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Changes in Breast Density and Circulating Estrogens in Postmenopausal Women Receiving Adjuvant Anastrozole

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Abstract

Factors associated with an increased risk of breast cancer include prior breast cancer, high circulating estrogens, and increased breast density. Adjuvant aromatase inhibitors are associated with a reduction in incidence of contralateral breast cancer. We conducted a prospective, single-arm, single-institution study to determine whether use of anastrozole is associated with changes in contralateral breast density and circulating estrogens. Eligible patients included postmenopausal women with hormone receptor-positive early-stage breast cancer who had completed local therapy, had an intact contralateral breast, and were recommended an aromatase inhibitor as their only systemic therapy. Participants received anastrozole 1 mg daily for 12 months on study. We assessed contralateral breast density and serum estrogens at baseline, 6, and 12 months. The primary endpoint was change in contralateral percent breast density from baseline to 12 months. Secondary endpoints included change in serum estrone sulfate from baseline to 12 months. Fifty-four patients were accrued. At 12 months, compared with baseline, there was a nonstatistically significant reduction in breast density (mean change: −16%, 95% CI: −30 to 2, $P = 0.08$) and a significant reduction in estrone sulfate (mean change: −93%, 95% CI: −94 to −91, $P < 0.001$). Eighteen women achieved 20% or greater relative reduction in contralateral percent density at 12 months compared with baseline; however, no measured patient or disease characteristics distinguished these women from the overall population. Large trials are required to provide additional data on the relationship between aromatase inhibitors and breast density and, more importantly, whether observed changes in breast density correlate with meaningful disease-specific outcomes.

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Introduction

One in 8 women in the United States will develop invasive breast cancer over her lifetime. Approximately three-quarters of these cases occur in the postmenopausal population and are hormone-receptor positive (1). New prevention strategies to decrease the incidence of breast cancer are critical, but large-scale randomized trials of preventive agents are costly, requiring thousands of participants and years of follow-up to detect statistically significant differences in cancer incidence. For example, the recently reported National Cancer Institute of Canada Clinical Trials Group (NCIC CTG) MAP.3 trial, which opened to enrollment in 2004, required 7 years and a sample size of more than 4,500 high-risk women to observe 43 invasive breast cancer events (2). Thus, the identification of surrogate endpoints predictive of response to promising chemopreventive agents, which could then be evaluated in large-scale prevention trials, would be ideal.

Recommendations regarding adjuvant therapy for breast cancer are based on risk estimates using prognostic factors such as staging; however, it is currently unknown which patients will recur despite treatment and which patients would remain cancer-free even in the absence of adjuvant therapy. Approximately 75% of invasive breast cancers and ductal carcinomas *in situ* (DCIS) express the estrogen receptor (ER) and/or progesterone receptor (PR). Women with hormone receptor-positive *in situ* or invasive breast cancer are generally recommended endocrine therapy; however, our ability to predict sensitivity or resistance to endocrine therapy for a given individual with hormone receptor-positive breast cancer is suboptimal. It has been hypothesized that surrogate biomarkers, such as circulating estrogen concentrations or breast density, which are known to correlate with breast cancer risk in population studies, may predict the likelihood of response to hormonal manipulations. In addition, it is possible that not only intratumoral characteristics, but also individual polymorphisms in drug-metabolizing enzymes or drug targets, may be responsible for sensitivity or resistance to individual therapies (3).

Percent mammographic density measures the proportion of epithelial and stromal tissue in the breast and is defined as the percent of the total breast area seen on the mammogram that appears dense. Mammographic breast density is strongly correlated with breast cancer risk (4). Only female gender, increasing age, and family history of breast cancer—none of which are modifiable risk factors—are better predictors of subsequent development of breast cancer (5). There is a graded, continuous increase in breast cancer risk associated with increasing mammographic density, such that the women who have a breast density of 75% or more have a 4- to 6-fold increase in breast cancer risk relative to those with very little (<5%) breast density (5, 6). Previous studies have reported that a 4% increase in absolute breast density is associated with a 10% increase in relative risk of developing breast cancer (6). Breast density varies according to the relative amount of fat, connective tissue, and epithelial tissue—all factors that are influenced by endogenous estrogen concentrations (7). Recently, investigators have reported that areas identified as mammographically dense on screening mammograms shown on core biopsy a 2-fold increase in aromatase expression relative to non-mammographically dense areas of the same breasts, suggesting a potential mechanism for the observed association between mammographic density, serum estrogens, and breast cancer risk (8).

Baseline mean breast percent density ranges from 14% to 26% in postmenopausal women (9). Fewer than 20% of untreated postmenopausal women will experience a 20% or greater relative reduction in breast density over a 12-month period (10). By contrast, women treated with combined estrogen plus progestin preparations commonly experience an increase in breast density. In the Women's Health Initiative trial, an absolute increase of 6% in mean percent breast density was observed in postmenopausal women receiving estrogen plus

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progestin hormone replacement therapy compared with an absolute decrease of 0.9% among postmenopausal women receiving placebo (11). Tamoxifen is associated with an 8% and 14% mean absolute reduction in breast density at 1.5 years and 4.5 years, respectively (12, 13). Raloxifene has been reported to decrease absolute breast density by 1.5% per year (10).

The NSABP P-1 trial investigators reported that tamoxifen, a selective estrogen receptor modulator (SERM), given for 5 years, reduces the relative incidence of invasive breast cancer by 50% in high-risk women (14). The NSABP P-2 trial compared 5 years of another SERM, raloxifene, to tamoxifen and showed the noninferiority of raloxifene for prevention of invasive breast cancer, retaining approximately 76% of tamoxifen's effect at a median follow-up of 81 months (15). To date, tamoxifen and raloxifene are the only agents U.S. Food and Drug Administration-approved for breast cancer prevention, and their use in this indication has been limited by patient concerns about tolerability and safety. In the Arimidex, Tamoxifen, Alone or in Combination (ATAC) trial, there was a 44% reduction in the relative incidence of new contralateral breast (CLB) cancers in the hormone receptor-positive women on the anastrozole monotherapy arm compared with the tamoxifen arm (16). This observation generated interest in the AIs as potential chemopreventive agents and provided the rationale for several large ongoing or recently reported randomized trials of AIs for chemoprevention. Indeed, results from NCIC CTG MAP.3, a randomized trial of exemestane versus placebo for primary prevention of breast cancer in postmenopausal women have recently been reported. With 35 months of median follow-up, there was a statistically significant 65% relative reduction in the incidence of invasive breast cancer favoring exemestane (2). Ongoing trials of AIs for chemoprevention include the NSABP B-35 trial of tamoxifen versus anastrozole for prevention of invasive breast cancer in postmenopausal women with a history of hormone receptor-positive DCIS, and the International Breast Cancer Intervention Study II (IBIS II) comparing anastrozole with placebo for primary prevention of breast cancer in postmenopausal women.

The overall goal of this study was to determine whether early (12 months) modulation in surrogate biomarker endpoints may be used to identify women who are most likely to benefit from long-term AI use, and to identify one or more surrogate endpoints of response to adjuvant AIs to define a "responder phenotype" that, if validated in future studies, could be incorporated into the design and execution of large-scale definitive chemoprevention trials. The primary objective of our study was to prospectively evaluate changes from baseline in CLB density in postmenopausal women with intraductal or invasive breast cancer following 12 months of adjuvant anastrozole. Secondary objectives were to correlate suppression of estrone sulfate with changes in breast density and with presence of wild type versus the rs4646 variant of the aromatase enzyme gene. We hypothesized that 12 months of adjuvant anastrozole would result in a significant decrease in both CLB density and serum estrone sulfate in women with a wild-type aromatase gene.

Materials and Methods

Study design and eligibility criteria

The protocol was approved by the Johns Hopkins Institutional Review Board, and registered prior to initiating enrollment (http://www.clinicaltrials.gov, NCT00244959). All patients provided a written informed consent to participate prior to study entry according to institutional guidelines. This prospective, single-arm, single-institution study was conducted at The Sidney Kimmel Comprehensive Cancer Center at Johns Hopkins.

We enrolled 54 postmenopausal women with histologically confirmed hormone receptor-positive DCIS or stage I–III invasive breast cancer who had completed local therapy no more than 6 months prior to study entry. Women were considered postmenopausal if any of

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the following criteria were met: age 60 or more years, amenorrhea lasting 12 or more months, history of bilateral oophorectomy, history of radiation castration with 6 or more months of amenorrhea, or amenorrhea lasting less than 12 months if luteinizing hormone and follicle-stimulating hormone values were within postmenopausal reference range. Tumors were considered to be hormone receptor-positive if immunohistochemical staining showed ER and/or PR staining of 5% or more.

Only subjects for whom endocrine therapy alone was planned were eligible; those receiving other adjuvant systemic therapy were not eligible. Subjects were required to have an intact CLB. Subjects who had previously been treated with hormone replacement therapy or a SERM were eligible as long as these agents had been discontinued at least 6 weeks prior to study enrollment. Prior use of an AI or current use of soy supplements was not permitted.

Anastrozole 1 mg per day was administered orally for a period of 12 months while on study with subsequent endocrine therapy determined by the patient and her medical oncologist.

Estrogen measurements

Circulating serum estrogen (estradiol and estrone sulfate) concentrations were measured at baseline, and at 6 and 12 months by radioimmunoassay (RIA) at the Dowsett Laboratory at the Royal Marsden Hospital, London, United Kingdom. Serum samples for estradiol analysis were preextracted with di-ethyl ether to remove water-soluble components followed by a radioimmunoassay procedure using methodology developed for the quantification of estradiol at concentrations found in postmenopausal women. At a concentration of 35 pmol/L, the within-batch and between-batch coefficients of variation were 5.9% and 13%, respectively (17–19). The lowest limit of detection was 3.0 pmol/L. The analytical range of the assay was 3.0 to 1500 pmol/L.

Estrone sulfate was also measured by radioimmunoassay. The procedure, which involves the preassay conversion of estrone sulfate to estrone using aryl sulfatase, followed by purification by ether extraction and column chromatography on lipidex 5000, has been described previously (20). The lowest limit of detection for the assay was 15 pmol/L. At a concentration of 130 pmol/L, the within-batch and between-batch coefficients of variation were 9.9% and 11%, respectively. The analytical range of the assay was 15 to 3,500 pmol/L.

Because the sensitivity limits of most radioimmunoassays for serum estrogen concentrations are in the vicinity of expected posttreatment concentrations, patients receiving AIs frequently have posttreatment hormone concentrations below the limit of detection (21). The ratio of mean plasma hormone concentration in postmenopausal women to the sensitivity limit of the assay varies greatly, from 1:10 for estradiol and estrone compared with 1:100 for estrone sulfate. Although both estradiol and estrone sulfate were measured, given the more favorable assay characteristics, estrone sulfate was selected as the preferred marker of estrogen suppression in this study (22).

Breast density

A film screen or digital mammogram of the CLB was obtained at baseline, 6, and 12 months for measurement of breast percent density. Film screen images were obtained using a Hologic LoRad machine, and digital mammography was done using GE 2000, 2000D, and 2000DS machines. All film screen images were digitized using the Kodak-LS80 following standard procedures, and DICOM copies of digital mammograms were obtained. A single investigator, blinded to the sequence (baseline vs. 6 months or 12 months) of the images, assessed mammographic breast density using the Cumulus software (University of Toronto, Toronto, ON Canada), from the processed digital images (23, 24).

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In brief, to assess breast density, the chest wall and outer edge of the breast were first outlined to define the area of the breast. A threshold on the grey scale was then selected that categorized the breast tissue as either dense or not dense. The number of pixels in the dense area divided by the number of pixels in the total breast area on the cranio-caudal mammogram quantified the proportion of the breast considered dense, and is referred to as percent mammographic breast density. This method has been shown to be highly reproducible and predictive of breast cancer risk (23–25). For each participant, the percent mammographic breast density from the cranio-caudal view of the CLB was assessed for all available time points within the same reading session (26). The difference between the percent mammographic breast density at baseline, 6 months, and 12 months produced the measure of change in breast density.

Genotyping

Genotyping of the aromatase enzyme gene was done at baseline. DNA isolated from whole blood was used for the genotyping of the rs4646 SNP in the CYP19A1 aromatase gene. The genotyping was done using the assay id# (C___8234730_1_) purchased from Applied Biosystems/Life Technologies. Assays were run on an iCycler (Bio-Rad, Inc.).

Statistical considerations

The primary endpoint of the study was change in CLB percent density from baseline to 12 months in postmenopausal women who received 12 months of adjuvant anastrozole. The secondary endpoints were change in estrone sulfate concentrations from baseline to 12 months and prevalence of wild type versus polymorphic aromatase enzyme gene.

The criterion of response for the primary endpoint was a 20% or greater relative decrease in CLB percent density at 12 months as compared with the baseline mammogram. Specifically, this relative reduction was calculated as: 100%* (follow-up CLB density—baseline CLB density)/(baseline CLB density) and is referred to as %ΔCLB. Patients with %ΔCLB less than 20% were considered nonresponders and those with values 20% or more were responders. Given that a 20% or greater relative decrease in breast percent density occurs in fewer than 20% of untreated postmenopausal women more than 1 year, a sample size of 54 evaluable subjects was selected to distinguish between population response rates of 0.20 and 0.38 with 80% power at a 2-sided significance level of 0.05 (10). Using an exact binomial test, we would need to observe 17 or more responses to conclude that the CLB response was significant (and reject the null hypothesis). The %ΔCLB was shown to have a skewed distribution; therefore, a log transform was applied to adhere to normality assumptions of parametric tests. This log-transformed value of log percent change is referred to as log (%ΔCLB).

Changes in CLB density and estrone sulfate were summarized using geometric means and CI and expressed as percent change from baseline values. Paired *t* tests were used to evaluate changes from baseline to follow-up. ANOVA of the %ΔCLB was used to test for the significance of differences in the change in CLB density and estrone sulfate from baseline between genotypes. Fisher's exact tests and Wilcoxon rank sum tests were used to determine whether any baseline characteristics were associated with achieving a response (i.e., 20% or greater relative reduction in breast density). Forty-three of the 54 women had both baseline and 12-month mammographic images. Of these women 7% had film mammograms at baseline and 12 months; 9% had a film mammogram at baseline and a digital mammogram at 12 months; and the remaining 84% had digital mammograms at baseline and the 12-month follow-up. A sensitivity analysis was done wherein patients with differing modalities (film vs. digital) for the baseline and follow-up CLB density scans were excluded from analyses and is described in the Results section.

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Results

Patient characteristics

From March 2004 to September 2006, 54 patients were enrolled and initiated study drug. All were eligible for analysis (Table 1). Mean age was 62.5 years, and 85% of participants were Caucasian. The majority of patients had a stage I or II breast cancer. Compliance with study drug was excellent, with 93% of participants completing 12 months of adjuvant anastrozole on study.

Breast density

Seventy-eight percent of participants had CLB percent density data available for analysis at all 3 time points, and 93% had baseline and at least one additional CLB percent density assessment available for analysis. Reasons for missing CLB percent density data included: patient discontinued study ($n = 3$), death unrelated to study drug ($n = 1$), ipsilateral breast inadvertently imaged ($n = 1$), CLB reduction for cosmesis ($n = 1$), radiotherapy to CLB ($n = 1$), magnification view precluded determination of breast density ($n = 1$), films lost ($n = 2$), and mammography not done, not otherwise specified ($n = 1$).

There was no change in mean CLB percent density, following 6 months of anastrozole [mean increase of 2%; 95% CI: −18 to 26, $P = 0.87$]. At 12 months, there was a nonstatistically significant reduction in mean CLB percent density relative to baseline CLB percent density (−16%; 95% CI: −30 to 2, $P = 0.08$; Table 2). There were 18/50 participants (36%, exact 95% CI: 23 to 51, exact $P = 0.006$) who met the criterion of response for the primary endpoint, showing a 20% or greater relative reduction in CLB percent density compared with baseline, indicating that the proportion of women who met the predefined response criteria is significantly greater than 20%. As seen in Table 3, no demographic or disease characteristics, including age, race, body mass index (BMI), disease stage, histology, extent of hormone receptor positivity (ER+/PR−, ER−/PR+, or ER+/PR+), baseline serum estrogen concentrations, baseline CLB density, or aromatase genotype, distinguished these participants from those who failed to achieve a significant reduction in CLB percent density. There was a significant weight gain observed at 6 months (median difference in BMI from baseline = 0.52, 95% CI: 0.16 to 0.85, $P = 0.008$), however, this difference did not persist out to 12 months (median difference in BMI from baseline = 0.04, 95% CI: − 0.13 to 0.56, $P = 0.27$). We also examined the change in BMI from baseline among participants who did and did not achieve a significant reduction in CLB percent density. Although those who met the response criterion exhibited more weight loss [median (range) change in BMI = −0.25 (−3.0 to 3.5)] than those who did not meet the response criterion [median (range) change in BMI = 0.25 (−1.7 to 2.5)], this difference was not statistically significant (exact $P = 0.52$).

There were 7 patients whose baseline and follow-up mammograms for evaluation of CLB density were done using differing methods of mammography (film mammography vs. digital mammography). To ensure that our results were not influenced by the change in mammographic technique, a sensitivity analysis was done wherein the 7 individuals identified were removed from all analyses. We found that the results presented above remained unchanged, in both direction and magnitude of the effects, as well as in their statistical significance.

Circulating estrogens

Table 2 also shows estrone sulfate, estradiol, and breast density as measured at baseline, 6, and 12 months. There were statistically significant reductions in mean estrone sulfate and estradiol concentrations observed at both 6 and 12 months. At 12 months, the mean change in estrone sulfate concentration, was −93% (95% CI: 91 to 94), $P < 0.001$.

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Genotyping

All participants underwent genotyping of the aromatase (CYP19: rs4646) gene. Of the 54 subjects enrolled, 33 (61%) were genotype CC, 17 (31%) were genotype AC, and 4 (8%) were genotype AA. There were no statistically significant differences in baseline breast density, estradiol, or estrone sulfate by genotype. There were also no statistically significant differences in change in breast density, estradiol, or estrone sulfate from baseline to 12 months by genotype (Table 4).

Discussion

Breast density and, in postmenopausal women, circulating estrogens are the only established risk factors for breast cancer that may be modifiable. Tamoxifen, which reduces the incidence of invasive breast cancer in high-risk women, is known to reduce breast density (14, 27). Postmenopausal hormone replacement therapy, which is associated with an increased incidence of invasive breast cancer, is associated with a modest increase in breast density (10, 11, 28–30). In view of these associations and the reductions in incidence of CLB cancer observed in adjuvant AI trials, we hypothesized that adjuvant anastrozole would reduce CLB density.

In our study, we found no change in CLB percent density from baseline following 6 months of adjuvant anastrozole. After 12 months of anastrozole use, there was a 16% relative reduction in CLB percent density compared with baseline that was not statistically significant ($P = 0.08$). At 12 months, study participants had a 93% mean reduction in serum estrone sulfate concentrations relative to baseline. Thus, the lack of a significant change in CLB density does not seem to be associated with noncompliance or failure to suppress circulating estrogens. There was no correlation between serum estrone sulfate concentrations and CLB density at 12 months. Although 36% of study participants did have a significant reduction in CLB percent density at 12 months relative to baseline, no particular patient or disease characteristics distinguished these women from the overall population of study participants. It is possible that younger women and those with a higher BMI would be more likely to have a greater reduction in breast density; however, additional data are required to test this hypothesis. It will be important to ascertain whether women who do achieve a significant reduction in breast density with AI use, are also those most likely to benefit from AIs for chemoprevention.

Breast cancer risk rises with increasing concentrations of circulating estrogens in postmenopausal women (31). Women with estradiol concentrations greater than 10 pmol/L have an approximately 7-fold greater risk of breast cancer relative to women whose estradiol concentrations are undetectable (32). The investigators of the Multiple Outcomes of Raloxifene Evaluation (MORE) trial evaluated the effects of raloxifene on breast cancer incidence. Women with high baseline serum estradiol concentrations had a 75% reduction in incidence of breast cancer when treated with raloxifene, whereas women with undetectable serum estradiol at baseline had no change in breast cancer risk whether assigned to raloxifene or placebo (32). These data obtained from larger studies suggest that the most effective preventive approach for future studies may involve targeting postmenopausal women with high circulating estrogen concentrations.

Our study is the largest prospective study to date to report the effects of AIs on breast density, and our results are consistent with other published reports. Fabian and colleagues reported the results of a single-arm trial in 42 postmenopausal women at increased risk of breast cancer who were on a stable dose of hormone replacement therapy. All participants were treated with letrozole 2.5 mg daily for 6 months. The primary endpoint was change in Ki-67 index in breast tissue at 6 months. Change in breast percent density at 6 months was

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one of several secondary endpoints assessed and showed no change from baseline values, despite a statistically significant reduction in Ki67 index (33). Cigler and colleagues reported the results of a randomized, placebo-controlled trial (NCIC CTG MAP.1) in which 67 postmenopausal women with a baseline breast density greater than 25%, with or without a history of breast cancer, were randomized 2:1 to receive letrozole 2.5 mg daily or placebo for 12 months and then followed for a total of 24 months. The primary endpoint was change in percent breast density from baseline to 12 months. The published report includes data for 67 participants, of whom 44 received letrozole. No significant changes in breast density from baseline, or between treatment arms, were observed at either 12 or 24 months of follow-up (34). Cigler and colleagues also recently reported the results of a second similar trial (NCIC CTG MAP.2), in which 98 postmenopausal women with any measurable baseline breast density and no prior history of breast cancer were randomized to exemestane 25 mg daily or placebo for 12 months and then followed for a total of 24 months. The primary end point was change in percent breast density from baseline to 12 months. Breast density data were available at baseline and 12 months for 65 participants, 34 of whom received exemestane. No significant changes in breast density from baseline, or between treatment arms, were observed at 12 or 24 months of follow-up (35).

At first glance, the results of our study and other reports suggest that modulation of breast density may not be an appropriate early biomarker of response for use in trials of AIs for chemoprevention. No statistically significant effect of 12 months of anastrozole on breast density was observed despite adequate suppression of circulating estrogens. Our results differ from those of the Cigler trials in that a numeric trend for reduction of breast density was observed with 12 months of anastrozole use, although this was not statistically significant. Although it is possible that anastrozole does not significantly modulate breast density, all of the available data come from small trials with AI exposure of 6 to 12 months. Therefore, it is also possible that larger sample sizes or longer durations of AI exposure, such as the 5 years routinely used in the adjuvant setting, would be required to show significant changes in breast density (36).

We did not observe an overall relative reduction of 20% or more in CLB density for the study population; however, 36% of participants met the criterion of response for the primary endpoint. We were unable to identify other specific patient or disease characteristics that distinguished these women from the study participants as a whole; however, it is possible that these are the women most likely to benefit from AIs for chemoprevention. This finding is intriguing in particular given a previous report by Cuzick and colleagues showing that 4.5 years of tamoxifen use was associated with a 13% absolute reduction in breast density among women aged 45 years or younger, compared with only a 1% reduction among women older than 55 years (13). Ongoing large randomized trials of AIs for prevention and as adjuvant therapy that include correlative substudies of breast density will provide additional data on the relationship between AIs and breast density and, more importantly, whether observed changes in breast density indeed correlate with meaningful disease-specific outcomes.

Although only a subset of patients will benefit from AIs, whether in the chemoprevention or adjuvant setting, virtually all compliant patients will experience suppression of circulating estrogens to below the limit of detection. Serum estrogens are therefore an inadequate biomarker for response to AIs, although pretreatment estrogen concentrations may be helpful, in combination with other risk factors, to identify postmenopausal women at increased breast cancer risk.

The rs4646 SNP of the aromatase gene was chosen as the first genetic biomarker to test because the aromatase enzyme is the direct target of anastrozole, and it has been associated

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with response to other AIs (37, 38). Indeed, we observed a trend in 12-month change in breast density associated with the genotype, but it was not statistically significant ($P = 0.10$), possibly due to the small sample size.

In conclusion, 12 months of anastrozole in the adjuvant setting did not result in a statistically significant reduction in CLB density despite adequate suppression of circulating estrogens. Although small, our study emphasizes the interpatient differences in early biomarker modulation and the need for correlative studies to be incorporated into future studies of chemoprevention agents.

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Table 1

Characteristics of participants at baseline

	All participants *n* = 54
BMI	27 (20.3–49.6)
Age	62.5 (47–79)
Race—no. (%)	
White	46 (85)
African-American	7 (13)
Other	1 (2)
Type of Surgery—no. (%)	
Mastectomy	13 (24)
Lumpectomy/radiation	41 (76)
Stage—no. (%)	
DCIS/LCIS	13 (24)
I	33 (61)
II	8 (15)
Nodal status—no. (%)	
Positive node	5 (9)
Negative node	38 (70)
Not done	11 (20)
Histology—no. (%)	
DCIS	12 (22)
Infiltrating ductal	34 (63)
Infiltrating lobular	6 (11)
Mixed	1 (2)
Other (colloid)	1 (2)
Grade—no. (%)	
1	18 (33)
2	29 (54)
3	7 (13)
Hormone receptors—no. (%)	
ER+/PR+	47 (87)
ER+/PR−	6 (11)
ER−/PR+	1 (2)
Her2 status (%) in invasive tumors (*n* = 42)	
HER2+	1 (2%)
HER2−	41 (98%)

NOTE: Values are *n* (%) for categories and median (range) for continuous measurements.

Abbreviation: LCIS: Lobular carcinoma *in situ*.

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Table 2

Changes in breast density, estradiol, and estrone sulfate, expressed as a percent change from baseline values

	Baseline—median (range)	6 mo—median (range)	% change at 6 mo, mean (95% CI)	*P*	12 mo—median (range)	% change at 12 mo, mean (95% CI)	*P*
Estradiol (pmol/L)	21 (6.9 to 112)	3 (3 to 9.1)	−84 (−89 to −82)	<0.001	3 (3 to 6.7)	−87 (−89 to −84)	<0.001
Estrone sulfate (pmol/L)	647 (115 to 3,500)	60 (8.4–248)	−92 (−93 to −90)	<0.001	55 (6.4 to 595)	−93 (−94 to −91)	<0.001
Breast density (%)	13.4 (0.1 to 66.2)	13.0 (1.0, 68.7)	2 (−18 to 26)	0.87	10.3 (0.3 to 55.7)	−16 (−30 to 2)	0.08

NOTE: *P* values for paired t tests, using log-transformed values to adhere to the normality assumption.

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Table 3

Characteristics of participants at baseline, by those who had a 20% or greater relative reduction in breast density at 12 months (or 6 months, if 12 month data not available) and those who did not

	<20% reduction or increase *n* = 32	**≥20% reduction *n* = 18**	***P***
Age—median (range)	63 (47–79)	60.5 (53–75)	0.26
BMI—median (range)	25.9 (20.6–41.7)	29.5 (23–49.6)	0.07
Race—no. (%)			
White	26 (81)	17 (94)	0.41
African-American	5 (16)	1 (6)	
Other	1 (3)	0 (0)	
Type of surgery–no. (%)			
Mastectomy	9 (28)	4 (22)	0.90
Lumpectomy/radiation	23 (72)	14 (78)	
Stage—no. (%)			
DCIS/LCIS	9 (28)	3 (17)	0.65
I	19 (59)	12 (67)	
II	4 (12)	3 (17)	
Nodal status—no. (%)			
Positive node	2 (6)	2 (11)	0.45
Negative node	22 (69)	14 (78)	
Not done	8 (25)	2 (11)	
Histology—no. (%)			
DCIS	8 (25)	3 (17)	0.74
Infiltrating ductal	18 (56)	13 (72)	
Infiltrating lobular	4 (12)	2 (11)	
Mixed	1 (3)	0 (0)	
Other (colloid)	1 (3)	0 (0)	
Grade—no. (%)			
1	12 (38)	6 (33)	0.45
2	17 (53)	8 (44)	
3	3 (9)	4 (22)	
Markers—no. (%)			
ER+/PR+	27 (84)	16 (89)	0.25
ER+/PR−	5 (16)	1 (6)	
ER−/PR+	0 (0)	1 (6)	
Genotype—no. (%)			
AA	2 (6)	2 (11)	0.63
AC	8 (25)	6 (33)	
CC	22 (69)	10 (56)	
Baseline CLB density (%)—median (range)	10 (0–70)	10 (0–50)	0.41
Estradiol (pmol/L)—median (range)	18 (6.9–102)	25 (7.2–112)	0.36
Estrone sulfate (pmol/L)—median (range)	603 (214–3,086)	736.5 (173–3,500)	0.90

NOTE: Values are *n* (%) for categories and median (range) for continuous measurements. *P* values for Fisher's exact test for comparisons of categorical characteristics and for Wilcoxon ranksum tests for comparisons of continuously measured characteristics.

Abbreviation: LCIS: Lobular carcinoma *in situ*.

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Table 4

Median (range) estrone sulfate, estradiol, and breast percent density at baseline, and changes in breast density, estradiol, and estrone sulfate, expressed as a percentage of baseline values, by CYP19 genotype

	Baseline value—median (range)			% change from baseline at 12 mo			
	AA ***n*** **= 4**	**AC** ***n*** **= 17**	**CC** ***n*** **= 33**	**AA** ***n*** **= 4**	**AC** ***n*** **= 17**	**CC** ***n*** **= 33**	***P***
Estradiol (pmol/L)	23.5 (6.9 to 35.0)	19.0 (7.2 to 53.0)	24.0 (7.3 to 112.0)	−84 (−95 to −48)	−85 (−89 to −80)	−88 (−91 to −84)	0.54
Estrone sulfate (pmol/L)	867 (358 to 968)	531 (115 to 3,086)	679 (214 to 3,500)	−95 (−98 to −86)	−92 (−95 to −86)	−94 (−95 to −92)	0.40
Breast density (%)	17.9 (5.5 to 66.2)	10.7 (0.1 to 58.6)	14.5 (0.7 to 46.1)	−58 (−97 to 483)	−19 (−47 to 25)	−7 (−23 to 12)	0.10

NOTE: Values in the right side of table represent percent changes [mean (95%CI)] from baseline (negative values indicate decreases from baseline). *P* values are based on ANOVA comparing genotypes where log-transformed values were analyzed to adhere to the normality assumption.