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ADJUVANT TAMOXIFEN AND LUTEINIZING HORMONE-RELEASING HORMONE AGONISTS IN PREMENOPAUSAL BREAST CANCER

On long-term benefits and side effects in a randomised study

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Life can only be understood backwards; but it must be lived forwards

Søren Kierkegaard

ABSTRACT

Adjuvant endocrine therapy improves breast cancer survival, unconditional of other treatment. In premenopausal breast cancer, tamoxifen for 5 years is the standard treatment, with or without the addition of ovarian ablative therapy. The optimal timing and duration of ovarian ablative treatment is not yet defined, and it is not clear if there is additional benefit from ovarian suppression in combination with cytotoxic chemotherapy. With improving survival and excellent prognosis, there is increasing need for prevention of long-term adverse effects, monitoring and treatment when appropriate. Premature ovarian failure is frequent from adjuvant treatment of young breast cancer patients with a following risk of accelerated bone loss and infertility. The possible ovarian protective effect of ovarian ablation from luteinizing hormone-releasing hormone (LHRH) agonists is debated.

Aims: The purpose of our study is to examine the efficacy of the LHRH agonist goserelin for adjuvant therapy of premenopausal breast cancer, the role of interaction between goserelin and tamoxifen and the impact of estrogen receptor (ER) content. We also examine long-term side effects in regard to ovarian function and bone health.

Patients and methods: The study was designed to determine whether the addition of the LHRH agonist goserelin and/or tamoxifen to adjuvant therapy provided benefit for premenopausal women with breast cancer. Patients were entered into a 2 x 2 factorial randomisation to tamoxifen 40 mg daily with or without concomitant goserelin, 3.6 mg every 28 days or goserelin alone for 2 years. Efficacy was analysed as well as the effects on ovarian function, bone mineral density and bone markers.

A total of 927 women were recruited to the Stockholm part of the ZIPP trial. At a median follow-up of 12.3 years, goserelin reduced the risk of first event by 32% ($P = 0.005$) in the absence of tamoxifen, and tamoxifen reduced the risk by 27% ($P = 0.018$)

in the absence of goserelin. The combined goserelin and tamoxifen treatment reduced the risk by 24% (P = 0.021) compared with no endocrine treatment. In highly ER-positive tumours, there were 29% fewer events among goserelin-treated patients (P = 0.044) and a trend towards greater risk reduction, depending on the level of ER content. The greatest risk reduction from goserelin treatment was observed among those not receiving tamoxifen (HR: 0.52, P = 0.007). In the study of ovarian function, 36% of the women in the goserelin group reported menses one year after completed CMF- and endocrine therapy, compared to 7% in the goserelin plus tamoxifen group, 13% in the tamoxifen group and 10% of the controls. Among women treated with goserelin, there was a statistically significant increase in the proportion of menstruating women one year after completed treatment, compared to at 24 months of treatment (P = 0.006), in contrast to all other treatment groups, who were unchanged or more often amenorrheic. The bone mineral study showed that after 2 years of treatment, there was a significant decline in bone mineral density (mean change, -5%; P < 0.001) in the women receiving goserelin. The combined goserelin and tamoxifen treatment, as well as tamoxifen alone, resulted in a lesser, but statistically significant, decrease in bone mineral density (mean change, -1.4%; P = 0.02; and -1.5%; P < 0.001). One year after cessation of treatment, the goserelin group alone showed partial recovery from bone loss (mean change, 1.5%; P = 0.02). In the study of bone turnover markers (BTM), there was a significant rise in Osteocalcin (RR: 1.57, p < 0.001), PINP (RR 1.65, p < 0.001) and CTX (RR 1.98, p < 0.001) among goserelin-treated patients. There were no significant changes in BTM among those treated with either goserelin and tamoxifen or tamoxifen alone. Among patients where bone mineral density measurements were available, change in BMD was inversely associated with change in BTM (r = -0.40 to -0.51).

Conclusions: Adjuvant tamoxifen in combination with the LHRH agonist goserelin is not superior to either tamoxifen alone or goserelin alone in regard to recurrence-free

survival in premenopausal endocrine responsive breast cancer. A significant interaction indicates that the effect of goserelin depends on whether tamoxifen is given or not, and the effect of tamoxifen depends on whether goserelin is given or not. In this study there is a trend towards greater efficacy of goserelin with increasing ER levels. A subgroup of women with strongly ER-positive tumours benefits more from goserelin treatment, whereas the benefit of tamoxifen does not seem to be dependent on ER content. This study shows some evidence of a protective effect of goserelin on ovarian function in CMF treated women. This effect was not observed where tamoxifen was given in addition to goserelin treatment. Further studies are needed to confirm this. Two years of ovarian ablation from goserelin treatment induces a significant reduction in bone mineral density, but there is partial recovery from the bone loss one year after stopped treatment. After six months of goserelin treatment, markers of both bone resorption and bone formation increase, whereas there is no change in bone turnover from tamoxifen alone or in combination with goserelin. Furthermore, there an inverse correlation of changes in BMD and bone markers. The addition of tamoxifen seems to counteract the effects of goserelin on BMD and BTM. In addition to BMD measurements, biochemical examinations of bone turnover markers may be useful for monitoring bone health, identifying women at risk for bone loss and making early interventions possible.

LIST OF PUBLICATIONS

I. Interaction between goserelin and tamoxifen in a prospective randomised clinical trial of adjuvant endocrine therapy in premenopausal breast cancer
Asgerdur Sverrisdottir, Hemming Johansson, Ulla Johansson, Jonas Bergh, Samuel Rotstein, LarsErik Rutqvist and Tommy Fornander
Breast Cancer Res Treat. 2011, 128 (3): 755-63

II. Adjuvant goserelin and ovarian preservation in chemotherapy treated patients with early breast cancer: results from a randomized trial
Asgerdur Sverrisdottir, Marianne Nystedt, Hemming Johansson and Tommy Fornander
Breast Cancer Research and Treatment 2009, 117 (3): 561-7

III. Bone Mineral Density Among Premenopausal Women With Early Breast Cancer in a Randomized Trial of Adjuvant Endocrine Therapy
Asgerdur Sverrisdottir, Tommy Fornander, Hans Jacobsson, Eva von Schoultz and LarsErik Rutqvist
Journal of Clinical Oncology 2004, 22 (18): 3694-9

IV. Bone turnover in goserelin and tamoxifen treated premenopausal breast cancer patients in a randomised adjuvant trial
Asgerdur Sverrisdottir, Janusz Gross, Hemming Johansson, Hans Jacobsson, Thomas Gustafsson, Samuel Rotstein and Tommy Fornander
Manuscript

CONTENTS

LIST OF ABBREVIATIONS

ABCSG	Austrian Breast Cancer Study Group
AC	Doxorubicin and cyclophosphamide
ACD	Doxorubicin, cyclophosphamide and docetaxel
ACT	Doxorubicin, cyclophosphamide and paclitaxel
AC+TD	Doxorubicin, cyclophosphamide and paclitaxel or docetaxel
AI	Aromatase inhibitor
ALP	Alkaline phosphatase
AMH	Anti-Müllerian hormone
ASCO	American Society of Clinical Oncology
BIG	Breast International Group
BMD	Bone mineral density
BSP	Bone sialoprotein
BTM	Bone turnover marker
C	Control
CMF	Cyclophosphamide, methotrexate and fluorouracil
CTX	C-terminal telopeptide
CYP2-D6	Cytochrome P450 2D6 enzyme
DNA	Deoxyribonucleic acid
EBCTCG	Early Breast Cancer Trialists' Collaborative Group
ER	Estrogen receptor
FAC	5-fluorouracil, doxorubicin and cyclophosphamide
FACT	Fulvestrant in Advanced Breast Cancer
FEC	5-fluorouracil, epirubicine and cyclophosphamide
Fmol	Femtomole
FSH	Follicle stimulating hormone
G	Goserelin
GT	Goserelin plus tamoxifen
Gy	Gray
HER-2	Human epidermal growth factor receptor 2
IFCC	International Federation of Clinical Chemistry and Laboratory Medicine
IHC	Immunohistochemical assessment
IOF	International Osteoporosis Foundation
Ki-67	Antigen Ki-67
LBA	Ligand-binding assay
LH	Leutinizing hormone
LHRH	Luteinizing hormone–releasing hormone
mTOR	Akt–mammalian target of rapamycin
OC	Osteocalcin
OPG	Osteoprotegerin
OS	Ovarian suppression
PINP	Pyridinoline cross-linked amino-terminal telopeptide of type I collagen
PI3K	Phosphatidylinositol 3-kinase
pN	Pathological nodal status

POF	Premature ovarian failure
PR	Progesterone receptor
pT	Pathological tumor size
QoL	Quality of life
RT	Radiotherapy
SERM	Selective estrogen receptor modulator
SNRI	Selective norepinephrine reuptake inhibitors
SOFT	Suppression of Ovarian Function Trial
SSRI	Selective serotonine reuptake inhibitor
STEPP	Subpopulation Treatment Effect Pattern Plots
T	Tamoxifen
Tam	Tamoxifen
TBBD	Total body bone density
TRAcP-5b	Tartrate-resistant acid phosphatase type 5b
ZEBRA	Zoladex Early Breast Cancer Research Association
ZIPP	Zoladex In Premenopausal Patients

1 INTRODUCTION

Breast cancer is the commonest cancer among women and the leading cause of death in women, aged 35-54 years [1]. Although the median age for breast cancer is over 60 years, around 25% of the women are below 50 years of age at the time of diagnosis [2]. Breast cancer at a young age has aggressive biological behaviour more often and is associated with poorer prognosis in comparison to older women [3, 4]. Tumours in younger women tend to have higher grades and proliferation markers and are more often hormone receptor negative and human epidermal growth factor receptor 2 (HER-2) positive, than tumours in older women [5-7]. Similarly to other Western countries, breast cancer incidence in Sweden has increased at an average of 1.3 per cent annually over the last 20 years [2]. At the same time, survival after breast cancer diagnosis has been constantly improving, due to several possible factors, such as the introduction of mammography screening, improved radiotherapy techniques, more potent cytotoxic and targeted drugs, as well as improved endocrine treatment. In spite of the continuously evolving treatment for early breast cancer in the last decades, resulting in better prognosis and better control of side effects, the optimal adjuvant therapy for premenopausal women is yet to be defined[8-10]. Furthermore, frequent long-term side effects among young women include premature menopause, infertility and bone loss, among other quality of life (QoL) issues needing to be addressed. As more women have excellent prognoses after adjuvant therapy, a greater effort is necessary to assure physical and psychological well being after treatment.

1.1 ADJUVANT BREAST CANCER TREATMENT

Adjuvant therapy in breast cancer has proven to be effective in regard to disease-free survival as well as overall survival, unconditional of age or menopausal status. International guidelines, such as the St. Gallen International Expert Consensus, recommend several prognostic and predictive factors in order to select optimal treatment after surgery[11-14]. In addition to assessment of tumour size and axillary lymph node status, analysis of estrogen- and progesterone receptor content, HER-2 expression, vascular invasion and the proliferative marker Ki-67 are recommended as standard assessment [12]. Using these clinicopathological parameters, breast cancer subtypes can be classified for systemic therapy recommendations (Table 1). According to the guidelines, hormone receptor positivity, HER-2 negativity and low Ki-67 are classified as Luminal A type, hormone receptor positive, HER-2 positive/negative and high Ki-67 as Luminal B type, hormone receptor negative, HER-2 positive as HER-2 positive (non luminal) type and hormone receptor negative, HER-2 negative as Triple negative type. In hormone receptor positive disease, endocrine therapy is a central part of the adjuvant treatment, with or without cytotoxic chemotherapy and/or radiotherapy. The efficacy of chemotherapy has been proven since the early studies of cyclophosphamide, 5-fluorouracil and methotrexate (CMF) therapy in the 1970s, later succeeded by anthracycline-based treatment and then with the addition of taxanes, survival has improved step by step [15-18]. In most studies, the benefit of chemotherapy is greatest among younger women, and is less as age advances [16, 17]. The recommended timing of cytotoxic chemotherapy is before endocrine and/or radiotherapy because the risk of increased adverse effects and possible interactions. Endocrine treatment is given concomitantly or after radiotherapy, according to local practise since concomitant treatment seems to be safe[19]. Radiotherapy, which is generally recommended following

breast-conserving surgery, and in some cases after mastectomy, is not only highly effective in regard to local recurrences, but improves survival as well [20].

SUBTYPE OF BREAST CANCER	RECOMMENDED THERAPY	
Luminal A	Endocrine therapy alone	Few high risk require cytotoxics
Luminal B (HER-2 negative)	Endocrine +/- cytotoxic therapy	Addition of cytotoxics dependent on hormone receptor content or other high risk factors
Luminal B (HER-2 positive)	Cytotoxics + anti-Her-2 + endocrine therapy	Very low risk patients may be observed without adjuvant treatment
HER-2 positive	Cytotoxics + anti-HER-2 therapy	
Triple negative	Cytotoxics	

Table 1. Systemic treatment recommendations for breast cancer subtypes (Adapted from St. Gallen International Expert Consensus on the Primary Therapy of Early Breast Cancer [12]).

1.2 STEROID HORMONE RECPETORS

Estrogen plays a fundamental role in the pathogenesis and development of breast cancer. Treatment aimed at reducing circulating estrogen has proven to be highly beneficial to women after breast cancer diagnosis and surgery. The estrogen receptor (ER) was discovered in the 1960s and led to subsequent findings that breast cancer growth is regulated by estrogen action via the ER [21]. The identification of ER has been of extreme measures in regard to the development of treatment options in breast cancer. Evaluation of ER status is recommended as a part of the routine assessment of a tumour, based on the ER content being a major prognostic as well as predictive biomarker for endocrine therapy [22, 23]. In premenopausal women, ER positive tumours are somewhat less frequent or approximately 60% [24, 25]. Around 75% of all tumours express steroid hormone receptors and about 60% of ER positive tumours respond to endocrine therapy [26, 27].

1.2.1 Methods of ER assessment

There have been mainly two methods used to assess the content of ER, i.e., quantitative analysis of ER (Figure 1) by ligand-binding assay (LBA) and immunohistochemical assessment (IHC).

1.2.2 Ligand-binding assay

LBA is based on isoelectric focusing of estradiol receptor protein from human mammary carcinoma in polyacrylamide gel or sucrose gradient analysis and requires fresh-frozen tumour tissue [28]. Values between 0 and 1000 femtomoles (fmol) per milligram (mg) of Protein have been reported, although a cut-off point of 3 to 10 fmol/mg Protein have often been used for defining ER positive tumours by this method, although some have used levels above 0.05 fmol/µg DNA. LBA was the former standard method and widely validated for estimating ER content. The main advantages of the LBA assay are the quantitative measurements with reproducibility as well as good quality control. There are, however, some disadvantages, namely that the method requires fresh tumour tissue, is relatively expensive and is not very specific for tumour cells only. On these grounds, LBA has mostly been replaced by IHC, which is currently the standard method [29].

1.2.3 Immunohistochemical assessment

IHC is based on the use of highly specific monoclonal antibodies binding to the ER. The IHC method is at present more commonly used than LBA, as it is less expensive, easier to perform, useful on a wider variety of tumour tissue and is more sensitive as well as specific for staining tumour cells only. As little as 1% staining cells are defined as ER positive by the American Society of Clinical Oncology (ASCO), although 10%

staining cells has been used as a cut-off for positivity in a variety of studies. Recently, the St. Gallen Consensus Conference declared a tumour with any staining cells as ER positive [12]. In spite of the differences in the techniques, there is high concordance between the LBA and IHC methods, both in relation to ER status and clinical outcomes [30-34].

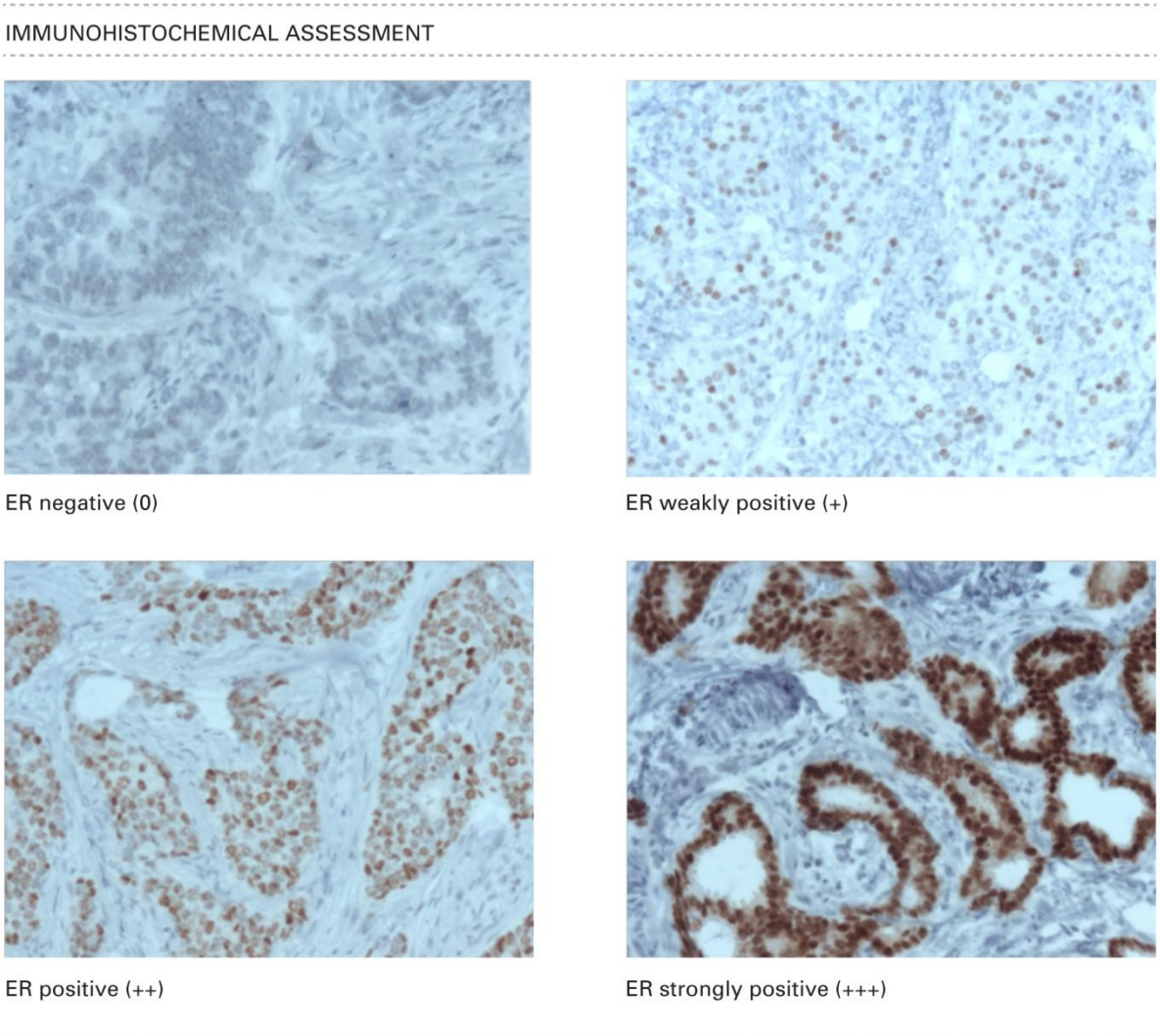

Figure 1. Immunohistochemical assessment of the estrogen receptor in tumour tissue. ER positive cells show a brown signal of nuclear intensity (score + to +++).

1.3 ADJUVANT ENDOCRINE THERAPY

The role of estrogen suppression has been known for a long time and goes back to the Scottish surgeon George Beatson's report in 1896 on the effect of bilateral oophorectomy in metastatic breast cancer [35]. However, there was over half a century of delay until the discovery of the ER [36], and it became clear that the presence of ER indicated that the tumour was dependent on estrogen and tumour response could be controlled by endocrine manipulation[21, 37]. Luteinizing hormone–releasing hormone (LHRH) synthetic agonists were developed in the 1970s, allowing reversible ovarian function suppression. Again, there was a delay until the 1990s before a definite survival advantage was established for adjuvant ovarian suppression (OS) in premenopausal breast cancer [38]. Tamoxifen was introduced in the late 1960s and has since been studied extensively. Tamoxifen, a drug originally intended for contraception but failed as such, interferes with the activity of estrogen by competitive binding to the ER. The anti-fertility properties in rats led to the hypothesis of the drug's anti-breast cancer properties [39, 40]. In later clinical trials, tamoxifen proved to be highly effective, and the drug has now been in clinical use for over 30 years. In spite of extensive research through the last decades, there are some unanswered questions regarding endocrine treatment in premenopausal women, such as whether OS in combination with tamoxifen is beneficial, the timing and duration of OS, as well as the additional benefit of OS in combination with cytotoxic chemotherapy.

1.3.1 Tamoxifen

Tamoxifen is the first successful targeted therapy in cancer and is listed as an essential medicine by the World Health Organisation. Tamoxifen is in a class of selective estrogen receptor modulators (SERMs), having tissue-dependent as well as species-dependent effects. For example, tamoxifen has a pure antiestrogenic effect in chicks [41].

In humans, however, tamoxifen has an antiestrogenic effect in some tissues and an estrogenic effect in other tissues. The antiestrogenic effects are most prominent in breast tissue and the vagina, where tamoxifen reduces glandular as well as epithelial development [42, 43]. The antiestrogenic effect on breast tissue decreases the risk of primary or contralateral breast cancer [44, 45]. Among the antiestrogenic effects of the drug are also vasomotor symptoms, such as hot flushes. However, in some tissues the estrogenic effects of tamoxifen are dependent on menopausal status. Among postmenopausal women, estrogenic effects are prominent in the uterus, the cardiovascular system as well as bone, whereas the main effect on bone in premenopausal women is antiestrogenic [46-54]. The enzyme cytochrome P450 2D gene (CYP2-D6) is responsible for the conversion of tamoxifen to endoxifen, which is the active metabolite of tamoxifen [55]. There are known inherited variants of CYP2-D6, which may influence both the magnitude of efficacy as well as side effects of tamoxifen [56]. In premenopausal women, estrogenic effects lead to decreased concentrations of the gonadotropins luteinizing hormone (LH) and follicle stimulating hormone (FSH) [57, 58]. Tamoxifen was early on shown to have antitumor effects in both pre- and postmenopausal breast cancer, but there was a several decades delay until it became a standard therapy in the premenopausal setting[59] . Tamoxifen with or without OS is still the standard endocrine treatment in premenopausal breast cancer [12]. The more recent aromatase-inhibitors (AIs) treatment is not effective in women with hormonal production from the ovaries [14]. The role of AIs in combination with OS in the premenopausal setting is under investigation.

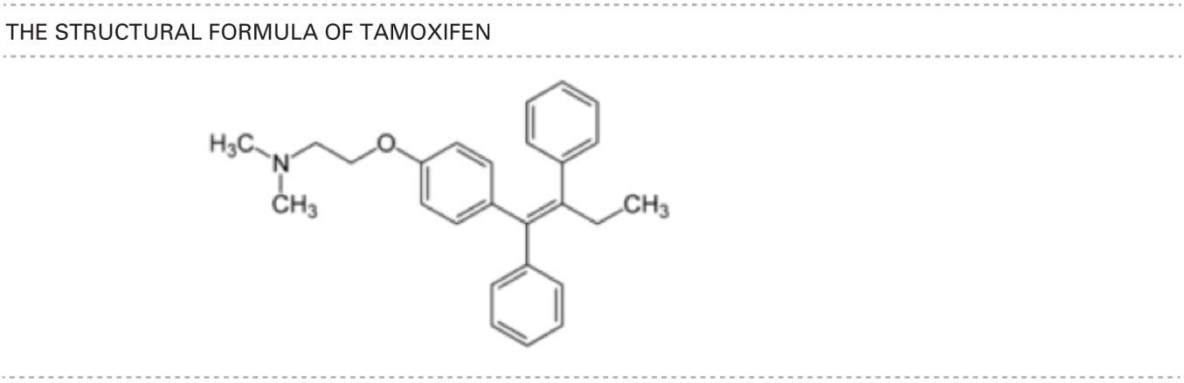

Figure 2. The structural formula of Tamoxifen

1.3.1.1 Duration and dosage of tamoxifen

Originally, tamoxifen was used for 1 year of adjuvant therapy after surgery, based on existing experience from advanced breast cancer treatment and average duration of response before drug resistance developed [60, 61]. Subsequent trials showed that 2 years were better than 1 year and later, 5 years were proven to be better than 2 years of treatment [61-63]. In the early clinical trials, the daily dose of tamoxifen varied from 10 to 40 mg. The dose mainly used in these studies for 1 and 2 years' treatment was 40 mg daily and 20 mg daily for 5 years. Treatment beyond 5 years, however, is not recommended as 10 years of tamoxifen has not yet shown net clinical benefit over 5 years of treatment because of the development of drug resistance and adverse side effects [64-66]. In the postmenopausal setting, a switch to AIs for 2 to 3 years after 5 years of tamoxifen has proven to be of additional benefit [67].

1.3.1.2 Efficacy of tamoxifen

In ER positive disease, tamoxifen improves disease-free survival as well as overall survival unconditional of other adjuvant therapies[15, 16, 44]. Tamoxifen has as well been shown to reduce the risk of breast cancer in women at high risk of developing breast cancer [65]. The efficacy of tamoxifen treatment has repeatedly been reported in the Early Breast Cancer Trialists' Collaborative Group (EBCTCG) overviews, and in a recent meta-analysis from 20 trials and 20,000 patients, tamoxifen reduces the rate of recurrence by 50% the first 5 years and 30% for up to 10 years.[44] Death rates are reduced by one third the first 15 years. The benefit of tamoxifen has been shown to be independent of factors, such as progesterone receptor (PR) status, age or nodal status. Interestingly, the efficacy of tamoxifen is independent of whether chemotherapy was given or not. Even for weakly positive ER, there is a substantial benefit of tamoxifen treatment and has only a marginally better effect in disease with much higher ER content[44, 68] . In ER-negative disease, there is no benefit from tamoxifen.

1.3.2 Aromatase inhibitors

Newer endocrine therapies, such as aromatase inhibitors (AI), have shown to be more beneficial in regard to recurrence-free survival than tamoxifen in postmenopausal women. AIs inhibit the estrogen synthesis from androgens in the postmenopausal endocrine milieu. AIs are however not recommended for premenopausal women unless combined with ovarian ablative (OA) treatment. Recently, trials have been designed to assess whether AIs are superior to tamoxifen in premenopausal women. So far, there seems to be no additional benefit of AIs compared to tamoxifen in terms of survival, but there are substantially more side effects from AIs [69].

1.3.3 Ovarian suppression

Apart from surgery, ovarian suppression is the oldest therapy still in use for breast cancer. Ever since Beatson's report more than a century ago, the association between the hormonal action of the ovaries and the proliferation of breast cells has been known [35]. Fifty years later, the first randomised trials on ovarian ablative (OA) treatment were launched, and, consequently, the benefit of suppression therapy was established by the EBCTCG [16, 70]. It is clear that OS improves survival in premenopausal women with early breast cancer [8]. Treatment-induced OA, whether by endocrine- or chemotherapy, radiation or surgery, results in increased disease-free survival and overall survival in premenopausal endocrine-responsive breast cancer. Results from trials using LHRH agonists in the adjuvant setting have indicated significant benefit in terms of prolonged disease-free survival and improved survival with 2 years of treatment, regardless of other systemic treatment. Overview data on the use of LHRH agonists have shown that LHRH agonists have efficacy similar as cytotoxic chemotherapy. Furthermore, the addition of LHRH agonist to cytotoxic chemotherapy, without tamoxifen, significantly reduces the risk of recurrence. There is however no therapeutic benefit from combination endocrine therapy versus tamoxifen or goserelin alone in women treated with cytotoxic chemotherapy [8]. Still, there are some unanswered questions regarding the role of OS. Among them is OS's potential added value to tamoxifen in the adjuvant setting for premenopausal women. The optimal timing and duration of OS treatment with LHRH agonist are yet to be defined. In addition, it is uncertain whether LHRH agonists have a role among those not achieving amenorrhea during cytotoxic chemotherapy [70].

1.3.3.1 LHRH agonists

A LHRH agonist is a synthetic peptide that interacts with the luteinizing hormone-releasing hormone receptor to elicit its biologic response, the release of the pituitary hormones follicle stimulating hormone (FSH) and luteinizing hormone (LH) [71]. Goserelin (Zoladex®) is a synthetic analogue of LHRH which binds to the LHRH receptor cells in the pituitary gland, thus leading to a short period of increased production of LH and production of the sex hormones testosterone and estrogen (Figure 3-4). After about 3 weeks, a profound decrease in FSH and LH secretion results through receptor downregulation. In women this results in severe hypoestrogenaemia, but the hypogonadal state is reversible [72, 73] . The recommended dose of goserelin for premenopausal breast cancer is 3.6 mg every 28 days subcutaneously for 2-3 years, as that has been the dose and duration used in most studies [74-79].

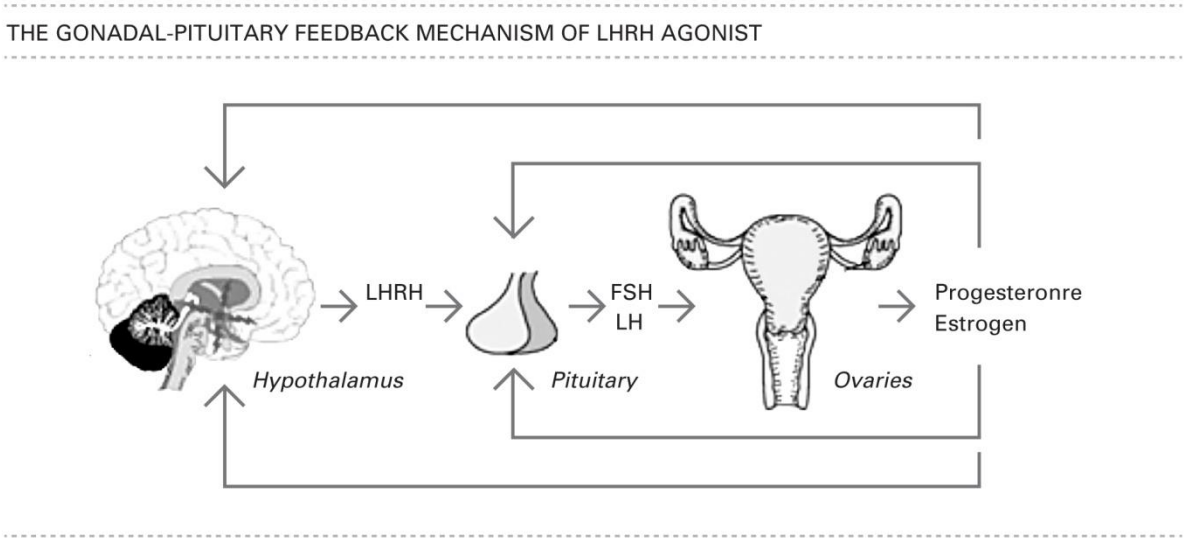

Figure 3. Schematic diagram of the gonadal-pituitary axis in women

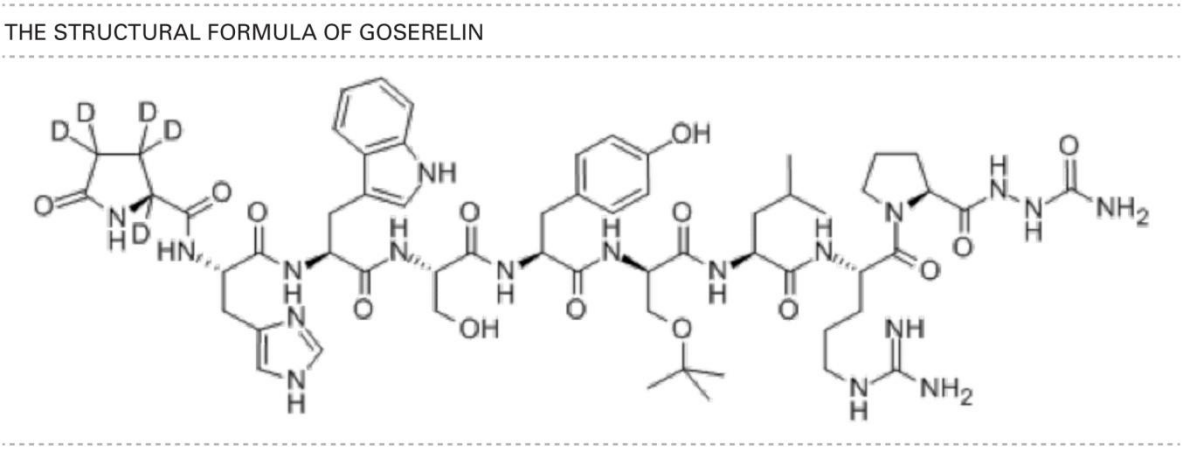

Figure 4.

1.3.4 Long-term side effects of adjuvant endocrine treatment

1.3.4.1 Side effects of tamoxifen

As toxicity is low and side effects generally well tolerated, tamoxifen has been widely used for the past decades. The most common side effects are vasomotor symptoms, such as sweating and hot flushes; although undesirable, they are not serious [80-82]. Life-threatening and serious side effects are few but include increased risk of uterine cancer and thromboembolic events. The risk of uterine cancer is considerable, but highly age-dependent, with risk low in younger women but increasing with age [44]. The risk of thromboembolic event with a fatal outcome is also low among younger women [83]. There is not a significantly increased risk of cerebral stroke or cardiac mortality among women treated with tamoxifen. Overall, tamoxifen has not been shown to increase mortality among patients without recurrence [84]. In a recent overview, there is 3% risk of dying from other causes than breast cancer in the age group 45 to 54 years [44]. Among premenopausal women, tamoxifen does not adversely affect sexual function [85]. Likewise, anxiety and depression have not been significantly increased. However, vaginal discharge and irregular menses are more frequent among women treated with tamoxifen [51, 86].

1.3.4.2 Therapy-induced ovarian failure

Premature ovarian failure (POF) and infertility is a frequent long-term result of cytotoxic chemotherapy in young women with breast cancer [74, 87-91]. The risk of ovarian failure is highly correlated to a woman's age, type of drug, dosage and duration [87, 91-96]. The rates of chemotherapy-related amenorrhea vary from 20% to 80%. In most studies, the majority of women over 40 years of age become amenorrheic [97]. The ovarian damage resulting from chemotherapy is not an "all or none" phenomenon and can present as irregular menses, amenorrhea or infertility. Cytotoxic chemotherapy reduces the number and quality of oocytes in the ovaries, i.e., the ovarian reserve [92, 93, 98-101]. Younger women require more chemotherapy to develop gonadal failure, which is probably related to the higher number of oocyte reserves in the ovaries, compared to women over 40 years. Older premenopausal women require shorter therapy for induction of amenorrhea than younger women and are more likely to develop permanent ovarian failure [90, 102]. Alkylating drugs, such as cyclophosphamide, mostly used in combination therapies, are known to be highly toxic to the gonads. The cumulative cyclophosphamide dose is a strong predictive factor of chemotherapy-induced amenorrhea in young cancer patients. Ovarian failure, induced by alkylating agents, is however not cell-cycle specific and rates vary mostly dependent on age. Amenorrhea from cyclophosphamide therapy in younger women may vary from 18% to 61%, whereas the rates range from 61% to 97% in older women [87, 97,90, 102]. The well-studied CMF chemotherapy in breast cancer is associated with a high risk of ovarian failure, but is also highly dependent on age [74, 87, 103]. Newer and more beneficial anthracycline-based regimens, with or without taxanes have not been shown to be more toxic to the ovaries (Table 2) [90, 94, 102, 104]. This is probably due to the lower cumulative

doses of anthracycline as well as cyclophosphamide in the taxane combination regimens. The addition of anti-HER-2 drugs, such as trastuzumab, are unlikely to affect fertility in the long term, but need to be studied.

CHEMOTHERAPY REGIMEN	RATE OF AMENORRHEA		
	AGE < 40 YEARS	AGE > 40 YEARS	AGE NOT DEFINED
CMF [11, 81, 91, 99-101]	36% (33 – 40)	78% (76 – 81)	60% (43 – 82)
FEC/FAC/AC [91, 98, 99, 102, 103]	44%	81%	56% (52 – 65)
ACD/ACT/AC+T/D [84, 85, 99, 103]	61%	85%	50% (13 – 62)

Table 2. Incidence of chemotherapy-induced amenorrhea by chemotherapy regimen and age

CMF: cyclophosphamide, methotrexate, and 5-fluorouracil, FEC: 5-fluorouracil, epirubicin and cyclophosphamide; FAC: 5-fluorouracil, doxorubicin and cyclophosphamide, AC: doxorubicin and cyclophosphamide, ACD: doxorubicin, cyclophosphamide and docetaxel, ACT: doxorubicin, cyclophosphamide and paclitaxel, AC+TD: doxorubicin, cyclophosphamide and paclitaxel or docetaxel.

1.3.4.3 Protective effect of LHRH agonists on the ovaries

Adjuvant cytotoxic chemotherapy is often recommended for premenopausal patients on grounds of young age and higher risk for recurrence or metastatic disease. For the last decades, it has been a steady trend to postpone the first childbirth, which is presently in the late twenties, and it has become quite common for women over the age of 40 to consider pregnancy. Pregnancies after breast cancer do not adversely affect the prognosis and should in general not be discouraged [110-113]. Although assisted fertility techniques have constantly been improving for the last decades, the need for ovarian protective therapies and prophylactic measures to prevent infertility is apparent. Since 2006, the American Society of Clinical Oncology (ASCO) recommends that oncologists should address the possibility of infertility and take measures to protect fertility [114]. Fertility consultation and treatment by cryopreservation of fertilized ova or

ovarian tissue can be offered to some of these patients, but this approach is not feasible for all, due to factors such as cancer treatment delay and cost. Some of the fertility techniques are still experimental and do not restore hormone production for a longer duration. Ovarian protection by endocrine manipulation could therefore render an important therapeutic option in the prevention of POF in young women with malignant disease, where adjuvant cytotoxic chemotherapy is recommended. The mechanism of possible LHRH agonist protective effect on the ovaries is not known but several hypotheses have been generated. One is that by gonadotropin suppression follicles are put in a resting state and thus are not as vulnerable to damage [115]. Another mechanism might be a decrease of utero-ovarian perfusion [116, 117]. Up-regulation of intra-gonadal anti-apoptotic molecules or protection of germline stem cells has been suggested as well [118]. Reports on this effect have mostly been non-randomised, retrospective, some made with historic controls [115, 116, 119-124]. There have been few prospective randomised trials of high quality, designed to examine the protective effects from LHRH agonists in combination with chemotherapy. Reports from these trials show a wide variety of results and are not conclusive [123, 125-128].

1.3.4.4 Bone mineral density

The skeleton undergoes constant remodelling throughout adult life. Estrogen receptors are present in bone, and estrogen plays a central role in the maintenance of bone. Estrogen stimulates osteoblasts, which in turn impair osteoclast activity and lead to decreased bone resorption [129]. Estrogen seems to be involved as well in remodelling bone by directly affecting osteoclast apoptosis, leading to increased bone resorption [130]. In premenopausal women these estrogenic effects act in delicate balance to define bone strength. However, when endogenous estrogen decreases dramatically after menopause,

bone resorption increases in proportion to bone formation, resulting in less bone strength [131]. Bone strength reflects bone mineral density (BMD) and bone quality. At a young age gonadal hormones act to increase bone mass, and this reaches a peak before age 30 [132]. Changes in hormonal balance induce bone metabolism disturbances and increase bone formation as well as bone resorption. Measurements of bone quality are not easily assessed, and BMD is generally used as a proxy for estimating bone strength. BMD is determined largely by genetic factors, hormonal status, body composition and muscle strength. In addition, lifestyle and environmental factors, such as exercise, smoking, vitamin-D, calcium intake and medication influence BMD [133]. Bone metabolism is highly affected by changes in ovarian function, and treatment with estrogen has been shown to prevent menopause-induced bone loss [134-136]. After the onset of menopause, decrease in estrogen levels is associated with an annual average loss of bone of 1-3% [137, 138]. The bone loss is accelerated in the first menopausal years but continues at a slower rate throughout life. Early menopause is one of the strongest predictors of osteoporosis [133]. Estrogen-sensitive changes in BMD are most rapidly seen in the lumbar spine and the hip, where osteoporotic fractures are also frequent and cause considerable morbidity and health economical consequences [138]. Osteoporosis can be classified as either primary or secondary. In women, primary osteoporosis often follows menopause, and secondary osteoporosis may be result of medication or disease. Current clinical guidelines recommend proactive monitoring and intervention for osteoporosis among women treated for breast cancer [139, 140]. More attention to conditions associated with secondary osteoporosis is needed, and measures to protect skeletal health are recommended [140]. Treatment with bisphosphonates has shown to be effective in preventing decrease in BMD from ovarian ablative therapy and should be considered when appropriate [141-144]. Therapy-induced bone loss is a well known long-term effect of OA [145-148]. Women developing chemotherapy-induced amenorrhea

undergo accelerated loss of bone mass, compared with women maintaining their ovarian function[142, 147, 148]. The effect of LHRH agonists on the ovaries is reversible, but there have been few reports on bone mass changes after stopped treatment. In postmenopausal women, tamoxifen has well-studied agonistic oestrogenic effects on bone [47, 53, 54, 149]. In contrast, tamoxifen induces bone loss in premenopausal women although the exact mechanism remains unclear [52, 53, 147]. In addition to LHRH-agonists tamoxifen results in less changes in BMD [52 69]. Hence, tamoxifen seems to modify somewhat the demineralising effect of LHRH-agonist in bone. The possible role of AI treatment in combination with LHRH agonist is not clear, but the addition of AIs is likely to exaggerate the bone effects. At present, there is limited data from the adjuvant setting, but AIs alone have shown a marked effect on bone metabolism[67, 69, 143, 150, 151]. Treatment with bisphosphonates has been shown to effectively reduce therapy-induced bone loss[141, 152, 153]. In addition, there is data from studies on bisphosphonate zoledronic acid, which effectively counteracts the demineralising effects from goserelin, as well as improves disease-free survival [69, 143].

1.3.4.5 Bone turnover markers

In spite of BMD being the standard for assessing bone mass changes, it does not provide information about the rate of bone turnover. Markers of bone formation and bone resorption can, in addition to BMD, give preciser information on bone quality and better predict fracture risk[154]. Changes in markers of bone turnover reflect changes in skeletal metabolism. Alkaline phosphatase **(**ALP) plays an important role in bone formation and mineralisation. ALP is produced in various tissues such as liver, bone, intestine, spleen, kidney and placenta. Around 50% of ALP in serum originates from bone. Bone-specific ALP isoenzyme assay can be used to detect bone ALP. Osteocalcin (OC) is a hydroxyapatite-binding protein synthesized by osteoblasts,

odontoblasts and hypertrophic chondrocytes. Osteocalcin is a well-established marker of bone formation but lacks both sensitivity and specificity [155]. OC is considered a specific marker of osteoblast function. Newer markers, such as pyridinoline, cross-linked amino-terminal telopeptide of type I collagen (PINP) and C-terminal telopeptide (CTX), which is a bone resorption marker, increase the predictability of bone turnover [134, 155].These markers are derived from collagen type I and can be measured by specific immunoassays. Bone turnover is easily affected by several factors, such as age, disease, drugs, recent fracture, circadian, menstrual or exercise effects, which need to be considered in clinical interpretation [156-158]. Bone markers are known to increase after the menopause, whereas bone protecting therapies, such as with calcium and bisphosphonates, have been shown to decrease levels of BTM [134-136, 141, 154]. High bone turnover has been associated as well with malignant bone disease and negative prognosis in metastatic disease [159]. Up to now, however, there is no consensus on the clinical use of bone markers.

2 AIMS OF THE STUDY

To evaluate the efficacy and some long-term side effects of adjuvant endocrine treatment in premenopausal breast cancer by testing the following hypotheses:

I: Adjuvant tamoxifen in combination with LHRH agonist is superior to tamoxifen alone, LHRH agonist alone or no endocrine therapy in regard to recurrence-free survival after radical surgery

II: The efficacy of the different endocrine therapies is dependent on the levels of estrogen receptor content.

III: LHRH agonist treatment have a protective effect on ovarian function in adjuvant cytotoxic chemotherapy.

IV: Treatment with LHRH agonist alone significantly increases bone loss.

V: LHRH agonist-induced bone loss is reversible after cessation of therapy and can be reduced by concomitant tamoxifen therapy.

VI: Biochemical markers of bone turnover may be useful for early detection of patients at risk of accelerated bone loss, as well as for monitoring and early intervention in prevention of osteoporotic fractures.

3 PATIENTS AND METHODS

3.1 PATIENT COHORT IN STUDIES I-IV

3.1.1 The Zoladex in Premenopausal Patients (ZIPP) trial

The ZIPP trial was a collaboration between four research groups: 1) Stockholm Breast Cancer Group, 2) South East (S-E) Sweden Breast Cancer Study Group, 3) Cancer Research UK and UCL Cancer Trials Centre (CRC), United Kingdom (UK) and 4) Gruppo Interdisciplinare Valutazione Interventi in Oncologia (GIVIO), Italy. The study was designed to determine whether the addition of goserelin and/or tamoxifen to adjuvant therapy provided benefit for premenopausal women with early breast cancer[77, 160]. The inclusion criteria were women, premenopausal or under 50 years of age, with stage I or II breast cancer, unconditional of ER status. Surgery of the breast/axilla and radiotherapy was according to local routines, as well as adjuvant chemotherapy. The protocol recommended CMF chemotherapy, but some centres used a regimen of 5-fluorouracil, epirubicin and cyclophosphamide (FEC). Initially, patients were to enter a 2 x 2 factorial randomisation. However, randomisation was not strictly factorial at all participating study sites. Randomisation was strictly 2 x 2 factorial throughout the study period only in the Stockholm and Italian centres. In the UK centres patients were initially entered into 2 x 2 factorial randomisation, but as the study proceeded, tamoxifen randomisation became optional and tamoxifen in 20 mg daily doses was allowed electively. In S-E Sweden patients were initially entered into a 2 x 2 randomisation to tamoxifen 40 mg daily, but later in the study all patients were given tamoxifen. The inconsistencies in the study design are mainly due to the uncertainty of the role of tamoxifen in the premenopausal setting at the time of the study planning. Goserelin was administered by subcutaneous injection of 3.6 mg every 28 days for 2 years at all sites[77].

3.1.2 The Stockholm cohort of the ZIPP trial (Papers I-IV)

In Stockholm all patients were included in a 2 by 2 factorial randomisation for goserelin (3.6 mg subcutaneously every 28 days), tamoxifen (40 mg daily), a combination of goserelin and tamoxifen or no endocrine therapy for 2 years. Node-positive women were allocated to adjuvant CMF chemotherapy (six cycles of cyclophosphamide 600 mg/m2, methotrexate 40 mg/m2 and 5-fluorouracil 600 mg/m2 intravenously administered on days 1 and 8, every 28 days), in addition to endocrine therapy. Patients with four or more positive lymph nodes received additional loco-regional radiotherapy, including the chest wall, axillary and supraclavicular lymph nodes, 46 Gy for 4.5 weeks (Figure 5). Endocrine treatment was given concurrently with chemotherapy. The randomisation was stratified in three groups, based on nodal status and use of other adjuvant therapies: node negative patients receiving no adjuvant chemotherapy, patients with one to three positive lymph nodes who received chemotherapy and patients with four or more positive lymph nodes who received both chemotherapy and loco-regional radiotherapy. Randomisation was carried out by telephone to a central office where the patient identifiers were recorded before the allocated treatment was revealed to the responsible physician. Treatment allocation was based on balanced lists, using the permuted block technique. The inclusion criteria were invasive breast cancer $\geq$ 10 mm, premenopausal menstrual status (defined as last menstruation $\leq$ 6 months earlier), primary surgery consisting of a mastectomy or a wedge resection plus axillary node dissection and no clinical evidence of distant metastases. The exclusion criteria were inoperable breast cancer, previous radiotherapy or neo-adjuvant chemotherapy and previous or concurrent endocrine therapy.

Patients from the Stockholm cohort were recruited to several studies, designed and conducted by the Stockholm Breast Cancer Study Group:

I: Study of the efficacy of and interaction between LHRH agonist and tamoxifen

II: Study of ovarian protection from LHRH agonist treatment

III: Study of bone mineral effects

IV: Study of bone turnover markers

V: Study of endocrine side effects (Quality-of-life)

VI: Study of chemo- and/or endocrine therapy's effects on sexuality

VII: Study of factors influencing return to work after adjuvant treatment

Studies V-VII have been described and reported separately [51, 85, 161]. Studies I-IV are included in this thesis.

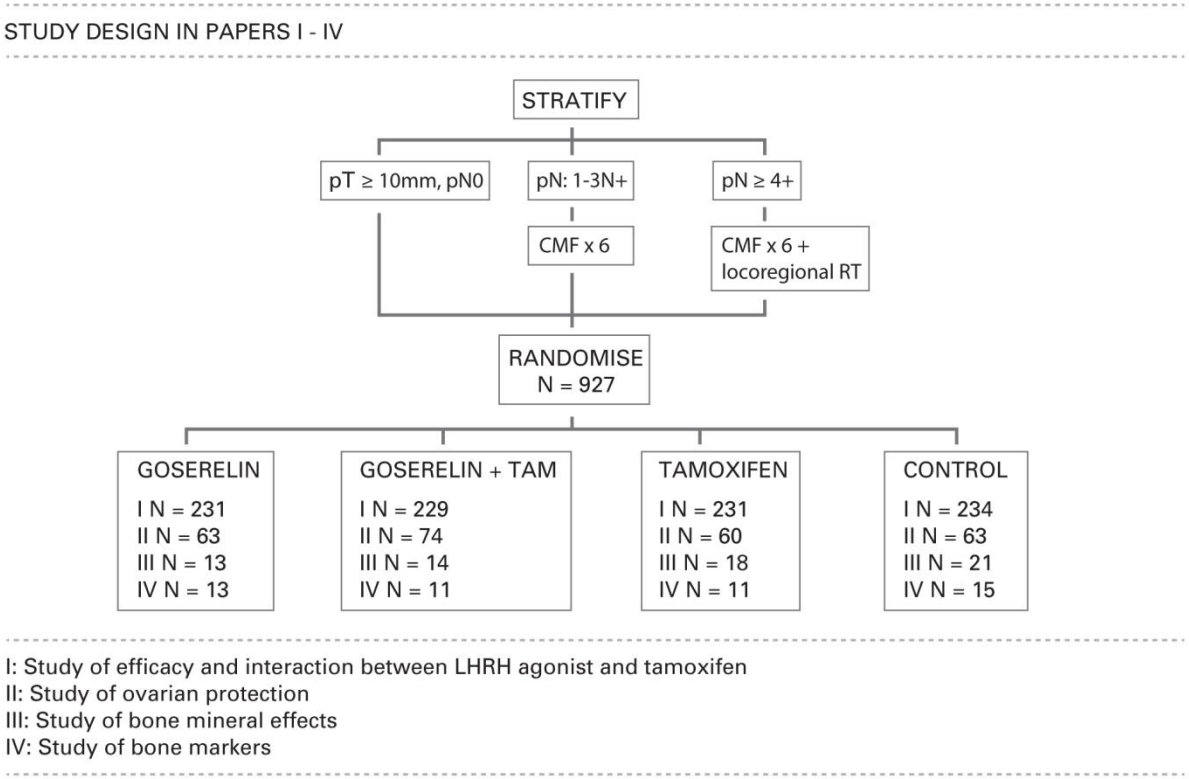

Figure 5. Study design and recruitment in studies I - IV.

All patients with breast-conserving surgery were assigned radiotherapy (RT) to the breast, 50 Gy's in 5 weeks. pT: pathological tumour size in millimetres, pN: pathological nodal status, CMF: cyclophosphamide, methotrexate and 5-fluorouracil, Tam: tamoxifen

3.1.3 Paper I

A total of 927 women in Stockholm were recruited to the study from May 31, 1990, to January 8, 1997. Of the 927 recruited women, 234 were randomised to the control (C) arm, 231 were randomised to receive goserelin (G), 231 to tamoxifen (T) and 231 to goserelin plus tamoxifen (GT) therapy. One woman was incorrectly randomised twice and one woman was diagnosed with recurrent disease at the date of randomisation. The remaining 925 women were included in the analysis (Figure 5). The common end of follow-up was January 1, 2006.

3.1.3.1 Efficacy

Time to event was calculated as time from the date of randomisation to the date of disease recurrence, contralateral breast cancer, other cancer or death without a reported recurrence, whichever came first. Time for alive and event-free patients was calculated from the date of randomisation to the common end-date for follow-up, December 31, 2005. The Kaplan–Meier technique was used to estimate failure probability, and the log rank test was used to test for difference in time to event between treatment groups. Hazard rate ratios and 95% confidence intervals were estimated using proportional hazards regression. When assessing the main treatment effect of tamoxifen or goserelin, the other treatment was used as control. Test for interactions was performed by inclusion of product terms in the regression models. We also analysed the effect of treatment on time to first recurrence according to level of ER content. Data on ER content were available on 793 patients (86%). Interaction between treatments and ER content were further investigated graphically by the use of Subpopulation Treatment Effect Pattern Plots (STEPP). The STEPP analysis was performed using the program stepp_tail, implemented in the statistical software Stata.

3.1.3.2 Hormone receptor content

All hormone receptor analyses on tumour samples were performed at a single laboratory, which participated in repeated control studies for receptor determination, using the same technique. The ER content was determined by isoelectric focusing on polyacrylamide gel. The receptor values were normalised to DNA content as measured by Burton and expressed as the binding capacity of estradiol in fmol/µg DNA [28]. Tumours with a receptor content equal to or more than 0.05 fmol/µg DNA were classified as ER-positive, whereas tumours with estrogen content less than 0.05 fmol/µg DNA were classified as ER-negative [162]. We further subdivided ER-positive tumours into intermediate ER: 0.05–0.59 fmol/µg DNA and high-ER: ≥ 0.60 fmol/µg DNA. The cut-off for the two ER-positive groups was made at a level which created equally sized groups.

3.2 PATIENT COHORT IN STUDIES II-IV

The Stockholm Breast Cancer Study Group initiated sub-studies of treatment-related side effects in the Stockholm cohort of the trial, such as effects on ovarian function, bone mineral density, return to work and quality of life. Between October 1990 and June 1994, patients were recruited to these studies.

3.3 PAPER II

285 out of 408 (70%) eligible patients in the randomised trial were recruited to the study of ovarian function. Patients were allocated to the study before randomisation to the treatment arms. The reason that 123 patients were not recruited was the patient's preference. Additionally 25 patients were excluded because of recurrent disease. The remaining 260 patients were included in the analyses (Figure 6).

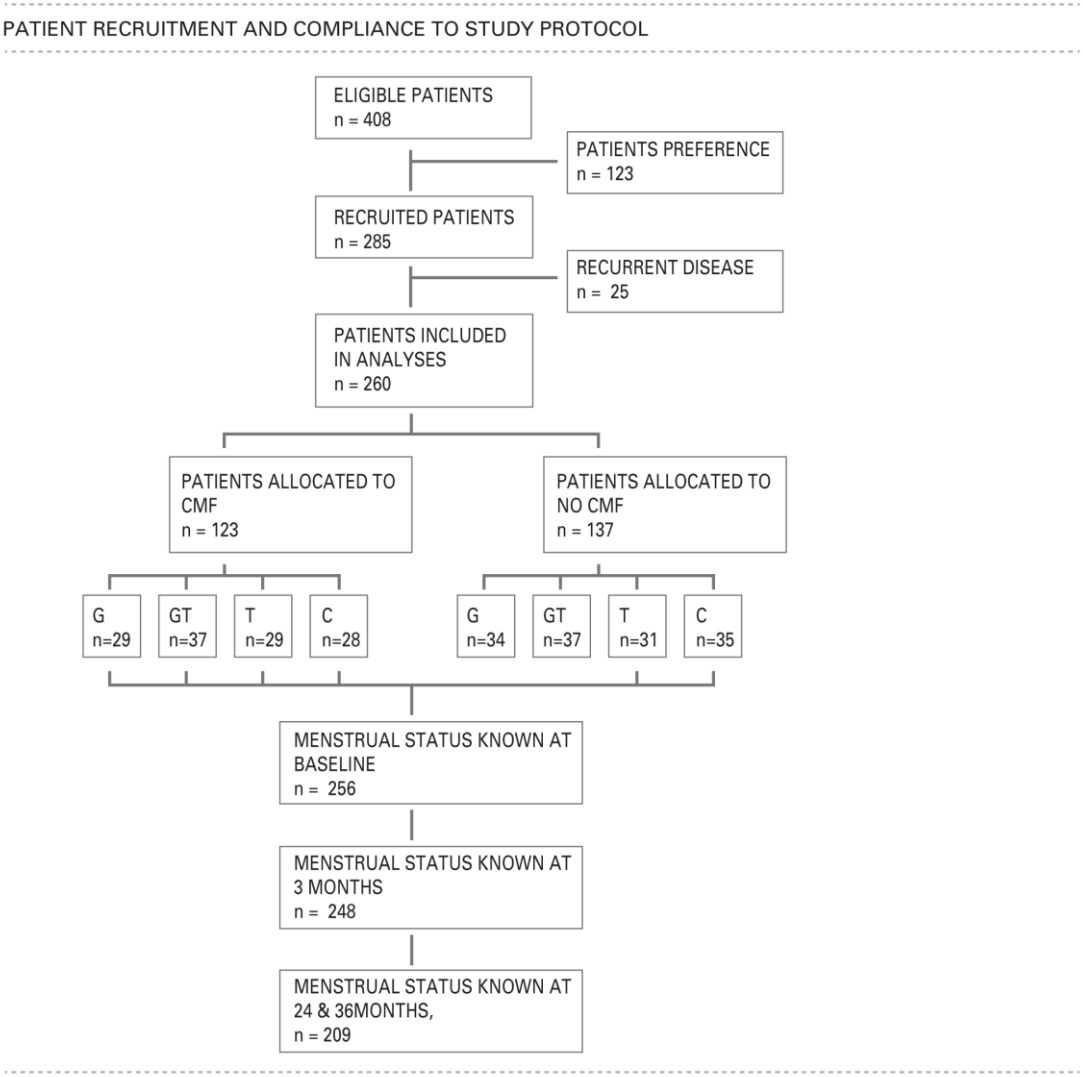

Figure 6. Study design and patient flow diagram

3.3.1 Instrument

Self-reporting questionnaires were obtained from patients on scheduled visits to the outpatient clinic at baseline, 3 months, 12, 24, 30 and 36 months after randomisation. The questions on menses were a part of the quality-of-life questionnaire designed for the study of side effects [163]. The women reported whether menses had ceased, had not ceased but become scanty or not ceased since the previous survey and visit to the outpatient clinic. The first survey was made at the time of randomisation and the period

since the previous survey, varied from 3 to 9 months. All analyses were performed according to the intention-to-treat principle. In analysing each time point, menses self-reported as regular or irregular were deemed as having menses, whereas women reporting absence of menses were assessed as being amenorrheic. Fisher's exact test was used to assess differences between the treatment groups.

3.4 PAPERS III-IV

Between October 1990 and June 1994, patients at the Department of Oncology, Karolinska University Hospital Södersjukhuset in Stockholm were recruited to the study of bone effects. Only patients from the strata not receiving chemotherapy were eligible for the study on BMD and bone markers. A total of 89 (81% of eligible patients at the two participating hospitals during the period of entry) node-negative patients from the four randomised groups were recruited to the bone mineral study. Of the eligible women, 27 patients were assigned to groups either receiving tamoxifen or tamoxifen plus goserelin; 26 patients were assigned to receive goserelin alone, and 30 patients were assigned to the control group (Figure 5).

3.4.1 Bone mineral density study (Paper III)

Women with recurrent or metastatic disease were withdrawn from further bone mineral examinations. Data for 23 patients were unavailable after 12 months. Of these, 3 patients died, 6 patients had missing data because of their preference, and 14 patients had missing data because of administrative errors. Complete data on all four measurements as defined in the protocol were available for 53 patients. The mean difference in BMD change between examinations at 24 months and baseline and within the treatment groups was tested by the paired t-test Differences in BMD change between treatment groups were estimated by multiple linear regression, controlling for

baseline values. Analyses were made according to the intention-to-treat principle and included women with at least three measurements of BMD available.

3.4.2 Bone Densitometry (Papers III-IV)

Bone density measurement was made before initiation of treatment and at 12 months, 24 months, and 36 months post-initiation (i.e., the last examination was made 1 year after treatment finished). Total body bone mass (TBBD) was measured by dual-energy x-ray absorptiometry, using a Lunar DPX-L device (Lunar Corporation, Madison, WI). The precision of the technique is 1%, and the accuracy is 10%. BMD of the lumbar spine (L2 to L4) was measured at baseline for the patients as well for validation of the equipment used. An experienced investigator blinded to the individual patients' identities analyzed the scans. All measurements were made with the same equipment and evaluation procedures.

3.4.3 Bone marker assays (Paper IV)

Blood sampling was performed on the planned visits to the out-patient clinic at baseline and every three months up to one year after stopped treatment. A total of 110 patients recruited to the examinations of bone markers were recruited to the study. Blood samples were collected at 6-month interval up to 36 months. All blood samples were drawn and stored at -70°C at the same centre (hospital, out-patient clinic). All blood analyses were performed at one laboratory, with the same equipment and technique. Of the 82 patients where blood samples were available at baseline, 17 were excluded from analysis (three because of metastatic or recurrent disease, two because of death and twelve because of patient preference). Of the remaining 65 patients, analysis included a total of 50 patients, where bone marker assays were available at baseline and 6 months.

BMD examinations were done at baseline and 6 months; in addition, bone marker assays were available for 40 patients.

The biochemical bone turnover markers osteocalcin (OC), pyridinoline cross-linked amino terminal telopeptide of type I collagen (PINP) and C-terminal telopeptide (CTX) were measured. The OC measurements were made with Elecsys(R) 1010/2010 System Osteocalcin Immunoassay. The assay uses 2 monoclonal antibodies against the N-MID-fragment and N-terminal fragment of the osteocalcin molecule. The method is non-dependent on the unstable C-terminal fragment. The sensitivity of the assay is 50 µg/L. The PINP analyses were made by immunoassay technique, using Roche Elecsys total PINP test with a sensitivity of 5 µg/L, and β –CTX was measured with Elecsys β-CrossLaps/serum assay. The assay uses 2 monoclonal antibodies against a cross-linked isomerised type I collagen fragment and has a sensitivity of 0.05 ng/ml. Values for OC, PINP and CTX are expressed in ng/mL. The intra- and interassay coefficient of variation (CV) was 2.0% and 4% for OC, 1.7% and 4.4% for PINP and 2.5% and 3.5% for CTX. The mean ratio (6 months/baseline) was calculated as the mean paired difference of the log-transformed variables and was tested using the t-test. The log-transformed differences were then back-transformed, where the antilog of the mean differences is an estimate of the geometric mean of the ratio of the variables. Results are presented as ratios together with 95% confidence intervals. Association between BMD and the bone markers OC, PINP and CTX was assessed by using the Spearman rank correlation coefficient. Confidence intervals for the correlation coefficients are based on Fisher's transformation.

4 RESULTS

4.1 PAPER I

The treatment groups were similar in regard to clinical and histopathological characteristics, as seen in Table 3.

PATIENTS CHARACTERISTICS BY ALLOCATED TREATMENT

	CONTROL N=234	GOSERELIN N=231	TAMOXIFEN N=231	GOSERELIN & TAMOXIFEN N=229
Age at randomization (SD), years	45.4 (5.5)	46.0 (4.9)	45.6 (5.1)	45.5 (5.0)
Histopathological nodal involvement				
N0	116 (50%)	118 (51%)	117 (51%)	114 (50%)
N1-3	81 (35%)	80 (35%)	79 (34%)	79 (35%)
N4+	37 (16%)	33 (14%)	35 (15%)	36 (16%)
Histopathological tumor size (mm)				
<20	152 (65%)	149 (65%)	153 (66%)	139 (61%)
21-50	73 (31%)	74 (32%)	72 (31%)	82 (36%)
>50	5 (2%)	4 (2%)	4 (2%)	4 (2%)
Unavailable	4 (2%)	4 (2%)	2 (1%)	4 (2%)
Estrogen receptor status				
Positive[1]	151 (65%)	147 (64%)	153 (66%)	141 (62%)
Negative[2]	51 (22%)	44 (19%)	50 (22%)	56 (25%)
Unavailable	32 (13%)	40 (17%)	28 (12%)	32 (14%)
Scheduled for chemotherapy	118 (50)	113 (49)	114 (49)	114 (50)

[1]>0.05 fmol/µg DNA [2]<0.05 fmol/µg DNA

Table 3. Patients characteristics by allocated treatment

After a median follow-up time of 12.3 years, 166 women presented with loco-regional recurrence as a first event, 159 had distant metastases, 54 had contra-lateral breast cancer, 50 women were diagnosed with other cancers, and there were 6 deaths as first event. The overall number of deaths from breast cancer was 225, and there were 26 non-breast cancer deaths. The total number of first events was 435. There were 128 (55%) first events in the C group, 98 (42%) in G treated, 101 (44%) in T treated and 108 (47%) in the GT group (Table 4).

TYPE OF EVENT BY ALLOCATED TREATMENT				
EVENT	CONTROL	GOSERELIN	TAMOXIFEN	GOSERELIN & TAMOXIFEN
Failures (1st events)	128 (55%)	98 (42%)	101 (44%)	108 (47%)
Deaths	75 (32%)	51 (22%)	59 (26%)	66 (29%)
Number of patients	234	231	231	229

[1]>0.05 fmol/µg DNA [2]<0.05 fmol/µg DNA

Table 4. Proportion of the type of first event in the different treatment groups

Three main sets of analyses were carried out: first the overall effect of endocrine therapy versus no endocrine therapy; second, the effect of goserelin with or without tamoxifen and, finally, the effect of tamoxifen with or without goserelin.

1. Compared to the controls, endocrine treatment with either goserelin alone or combined with tamoxifen or endocrine treatment with tamoxifen alone reduced the risk of first recurrence by 32% (CI, 0.52–0.89), 24% (CI, 0.59–0.98) and 27% (CI, 0.56–0.94), respectively (P = 0.021).
2. Goserelin treatment reduced the risk of first recurrence by 16% (CI, 0.68–1.02). There was no additional beneficial effect of goserelin with tamoxifen, but in the absence of tamoxifen, first recurrence was reduced by 32% (CI, 0.53–0.89).
3. The main effect of tamoxifen was not statistically significant (HR: 0.89, CI, 0.74–1.08), but when examined separately for those not treated with goserelin, there was a 27% benefit from tamoxifen (CI, 0.56–0.95). A test for interaction between goserelin and tamoxifen was statistically significant (p = 0.025) (Figure 7).

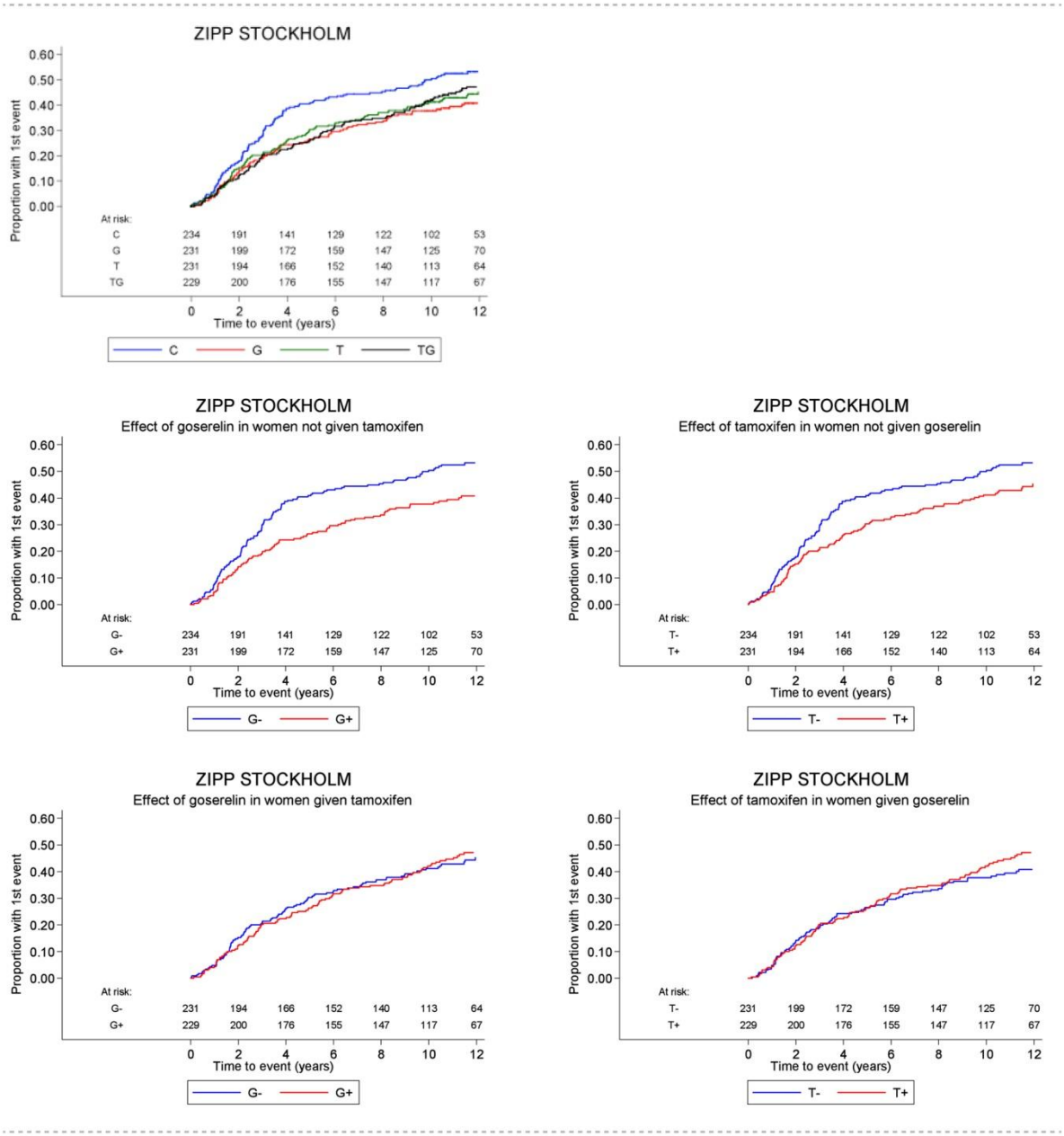

Figure 7. First event according to treatment groups.

C: controls, G: goserelin, T: tamoxifen, TG: tamoxifen and goserelin, G-: without goserelin, G+: with goserelin, T-: without tamoxifen, T+: with tamoxifen

When we examined the treatment effect for different levels of ER content, there was an overall effect of goserelin in the high-ER group (HR: 0.71, CI, 0.50–0.99, P = 0.044) and a trend towards greater risk reduction with increasing levels of ER content. The greatest risk reduction from goserelin treatment in the group with high ER was observed among those not receiving tamoxifen (HR: 0.52, CI, 0.32–0.84, P = 0.007). The main effect of tamoxifen in the high-ER group was not significant (HR: 0.85, CI, 0.61–1.18). Neither was there a significant risk reduction for tamoxifen alone (HR: 0.68, CI, 0.44–1.05, P = 0.081). For the intermediate ER group, there was no main effect from either goserelin (HR: 0.80, CI, 0.57–1.14) or tamoxifen (HR: 0.97, CI, 0.68–1.38). Finally, among those classified as ER negative, there was no main effect from goserelin (HR: 1.25, CI, 0.85–1.82), tamoxifen (HR: 0.85, CI, 0.59 to 1.24) or combined goserelin and tamoxifen (HR: 1.03, CI, 0.63–1.67). In the intermediate and ER-negative groups, the treatment effects of goserelin and tamoxifen did not modify each other (Fig. 8).

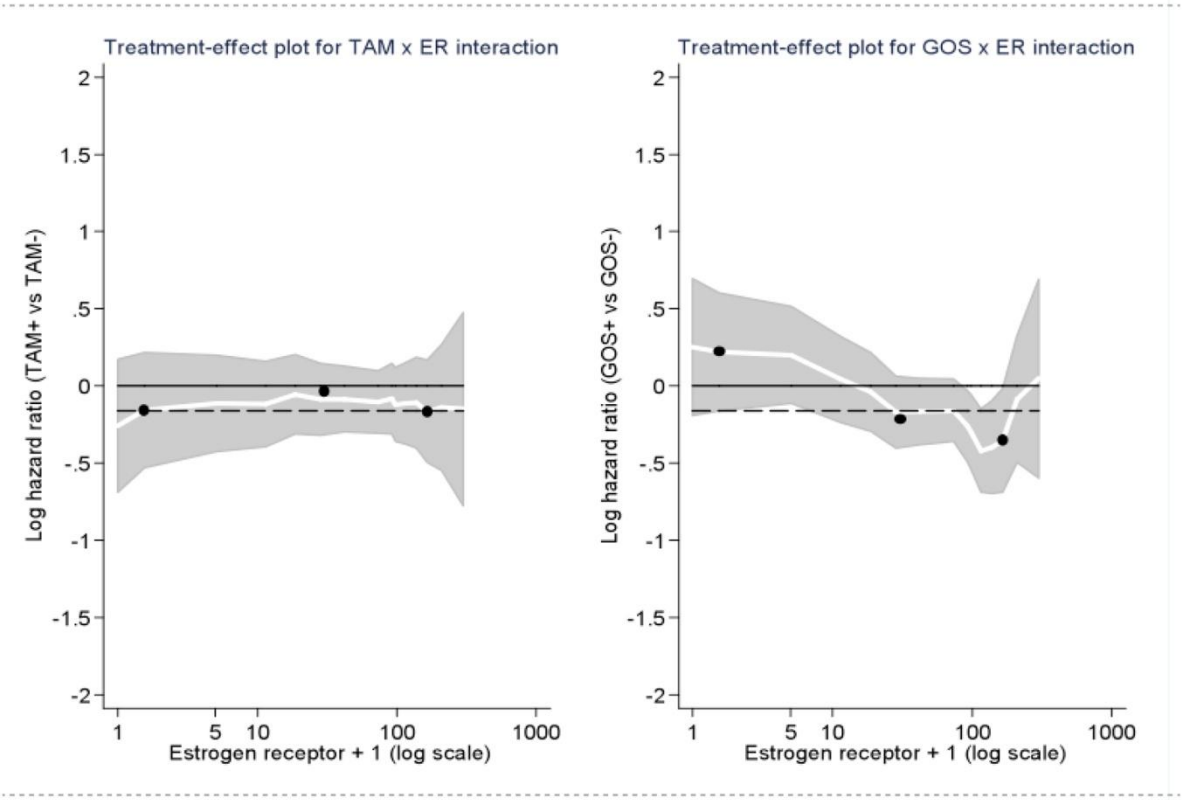

Figure 8. Subpopulation treatment effect pattern plot at different ER levels.

The curves show hazard ratios on a logarithmic scale for treatment interactions. Circles represent the treatment effect (plotted at the category mean) in the ER categories: < 0.05, 0.05–0.59 and ≥ 0.60 fmol/µg DNA.

4.2 PAPER II

4.2.1 Women treated with CMF chemotherapy

At baseline, 6% (7/119) women reported absence of menses, which was in accordance with inclusion criteria, as premenopausal status was defined as the last menses within less than 6 months. At 3 months, 50% (13/26) of the women in the C group reported amenorrhea. For women in the T group, the figure was 73% (19/26). Women in G or GT groups, reported amenorrhea in 93–94% (26/28 and 34/36 respectively) of cases. At 24 months, i.e., after 2 years of endocrine treatment, 85% (17/20) of the controls, 95% (19/20) of the T group, 97% (29/30) of the GT and 92% (22/24) of the G group were amenorrheic. At this time point, there was no statistically significant difference in amenorrhea for goserelin-treated patients, compared to all other treatment groups (P = 1.00). Six months after cessation of endocrine treatment, the proportion of amenorrheic women continued to increase to 94% (16/17) for the controls, was 87% (20/23) for the T group, unchanged at 92% (24/26) for the GT group, but only 67% (14/21) of the women treated with goserelin reported absence of menses at this time point. At 36 months, i.e., 1 year after stopped endocrine treatment, the proportion of amenorrheic women in the control group was 90% (18/20), 87% (20/23) for the T group, 93% (27/29) for the GT group but had decreased to 64% (14/22) for the G group. The increase in the proportion of menstruating women in the G group was statistically significant, compared to all other groups where menses were unchanged or decreasing between 24 and 36 months (P = 0.006) (Fig. 9).

4.2.2 Women not treated with CMF chemotherapy

All randomized patients reported having menses at baseline. At 3 months, 9% (3/34) of the controls, 21% (6/29) of the T group, 86% (31/36) of the GT group and 97% (32/33) of the G group were amenorrheic. At 2 years after randomization, 13% (4/31) of the controls, 21% (5/24) of the T-group, 69% (22/32) of GT-group and 82% (23/28) of the G-group reported amenorrhea. Six months after completed endocrine treatment, 17% (5/29) of the controls, 12% (3/24) of the T group, 28% (8/29) of the GT group and 37% (10/27) of the G group were amenorrheic. At 36 months, i.e., 1 year after completed endocrine treatment, 20% (6/30 and 5/25 respectively) of the controls and T group, 32% (10/31) of the GT group and 41% (12/29) of the G group were amenorrheic. At 36 months, i.e., 1 year after stopped endocrine therapy, there was no statistically significant difference in the menstrual status of women treated with goserelin, compared to all other treatment groups (P = 0.15).

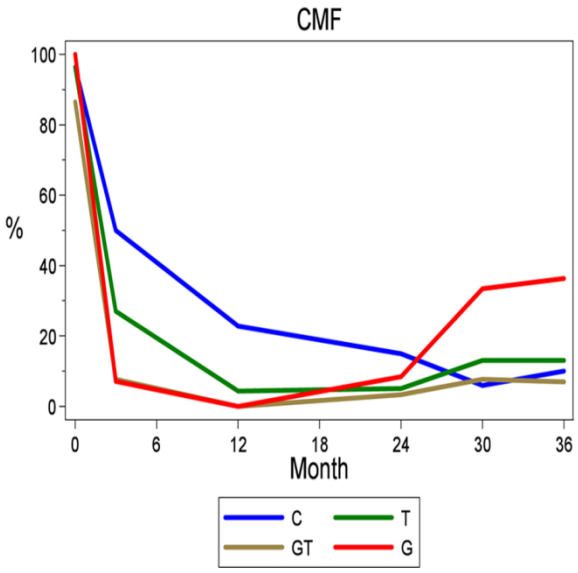

Figure 9. Ovarian function according to treatment. Percentage of CMF treated women menstruating at different time points

4.3 PAPER III

During the first 2 years, all three endocrine treatment groups showed a significant decrease in BMD, and the greatest changes in all measurements were seen in patients allocated to goserelin treatment. The groups receiving tamoxifen alone or tamoxifen plus goserelin showed a less but continuous decline in BMD. For patients allocated to the control group, no significant change in BMD was found. The G group showed an average change from baseline in BMD (in grams per centimetres squared) of - 0.057 (P < 0 .001). In the TG group the change was -0.015 (P = 0 .02), and in the T group the change was -0.018 (P < 0.001). In the group receiving no endocrine treatment, the change was -0.002 (P = 0.76). These absolute changes correspond to decreases in BMD of 5.0%, 1.4%, 1.5%, and 0.3% for the G group, TG group, T group, and control group, respectively. Of the endocrine-treated groups, only the G group significantly differed in mean change in BMD at 2 years, compared to the control group (-0.058 g/cm^2; 95% CI, -0.078 to -0.039; P < 0.001). At 3 years (1 year after cessation of treatment), the G group alone showed a partial recovery from bone loss, with a change of 0.017 (P = 0.02). This corresponds to an increase in BMD of approximately 1.5%. None of the other groups showed significant changes in BMD during this period. Changes in BMD are presented in Figure 10. Baseline measurements of BMD of the lumbar spine showed a correlation with TBBD (r = 0.8).

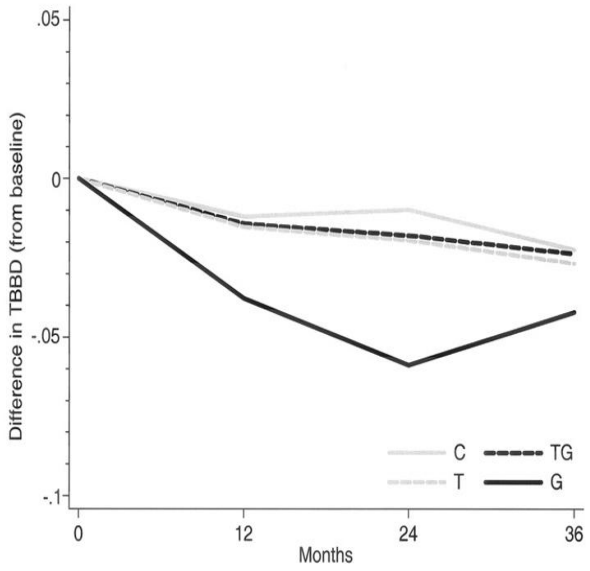

Figure 10. Change in bone mineral density in the different treatment groups

Mean change in BMD (g/cm^2) in different treatment groups from baseline up to 3 years of treatment for 53 patients. C: control, T: tamoxifen, TG: tamoxifen plus goserelin, G: goserelin

4.4 PAPER IV

Among women treated with goserelin (G), there was a statistically significant elevation of all bone markers at 6 months. The mean increase in OC was 57% (CI, 1.31-1.89, $p < 0.001$); PINP increased by 65% (CI, 1.29-2.11, $p = 0.001$), and there was a 98% increase in CTX (CI, 1.55-2.53, $p < 0.001$). Among women treated with goserelin in combination with tamoxifen (TG), there was a 5% decrease in OC (CI, 0.83-1.08, $p = 0.35$), a 13% decrease in PINP (CI, 0.75-1.02, $p = 0.087$), and a 10% increase in CTX (CI, 0.84-1.42; $p = 0.45$). In women treated with tamoxifen alone (T), OC decreased by 13% (CI, 0.54-1.40, $P = 0.52$), PINP by 13% (CI, 0.54-1.40, $P = 0.52$), and there was an increase in CTX of 4% (CI, 0.83-1.30, $P = 0.71$). Among the control (C) group of patients, OC decreased by 3% (CI, 0.83-1.12, $P = 0.64$), PINP by 22% (CI, 0.67-0.91, $P = 0.003$), and there was a 15% increase in CTX (CI, 0.98-1.36, $P = 0.076$). Among the 40 patients where BMD examinations were available at baseline and at 6 months, there

was on average a 4% decrease in total body bone mass among G-treated patients (CI, 0.95-0.97, P < 0.001), a 2% decline among TG-treated patient as well as those treated with T alone (CI, 0.97-0.99, P < 0.001 and 0.97-1.00, P = 0.012, respectively). There was no statistically significant change in BMD in the C group (CI, 0.98-1.01, P = 0.86) (Table 4). Spearman rank correlation analysis showed that change in BMD was inversely correlated to change in all bone markers. After 6 months of treatment, there was a statistically significant association between change in BMD and OC (r = -0.51, CI, -0.71 to -0.23), PINP (r = -0.40, CI, -0.63 to -0.10) and CTX (r = -0.41, CI: -0.64 to -0.10) (Table 5).

MEAN BLOOD VALUES AND BONE MINERAL DENSITY BY ALLOCATED TREATMENT

		EXAMINATIONS AT BASELINE AND AT 6 MONTHS MEAN VALUES (SD)			
TREATMENT	N	BASELINE	6 MONTHS	RATIO[A] (95% confidence interval)	P-VALUE[B]
CTX					
Goserelin	13	0.37 (0.15)	0.73 (0.32)	1.98 (1.55-2.53)	<0.001
Goserelin and tamoxifen	10	0.36 (0.16)	0.38 (0.13)	1.10 (0.84-1.42)	0.45
Tamoxifen	11	0.24 (0.14)	0.23 (0.09)	1.04 (0.83-1.30)	0.71
Control	15	0.25 (0.13)	0.29 (0.14)	1.15 (0.98-1.36)	0.076
PINP					
Goserelin	13	58.3 (21.5)	102.7 (56.7)	1.65 (1.29-2.11)	0.001
Goserelin and tamoxifen	11	46.0 (14.5)	40.1 (11.5)	0.87 (0.75-1.02)	0.087
Tamoxifen	11	43.9 (21.6)	32.9 (9.5)	0.87 (0.54-1.40)	0.52
Control	15	51.7 (16.1)	40.9 (14.5)	0.78 (0.67-0.91)	0.003
OSTEOCALCIN					
Goserelin	13	25.0 (9.9)	38.6 (11.6)	1.57 (1.31-1.89)	<0.001
Goserelin and tamoxifen	11	20.6 (5.2)	19.4 (4.3)	0.95 (0.83-1.08)	0.35
Tamoxifen	11	17.7 (3.8)	15.5 (4.5)	0.87 (0.75-1.01)	0.058
Control	15	19.0 (5.3)	18.7 (6.5)	0.97 (0.83-1.12)	0.64
BMD					
Goserelin	11	1.15 (0.06)	1.11 (0.05)	0.96 (0.95-0.97)	<0.001
Goserelin and tamoxifen	8	1.10 (0.05)	1.08 (0.05)	0.98 (0.97-0.99)	<0.001
Tamoxifen	9	1.22 (0.07)	1.20 (0.06)	0.98 (0.97-1.00)	0.012
Control	12	1.13 (0.04)	1.13 (0.06)	1.00 (0.98-1.01)	0.86

Table 5. Mean blood values of bone markers and bone mineral density by allocated treatment.

[A]Mean ratio (6 months / baseline) of the paired values. [B]Paired t-test on log-transformed data.

5 DISCUSSION

This thesis focuses on the efficacy as well as long-term side effects in regard to bone health and ovarian preservation from ovarian ablative therapy in premenopausal breast cancer. All results are based on the Stockholm cohort of the larger ZIPP trial.

5.1 PAPER I:

The Stockholm cohort was exceptional in several aspects in comparison to the main ZIPP trial. In our study, patients were randomly assigned to endocrine therapy by a strict 2 x 2 factorial design, as was initially planned for the main trial. This cohort was also well defined according to hormone receptor content, nodal status and whether chemotherapy and/or radiotherapy were given or not. In addition, our strictly randomised data, based on all ER measurements made in a single laboratory, were suited for examining the significance of ER content for predictability of endocrine therapy. The EBCTCG Overview group has concluded that LHRH agonists alone improve survival in the adjuvant setting in hormone receptor positive breast cancer [8, 70]. There are, however, some remaining questions regarding their optimal use. Among these is the uncertainty over the added value of combining tamoxifen with a LHRH agonist. As there is lack of data from randomised trials where LHRH agonists are tested against chemotherapy, with or without tamoxifen in both arms, a separate study of our cohort therefore seemed reasonable. Furthermore, the analyses of ER content, analysed at a single, highly qualified laboratory with a quantitative cytosol method, allowed analysis of the effect of different receptor levels on outcome. Our study shows that the effect of goserelin on recurrence is considerable. The most marked effect of goserelin is seen among those not concomitantly receiving tamoxifen. In a recent update of the ZIPP trial, there was also a survival benefit, not possible for us to examine in the Stockholm cohort because of the small sample size [160]. Our study

allows formal testing of interaction that is not possible for the overall trial because of inconsistencies in the study design. This formal test of the interaction between goserelin and tamoxifen in our randomised cohort confirms what was indicated in the overall trial report, i.e., that tamoxifen provides no additional benefit among women treated with goserelin. The same is true for the opposite, where goserelin provides no additional benefit for those treated with tamoxifen. Tamoxifen is effective in extending the time to first recurrence, but only among those not receiving goserelin. Our results, showing no additional benefit from combined endocrine therapy, are consistent with Overview data [164]. Former reports on combination endocrine therapy in postmenopausal adjuvant setting, such as the ATAC and ABCSG-12 trials, as well as the FACT trial in the metastatic setting, have also shown a lack of benefit [69, 150, 165]. Interestingly, the principle of combining endocrine drugs for improved efficacy has therefore not been proven. Sequential endocrine therapy, on the other hand, has proven to be beneficial, as seen in the BIG 1-98 and MA-17 trials [67, 166]. Survival analysis of the effect from different endocrine treatment overall, or in the subset of women developing chemotherapy-induced amenorrhea, was not possible in our study due to the limited cohort size. The ongoing Suppression of Ovarian Function Trial (SOFT) is expected to clarify the effect of combination endocrine therapy in the premenopausal setting more decisively. Our analysis of ER levels confirms the lack of effect on ER-negative women, in line with previous reports [16, 44]. An intriguing finding is, however, that the effect of goserelin increases with higher ER levels, whereas the effect of tamoxifen is unconditional of ER content. This may be a finding of chance, but the data indicate a clear difference, which may be based on biological grounds. Goserelin, which has a stronger estrogen suppressor effect than tamoxifen, is potentially more effective when ER content is high. In this group, tamoxifen may not have sufficient effect to counteract the highly estrogenic milieu of premenopausal

women. The STEPP plots demonstrating this effect are, however, based on small subsets of patients, and the data therefore have to be interpreted conservatively. Among postmenopausal women earlier studies have shown a significant trend in reduction of recurrence rate with higher ER levels among women treated with tamoxifen [167] [27, 30, 162]. This supports the significance of stronger ER content in endocrine treatment where ovarian function is absent. A recent overview of pre- and postmenopausal patients shows unequivocal benefit of tamoxifen in all ER-positive categories, and the benefit is not dependent on the strength of ER content as formerly suggested [15, 44]. This supports our data in regard to the predictability of a tamoxifen effect related to ER content.

Quality of life aspects and long-term side effects are important factors to be taken into consideration when assessing improvements in adjuvant therapy. The Overview group earlier showed that goserelin is an option for women strongly opposed to cytotoxic drugs, or where chemotherapy is contraindicated [8]. Premature menopause is highly probable after chemotherapy, and desire for pregnancy is not uncommon after completed treatment. Moreover, some women may be reluctant to risk the adverse effects of permanent ovarian failure, such as infertility and accelerated bone loss. In addition to the studies presented here, the Stockholm cohort has been studied extensively concerning quality of life aspects and reported in several of publications [51, 85, 161]. Berglund et al. have earlier shown that sexual dysfunction from goserelin was substantial the first 2 years but diminished over time, whereas chemotherapy-related symptoms were on-going at follow-up at 3 years[85]. Nystedt et al. have similarly shown in the same cohort that menopausal side effects from goserelin the first 2 years are worse than from CMF. There were no differences in side effects from goserelin and/or tamoxifen among those receiving CMF chemotherapy. Only patients who did not receive chemotherapy had various effects from endocrine treatment. Among those,

treatment with goserelin was worse than therapy with combined goserelin-tamoxifen or tamoxifen alone. Anxiety and depressive symptoms were not significantly affected by endocrine treatment or chemotherapy. At 3 years of follow-up, the menopausal symptoms from goserelin reversed, whereas physical symptoms of CMF therapy effects were persistent [86]. Tolerability of goserelin in the adjuvant setting has also been reported by the Zoldex Early Breast Cancer Research Association (ZEBRA) with similar results. Side effects related to therapy-induced menopause were worse for goserelin, compared to CMF the first 2 years of treatment. However, one year after stopped treatment, side effects increased among patients treated with CMF, whereas side effects reversed when menses returned after goserelin treatment [74, 168]. Sick leave and factors associated with returning to work after treatment were examined by Johnson et al. Their study showed that endocrine therapy was associated with a twofold increase of risk of not having returned to work after 2 years [161]. Compliance in the studies described here (papers I-IV) was good in all study groups. Compliance to adjuvant endocrine therapy is a factor that has recently come increasingly into focus. Adherence to 5 years of treatment can be a challenge for patients suffering from menopausal symptoms, and lack of compliance translates into inferior survival rates [169]. Clearly, this presents a great challenge for health professionals in regard to patient information as there is need for adequate follow-up and appropriate treatment of adverse effects. Lately, the use of selective serotonin reuptake inhibitors (SSRIs) that block the enzyme CYP2-D6 has been used against hot flushes, but there is concern that blocking the enzyme which metabolically activates tamoxifen to the potent antiestrogenic endoxifen reduces the benefits of the drug [170]. Selective norepinephrine reuptake inhibitors (SNRIs), such as venlafaxine, may be a safer alternative. The genetic variants of CYP2-D6 may also influence outcome among tamoxifen treated, but further research is needed before a routine assessment of the enzyme is recommended.

A limitation of the design of the Stockholm study may be the 2 years of 40 mg/day tamoxifen, which was standard at the time, instead of 5 years of 20 mg/day treatment as currently recommended. In contrast to tamoxifen, 2 years of goserelin is still within the recommended treatment period of 2 to 3 years although the optimal duration of treatment is not yet determined. Another limitation is the concurrent use of chemotherapy in the trial, which may not be optimal, based on the risk of endocrine therapy interacting with chemotherapy and sequential treatment currently being recommended as standard. This concern, however, is primarily based on a single report by Albain et al. [171]. In all studies presented here (Papers I-IV), randomisation minimized the risk of uneven distribution of such factors in the different treatment groups.

5.2 PAPER II:

Our study on ovarian function shows, that amenorrhea is significantly less frequent one year after completed treatment among patients treated with goserelin as the only additive endocrine therapy during chemotherapy. This suggests that goserelin has a role in protecting ovarian function in a cytotoxic milieu, as has been reported in a recent review of randomised trials by the Cochrane group. There it is concluded that the use of LHRH agonists seems to be effective in protecting ovaries during chemotherapy and should be considered in women of reproductive age receiving chemotherapy [172]. Previous data have nevertheless been conflicting. Several non-randomised studies have shown benefit from LHRH agonist treatment [96, 122-124, 173]. Other studies have shown no difference in the menses restoration rates [119, 121, 127]. The ZORO trial by the German Breast Group, where goserelin prophylaxis during taxane-based chemotherapy was investigated, showed no protective effect from goserelin on ovaries function [126]. In our study, the benefit is moderate and may be due to several effect modifiers, such as high

age and the timing of LHRH agonist treatment. Here, the age is rather high for a premenopausal population, with a mean age of 45 years. This is an age where natural menopause approaches, and age above 40 years is undisputedly a strong risk factor for permanent chemotherapy-induced amenorrhea [174]. The timing of goserelin treatment is another factor, which possibly may not be optimal concerning a potential effect on preservation of ovarian function. Theoretically, LHRH agonist treatment should preferably start at least 2 weeks before initiation of chemotherapy, as an approach to optimising ovarian suppression in advance of cytotoxic chemotherapy effects. In our study, however, goserelin injections were started simultaneously with the first CMF course, which may be too late for achieving full effect. On the other hand, there are concerns of possible hazards from concomitant LHRH agonist and cytotoxic chemotherapy. Theoretically, there is a risk of an LHRH agonist affecting a receptor level, inducing cell cycle arrest, inhibiting proliferation and apoptosis and thereby reducing the efficacy of cytotoxic drugs. This negative effect, however, was not confirmed by the EBCTCG meta-analysis of the effect of LHRH agonists, which showed no significant difference in efficacy between chemotherapy used alone versus chemotherapy used in addition to an LHRH agonist [8]. Nevertheless, LHRH agonists should not be recommended routinely but used highly selectively, preferably in hormone receptor negative tumours, until data are conclusive. When tamoxifen was added to goserelin in CMF-treated women, there was no statistically significant change in the proportion of menstruating women one year after stopped treatment, in contrast to the CMF-treated women receiving goserelin alone. The mechanism behind this is unclear but can be explained by the SERM properties of tamoxifen, which acts as an estrogen agonist or antagonist, depending on the endogenous estrogen milieu. The rates of amenorrhea in our study are comparable to several earlier studies, where CMF frequently induces early and irreversible amenorrhea [74, 87, 103, 148]. The ZEBRA study

showed results comparable to ours, in that 65% of CMF-treated women became permanently amenorrheic [74]. CMF was the standard chemotherapy regimen at the time of the study, whereas anthracycline based chemotherapy, with or without taxanes, have later replaced CMF. The extent of ovarian suppression from these agents seems similar or even less than from CMF [90, 91, 97, 104, 105, 108, 109]. Our study, like most previous studies, used amenorrhea as a surrogate for ovarian failure. However, absence of menses is not an accurate measure of ovarian function. A woman can thus retain premenopausal status as well as fertility in spite of being amenorrheic. Likewise, a woman with regular menses can be infertile. Tamoxifen treatment for 5 years after cytotoxic chemotherapy presents a special dilemma, as tamoxifen can affect menses to a variable degree. Amenorrhea while on tamoxifen therapy does not equal premature menopause and infertility, and the effect of tamoxifen is reversible. Additional parameters of ovarian function, such as serum analysis of FSH, LH, anti-Müllerian hormone (AMH) and inhibin B, have been studied as markers of ovarian reserve but are not yet integrated into clinical practice [99, 100, 175]. Poikonen et al. showed that post-chemotherapy menstrual status is a clinically useful marker of menstrual status, and that FSH and LH are less reliable [176] . The evaluation of serum markers, such as FSH and LH, after chemotherapy is therefore still under investigation. Furthermore, their use is not appropriate during goserelin and/or tamoxifen treatment because of gonadal suppression resulting in decreased levels of FSH and LH [175, 177]. Possible confounders, such as the previous use of contraceptive pills as well as hormone replacement therapy due to menopausal symptoms, or prolonged tamoxifen treatment beyond 2 years, were not examined in this study.

5.3 PAPERS III-IV:

Our study on bone mineral density shows that goserelin causes a 5% loss of BMD after 2 years of treatment, but BMD partially recovers one year after stopped treatment. This represents rapid bone loss in young women, often many years prior to natural menopause, and may be an important determinant of fracture risk, as several other studies have shown [132, 137, 138]. This BMD study is consistent with other reports on LHRH agonist and/or tamoxifen effects among premenopausal women [142, 145, 146]. Treatment with tamoxifen alone resulted in a mild, yet statistically significant, decrease in BMD. This apparent menstrual status–dependent effect can be explained by the difference in endocrine milieu in which tamoxifen is acting. In premenopausal women a demineralising effect on bone may be caused by tamoxifen antagonizing the more potent activity of endogenous estrogen [178]. Among patients treated with goserelin in addition to tamoxifen, the effect on bone mass was similar to that in the group treated with tamoxifen alone. In this group, it seems that tamoxifen at least partially counteracts the demineralising effects of goserelin. In premature menopause from LHRH agonist treatment, it seems that tamoxifen when added to goserelin has similar agonistic estrogenic effects on bone as in postmenopausal women. In order to estimate the clinical impact of a 5% bone loss from goserelin, as in our study, a 10% loss in BMD is equivalent to a drop in T score by 1 which in turn increases fracture risk by 2.6 times. Therefore, we conclude that there is increased fracture risk from goserelin treatment although other factors play a role in bone strength [179, 180].

In the study of bone turnover, the LHRH agonist goserelin increases bone turnover. Markers of both bone formation and bone resorption increase after 6 months of goserelin treatment, whereas other endocrine therapies in our study do not show significant bone marker changes. Tamoxifen alone or in combination with goserelin is

not associated with significant changes in BTM. When compared to changes in BMD, the changes in bone markers show an inverse association, i.e., a decrease in BMD is correlated to elevation of serum bone markers. The effects of goserelin on BTM and BMD are distinct in comparison to other endocrine therapies. The increase in markers of both bone formation and bone resorption is consistent with other studies, and reflect the instability of the microstructure of bone induced by increased bone metabolism. The negative effect on bone mass from LHRH agonists and tamoxifen treatment among premenopausal women has been reported in several studies. In a tamoxifen chemoprevention study Powles et al. showed an annual BMD loss of 1.4% in the lumbar spine [53]. Our previous BMD study showed 5% decrease in BMD on average among goserelin-treated patients, whereas treatment with a combination of goserelin and tamoxifen or tamoxifen alone resulted in only a mild (1.4 - 1.5%) decline in BMD [52]. Vehmanen et al. showed a similar effect of tamoxifen on bone loss, where women not achieving amenorrhea after chemotherapy developed bone loss from tamoxifen, whereas tamoxifen decreased bone loss among those achieving amenorrhea [147]. The ABCSG-12 study showed an even greater loss of bone (11% in the lumbar spine and 7% in the hip) for those treated with combined goserelin and tamoxifen, and tamoxifen did not seem to have a counterbalancing effect to the same extent as seen in our study [69]. Moreover, the ABCSG-12 study showed a survival benefit from the use of the bisphosphonate zoledronic acid, an effect which needs to be confirmed in further studies [143]. Several studies on bone effects from AIs in postmenopausal women have shown an inverse correlation between BMD and bone markers similar to that presented here [155, 181, 182]. These reports show that bone loss is manageable, and the use of goserelin in addition to AIs should not be restricted. However, more aspects must be taken into consideration before recommending AIs in this setting. AIs have also been shown to have significantly increased association with hypercholesterolaemia and

cardiovascular morbidity in comparison to tamoxifen [183]. Studies of the AIs anastrozole and exemestane have shown consistent and significantly more gastrointestinal symptoms, vaginal dryness, dyspareunia and diminished libido among women treated with AIs in comparison to tamoxifen [184-186]. At present, BMD measurements of the spine and hip are generally regarded as the method of choice for assessing fracture risk because of the frequency of fractures and their morbidity at these sites. This study was initiated before this was widely accepted. Therefore, it may be a limitation of our study that we did not measure BMD at the spine or hip. However whole-body measurements have advantages, such as detection of small changes with higher sensitivity than regional measurements [187]. Several investigators have shown a strong correlation on group level between whole-body BMD and BMD measurements at the hip or the spine [188, 189]. Whole-body measurements of BMD can therefore be used to detect bone loss as a systemic disease that is not limited to the axial skeleton [179, 190, 191]. In the past, a variety of bone markers have been examined besides OC, PINP and CTX as in our study. Bone-specific alkaline phosphatase (bone ALP), a relatively specific marker of bone formation, has also been widely used. Newer markers, such as such as tartrate-resistant acid phosphatase type 5b (TRAcP-5b), osteoprotegerin (OPG) as well as bone sialoproteins (BSP), have been described. There has however been a lack of consensus on the use of bone markers in clinical practice, and BMD examinations remain to date the standard assessment of bone mass changes. Nevertheless, the International Osteoporosis Foundation (IOF) and the International Federation of Clinical Chemistry and Laboratory Medicine (IFCC) have recently recommended the use of PINP as a marker of bone formation and CTX as a marker of bone resorption for BTM in clinical studies [192]. A strength of our study, despite its limited sample size, is the use of these markers. In addition, all analyses were performed at one laboratory, which minimizes the risk of laboratory inconsistencies. The blood samples, however, were not collected

in the fasting state, which may have influenced results, as CTX in particular is highly influenced by dietary intake and circadian rhythm. In the studies of bone mineral density and bone turnover, data on possible confounders, such as smoking, calcium, vitamin D intake, and physical exercise, were unavailable. Another limitation of our study on bone markers is that due to the small number of patients undergoing blood tests at the predefined interval, analysis past 6 months of treatment was impossible. Although the follow-up was short in our study, Rogers et al. have shown earlier that the rate of bone loss is most rapid in the early menopausal period [193]. Furthermore, studies of women with endometriosis have shown high bone turnover after only 3 to 6 months of goserelin treatment, followed by a decline in BMD [194, 195]. A variety of earlier studies of HRT-treated postmenopausal women have similarly shown that a decrease in bone turnover markers within 6 months correlates significantly with an increase in BMD after 1-2 years [134-136, 154]. The 6 months of follow up presented here may therefore be adequate and give clear indications for evaluating therapy-induced bone loss. Endocrine-induced bone loss with subsequent risk of osteopenic fractures is among the side effects that can be prevented by early use of interventions. Several studies have shown the positive effect of bisphosphonate treatment, especially if used from the start of OA therapy [142, 143, 147, 152].

In summary, goserelin is effective in reducing the risk of recurrence and improving survival in endocrine-responsive premenopausal breast cancer. These effects are not enhanced by the addition of tamoxifen, and there is a significant interaction between goserelin and tamoxifen. Quality of life is affected by goserelin, and its side effects are considerable, but they are manageable and, moreover, reversible. A meta-analysis from the Overview group has earlier suggested that younger women may benefit more than older premenopausal women from LHRH agonist treatment [8].

Our study indicates that there may be an additional subgroup of women besides the very young, i.e., those with strongly ER-positive tumours, who benefit more from goserelin treatment, whereas the effect of tamoxifen does not seem to be modified by ER content. A significant interaction indicates that the effect of goserelin depends on whether tamoxifen is given or not, and the effect of tamoxifen depends on whether goserelin is given or not. Our data support that there is no additional benefit from combination endocrine therapy in the premenopausal setting. Within the limitations of the exploratory approach and its limited power as a stand-alone trial, our results should be viewed mainly as hypothesis-generating, awaiting data from ongoing trials.

Our study shows that the addition of LHRH agonist to goserelin alone may prevent permanent amenorrhea for some women receiving cytotoxic chemotherapy. Young breast cancer patients should therefore not only receive adequate information on possible long-term risks of chemotherapy therapy but should as well be considered for optional goserelin treatment when future fertility is strongly desired. Our studies on bone effects show that treatment with the LHRH agonist goserelin results in substantial decrease of BMD and higher bone turnover in breast cancer patients. Tamoxifen seems to neutralize the effect of goserelin in regard to BTM as well as BMD. Furthermore, our results show a clear association of changes in BTM and BMD after only 6 months of endocrine treatment. We therefore conclude that bone marker examinations may predict bone loss in therapy-induced OA. These markers, in addition to BMD, can be used to improve the identification of women at high risk for rapid bone loss and make early interventions possible.

6 CONCLUSIONS

I: Adjuvant tamoxifen, in combination with the LHRH agonist goserelin, is not superior to either tamoxifen alone or goserelin alone in regard to recurrence-free survival in premenopausal endocrine-responsive breast cancer. A significant interaction indicates that the effect of goserelin depends on whether tamoxifen is given or not, and the effect of tamoxifen is dependent on whether goserelin is given or not.

II: In our study, there is a trend towards greater efficacy of goserelin with increasing ER levels. A subgroup of women with strongly ER-positive tumours benefits more from goserelin treatment, whereas the benefit of tamoxifen does not seem to be dependent on ER content.

III: In our study, there is some evidence of goserelin's protective effect on ovarian function in CMF-treated women. This effect was not observed where tamoxifen was given in addition to goserelin treatment.

IV**:** Two years of ovarian ablation from goserelin treatment induces a significant reduction in bone mineral density, but there is partial recovery from the bone loss one year after stopped treatment.

V: The addition of tamoxifen partially counteracts the demineralising effects of goserelin.

VI: After six months of goserelin treatment, there is an increase in markers of both bone resorption and bone formation, whereas there is no change in bone turnover from

tamoxifen alone or in combination with goserelin. In our study, there is an inverse correlation of changes in BMD and bone markers. In addition to BMD measurements, biochemical examinations of bone turnover markers may be useful for monitoring bone health, identifying women at risk for bone loss and making early interventions possible

7 FUTURE ASPECTS

In spite of extensive earlier research, there are still many unanswered questions and challenges remaining regarding estrogen effects and endocrine treatment. Regarding the Stockholm cohort of the ZIPP study, there are plans to further analyse frozen tumour material in search of possible predictive factors. Another research area is how the development of resistance to endocrine therapy in breast cancer can be prevented or reversed. Intracellular signalling pathways, such as the phosphatidylinositol 3-kinase (PI3K)–Akt–mammalian target of rapamycin (mTOR), play a role in tumour progression in breast cancer. Ligand-independent receptor activation of the ER is regulated by a substrate of mTOR [196, 197]. Drugs targeted to inhibit these pathways in order to reverse resistance are in development, and some already have proven clinical benefit, such as the selective inhibitor of the (mTOR) drug everolimus[198]. Src family tyrosine kinase inhibitors are also involved in several signalling pathways in breast cancer, such as ER and HER-2 under investigation [199, 200]. Drugs of this class, such as dasatinib, are being tested in ongoing trials among hormone receptor positive and HER-2 normal/positive patients. Among hypotheses tested in ongoing trials are endocrine therapy "drug holidays", designed to examine if endocrine resistance development can be prevented and efficacy increased. This concept may possibly impact long-term side effect profiles as well as compliance.

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