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A short-term biomarker modulation study of simvastatin in women at increased risk of a new breast cancer

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Abstract

Observational studies have demonstrated a decreased incidence of cancers among users of HMG CoA reductase inhibitors (statins) and a reduced risk of recurrence among statin users diagnosed with early stage breast cancer. We initiated a prospective study to identify potential biomarkers of simvastatin chemopreventive activity that can be validated in future trials. The contralateral breast of women with a previous history of breast cancer was used as a high-risk model. Eligible women who had completed all planned treatment of a prior stage 0–III breast cancer received simvastatin 40 mg orally daily for 24–28 weeks. At baseline and end-of-study, we measured circulating concentrations of high-sensitivity C-reactive protein (hsCRP), estrogens, and fasting lipids; breast density on contralateral breast mammogram; and quality of life by Rand Short Form 36-Item health survey. Fifty women were enrolled with a median age of 53 years. Total cholesterol, LDL cholesterol, triglyceride, and hsCRP fell significantly during the study (P values < 0.001 , < 0.001 , 0.003 , and 0.05 , respectively). Estrone sulfate concentrations decreased with simvastatin treatment ($P = 0.01$ overall), particularly among post-menopausal participants ($P = 0.006$). We did not observe a significant change in circulating estradiol or estrone concentrations, contralateral mammographic breast density, or reported physical functioning or pain scores. This study demonstrates the feasibility of short-term biomarker modulation studies using the contralateral breast of high-risk women. Simvastatin appears to modulate estrone sulfate concentrations and its potential chemopreventive activity in breast cancer warrants further investigation.

Keywords

Simvastatin; Breast density; Chemoprevention; Breast cancer; Contralateral breast

Introduction

Large-scale randomized trials of potential breast cancer prevention agents require thousands of patients and many years to complete as exemplified by the National Surgical Adjuvant Breast and Bowel Project P1 Trial, which enrolled more than 13,000 women from 300 centers who have been followed for more than 15 years at a cost of 60 million dollars [1–3]. There is therefore a pressing need to identify surrogate endpoints predictive of response to promising chemopreventive agents, which could then be taken to definitive randomized trials. The contralateral breast in women with a history of breast cancer can be used as a high-risk model to test promising chemopreventive agents given their known 0.5–1% per year risk of developing a contralateral metachronous breast cancer [4, 5]. In addition, women with a prior diagnosis of cancer are motivated and often eager to participate in chemopreventive trials [6].

Several lines of evidence suggest a potential chemopreventive role for HMG CoA reductase inhibitors (statins) in breast cancer. Observational studies have reported a decreased incidence of cancer in users of statins [7–9] although the data from subsequent meta-analyses have been conflicting [10–13]. One proposed mechanism of this anticancer effect is statin-induced blockade of the formation of cholesterol precursors, such as farnesyl pyrophosphate that are required for the activation of Ras and Rho and which, in turn, play critical roles in cell signaling survival and proliferation pathways [14, 15].

While several meta-analyses have failed to demonstrate a specific effect of statins on breast cancer incidence [10–13], a study of 4,383 incident cases of invasive breast cancer within the Women's Health Initiative cohort investigators demonstrated that use of lipophilic statins (such as simvastatin, fluvastatin, and atorvastatin) specifically, but not statins generally (which include hydrophilic statins such as pravastatin), was associated with a significant reduction in overall breast cancer risk [16]. Concordantly, a recent paper has shown that simvastatin use was associated with a reduced risk of breast cancer recurrence among a large cohort of Danish women whereas no association between hydrophilic statin use and breast cancer recurrence was observed [17]. It is possible that lipophilic statins have the potential to decrease the incidence of both hormone receptor-negative and hormone receptor-positive breast cancers in contrast to the two agents currently approved by the Food and Drug Administration for chemoprevention of breast cancer, tamoxifen and raloxifene, which reduce the incidence of only hormone receptor-positive breast cancer [1, 2, 18]. This evolving body of preclinical and clinical evidence suggested that the lipophilic HMG CoA reductase inhibitors may have chemopreventive activity in breast cancer.

We therefore performed a prospective short-term biomarker modulation study of simvastatin in women at increased risk for breast cancer. The primary objective of this study was to assess changes in a panel of potential surrogate biomarkers of simvastatin chemopreventive activity for further evaluation and validation in larger prospective chemoprevention trials. Breast cancer risk-associated markers, such as serum estrogens and mammographic breast density, were evaluated as both circulating estrogens and mammographic breast density are positively correlated with risk of developing new breast cancer in women [19, 20]. This study also examined agent-specific biomarkers of simvastatin activity. An inexpensive and well-standardized assay is available for high-sensitivity C-reactive protein (hsCRP) and is associated with cardiovascular risk [21]. Although a relationship between hsCRP and breast

cancer risk has not been reported, elevated hsCRP has been associated with reduced survival among breast cancer patients [22]. It is possible that effects on pathways involved in inflammation and cholesterologenesis are responsible for the decrease in cancer risk observed in statin users.

Patients and methods

Study setting, participants, and recruitment

Women were enrolled at two study sites; The Sidney Kimmel Comprehensive Cancer Center at Johns Hopkins, Baltimore, MD and the Dana-Farber Cancer Institute, Boston, MA. The study was approved by the participating center's institutional review boards, and all participants provided written informed consent. The study was registered prior to initiating enrollment (<http://www.clinicaltrials.gov>, NCT00 334542).

Eligible participants were women 18 years of age, with an Eastern Cooperative Oncology Group performance status of 0–2, with at least one intact breast (no radiation therapy, mastectomy, or implant; prior benign biopsies permitted) and a history of histologically confirmed ductal carcinoma in situ (DCIS) or stage I–III invasive breast cancer of any hormone receptor and HER2/Neu status who were at least 3 months after completion of all planned surgery, radiation, adjuvant chemotherapy, and/or endocrine therapy. Patients with prior contralateral breast cancer were ineligible.

Use of any other cholesterol-lowering drugs, including a statin, selective estrogen receptor modulators, aromatase inhibitors, or hormonal contraception (e.g., oral contraceptives, patches, or injections) within 3 months of initiation of simvastatin or while on study was not allowed, nor was use of hormone replacement therapy within the 5 years before initiation of simvastatin. Vaginal estrogen preparations were permitted. Other exclusion criteria included: pregnancy or lactation, active liver disease, serum AST or ALT > 3 times the upper limit of normal, calculated creatinine clearance <30 ml/min using the modified Cockcroft-Gault equation, or history of hypersensitivity to any HMG CoA reductase inhibitor. The study protocol prohibited alcohol use of more than three standard drinks per day, daily grapefruit juice consumption of more than 8 oz per day, and concomitant use of cytochrome P450 (CYP) 3A4 inhibitors. Women with other infectious, inflammatory, or autoimmune diseases were excluded at the discretion of the principal investigator.

Women were deemed 'post-menopausal' using the World Health Organization definition of amenorrhea of >12 months, or a history of bilateral oophorectomy, and serum estradiol concentration less than 100 pmol/L or less. Participants were provided with simvastatin at a dose of 40 mg for 24–28 weeks. Pill diaries were used to assess compliance. Simvastatin was provided to participants free of cost by Merck and Company Ltd.

Correlative studies

At baseline and upon completion of 24–28 weeks of simvastatin therapy, blood samples were collected for assessment of hsCRP, circulating estrogens, and fasting lipid concentrations. The fasting lipid concentrations were determined at the clinical laboratories at Johns Hopkins Hospital and the Dana-Farber Cancer Institute. All hsCRP concentrations were analyzed at the clinical laboratory at the Johns Hopkins Hospital using a Roche-Hitachi Modular P immunoturbidimetric endpoint assay for hsCRP with a linearity of 0.1–20.0 mg/l [23]. Plasma was sent to the Dowsett laboratory at the Royal Marsden Hospital, London, UK for determination of estradiol concentrations via a highly sensitive radioimmunoassay (RIA) after ether extraction as previously described [24–26]. The within- and between-batch coefficients of variation were 5.9 and 13.0%, respectively, at a concentration of 35 pmol/l. The lowest limit of detection was 3.0 pmol/l. Estrone was measured by RIA after extraction

with diethyl ether and liquid column chromatography using Lipidex 5000 (Perkin Elmer, Boston, MA) with elution using chloroform:hexane:methanol (50:50:1). Within and between-batch coefficients of variation were 6.7 and 8.2 at 81 pmol/l. The sensitivity of the assay was 15 pmol/l [25]. Estrone sulfate was measured using RIA after conversion to estrone by incubation with aryl sulfatase from abalone entrails type VIII as previously described [27]. Within- and between-batch coefficients of variation were 9.9 and 11% at 130 pmol/l ($n = 8$), and the sensitivity limit was 15 pmol/l. The Rand Short Form (SF) 36-Item health survey was completed by participants at baseline and end-of-study [28].

Digital mammography was performed on the contralateral breast at baseline and end-of-study. One investigator (CB), who was blinded to the timing (baseline vs. end-of-study) of the images, used the Cumulus software (University of Toronto, Toronto, ON Canada), for a computer-assisted assessment of mammographic breast density from the processed digital images [29, 30]. This method has been shown to be highly reproducible and predictive of breast cancer risk [30, 31]. Mammographic breast density from the cranio-caudal view of the contralateral breast was assessed from both time periods within the same batch [32]. Percent mammographic breast density was calculated from the ratio of the total number of pixels identified as dense on the gray-scale to the total number of pixels within the breast area. The difference between the percent mammographic breast density at baseline and end-of-study was the measure of change in breast density. As a safety measure, creatine kinase (CK) levels were drawn at baseline; repeat assessments were not required unless a participant reported muscle pain or other symptoms.

We collected whole blood from each participant at baseline for genotyping of candidate genes that may impact simvastatin-associated modulation of biomarker or adverse events. Simvastatin is a prodrug that is absorbed in the small intestine and undergoes first pass metabolism in the liver. It is metabolized via the CYP3A4 pathway and its subsequent metabolites are metabolized via CYP2D6. The following genes and variants were evaluated: (1) CYP3A5*3 rs776746, a null allele with a minor allele frequency (MAF) of 3–6% in Caucasians which has been associated with a significantly smaller mean percentage reduction in serum total cholesterol from baseline in expressors than non-expressors [33], (2) 3-hydroxy-3-methylglutaryl CoA reductase (*HMGCR*) rs12654264, which has a MAF of 57–60% in Caucasians. This variant has been associated with a reduced risk of colorectal cancer in statin users (odds ratio [OR] 0.3, 95% confidence interval [CI], 0.18–0.51) [34], (3) Common *CYP2D6* variants including *2,*3,*4,*6,*10, and *41. Carriers of the null alleles (*3,*4, and *6) are more likely to benefit from simvastatin as demonstrated by reduction in total cholesterol reduction compared to those with wild type genotype, and those with two wildtype alleles were more likely to discontinue statin treatment [35].

Statistical considerations

This study was designed with a sample size of 45 evaluable participants, in order to detect a minimal standardized difference of 0.5 in continuous measurements with 90% power and a two-sided type I error rate of 5%. Baseline to 24 week changes in hsCRP, fasting lipid profile, circulating estrogens and contralateral breast density were measured for all patients on a continuous scale. Due to skewness in the distributions of these variables, Wilcoxon signed rank tests were used to determine if each of these continuous differences was significantly different from zero. All tests were two-sided. Analyses were completed using statistical freeware R version 2.10.1.

Results

Patient characteristics

Fifty patients enrolled on the study between January 2005 and November 2009 and were evaluable if they took at least one dose of simvastatin. The main reasons for screen failure were recent use of hormonal therapies or current use of a statin. A proportion of eligible patients declined study participation due to geographical concerns and a reluctance to take 'unnecessary' medication. Characteristics of the patients and their prior breast tumors are shown in Table 1. The median age was 53 (range 38–74). The majority of women enrolled were Caucasian (94%) and post-menopausal (80%). Almost three quarters (74%) of participants had a history of a previous stage I or II invasive breast carcinoma, and the majority of invasive tumors were of a high grade (78%). The previous tumors were hormone receptor-negative in 71% of cases, and 70% of participants had received either neoadjuvant or adjuvant systemic chemotherapy. Thirty-two percent of participants had previously received adjuvant endocrine therapy. Forty-five participants (90%) completed the prescribed 24–28 weeks of simvastatin.

Fasting lipid values

Paired baseline and post-simvastatin treatment fasting lipid samples were available for 47 participants, including 45 women who completed the study and from two who discontinued the drug prior to the completion of the 24–28 weeks of drug, and are integrated in the intention-to-treat analyses (Fig. 1a). The median total cholesterol was 213 mg/dl (range 157–297) at baseline and fell to 164 mg/dl (range 102–261) at end of study (median difference –54 mg/dl, 95% CI: –58.5 to –39, $P < 0.01$). We observed significant reductions in triglycerides and low-density lipoprotein cholesterol (LDL, $P = 0.003$ and $P < 0.001$, respectively). As expected with statin therapy, high-density lipoprotein cholesterol (HDL) concentrations did not fall significantly (median difference –1 mg/dl, 95% CI: –2 to 3, $P = 0.53$). These data suggest a high level of study drug compliance among participants.

High-sensitivity C-reactive protein

Paired samples for comparison of baseline and end-of-study hsCRP concentrations were available for 48 participants. The median hsCRP concentration at baseline was 1.6 mg/l (range 0.2 to 21.2) and fell at end of study to 1.2 mg/l (median difference: –0.15, 95% CI: –1 to 0, $P = 0.05$). These results are depicted in Fig. 1b.

Circulating estrogens

Forty-eight matched pre- and post-simvastatin-treatment samples were available for evaluation of changes in circulating estradiol and estrone sulfate concentrations and 47 pairs for estrone concentration (Table 2). Among pre-menopausal women, the median pre-simvastatin plasma concentrations of estradiol, estrone, and estrone sulfate were 433 pmol/l (range 118–1,304), 323 pmol/l (range 131–2210), and 3,806 pmol/l (range 2,326–33,336), respectively. After 24–28 weeks of treatment with simvastatin, these had not changed significantly. Among post-menopausal study participants, the median pre-simvastatin concentrations of estradiol, estrone, and estrone sulfate were 27 pmol/l (range 5.5–87), 90 pmol/l (range 41–216), and 746 pmol/l (range 152–3,067), respectively. We observed a statistically significant decrease in estrone sulfate [median change: –81.5 pmol/l (95% CI: –225.5 to –40.5, $P = 0.006$)] but not in the median change in estradiol and estrone concentrations from baseline to end of study.

Breast density

Forty-three paired mammograms were available for evaluation of simvastatin-induced changes in contralateral mammographic breast density (Table 2). The median percentage breast density among premenopausal women did not change significantly after simvastatin treatment; it was 17.1% (range 2.3–40.9%) pretreatment and 19.2% (range 10.8–43.8%) following 6 months of simvastatin. The median percentage breast density among the post-menopausal women was 19.5% at baseline (range 0.2–49.5%) and did not change significantly after 6 months of simvastatin treatment (median difference -1.03 , 95% CI: -2.33 to 0.85 , $P = 0.342$).

Drug tolerability and quality of life

Forty-five out of 50 patients completed 24–28 weeks of simvastatin without complications. Five patients discontinued drug early for the following reasons: unrelated personal concerns ($n = 1$); adverse events of uncertain relation to study drug ($n = 3$); and a diagnosis of a new breast cancer ($n = 1$).

All adverse events were assessed using the Common Toxicity Criteria for Adverse Events, version 3.0 (CTCAE v.3). Overall, this was a well-tolerated regimen with the majority of adverse events reported as mild (grade 1). Possibly drug-related adverse events included: constipation ($n = 7$), headache ($n = 4$), rash ($n = 1$), and muscle pain/weakness ($n = 5$). Several patients reported grade 1 symptoms at baseline including joint aches/pains and headaches secondary to allergies or other underlying conditions; for most, these symptoms were not exacerbated by the 6 months of simvastatin, although one patient did report increase in muscle pain from grades 1 to 2; and one reported increased constipation from grades 1 to 2 and stopped drug. In addition, 6 months of simvastatin did not exacerbate the following baseline grades 1–2 unrelated symptoms: bone/joint aches/pain, mood issues, post-chemotherapy neuropathy, seasonal allergies, cough/congestion, or hot flashes. Three patients reported new musculoskeletal symptoms while receiving simvastatin without an elevation in CK values from baseline. A fourth patient who reported musculoskeletal symptoms at her off-study visit was found to have an elevated CK of 860 IU/l (compared to 79 IU/l at baseline); both the symptoms and CK value returned to baseline within 4 weeks of discontinuation of simvastatin.

Forty-six study participants completed the Rand SF 36-Item health survey at baseline and end-of-study. A summary of baseline and change in participant scores after therapy is shown in Table 3. The median physical functioning score was 100 (range 75–100) at baseline and remained unchanged after simvastatin treatment (median 95, range 70–100). Pain scores and energy/fatigue scores were similarly high at baseline (median 90 and 80, respectively) and there were no changes observed after treatment [median difference in energy/fatigue score 0 (95% CI: -10 to 0 , $P = 0.04$) and median difference in pain score 0 (95% CI: -11.25 to 5 , $P = 0.36$)].

Genotyping

Of 45 evaluable samples, only one individual (2%) was a CYP2D6 poor metabolizer. All 45 participants were non-expressors of CYP3A5 enzyme. Overall allelic frequencies were consistent with those expected in this study population. Twenty (44%) of the patients had the *HMGCR* A/A genotype. Three women who had developed musculoskeletal complaints 2, 12, and 24–28 weeks following drug initiation were carrier of the *HMGCR* A/A genotype, and one additional woman who reported symptoms at 24 months was not evaluable. *HMGCR* genotype was not predictive of changes in lipids or hsCRP (Data not shown). However, our sample size is too small to show an association or draw conclusions.

Discussion

We have prospectively assess the effects of simvastatin treatment on potential biomarkers of breast cancer chemopreventive activity. As expected, we observed significant decreases in serum hsCRP, total cholesterol, LDL cholesterol, and triglyceride concentrations, which suggests good compliance with the study intervention. The median estrone sulfate concentration fell in the overall study population and among post-menopausal study participants; however, no significant change was observed in other circulating estrogens or mammographic density. There are several possible explanations for our observations that warrant discussion.

Better surrogate biomarkers of chemopreventive activity are urgently needed—we selected rational breast cancer risk-specific markers (circulating estrogen concentrations and mammographic breast density) and drug-specific markers (hsCRP, fasting lipids). The inability to overall observe significant changes in breast cancer-specific biomarkers after 6 months of simvastatin may reflect inactivity of this drug as a chemopreventive agent in breast cancer. It is also possible that the drug has chemopreventive activity, but the selected biomarkers were not appropriate. An ideal marker would correlate accurately with a reduction in breast cancer incidence, demonstrate compliance, and change significantly within the period of drug treatment on study.

We observed a reduction in estrone sulfate but not estradiol or estrone. Estrone sulfate is the most abundant estrogen in post-menopausal women and has the longest half-life [36]. The majority of study participants (68%) had never received adjuvant endocrine therapy and all were required to have discontinued any hormonal therapies for at least 3 months at study entry. Based on the known elimination half-lives of commonly used adjuvant hormonal therapies we believe this interval provided an ample wash-out period to protect our observed findings from the potential influences of these drugs [37–39]. It is possible however that some of the fluctuations observed may have been related to resumption of ovarian activity following chemotherapy in these women. In addition, due to the superior sensitivity of the assay for estrone sulfate relative to other circulating estrogen concentrations (1:100 for the ratio between mean plasma hormone concentration of estrone sulfate in untreated post-menopausal patients and the sensitivity limit of the assay vs. 1:10 for the assays of estradiol and estrone) estrone sulfate is regarded by some as a more sensitive marker of estrogen suppression [40]. This study identified a modest but statistically significant reduction in estrone sulfate among post-menopausal study participants with simvastatin treatment. Post-menopausal breast tissue has the ability to maintain concentrations of estrone and estradiol that are 2–10- and 10–20-fold higher than the corresponding plasma estrogen levels. Breast tissue expresses not only aromatase, but also estrogen sulfotransferase, sulfatase, and dehydrogenase, which allow estrogen storage and release in the cells as well as conversions between estrone sulfate, estrone, and estradiol [41]. Estrone sulfate is therefore considered by some to be a ‘prohormone’ in post-menopausal breast tissues. Previous studies have shown that drugs such as aminoglutethimide and rifampicin induce P450-dependent mixed-function oxygenases that selectively suppress plasma estrogen sulfate levels through enhanced clearance. We hypothesize that simvastatin exerted a similar, estrone sulfate-specific effect in our study [42, 43].

High breast density is a known, strong predictor of breast cancer risk. Baseline mean breast percent–density ranges from 10 to 20% in healthy post-menopausal women. In our study, we observed a broad range in baseline percent–density (0.2–49.5%). The median baseline breast density was toward the upper end of the expected normal range at 19.5%, which may reflect the inclusion of high-risk women who had already experienced a breast cancer event; however, the wide range suggests that there were women enrolled in the study who had

predominantly fatty breasts which may have decreased the ability to detect significant changes in breast density over time. This study is also one of the first to evaluate change in breast density based on digital mammograms. Unknown differences in image processing between baseline and 6 months may diminish the ability to detect change over time. In addition, studies published after initiation of our trial that have identified a significant change in percent mammographic breast density have reported these changes following treatment periods of at least 1–2 years, in contrast to the 6-month intervention period of our study [44, 45]. An ongoing randomized cooperative group trial of atorvastatin 40 mg daily versus placebo in premenopausal women at high risk for breast cancer will evaluate the effect of 1 year of treatment on several breast cancer biomarkers including breast density, plasma insulin-like growth factor 1 (IGF1), and proliferation markers (NCT00914017). Interestingly, we and others have reported that aromatase inhibitors do not appear to modulate mammographic breast density when given for up to 1 year despite being associated with a significant decrease in the incidence of contralateral breast cancer events when used for 5 years in the adjuvant setting [46]. Thus, a biomarker that predicts response to one or more endocrine interventions may not necessarily be an appropriate biomarker for other agents with potential chemopreventive activity.

In addition, genetic variants in drug target or metabolizing enzymes may influence its efficacy or safety. In our study we did not observe a correlation between candidate genotypes and change in biomarkers. However, the sample size was small and all results should be regarded as exploratory and hypothesis generating for future studies.

What better biomarkers of breast cancer chemopreventive activity are available? Change in the proliferative index Ki-67 is a potentially useful marker and has been used by several investigators; however, the low baseline level seen in healthy breast tissue and the variability of the marker at baseline among premenopausal women in particular makes interpretation of observed changes challenging [47–50]. Decensi et al. [51] recently reported that low-dose tamoxifen and a combination of tamoxifen and fenretinide successfully modulated changes in plasma IGF1 levels, but these changes were not associated with a reduction in breast cancer events. Other tissue-based, imaging, and circulating biomarkers that may predict chemoprevention activity of agents are under investigation.

Finally, a further possible explanation for our inability to detect changes in the selected biomarkers of breast cancer risk is that the study may have been underpowered. The study was originally powered for an overall analysis not an analysis by menopausal status. We acknowledge that the small size and heterogeneity of the cohort limits interpretation of our results. While the observations of this study are not definitive regarding the chemopreventive activity of simvastatin, this is to our knowledge the first prospective, systematic evaluation of biomarker modulation following simvastatin administration in the chemoprevention setting. We have demonstrated that certain biomarkers can be modulated within a brief period and that the contralateral breast of breast cancer survivors may be a valuable and acceptable high-risk model. There is a pressing need for chemopreventive agents that reduce the incidence of both hormone receptor-positive and hormone receptor-negative breast tumors. Combinations of drugs, such as a selective estrogen receptor modulator or an aromatase inhibitor added to a novel agent, to reduce hormone receptor-negative tumors should be considered. The rational selection and systematic validation of appropriate biomarkers of chemopreventive activity in models such as the one presented here will facilitate large-scale clinical evaluation of promising new agents in a time of limited resources.

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Fig. 1. Box plot demonstrating (a) changes in total, LDL, and HDL cholesterol and triglycerides at baseline and after 24–28 weeks of treatment with simvastatin ($N = 47$) and (b) change in hsCRP at baseline and after 24–28 weeks of treatment with simvastatin ($N = 48$)

Table 1Patient characteristics (*N* = 50)

	Number (%)
Age—median (range)	53 (38,74)
Race	
White	47 (94)
African American	1 (2)
Other	2 (4)
Menopausal status	
Pre	10 (20)
Post	40 (80)
Body mass index (BMI)	
24.9 kg/m ²	15 (30)
25–29.9 kg/m ²	21 (42)
30–34.9 kg/m ²	10 (20)
35 kg/m ²	4 (8)
Stage of prior breast tumor	
DCIS	9 (18)
I	15 (30)
II	22 (44)
III	4 (8)
Hormone receptor status	
ER+/PR–	1 (2)
ER+/PR+	11 (26)
ER–/PR–	30 (71)
Unknown	8
Prior adjuvant hormonal therapy	
None	34 (68)
Tamoxifen	10 (20)
Aromatase Inhibitor	1 (2)
Tamoxifen and Aromatase Inhibitor	5 (10)
Prior chemotherapy (Neo and Adjuvant)	
No	15 (30)
Yes	35 (70)
Characteristics of invasive tumors (<i>N</i> = 41)	
Histology	
Infiltrating ductal carcinoma	32 (80)
Infiltrating lobular carcinoma	1 (2)
Other	7 (18)
Unknown	1 (0.5)
Grade	
I	4 (11)

	Number (%)
II	4 (11)
III	29 (78)
Unknown	4
Nodal status	
Positive	19 (50)
Negative	19 (50)
Unknown	3
HER 2 status	
Positive	7 (19)
Negative	29 (81)
Unknown	5

ER estrogen receptor, *DCIS* ductal carcinoma in situ, *PR* Progesterone receptor

Table 2

Change in circulating estrogens and breast density from baseline to end-of-study

	Before median (range)	After median (range)	N	Median difference [95% CI], <i>P</i>
Estradiol (pmol/l)				
All patients	33.5 (5.5, 1304)	24 (7, 1028)	48	-2 [-11.5, 0.25], <i>P</i> = 0.07
Pre-menopausal	433 (118, 1304)	391.5 (13, 1028)	10	-207.5 [-417.5, 173], <i>P</i> = 0.322
Post-menopausal	27 (5.5, 87)	22 (7, 508)	38	-2 [-6.25, 1.5], <i>P</i> = 0.267
Estrone (pmol/l)				
All patients	101 (41, 2210)	98 (48, 1043)	47	4 [-22.5, 4.5], <i>P</i> = 0.24
Pre-menopausal	323 (131, 2210)	336 (53, 1043)	9	-78 [-564.5, 161], <i>P</i> = 0.496
Post-menopausal	90 (41, 216)	87.5 (48, 351)	38	-3.5 [-16.5, 3.5], <i>P</i> = 0.242
Estrone sulfate (pmol/l)				
All patients	875.5 (152, 33336)	728.5 (205, 24307)	48	-109.5 [-302, -43.5], <i>P</i> = 0.01
Pre-menopausal	3806 (2326, 33336)	4201.5 (674, 24307)	10	-1017.5 [-4543, 2356.5], <i>P</i> = 0.432
Post-menopausal	746 (152, 3067)	612.5 (205, 3998)	38	-81.5 [-225.5, -40.5], <i>P</i> = 0.006
Breast density (%)				
All patients	19.5 (0.2, 49.5)	16.9 (0, 48.3)	43	-0.78 [-2.0, 1.2], <i>P</i> = 0.53
Pre-menopausal	17.1 (2.3, 40.9)	19.2 (10.8, 43.8)	8	1.1 [-4.97, 9.85], <i>P</i> = 0.844
Post-menopausal	19.5 (0.2, 49.5)	16.9 (0, 48.3)	35	-1.03 [-2.33, 0.85], <i>P</i> = 0.342

Table 3Change in RAND-36 healthy survey scores from baseline to end-of-study ($N = 46$)

Category	Before median (range)	After median (range)	Median difference [95% CI], P
Physical functioning	100 (75, 100)	95 (70, 100)	0 [-7.5, 2.5], $P = 0.248$
Energy/fatigue	80 (40, 100)	70 (35, 95)	0 [-10, 0], $P = 0.043$
Pain	90 (57.5, 100)	90 (45, 100)	0 [-11.25, 5], $P = 0.357$