
	Former smoker	17	43.6	40	60.6	37	63.8	3	37.5
	Pack years	7 (0-91)		12 (0-44)		12 (0-44)		12 (4-39)	
	Missing	1	2.6	0	0	0	0	0	0
Medical Cardiac History	Hyperlipidemia	1	2.6	11	16.7	11	19	0	0
	Hypertension	10	25.6	13	19.7	12	20.7	1	12.5
	Cardiovascular	3	7.7	4	6	4	6.9	0	0
	Diabetes Mellitus type I	0	0	0	0	0	0	0	0
	Diabetes Mellitus type II	0	0	3	4.5	3	5.2	0	0
	Chronic Obstructive Pulmonary Disease	0	0	0	0	0	0	0	0
	Asthma	4	10.3	4	6	4	6.9	0	0
Other significant Medical history	(ulcerative colitis; sickle cell anemia, melanoma, cervix carcinoma, lymphoma, idiopathic thrombocyto-penia, sarcoidosis, colon carcinoma)	2	5.1	6	9.1	6	10.3	2	25
	Breast cancer	0	0	8	11.8	7	12.1	1	12.5
Family History	First members of cardiovascular disease by age < 60 years	7	17.9	20	30	18	31	2	25
Co-medication at baseline	Any cardiac co-medication at baseline	5	13.2	11	16.2	10	17.2	1	12.5
	ACE inhibitors	0	0	3	4.5	3	5.2	0	0
	Statins	0	0	5	7.6	5	8.6	0	0
	Anti-thrombotic therapy	1	2.6	2	3.0	2	3.4	0	0

		No anthracyclines		Anthracyclines		Anthracyclines without Cardiac Event		Anthracyclines with Cardiac Event	
	Diuretics	2	5.1	5	7.6	5	8.6	1	12.5
	Beta blockers	3	7.7	1	1.5	1	1.7	0	0
	Angiotensin blockers	2	5.1	3	4.5	3	5.2	0	0
	Calcium antagonists	0	0	0	0	0	0	0	0
Type of anthracycline derivates	Epirubicin	0	0	9	13.6	8	13.8	1	12.5
	Doxorubicin	0	0	57	86.4	50	86.2	7	87.5
Cumulative dose in milligram	Anthracyclines	0	0	436 (100-754)		436 (100-754)		430 (379-575)	
	Epirubicin	0	0	550 (400-754)		550 (400-754)		420	
	Doxorubicin	0	0	432 (100-738)		432 (100-738)		440 (379-575)	
Interval between anthracyclines and trastuzumab	Days	0	0	21 (12-1694)		21 (12-1694)		21 (14-43)	
Concurrent epirubicin and trastuzumab		0	0	6	9.1	5	8.6	1	12.5
Trastuzumab total dose	Milligram	7085 (3756-11500)		7010 (1400-11500)		6777 (1400-10464)		3315 (1690-6308)	
Resean for trastuzumab interruption	Cardiac related	4	10.3	15	22.7	11	19	4	50
Stop reason of trastuzumab treatment	According treatment plan	36	92.3	49	74.2	48	82.8	1	12.5
	Cardiac related	3	7.7	13	19.7	6	10.3	7	87.5
Endocrine therapy	Total	16	41	34	51.5	29	50	5	62.5
	Aromatase inhibitor	8	20.5	33	50	28	48.3	5	62.5
	Estrogen receptor down regulators	8	20.5	2	3	2	3.4	0	0
	LHRH agonists	3	7.7	8	12.1	5	8.6	3	37.5

*Patient characteristics overall by treatment group and by anthracycline treatment with or without Cardiac Event

Abbreviations: Congestive Heart Failure (CHF); Left ventricular ejection fraction (LVEF)

Table 3. Severity of cardiotoxicity and Cardiac Events

		No anthracyclines N=39		Anthracyclines N=66		P values
Cardiac toxicity during trastuzumab treatment according CTC NCI – CTC	Grade 0: 60% or higher	22	56.4	15	22.7	0.003
	Grade I: LVEF < 60 - 50%	16	41	40	60.7	
	Grade II: LVEF < 50 - 40%	1	2.6	9	13.6	
	Grade III: LVEF: < 40 - 20%	0	0	2	3	
Cardiac toxicity long-term after trastuzumab treatment according CTC NCI – CTC	Grade 0: 60% or higher	32	82	45	68	0.044
	Grade I: LVEF < 60 - 50%	6	15.4	17	26	
	Grade II: LVEF < 50 - 40%	0	0	3	4.5	
	Grade III: LVEF: < 40 - 20%	0	0	1	1.5	
	Missing	1	2.6	0	0	
Cardiac events after systemic therapy	Myocardial infarction	0	0	1	1.5	

Abbreviations: Left ventricular ejection fraction (LVEF); National Cancer Institute Common Toxicity Criteria for Adverse Events, version 3.0 (CTC NCI – CTC)

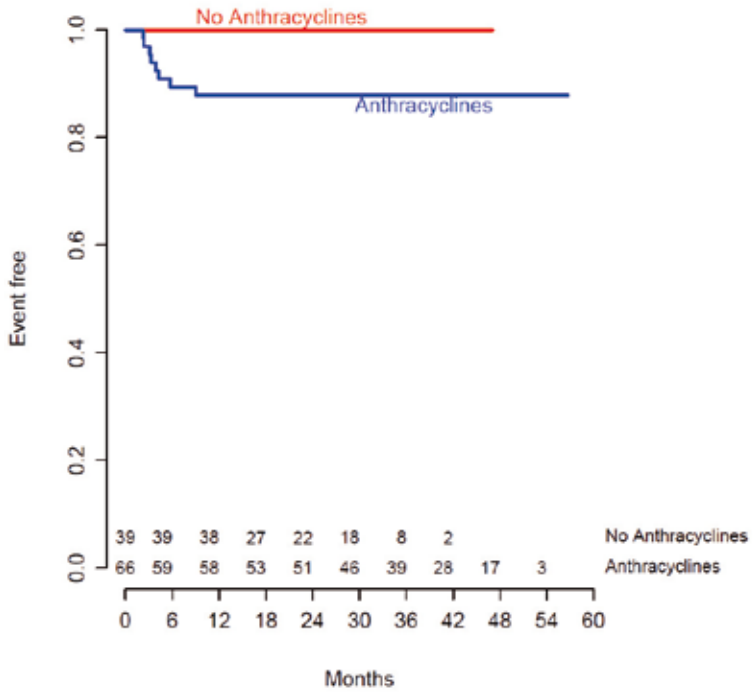


Figure 1. Number of Cardiac Events by treatment group (p=0.026)

Cardiac Events and patient characteristics

The incidence of cardiac events was not statistically significantly associated with potential risk factors at baseline for the development of cardiotoxicity. Table 4 shows the estimated hazard ratios of a cardiac event for potential risk factors.

Table 4. Hazard Ratios for Cardiac Event

Variable	Events		No	Hazerd Ratio	95% CI	P values
Age at start of trastuzumab	8		105	0.945	0.87 to 1.02	0.16
Age, years	31-50	5	48	0.50	0.12 to 2.09	0.34
	50-71	3	57			
Body Mass Index	8		104	0.85	0.69 to 1.06	0.14
Baseline LVEF value	8		105	0.93	0.86 to 1.01	0.08
History of hypertension	No	7	81	0.50	0.06 to 4.05	0.52
	Yes	1	23			
Doxorubicin exposure	No	1	46	5.70	0.70 to 46.26	0.10
	Yes	7	59			
Epirubicin exposure	No	7	98	2.05	0.25 to 16.63	0.50
	Yes	1	7			
Cumulative anthracycline dose, mg	8		66	1.00	0.99 to 1.01	0.82
Concomitant anthracyclines and trastuzumab	No	7	60	1.45	0.18 to 11.77	0.73
	Yes	1	6			
Paclitaxel treatment	No	1	11	0.80	0.10 to 6.57	0.84
	Yes	7	94			
Previous Radiotherapy	No	3	19	0.34	0.08 to 1.43	0.14
	Yes	5	86			
Time to the last anthracycline administration, days	7		60	0.98	0.93 to 1.04	0.55

Abbreviation: Left ventricular ejection fraction (LVEF)

Reversibility of cardiac events and severe asymptomatic systolic dysfunction

In four patients (3.8%) the LVEF was below 50% (grade II cardiac toxicity) which in one patient (1.5%) the LVEF value was 31% (grade III cardiac toxicity) long-term after trastuzumab treatment. Among the two patients who experienced a grade III cardiac toxicity during trastuzumab treatment, the LVEF value in one patient recovered partially after trastuzumab discontinuation and start of cardiac medication (ACE-inhibitors, beta-blockers and diuretics) and was completely recovered at long term; and in one other patient the LVEF value did not recover despite medical treatment, even not three years after discontinuation of trastuzumab treatment. At that moment the LVEF value was 31%. Among the eight patients who experienced a cardiac event, the LVEF values in one patient (12.5%) recovered partially,

in another patient (12.5%) the LVEF value did not recover and in six patients (75%) the LVEF values recovered completely based on the applied definition of recovery. Patients who recovered completely were re-treated with trastuzumab. After re-treatment the LVEF values were below 50% or lower than baseline LVEF in two patients long-term after trastuzumab treatment.

Signs and symptoms of CHF and recovery

Four patients (3.8%) experienced signs and symptoms of CHF during trastuzumab treatment; these patients were previously treated with anthracyclines. Among these patients with symptomatic cardiotoxicity, one patient discontinued trastuzumab treatment temporarily and three patients discontinued trastuzumab treatment permanently. Two patients recovered completely after trastuzumab discontinuation. Two other patients recovered partially, and symptoms of CHF were less severe one year after discontinuation of trastuzumab treatment. These patients were suffering from symptoms as dyspnoea d'effort, palpitations, fatigue and edema of the ankle long term after trastuzumab treatment. Cardiac medication (ACE-inhibitors) was used by one of these two patients. Thirteen patients (12.5%) were suffering from physical symptoms such as dyspnea d'effort, palpitations, fatigue and ankle edema long-term after trastuzumab treatment. However, these symptoms were not evidently related to cardiotoxicity.

Trastuzumab discontinuation because of cardiac events

Of the 105 women, 19 patients (18.1%) discontinued trastuzumab treatment temporarily and 16 patients (15.2%) discontinued trastuzumab permanently because of a cardiac event. Premature discontinuation of trastuzumab treatment was not statistically significantly associated with prior anthracycline-based chemotherapy (p=0.16).

Cardiac Markers: troponines and NT-proBNP

Eighty-five patients were available for analyses of the cardiac markers cTnI, cTnThs and NT-proBNP. These patients presented 81% of the total cohort of 105 patients. One hundred-four patients were available for analysis of the cTnT and NT-proBNP values long-term after trastuzumab treatment. These patients presented 99% of the total study population. In 84 out of the 85 patients (98.8%) in which cTnI was measured the cTnI values were below the LoD at baseline. An elevated cTnI value at baseline was found (1.2%; 0.209 µg/L) in one patient. This patient experienced a decrease in LVEF of 15 percentage points compared to baseline and to an absolute value of below 50%. The LVEF value had not recovered after two years of follow-up. Elevated cTnThs values were not found at baseline and elevated cTnT values were not found at long-term. The troponine values at baseline and long-term were not statistically significantly associated with changes in LVEF values or with cardiac events.
In this patient population (n=85), changes in LVEF values were statistically significantly associated with changes in NT-proBNP values (Spearman's rho = -0.275, p=0.011). Furthermore, in patients with anthracycline-based chemotherapy (n=55), changes in LVEF values were statistically significantly associated with changes in NT-proBNP values
(rho = -0.439, p=0.001). However, in patients without prior anthracyclines (n=30), the NT-proBNP values were not statistically significantly associated with changes in LVEF values. The

Table 5. Cardiac markers at baseline

	No anthracyclines N=30	Anthracyclines without cardiac event N=48	Cardiac Events N=7
NT-proBNP pmol/L Median (range)	7 (5-23)	5.5 (5-36)	5 (5-11)
Troponin I ug/L Median (range)	≤0.006 (≤0.006 -0.022)	≤0.006 (≤0.006-≤0.006)	≤0.006 (≤0.006 -0.209)
Troponin T (high sensitivity) ug/L Median (range)	≤0.003 (≤0.003 -0.005)	≤0.003 (≤0.003-≤0.003)	≤0.003 (≤0.003 -0.021)

No significant differences between any of the groups at baseline were observed

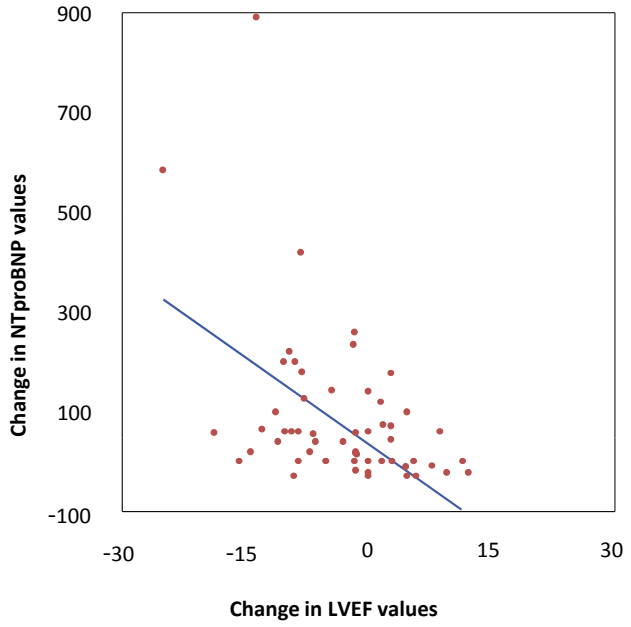


Figure 2. Scatter plot with changes in LVEF values and changes in NT-proBNP values in the anthracycline-based treatment group (rho =-0.439, p=0.001)

baseline values of these cardiac markers are presented in Table 5 and a scatter plot of LVEF values and changes in NT-proBNP values are presented in Figure 2.

Genotyping

We analyzed the six SNPs in 105 patients of this study population. No statistically significant association was observed with the risk of a cardiac event or with changes in LVEF values. Results of the genotype analyses are shown in Table 6 and Figure 3. Preliminary haplotype analysis did not show significant associations between the studied *Her2* genetic variability and the risk of a cardiac event.

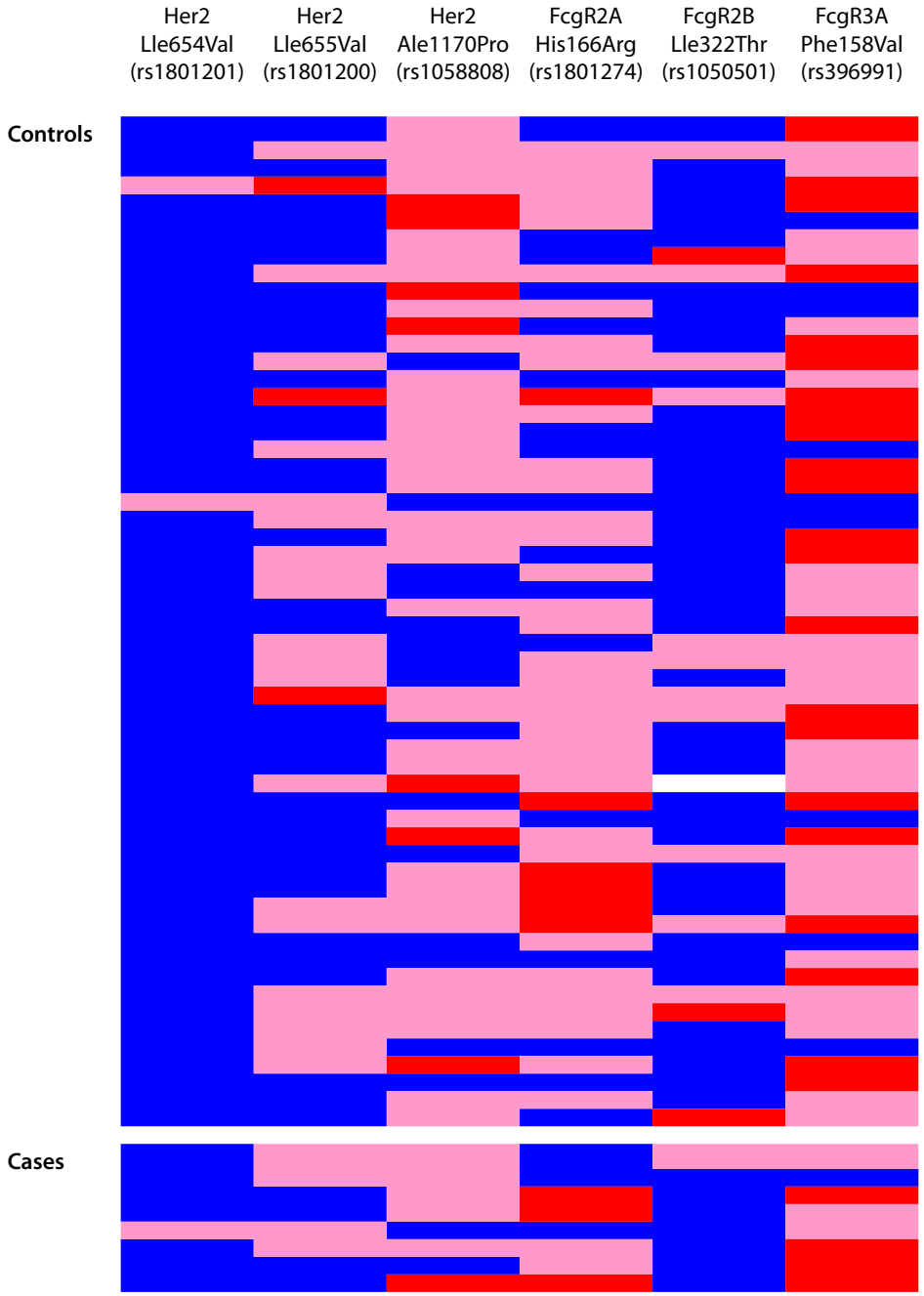
Electrocardiogram

In three patients there were ECG changes in comparison with the baseline ECG findings and in two of these patients the QTc time was ≥ 440 msec long-term after trastuzumab. These ECG findings were likely related with the adjuvant systemic treatment. These patients had been treated with anthracyclines and trastuzumab.

Physical examination, vital signs and laboratory

Potential risk factors long-term after trastuzumab treatment were tested by laboratory parameters and measurements of vital signs. Patients with elevated TSH, glucose, triglycerides, cholesterol, or LDL-C, HDL-C blood values or an elevated blood pressure (according to the

Figure 3. Genotyping anthracyclines without cardiac events (controls) and anthracyclines with cardiac events (cases)



Blue= wild-type, pink=heterozygeous, red=homozygeous, white =missing

Dutch guidelines of general practitioners) were referred to their general practitioners for follow-up measures, or treatment. In two patients the addition at diagnostic measurements were performed because of elevated serum values of transaminases. However, overall no statistically significant association between cardiac events and laboratory values or measurements of vital signs at long-term were found.Results of the measurements in the prospective part of our study are presented in Table 7.

Table 6. SNPs and risk of Cardiac Events

SNP	Genotype	Controls		Cardiac Event		OR	P-value
		n	%	n	%		Chi-square
Prior anthracyclines		**57**		**8**			
Her2lle654Val	Wild-type	55	98.2	6	75.0	1.00	0.09
	Heterozygous	2	3.6	1	12.5	3.06	
	Homozygous	0	0	0	0	n.a.	
Her2 lle655Val	Wild-type	34	58.9	4	50.0	1.00	0.68
	Heterozygous	20	35.7	4	50.0	1.7	
	Homozygous	3	5.4	0	0	n.a.	
Her2 Ala1170Pro	Wild-type	15	26.8	2	25.0	1.00	0.85
	Heterozygous	35	60.7	5	62.5	1.07	
	Homozygous	7	12.5	1	12.5	1.07	
FigR2A His166Arg	Wild-type	17	30.4	3	37.5	1.00	0.27
	Heterozygous	34	58.9	2	25.0	0.33	
	Homozygous	6	10.7	3	37.5	2.83	
FigR2B lie232Thr	Wild-type	44	78.6	7	87.5	1.00	0.68
	Heterozygous	10	17.9	1	12.5	0.63	
	Homozygous	2	3.6	0	0	n.a.	
	Missing	1	1.8	0	0	n.a	
FigR3A Phe158Val	Wild-type	9	16.1	1	12.5	1.00	0.90
	Heterozygous	25	44.6	3	37.5	1.08	
	Homozygous	22	39.3	4	40	1.64	
	Missing	1	1.8	0	0	n.a	
No prior anthracyclines		**39**		**0**			
Her2lle654Val	Wild-type	39	100	0	0	n.a.	
	Heterozygous	0	0	0	0	n.a.	
	Homozygous	0	0	0	0	n.a.	
Her2 lle655Val	Wild-type	16	41.0	0	0	n.a.	
	Heterozygous	19	48.7	0	0	n.a.	
	Homozygous	4	10.3	0	0	n.a.	
Her2 Ala1170Pro	Wild-type	19	48.7	0	0	n.a.	
	Heterozygous	17	43.6	0	0	n.a.	
	Homozygous	3	7.7	0	0	n.a.	
FigR2A His166Arg	Wild-type	10	25.6	0	0	n.a.	
	Heterozygous	17	43.6	0	0	n.a.	
	Homozygous	12	30.8	0	0	n.a.	
FigR2B lie232Thr	Wild-type	27	69.2	0	0	n.a.	
	Heterozygous	7	17.9	0	0	n.a.	
	Homozygous	3	7.7	0	0	n.a.	
	Missing	2	5.1	0	0	n.a	
FigR3A Phe158Val	Wild-type	4	10.3	0	0	n.a.	
	Heterozygous	18	41.0	0	0	n.a.	
	Homozygous	16	46.2	0	0	n.a.	
	Missing	1	2.6	0	0	n.a.	

Abbreviation: single nucleotide polymorphisms (SNPs); not applicable (n.a.)

Table 7. Long-term follow-up data

		No N=39		Yes N=58		Cardiac Events N=8	
Performance status	WHO: 0	25	64.1	44	76	7	87.5
	WHO: 1	11	28.2	13	22.4	0	0
	WHO: 2	2	5.1	1	1.6	1	12.5
	Missing	1	2.6	0	0	0	0
NYHA	1	34	87.2	51	87.9	7	87.5
	2	4	10.2	7	12.1	1	12.5
	Missing	1	2.6	0	0	0	0
Blood pressure mmHg	Systolic	115 (90-180)		1157 (95-155)		110 (105-135)	
	Diastolic	75 (60-100)		75 (55-90)		75 (70-80)	
	Missing	1	2.6	0	0	0	0
Pulse	Per minute	68 (50-88)		70 (52-100)		71 (60-100)	
Current use of co-medication	ACE-inhibitor	2	5.1	5	8.8	1	12.5
	Treatment of hyperlipidemia	1	2.6	6	10.3	0	0
	Antiocoagulantia	2	5.1	3	5.2	0	0
	Diuretics	4	10.3	6	10.3	2	25
	Beta-blocker	2	5.1	5	8.6	1	12.5
	Angiotensin blockers	1	2.6	4	6.9	1	12.5
	Calcium antagonist	1	2.6	1	1.6	0	0
	Missing	1	2.6	0	0	0	0
Laboratory	Cholesterol	5.6 (3.6-7.1)		5.8 (3.8-9.1)		5.8 (4.4-6.9)	
	LDL-Cholesterol	3.2 (1.7-4.8)		3.45 (1.9-6.9)		3.15 (2.6-4.6)	
	HDL-Cholesterol	1.75 (1-2.5)		1.65 (0.6-3.4)		1.75 (1.1-2.4)	
	Triglycerides	1.2 (0.5-3.7)		1.2 (0.5-4.8)		0.75 (0.5-1.7)	
	TSH	1.7 (0.3-10.5)		1.8 (0.3-14.7)		0.9 (0.8-4.6)	
	NT-proBNP pmol/L	7.5 (5-30)		10 (5-126)		11.5 (6-41)	
	Troponin T ug/L	0.02 (0.02-0.02)		0.02 (0.02-0.02) u		0.02 (0.02-0.02)	
	Bilirubin	7 (3-83)		6 (3-66)		6 (4-12)	
	Alkaline phosphatase	85 (48-145)		81 (50-246)		78 (50-111)	
	ASAT	22 (14-32)		22 (12-133)		22 (19-31)	
	ALAT	18 (7-56)		19 (7-165)		18 (14-38)	
	LDH	179 (109-234)		176 (119-1011)		161 (145-187)	
	Sodium	141 (132-149)		141 (14.2-143)		140 (137-143)	
	Potassium	4.6 (3.7-5.4)		4.6 (3.7-5.4)		4.6 (4.1-4.7)	
	Glucose	5.1(3.4-7.3)		5.3 (3.2-11.9)		5.7 (4.5-7.2)	
	Albumin	46 (40-50)		46 (41-50)		47 (43-49)	
	Calcium	2.4 (2.19-2.69)		2.4 (2.15-2.60)		2.38 (2.2-2.46)	
	Serum Creatinine	65 (50-104)		65.5 (46-102)		60 (50-70)	

Abbreviations: high-density lipoprotein cholesterol (HDL-Cholesterol); low-density lipoprotein cholesterol (LDL-Cholesterol); New York Heart Association Classification (NYHA); thyroid stimulating hormone (TSH)

Discussion

Cardiac dysfunction is an important side-effect of trastuzumab treatment. After a median follow up of 36 months, the incidence of cardiac events was 12% and 3.8% of the patients experienced signs and symptoms of CHF. All occurrences of asymptomatic cardiac events and CHF manifested during trastuzumab treatment and all of these patients had been treated with anthracyclines. The results of this trial are in accordance with earlier trials in which both drugs, anthracyclines and trastuzumab, have been studied. Anthracyclines are associated with the risk of dose-related cardiac dysfunction, defined as a destruction of myocytes, and investigators recognize structural abnormalities on myocardial biopsies in anthracycline-treated patients (22, 23). Trastuzumab-related cardiotoxicity is associated with a loss of contractility of myocytes and has been reported in patients who received trastuzumab as monotherapy or in combination with chemotherapy. Clinical data suggested a higher incidence of cardiac dysfunction if both drugs were used concurrently or sequentially (2). The exact causal synergistic mechanism of these drugs remains unclear, but investigators suggest that anthracyclines induce myocardial oxidative stress whereas trastuzumab blocks the HER2 receptor and initiation of cell survival pathways that modulate myocyte damage as a result of oxidative stress and myocyte repair (14, 24, 25). The prevalence of trastuzumab-related cardiac dysfunction varies widely between studies and an indirect comparison between our study and earlier clinical studies is hampered by differences in the applied treatments, in inclusion and exclusion criteria and the applied definition of cardiotoxicity (4, 5, 9, 10). However, based on the findings presented here we may conclude that trastuzumab-associated cardiotoxicity is related with the prior exposure to anthracyclines.

The European HERA trial, in which 94.1% of patients had been treated with anthracyclines, found an incidence of severe CHF and left ventricular (LV) dysfunction of 0.8% and 9.8%, respectively, after a median follow-up of 3.6 years (9). These results are comparable with data from patients who had received anthracyclines in this study. After a median follow-up of 3.6 year in the HERA trial and a median follow-up of 3 year on this study, the incidence of LV dysfunction remained at 9.8% and 12%, respectively. The higher rate of asymptomatic cardiotoxicity in this study compared with the HERA trial might be explained by the lower baseline LVEF values. In the HERA trial patients with a LVEF value ≥ 55% were eligible, while in our study all trastuzumab treated patients were included, even patients with a baseline LVEF value of below 55%. Low baseline LVEF values might be a potential independent predictor for trastuzumab-induced cardiotoxicity (5, 26, 27). A comparison of the prevalence of CHF in both studies is hampered by differences of the applied definition of CHF.
Among the eight patients who developed a cardiac event, in six patients the LVEF values recovered completely. Of these patients, two patients were re-treated with trastuzumab. After re-treatment the LVEF values were below 50% or lower than baseline LVEF in two patients long-term after trastuzumab treatment. Based on these findings, strict cardiac monitoring even long-term after trastuzumab treatment might be recommended and longer follow-up will be needed.

Clinical evidence suggests that clinical detection of cardiotoxicity is best accomplished via sequential measurements of LVEF by MUGA or echocardiography, although actual cardiac damage may not be detectable by measurements of the LVEF, which can result in an underestimation of trastuzumab-induced cardiotoxicity (28). Therefore, there is a need for prospective validation of specific and sensitive markers in the prediction of trastuzumab-induced cardiotoxicity, which might be useful to identify high-risk patients before a decrease in LVEF or heart failure becomes manifest. Serum biomarkers such as NT-proBNP and the troponines are sensitive cardiac markers in the detection of acute myocardial stress or injury and might be parameters in the detection or prediction of trastuzumab-induced cardiotoxicity. In our study we found a statistically significant association between changes in NT-proBNP values and changes in LVEF values in the total patient population ($p=0.011$) and in the anthracycline-based treatment group ($p=0.001$). Several clinical trials have reported a relationship between an increase of NT-proBNP values and anthracycline-induced cardiotoxicity. A study by Sandri et al. reported that patients with increased NT-proBNP values developed more severe cardiotoxicity, one year after high-dose chemotherapy, than patients with stable NT-proBNP values (9, 29). In a study of 40 primary breast cancer patients, increased NT-proBNP values were not associated with changes in LVEF values, while in another small clinical study of 14 advanced breast cancer patients, pre-treatment NT-proBNP levels were higher in patients who developed CHF during trastuzumab treatment than those without CHF ($p=0.009$) (30).

The value of NT-proBNP as a marker for the development of cardiotoxicity is still a matter of debate; however, also other serum markers have been clinically evaluated. In a prospective study of 251 trastuzumab treated patients, cTnI levels were assessed before, after each trastuzumab cycle, every three months during the first year after trastuzumab discontinuation, and every six months thereafter. These values were related to the development of cardiotoxicity. Cardiotoxicity occurred more frequently in patients with elevated cTnI levels prior to trastuzumab treatment (i.e. after chemotherapy with or without anthracyclines) or during trastuzumab treatment ($p<0.001$) and recovery of cardiotoxicity was less likely (11). In contrast with the study of Cardinale et al., only one patient had an elevated cTnI prior to anthracycline treatment. In this patient baseline cTnI levels were related to trastuzumab-associated cardiotoxicity and absence of recovery. Differences between our results and the study of Cardinale et al. might be explained by differences in timing of blood sample collection. In our study baseline blood samples for cTnI and cTnThs measurement were collected prior to anthracycline treatment, while in the study of Cardinale et al. blood was collected after anthracycline- but prior to trastuzumab treatment. More clinical evidence is needed to establish the value of NT-proBNP, cTnI or cTnT(hs) as markers for the development of trastuzumab-induced cardiotoxicity. Differences in timing of blood sampling might be critical in detection of cardiotoxicity and should be taken into account in the design of future clinical studies.

Polymorphisms of the *Her2* gene seem to be related to the functionality of the receptor and the development of cardiotoxicity in trastuzumab treated patients. In a study of 61 advanced breast cancer patients, the presence of the Ill655Val alle was found to be a risk factor for trastuzumab-related cardiotoxicity (16). All cases of cardiotoxicity defined as a $\geq$ 20% reduction in LVEF, were found in the Ile/Val group, while there was no cardiotoxicity in the Val/Val or Ile/

Ile group of patients. Since there is clinical evidence that genetic variability in the *HER2* gene might affect trastuzumab-related cardiotoxicity, we analysed the prevalence of six SNPs in 105 trastuzumab treated breast cancer patients. The Ile655Val SNP was included in our panel and also FcγRIIIa-158 valine (V)/phenylalanine (F), FcγRIIIa-131 histidine (H)/arginine (R), FcγRIIIa-232 isoleucine (I)threonine (T), Val654Ile and P1170A. There are no previous reports on the possible role of the latter five SNPs in the development of CHF during trastuzumab treatment. Moreover, also in patients with a Val allele at codon 655 no relationship with cardiac toxicity was found. Although we observed no significant relationship between these SNPs and trastuzumab-associated cardiotoxicity, the retrospective nature of our study and the limited number of cardiac events might have resulted in an underestimation of these SNPs as predisposing factor for trastuzumab-induced cardiotoxicity.

A drawback of a retrospective study design is the possible bias of patient selection. Selection bias might have been introduced if patients had died early from trastuzumab-related toxicity, however upon review of patient files no early deaths were encountered, hence this possible selection bias was considered to be absent.

In conclusion, the severity and incidence of trastuzumab-related cardiotoxicity is associated with prior or concomitant anthracycline-based chemotherapy. Cardiac dysfunction is only partly reversible despite medical treatment. Based on currently presented data, more and longer follow-up studies are needed before the cardiac markers such as the troponines and NT-proBNP can be routinely applied as useful clinical measurements in the detection of trastuzumab-related cardiotoxicity. It is advisable to monitor patients strictly during trastuzumab treatment and in patients who developed cardiotoxicity during trastuzumab even after discontinuation of treatment.

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Chapter 3.4

Safety of trastuzumab treatment in patients with a low baseline Left Ventricular Ejection Fraction

Submitted for publication

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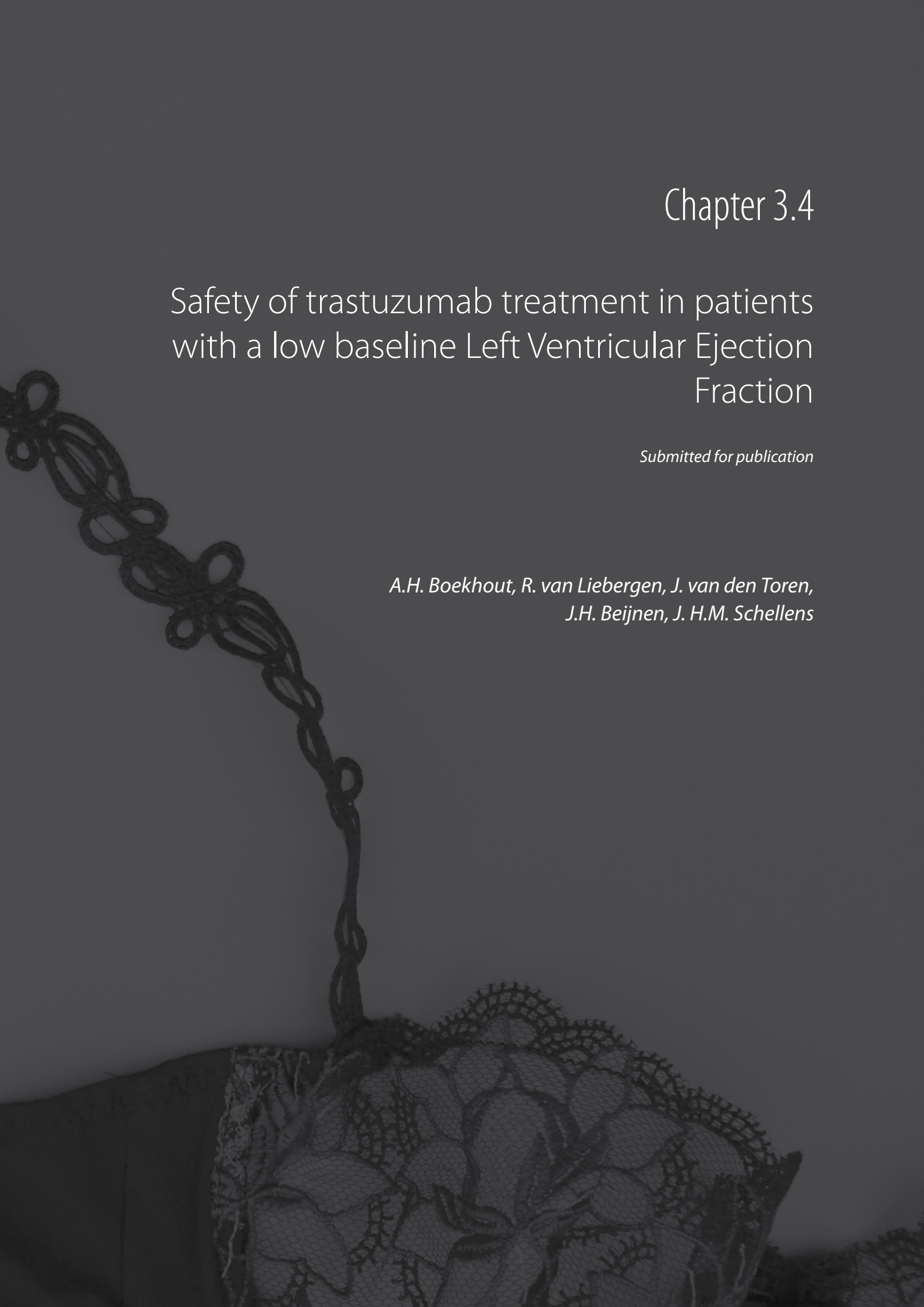

Abstract

Background

Trastuzumab treatment for early or advanced breast cancer may induce cardiac events. Safety of trastuzumab therapy at low baseline left ventricular ejection fraction (LVEF) value is unknown. Therefore, we evaluated the cardiac safety of trastuzumab treatment in patients with a baseline LVEF of < 50%.

Methods

Patients with a baseline LVEF below 50% prior to start of trastuzumab treatment were identified retrospectively. Cardiac toxicity was evaluated according to the National Cancer Institute Common Toxicity Criteria for Adverse Events. Cardiac events were defined as a decrease of 10 percentage points in LVEF compared with baseline.

Results

In total 11 breast cancer patients with a baseline LVEF < 50% were treated with trastuzumab. The median follow-up time was 9 months (range 2 to 52 months). Eight patients started trastuzumab treatment with a grade II decline in LVEF value (<50-40%) and three patients with a grade III decline in LVEF value (<40-20%). During treatment, four patients developed grade II (LVEF value 42-49%) and seven patients grade III cardiac toxicity (LVEF value 24-39%). Three patients experienced a cardiac event. LVEF values recovered partially after treatment discontinuation. There were no cardiac related deaths.

Conclusion

Patients with a low baseline LVEF of < 50% experienced significant and sometimes severe and only partially reversible cardiotoxicity based on LVEF measurement during trastuzumab treatment. No cardiac related deaths were reported. Clinical benefit and trastuzumab related cardiac toxicity should be carefully weighted in patients with a low baseline LVEF value before start of trastuzumab treatment.

Introduction

Trastuzumab (Herceptin ®) is a humanized monoclonal antibody against the extracellular domain of the HER2 transmembrane growth factor receptor that improves outcome in early and advanced HER2 positive breast cancer and advanced gastric cancer (1-5) Cardiotoxicity, however, is one of the side-effects of trastuzumab treatment; usually manifested by occurrence of asymptomatic declines in left ventricular ejection fraction (LVEF) or even congestive heart failure (CHF). The risk of a cardiac event, defined as a decrease in LVEF of 20 percentage points compared with baseline or a decrease in LVEF < 50% or signs and symptoms of CHF, was related with baseline LVEF values in a retrospective clinical trial (6). A higher incidence of cardiotoxicity in the National Surgical Adjuvant Breast and Bowel Project Trial B-31 (NSABP-B31), evaluating the use of trastuzumab in the adjuvant setting, in comparison with the European trial (HERA), has been attributed to the allowed lower baseline LVEF value (4, 7). In the NSABP-B31 trial a LVEF value > 50% and in the HERA trial a LVEF value ≥ 55% were eligibility criteria. For safety reasons all clinical trials strictly excluded patients with baseline LVEF values below 50%. Currently, guidelines derived from these trials are being followed in clinical practice, possibly resulting in exclusion of patients from receiving trastuzumab even though they may benefit from treatment, however at the cost of an uncertain risk of developing long-term cardiac side-effects. Therefore, data are needed about the cardiac safety of trastuzumab treatment in HER2 positive breast cancer patients with a baseline LVEF value below 50%.

Materials and methods

Patients

Women treated with trastuzumab for HER2 positive early or advanced breast cancer with a baseline LVEF below 50% and treated at the Netherlands Cancer Institute between 2004 and 2010 were included in this study. Other criteria for inclusion were: available baseline LVEF value, at least one LVEF value during trastuzumab treatment; trastuzumab treatment for at least four weeks.

Methods and Statistical analysis

Medical records were reviewed for baseline patient and treatment characteristics. Baseline was start of trastuzumab treatment. The follow-up time was calculated from the start date of trastuzumab to the last LVEF measurement. The following parameters were registered: age; history of hypertension, cardiac disease, pulmonary disease (COPD, asthma); history of hyperlipidemia; type 1 or 2 diabetes mellitus; use of co-medication at baseline or during trastuzumab treatment such as angiotensin-converting enzyme (ACE) inhibitors; angiotensin blockers; diuretics; calcium antagonists and ß-blockers; cumulative time, dose and schedule of trastuzumab treatment; type and (cumulative) dose of anthracycline-based chemotherapy and the time-period between anthracycline-based chemotherapy and the first administration of trastuzumab.

Cardiac evaluation

Cardiac evaluation included LVEF measurements assessed by multiple-gated acquisition scanning (MUGA) and an evaluation of signs and symptoms of congestive heart failure (CHF). Asymptomatic cardiotoxicity was defined as: a decrease of 10 percentage points in LVEF compared with baseline based on the definition of the HERA trial (8). Complete recovery of asymptomatic cardiotoxicity was defined as a LVEF value equal to the baseline LVEF value with a margin of 5 absolute points. Partial recovery of asymptomatic cardiotoxicity was defined as an increase in LVEF but not equal to the baseline LVEF value. The severity of cardiac toxicity was graded according to the National Cancer Institute Common Toxicity Criteria for Adverse Events, version 3.0 (NCI-CTC). CHF was defined as reported signs and symptoms confirmed by a cardiologist. Complete recovery of symptomatic cardiotoxicity was defined as the disappearance of all clinical signs and symptoms.

Results

Baseline patient characteristics

In total 11 patients were eligible for this study. Two patients (18%) were treated for HER2 positive early breast cancer, three patients (27%) for locally advanced breast cancer, one patient (9%) for oligometastatic disease and five patients (46%) were treated for HER2 positive advanced breast cancer. Distant metastases were localized in bone (n=1), lung (n=2), brain (n=3), and liver (n=3). Median follow-up time was 9 months; range 2-52 months. Median age at the start of trastuzumab treatment was 55 years; range 32-68 years. Two patients (18%) had a history of hypertension; one patient (9%) was diagnosed with cardiac arrhythmia; one patient (9%) was diagnosed with cardiomyopathy and type 2 diabetes mellitus and one patient (9%) was diagnosed with signs and symptoms of CHF after anthracycline-based chemotherapy, prior to trastuzumab treatment. Four patients (36%) used co-medication at baseline; ACE-inhibitors, diuretics, beta-blockers and cardiac glycoside (digoxin). The median baseline LVEF was 45%; range 35-49%. Eight patients started trastuzumab treatment with a grade II decline in LVEF value (< 50-40%) and three patients with a grade III decline in LVEF value (<40-20%).

Treatment characteristics

Ten patients (91%) were treated with anthracycline-based chemotherapy prior to trastuzumab treatment. Five patients (46%) received epirubicin with a cumulative dose of 952 mg (range 725-980 mg) and five patients (46%) received doxorubicin with a cumulative dose of 560 mg (range 390-840 mg). Time interval between the last anthracycline infusion and trastuzumab treatment was 756 days; range 117-2228 days. Median time of trastuzumab treatment was 8 months; range 1-47 months. Median cumulative dose of trastuzumab was 4880 milligram; range 980-18010 milligram. Trastuzumab was given weekly, two-weekly or tri-weekly. Fifty-five percent of patients received concomitant vinorelbine, 27% paclitaxel and 18% capecitabine. Four patients (36%) started with co-medication during trastuzumab treatment; one of these patients started with an ACE-inhibitor one week after start of trastuzumab. Three patients (27%) started with co-medication because of a further decline

in LVEF; ACE-inhibitors, diurectics, beta-blockers or angiotensin blockers. Eight patients (73%) were also treated with endocrine therapy and six patients (55%) were treated with radiotherapy.

Cardiac Events and severity

Three patients (27%) experienced a cardiac event after a median time-period of trastuzumab treatment of 2 months (1 to 3 months). Four patients (36%) experienced grade II cardiac toxicity (LVEF value 42-49%) and seven patients (64%) grade III cardiac toxicity (LVEF value 24-39%) during trastuzumab treatment. Changes in LVEF from baseline in individual patients are shown in Figure 1.

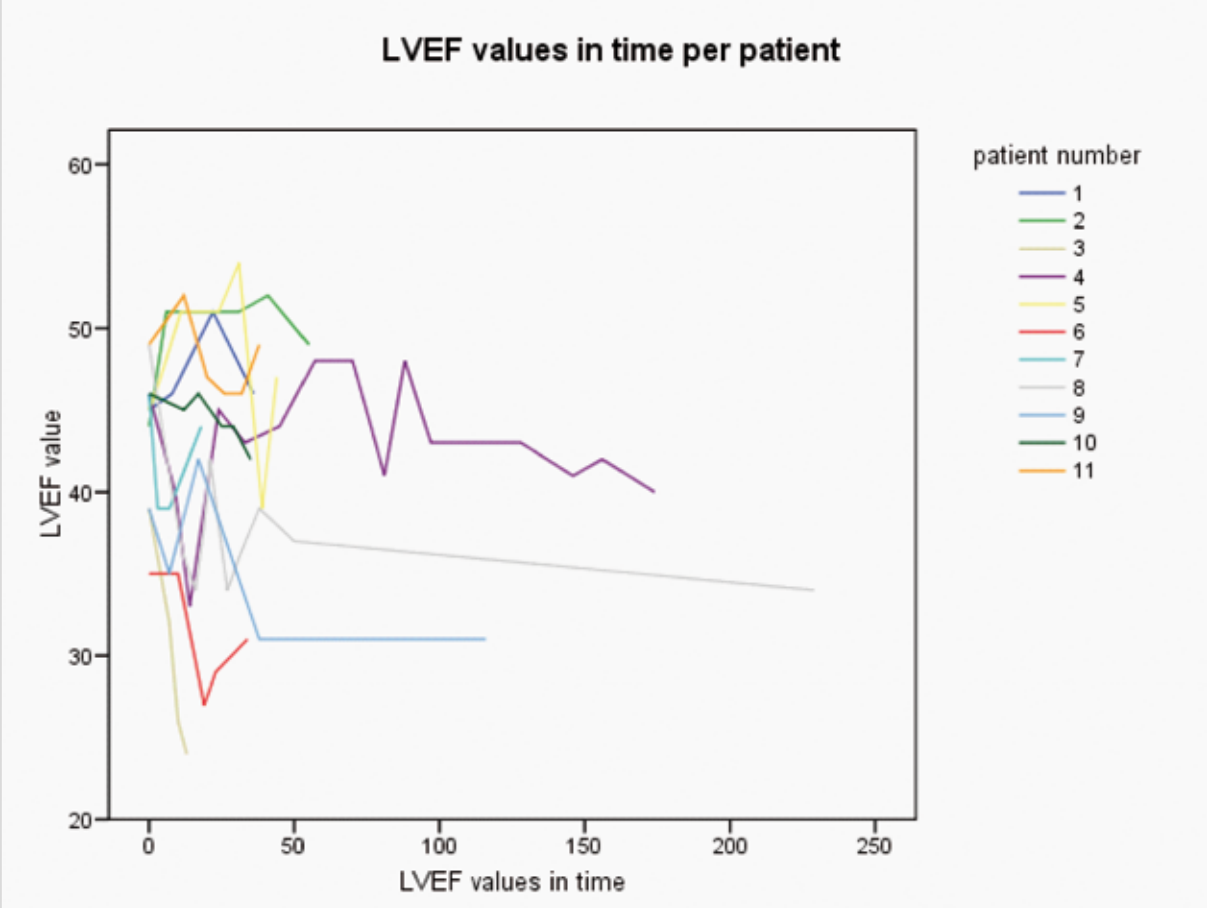

Figure 1. Changes in LVEF from baseline

Cardiac Reversibility

In four patients (36%), trastuzumab treatment was discontinued temporarily, of which two more than once, due to asymptomatic decline in LVEF. Of these patients, three recovered after discontinuation of trastuzumab and the addition of specific cardiac medication (ACE inhibitor, beta-blocker). One patient recovered after an interruption of trastuzumab treatment; this patient used already an ACE-inhibitor as co-medication at baseline. All these patients were re-treated with trastuzumab after on average interval of 18 weeks.

Of 11 patients with a baseline LVEF below 50%, three patients (27%) completed trastuzumab

treatment according to treatment guideline, in six patients trastuzumab treatment was discontinued prematurely, one patient (9%) was still on trastuzumab treatment at the time of this analysis and one patient (9%) was lost to follow-up (other hospital). Reasons for permanently discontinuation were progressive disease in two patients (18%) and an asymptomatic decline in LVEF in four patients (46%). Of these four patients, three patients did not recover with or without cardiac medication after a median follow-up time of 421 days; range 51-1397 days, in one patient no LVEF value was evaluable after discontinuing trastuzumab. At time of this analysis, five patients had died of progressive disease, one patient was lost to follow-up and five patients were alive (with or without anti-cancer treatment). No cardiac deaths were reported.

Discussion

Trastuzumab treatment is associated with a decline in LVEF and cardiac events especially in patients with prior anthracycline-based chemotherapy (9). Evidently, the small sample size of this study limits comparison with the large adjuvant clinical studies (4, 10). In contrast to these studies, in which only patients with a LVEF value within the normal range were included, we analyzed patients with a low baseline LVEF value who were treated with trastuzumab. In this special population we observed a high percentage of cardiac events and grade III cardiac toxicity. However, there were no cardiac deaths. Despite the long follow-up of close to four years in one patient, considering the median follow-up of slightly more than one year in the total population of this retrospective study long term cardiac toxicity is still uncertain in these patients.

The LVEF in three patients recovered partially after an interruption of trastuzumab of on average 21 weeks and start of co-medication. Also in the large randomized trials up to 86% of the patients reached partial or complete recovery after discontinuation of trastuzumab treatment (8, 11). ACE-inhibitors are standard of care in patients with CHF. In patients with an anthracycline-induced cardiotoxicity, ACE-inhibitors can ameliorate symptoms of CHF and prevent a decline in LVEF (12-14). The majority of patients that developed symptomatic cardiotoxicity during trastuzumab treatment responded well to standard medical management for CHF (11). However, no evidence is available that treatment with ACE-inhibitors to prevent or treat trastuzumab-associated cardiotoxicity is effective on the long-term.

Although, all the cardiac events were reported as asymptomatic cardiotoxicity, this patient population may suffer from significant clinical signs and symptoms. In this study LVEF was used as intermediate measure of outcome and only signs and symptoms that were confirmed by a cardiologist were reported as symptomatic cardiotoxicity. This could have resulted in underestimation of the severity of trastuzumab-induced cardiotoxicity in this patient population.

In conclusion, in indirect comparison with trastuzumab treated patients with early or advanced breast cancer and a baseline LVEF value above 50%, cardiac events are more common and cardiotoxicity is more severe in patients with a decreased LVEF at start of treatment with trastuzumab. Nonetheless, all observed cardiac events were considered asymptomatic and

there were no cardiac related deaths. Since a proportion of patients might benefit from trastuzumab treatment, patients should be informed about the potential advantages and disadvantages of treatment. The decision to treat should be weighed against the safety risk. The adjuvant or palliative setting of trastuzumab treatment will be critical in this evaluation. Based on current data, treatment of patients with trastuzumab and a low baseline LVEF appears to be feasible, however referral to a cardiologist and strict monitoring of cardiac function are recommended. Longer follow-up of larger cohorts and more prospective data will be needed for a balanced benefit/risk assessment of trastuzumab in patients with a low baseline LVEF value.

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Chapter 3.5

Population pharmacodynamic analysis of trastuzumab-related cardiotoxicity

Submitted for publication

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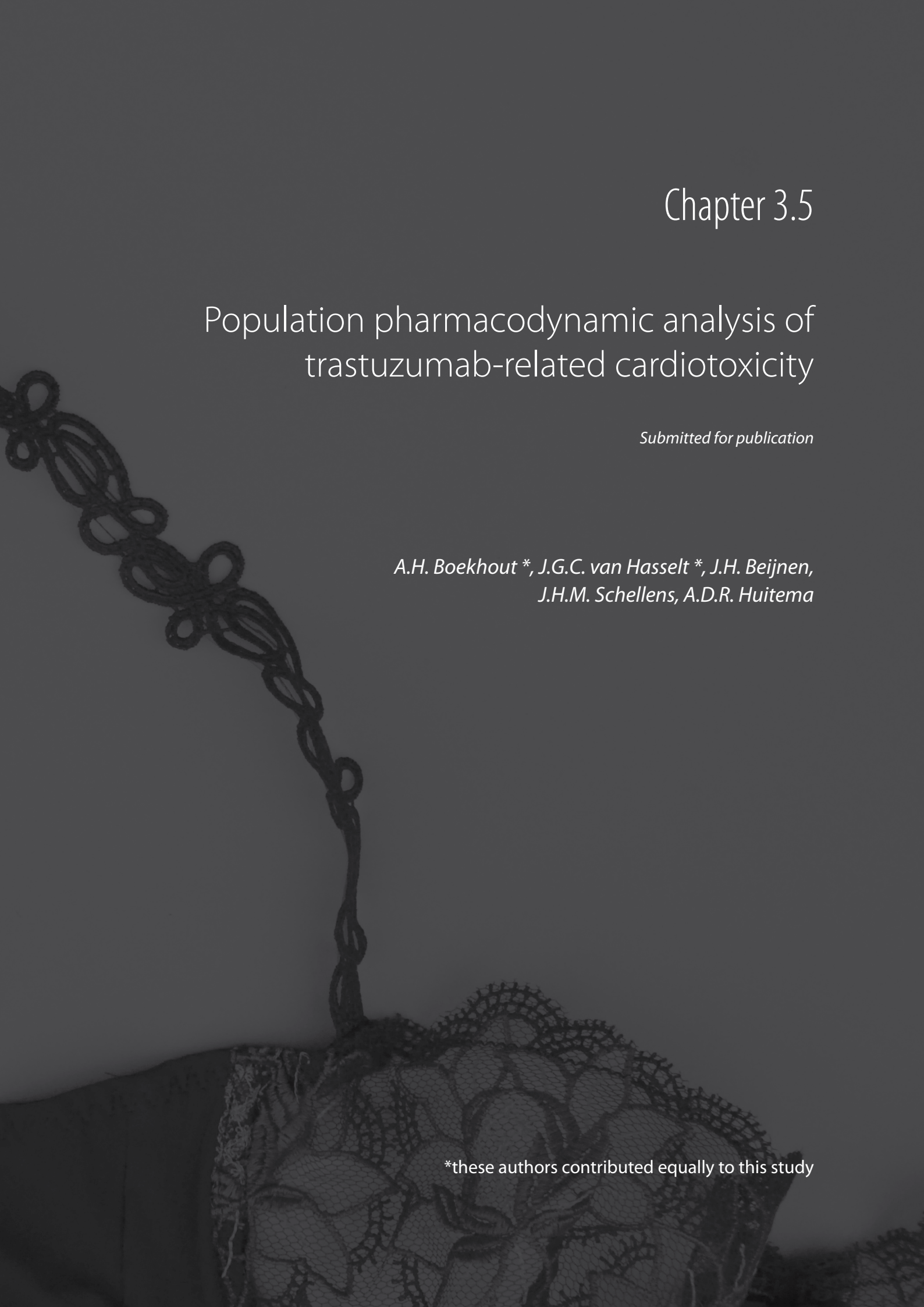

*these authors contributed equally to this study

Abstract

Purpose

Trastuzumab treatment is associated with cardiac dysfunction. The aim of this study was the development of a population pharmacokinetic-pharmacodynamic (PK-PD) model to unravel the dynamics of left ventricular ejection fraction (LVEF) decline and recovery in relation to trastuzumab exposure. Moreover, we aimed to identify and quantify clinically relevant determinants for trastuzumab cardiotoxicity.

Patients and methods

An unselected cohort of HER2 positive primary and advanced breast cancer patients treated with trastuzumab, with and without prior anthracycline treatment was included in this analysis. The data analysis was conducted using the nonlinear mixed effect modelling platform NONMEM. PK was described using trastuzumab dosing history in combination with a previously published PK model. Model evaluation was conducted using goodness of fit diagnostics, normalized prediction distribution errors, and a non-parametric bootstrap.

Results

A total of 1651 LVEF values from 240 patients were available. The data were best described by an effect-compartment model. The population recovery half-life after trastuzumab treatment ($T_{1/2rec}$) was estimated at 49.7 days, with high between subject variability (BSV), estimated at 79.4 CV%. Also, the sensitivity to LVEF decline (EC_{50}) was associated with a high BSV (103 CV%). The cumulative anthracycline dose was a significant determinant of the EC_{50}, causing a 45.9% increase in sensitivity (EC_{50}) at the maximum cumulative anthracycline dose.

Conclusion

A population PK-PD model describing trastuzumab associated cardiotoxicity was developed. The cumulative anthracycline dose was a significant determinant for between-subject variability on EC_{50}. The developed model can be used to establish optimal treatment and monitoring strategies for trastuzumab.

Introduction

Trastuzumab is a humanized monoclonal antibody that selectively binds to the extracellular domain of HER2, and improves outcome in early and advanced HER2 positive breast cancer and advanced gastric cancer (1-3). Trastuzumab treatment is associated with the occurrence of asymptomatic declines in left ventricular ejection fraction (LVEF) and in the development of congestive heart failure (CHF) in a small proportion of patients (4, 5). Trastuzumab-associated cardiotoxicity is likely to be related to the HER2 receptor involvement in the physiology and pathophysiology of heart muscle contractility, but the exact mechanism is still unclear (6, 7).

Unlike anthracycline-induced cardiac dysfunction, trastuzumab-induced CHF and decline in LVEF values are at least partly reversible when trastuzumab is discontinued (8-10), with a reported mean time to normalization of 1.5 months as established in a small study (10).

Clinical data suggested a higher incidence of cardiac dysfunction in patients treated with anthracyclines prior to (9, 11, 12) or concurrently with trastuzumab(2).

Several other potential independent predictors for cardiac events have been reported, such as low baseline LVEF value, older age, history of cardiac disease, or radiotherapy to the chest (5, 13, 14).

The optimal monitoring and treatment strategy for trastuzumab-related cardiac toxicity remains to be elucidated. Results of clinical studies are conflicting and several unanswered questions remain, including whether and how reported risk factors should guide trastuzumab treatment, what the optimal cardiac monitoring strategy is and uncertainty regarding optimal stopping and re-initiation algorithms of trastuzumab in relation to recovery time after a cardiac event.

Knowledge about risk factors and incidence of cardiac toxicity is difficult to translate into clinical practice in a quantitative manner. Moreover, prospective clinical trials with strict criteria for in- and exclusion and strict guidelines for management of LVEF changes may not be fully representative for routine clinical practice.

In order to answer these clinically important questions it is essential to be able to make quantitative inferences on the sensitivity to, and recovery from trastuzumab-associated cardiotoxicity, the relationship with important determinants such as anthracycline therapy and the magnitude of between-subject variability on these effects, based on a representative patient cohort eligible for trastuzumab treatment. To date no integrated pharmacokinetic-pharmacodynamic (PK-PD) model to characterize the relationship between PK and trastuzumab-associated cardiotoxicity has been reported.

The objectives of the present study were to i) develop a population PK-PD model that describes the relationship between trastuzumab exposure and changes in LVEF values, in terms of sensitivity to trastuzumab and recovery of trastuzumab-induced LVEF changes, and ii) to identify relevant determinants that explain between-subject variability on the model PK-PD parameters. Using a simulation platform, the developed model can then help to address questions regarding optimal clinical management of trastuzumab associated cardiotoxicity in a quantitative manner.

The ultimate aim of this work would be to develop and prospectively validate treatment guidelines for trastuzumab in clinical practice to improve the safety of the therapy with trastuzumab.

Methods

Patients and treatment

Patients (n=240) were treated with trastuzumab for HER2+ primary or metastatic breast cancer at the Netherlands Cancer Institute between 2005-2009. The inclusion criteria were: histologically confirmed HER2+ breast cancer treated with trastuzumab according to local treatment guidelines for at least one month or longer. Trastuzumab was administered weekly, two-weekly, or three-weekly, according to the summary of product characteristics.

LVEF measurements

LVEF was used as pharmacodynamic outcome measure for cardiotoxicity. LVEF values were assessed by multiple-gated acquisition (MUGA) scan or echocardiography. For MUGA scans, 400 MBq Tc-99m pertechnetate was used, and acquisition was done in 6 min with a large-field-of-view gamma camera with a low energy all-purpose parallel-hole collimator. A 3-dimensional echocardiography was performed by an echocardiographist. During the course of this retrospective study, the LVEF image analysis software was switched from (Pegasys-X Gated Analysis (v.3.40, ADAC) to Syngo (version 7.5.9.4 SP1, Siemens). For this new calculation method, LVEF values were known to be slightly higher compared to the old method. For 245 LVEF measurements, paired values for the old and new analysis method were available, and these pairs were used to support a simultaneous analysis of the full dataset.

Data analysis

Data management and graphical evaluation were performed using R (version 2.9.0) (15). Nonlinear mixed effect modelling was performed using NONMEM (version 7.1.0, ICON Development Solutions, Ellicott City, MD, USA) (16). All models were estimated using First Order Conditional Estimation method with η-ε Interaction (FOCE-I). Discrimination between hierarchical models was based on the likelihood ratio test (LRT), for which a change in objective function value (OFV) of 3.84 was considered statistically significant ($\alpha = 0.05$).

Structural model

Individual PK profiles for trastuzumab were obtained using a previously published PK model (12) for trastuzumab together with the individual dosing history, and these profiles were subsequently linked to the PD model. Indirect and direct effect compartment models, with and without recovery of the LVEF were evaluated. The PK-PD relation between the effect compartment concentration (C_{EF}) and the observed LVEF was modeled using either an Emax or simplified Emax model.

Statistical model

Modeling of between and residual unexplained variability

Between-subject variability (BSV) was modeled using an exponential error model (Eq. 1):

$$P_i = P_g . \exp(\eta_i) \qquad (1)$$

where P_i represents the individual parameter for the i^{th} subject, P_g the covariate-scaled population parameter value, and η_i the individual value of between-subject variability of the i^{th} subject, obtained from the BSV distribution with mean 0 and variance ω^2. Off-diagonal elements accounting for correlation between BSV estimates were also evaluated.

Residual unexplained variability (RUV) was modeled using a proportional error model as depicted in Eq. 2:

$$LVEF_{obs,ij} = LVEF_{pred,ij} \times (1+\varepsilon_{ij}) \qquad (2)$$

where $LVEF_{obs,ij}$ represents the observed LVEF for the *ith* individual and the j^{th} measurement, and $LVEF_{pred,ij}$ represents the predicted LVEF for the i^{th} individual and the j^{th} measurement, obtained from the RUV distributions with mean 0 and variance σ^2.

Handling of the two LVEF analysis methods

Separate RUV estimates for the old and new LVEF calculation methods were determined. Correlation between paired samples were accounted for by using the L2-method as implemented in NONMEM and described by Karlsson *et al* (17). Moreover, a scaling factor was estimated to scale observations calculated using the old method to the new analysis method.

Baseline value transformation

In order to restrict LVEF baseline ($LVEF_0$) values between 0 and 1, a logit transformation was used for the individually predicted baseline value for individual *i* (Eq. 3):

$$LVEF_{0i} = \frac{\exp(\varphi)}{1+\exp(\varphi)}, \text{ with } \varphi = \log\left(\frac{LVEF_0}{1 - LVEF_0}\right) + \eta_i \qquad (3)$$

Covariate analysis

Covariates

Only clinically relevant and biologically plausible covariate-parameter relationships were explored in the covariate analysis. Available covariates were: age, body mass index (BMI), radiotherapy (yes/no), adjuvant or metastatic indication, cumulative normalized dose of anthracyclines (doxorubicin, epirubicine), cumulative dose of cyclophosphamide, cumulative normalized dose of taxanes (paclitaxel, docetaxel).

Dose normalization of anthracyclines and taxanes

For anthracycline drugs, both epirubicin and doxorubicin were administered, while for

taxanes both docetaxel and paclitaxel were used in this patient population. In order to assess the effects of anthracyclines and taxanes as a single covariate effect, the individual cumulative doses administered for these drugs was divided by their maximum cumulative dose (anthracyclines) or the maximum 3-weekly doses (taxanes), as described in Eq. 4.

$$D_{norm} = \frac{\sum D_1}{MD_1} + \frac{\sum D_2}{MD_2} \qquad (4)$$

D_1 and D_2 represent the sum of the individual doses administered for the drugs within a drug class (e.g. for anthracyclines D_1=doxorubicin and D2=epirubicin). MD_1 and MD_2 represent the usually accepted maximum 3-weekly dose for taxanes (100 mg/m^2 for docetaxel, 175 mg/m^2 for paclitaxel) or maximum cumulative doses for anthracyclines (550 mg/m^2 for doxorubicin (18), 950 mg/m^2 for epirubicin) (19), that are associated with significant increases in the risk for CHF. D_{norm} represents the normalized combined dose level for each drug class.
The cumulative dose of cyclophosphamide was divided by its median cumulative dose.

Covariate testing
Initial covariate screening was performed by plotting empirical Bayes estimates versus the covariates.
Covariates that gave indication for a potential relationship were formally tested. However, for BSV parameters with more than 20% η-shrinkage, plausible parameter-covariate relationships were also formally tested. Formal testing of models was performed using the LRT.

Parameter-covariate relationships
Parameter-covariate relationships were implemented according to equations 5-7, were COV represents the covariate value, and θ_{COV} represents the covariate effect estimator. Cumulative dose effects were incorporated using the following equation (Eq. 5):

$$P_g = P_{pop} \times \left(1 - \frac{COV}{\theta_{COV} COV}\right) \qquad (5)$$

Continuous covariates were implemented using a power function (Eq. 6):

$$P_g = P_{pop} \times \left(\frac{COV}{median(COV)}\right)^{\theta_{COV}} \qquad (6)$$

Dichotomous covariates were implemented as following (Eq. 7):

$$P_g = P_{pop} \times \left(1 + \theta_{COV}\right)^{COV} \qquad (7)$$

Model evaluation
Candidate models were evaluated using plots of observed LVEF values versus individual- and population predicted LVEF values.
Also, η-shrinkage (20) on all BSV parameters was calculated.
Due to the heterogeneity of the data, the predictive performance of the model could not be evaluated using a visual predictive check. Therefore, normalized prediction distribution

errors (NPDE) were used to asses predictive performance (21). Ideally, the NPDE distribution should be normally distributed with a mean of 0 and a variance of 1. The NPDE distribution can be plotted against the dependent variable LVEF to detect model misspecification.
A non-parametric bootstrap analysis was conducted to determine parameter estimate precision.

Results

Patients and data

The total number of available LVEF measurements was 1651, with a median of 6 measurements per subject. Baseline measurements were available for 214 subjects. An overview of patient characteristics is given in Table 1. The median value of observed baseline values of LVEF was 0.64 (inter-quartile range 0.58 – 0.70).

Structural model

Indirect and direct effect models with and without recovery were evaluated. An indirect effect model with recovery best described the data, with stable and adequate parameter estimation, whereas other models were not reliably identifiable and/or showed considerable misspecification. The link between the C_{EF} and the observed LVEF was initially modeled using an Emax equation. During model development, it was observed that the parameter Emax was highly correlated with EC_{50}, and thus could not reliably be identified. Therefore, a simplified Emax model (Eq. 8) was used.

$$LVEF = LVEF_0 \times \left(1 - \frac{C_{EF}}{EC_{50} = C_{EF}}\right) \qquad (8)$$

In this equation, LVEF0 represents the baseline LVEF value prior to trastuzumab treatment, and EC_{50} represents the sensitivity to changes in LVEF.
The final model is schematically depicted in Figure 1. The parameter estimates of the final model are shown in Table 2. The population recovery half life- was estimated at 49.7 days (RSE 28.2%).

Statistical model

Between-subject variability (BSV) could be estimated for $LVEF_0$, $T_{1/2rec}$ and the EC_{50}. A full covariance matrix that accounts for correlations between BSV on each parameter could be estimated. The BSV on EC_{50} and recovery half-life was precisely estimated (RSE<30%) but high, with 103% and 79.4% respectively.
Correlation between BSV for baseline LVEF and EC_{50} was estimated, but negligible (r = -0.014). The BSV for baseline LVEF was negatively correlated with the recovery half-life (i.e. *r* = 0.616), indicating that patients with a low baseline value tend to recover more slowly from a decreased LVEF after end of trastuzumab treatment. There was also a relevant correlation between BSV in EC_{50}, and the recovery half-life (r = -0.739), where patients with lower EC_{50} tend to show a higher recovery half-life.

Table 1. Patient characteristics

Description	Nr of patients (%)	Median	Inter-quartile range
Demographics			
Age (years)		50.0	43 - 59
Body mass index (kg/m^2)		24.4	22.2 - 27.5
Adjuvant / metastasized patients	164 (68%) / 76 (32%)	-	-
Treatments			
Cumulative anthracycline dose (mg/m^2)*	141	0.436	0.421 – 0.600
Cumulative cyclophospamide dose (mg/m^2)	123	2400	2329 - 3000
Cumulative taxanes dose (mg/m^2)*	62	7.11	5.04 - 7.78
Prior radiotherapy (yes/no)	196	-	-

* Normalized dose based on maximum cumulative dose (anthracyclines) or maximum 3-weekly dose (taxanes)

Table 2. Population PK-PD parameter estimates from the full covariate model for trastuzumab

Parameter					*Bootstrap estimates* [a]	
Population parameters	*Units*	*Estimate*	*RSE*		*Median*	*90%CI*
Baseline LVEF (θ_{LVEF0})	-	0.636	0.904		0.636	0.627 – 0.645
Recovery half-life ($\theta_{T1/2rec}$)	day	49.7	28.2		53.7	27.8 – 107
Sensitivity (θ_{EC50})	mg/mL	4.82	19.6		4.71	3.46 – 6.82
Anthracycline effect on θ_{EC50} [b]	-	0.848	58.8		0.779	0.336 – 2.96
Inter-methods slope effect ($\theta_{SL\text{-}MET}$)	-	0.948	0.713		0.948	0.938 – 0.959
Between-subject variability	*Units*	*Estimate*	*RSE*	*Shrinkage (%)*	*Median*	*90%CI*
Baseline LVEF (ω_{LVEF0})	CV%	30.0	6.50	9.40	30.0	26.6 – 33.4
Recovery half-life ($\omega_{[ln(2)/T1/2rec]}$) [c]	CV%	79.4	27.6	36.8	86.9	38.1 – 155
Sensitivity (ω_{EC50})	CV%	103	13.8	34.9	102	71.2 – 134
Correlation ω_{LVEF0} – $\omega_{[ln(2)/T1/2rec]}$ [d]	-	-0.616				
Correlation ω_{LVEF0} – ω_{EC50} [d]	-	-0.014				
Correlation ω_{EC50} – $\omega_{[ln(2)/T1/2rec]}$ [d]	-	0.739				
Residual unexplained variability	*Units*	*Estimate*	*RSE*	*Shrinkage (%)*	*Median*	*90%CI*
Proportional residual error new method (σ_{new})	%	9.11	17.1	8.3	9.09	0.0842 – 0.0972
Proportional residual error old method (σ_{old})	%	7.35	8.40	5.7	7.26	0.0630 – 0.0839
Correlation between old and new ($\sigma_{old} \sim \sigma_{new}$)	-	0.512				

a Obtained from 871 non-parametric bootstrap runs.
b Proportional covariate effect of the cumulative normalized anthracycline dose.
c Between-subject variability was estimated on the recovery rate constant, i.e. $ln(2)/T_{1/2rec}$.
d Obtained from a full OMEGA BLOCK covariance matrix RSE=Relative asymptotic standard error (%); CV=Coefficient of Variation (%). CI=Nonparametric bootstrap confidence interval.

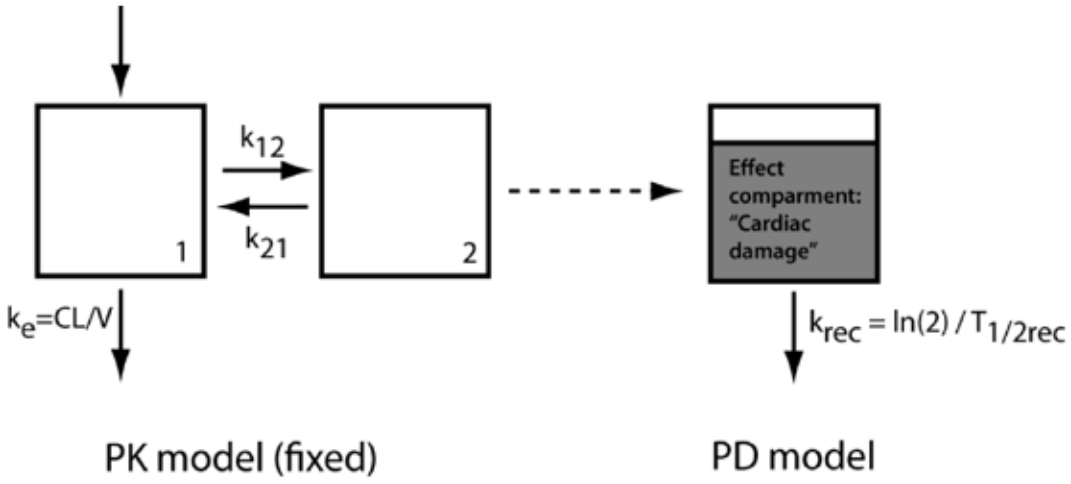

Figure 1. Schematic representation of the PK-PD model.

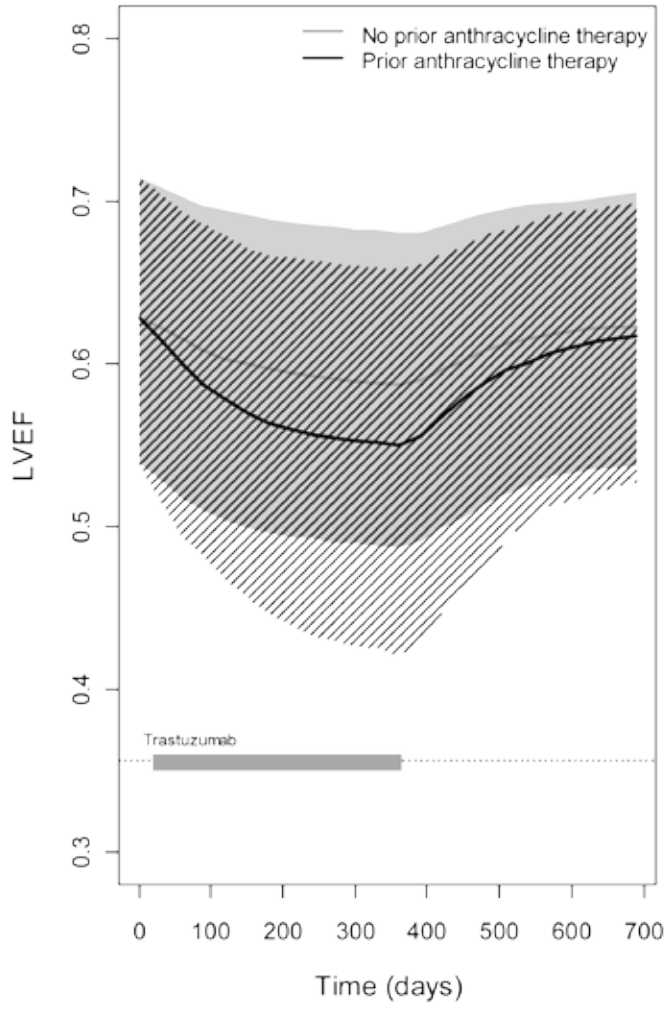

Figure 2. Stochastic simulations of left ventricular ejection fraction (LVEF) versus time (days) using the final PK-PD model, for 1000 patients receiving a weakly dose of 150 mg trastuzumab for 1 year, with (black) and without (gray) a prior maximum cumulative anthracycline dose. The solid lines represent the median; the area represents the 80% prediction interval. Trastuzumab dosing is indicated with the horizontal gray bar.

Covariate analysis

For the parameters EC_{50} and $T_{1/2rec}$ all covariates were tested because of η-shrinkage between 30-40%. For baseline, only age was formally tested. Although the relationship with age was significant, the effect size was marginal and therefore excluded from the model.

The cumulative anthracycline dose was a significant determinant ($p < 0.02$) of the EC_{50}, causing a 45.9% increase in sensitivity (EC_{50}) at the maximum cumulative anthracycline dose. Figure 2 depicts the predicted LVEF-time profiles for 1000 individuals using the final model, who received either the maximum cumulative dose of anthracyclines prior to trastuzumab treatment, or no prior anthracyclines.

Model evaluation

Typical observed and predicted LVEF profiles were depicted for a patient with long-term data available (Figure 3A) and short-term data available (Figure 3B). Figure 3C shows the prediction for an individual with paired observations using the old and new LVEF calculation method together. Figure 3D shows an individual with good tolerance for trastuzumab during the course of treatment.

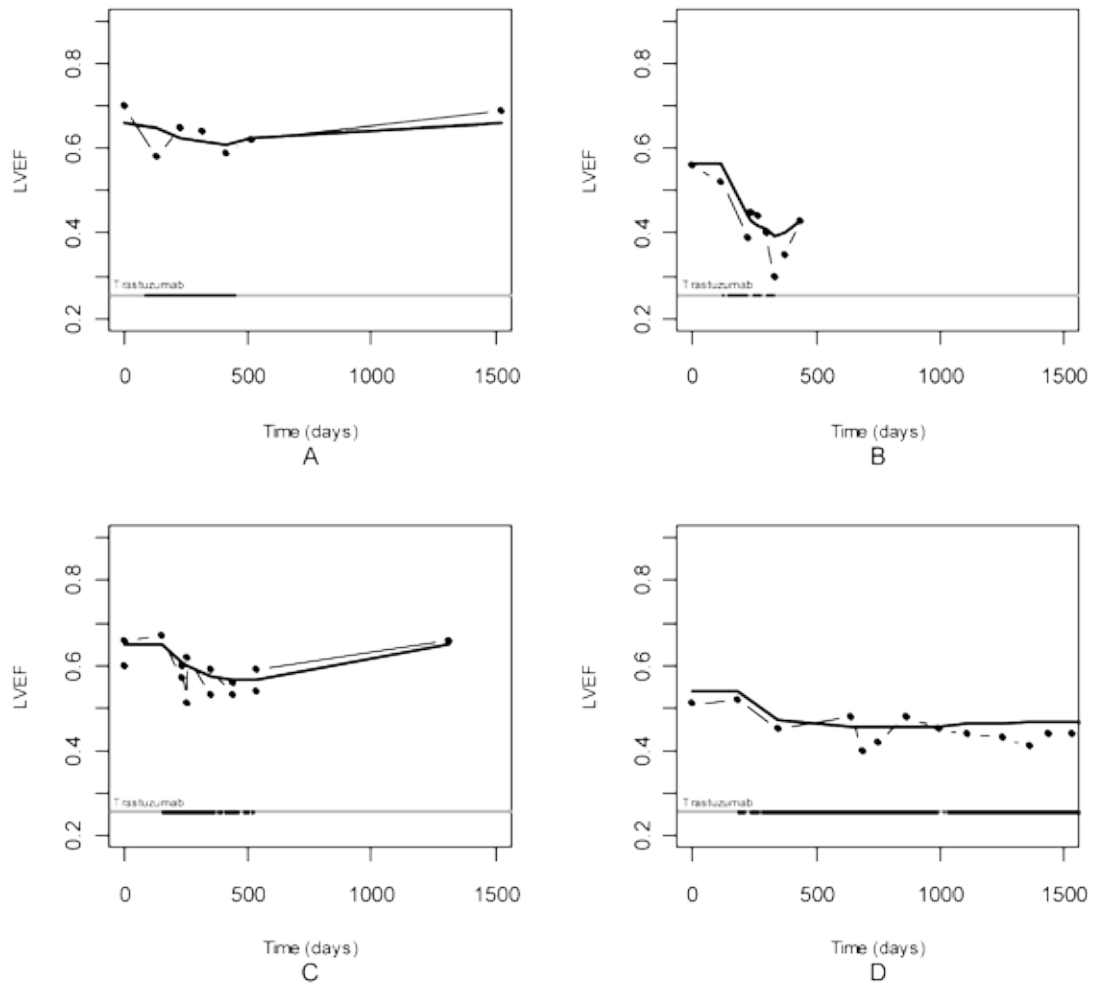

Figure 3. Observed (solid circles), individual predicted (solid line) left ventricular ejection fraction (LVEF) versus time (days) for four typical patients. Timing of trastuzumab dosing is indicated using the horizontal black bars. Sub-figure 2A) patient for which the long-term recovery is visible; 2B) patient for which no long-term recovery data is available; 2C) patient with LVEFs measured using both methods; 2D) patient under prolonged trastuzumab treatment.

Goodness of fit plots of the individual predicted (Figure 4A) and population predicted values versus observations (Figure 4B) showed adequate predictions.
The model performance was further evaluated using the plots of the NPDE distribution versus the predicted LVEF values (Figure 4C) and versus time (Figure 4D), which did not show any significant trends.
Non-parametric bootstrap indicated that model parameters could be estimated with acceptable precision (see Table 2).

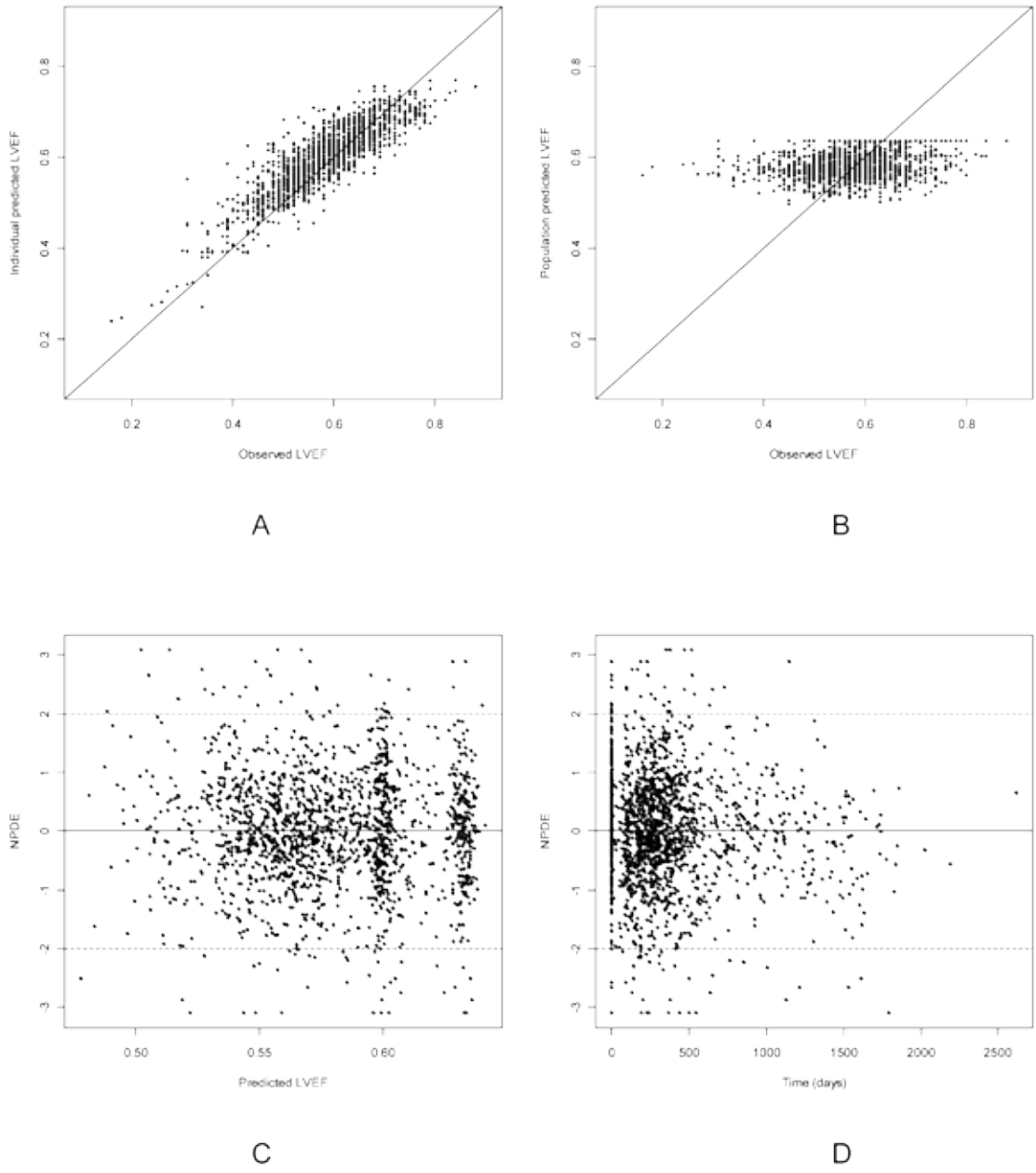

Figure 4. Diagnostic graphics displaying the individual (3A) and population (3B) predictions versus the observed left ventricular ejection fraction (LVEF) values, and the normalized prediction distribution errors (NPDE) versus the predicted LVEF values (3C) and versus time (3D).

Discussion

A population PK-PD model describing the relationship between trastuzumab exposure and LVEF decline, in patients with early and advanced breast cancer was successfully developed. The cumulative anthracycline dose was found as important determinant for the sensitivity to LVEF decline (EC_{50}).

Cardiac damage was modeled using an effect compartment model that was linked to the central PK compartment. The hypothetical concentration in this effect compartment may be interpreted as the degree of cardiac damage. Recovery of cardiac damage is occurring at the rate described by $\ln(2)/T_{1/2rec}$ A simplified Emax model was used to translate the cardiac damage to the observed LVEF.

The structural model strongly supported identification of recovery of the LVEF after end of trastuzumab treatment. The population recovery half-life ($T_{1/2rec}$) for the LVEF was estimated at 49.7 days (RSE 28.2%). Also, a high degree of between-subject variability of 69% was observed on recovery. When taking the PK half-life into account of 28.5 days (12), the overall effective half-life for trastuzumab associated cardiotoxicity after cessation will be somewhat longer. This value is higher than the value reported by Ewer et al., who reported a mean observed time to full recovery of 1.5 months (10). This difference may be explained by the large amount of between-subject variability on the recovery half-life, the much larger sample size and more heterogeneous study population.

The cumulative prior anthracycline dose was found to be a significant and relevant covariate for sensitivity to cardiotoxicity (EC_{50}). Hence, patients who have received prior anthracycline therapy will show more intensive LVEF declines. The magnitude of the covariate effect is illustrated in Figure 2 depicting model simulations for patients with and without the maximum prior dose of anthracyclines prior to trastuzumab treatment.

The identification of the cumulative dose of anthracyclines as the single covariate in the developed model is relevant for the fact that anthracyclines are frequently included in the treatment of HER2 positive breast cancer.

Anthracycline-induced decline in LVEF has been reported to be irreversible in contrast to what is observed for trastuzumab. Hence, when a high dose of anthracyclines is administered shortly before trastuzumab treatment, patients may not fully recover to their original baseline LVEF. This is not accounted for in the current model. The current dataset did however not allow reliable estimation of anthracycline-associated reduced baseline effect.

In the various clinical studies conducted, cardiotoxicity only occurs in a subset of all patients. Cardiotoxicity if often defined as a minimum decline in LVEF combined with an absolute LVEF value lower then a specific threshold. In this PK-PD model, cardiotoxicity is not categorized as such, but the change in LVEF is described as a continuous variable. The past observation of the occurrence of cardiotoxicity in only a subset of patients in comparison with our analysis can be explained by the high between-subject variability in PD parameters and the lack of categorization of occurrence of cardiac toxicity events. Therefore, changes in LVEF appear to be consistent across all patients, the magnitude of change however, might not be clinically relevant in patients in whom the baseline is high, or the sensitivity to cardiotoxicity (EC_{50}) is low.

The model was based on a representative patient population in daily clinical practice. No data from patients treated in a clinical study were used, thus restrictive inclusion and exclusion criteria did not apply. Moreover, data from patients with and without prior anthracycline treatment were included. For patients with metastatic breast cancer with no other treatment options, lower LVEF values for interruption of trastuzumab are often accepted. In the current analysis these patients were highly informative for identification of the PK-PD parameters, while these patients are usually not represented in clinical trials.

Population pharmacokinetic-pharmacodynamic (PK-PD) models are increasingly recognized as valuable tools in drug development (22). This approach allowed analysis of data that was informative, but also highly heterogeneous in terms of individual dosing histories, LVEF recording times, and patient characteristics. Moreover, the developed PK-PD model can be used to simulate novel clinical scenarios with alternative dosing regimens or toxicity management guidelines. For example, the developed PK-PD model can be used to address clinical questions regarding optimal clinical management of trastuzumab-associated cardiotoxicity. This can be accomplished by simulation of large virtual patient cohorts, and subsequent calculation of relevant clinical statistics, or by application of a cardiac monitoring protocol to the simulated dataset. Hence, this approach would allow for calculation of overall optimal treatment and monitoring strategies in a quantitative manner, rather than proposal and subsequent clinical validation of somewhat arbitrarily designed monitoring strategies.

Examples of clinical questions that could potentially be addressed using this model might be identification of high-risk and low-risk patient and subsequent adaptations to monitoring protocols, impact of dose reductions, monitoring strategies based on risk profiles (i.e. the cumulative anthracycline dose received).

This is the first report of a population PK-PD model that describes the relationship between trastuzumab exposure and decline in LVEF, incorporating the effect of the cumulative dose of prior anthracycline therapy. The developed model adequately described the data. A highly informative and representative dataset from routine clinical practice was used. Population PK-PD modelling demonstrated to be an effective data analysis method that was able to analyze a highly heterogeneous dataset in an efficient manner. The PK-PD model can be used in a simulation framework, to guide optimization of cardiac monitoring strategies during trastuzumab treatment. Ultimately, this work should lead to optimal monitoring guidelines that are prospectively validated.

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Chapter 3.6

Prevention of trastuzumab-associated cardiotoxicity in early breast cancer patients by the angiotensin II-receptor blocker candesartan: a prospective, randomized, placebo-controlled pharmacological intervention (CANDY) study

Interim analysis of blinded data

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Abstract

Purpose

Trastuzumab treatment is associated with cardiac dysfunction. We undertook a double-blind, placebo controlled trial. The aim of this study was to investigate whether the concurrent use of an angiotensin II-receptor blocker (ARB) in patients treated with adjuvant trastuzumab for HER2 positive breast cancer can prevent or ameliorate trastuzumab-related cardiotoxicity.

Methods and design

In total 175 patients with HER2 positive breast cancer had been randomly assigned to candesartan or placebo. Cardiac events were defined as a decline in LVEF of more than 15 percentage points compared to baseline or a decrease in LVEF to an absolute value below 45%. Four interim analyses were planned after 10, 20 and 30 cardiac events, with the final look at 200 evaluable patients.

Results

The tentative results of this study at its second safety analysis after 20 cardiac events are presented but results are not available per treatment arm. A total of 107 patients were evaluable for this study. In total 20 cardiac events were observed after a median time on trastuzumab of 25 weeks; range 11-92 weeks. The median follow-up was 8.5 months. In total 25 Serious Adverse Events (SAEs) were reported as not related with the study treatment and 6 SAEs were reported as unlikely related with the study treatment. No Suspected Unexpected Serious Adverse Reaction (SUSAR) was reported.

Conclusion

The rate of trastuzumab-related cardiac events observed at the first and second interim analysis is in line with the rate of cardiac events observed in other trastuzumab trials. There is no evidence thus far that intervention with candesartan treatment during trastuzumab treatment is associated with an increased rate of cardiac events.

Introduction and background

Introduction

Trastuzumab is a humanized monoclonal antibody that targets the extracellular domain of the human epidermal growth factor receptor 2 (HER2). It has been shown to benefit patients with early and advanced HER2 positive breast cancer and patients with advanced gastric cancer (1-4). Trastuzumab as monotherapy or in combination with chemotherapy in HER2 positive breast cancer patients markedly improves treatment outcome. Trastuzumab treatment in the adjuvant setting results in an absolute reduction in the risk of recurrence or death (5, 6). One year of trastuzumab treatment after adjuvant chemotherapy is currently standard of care in HER2 positive primary breast cancer patients. However, trastuzumab treatment can be associated with cardiac dysfunction, usually manifested by asymptomatic reduction in left ventricular ejection fraction (LVEF), and in a small proportion of patients in symptomatic congestive heart failure (CHF). Results of large adjuvant clinical trials suggest that trastuzumab-associated asymptomatic declines in LVEF and CHF have a high rate, up to 86% of the patients, of reversibility (7, 8). However, study results of trastuzumab-induced cardiotoxicity are controversial. Follow-up data from a large advanced clinical trial show that many patients did not reach their baseline LVEF value after discontinuation of trastuzumab treatment and that up to two third of the patients continued anti-congestive medication even after complete recovery of asymptomatic cardiotoxicity (9). Still, risk factors for the development of trastuzumab-related cardiotoxicity and outcome of patients with cardiac dysfunction at long term are unknown. Therefore, development of methods to prevent or alleviate cardiac dysfunction is of major relevance.

ACE-inhibitors and Angiotensin II-receptor blockers

A rationale approach to decrease trastuzumab-induced cardiotoxicity could be influencing the Renin-Angiotensin-Aldosterone system (RAAS), e.g. with angiotensin converting enzyme (ACE) inhibitors or angiotensin II-receptor blockers (ARBs). ACE-inhibitors are standard of care in the treatment of patients with CHF, significantly reducing the development of heart failure in asymptomatic patients with cardiomyopathy of various origins (10, 11). In patients with anthracycline-induced cardiotoxicity, ACE-inhibitors can ameliorate symptoms of CHF and can prevent a decline in LVEF (12, 13). A randomized, double-blind, clinical trial compared an ACE-inhibitor (enalapril) and placebo in 135 survivors of childhood malignancy (14). The enalapril group showed markedly reduced declines in LVEF compared to the placebo group, presumably via reductions in afterload (15). The ACE-inhibitors are increasingly being replaced by ARBs, because of the experienced intolerance to ACE-inhibitors. Approximately 10% of patients with CHF are intolerant to ACE-inhibitors, with as most common side-effect the development of cough. ARBs may induce a more complete inhibition of the renin-angiotensin system than ACE-inhibitors, they do not affect the response to bradykinin, and are less likely to be associated with nonrenin-angiotensin effects such as cough and angioedema. The ARBs are as effective as the ACE-inhibitors in CHF patients(16-18). Clinical trials showed that a large population of patients who were intolerant of ACE-inhibitors do tolerate the ARBs, candesartan or losartan (19).

On the bases of these results we conducted a prospective, randomized, double-blind,

placebo controlled trial of candesartan and placebo in women with primary HER2 positive breast cancer. Currently, no evidence is available that ACE-inhibitors or ARBs can prevent trastuzumab-related cardiotoxicity. We hypothesized that the concurrent use of ABRs in patients treated with adjuvant trastuzumab for HER2 positive breast cancer can limit the development of trastuzumab-related impairment of cardiac function. Endpoints included the occurrence of cardiotoxicity during one-year trastuzumab therapy and 40 weeks after discontinuation of trastuzumab treatment, defined as a decline in LVEF of more than 15% or a decrease to an absolute value below 45%.

Biochemical cardiac markers

Plasma levels of N-Terminal pro-Brain Natriuretic Peptide (NT-proBNP) and troponin I and troponin T are sensitive cardiac biomarkers, which are used as markers to detect estimate the severity of left ventricular systolic dysfunction (20). The plasma concentration of NT-proBNP is increased in patients with cardiac dysfunction (asymptomatic or symptomatic), in response to the high ventricular filling pressure. The troponins I and T, are both well established specific and sensitive markers, for the extent of acute cardiomyocyt injury (21, 22).

Data suggest that NT-proBNP and the troponins might be useful parameters to detect or predict trastuzumab induced cardiotoxicity (23, 24). In a study of Perik et al., pre-treatment plasma NT-proBNP levels were higher in patient who experienced heart failure during trastuzumab treatment than in patients without heart failure (25). A recently published trial in 251 patients revealed a significant relationship between troponin I and trastuzumab-associated cardiotoxicity. Patients with elevated troponin I levels during treatment were at risk for development of cardiotoxicity and recovery of trastuzumab-associated cardiotoxicity was found to be unlikely (26).

Serum biomarkers are an attractive monitoring strategy for cardiotoxicity, as samples can be obtained in a minimally invasive way and the interpretation of their measurement is subjected to only low inter-observer variability. However, more evidence is needed before these parameters can be applied as routine screening measurement for trastuzumab-associated cardiotoxicity. In this study we prospectively evaluated whether NT-proBNP and troponin T, measured before, during, and after trastuzumab treatment, might be a useful marker to predict or detect trastuzumab-associated cardiotoxicity.

Genetic variations in the HER2 gene

Genetic variations in the *HER2* gene may modulate the risk for developing trastuzumab-associated cardiotoxicity. Most of these variations are single nucleotide polymorphisms (SNPs). Several SNPs in the extracellular, transmembrane and intracellular region of HER2, have been studied to examine the impact of these polymorphisms on disease outcome and on trastuzumab-related cardiac toxicity. In addition many epidemiological studies have been conducted to explore the association between the HER2 Ile655Val polymorphism and the risk of breast cancer however, study results are inconclusive (27). In a prospective clinical study of 61 advanced breast cancer patients, the presence of the Val655Ile polymorphism was found to be a risk factor for trastuzumab-related cardiotoxicity. All cases of cardiotoxicity defined as a ≥ 20% reduction in LVEF, were found in the Ile/Val group, while there was no

cardiotoxicity observed in the Val/Val or Ile/Ile group of patients. Study results suggest that the Val allele is a predisposing factor of trastuzumab-associated cardiotoxicity (28). In the current study we determined genetic variability in the extracellular domain of the HER2 receptor; FcγRIIIa-158 valine (V)/phenylalanine (F), FcγRIIIa-131 histidine (H)/arginine (R) and FcγRIIIa-232 isoleucine (I)threonine (T), in the transmembrane domain; Val654Ile, Val655Ile and in the intracellular domain; P1170A. We hypothesized that SNPs in the HER2 gene might identify patients who are at risk for cardiac morbidity in trastuzumab treatment.

Methods

Patients

This multicenter study was performed at 19 Dutch hospitals. The participating centers are summarized in Table 1. Eligibility criteria were: women aged ≥18 years; WHO performance score: ≤ 2; strongly HER2-positive breast cancer, defined as an immunohistochemistry score of 3+ using the HercepTestTM or gene amplification by fluorescence in situ hybridization, or chromogenic in situ hybridization (CISH); serum creatinine <140 µmol/l or creatinine clearance > 50 ml/min (by Cockcroft-Gault formula); thyroid stimulating hormone (TSH) between 0.5 - 3.9 MU/l or thyroid hormone FT4 between 8 – 26 pmol/l; blood pressure systolic ≥ 100 mmHg and ≤ 180 mmHg and diastolic ≥ 60 mmHg and ≤ 100 mmHg; LVEF ^{3} 50% assessed by multiple gate acquisition(MUGA) scan or cardiac ultrasound at baseline of trastuzumab

Table 1. Participating centers in the Netherlands

Netherlands Cancer Institute - Antoni van Leeuwenhoek Hospital	A.H. Boekhout, MA
	J.H.M. Schellens, MD PhD
University Medical Center Groningen, Groningen	R. Altena, MD
	J.A. Gietema, MD PhD
	E.G.E. de Vries, MD PhD
Medisch Spectrum Twente, Enschede	W.M. Smit, MD PhD
University Medical Center St. Radboud, Nijmegen	P.B. Ottevanger, MD PhD
Isala klinieken, Zwolle	A. Honkoop, MD PhD
Medisch Centrum Leeuwarden, Leeuwarden	W.E. Fiets, MD PhD
Slotervaart Hospital, Amsterdam	M. Soesan, MD
Canisius Wilhemina Hospital, Nijmegen	C.M.P.W. Mandigers, MD PhD
Wilhelmina Ziekenhuis, Assen	P. Nieboer, MD PhD
Deventer Ziekenhuis, Deventer	L. Kessels, MD PhD
Onze Lieve Vrouwe Gasthuis, Amsterdam	O. C. Leeksma, MD PhD
Jeroen Bosch Hospital, 's Hertogenbosch	T. Smilde, MD PhD
Medisch Centrum Alkmaar, Alkmaar	C.H. Smorenburg, MD PhD
Martini Ziekenhuis, Groningen	A.W.G. van der Velden, MD
Antonius Ziekenhuis, Nieuwegein	M. Los, MD, PhD
Ziekenhuis de Tjongerschans, Heerenveen	J. De Boer, MD
Streekziekenhuis Koningin Beatrix, Winterswijk	P.P.J.B.M. Schiphorst, MD
VieCuri Medisch Centrum voor Noord-Limburg, Venlo	A.J. van der Wouw, MD PhD
Flevoziekenhuis, Almere	V. Lustig, MD

treatment; (neo-) adjuvant treatment setting; at least an approved anthracycline-based chemotherapy regimen prior to trastuzumab treatment; first trastuzumab infusion at least 3 weeks after day 1 of the last anthracycline infusion. Exclusion criteria were: previous malignancy requiring anthracycline-based chemotherapy, anti-HER2 therapy, other prior biologic or immunotherapy or mediastinal radiotherapy; uncontrolled serious concurrent illness; New York Heart Association (NYHA) class II/III/IV congestive heart failure; myocardial infarction < 6 months before registration; treatment with an ACE- inhibitor, ARB or lithium; history of hypersensitivity to the study medication; pregnancy or breast feeding. In case of uncontrolled hypertension, or when patients were already on treatment with ACE- inhibitors or ARBs at the time of registration, blood pressure regulation guidelines were recommended, as summarized in Table 2.

The study protocol was approved of the Medical Ethics Committees of all participating centers and all participants had to give written informed consent.

Table 2. Algorithm for medical treatment of hypertension

Algorithm for the treatment of hypertension without ACE inhibitors or ATII blockers with the goal to achieve a blood pressure equal to or below 140/90 mmHg;
Patients who are using:
ACE inhibitor or ATII blocker Stop ACE inhibitor or ATII blocker Start β-blocker: metoprolol ZOC 100 mg daily After 1 week, systolic blood pressure (BP) > 140 mmHg and/or diastolic BP > 90 mmHg increase dose to metoprolol Retard 200 mg daily, unless heart rate at rest ≤ 60 minute then keep the 100 mg dose and add hydrochlorothiazide 25 mg daily
ACE inhibitors or ATII blocker and contraindication for β-blocker Stop ACE inhibitor or ATII blocker Start hydrochlorothiazide 25 mg daily
ACE inhibitor or ATII blocker with β-blocker Stop ACE inhibitor or ATII blocker Continue β-blocker Start hydrochlorothiazide 25 mg daily
Hydrochlorothiazide and systolic BP > 140 mmHg and/or diastolic BP > 90 mmHg Continue hydrochlorothiazide 25 mg daily Start amiloride 5 mg daily
Hydrochlorothiazide and amiloride and systolic BP > 140 mmHg and/or diastolic BP > 90 mmHg Increase amiloride to 10 mg daily
Hydrochlorothiazide and hypokalemia (potassium <3.5 mmol/l) Stop hydrochlorothiazide monotherapy Start hydrochlorothiazide-amiloride 25/2.5 mg; if no normalization of serum potassium: increase dose of amiloride to 5 mg.
Hydrochlorothiazide 25 mg and amiloride 10 mg and systolic BP still > 140 mmHg and/or diastolic BP > 90 mmHg: Increase dose of hydrochlorothiazide to 50 mg
Hydrochlorothiazide 50 mg and amiloride 10 mg and systolic BP still > 140 mmHg and/or diastolic BP > 90 mmHg: Patient will not enter the study

Procedures

After patients had given informed consent, they were registered at the Trial Office of the Data Centre of the Netherlands Cancer Institute (DC-NKI). Patients were registered before start of chemotherapy, during the chemotherapy treatment period or after completion of chemotherapy. Patients were randomized when the blood pressure and the LVEF value after the end of anthracycline-based chemotherapy were according to the inclusion criteria. Eligible participants were randomly assigned (1:1) of double-blind therapy with either candesartan or placebo. The pharmacy supplied and labeled the study medication for patients enrolled on this trial. Tablets were prepared identical and consisted of 16 mg candesartan or placebo. Each bottle of investigational product included an investigational-use label, box-code and space for patient name. Treatment allocations were kept in sealed envelopes to be opened only at an imperative need to identify the actual treatment given to a certain patient such as in medical emergencies. During the treatment period patients received study medication three-monthly and were instructed to return unused study medication to the hospital at each next follow-up visit. The pharmacy maintained a complete drug accountability record, including the number of tablets dispended to each patient. The DC-NKI conducted the registration and randomization procedure and assigned box-numbers to individual patients.

Study intervention

Dosing schedule and dose escalation

Patients started at a dose of candesartan of 16 mg daily for one week. From week two until 26 weeks after completion of treatment with trastuzumab patients took 32 mg candesartan daily. The dose of candesartan treatment was based on results of earlier clinical trials. The optimal dose of candesartan in the treatment of cardiac diseases is 32 mg per day and is well tolerated (29). The duration of candesartan treatment was based on the half-life of trastuzumab. The half-life of trastuzumab, is approximately 28 days (95% confidence interval, 25-33 days) and trastuzumab may persist in the circulation for up to 24 weeks after stopping of trastuzumab treatment (95% confidence interval, 18-24 weeks)(30). Patients were treated with candesartan 32 mg daily if the patient experienced no disabling candesartan-associated side-effects.

Trastuzumab treatment

Trastuzumab treatment was given weekly or tri-weekly. According to the national guideline for the treatment of early breast cancer trastuzumab was commonly used in combination with taxane-based chemotherapy followed by trastuzumab as single agent or with endocrine therapy in case of ER+ and/or PR+ breast cancer. Endocrine therapies in premenopausal women included selective estrogen receptor downregulators optionally in combination with suppression of ovarian function by surgery or luteinizing hormone-releasing hormone (LHRH) agonists. The treatment of early ER+ and/or PR+ breast cancer in postmenopausal women included selective estrogen receptor downregulators or aromatase inhibitors. The combination of trastuzumab and these types of therapies were acceptable for participation in this trial.

Clinical assessments

Physical examinations, vital signs, performance status, adverse events, hematology and clinical chemistry were performed at baseline, in week 12, 24, 36, 52, 78 and 92. At each study assessment, cardiac questionnaires were used to estimate presence of signs or symptoms of CHF, including assessment of the New York Heart Association classification (NYHA), as presented in Table 3. An electrocardiogram (ECG) was performed at baseline, on week 52 and week 78. The timing and contents of all study-related assessments are summarized in Figure 1.

LVEF measurements

LVEF values were assessed by MUGA scan or echocardiography. The same technique used for the LVEF measurement the first time, was used also for follow-up measurements. For MUGA scans, 400 MBq Tc-99m labeled autologous red blood cells were injected and acquisition was done in 6 min with a large-field-of-view gamma camera with a low energy all-purpose parallel-hole collimator. A conventional 2-dimensional echocardiogram was performed by a skilled echocardiographist.

Laboratory assessments and analyses of the cardiac markers

Blood samples were taken for hematological and serum biochemical monitoring. These analyses were performed by the local laboratories. Analyses of the cardiac markers were performed at the laboratory of the UMCG. cTnT levels were assessed by the CARDIAC T immunoassay on the Cobas h232 instrument with an Upper Limit of Normal (ULN) of 0.05 µg/l and limit of detection (LoD) of 0.03 µg/l. Serum NT-proBNP levels were assessed in serum using an immunoassay with an ULN of 18 pmol/l and LoD of 5 pmol/l (Roche Diagnostics GmbH, Mannheim, Germany).

Genotype analysis

PCR amplification and sequencing

Genomic DNA was isolated from whole blood samples using QIAamp® DNA Mini Kit (Qiagen Benelux B.V., The Netherlands) and amplified by PCR reaction in a PTC-200 thermocycler (MJ Research, Inc., Waltham, MA, USA). The used DNA primers and PCR conditions are listed in Table 4. DNA sequencing was carried out for HER2 I654V, I655V, FcγR2B I232T, and FcγR3A F158V. First, amplified DNA samples were purified using ExoSAP-IT® (USB, Germany). Secondly, purified DNA sample, DNA Sequencing Kit Big Dye TerminatorTM Cycle sequencing mix v3.1 (Applied Biosystem, Foster City, CA, USA), and a specific primer (forward or reverse, same as PCR primer) were mixed in a final volume of 20 µl, the reaction was performed in a PTC-200 thermocycler. Analysis of the DNA sequence reaction product was carried out on an Applied Biosystem 3730/3730xl DNA analyzer (Applied Biosystem).

SNP genotyping assay

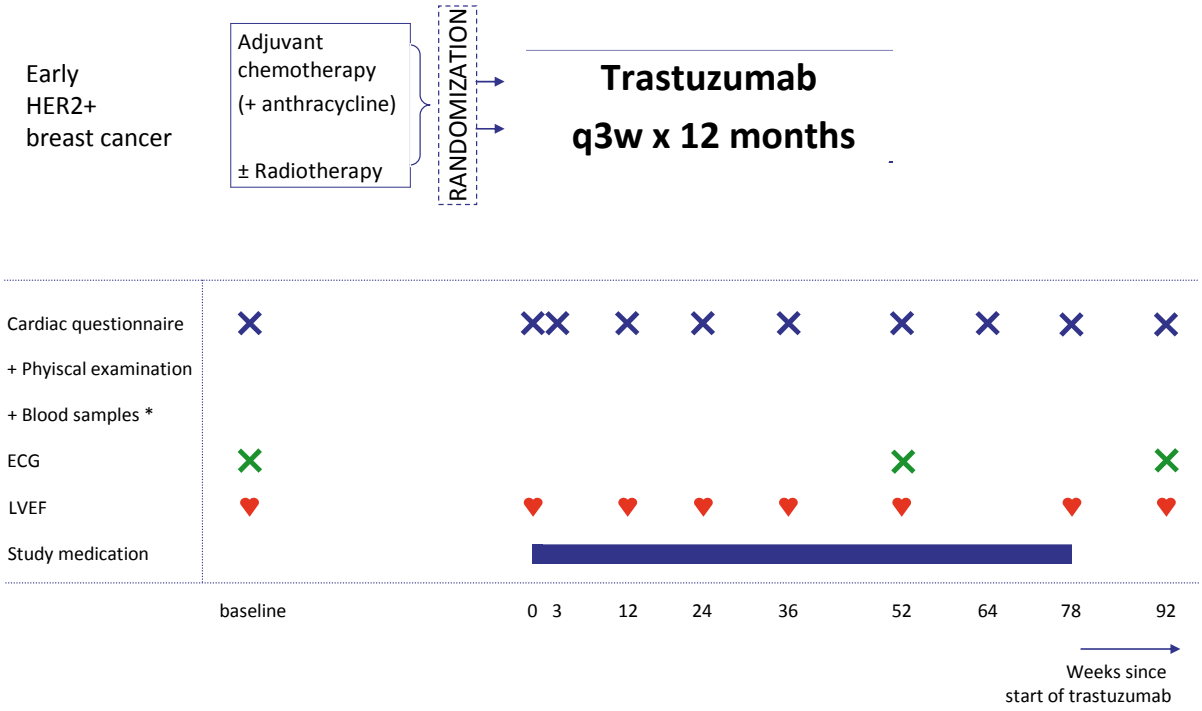
The allelic discrimination analysis was performed for HER2 A1170P and FcγR2A H166A according to the manufacturer's protocol (Applied Biosystems). The assay contained Taqman® Universal PCR Fast Master Mix No AmpErase® UNG (2x), genotyping assay mix (40x) (SNP

Table 3. Cardiac questionnaire

Symptoms of cardiac disease	**YES**	**NO**
Does the patient have any new symptoms of cardiac disease since the last visit?	☐	☐
Chest pain	☐	☐
Dyspnea on excertion	☐	☐
Palpitations	☐	☐
Syncope (fainting)	☐	☐
Dizziness	☐	☐
Other signs of cardiac disease	☐	☐
If other, specify	☐	☐
Physical findings of cardiac disease		
Does the patient have physical findings of cardiac disease?	☐	☐
Pulmonary congestion	☐	☐
S3 gallop	☐	☐
Tachycardia	☐	☐
Jugular venous distention	☐	☐
Edema	☐	☐
New murmur	☐	☐
Other physical findings of cardiac disease	☐	☐
If other, specify:	☐	☐
Does the patient have any other new symptoms since the last visit?	☐	☐

New York Heart Association (NYHA) classification

NYHA Class	*Symptoms*	
I	No symptoms and no limitation in ordinary physical activity, e.g. shortness of breath when walking, climbing stairs etc	☐
II	Mild symptoms (mild shortness of breath and/or angina) and slight limitation during ordinary activity	☐
III	Marked limitation in activity due to symptoms, even during less-than-ordinary activity, e.g. walking short distances (20–100 m). Comfortable only at rest.	☐
IV	Severe limitations. Experiences symptoms even while at rest. Mostly bedbound patients.	☐



* Including N-terminal pro brain Natriuretic Peptide, Troponin T

Abbreviations: Human Epidermal Receptor 2 (HER2); Electocardiogram (ECG); Left Ventricular Ejection Fraction (LVEF)

Figure 1. Candy study design and timing of study-related investigations

Table 4. Polymerase chain reaction, sequencing and genotyping assay

SNP	Primer Probe	PCR and sequencing primers, 5′→3′ Taqman® SNP genotyping primers and probes, 5′→3′	PCR conditions
I654V (rs1801201) I655V (rs1801200)	Fwd Rev	CCTTTCCGAATGCCAAACA CGCCTCACCTCCGTTTCCT	95°C 9 min, 95°C 1 min, 63°C 1 min, 72°C 1 min, 39 cycles, 72°C 10 min
I232T (rs1050501)	Fwd Rev	CTAAGAGGAGCCCTTCCCTATGT AATACGGGCCTAGATCTGAATGTG	95°C 5 min, 94°C 30 sec, 54°C 45 sec, 72°C 1 min, 35 cycles, 72°C 7 min
F158V (rs396991)	Fwd Rev	AGCTGGAAGAACACTGCTCTGCA AACTCAACTTCCCAGTGTGAT	95°C 5 min, 94°C 30 sec, 55.7°C 30 sec, 72°C 30 sec, 45 cycles, 72°C 7 min
A1170P (rs1058808)	Fwd Rev Probe Probe	CCTGCTGGTGCCACTCT CGTCTTTGACGACCCCATTCTT AGTCTTGG**G**CCTTTC (VIC)(mutant) AGTCTTGG**C**CCTTTC (FAM)(wild type)	Manufacturer's Fast RT-PCR settings, Applied Biosystems
H166A (rs1801274)	Fwd Rev Probe probe	CTGGTCAAGGTCACATTCTTCCA GCTTGTGGGATGGAGAAGGT CAGAAATTCTCCC**A**TTTGGA (VIC)(wild type) AGAAATTCTCCC**G**TTTGGA (FAM) (mutant)	Manufacturer's Fast RT-PCR settings, Applied Biosystems

Abbreviation: Polymerase chain reaction (PCR)

specific unlabeled PCR primers and TaqMan® MGB probes [FAM™ and VIC® dye-labeled]) and a sample DNA. The genotyping assay details are listed in Table 4.
Haplotype analysis was performed for HER2 I654V, I655V and A1170P SNPs.

Safety

Reporting

Patients were monitored for toxicity and all toxicities were recorded and graded according to the National Cancer Institute Common Terminology Criteria of Adverse Events version 3.0 (NCI-CTCAE). All cardiac failures, defined as a decrease in LVEF of more than 15 percentage points compared to baseline or an absolute value of LVEF < 45%, Suspected Unexpected Serious Adverse Reactions (SUSAR) and Serious Adverse Events (SAE) were reported from start of the study treatment until the end of the study period at the DC-NKI.

Monitoring

The study was monitored by an independent Clinical Research Associate (CRA) of the DC-NKI. The primary task CRA visited the medical centers and controlled the medical files and Case Report Forms (CRFs). The mission of the CRA was to ensure the safety of patients entered on the study and to guarantee the quality of this study.

Management of side effects

In case of > grade 1 side-effects from candesartan or placebo at the full dose, the dose was halved (i.e. to one tablet per day). When patients developed candesartan associated side-effects > grade half the dose, the patients went off study and stopped taking the study drug. If patients developed an acute worsening or new onset of angioedema or hypersensitivity of the study drug, patients stopped taking the study drug.

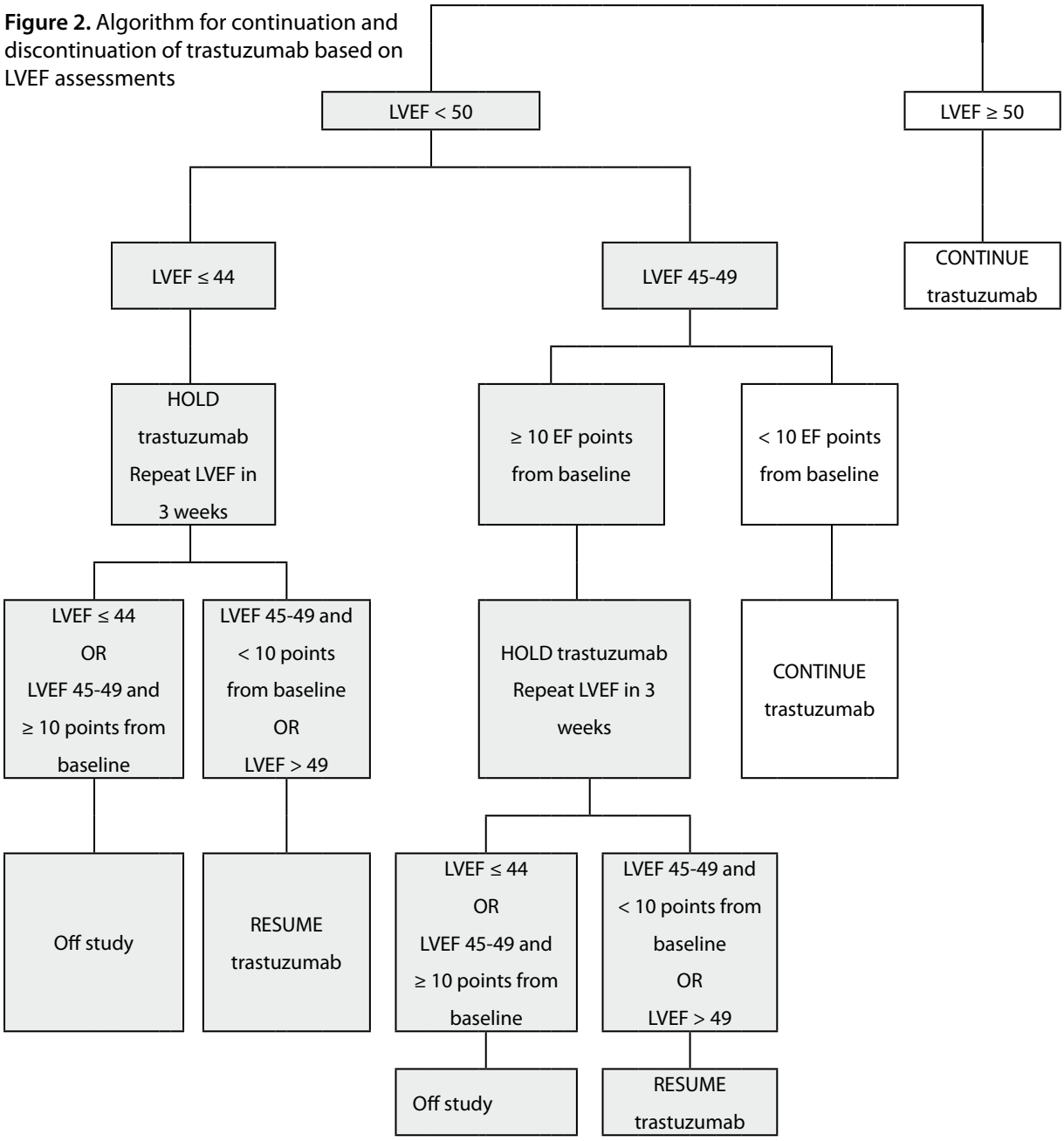
Management of asymptomatic cardiac events

If patients experienced a drop in the LVEF to below 45% or a second (3 weeks after the first assessment) significant drop in the LVEF between 45 – 49%, trastuzumab treatment was discontinued. The decision to (dis)continue trastuzumab treatment was based on the algorithm, as depicted in Figure 2. When patients experienced a significant drop in LVEF, patients were intensively monitored by LVEF, laboratory assessments (cardiac biomarkers) and NYHA score. Patients went off study if trastuzumab treatment was withheld for in total 6 weeks or more because of decline in the LVEF.

Management of congestive heart failure

If patients experienced CHF or NYHA class III or IV cardiac toxicity during trastuzumab treatment, trastuzumab was discontinued. The diagnosis CHF was defined, treated and followed adequately according to the guidelines of the American College of Cardiology/ American Heart Association (31, 32). Patients were evaluated by a cardiologist. Patients who experienced CHF were intensively monitored by LVEF, laboratory assessments (cardiac biomarkers) and NYHA score. CHF confirmed by a cardiologist was reported as a study related SAE.

Figure 2. Algorithm for continuation and discontinuation of trastuzumab based on LVEF assessments



Abbreviations: Left Ventricular Ejection Fraction (LVEF)

Interim analyses

Four interim analyses including the final analysis were planned after 10, 20 and 30 LVEF failures, with the final look at 200 evaluable patients. Treatment allocations were unblinded at the time of planned interim analyses. These interim analysis data were available for an external independent Data Safety Monitoring Committee (DSMC). The DSMC was composed of one statistician, one medical oncologist and one cardiologist. Only the DSMC had access to unblinded data during the study and advised the principal investigators regarding the safety of current participants, those yet to be recruited, as well as the continuing scientific validity of the trial. The statistician at the DC-NKI had access to the assignment of the patients to which treatment arm. However, the statistician was not able to identify whether arm I or

arm II was receiving candesartan at the time of the interim analysis. The statistician prepared the analysis and wrote a confidential report to the DSMC.

Statistical considerations

The primary endpoint of the study was deterioration of the cardiac function defined as a decline in LVEF of more than 15 percentage points from baseline or a decrease of less than 15 percentage points to an absolute value below 45%, during the year of treatment and during the 40 weeks after discontinuation of trastuzumab treatment. From previous studies it was estimated that about 30% of the patients treated with trastuzumab will show cardiac deterioration (33).

A sample size of 100 patients in each study arm would provide 80% power (α 0.05) to detect a significant reduction in cardiotoxicity rate from 0.30 to 0.13 (odds ratio 0.35). The EAST software package was used to calculate the stopping boundaries for the interim tests. The Lan and DeMets error spending function resembling the Pocock boundary, were used to find the stopping boundaries for safety. The overall one-sided significance level of the safety tests was set to be 0.05. The stopping boundaries are depicted in Table A.

Table A

Information Fraction	α -Spent	Boundary to Reject H0
0.250	0.018	-2.100
0.500	0.031	-2.077
0.750	0.041	-2.053
1.000	0.050	-2.035

For each interim analysis the logrank test on all available patient data was used. The final analysis will be after the last patient has been followed for 92 weeks. The final analysis will use the Chi square test with the squared boundary from the logrank test.

Results

Patient characteristics

A total of 196 patients were enrolled between October 2007 and October 2010 in 19 Dutch hospitals. The accrual rate is presented in Figure 3. At the time of the first interim analysis, 63 patients had been randomly assigned to candesartan or placebo and, the locked database file of June 30, 2009 was used. On 7 December 2009, the DSMC advised the principal investigators to continue this clinical trial. At the time of this report, 175 patients had been randomly assigned to candesartan or placebo. For the current report however the locked database file of July 13, 2010 was used. Our report is based on the data evaluation at the second interim analysis but results are not available by treatment arm. One hundred-ten patients were randomized before 1 January 2010. Three patients were not evaluable because their LVEF value was not measured after randomization. A total of 107 patients were evaluable for this study. The LVEF value was evaluated at least once after start of the study treatment. The median follow-up (i.e. time between randomization and last LVEF measurement) was 8.5

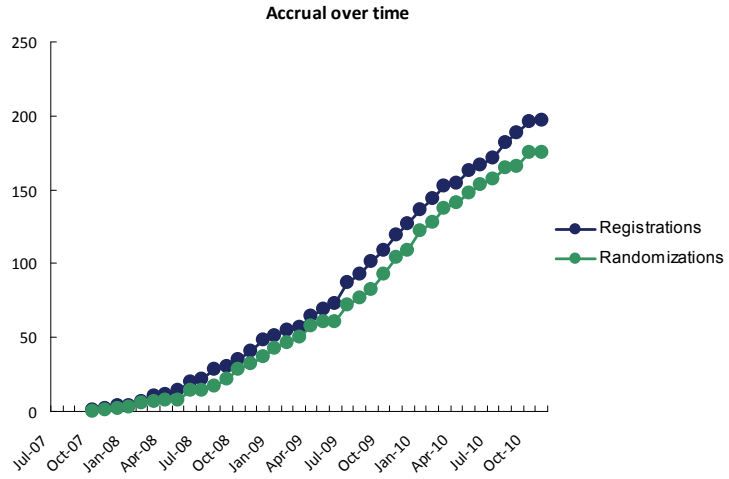


Figure 3. Patient Accrual

months. The median age at baseline was 50 years; range 25-67 years. The median baseline (after anthracyclines and prior to start of trastuzumab treatment) LVEF value was 60; range 50-82. After 12 weeks of trastuzumab, the median LVEF value was 58; range 26-77. Changes in LVEF values from baseline to week 52 in the total study population are shown in Figure 4.

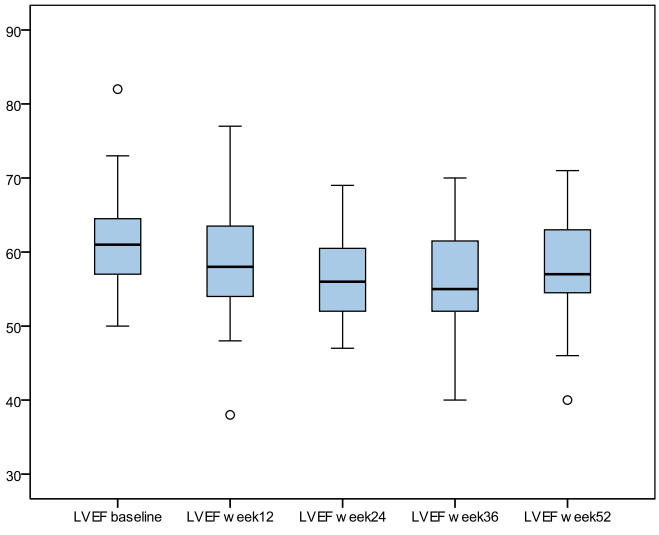


Figure 4. Changes in LVEF values from baseline

Cardiac events

In total 20 cardiac events were observed in 107 evaluated patients, defined as a decline in LVEF of more than 15 percentage points compared to baseline or a decrease in LVEF to an absolute value below 45%, after a median time on trastuzumab of 25 weeks; range 11-92 weeks. The number of cardiac events is shown in Figure 5.

Serious Adverse Events

In total 31 SAEs were reported at the DC-NKI. Twenty-five SAEs were reported as not related with the study treatment and 6 SAEs were reported as unlikely related with the study

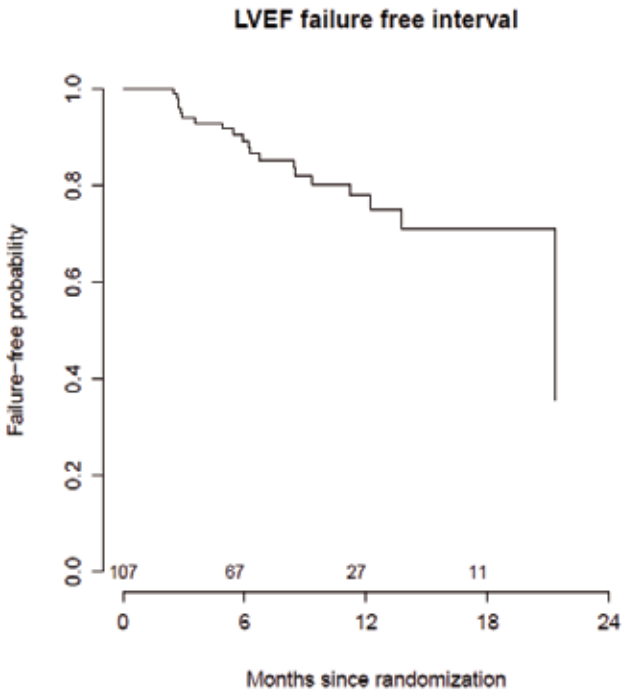


Figure 5. Number of cardiac events by CANDY definition

treatment. Unlikely related SAEs were febrile neutropenia (n=2), erysipelas (n=1), embolism (n=1) and infection (n=1). There was no SUSAR reported.

Off study reasons

In total 33 patients went off study prematurely. Eleven patients completed study treatment according to the study protocol. Other reasons were a significant decrease in LVEF (n=10), advanced disease (n=3), adverse events (n=3) such as hypotension, dizziness, myalgia and peripheral tibial pitting edema, non-compliance with study medication (n=1), withdrawal of informed consent (n=1), unknown (n=1) and 2 patients wished not to continue study treatment.

Discussion

The tentative results of the CANDY study at its second safety analysis after 20 cardiac events are presented. These results are based on the total study population and are not presented by treatment arm, as this is an ongoing study and unblinding would introduce an unacceptable bias. At the time of the first interim analysis, in total 11 cardiac events were observed in 63 patients treated with candesartan or placebo. The second interim analysis was assessed after 20 reported LVEF failures in, in total 107 patients treated with candesartan or placebo. The demographic characteristics such as age and median baseline LVEF value in our study did not differ from those reported in previous adjuvant clinical studies (5, 6).

The rate of trastuzumab-related cardiac events observed at the first and second interim analysis is in line with the rate of cardiac events observed in other trastuzumab trials (5-7). Evidently, the blinded data of this study limits comparison with the published study results. At

the first and second interim analysis the statistical stopping boundaries were not exceeded. Therefore, the DSMC advised the principal investigators to continue this clinical trial. Based on these blinded data, the effect of candesartan in the prevention of trastuzumab-associated cardiotoxicity in early breast cancer patients is not disclosed. The database will be unblinded 92 weeks after the last included patient, which will allow drawing conclusions on the activity of candesartan in limiting trastuzumab-related cardiotoxicity.

In conclusion, trastuzumab treatment is related with asymptomatic cardiotoxicity in patients treated according to the CANDY study protocol. The number of cardiac events is in line with that reported in previous clinical trials. Therefore, there is currently no evidence so far that intervention with candesartan treatment during trastuzumab treatment is associated with an increased rate of cardiac events. The next, and last, safety analysis will be performed after 30 cardiac events. Unblinding of the database of the CANDY study is expected in May 2013. Longer follow-up of more patients and unblinding of the database is expected to allow a balanced risk-benefit assessment of the use of candesartan in patients treated with trastuzumab.

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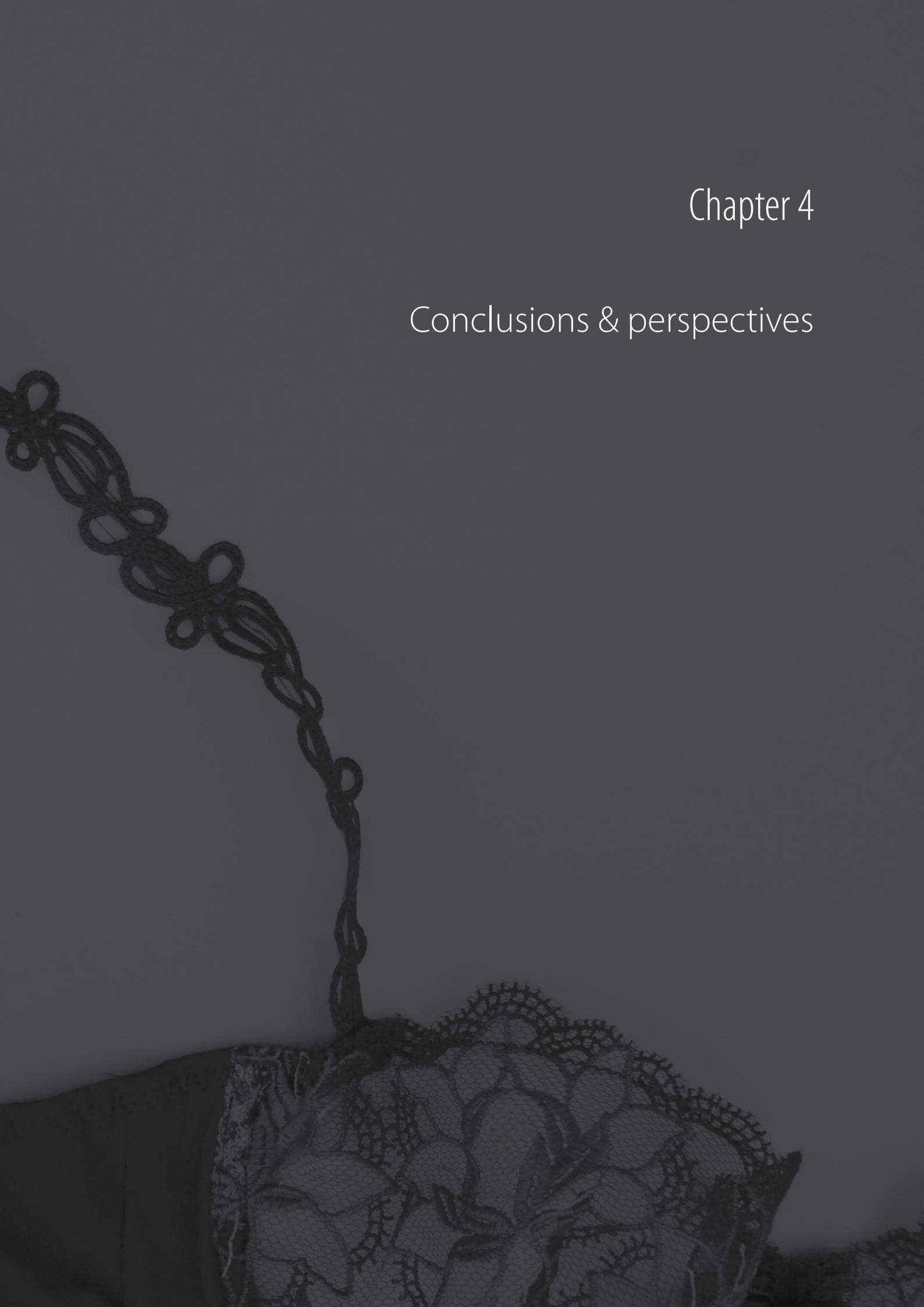
Chapter 4

Conclusions & perspectives



Conclusions and Perspectives

Studies in this thesis are focused on two main topics in the palliation of symptoms and the treatment of early and advanced breast cancer, i.e. the evaluation and management of treatment-induced hot flashes and the investigation of new options in the prevention and detection of trastuzumab-related cardiotoxicity.

Hot flashes are the most often occurring complaints in pre- and postmenopausal breast cancer patients. They are significantly more frequent and more severe in breast cancer patients than in women without breast cancer. Many agents have been investigated as potential means for alleviating hot flashes in survivors of breast cancer (chapter 2.1). However, limitations of most trials were the absence of a placebo group and short follow-up. This emphasized the need for larger-scale and long-term prospective trials to test the efficacy and safety of selected non-pharmacological and pharmacological strategies in the management of hot flashes. Chapter 2.2 describes a prospective randomized double blind placebo-controlled three-armed trial comparing clonidine, venlafaxine and placebo in the management of hot flashes in women with a history of breast cancer. The main conclusion of this study is that both drugs reduce the hot-flash score in women after a diagnosis of breast cancer. Venlafaxine treatment results in a more immediate reduction of hot-flash scores versus clonidine and placebo and is more effective in comparison to clonidine during 12 weeks of treatment. The main side-effects are nausea, constipation and appetite loss. On the basis of our results we advise the following treatment strategy: start with venlafaxine 75 mg daily. However, in case of side-effects it is advised to reduce the venlafaxine dose to 37.5 mg or to start with clonidine at a daily dose of 0.1 mg.

Venlafaxine and clonidine are both often investigated and prescribed treatments in the management of hot flashes. There are well controlled published clinical trials assessing the role of venlafaxine and clonidine in the management of hot flashes. However currently there are no published data of a three-arm trial comparing clonidine, venlafaxine and placebo in breast cancer patients, nor are data available concerning longer term duration of treatment effect. Therefore, we investigated two often prescribed treatments. The duration of our study was based on guidance of the United States Food and Drug Administration for development of hormone products for menopausal symptoms. We demonstrated a dramatic change of the efficacy of both treatments in our study during a study period of 12 weeks. Based on these findings, hot flash trials with two active drugs and placebo should be continued at least 12 weeks due to changes in efficacy over longer treatment periods. However, the effectiveness of both treatments for durations of longer than 12 weeks is unknown. A study period longer than 12 weeks might be evidently better. The study duration might be critical in investigating the efficacy of interventions in the management of hot flashes and should be taken into account in the design of future clinical studies.

We observed a substantial placebo effect as the reduction of hot-flash scores over the entire 12 week period was 29% in the placebo group. In clinical trials this observation is a consistent effect, which provides further evidence that a placebo group as control is required in hot flash trials for interpreting the effects of the medication.

This study may be judged as too small in view of the limited sample size. However, on the basis of the predicted size of the treatment effect statistically significant and clinically relevant differences could be demonstrated, thereby supporting the adequacy of the a priori selected statistical power and sample size of this study. A challenge in the investigation of pharmacological interventions in the management of hot flashes will be the recruitment of patients for clinical studies. Recruiting patients for clinical studies is often a limiting factor in the speed of the execution of the trial. Small sample sizes undermine statistical power and increase the probability of bias.In many studies on the management of hot flashes, recruitment of patients was difficult and slow. As our study largely was a single-center study, with help of one other regional hospital, it is recommended to start directly with a large multicenter study.

Currently, gabapentin and venlafaxine have been shown to be both effective and well tolerated treatments in the management of hot flashes. Recently, a study by Bordelau et al. concluded that patients preferred venlafaxine treatment at a dose of 75 mg daily over gabapentin treatment at a dose of 900 mg daily (1). Further, long-term, well-controlled clinical trials are needed to investigate the efficacy of venlafaxine and gabapentin versus placebo in the management of hot flashes in breast cancer patients.

This finding brings us to an important point: The Dutch guideline on the management of hot flashes in patients who are contraindicated for the most effective pharmacological therapy, hormone replacement therapy. Currently, venlafaxine is considered the drug of choice and appears to be the most potent drug in the management of hot flashes, which effect is combined with an acceptable safety profile. Although, venlafaxine treatment is often recommended in international guidelines, only clonidine is recommended as pharmacological intervention in the management of hot flashes in the Dutch guidelines of general practitioners. Findings of this study, might eventually lead to a prioritization of venlafaxine as pharmacological intervention in the treatment of hot flashes in Dutch guidelines. Preclincial studies have extensively addressed the interaction between venlafaxine, a known albeit weak CYP2D6 inhibitor, and the important SERM tamoxifen, a prodrug activated partly via CYP2D6. The interaction does not appear to be clinically relevant. Thus, also in patients on tamoxifen venlafaxine can be safely prescribed in the treatment of hot flashes.

Trastuzumab has been shown to benefit patients with HER2 positive advanced breast cancer in terms of longer time to disease progression, longer duration of response and a longer overall survival. The addition of trastuzumab to the (neo) adjuvant treatment setting resulted in an absolute reduction in the risk of recurrence or death. Side-effects of trastuzumab treatment are often mild and mostly manageable. The major side-effect of trastuzumab is decline in left ventricular ejection fraction (LVEF), in a small proportion of patients even leading to advanced congestive heart failure (CHF). In chapter 3.1, the indications for trastuzumab treatment, the clinical application, the registered side-effects and clinical guidelines of trastuzumab treatment were reviewed. Chapter 3.2 describes a retrospective study of trastuzumab treatment in an unselected patient population. In this study, we found a statistically significant association between prior anthracycline-based treatment and trastuzumab-

induced asymptomatic cardiac dysfunction. Reversibility of declines in LVEF values was not complete in 22% of patients after discontinuation of trastuzumab for 2.5 months. Several factors might have attributed to these study results. First, the results of the LVEF values are sensitive for identifying asymptomatic cardiotoxicity, however, actual cardiac damage may not be detectable by measurements of the LVEF which can result in an underestimation of trastuzumab-induced cardiotoxicity. Even though this study is representative for estimating the incidence of trastuzumab-associated cardiotoxicity in clinical practice, the retrospective design of this study could have resulted in an under- or overestimation of the changes in LVEF values. Incomplete follow-up information of the patient, an unknown preexisting cardiac disease and the use of anthracyclines can influence the cardiotoxicity determination. However, clinical trials adhere to strict eligibility criteria and patients treated in clinical trials are fit and often not representative of a general unselected patient population. Data of this study demonstrate the implications of trastuzumab treatment in a patient population that may suffer from significant co-morbidity. Clearly, the incidence of cardiotoxicity as measured by the LVEF in the patients treated with trastuzumab is high and higher than in the well selected patient population of the registration trials.

Findings of this study were confirmed in the long-term safety study of trastuzumab treatment in primary breast cancer patients (chapter 3.3). The severity and incidence of trastuzumab-related cardiotoxicity is associated with prior or concomitant anthracycline-based chemotherapy. Cardiac dysfunction is only partly reversible despite medical treatment. Troponin values were not statistically significantly associated with changes in LVEF values or cardiac events. In the total patient population and in the anthracycline-based treatment group, changes in NT-proBNP values were related with changes in LVEF values. We noticed that the retrospective part (the chemotherapy- and trastuzumab treatment period), was to some extend a limitation of this study. Several patients were suffering with physical symptoms such as dyspnea d'effort, palpitations, fatigue and ankle edema during trastuzumab treatment. In this study CHF was defined as, clinical signs and symptoms of CHF, however, traditional endpoints of signs and symptoms of CHF were not consequently reported in the medical records. The lack of prospective data during the chemotherapy- and trastuzumab treatment might result in an under- or overestimation of CHF. The lack of the association between troponines and changes in LVEF values might be related with the timing of blood sample collection. The use of the troponines was more successful in studies where blood sampling was obtained prior to chemotherapy, prior to-, during and after trastuzumab treatment. Nevertheless, the prospective long-term data gives a good approximation of trastuzumab-related cardiotoxicity in unselected patients long-term after trastuzumab treatment. We demonstrated a significant association between changes in NT-proBNP and changes in LVEF values however larger and longer follow-up studies are needed to translate these findings into guidelines to be used in clinical practice.

Chapter 3.4 describes the safety of trastuzumab treatment in patients with a low LVEF value prior to trastuzumab treatment. For safety reasons clinical guidelines exclude patients with baseline LVEF values below 50%, possibly resulting in exclusion of patients from receiving trastuzumab even though they might benefit from treatment.The main conclusion of this study is that treatment of patients with trastuzumab and a low baseline LVEF value appears

to be feasible, however referral to a cardiologist and strict monitoring of cardiac function are recommended. Cardiac events are significantly more common and cardiotoxicity is sometimes severe in these selected patients. A limitation of this study is the small sample size and the retrospective design. Follow-up of large cohorts and more prospective data are needed for a balanced benefit- and risk assessment of trastuzumab treatment in patients with a low baseline LVEF value.

Several clinical questions in relation to trastuzumab-associated cardiotoxicity are described in this thesis. These and previously reported findings suggest a higher incidence of cardiac dysfunction in patients treated with anthracyclines prior or concurrently with trastuzumab. However, several unanswered clinical questions remain 1) how to translate reported risk factors of trastuzumab treatment?, 2) what are optimal cardiac monitoring strategies?, 3) what are the optimal stopping and re-initiation algorithms of trastuzumab in relation to recovery time after a cardiac event?, 4) what is the effect of dose reduction of trastuzumab? and, 5) what is the cardiac safety of trastuzumab treatment at long-term (i.e. progressive disease)? Clearly, there is a need for guidelines to select patients who are at risk for development of cardiac dysfunction. A pharmacokinetic-pharmacodynamic (PK-PD) model that we have successfully developed could be used for an optimization of cardiac monitoring strategies during trastuzumab treatment (chapter 3.6). The main conclusion of this study is that this proposed PK-PD model adequately described the relationship between trastuzumab exposure and decline in LVEF values, incorporating the effect of the cumulative dose of prior anthracycline therapy. This PK-PD model can already be used to investigate optimal cardiac monitoring strategies during trastuzumab treatment. This model was based on a large representative patient population in daily clinical practice. Data of patients treated with or without anthracycline-based chemotherapy both in the metastatic and adjuvant setting were included. Multiple covariates were evaluated for a possible role in a decrease in LVEF during trastuzumab treatment. The only covariate predicting cardiotoxicity and recovery of cardiotoxicity was the cumulative dose of anthracyclines of prior chemotherapeutic treatment. However, a possible limitation of this model is the absence of data on the LVEF prior to anthracycline treatment, which might overestimate the cardiotoxicity of trastuzumab treatment, since a significant portion of the damage might be inflicted already during anthracycline treatment. Prior to implementation into clinical practice, the current PK-PD model should be prospectively validated. Simulation of large 'virtual' patient cohorts is needed to investigate optimal clinical management of trastuzumab-related cardiotoxicity such as alternative dosing regimens or toxicity management guidelines.

Chapter 3.6 describes the second interim analysis of a prospective pharmacological intervention study with candesartan treatment or placebo during adjuvant trastuzumab treatment (CANDY study). Since data remains blinded, the effect of candesartan in the prevention of trastuzumab-associated cardiotoxicity in early breast cancer patients is not disclosed and only known to the members of the Drug Safety Monitoring Board. The rate of trastuzumab-related cardiac events observed at the first and second interim analysis of the CANDY study is in line with the rate of cardiac events observed in other trastuzumab trials.

This multicenter trial need to be continued, which will hopefully allow drawing conclusions on the activity of candesartan in limiting trastuzumab-related cardiotoxicity.

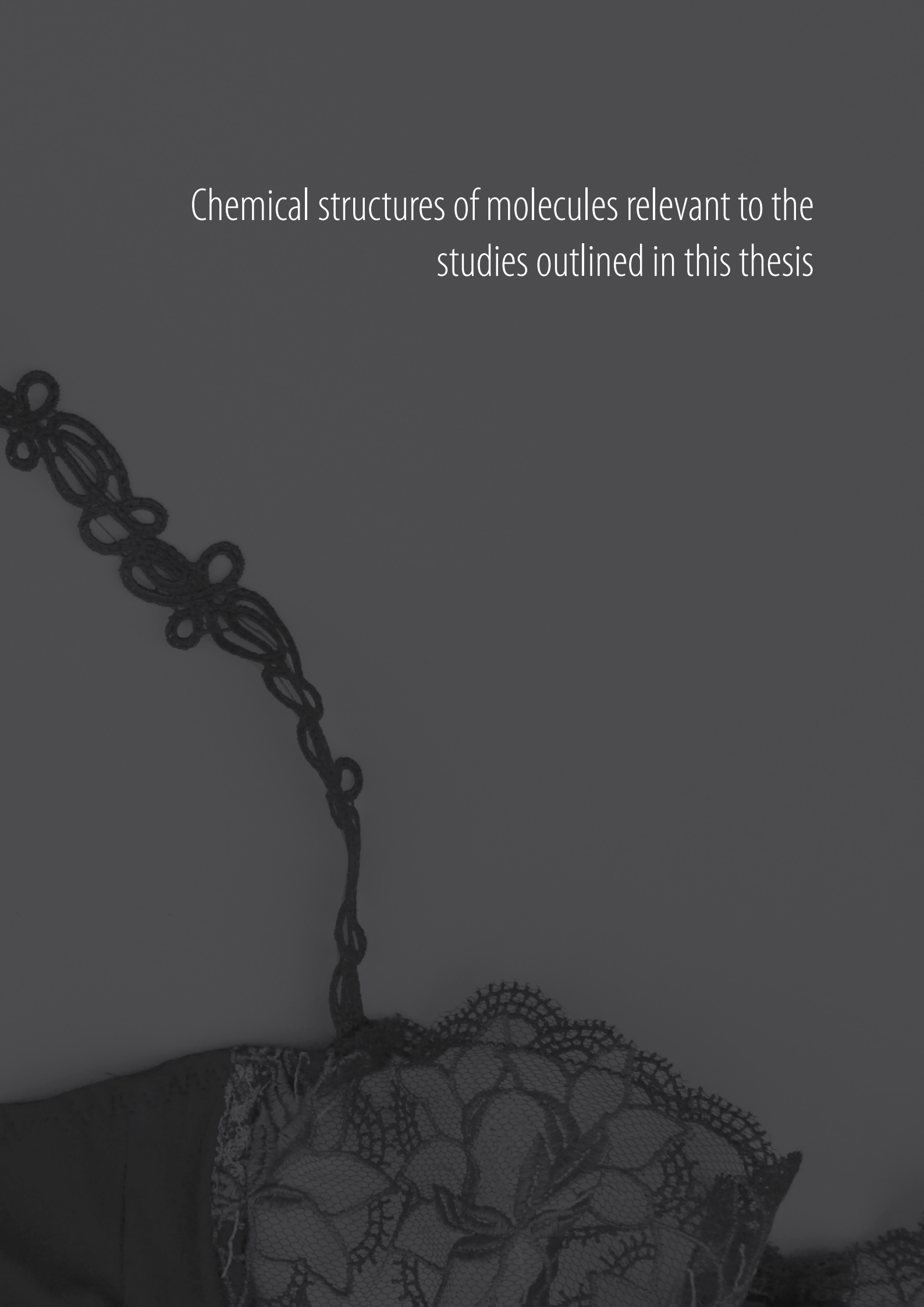
In this two armed study, 100 patients per arm will be evaluated. The sample size is based on the assumption that 30% of patients in the placebo group will develop a cardiac event and 13% in the candesartan groep (α=0.05, β=0.8). However, in one of the above described studies, the incidence of cardiac events was lower than 30%, as in several other studies. This might hamper detection of a significant effect of candesartan treatment on the reduction of cardiac events and might necessitate to increase the sample size, which will be considered after the current study has been completed.

Finally, important limitations in the interpretation of trastuzumab-related cardiotoxicy in the reported trials are differences in the applied treatments, in inclusion and exclusion criteria and in the applied definition of cardiotoxicity or study endpoints. In order to fully understand trastuzumab-related cardiotoxicity, there is a need for more standardization, prospective validation and integration of novel end-points and of simple and reproducible methods for the diagnosis, such as cardiac biomarkers. New cardiac imaging techniques and biomarkers could enable more accurate monitoring of the short- and long-term trastuzumab treatment related cardiac side-effects.

Reference

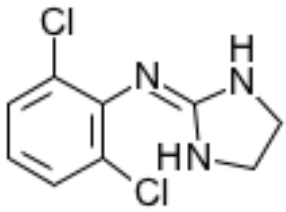
(1) Bordeleau L, Pritchard KI, Loprinzi CL et al. Multicenter, Randomized, Cross-Over Clinical Trial of Venlafaxine Versus Gabapentin for the Management of Hot Flashes in Breast Cancer Survivors. J Clin Oncol 2010 Nov 8 (Epub ahead of print).

Chemical structures of molecules relevant to the studies outlined in this thesis

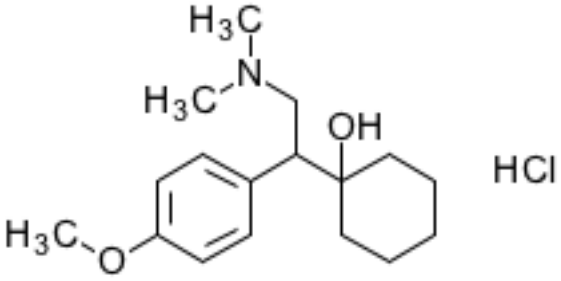


Chapter 2.2: clonidine and venlafaxine

Clonidine

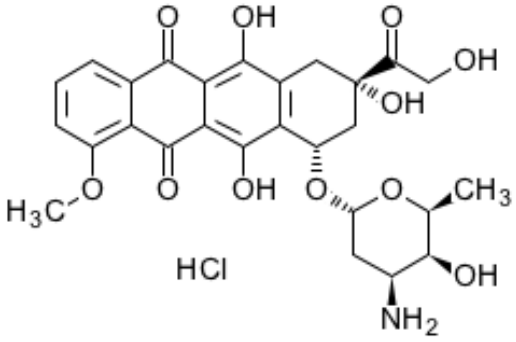


Venlafaxine

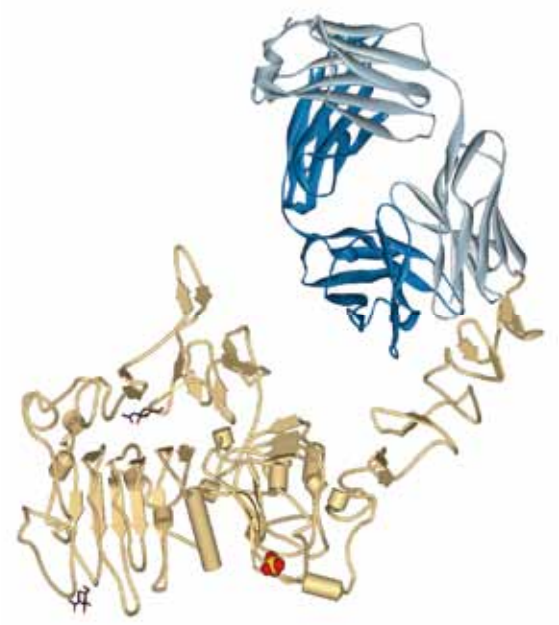


Chapter 3.1: doxorubicin, epirubicin and trastuzumab

Doxorubicin, Epirubicin

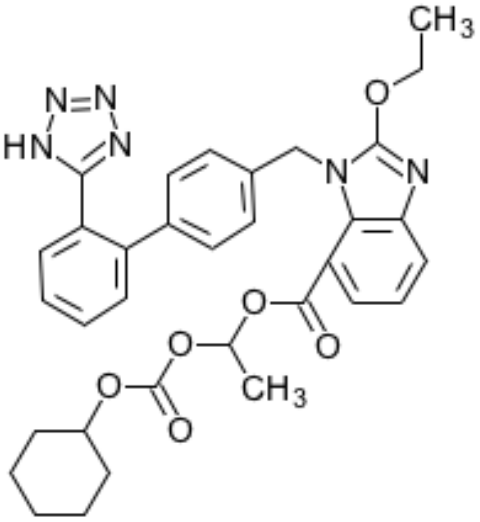


Trastuzumab

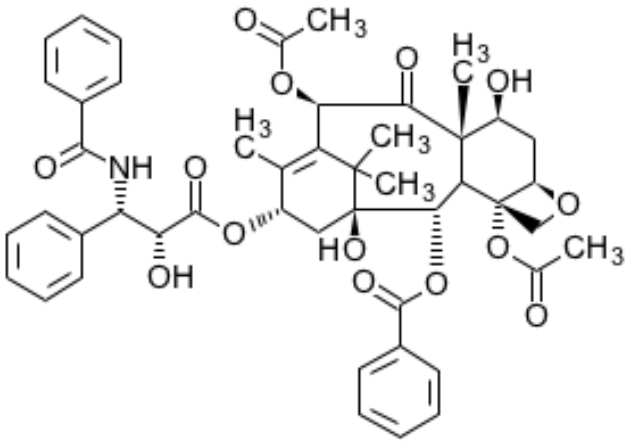


Chapter 3.6: candesartan, paclitaxel, tamoxifen, anastrozole, exemestane and letrozole

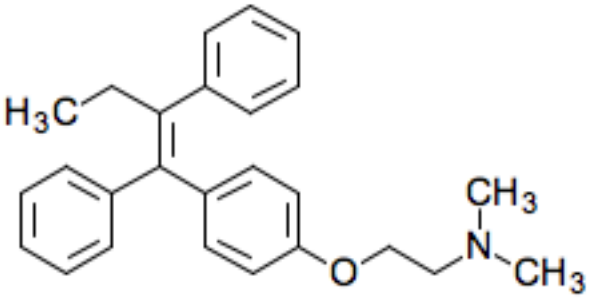
Candesartan



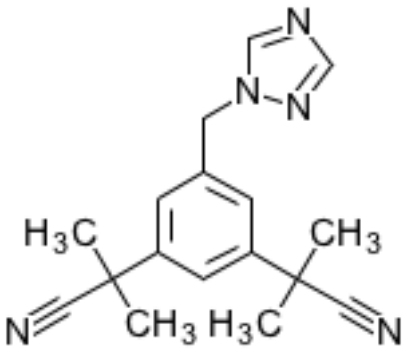
Paclitaxel



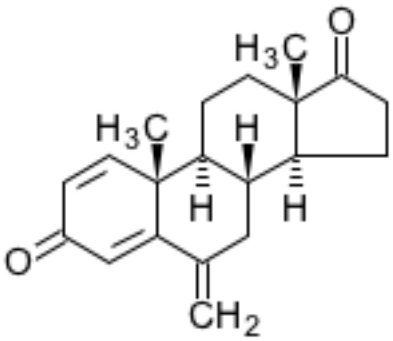
Tamoxifen



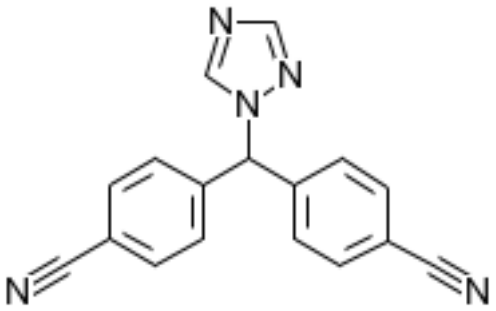
Anastrozole



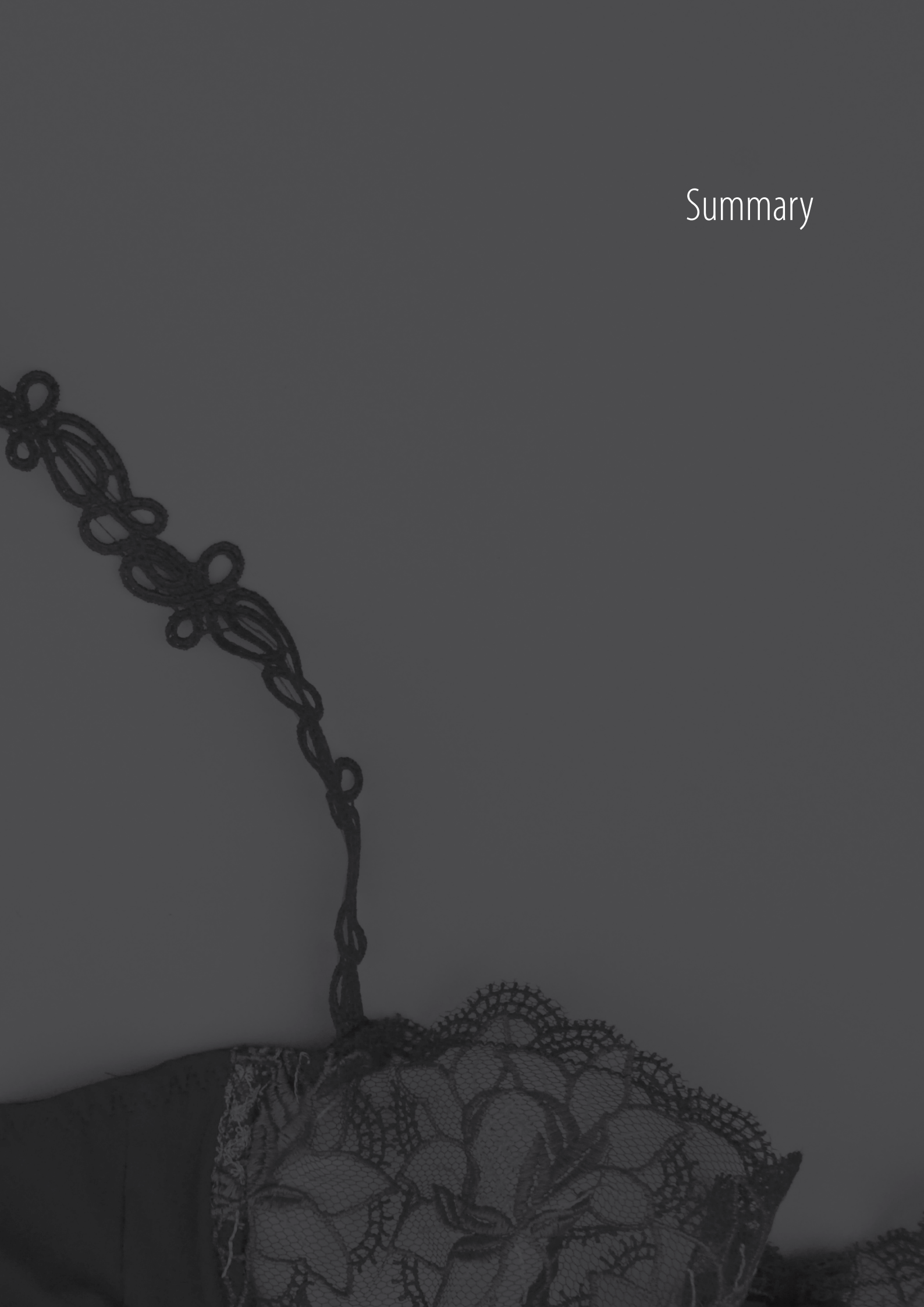
Exemestane



Letrozole



Summary



Summary

Breast cancer is one of the most common malignant diseases. Adjuvant systemic therapies such as chemotherapy, immunotherapy and endocrine therapy play an important role in the treatment of breast cancer. These therapies reduce the risk of relapse of breast cancer and increase cure rates. However, these therapies are associated with short- and long-term side-effects. Sometimes these side-effects are irreversible and disabling, which may affect the quality of life, treatment continuation and treatment adherence.

Menopausal complaints such as hot flashes, genitourinary atrophy and mental distress occur in pre- and postmenopausal breast cancer patients. Hot flashes are among the most frequent menopausal symptoms. Several pharmacological and non-pharmacological interventions in the management of hot flashes in survivors of breast cancer have been investigated. Well-controlled clinical trials testing new treatments that moderate hot flashes are increasingly important. Effective treatments may improve quality of life and continuation of anti-cancer therapies and may thereby improve treatment outcome.

Trastuzumab improves treatment outcome in early and advanced HER2 positive breast cancer and advanced gastric cancer. However, this humanized monoclonal antibody, is associated with the development of cardiac dysfunction. Risk factors for acute and long-term trastuzumab-related cardiotoxicity are unknown, although anthracycline treatment has been identified as a contributing factor. The development of methods to prevent or alleviate cardiac dysfunction is of major relevance.
In this thesis new treatment options for the management of hot flashes, the cardiac safety of trastuzumab treatment and new modalities for the detection and prevention of trastuzumab-related cardiotoxicity are described. The subjects of this thesis, i.e. menopausal-induced symptoms and trastuzumab-induced cardiotoxicity, are closely related, as they are both induced by the treatment of breast cancer, may be severe and disabling and could lead to reduced quality of life, treatment adherence, and negatively affect the outcome of breast cancer treatment.

After a general introduction (**Chapter 1**), **Chapter 2.1** reviews the epidemiology and diagnosis of hot flashes and the non-pharmacological and pharmacological strategies in the management of hot flashes described thus far. Several complementary and alternative medicines have been investigated as potential means for alleviating hot flashes in survivors of breast cancer, such as black cohosh, phytoestrogens, homeopathy, vitamin E, acupuncture and behavioural strategies. Many frequently used pharmacological agents have also been studied such as progestagens, clonidine, gabapentin and antidepressants. However, evidence on the efficacy of complementary and alternative therapies is limited and studies are limited by the absence of a placebo control group and short study durations. Therefore, there is a need for larger-scale and long-term placebo controlled prospective trials to test the efficacy and safety of non-pharmacological and pharmacological strategies in the management of hot flashes.

Chapter 2.2 describes the results of a prospective randomized double blind three-armed trial comparing clonidine, venlafaxine and placebo in the management of hot flashes in women with a history of breast cancer. Patients were randomly assigned to venlafaxine 75 mg, clonidine 0.1 mg or placebo in a daily dose for 12 weeks. Venlafaxine treatment resulted in a more immediate reduction of hot-flash scores when compared with clonidine, however hot-flash scores at week 12 were lower in the clonidine group than in the venlafaxine group. Frequencies of treatment-related side effects were higher in the venlafaxine group. The main conclusion of this study was that both drugs reduced the hot-flash scores in women after a diagnosis of breast cancer. However, since venlafaxine showed a better reduction of hot flashes score over the whole treatment period of 12 weeks, venlafaxine might be the preferred treatment.

In **Chapter 3.1** an overview of the pharmacokinetics, pharmacodynamics, pharmacogenomics, clinical monitoring, side-effects and efficacy of trastuzumab treatment is presented. Trastuzumab is a humanized monoclonal antibody and is only effective in patients with an overexpression of the HER2 protein. Overall, trastuzumab treatment is well tolerated, however cardiac dysfunction is one of the clinically relevant side-effects manifested by left ventricular systolic dysfunction and in a small proportion of patients even in advanced congestive heart failure. Although, trastuzumab treatment improved disease-free and overall survival, a part of the HER2 positive breast cancer patients did not respond to trastuzumab treatment. There are few data on treatment modalities for patients with HER2 positive breast cancer that progressed after trastuzumab treatment. Several possibilities to treat patients beyond progression and new HER2 directed therapies are discussed. However, further research into the long-term side-effects of trastuzumab treatment and the development of robust, reproducible, sensitive and easily assessable parameters to detect or predict trastuzumab-induced cardiotoxicity is needed.

Chapter 3.2 describes the incidence and reversibility of trastuzumab-related cardiotoxicity and the association between risk factors and the development of trastuzumab-associated cardiotoxicity in an unselected early breast cancer patient population. In this study, trastuzumab treatment was associated with significant and partly reversible cardiotoxicity. The combination of anthracyclines and trastuzumab increased the incidence of asymptomatic declines in left ventricular ejection fractions (LVEF). Furthermore, the baseline LVEF value was associated with the incidence of cardiac events. However, we found no statistically significantly association between potential risk factors for the development of cardiotoxicity, such as age, high body mass index and use of antihypertensive drug or co-morbidity and the incidence of cardiac events. More research is needed to evaluate, predict and improve long-term cardiac safety after trastuzumab treatment.

Chapter 3.3 describes results of the safety of trastuzumab treatment in unselected primary breast cancer patients. In this study we recruited breast cancer patients after adjuvant trastuzumab treatment, to determine long-term tolerability, cardiac safety, and parameters to detect or predict trastuzumab-induced cardiotoxicity. The severity and incidence of trastuzumab-associated cardiotoxicity was related with prior or concomitant exposure to

anthracyclines. Cardiac dysfunction was partly reversible. In this study, serum biomarkers such as NT-proBNP and the troponines were not statistically significantly related to trastuzumab-associated cardiotoxicity or recovery. Moreover, we found no significant relationship between selected single nucleotide polymorphisms in the HER2 gene and trastuzumab-associated cardiotoxicity. However, longer follow-up studies in large patient populations are needed to evaluate long-term tolerability and cardiac safety of trastuzumab treatment.

Chapter 3.4 describes the cardiac safety of trastuzumab treatment in patients with a low baseline LVEF value prior to start of trastuzumab. Patients treated with trastuzumab for HER2 positive early or advanced breast cancer with a baseline LVEF below 50% were retrospectively identified. In this study population patients experienced significant and sometimes severe and only partially reversible cardiotoxicity based on LVEF measurements during trastuzumab treatment. However, all observed cardiac events were considered asymptomatic and there were no cardiac related deaths. Longer follow-up of larger cohorts and more prospective data will be needed to evaluate the cardiac safety of trastuzumab in patients with a baseline LVEF below 50%. However, our preliminary results indicate that trastuzumab could be offered under strict cardiac monitoring to patients with a low baseline value of LVEF in order to improve the prognosis of HER2 positive breast cancer.

The development of a pharmacokinetic-pharmacodynamic (PK-PD) model is described in **Chapter 3.5.** This PK-PD model describes the relationship between trastuzumab exposure and LVEF declines, in patients with early and advanced breast cancer. The main finding of this PK-PD model was the unique significant relationship between the cumulative anthracycline dose and the sensitivity to development of cardiotoxicity defined, by a decline in the LVEF value. Ultimately, this developed PK-PD model can be used to address clinical questions regarding optimal management of trastuzumab-associated cardiotoxicity and to develop monitoring strategies.

Chapter 3.6 presents the tentative blinded results of a prospective, randomized, placebo-controlled, pharmacological intervention study (CANDY) up to its second interim analysis. In this study, patients were treated concurrently with adjuvant trastuzumab and an angiotensin II-receptor blocker (candesartan) for HER2 positive breast cancer. The aim of this study was to investigate whether the concurrent use of candesartan in patients treated with trastuzumab can prevent or ameliorate trastuzumab-associated cardiotoxicity. Since an interim analysis is described in this chapter, the results of this study were based on the total study population and were not presented by treatment arm. Trastuzumab-related cardiac events were observed in patients treated according to the CANDY study protocol. Statistical stopping boundaries for safety were not exceeded at the first and second safety interim analysis. Therefore, the data safety monitoring committee advised the principal investigators to continue this clinical trial. Currently, based on these blinded data, the effect of candesartan in the prevention of trastuzumab-associated cardiotoxicity in early breast cancer patients can not be disclosed. Unblinding and final results of this study are expected in May 2013.

In conclusion, although effective, breast cancer treatment is associated with multiple side effects, such as hot flashes in premenopausal women and cardiac dysfunction. These side effects can be severe and might result in discontinuation of treatment, which may be associated with worse outcome. Hot flashes induced by systemic therapy of breast cancer can be alleviated by clonidine and venlafaxine treatment. Although clonidine treatment showed a superior reduction in hot flash scores at the primary endpoint, the twelfth week of treatment, venlafaxine was more effective during the whole treatment period.
Several studies into the cardiac safety of trastuzumab treatment are described in this thesis. Trastuzumab treatment can result in decreased left ventricular function and in a small proportion of patients in congestive heart failure. Only prior or concomitant treatment with anthracyclines was identified as a significant predictive factor of cardiac dysfunction. However, trastuzumab induced cardiac toxicity was partly reversible also in patients with a decreased left ventricular function prior to treatment with trastuzumab. A study into the efficacy of an angiotensin II-receptor blocker to prevent myocardial damage is currently ongoing.

Nederlandse samenvatting

Samenvatting

Borstkanker is één van de meest voorkomende vormen van kanker. Aanvullende (adjuvante) systemische therapieën zoals chemotherapie, immunotherapie en hormonale therapie spelen een belangrijke rol in de behandeling van borstkanker. Deze behandelingen reduceren het risico op terugkeer van de ziekte en verhogen de kans op genezing. Echter, deze behandelingen kunnen zowel op de korte als ook op de lange termijn bijwerkingen veroorzaken. Deze bijwerkingen zijn soms irreversibel wat invloed kan hebben op de kwaliteit van leven, de voortzetting van de behandeling en de therapietrouw.
Menopausale klachten komen vaak voor bij pré- en postmenopausale borstkankerpatiënten, zoals opvliegers, veranderingen van genitale slijmvliezen (atrofie) en psychische klachten. Van deze menopausale klachten komen opvliegers het meest frequent voor. Verschillende farmacologische en niet-farmacologische interventies zijn op hun werkzaamheid tegen klachten van opvliegers bij borstkankerpatiënten onderzocht. Het wordt steeds belangrijker om voor de behandeling van opvliegers nieuwe middelen te onderzoeken binnen klinische studies. Effectieve behandelingen zullen de kwaliteit van leven, de therapietrouw en de mogelijkheid tot het continueren van een anti-kanker behandeling verbeteren en daardoor uiteindelijk een verbetering van de behandeluitkomst bewerkstelligen.
Trastuzumab, een monoklonaal antilichaam, verbetert de behandeluitkomst van uitgezaaide maagkanker en van een primaire of uitgezaaide HER2-positieve borstkanker. Het ontwikkelen van hartschade is één van de bijwerkingen van een behandeling met trastuzumab. De belangrijkste risicofactor voor het ontwikkelen van trastuzumab-gerelateerde hartschade is een voorafgaande of gelijktijdige behandeling met anthracyclines. Echter, mogelijk zijn er andere risicofactoren. Het is van groot belang om nieuwe methoden te ontwikkelen waardoor hartschade kan worden voorkomen, of waardoor de ernst van de hartschade kan worden beperkt.
In het eerste deel van dit proefschrift worden nieuwe behandelopties in de bestrijding van opvliegers beschreven en in het tweede deel de veiligheid van een behandeling met trastuzumab en nieuwe mogelijkheden voor het diagnosticeren en voorkomen van trastuzumab-gerelateerde hartschade. De onderwerpen van dit proefschrift, te weten menopauze-geassocieerde klachten en trastuzumab-geinduceerde hartschade, vertonen een belangrijke samenhang, nl dat het bijwerkingen betreffen van borstkankerbehandeling die soms ernstig en invaliderend zijn, de kwaliteit van het leven en therapietrouw aantasten en een ongunstig effect kunnen hebben op de uitkomst van de therapie van borstkanker.

Na de inleiding (**hoofstuk 1**), wordt in **hoofdstuk 2.1** een overzicht gegeven van de epidemiologie en diagnostiek van opvliegers inclusief een overzicht van de niet-farmacologische en farmacologische behandelmogelijkheden in de bestrijding van opvliegers. Er zijn verschillende complementaire en alternatieve behandelingen onderzocht in de bestrijding van opvliegers, zoals black cohosh, phyto-oestrogenen, homeopathie, vitamine E, acupuntuur en gedragsstrategieën. Veel frequent voorgeschreven farmacologische middelen zijn onderzocht, zoals progestagenen, clonidine, gabapentine en anti-depressiva. Echter, gegevens met betrekking tot de effectiviteit van de verschillende complementaire en alternatieve behandelingen zijn beperkt en daarnaast worden studies

beperkt door het ontbreken van een placebo controlegroep en door veelal een te korte studieperiode. Lange termijn follow-up, placebo-gecontroleerd, prospectief onderzoek is nodig om de effectiviteit en veiligheid van niet-farmacologische en farmacologische interventies in de behandeling van opvliegers te onderzoeken.

Hoofdstuk 2.2 beschrijft de resultaten van een prospectief, dubbelblind, gerandomiseerd, drie-armig onderzoek waarin de effectiviteit van clonidine, venlafaxine en placebo in de behandeling van opvliegers met elkaar werden vergeleken. Een behandeling met venlafaxine veroorzaakte een snellere reductie van de opvliegerscore ten opzichte van clonidine, echter in de 12e week van de studieperiode waren de opvliegerscores lager in de clonidinegroep dan de venlafaxinegroep. Bijwerkingen ten gevolge van de behandeling kwamen vaker voor in de venlafaxinegroep. De belangrijkste conclusie van deze studie was dat door beide middelen de opvliegerscores verminderden bij vrouwen na borstkankerbehandeling. Echter, een behandeling met venlafaxine wordt aanbevolen, aangezien venlafaxine over de gehele 12 weken durende studieperiode effectiever was in het reduceren van de opvliegerscores.

In **hoofdstuk 3.1** wordt een overzicht gepresenteerd van de farmacokinetiek, farmacodynamiek, farmacogenetica, klinische monitoring, het bijwerkingenprofiel en de effectiviteit van een behandeling met trastuzumab. Trastuzumab is alleen effectief bij patiënten met een verhoogde expressie van het HER2 eiwit in de kankercel. Over het algemeen wordt een behandeling met trastuzumab goed verdragen, echter het ontstaan van hartschade bestaande uit linker ventrikel dysfunctie, en in een klein gedeelte van de patiënten hartfalen, is de klinisch meest relevante bijwerking. Een behandeling met trastuzumab verbetert de (ziekte-vrije) overleving, echter een gedeelte van de patiënten heeft geen baat bij deze behandeling. Er zijn voor deze groep HER2 positieve borstkankerpatiënten nog onvoldoende gegevens met betrekking tot optimale behandelmogelijkheden. Verder onderzoek naar de bijwerkingen op de lange termijn en de ontwikkeling van gevoelige, gemakkelijk en eenduidig interpreteerbare meetmethoden voor de diagnostiek en voorspelling van trastuzumab gerelateerde hartschade is van groot belang.

Hoofstuk 3.2 beschrijft de incidentie, reversibiliteit en relatie tussen risicofactoren en de ontwikkeling van trastuzumab gerelateerde hartschade in een niet geselecteerde patiëntenpopulatie met primaire borstkanker. In dit onderzoek was er sprake van een significante relatie tussen een behandeling met trastuzumab en de ontwikkeling van hartschade, welke slechts gedeeltelijk reversibel was. Een combinatie van een behandeling met anthracyclines en trastuzumab bleek de incidentie van de ontwikkeling van hartschade, gemeten als een verminderde linker ventrikel ejectiefractie (LVEF), te verhogen. Bovendien was er een significante relatie tussen de incidentie van hartschade en de uitgangswaarde van de LVEF. Wij vonden geen statistisch significante relatie tussen het ontstaan van hartschade en mogelijke andere risicofactoren voor de ontwikkeling van hartschade, zoals leeftijd, overgewicht, het gebruik van antihypertensiva, of het aanwezig zijn van ziekten (comorbiditeit). Er is meer onderzoek nodig met betrekking tot het voorkómen en beperken van trastuzumab-gerelateerde hartschade.

Hoofdstuk 3.3 beschrijft de resultaten van de veiligheid van een trastuzumab-behandeling in een niet-geselecteerde patiëntenpopulatie met een primaire borstkanker. Wij hebben in deze studie borstkankerpatiënten geïncludeerd die eerder adjuvant waren behandeld met trastuzumab. Tolerantie op de lange termijn en de cardiale veiligheid werden in deze studie onderzocht en factoren voor diagnostiek en voorspelling van trastuzumab-gerelateerde hartschade geidentificeerd. De ernst en incidentie van trastuzumab-geïnduceerde hartschade was gerelateerd aan voorafgaande of gelijktijdige anthracycline blootstelling. De hartschade was gedeeltelijk reversibel. In deze studie waren biomarkers, zoals NT-proBNP en de troponines, niet statistisch significant gerelateerd aan trastuzumab-geïnduceerde hartschade en herstel van deze hartschade. Bovendien vonden wij geen significante relatie tussen trastuzumab-gerelateerde hartschade en geselecteerde zogenaamde single nucleotide polymorphisms in het HER2 gen. Echter, langere follow-up studies in grotere patiëntenpopulaties zijn nodig om de tolerantie en cardiale veiligheid van behandeling met trastuzumab te evalueren.

Hoofdstuk 3.4 beschrijft de cardiale veiligheid van een behandeling met trastuzumab bij patiënten die starten met een lage LVEF-waarde. Patiënten met een primaire of uitgezaaide borstkanker waarbij de baseline LVEF-waarde beneden de 50% was bij aanvang van de behandeling met trastuzumab werden retrospectief geïdentificeerd. Patiënten ontwikkelden, soms ernstige, gedeeltelijk reversibele hartschade. Echter, alle gemeten zogenaamde cardiac events werden beschouwd als asymptomatisch en er waren geen aan hartschade gerelateerde doden. Een langere follow-up periode, grotere patiëntenpopulaties en prospectieve data zijn nodig om de cardiale veiligheid van een behandeling met trastuzumab bij patiënten met een baseline LVEF-waarde beneden 50% te evalueren. Echter, uit onze voorlopige onderzoeksresultaten blijkt dat ter verbetering van de prognose een behandeling met trastuzumab onder strikte cardiale controle overwogen kan worden bij patiënten met een HER2-positieve borstkanker en een lage uitgangswaarde van de LVEF.

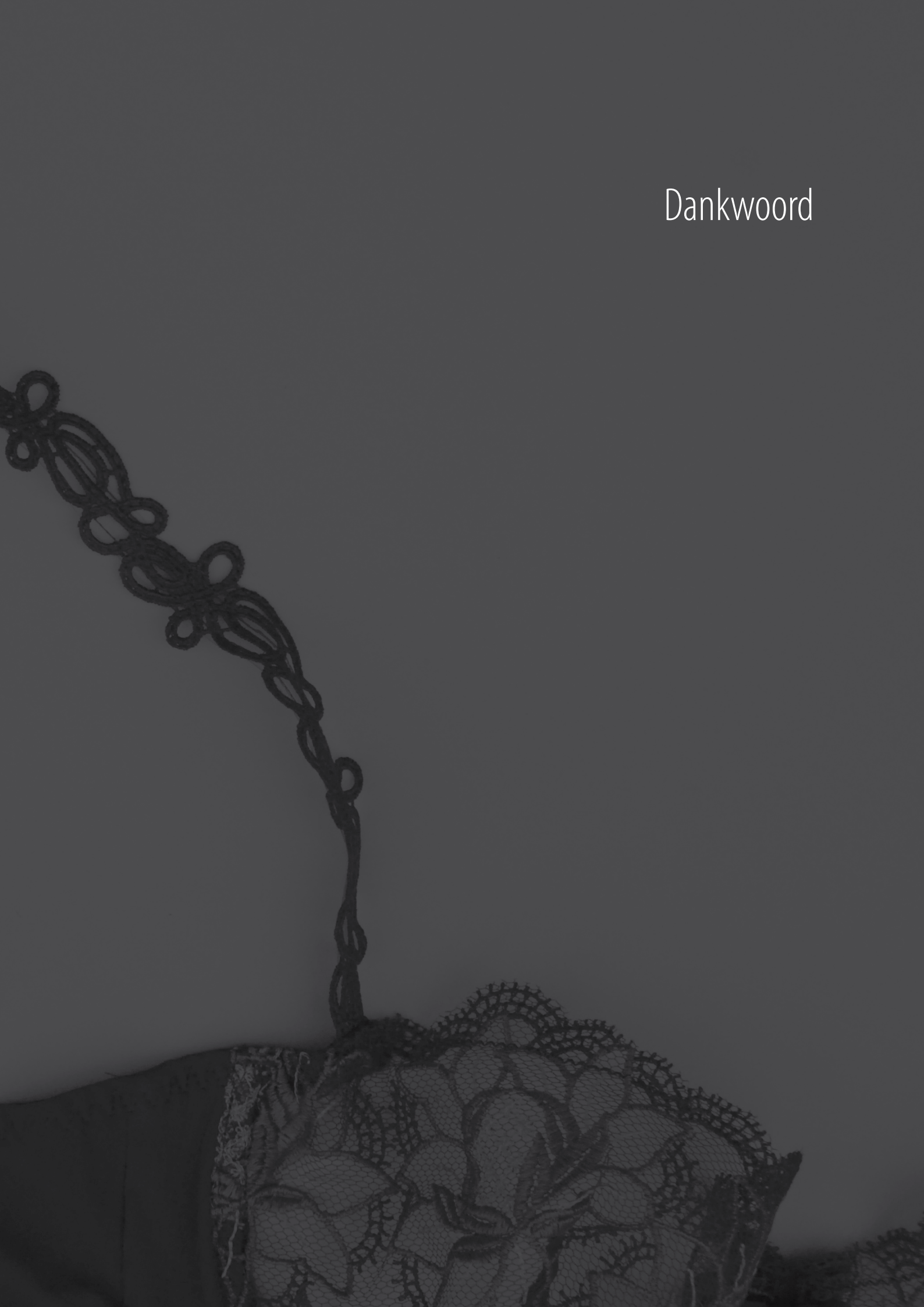
De ontwikkeling van een farmacokinetisch-farmacodynamisch (PK-PD) model is beschreven in **hoofdstuk 3.5**. Dit PK-PD model beschrijft de relatie tussen de trastuzumab blootstelling en daling van de LVEF-waarde in patiënten met een primaire of uitgezaaide borstkanker. De belangrijkste conclusie van dit PK-PD model was dat er alleen een significante relatie bestond tussen de cumulatieve dosis anthracyclines en gevoeligheid voor de ontwikkeling van hartschade. Uiteindelijk kan dit PK-PD model worden gebruikt voor de beantwoording van klinische vragen en de ontwikkeling van richtlijnen met betrekking tot een optimale behandeling van trastuzumab-gerelateerde hartschade.

In **Hoofdstuk 3.6** worden de voorlopige geblindeerde resultaten gepresenteerd tot en met de 2e interimanalyse, van een prospectief, gerandomiseerd, placebo-gecontroleerd interventie onderzoek (CANDY). In deze studie worden patiënten met HER2-positieve borstkanker behandeld met gelijktijdig adjuvant trastuzumab en een angiotensine II-receptor blokker (candesartan) of placebo. Het doel van deze studie is het onderzoeken of het gelijktijdig gebruik van candesartan in trastuzumab-behandelde patiënten de ernst van trastuzumab-gerelateerde hartschade kan verminderen of voorkomen. De studieresultaten

van deze tussentijdse analyse zijn gebaseerd op de gehele patiëntenpopulatie en niet per behandelarm uitgesplitst. Er is trastuzumab-gerelateerde hartschade geobserveerd in patiënten die zijn behandeld volgens het CANDY studieprotocol. Vooraf vastgestelde statistische grenzen met betrekking tot de veiligheid zijn tijdens de 1e en 2e interimanalyse niet overschreden. De zogenaamde data monitoring commissie heeft op basis van deze gegevens de hoofdonderzoekers geadviseerd om deze klinische studie te continueren. Momenteel is de effectiviteit van een behandeling met candesartan in de preventie van trastuzumab-geïnduceerde hartschade bij patiënten met een primair mammacarcinoom onbekend. De definitieve gedeblindeerde studieresultaten worden verwacht in mei 2013.
Samenvattend, behandelingen van borstkanker zijn veelal effectief, echter deze behandelingen leiden vaak tot verschillende bijwerkingen, zoals opvliegers en hartschade. Deze bijwerkingen kunnen ernstig zijn waardoor behandelingen voortijdig worden gestaakt wat uiteindelijk kan resulteren in een slechte behandeluitkomst. Opvliegers kunnen worden behandeld met clonidine of venlafaxine. Een behandeling met clonidine verminderde de opvliegerklachten beter dan venlafaxine ten tijde van het primaire studie eindpunt na 12 weken behandeling. Echter, een behandeling met venlafaxine was effectiever gedurende de gehele studieperiode.

In dit proefschrift zijn verschillende studies beschreven met betrekking tot de cardiale veiligheid van een trastuzumab-behandeling. Een behandeling met trastuzumab kan een daling van de hartfunctie, gemeten als de linker ventrikel ejectiefractie, veroorzaken en in een klein gedeelte van de patiënten kan dit resulteren in manifest hartfalen. Een belangrijke voorspellende factor voor de ontwikkeling van trastuzumab-gerelateerde hartschade is een voorafgaande of gelijktijdige behandeling met anthracyclines. Trastuzumab-gerelateerde hartschade was gedeeltelijk reversibel ook bij patiënten met een lage LVEF-waarde voorafgaande aan de behandeling met trastuzumab. Momenteel loopt er een onderzoek waarin de effectiviteit van een angiotensine II-receptor blokker wordt onderzocht in de preventie van trastuzumab- gerelateerde hartschade.

Dankwoord



Dankwoord

Promotieonderzoek doen lukt niet in je eentje. Het waren de mensen om mij heen, die zowel op het werk als thuis mij stimuleerden, aanhoorden en bijstonden met raad en daad. Ik wil graag een ieder om mij heen bedanken die heeft bijgedragen aan het tot stand komen van dit proefschrift waarvan een aantal mensen in het bijzonder.

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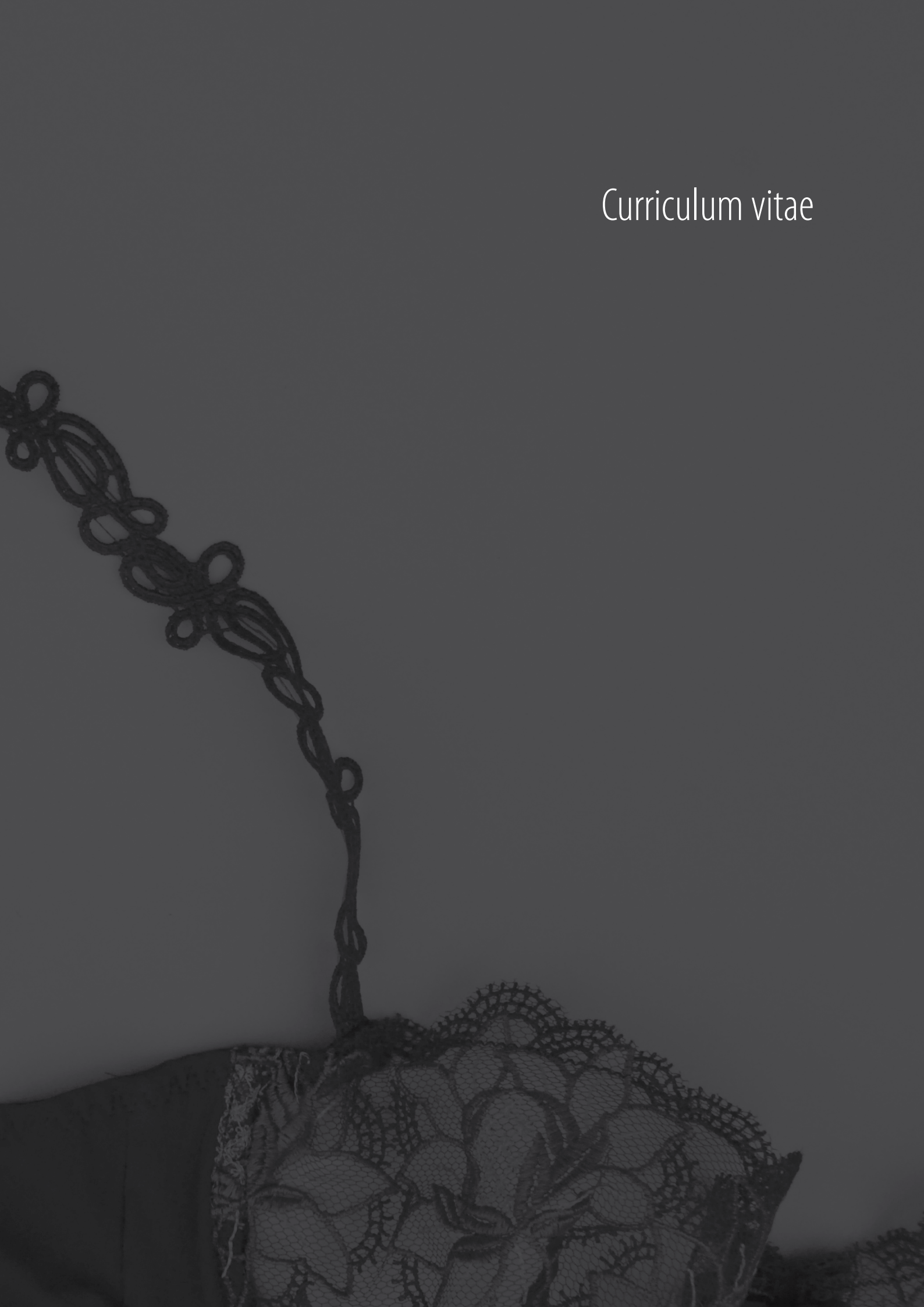
Annelies

Curriculum vitae



Curriculum Vitae

Annelies Hendrikje Boekhout was born on January 29, 1975 in Houten. In 1992 she graduated from secondary school (HAVO) and in the same year she started her Nursery study and graduated in 1996. Between 1996 and 1998 she worked as a nurse at the Elizabeth Hospital in Amersfoort. In 1998 she started her Oncology Nursing study at the Netherlands Cancer Institute-Antoni van Leeuwenhoek Hospital (NKI-AVL) and graduated in 1999. Between 1999 and 2001 she worked as an oncology nurse at the NKI-AVL, which was interrupted in 2000 by a three months period as a volunteer at the Bethesda Hospital in Nigeria. In 2001 she started to study Advanced Nursing Practice and received her Master's degree in 2003. The PhD project described in this thesis started in 2005 at the division of Clinical Pharmacology at the NKI-AVL under supervision of prof. dr. J.H.M. Schellens and prof. dr. J.H. Beijnen. During her PhD project she part time continued to work as a nurse practitioner at the division of medical oncology.