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Exemestane use in postmenopausal women at high risk for invasive breast cancer: evaluating biomarkers of efficacy and safety

Margaret Gatti-Mays¹, David Venzon², Claudia Galbo³, Andrea Singer¹, James Reynolds⁴, Erini Makariou⁵, Bhaskar Kallakury⁶, Brandy Heckman-Stoddard⁷, Larissa Korde⁸, Claudine Isaacs⁹, Robert Warren⁹, Ann Gallagher⁹, and Jennifer Eng-Wong⁹

¹ Department of Medicine, Medstar Georgetown University Hospital (Washington, DC)

² Biostatistics and Data Management Section, National Cancer Institute, National Institutes of Health (Bethesda, MD)

³ Department of Radiology, Walter Reed National Military Medical Center (Bethesda, MD)

⁴ Depart of Nuclear Medicine, National Institutes of Health (Bethesda, MD)

⁵ Division of Neuroradiology and Breast Imaging, Department of Radiology, Medstar Georgetown University Hospital (Washington, DC)

⁶ Department of Laboratory Medicine, Medstar Georgetown University Hospital (Washington DC)

⁷ Division of Cancer Prevention, National Cancer Institute, (Rockville, MD)

⁸ Division of Oncology, University of Washington (Seattle, WA)

⁹ Lombardi Comprehensive Cancer Center, Georgetown University Hospital (Washington, DC).

Abstract

This phase II trial evaluated clinical markers of efficacy and safety of exemestane in postmenopausal women at increased risk for breast cancer.

Postmenopausal women (n=42) at risk for invasive breast cancer received 25mg exemestane daily for two years along with calcium and vitamin D. The primary outcome was change in mammographic density (MD) after one year. Secondary outcomes included change in serum steroid hormones as well as change in trefoil protein 1 (TFF1) and proliferating cell nuclear antigen (PCNA) in breast tissue. Safety and tolerability were also assessed.

Corresponding Author Full Name: Margaret E Gatti-Mays MD MPH, **Contact Information:** Phone: 202-444-8683; Fax: 202-444-7797, **Mailing Address:** 3800 Reservoir Road, PHC 5th Floor, Washington, DC 20007, Margaret.gatti@gmail.com,

Affiliation: Department of Internal Medicine, Medstar Georgetown University Hospital, Washington, DC.

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Dr Andrea Singer receives Grant funding from Amgen. She consults for Amgen, Eli Lilly, Mission Pharmacal, Actavis. She also serves on the Advisory Board/Teaching/Speaker's Bureau for Amgen and Eli Lilly.

Drs Gatti-Mays, Venzon, Galbo, Reynolds, Makariou, Kallakury, Heckman-Stoddard, Korde, Isaacs, Warren, and Eng-Wong as well as Ms Gallagher do not report any conflicts of interest.

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MD decreased at 1 year and was significant at 2 years (mean change = -4.1% ; 95% CI $-7.2, -1.1$; $p = .009$). Serum estradiol and testosterone levels significantly decreased at three months and remained suppressed at 12 months. After 1 year of treatment, TFF1 intensity decreased (mean change -1.32 ; 95% CI -1.87 to -0.76 ; $p < 0.001$). Exemestane was safe and well tolerated.

Exemestane decreased MD and expression of breast tissue TFF1. It was well tolerated with few clinically relevant side effects.

MD and breast tissue TFF1 are potential biomarkers of breast cancer preventive effects of exemestane in high-risk postmenopausal women.

Keywords

Breast Cancer; Prevention; High-risk; Exemestane

Introduction

Aromatase inhibitors (AI), in addition to selective estrogen receptor modulators (SERMs), reduce breast cancer incidence in postmenopausal women with increased risk for hormone-receptor positive breast cancer, and 5 year therapy is recommended by the American Society of Clinical Oncology based on evidence from large clinical trials (1-4). The phase III MAP.3 trial found that postmenopausal women taking exemestane had a 65% relative reduction in the incidence of invasive breast cancer. Based on these results, it is estimated that the number of healthy women needed to treat to prevent one case of breast cancer is 94 over 3 years and 26 over 5 years of therapy (1). Although the findings are favorable, most women do not develop breast cancer while taking exemestane. A surrogate biomarker of effect could help to determine which women will benefit from exemestane to maximize benefit and minimize potential risks.

Increased mammographic density (MD) is a risk factor for breast cancer. Compared with women who have the lowest MD, those with the highest MD have a 4-6 fold risk of invasive breast cancer (5-9). Studies evaluating the effect of AIs on MD have yielded conflicting results and cross-trial comparison of results is limited by multiple factors (e.g., study population, intervention duration, concomitant therapies). One recent large study showed a non-statistically significant 1% decrease in MD at 12 months in the non-affected breast of women with early breast cancer compared to matched controls and concluded that changed in MD is not a biomarker of AI effect (10). Overall, studies have shown no or modest declines in MD among women taking AIs (11-13).

The decline in serum estrogens in postmenopausal women on AI therapy is well documented and expected given the drug's mechanism of action. Within breast tissue, a potential biomarker of effect is trefoil protein 1 (TFF1), also known as pS2. TFF1 is an estrogen response gene present in normal mammary tissue with increased expression in estrogen receptor positive breast cancer (14-16). Since exemestane binds to the aromatase enzyme and decreases circulating estrogen levels, TFF1 down-regulation could possibly preclude tumor development (16). In addition, proliferating cell nuclear antigen (PCNA) is a marker of proliferation and has been positively correlated with the use of exogenous hormones in

the normal postmenopausal breast as well as with an increase in mammographic density in women on exogenous estrogen and progesterone (17). Bernardes et al demonstrated that the number of cells expressing PCNA declined after a 22 day course of tamoxifen in normal women with breast fibroadenomas (18). Increase in proliferation, specifically in the terminal duct lobular unit, as measured by PCNA may represent the underlying histology of radiographically dense breast tissue. PCNA was chosen in lieu of Ki-67 as the marker of proliferation because Ki-67 is often undetectable in normal post-menopausal breast tissue.

Safety and tolerability are of particular importance in treating healthy populations. Use of AIs in the adjuvant setting has raised concern due to decreased bone mineral density (BMD) (12, 19) and an increase in the bone reabsorption marker urine N-telopeptide (11). Ten-year data from Arimidex, Tamoxifen, Alone or in Combination (ATAC) trial (20) showed increased fractures in women receiving anastrozole when compared with women receiving tamoxifen. However, the fracture risk was similar in post-treatment follow-up. In addition, the recent MAP.3 study found that 2 years of exemestane worsened age-related bone loss (19).

Another concern regarding the safety of exemestane is the potential to increase risk of cardiac events. Estrogens have long been thought to be cardioprotective and decreased circulating estrogen levels could increase risk of cardiovascular disease (21). Inconsistencies among studies regarding clinically significant changes in lipid profiles have raised questions about the cardiovascular safety of AIs in the prevention setting (1, 11, 12, 22). Finally, AIs have been linked to side effects including arthralgias, hot flashes, insomnia, and mood instability. Severe side effects are often responsible for drug discontinuation in the adjuvant setting (20, 23-25). However, most women report minimal changes in quality of life (1).

To address these research gaps, this study was a multi-site, single-arm, phase II trial of exemestane in postmenopausal women at increased risk for breast cancer (NCT00073073). The primary outcome was change in MD, and additional outcomes included change in breast tissue biomarkers and serum steroid hormones. Safety and tolerability of exemestane were examined via BMD, lipid levels, and subject reported outcomes and adverse events.

Materials and Methods

Participants and Treatment

Postmenopausal women at increased risk for breast cancer were enrolled at two clinical research hospitals in the eastern United States. Women were defined as postmenopausal if they had no menses for at least 12 months or bilateral oophorectomy. In unclear cases, (e.g. 50 year old who has had hysterectomy) chemical confirmation of postmenopausal status may be confirmed with FSH>35 U/L. The trial protocol was approved by IRBs at both participating sites (NCI 04-C-0044, GT 07-313). Women were considered high risk by any one of the following criteria: Gail Model (26) risk 1.7 over 5 years; history of lobular neoplasia or atypical ductal hyperplasia; DCIS treated with mastectomy or lumpectomy and radiation; stage I/II breast cancer at least two years from completing treatment for invasive disease; or *BRCA1/2* mutation carrier. Additionally, women were required to have no osteoporosis on BMD scan (AP spine T score > -2.5) and no prior AI therapy, but prior use

of menopausal hormone therapy (MHT) and SERMs was allowed. Women were required to have stopped MHT, tamoxifen, or raloxifene for at least three months before enrollment. Women were given 25mg of exemestane (Pfizer, Inc.) daily for two years with calcium carbonate 1200 mg and Vitamin D 400 international units daily.

Mammography

Women had a mammogram at baseline and after one and two years. Film screen images were collected at the start of the study; however, due to clinical practice changes digital mammography was allowed starting September 2007. The craniocaudal view was evaluated. For subjects who had no prior surgery, the side with the greater density was selected and was followed over the course of the study. For subjects who had undergone breast surgery for noninvasive or invasive cancer, the opposite side was followed. A single breast radiologist experienced in evaluating MD who was blinded to time on treatment (C.G.) read each image twice in the same setting and results were averaged. MD was assessed by semi-quantitative Cumulus method using the scripting language of the MEDx software (27). For digital mammograms, raw data files were used for analysis.

Steroid Hormone Levels

Steroid hormone levels were obtained at baseline, and after 3 and 12 months on exemestane. To decrease inter-assay variability, blood samples were drawn, processed and frozen at -70°C until all samples could be analyzed. Serum estradiol, androstenedione, testosterone and progesterone were purified by HPLC and then analyzed by RIA (Taylor Technology Inc Princeton NJ). Serum prolactin was evaluated by RIA (Cephac Europe SA, France).

Breast Tissue Collection and Immunohistochemistry

Image guided breast biopsies of mammographically dense tissue were performed at baseline and after 12 months. Five core specimens were obtained under local anesthesia at each time point. At the follow-up biopsy visit, the radiologist localized the previous biopsy site (biopsy clip placed at baseline) so that tissue was obtained from the same area of the breast. One core biopsy sample was formalin-fixed, paraffin-embedded, and examined for pathologic abnormalities. Immunohistochemistry (IHC) staining of breast tissue was performed for PCNA (Invitrogen) and TFF1 (Histostain Plus Kit from Zymed/Invitrogen) according to the manufacturer's instructions. Tissue was exposed to primary antibodies for PCNA (1:14000, Dako, M0879) and for TFF1 (1:50). Negative controls for both stains were included. TFF1 was assessed by both intensity of stain (0 to 3+) and percent of cells with any staining. PCNA was assessed by the percent of positive epithelial cells in the ductal/lobular component of the breast tissue and the intensity of the stain (0 to +3). The pathologist (B.K.) was blinded to the time of biopsy.

Bone Mineral Density

BMD was assessed using dual-energy x-ray absorptiometry (DXA; Hologic QDR 4500W or Hologic Discovery W Bedford MA). Each subject was assessed on the same scanner for all assessments. Anterior posterior (AP) views of the lumbar spine and one view of the hip were

evaluated. The AP lumbar spine measurement was repeated at each time point and results were averaged; total hip was measured once at all evaluations.

Lipid Profile

Fasting serum total cholesterol, HDL, LDL, triglycerides, and homocysteine were collected at baseline, 3, 12, and 24 months after initiation of exemestane therapy. Apolipoprotein A and B were collected at baseline, 3 and 12 months. Lipid analysis was completed at each institution's Clinical Laboratory Improvement Amendments (CLIA) approved laboratory.

Adverse Events and Quality of Life

Participants were asked to track adverse events (AEs) on calendars and were evaluated in clinic every 3 to 6 months. AEs are reported as per NCI Common Terminology Criteria (NCI CTCAE) 3.0 grading criteria. In calculating the overall incidence of AEs, subjects who experienced the same event on more than one occasion were counted once in the calculation of the event frequency at the highest intensity observed.

Quality of life (QOL) was evaluated with the Menopause-Specific Quality of Life Questionnaire (MENQOL) (28).

Statistical Analysis

The trial was designed to have 88% power to detect a MD decrease with a mean of 5% or larger, using a one-tailed Wilcoxon signed rank test at the $p < 0.05$ level with 35 subjects evaluable at one year. Potential confounders of increased breast density such as prior use of MHT or SERMs were assessed using the Wilcoxon rank sum test and Spearman rank correlation. Since the denser breast was followed, regression to the mean was also investigated.

Changes in steroid hormone levels were assessed by the Wilcoxon signed rank test, and the Hochberg p-value adjustment was applied to account for multiple hypothesis testing. Breast tissue TFF1 and PCNA change in intensity and percent positive cells were evaluated by the Wilcoxon signed rank test.

Changes in BMD from baseline to 12 months and from baseline to 24 months were calculated for AP lumbar spine and total hip and summarized using means and standard deviations (SD). Paired t-tests and confidence intervals (CI) were used given percent changes consistent with normal distributions. Change in BMD was analyzed by Student's t-test.

Changes in serum lipid levels were evaluated by the Wilcoxon signed rank test and the sign test and were assessed for the use of cholesterol-lowering medications. The Hochberg p-value adjustment was used to account for multiple hypothesis tests.

MENQOL mean scores for each of the domains were compared at baseline, 12 months and 24 months using analysis of variance. Analyses were conducted using SAS/STAT software version 9.3.

Results

Participants and Medication Adherence

Forty-six women enrolled in the study, and between October 2004 and December 2009; 42 started exemestane. Three women were ineligible for the study after baseline DXA scans demonstrated osteoporosis. One did not return for scheduled appointments after enrollment. Of the 42 women who received exemestane, 36 completed one year of treatment and 35 completed two years (Fig 1). Of the 7 women who did not complete the study, 3 cited AEs as the primary reason for drop-out (see below) and 4 lost interest in participating.

Participants averaged 59.1 years of age (Table 1). The majority were eligible due to high risk pathologic lesions (DCIS 45%; atypical ductal hyperplasia 19%; lobular neoplasia 12%). Twelve women (29%) previously used SERMs (but had stopped 3 months prior) for treatment of invasive cancer (n = 1), DCIS (n = 4), and for breast cancer prevention (n=7).

Nearly all (95%) women maintained satisfactory exemestane adherence (pre-defined as taking 80% of scheduled pills) measured based on patient-report calendars and verified pill counts. More than 90% also maintained satisfactory adherence with calcium (1200 mg daily) and vitamin D (400 IU daily).

Mammographic Density

Clinical standards for mammography evolved during the study. Of the 36 women completing year one, 35 had paired mammograms available for analysis (1 subject had no mammographic image at year 1 and was excluded from analysis at all time points). Fourteen women had film mammograms at all time points; 14 had digital mammograms at all time points and 7 had film mammograms at baseline and digital at 1 year (n=4) or 2 year (n=3). To determine if mammogram technique affected change in MD, analyses of changes among women with film screen only, digital only, or film followed by digital were completed and did not differ in regard to magnitude or direction of change.

The mean baseline MD was 32.5% (SD 13.5%). The highest and lowest baseline MD values had similar changes over the course of the study, so there was no evidence that the distribution of the baseline values affected the distribution of changes. The mean change in MD from baseline was -2.4% at year 1 (95% CI: -5.0% to 0.1%; p-value = 0.055) and -4.1% at year 2 (95% CI: -7.2% to -1.1%; p = 0.009; Table 2). The four women who had taken MHT had significantly denser breasts at baseline (median 43% vs 31%; p = 0.04) but all had stopped MHT at least 20 months prior to trial enrollment. The change in MD for these women at 1 year and 2 years was not significantly different from women who had not used MHT. Prior SERM use (n=12) did not significantly affect baseline MD or change in MD. Ten subjects had stopped SERMs within 12 months of starting on the trial. These 10 participants had slightly higher baseline MD than those who did not use SERM or MHT (33% vs 29%) but this difference was not significant. The change in MD for these women at 1 year and 2 years was not significantly different from women who had not used SERMs.

Serum Hormone Levels

Seventy percent of women had serum samples that could be assessed for changes in hormones. Twenty-five serum samples were available for paired analyses. At baseline, the mean estradiol level was 5.54 pg/ml (SD =3.61). After 3 months of exemestane, 84% (n=21) of subjects had undetectable (<0.625 pg/ml) estradiol levels ($p = 0.001$). Similarly, at 12 months, 20 women (80%) had undetectable estradiol levels ($p = 0.001$). Testosterone levels also significantly decreased from baseline (mean = 184.47 nmol/L; SD = 84.63) values at 3 months ($\Delta = -18$ nmol/L; $p=0.01$) and this decrease was sustained through 12 months of study drug exposure ($\Delta = -19$ nmol/L; $p=0.002$). Prolactin and androstenedione levels did not significantly change.

Breast tissue biomarkers

Thirty five women (97%) underwent baseline and twelve month breast biopsies. One woman declined the 12 month biopsy. Seven biopsies at baseline and 5 biopsies at 12 months did not contain any ductal or lobular tissue, which is not unexpected given the fatty replacement of glandular tissue that occurs during menopause. No high risk lesions or invasive cancers were identified. Twenty-two women had evaluable breast tissue at both time points for TFF1 and 23 subjects for PCNA analysis.

Of the baseline specimens, 96% were positive for TFF1: 59% (13 of 22) were scored as 3+ (intense), 32% (7 of 22) were 2+ (moderate) and 5% (1 of 22) were 1+ (weak). Percent of cell cytoplasm staining for TFF1 ranged from 0 to 20% (median=5%). After 1 year on exemestane, TFF1 intensity decreased in 17 women (77%), 4 had no change and 1 increased. Mean TFF1 intensity change was -1.32 (95% CI: -1.87 to -0.76 ; $p < 0.001$; Fig 2a and 2b). The 17 participants with a TFF1 intensity decline had slightly larger decreases in MD compared to the total study population with mean changes -3.1% from baseline to year 1, -7.4% from baseline to year 2, where the total population has declines of -2.4% , and -4.1% , respectively. As in the total population, the first was not significantly different from a zero mean, but the change at year 2 was significant ($p=0.0013$). There was no significant association between changes in TFF1 and serum estradiol, prolactin, testosterone, or androgen. At baseline, the percent of cell cytoplasm staining for PCNA ranged from 15 to 50%. There was no significant change in PCNA IHC after 1 year of exemestane therapy.

BMD Measurements

No significant change in the AP spine was seen at 12 or 24 months. Statistically significant decreases in total hip bone density occurred at both 12 (mean % change = -1.4 ; $p = 0.01$; Table 3) and 24 months (mean % change = -2.7 ; $p=0.0004$), although these are not clinically significant per the International Society for Clinical Densitometry (ISCD)(29-31). There were also no new diagnoses of osteoporosis or fractures during the study. Percent changes did not differ significantly between the trial sites ($p>0.25$ for each). Changes in BMD among 4 women taking bisphosphonates at baseline did not significantly differ from women who were not taking bisphosphonates. For the short term precision determination, duplicate measurements of the AP lumbar spine with repositioning of the subject yielded a coefficient of variation of 1.6%.

Serum Lipids Levels

Change in HDL decreased from baseline at 3, 12 and 24 months (all p-values = 0.001 after Hochberg adjustment; Table 4). Total cholesterol also significantly decreased from baseline at 3 months (−13.6 mg/dL, $p = 0.007$) but was no longer significant at 12 and 24 months (−9.6 mg/dL and −12.0 mg/dL, respectively; p -values = 0.19). A majority ($n = 19$) of women were on lipid-lowering medications and there were no significant differences in mean lipid values for each of the 4 assessment points or in the mean changes from baseline between women who were and were not taking lipid-lowering medications.

Adverse Events and QOL Results

The majority of subjects ($n = 38$, 90%) reported at least one AE to be possibly, probably, or definitely attributed to treatment. Of these AEs, 95% were grade 1 or 2. The most common drug-related grade 1 and 2 adverse events were hot flashes ($n = 14$) and arthritis/joint or bone pain ($n = 13$). Other reported adverse effects included vaginal dryness, fatigue, myalgias, diarrhea, libido changes, and headache. Events were typically self-limited with resolution prior to the next assessment point. One subject had a persistent transaminitis and it was unclear if it was related to treatment. There were 5 grade 3 AEs: migraine (possibly related), vaginal dryness (possibly related), fatigue (possibly related), uterine prolapsed resulting in hysterectomy (not related) and severe arthritis precipitating a knee replacement (not related). There was no association between duration of exemestane therapy and onset of AEs.

Of the seven women who dropped out after starting treatment, three cited AE as the primary reason for discontinuation. One reported grade 2 joint pain; one reported grade 3 myalgias (later attributed to statin use), and one reported grade 1 hot flashes.

Despite the large percentage of participants who reported AEs during the study, the effect on quality of life was minimal. There was no statistically significant change in quality of life reported in any of the four domains (sexual, physical, psychosocial or vasomotor) while taking exemestane (*data not shown*).

Discussion

In this study of exemestane in healthy women at increased risk for breast cancer, we found that exemestane use is associated with a decreasing trend in MD at one year (−2.4%; $p = 0.055$) that becomes statistically significant at 2 years (−4.1%; $p = 0.009$). While the strength of our conclusions is limited by the lack of a control arm, comparable control groups of postmenopausal women have a MD change of +0.58 to −0.8 at one year follow-up (10-12) suggesting that the observed decline of 2.4% at 1 year and 4.1% at 2 years in our study may be significant. Henry et al noted (13) a similar decline of 5.8% at 2 years in the contralateral breast in women with early breast cancer on exemestane or letrozole. A small pilot study by Smith et al (32) found that letrozole use was associated with a reduction in MD over 12 months in 11 of their 16 patients. However, other larger studies have not found a significant decrease in MD with the use of AIs (10-12, 33). A prospective, matched case-control study found no difference in MD decrease in post-menopausal women treated with

AIs when compared to matched controls (34). Many of the studies which have no change in MD limited AI therapy to 12 months (10-12, 35). However, our study along with at least one other study (13) which evaluated AI therapy for 24 months did note a significant decrease in MD at the 24 month evaluation point. Unfortunately, our study was powered to evaluate MD at 12 months and not at 24 months. In addition, while MD is an accepted and well recognized indicator of breast cancer risk, inconsistencies among recent studies suggest that it may not be as robust when used as a biomarker of effect in the prevention setting with AIs.

We found exemestane decreased serum estradiol as well as decreased TFF1 intensity in breast tissue. Fabian et al (36) evaluated the effect of letrozole in women concurrently on MHT and similarly found that pS2 (also known as TFF1) as well as Ki-67 (a marker of proliferation) were decreased at 6 months. While numbers in both studies are small, the biologic plausibility and concordance of results (using different technology) suggest TFF1 is a promising biomarker of effect. Furthermore, prior studies have also found TFF1 expression in breast tissue to be a strong prognostic indicator of hormone responsiveness (15, 36-38). To be a proven biomarker, long term studies evaluating the change in TFF1 intensity with breast cancer incidence are needed. In addition, since prevention agents are not universally protective, determining biomarkers of effects may allow tailored therapy.

Down-regulation of estrogen by AIs has created concern about the safety of AI use in the prevention setting and the potential for osteoporotic fractures. Exemestane was initially hypothesized to be potentially bone sparing due to its steroidal structure; however, subsequent studies have not shown any advantage in regard to bone density and skeletal events with exemestane compared with non-steroidal AIs (39). While the decrease in hip BMD reached statistical significance, the change is not clinically significant according to the 2002 position statement by the ISCD (31). The ISCD recommends that changes in bone density be considered significant if it is equal to or greater than the lower 95% confidence interval of the precision of measurement at each physiologic site. Based on this recommendation and data from published studies (29, 30), a clinically significant change in bone density is defined as 5 to 10% at the femoral neck and 3% at the lumbar spine.

Both vitamin D and calcium supplementation were required on this study due to the concerns of exemestane's potential effect on BMD. Vitamin D has generated interest as a breast cancer risk reduction agent that may also modulate MD (40, 41), however controlled trials with vitamin D and calcium supplementation have not been associated with a decline in MD (42).

Another concern about AI use is the potential to increase cardiac events due to the down-regulation of estrogens, which have long been felt to be cardioprotective. In agreement with prior studies, exemestane decreased HDL over time (11, 43) while leaving the rest of the lipid panel relatively unchanged. The mean HDL in our study population was 61 mg/dL. A decrease of 8.9mg/dL (15%) over the two year period, may be a clinically significant decrease. The strength of HDL as a cardioprotective factor is not as strong as previously thought, (44) but there are still concerns about the potential increase in cardiovascular risk in this healthy population. Younus et al (45) evaluated 22 papers that reported data regarding cardiovascular event incidence in women taking AIs from 8 randomized control trials. To

date, no studies have shown that AIs increase cardiovascular risk but follow-up has been limited.

Exemestane was generally well tolerated by our subject population. While most subjects did report grade 1 adverse events at some point during the trial, over 90% were transient and most of the reported symptoms were similar to menopausal symptoms as well as symptoms experienced while on SERMs (e.g., musculoskeletal pain, hot flashes, fatigue, etc). More importantly, there was no significant change in quality of life while taking exemestane. Our high compliance rate over the two year trial as well as the high retention rate (83%) demonstrates the limited impact of the AEs of exemestane in this population. The larger MAP.3 trial found similar results (1).

Our study had multiple strengths. Participants were highly compliant with exemestane, calcium and vitamin D. Our study evaluated the willingness of healthy women to undergo breast core biopsy collection procedures for assessment of tissue based biomarkers. While previous studies have evaluated other techniques for collecting cells (46-48) this study allowed evaluation of both ductal epithelium and stromal cells. Only one participant declined the post-exemestane biopsy citing personal concerns about the procedure's safety. Otherwise the procedure was well tolerated by subjects.

As with all phase II clinical trials, one of the major limitations is the small study size. Although originally designed to assess exemestane with or without celecoxib, due to safety concerns regarding cyclo-oxygenase 2 inhibitors, the trial was amended to a single-arm design prior to the first patient enrollment. Study practices were put in place to in order to improve rigor, e.g. BMD follow up assessment were evaluated at the same center on the same imager and biopsy clips were placed to sample the same breast tissue area.

Exemestane decreased MD, serum estrogen levels and the expression of breast tissue TFF1. It was well tolerated with few clinically relevant side effects. The results from our small study are in agreement with larger clinical trials. In 2013, the American Society of Clinical Oncology released guidelines recommending the use of exemestane as an alternative to SERMs for cancer prevention in high-risk, post-menopausal women (3). Defining which women will have the optimal benefit-risk profile for this agent still needs to be determined. Our findings suggest potential surrogate biomarkers of prevention benefit and risk. However, given that a phase 3 prevention trial of exemestane has already been completed, additional information about the utility of these potential biomarkers with exemestane use is not expected. In the future, consideration should be given to stratification by MD, estrogen level and/or TFF1 expression to determine who may benefit from the next generation endocrine therapy in the primary prevention setting.

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* Osteoporosis was identified on baseline DXA scans

Figure 1.
Study Enrollment

A

Baseline

12 Months

Patient 34

Patient 35

Figure 2a.

Down-regulation of TFF1 in normal breast tissue after 12 months of treatment with exemestane.

Figure 2b.

Down-regulation of TFF1 intensity in normal breast tissue after 12 months of exemestane.
(n=22; 1 bar = 1 subject)

TFF1 is down-regulated after 12 months of treatment with Exemestane. Pre-treatment images were scored +3 (intense) TFF1 staining and post-treatment images as +1 (weak) TFF1 staining. All images magnified at 40x.

Table 1

Baseline Demographics and Eligibility Risk Data (n = 42)

Age, mean	59.1
BMI, mean	29.3
Race	
White	35 (83)
African American	6 (14)
Ethnicity: Hispanic	1 (2)
Prior SERM Use	12 (29)
Mean duration of use in months	47
Median duration between cessation and start of trial in months	5.1
Prior MHT Use	4 (10)
Mean duration of use in months	51
Median duration between cessation and start of trial in months	45
Risk Factors for Inclusion	
Gail model risk of 1.7% over 5 yrs preceding study entry	6 (14)
Lobular neoplasia	5 (12)
Atypical ductal hyperplasia	8 (19)
DCIS that has been previously treated with mastectomy or lumpectomy and radiation, +/- tamoxifen	19 (45)
Deleterious mutations in <i>BRCA1</i>	1 (2)
Prior Stage I or II at least two years out from treatment for invasive disease	3 (7)

All values are n(%) unless otherwise stated.

Table 2

Change in Mammographic Breast Density on Exemestane

	n	Mean	Median	SD	95% CI	p-value
Baseline → Year1	35	−2.4%	−3.4%	7.5%	−5.0% to 0.1%	0.055
Baseline → Year2	34	−4.1%	−2.8%	8.7%	−7.2% to −1.1%	0.009 *

Note: One subject had no mammographic image at year 1 and was excluded from analysis at all time points

* Significant at p = 0.05

Table 3

Change in Bone Mineral Density on Exemestane

	Baseline Mean g/cm ² (SD) Range	Mean % Change (95% CI)	
		12 Months	24 Months
AP Lumbar Spine	n = 42 1.05 (0.16) Range: 0.80 to 1.50	n = 37 -0.8 (-2.1 to 0.4) p = 0.18	n = 34 -0.8 (-2.5 to 1.0) p = 0.37
Hip	n = 42 0.97 (0.14) Range: 0.76 to 1.48	n = 36 -1.4 (-2.5 to -0.3) p=0.01 *	n = 33 -2.7 (-4.0 to -1.3) p= 0.0004 *

* Statistically significant at p = 0.05

Table 4

Mean Values for Lipids at Baseline and 3 Months, 12 Months and 24 Months after Initiation of Exemestane Therapy

	Baseline	3 Months	12 Months	24 Months
Apolipoprotein A (mg/dL)	118.3 (58.4)	111.4 (52.0) = -9.6	125.7 (46.9) = +7.4	n/a
Apolipoprotein B (mg/dL)	86.1 (19.3)	81.5 (17.7) = -5.0	80.7 (18.5) = -5.5	n/a
Homocysteine (umol/L)	8.4 (2.2)	9.7 (2.7) = +1.2 *	9.4 (3.1) = +0.9	9.6 (3.1) = +1.3
Total Cholesterol (mg/dL)	190.2 (31.8)	177.5 (32.2) = -13.6 *	181.6 (35.9) = -9.6	179.4 (32.8) = -12.0
HDL (mg/dL)	62.6 (16.6)	54.6 (14.5) = -8.0 *	54.0 (14.0) = -8.5 *	53.1 (15.1) = -8.9 *
LDL (mg/dL)	113.7 (29.7)	110.5 (30.0) = -4.3	110.1 (31.4) = -4.7	105.1 (27.1) = -10.4
Triglycerides (mg/dL)	107.5 (52.8)	101.1 (55.5) = -6.8	102.6 (54.2) = -5.3	107.0 (53.2) = -2.3

All values are mean (SD).

= Repeated Lipid Value – Baseline Lipid Value. Analyzed via Wilcoxon Signed Rank test ($H_0: \mu = 0$).

* p-values adjusted for multiple comparisons using the Hochberg adjustment. p-values are significant 0.05.