Short-Term Biomarker Modulation Prevention Study of Anastrozole in Women at Increased Risk for Second Primary Breast Cancer

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Abstract

The selective estrogen receptor modulators (SERM), Tamoxifen and raloxifen reduce risk breast cancer. Patient acceptance of SERMs for breast cancer prevention is low due to toxicities. New agents with a better toxicity profile are needed. Aromatase inhibitors (AI) reduce the risk of contralateral breast cancer and risk of new breast cancer in high risk women. However, the mechanism by which AIs reduce breast risk is not known. Surrogate biomarkers are needed to evaluate the effect of preventive agents. The objective of this prospective short-term prevention study was to evaluate the effect of anastrozole on biomarkers in breast tissue and serum of women at increased risk for developing a contralateral breast cancer. Women with a history of stage I, II breast cancer who started anastrozole for standard adjuvant treatment were eligible. Patients underwent baseline fine needle aspiration of the unaffected breast and serum collection for biomarker analysis before starting anastrozole at 1 mg per oral/day and again at 6 months. Biomarkers included changes in cytology, insulin-like growth factor 1 (IGF-1), IGF-binding protein 1 (IGFBP-1), and IGFBP-3. Thirty-seven patients were enrolled. There was a significant modulation in serum IGFBP-1 levels between pre- and post-samples ($P = 0.02$). No change was observed in IGF-1, IGFBP-3, and breast cytology. We showed a significant modulation of IGFBP-1 levels with six months anastrozole. Anastrozole is currently being studied as a prevention agent in a large phase III trial and our results provide support for continued evaluation of IGFBP-1 as a surrogate endpoint biomarker in prospective breast chemoprevention studies. Cancer Prev Res; 5(2): 276–82. ©2011 AACR.

Introduction

The selective estrogen receptor modulators (SERM) tamoxifen and raloxifen have been studied as breast cancer preventive agents over the past 20 years in large prospective phase III trials. In the National Surgical Adjuvant Breast and Bowel Project (NSABP) P-1 trial tamoxifen was shown to reduce breast cancer risk by 50% (1). The second study, NSABP P-2, randomized about 19,000 high risk women to tamoxifen or raloxifene and showed that tamoxifen was more effective than raloxifene in reducing the risk of invasive breast cancer (2). Furthermore, large and prospective long-term breast cancer adjuvant trials of aromatase inhibitors have shown that these agents, while improving outcome of established breast cancer, also reduce risk of contralateral breast cancer more than tamoxifen (3). Therefore, several large prospective studies are underway evaluating aromatase inhibitors (AI) as potential breast cancer risk reductive agents (3). One of the AI breast cancer prevention studies conducted in 4,560 high risk patients was recently published and showed a 65% reduction in the incidence of invasive cancer in patients randomized to 5 years of exemestane compared with placebo (4). However, the mechanism(s) by which these agents can achieve this preventive effect and whether there are certain biomarkers that could be used as surrogates to predict this beneficial effect remains unknown.

The conduct of large long-term phase III breast cancer prevention trials is costly, requires a very large number of participants and the results are not obtained for almost a decade. Therefore, short-term studies are needed that are designed to identify biomarkers that could be used in future larger studies that evaluate potential preventive agents.

Several breast cancer risk biomarkers could be considered for short-term phase I and phase II breast cancer prevention trials. It is known that sex steroidal hormones, including estradiol, are associated with increased risk of breast cancer (5). Furthermore, studies have shown that dysregulation of
the IGF pathway and an increase in insulin-like growth factor 1 (IGF-1) also result in increased risk of breast cancer (6). And finally, cytomorphology has also been studied as a potential biomarker for breast cancer risk and has been shown to be suitable to be used in the setting of short-term prevention studies (7).

In this study, our hypothesis was that anastrozole would induce a decrease in breast cancer risk biomarkers. The aim was to evaluate whether anastrozole would induce favorable modulation in breast cancer risk biomarkers, such as cytology, IGF pathway and estradiol, with the ultimate aim to validate these biomarkers in large prospective breast cancer prevention trials.

**Materials and Methods**

**Patient eligibility and study design**

Patients who were diagnosed with invasive breast cancer at the University of Texas MD Anderson Cancer Center and who were found to be eligible to receive anastrozole adjuvant endocrine therapy as part of standard of care were offered participation in this prospective study. Women with a history of invasive breast cancer have a 0.5% to 1% year risk of developing a second contralateral primary breast cancer (8, 9), hence this population is one of the ideal cohorts for short-term breast cancer prevention trials and has been studied previously as such (10). Eligibility criteria for our study included diagnosis of unilateral invasive, ER-positive breast cancer, completion of all adjuvant therapy, including surgery, chemotherapy and radiation therapy if indicated, and having an intact contralateral breast. Patients were approached by the study research nurse before starting anastrozole. After signing informed consent, patients underwent baseline blood draw and random periareolar fine needle aspiration (FNA) of the contralateral unaffected breast for biomarker evaluation. Patients then started anastrozole therapy and underwent second blood draw and breast FNA after 6 months of therapy (±7 days) to evaluate modulation of biomarkers. This study was approved by the Institutional Review Board.

**FNA and cytologic evaluation**

The FNA procedure was carried out as previously described (11). Briefly, patients with previous history of breast cancer underwent the procedure in their intact opposite breast. The FNA was carried out 4 times each at the 3 o’clock and 9 o’clock positions. A 1.5-inch, 23-gauge needle attached to a 10-mL syringe was used. The needle was inserted approximately 1 to 2 cm away from the areola, at 3 o’clock and later at 9 o’clock. Following injection of 2 mL of 1% lidocaine, the aspiration needle was moved in multiple directions to ensure sampling of most of the breast tissue, with emphasis on areas of dense breast tissue, where proliferative glandular tissue is often present. All of the FNA samples were pooled in 5 mL of Cytolyte solution. After aspiration, firm pressure was applied to the aspiration site to prevent hematoma formation. A cold pack was also applied to the breast for approximately 10 minutes after completion of FNA.

Cytologic specimens were prepared using the thin preparation (ThinPrep) technique (Cytyc Corporation). One slide was stained with Papanicolaou stain for cytologic diagnosis. Sample adequacy was defined as having 10 or more epithelial cells per slide.

All slides were assessed by a single cytopathologist (N.S.). Cytologic diagnosis was made on the basis of previously published criteria (12). The categories used were nonproliferative epithelium (NPE), hyperplasia (with or without atypia), and malignant lesion.

**IGF pathway biomarkers and estradiol**

Blood was obtained and serum was frozen at −80°C for analysis of IGF-1 and its binding proteins IGFBP-1 and IGFBP-3, and estradiol. Serum was frozen at −80°C until analysis. For estradiol, commercial kits for enzyme immunoassay (EIA) from ALPCO Diagnostics, Salem, New Hampshire, were used according to manufacturer’s instructions. The sensitivity of this essay is reported to be 10 pg/mL, range: 20 to 3,200 pg/mL. IGF-1, IGFB-1, and IGFBP-3 were measured by ELISA using kits from Diagnostic Systems Laboratories according to the manufacturer’s instructions. Baseline and 6 months samples were analyzed at the same time.

**Aromatase enzyme genotyping**

DNA isolated from whole blood was used for the genotyping of the rs4646 SNP in the CYP19A1 aromatase gene. The genotyping was carried out using the assay id# (C__8234730_1_) purchased from Applied Biosystems/Life Technologies.

**Statistical considerations**

We planned to accrue a total of 37 patients, so that after attrition we would have evaluable pre-and posttreatment samples from 30 patients. For those markers measured on a continuous scale, the sample size of 37 would provide a 75% power to detect an effect size of 0.5 in the change from pretreatment to 6-month measurements (assuming 2-sided 1-sample t test with significance level of 0.05). Results from these studies will be important for determination of sample sizes required when definitive studies are undertaken.

Summary statistics using mean, SD, median, and range were employed to describe continuous variables including age, body mass index (BMI), and marker measurements and tabulation was used to present distribution of categories for patients’ characteristics that were discrete. The modulation in continuous markers was calculated by subtracting baseline measures (Pre-) from the posttreatment measures. Signed-rank test was used to test if the modulation was significantly different from 0. We have also compared baseline markers and modulation of the markers between or among various characteristics groups, using Wilcoxon rank sum test or Kruskal–Wallis test, when appropriate. McNemar test is used to compare if there is any difference in cytology before and after treatment.
Results

Patient characteristics

Thirty-seven patients were enrolled to this prospective study. Patient and tumor characteristics are shown in Table 1. Median age was 58 years (range: 45–75 years). Sixty-five percent of patients used hormonal replacement therapy, 45.9% for more than 5 years. The median BMI was 25.3 (18.8–47.4). Majority of the patients underwent segmental mastectomy and the majority of the patients did not receive adjuvant chemotherapy.

Compliance during the study was very good, with 84% (31/37) of the patients undergoing month 6 repeat FNA. Reasons for not undergoing a repeat FNA included discontinuation of anastrozole due to recurrent breast cancer (n = 1) or drug intolerability (n = 1), and for the remainder of the patients included self rescheduling their 6 months follow-up that placed them out of the window period (+/7 days) to undergo repeat FNA (n = 4). Out of the 31 patients that underwent month 6 FNA, 2 had inadequate samples, leaving 29 paired FNA samples for cytologic evaluation. For serum biomarkers, paired samples were available in 29 patients for IGFBP-1, in 22 patients for IGFBP-3 and IGF-1 and in 24 patients for estradiol.

Baseline biomarkers by prior chemotherapy use

To account for the possible effect of chemotherapy on baseline biomarkers, we have stratified baseline cytology, IGF-1, IGFBP-1, IGFBP-3, and estradiol levels by prior chemotherapy use and have found no difference (Table 2).

Changes in breast cytology

Out of 31 patients who underwent who underwent a second 6 months follow-up FNA, we had 29 paired samples available. The epithelial mean cell number retrieved was 625 per FNA (range: 10–2412). The rate of hyperplasia (H) in baseline FNA samples was 24.3%, atypical hyperplasia (AH) rate 2%. After 6 months of anastrozole therapy the H rate was 7% and AH 24%. The baseline AH rate was too small to run statistical analysis (Table 3).

IGF pathway biomarker changes in serum

Pre- and posttreatment IGF-1, IGFBP-1, IGFBP-3 levels are shown in Table 4. Anastrozole induced significant modulation IGFB-1 between pre- and posttreatment levels (median, range) being 6.37 ng/mL (0.01–35.5) and 11.9 ng/mL (0.01–45.6), respectively (P = 0.02; Fig. 1). No changes were observed in IGF-1 and IGFBP-3 levels (Table 4).

Estrogen levels

Pre- and posttreatment estradiol levels are shown in Table 4. Anastrozole did not induce a significant modulation in estradiol levels (P = 0.95).

We further evaluated whether certain patient and tumor characteristics were associated with significant changes induced by anastrozole in the biomarkers that were analyzed. Characteristics included age, BMI, HRT use, breast feeding, tumor grade, and chemotherapy use; none of these characteristics were associated with changes in biomarkers (data not shown).

Aromatase genotype

We evaluated the aromatase genotype (CYP19: rs4646) in 33 patients. In 4 patients baseline blood did not contain optimal DNA to conduct the analysis. Twenty-one (63.6%) patients had genotype CC, 9 (27.3%) had genotype AC, and 3 (91%) had genotype AA (Table 1). Furthermore, we evaluated whether a certain genotype was associated with changes in biomarkers induced by anastrozole (Table 5). Genotype was not associated with changes in biomarkers that were evaluated. However, a
trend ($P = 0.06$) toward a significant association between genotype and changes in IGFBP-3 levels was observed (Table 4).

Discussion

In our current study, we show that 6 months of anastrozole therapy induces a favorable modulation of IGFBP-1 in women who are at increased risk of developing second primary breast cancer and that IGFBP-1 could be used as a surrogate for anastrozole and other potential agents' preventive effect. We have not observed changes in estradiol and cytology.

It was previously shown that blood concentrations of IGF-I and insulin-like growth factor binding proteins are associated with breast cancer risk (6). Furthermore, it has also been shown that risk for breast cancer increases significantly with increasing concentrations of all sex hormones including estradiol (5). And finally, Fabian and colleagues have shown that cytormorphology from breast random periareolar fine-needle aspirates can be used with the Gail risk model to identify a cohort of women at very high short-term risk for developing breast cancer. The authors of this study concluded that cytormorphology can be used as a potential surrogate endpoint in breast cancer prevention trials (7).

These risk biomarkers have been evaluated in several phase I and II breast cancer prevention trials using different agents including aromatase inhibitors.

No changes in IGF-1/IGFBP-3 ratio were reported in a study evaluating DFMO versus placebo for 6 months in 119 women (13) and 2 other studies using letrozole for 3 or 6 months (14, 15). However, Bonanni and colleagues evaluated the effect of 2 months of tamoxifen in different doses versus placebo in 110 healthy women and showed a reduction in IGF-1/IGFBP-3 (16). In another study of 75 postmenopausal patients that were randomized to tamoxifen 10 mg/week, anastrozole at 1 mg/d or the combination for 12 months showed also a reduction in IGF-1/IGFBP-3 levels in the combination arm compared with the anastrozole arm (17). Similar to these results, our study also showed a significant modulation in the IGF pathway, specifically, a significant increase in IGFBP-1 levels. To the best of our knowledge a change in this biomarker has not been reported previously. This might be a desired effect, because it is known that IGFBPs, including IGFBP-1, bind to IGF-1 with high affinity and thereby exhibit antiproliferative effects (18). Furthermore, it was shown that IGFBP-3 can also act independently from IGF-1, by regulating known growth suppressing factors such as retinoic acid and antiestrogens in breast cancer cells (18). Whether IGFBP-1 can induce similar effects with antiestrogen therapy (such as anastrozole) is unknown at this time. However, from the practical point of view, the fact that modulation in the IGF pathway can be achieved with different agents (as summarized above) might indicate that IGFBPs can be used as surrogate biomarkers in short-term prevention trials regardless of what agent is used.

Controversial results have been reported in studies evaluating changes in estradiol. Fabian and colleagues evaluated the effect of 6 months of letrozole in 42 postmenopausal women taking hormone replacement therapy and, as in our study, no reduction in estradiol levels were seen (15). In contrast, another small study of 32 patients with letrozole did show a reduction in estradiol levels (14). Similarly, Bonanni and colleagues showed a reduction in estradiol levels with anastrozole when combined with tamoxifen (17). Finally, a study by Prowell and colleagues that had a very similar cohort and design to our study, showed a reduction in estradiol levels after 6 months of anastrozole therapy (19). In our study, even though not statistically significant, there was a small trend toward decreased levels after anastrozole treatment. Furthermore, a large 

<table>
<thead>
<tr>
<th>Table 2. Baseline biomarkers stratified by previous chemotherapy exposure</th>
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<tr>
<td><strong>Covariate</strong></td>
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<tr>
<td>----------------</td>
</tr>
<tr>
<td>Pre_IGFBP1</td>
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<tr>
<td></td>
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<tr>
<td>Pre_IGFBP3</td>
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<tr>
<td></td>
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<tr>
<td>Pre_estradiol</td>
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<th>Table 3. Changes in cytology</th>
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<td><strong>Posttreatment</strong></td>
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<tr>
<td></td>
</tr>
<tr>
<td>Pretreatment</td>
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<tr>
<td>Normal</td>
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<td>Hyperplasia atypical</td>
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<tr>
<td>Hyperplasia</td>
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study with anastrozole has shown that there is a significant decrease in estradiol levels. Taken together, the results of biomarkers studies, including ours, need to be interpreted cautiously as these studies are small and the assay sensitivities might be different.

Similar to our current study, several studies have reported no improvement in cytology with a given intervention. In one study, 119 high risk women treated with DFMO versus placebo for 6 months: it did not show a significant cytologic improvement in samples obtained via breast FNA (13). Likewise, a similar study using letrozole for 6 months in 42 healthy women did not improve cytologic atypia. However, this particular study did show a significant reduction in Ki-67 in breast FNA samples (15). Finally, in a study of 45 women without breast cancer treated with tamoxifen versus placebo for 1 year, no change in hyperplasia was observed in breast tissue samples obtained by breast core biopsy (20).

Recently, it has been suggested that certain single nucleotide polymorphisms (SNP) might play a role in drug metabolism that could account for side effects and benefits from therapy with a given agent. The aromatase genotype was evaluated in several studies and correlated with response to aromatase inhibitors (21). Therefore, we aimed to evaluate whether a certain genotype would be associated with a favorable modulation of biomarkers studied. Our results indicate a trend toward a favorable change in IGFBP-3 by genotype. To the best of our knowledge this is the first observation of this interaction and this finding needs to be validated in subsequent larger cohorts. The study by Prowell and colleagues also evaluated the interaction of genotype and biomarkers changes induced by anastrozole for 6 months in a very similar cohort (19). Biomarkers were very similar that included serum markers such as estradiol and tissue makers such as mammographic density. No statistically significant changes were seen in biomarkers by genotype in either study.

The differing results in biomarker changes (or no changes) among these studies might be due to differences in patient populations. In some studies, the cohort consisted of healthy individuals (14), in some, of women on hormonal replacement therapy (HRT; ref. 15) and in some, as in ours and the study by Prowell and colleagues (19), of patients with a history of previous breast cancer of which some had received adjuvant chemotherapy that might have affected baseline biomarkers and changes in these biomarkers. Furthermore, it needs to be acknowledged that many of these studies have a small samples size. However, we all acknowledge the fact that prospective accrual to these types of prevention trials that require paired breast tissue sampling is challenging. And finally, it is also possible that methodologies used for biomarker assays were different in these studies, and some of the assays are not standardized. As many of these studies, our study was also a pilot study designed to provide information and estimation for future placebo controlled trials.

<table>
<thead>
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<th>Table 4. Changes in serum biomarkers</th>
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<tr>
<td>Biomarker (n)</td>
</tr>
<tr>
<td>IGF-1 ng/mL (22)</td>
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<tr>
<td>IGFBP-1 ng/mL (29)</td>
</tr>
<tr>
<td>IGFBP-3 ng/mL (22)</td>
</tr>
<tr>
<td>Estradiol Pg/mL (24)</td>
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aStatistically significant.
Anastrozole and Biomarker Modulation Breast Cancer Prevention Study

Table 5. Biomarker changes by aromatase genotype

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<th>Baseline</th>
<th>Change at 6 months</th>
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<tbody>
<tr>
<td></td>
<td>AA</td>
<td>AC</td>
</tr>
<tr>
<td>IGF-1 ng/mL, median</td>
<td>256</td>
<td>173</td>
</tr>
<tr>
<td>IGFBP-1 ng/mL, median</td>
<td>9.17</td>
<td>8.95</td>
</tr>
<tr>
<td>IGFBP-3 ng/mL, median</td>
<td>4,114.87</td>
<td>4,869.22</td>
</tr>
<tr>
<td>Estradiol Pg/mL, median</td>
<td>7.79</td>
<td>7.97</td>
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Previous studies of tamoxifen have shown that acceptance to take chemopreventive agents is as low as 20% to 30%, mainly due to the side effects. Although not the same as tamoxifen, AIs also have their unique side effects that sometimes even for a breast cancer patient, who is taking it with curative intent, is not acceptable and drug discontinuation in these patients can be equally high. One might assume that the acceptance rate of AIs in high-risk women who do not have cancer might be even lower. Therefore, it would be very important to be able to select those individuals who would benefit most and have a favorable benefit/side effect ratio with a given preventive agent.

In conclusion, AIs are currently being studied in large prospective prevention studies in high-risk women. Given the motivation of many high-risk women to reduce their breast cancer risk while maintaining their quality of life, it is important to select those individuals who would benefit most and have the least side effects. IGFBP-1 is a candidate surrogate marker for AI (and perhaps other) breast cancer risk reductive therapies and should be, along with other potential markers, validated in other studies and then incorporated as a marker in larger prospective breast cancer prevention trials. Furthermore, larger studies are needed to study the association between genotype and benefit of a preventive agent.

Disclosure of Potential Conflicts of Interest

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