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Clinical Protocol UCI 03-16: Phase II Chemoprevention Trial - Anastrozole in the DCIS and early invasive breast cancer in postmenopausal women

1.0 OBJECTIVE

1.1 To assess the efficacy in terms of reduction in Ki-67(as a surrogate endpoint to breast cancer risk reduction) in patients treated with anastrozole.

1.2 To measure the histopathological response and correlate the degree of histopathological response with the degree of Ki-67 response.

1.3 To compare pretreatment vascular density with post treatment vascular density using MRI

1.4 To compare pretreatment markers of angiogenesis with post treatment marker of angiogenesis.

1.5 To correlate MR imaging response to markers of angiogenesis response.

1.6 To correlate ER, Ki-67, angiogenesis markers and MRI response to histopathological changes

2.0 BACKGROUND

A. SPECIFIC AIMS

Breast cancer is a genetically heterogeneous disease. Hormone receptor expression occurs in the majority of postmenopausal breast cancer and defines the subset that can be treated and prevented with hormone modulators. Hormone modulators include antiestrogen like tamoxifen and aromatase inhibitor that block the formation of estrogens in the adrenal glands and peripheral tissue. Hormone modulators have been established in treatment of invasive breast cancer. These agents not only been established as treatment for breast cancer but also have demonstrated efficacy as chemopreventive agents. Tamoxifen has been established as a chemopreventive agent in reducing the development of breast cancer in patients who are at high risk of developing breast cancer. Intriguing results have been obtained in the reduction of breast cancer event rates in established breast cancer patients treated with anastrozole as compared to patients treated with tamoxifen, providing the first glimpse of its efficacy as a chemopreventive agent. Similarly, comparable results were obtained in the study of use of letrozole that is another aromatase inhibitor. It reduced the incidence of contralateral breast cancer in patients with established breast cancer given letrozole as opposed to placebo for 2-3 years post completion of 5 years of tamoxifen treatment as adjuvant treatment. However, costly, long trials are needed to establish the use of aromatase inhibitors as chemopreventive agents if breast cancer events are used as the endpoint. Such trials include NSABP 35 that is comparing anastrozole versus tamoxifen in treatment of DCIS and IBIS -2 that is comparing tamoxifen, anastrozole versus placebo in

patients at high risk of developing breast cancer. A more efficient method is to use intermediate markers of response in testing multiple agents as chemopreventive agents in short pilot studies. The surrogate markers widely accepted include mammographic density and reversibility of preinvasive lesion.

Specific Aim 1: We hypothesize that a proliferative marker Ki-67 is reduced in patients with preinvasive (DCIS) and very early breast cancer treated with anastrozole. To establish reduction in Ki-67 as a primary surrogate endpoint to breast cancer risk reduction in patients treated with anastrozole we will measure Ki-67 before and after treatment with anastrozole. Consistent with this, it has been demonstrated by Geisler et al that patients with invasive breast cancer show a decrease in Ki-67 on lumpectomy/mastectomy samples when anastrozole is administered for few weeks prior to definitive surgery. In addition, there is a trend for a more profound suppression in those achieving an objective response. Ki-67 will be measured by routine immunohistochemistry.

Specific Aim 2: We hypothesize that histopathological tumor response will be demonstrated in 30-40 percent of patients with preinvasive (DCIS) and early invasive (less than 2 cm) breast cancer treated with anastrozole. The percent ability to reverse early breast cancer lesions in patients treated with anastrozole will be qualified as a secondary surrogate endpoint to breast cancer risk reduction. Consistent with this, it has been demonstrated that 30-40 percent of patients with invasive breast cancer show an infiltration of foamy macrophages and fibrosis on lumpectomy/mastectomy samples when chemotherapy is administered for few months prior to definitive surgery. Further, there is a trend for a more profound change in those achieving a complete clinical response. Importantly, a complete pathological response in these invasive breast cancer has been shown to correlate with improved disease free survival and overall survival in breast cancer patients. A corollary is that if reversibility of early carcinogenic lesions is reliably demonstrated in our present proposal, it would translate into chemoprevention of breast cancer.

Specific Aim 3: To compare the pretreatment MRI with post treatment MRI (as a secondary surrogate endpoint to breast cancer risk reduction). We hypothesize that tumor response can be measured by contrast washout characteristic in patients with preinvasive and very early breast cancer treated with aromatase inhibitor. Consistent with this, we have previously demonstrated that patients with invasive breast cancer show a reduction in vascularity in response to chemotherapy. Further, there is a trend for a more profound suppression in those achieving a pathological response on lumpectomy/mastectomy specimen.

Specific Aim 4: To compare the pretreatment markers of angiogensis with post treatment markers of angiogenesis (as a secondary surrogate endpoint to breast cancer risk reduction). We hypothesize that tumor response can be measured by reduction in CD31 (microvessel count), CD105 (endoglin) and VEGF in response to hormonal therapy. There may be upregulation of TSP-1, an angiogenesis inhibitor in response to anastrozole. Angiogenic activity has been reported for ligands of the nuclear hormone receptor superfamily such as estrogens. Inhibition of the proangiogenic effects of estrogens could underlie the chemopreventive action of hormone modulators on mammary carcinogenesis. A group of investigators have indeed coined the word angioprevention as a mechanism of chemoprevention that reverses the angiogenic switch from

preinvasive to invasive cancer. Additionally, it has been demonstrated that patients with various cancers whose tumor vascularity is targeted with VEGF inhibitor show higher response than patients who are treated with chemotherapy alone. Our present proposal capitalizes on the data obtained in invasive breast cancer as to the efficacy of antiangiogenesis mechanism as an option in treatment and prevention .

Further, we will correlate MR imaging response to markers of angiogenesis response. Similarly, we will correlate angiogensis marker response and MR response to Ki-67 and histopathological changes of response.

The successful completion of specific aims 1 and 2 will allow us to determine whether there is reduction of Ki-67 in response to 4 weeks of anastrozole and what percentage of patients show reversibility of carcinogenesis process by histopatholgical evaluation of lumpectomy/mastectomy specimen. Specific aim 3 and 4 will define angiogenesis response to hormonal manipulation as reflected by MRI and IHC. In this manner the best surrogate endpoint will be established to be used in future trials of hormonal agents, angiogenesis inhibitor, biological response modifiers etc. This will further allow a fast screening of chemopreventive agents by using the intermediate endpoint of breast cancer risk reduction instead of using the breast cancer risk reduction as the endpoint.

Breast cancer is one of the most common cancers seriously afflicting women in the United States. Of the one million incident cases that are reported annually there are approximately 193,000 new cases of breast cancer (Greenlee, 2001). Although significant advances have been made both in early detection and treatment of breast cancer, the impact of these on reduction in mortality has been modest (Peta, 2000). Furthermore, despite data implicating diet and other environmental risk factors, no lifestyle changes have yet been shown to significantly reduce the risk of breast cancer. Therefore, chemoprevention of breast cancer is a worthwhile approach to reduce the incidence of breast cancer.

There is every reason to believe that a detailed understanding of the initiation, promotion and growth of breast cancer will ultimately provide a rational strategy upon which to base prevention strategies. While the pathways of breast cancer development are not yet fully understood, a role for estrogens in breast cancer etiology has been well established (Hulka, 1995; Kreiger, 1999).

While many pathways are involved in breast cancer etiology, including loss of tumor suppressor function by p53 or BRCA1 and gain of HER2 oncogene expression, their exact role in an individual patient's cancer development may vary (Marquis, 1995; Boggio, 1998).

Therefore, it may be advantageous to focus on a chemoprevention strategy that may have a more uniform impact on breast cancer development, such as estrogen exposure. Estrogen and its metabolites, both in the circulation and locally synthesized in the breast, are important in the pathogenesis of breast cancer. High levels of circulating estrogen in postmenopausal women have been associated with an increased risk of breast cancer (Clemons, 2001). Furthermore, local estrogen synthesis, i.e. aromatase activity, in the breast may also be important in the development of breast cancer (Brodie, 1998).

Chemoprevention Strategies

Lowering estrogen by premenopausal oophorectomy has for a long time been known to reduce breast cancer risk. Attempts to block estrogen's effects on the breast with selective estrogen receptor modulators (SERMS) are ongoing. Based on findings in the adjuvant setting, which suggested a preventive effect of tamoxifen on the development of contra lateral breast cancer (Fisher, 1989), several large chemoprevention trials have been conducted. Both tamoxifen and raloxifene have now been shown to reduce breast cancer risk (Fisher, 1998). The randomized Study of Tamoxifen and Raloxifene (STAR) study, designed to compare raloxifene to tamoxifen in over 20,000 postmenopausal women at increased risk of breast cancer, as well as ongoing and proposed placebo-controlled studies of tamoxifen, the aromatase inhibitor anastrozole, and other antiestrogens in high- or average-risk postmenopausal women, should provide further data on optimal prevention strategies (Pritchard, 2001).

Aromatase inhibitors that antagonize estrogen by blocking its synthesis from androgens offer an alternative way of preventing the effects of estrogen and its metabolites on the breast. Aromatase is found in breast tissue in addition to the peripheral tissues, and importance of intratumoral aromatase and local estrogen production has been pointed out by several investigators (Miller, 1987: Dowsett, 1993). Prevention of tumor formation in carcinogen induced and spontaneous breast tumor animal bearing models has been demonstrated previously (Gunson, 1995; Schieweck, 1993). However, the first proof of clinical chemoprevention with aromatase inhibitors was obtained in the adjuvant study (ATAC) where adminstration of anastrozole (an aromatase inhibitor) was associated with a striking reduction in primary contralateral breast cancer as compared to patients given tamoxifen (odds ratio 0.42 [.22-.79], p=0.007). The inhibitors have also been shown to be of superior efficacy to tamoxifen in a full spectrum of clinical settings in breast cancer patients and to have a more favorable toxicity profile.

However, chemoprevention by non-steroidal anti-aromatase Anastrozole in DCIS and early invasive breast cancer prior to surgery has not been investigated previously. This offers a window of opportunity and is the rationale of our proposed study.

Surrogate Endpoints of chemoprevention

Large cohorts and a long time of follow-up are needed to obtain significant results in a breast cancer chemoprevention trial, if breast cancer incidence is used as an endpoint. The strategy of using surrogate markers of breast cancer occurrence as intermediate endpoints should permit short and simple pilot trials to evaluate a large number of potential chemopreventive agents and trial designs (Kelloff, 2000). The surrogate markers already in use in chemoprevention pilots include histo-pathologic changes of breast tissue and a higher than average density on a screening mammogram (Atkinson, 1999). Ultimately, long and costly phase III trials with breast cancer occurrence as an endpoint will probably be required unless truly validated surrogate markers can be identified (Lippman, 1990). An alternative strategy for development of chemopreventive compounds would be to seek approval for a drug for the treatment of a preinvasive lesion of the breast, if reversibility of such a lesion could reliably be shown in a clinical trial.

The plasma levels of free estradiol are very low in postmenopausal women. However, concentrations of estrogens within breast tissue have been reported to be higher than in plasma

and similar to plasma concentrations in premenopausal women. One mechanism by which this may occur is for breast cells to synthesize estrogens themselves and produce high concentrations locally. An investigation by Brodie et al. suggests that estrogen produced locally is important in enhancing proliferation of the tumor (Brodie, 1998). In this study, they used immunocytochemical method using a monoclonal antibody to determine the expression of aromatase (Brodie, 1998). The method was applied to sections of tumors embedded in paraffin blocks as routinely prepared for pathology. Both immunocytochemistry and in situ hybridization identified aromatase enzyme and mRNA expression in the epithelial cells of the terminal ductal lobula units (TDLU) and surrounding stromal cells of the normal human breast, and in the tumor epithelial cells and stromal cells of breast cancers. Thus, tumor aromatase may be a significant source of estrogen which stimulates tumor growth (Brueggemeier, 2001). The finding that significantly higher levels aromatase expression levels were found in DCIS than in infiltrating ductal carcinoma indicates that it may be possible to treat DCIS patients with aromatase inhibitors (Zhang 2002).

We propose to undertake a Phase II a chemoprevention clinical trial in women with ductal carcinoma in situ (DCIS) and early breast cancer using aromatase inhibitors. In order to assess efficacy of the chemoprevention of such drugs, we propose to employ reduction in tumor Ki-67 as the primary surrogate marker with dynamic contrast enhanced MRI as a secondary marker. Other immunohistochemical markers such as p53, thrombospondin-1, CD31, CD105, and VEGF will also be used as exploratory markers for changes in angiogenesis in response to therapy. Histopathological reversal of preinvasive and early invasive breast cancer will also be evaluated.

Angiogenesis plays a fundamental role in tumor growth and metastasis. Investigations by Teruyama et al. and Mastyugin et al. have established a relationship between VEGF expression and p450 aromatase activity (Teruyama, 2001; Mastyugin, 2002). We have previously shown that dynamic contrast enhanced MRI in conjunction with compartmental modeling may be used to assess the angiogenic status of tumors while enabling the study of both breasts (Su, 2002). Additionally, image based measurement techniques do not suffer from the errors due to tissue heterogeneity and use of limited spatial sampling imposed in methods such as histology. Furthermore, MRI based parameters that describe breast physiology have been found to correlate well with immunohistochemically measured angiogenesis markers (Su, 1995; Su, 1994; Su, 1996). *Thus, dynamic contrast enhanced MRI will be used as a secondary surrogate marker in the proposed study.*

Methodology to test the hypothesis: Core biopsies will be obtained from patients whose mammographic findings are suspicious for DCIS. Patient will undergo MRI study prior the biopsy. At the time of biopsy, a portion of the tissue retrieved will be sent in formalin for histopathological examination for Ki-67, ER, PR and angiogenesis markers evaluation.

1 mg of anastrozole administered orally daily for 4 weeks after the diagnosis of DCIS/invasive breast cancer. The reason for using a 4-week regimen is that this is the average time between diagnostic biopsy and definitive lumpectomy or mastectomy.

Patients will undergo MRI at baseline before therapy and then again after anastrozole treatment that will be followed immediately by definitive surgical procedure, i.e. lumpectomy or mastectomy. Lumpectomy/mastectomy specimen will be again evaluated for the level of tumor

Ki-67 decline and the level of angiogenesis markers. The specimen will be subjected to the routine histopathological, and ER and PR evaluation. Histopathological changes pre and post anastrozole will be evaluated in an exploratory analysis.

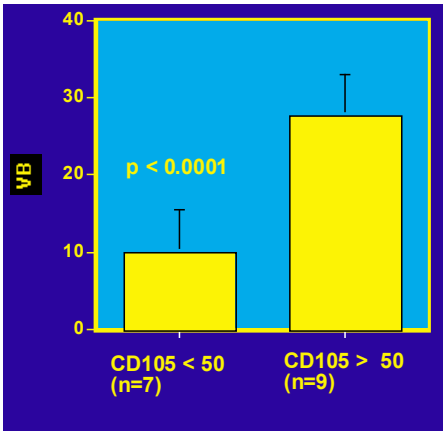
1. **Quantification of Ki-67 as a Primary Marker to Monitor Response to Chemoprevention by Aromatase Inhibitor Anastrozole**

Measurement of Ki-67 Levels.
Measurement of **Ki67** will be performed with the MIB1 mouse monoclonal antibody. To ensure acceptable precision, quantitation of Ki67 will involve the counting of 1000 cells.

Other Tissue Biomarkers.
ER expression will be demonstrated with the DAKO 1D5 mouse monoclonal and PgR with the Novocastra antibody NCL-PgR clone 1A6 For all other analytes, 10 high-powered fields (chosen randomly) will be assessed. ER and PgRwill be reported as percentage of positively stained cells.

2. **Dynamic Contrast Enhanced MRI as a Secondary Marker to Quantify Chemoprevention by Anastrozole**

We have investigated the relationship between endoglin (CD105), a marker selectively associated with neo-vascular endothelium, and vascular volume density in early breast cancer. The patients had a preoperative contrast enhanced dynamic MRI and the tissue blocks obtained at surgery were immunohistochemically (IHC) stained for CD105. The dynamic contrast enhanced MRI images were then analyzed by using the compartmental modeling technique as described by us previously to quantify vascular density VB (Su, 1995; Su, 1994; Su, 1998; Su, 1999). The result shown in the next figure summarizes our finding that shows a strong correlation between vascular density (VB) and CD105 score suggesting that the MRI technique to be used in the current investigation provides a measure of tumor angiogenesis in breast cancer.



In another study, Gilles et al. preformed preoperative contrast material-enhanced subtraction

dynamic MR imaging in thirty-six women with DCIS. Concomitant early contrast enhancement in the breast parenchyma with normal vessels was considered a positive finding. The size and shape of early enhancement were correlated with the size and density packing of ducts involved by DCIS. Their results indicate that early contrast enhancement was demonstrated in 34 patients with DCIS but not in two patients with comedo-type DCIS. Tumor angiogenesis was demonstrated in the stroma. The size and morphology of contrast-enhanced lesions significantly correlated with the size (P = .0085) and density packing of ducts involved by DCIS (P = .012). They conclude that enhancement on dynamic MR images of DCIS may be due to the presence of tumor angiogenesis in the stroma (Gilles, 1995).

MRI study that is described below will be repeated twice, once before the therapy at baseline and once after the therapy.

2.a Imaging Technique:

An IV line will be set up by placing an indwelling cannula in an antecubital vein before patient is placed into the scanner. The patient will then be placed in the prone position with both breasts hanging in a double breast coil. The following image sequences will be performed.

First a 3D slab covering the entire area of interest will be selected for shimming. The study protocol consists of the following pulse sequences:

T1-weighted spin echo pulse sequence. TR= 928 ms, TE=14 ms, matrix size= 256x256, 32 slice with 4 mm thickness, no gap. The scan time is 3 min 07 s.

Fat Sat T2-weighted pulse sequence. TR= 5930 ms, TE=90 ms, matrix size= 256x252, 32 slice with 4 mm thickness, no gap. The scan time is 3 min 39 s.

3D Turbo Flash pulse sequence for sagittal dynamic imaging. TR= 10 ms, TE=3.0 ms, flip angle = 20-degrees, 24 slices with 5 mm thickness. The scan time is 31.8 s per acquisition. Repeat the sequence 16 times for dynamic acquisition, 4 pre-contrast, and 12 post-contrast. The contrast agent (Magnevist®, 0.1 mmol/kg) will be calculated based on patient's body weight. The injection starts after 4 pre-contrast acquisitions have been completed, and the total injection time will be fixed at 15 sec, i.e. all contrast agents will be administered at constant rate and finish in exactly 15 sec.

For ease of comparison of the images acquired using different pulse sequences, the number of slices, thickness, and location in all 3 pulse sequences are kept the same.

2.b Image Analysis:

Based on the Gd-DTPA enhanced images at 3 minutes after the injection, the tumor regions will be manually outlined slice by slice. Then the enhancement time course of Gd-DTPA from the tumor ROI in each imaging slice will be obtained. For each tumor a mean enhancement time course will be calculated. Then the data will be analyzed following the procedure described below to generate the pixel population distribution curves of each vascular volume parameter (VB). The enhancement time course of Gd-DTPA will be analyzed with the pharmacokinetic model to derive the parameters for vascular volume (VB) and permeability (K2) information. In each tumor the analysis will be performed on a pixel-by-pixel basis. After the analyses are completed, the derived parameters from all pixels in an imaging slice will be reconstructed into the parameter maps. From all pixels in each tumor, the pixel population distribution curves of

the two parameters V_b and K_2 obtained from the kinetics of Gd-DTPA will be calculated. For each parameter, the values from all pixels will be sorted in a descending order, then the cut-off values of the top 90% pixels, 80% pixels, 10% pixels will be determined to generate the pixel population distribution curve. In addition to the full spectrum of the population, each data point in the population distribution curve (representing a subgroup of pixels from a tumor) can also be separately used in the correlation analysis. As demonstrated in a previous study, the out-flux transport rate K_2 could be related to the vascular permeability and was correlated well with the aggressive growth of tumors, but the relationship can only be revealed from the top half pixel population where the blood supply was sufficient, not in entire pixel population. Therefore, the unique analysis of pixel population distribution curve allows the analysis in a certain subgroup of interest. With the full population spectrum available, the problems associated with the limit of the pharmacokinetic analysis and the problem of tumor heterogeneity can be handled properly. We will use the 50% pixel population of the parameter in each tumor.

3. **Immunohistochemistry of p53, TSP-1, CD31, CD105, and VEGF as Exploratory Markers of Angiogenesis in neoadjuvant setting.** We reported recently that mutant p53 (mp53) was associated with decreased expression of an endogenous inhibitor of angiogenesis, thrombospondin-1 (TSP-1), and increased microvessel density in breast cancer. Carcinoma-specific levels of p53, TSP-1, and tumor angiogenesis were determined using semiquantitative immunohistochemistry (IHC) methods. Acquisition of mp53 was significantly associated with decreased TSP-1 and increased angiogenesis. An angiogenesis index integrating mp53, TSP-1, and angiogenesis (CD31) scores was found to be an independent predictor of survival in univariate and multivariate analyses that included lymph node status. We have also shown increased angiogenesis is associated with increased VEGF.

   Immunohistochemical detection of p53, TSP-1, and microvessel counts will be performed .Five-µm tissue sections of fixed, paraffin-embedded specimens will be cut, mounted on poly-L-lysine slides (VWR Superfrost Plus), and then deparaffinized in Histoclear. Specimens will be rehydrated by sequential washing in ethanol solutions. Antigen retrieval for CD31 used Pronase digestion for 20 min or microwave boiling in citrate buffer for 15 min for p53. No antigen retrieval was necessary for TSP-1 detection. Slides were then incubated in 3% hydrogen peroxide for 10 min to block endogenous peroxidase activity and rinsed in tap water, followed by distilled water. Each slide was subsequently incubated in 100 µl of goat serum (Protein Block; Biogenex, San Ramon, CA) for 10 min at room temperature. Excess blocking buffer was shaken off; the slide was incubated with primary antibody solution for 30 min at room temperature and then rinsed twice in PBS for 5 min. Tissue sections were then incubated in biotinylated goat antimouse immunoglobulin for 20 min at room temperature in a humidified chamber. After rinsing for 5 min in PBS, each section was exposed to peroxidase-conjugated streptavidin and incubated for 20 min at ambient room temperature, rinsed in PBS, and exposed to diaminobenzidine for 3 min. Slides were then rinsed in PBS for 5 min, exposed to hematoxylin for 1 min, rinsed for 10 min in tap water, dehydrated in ascending ethanol series, cleared in xylene, coverslipped in Permount, and viewed at x40. All IHC procedures were optimized in preliminary experiments. All antibody reagents were commercially available; p53 was detected with antibody DO1 (Santa Cruz

Biotechnology, Santa Cruz, CA); TSP-1 was detected with clone p12 (Immunotech, Marseille, France), and CD31 was detected with clone JC/70A (Dako, Carpinteria, CA). Paraffin-embedded MCF-7 40F and MCF-7 wt breast cancer cells were used as positive controls for p53 and TSP-1 IHC staining, respectively. Titrations were performed for all antibody reagents to insure minimal background staining and optimal antigen detection. Invasive breast carcinoma specimens that stained negative for p53 or TSP-1 were used as negative controls. Entire tissue sections were evaluated for intensity and percentage of tumor cells staining positively. Only the malignant component was scored for each case. The intensity of p53 staining was scored as follows: negative when <5% of tumor cells displayed staining; 1+ when intensity was mild; 2+, moderate; 3+, when intensity was equal to the positive control; and 4+ when intensity was greater than the positive control . The 5% cutoff used to delineate a positive staining result was based on the previously published cutoff values by Grant *et al.* in melanoma, and Poller *et al.* in mammary ductal carcinoma *in situ* and on observations that tumors showing only weak focal p53 protein expression had expression of p53 equivalent to that sometimes seen in normal cutaneous basal cells. HScores were assigned on the basis of multiplying the percentage of cells staining positive by the intensity of staining plus 1 [HScore = % positive x (intensity + 1)]. HScores are an objective measurement of tumor heterogeneity reflecting the variability in percentage and intensity of staining within a tumor and provide a single unit of measure for the amount of marker present in the field examined, as originally described in early work to quantitate estrogen receptor content in breast cancer by IHC . (Mehta, 2001).All procedures will be performed by pathologists who had no previous knowledge of the clinical outcomes for this series of cases.

Angiogenesis controls with intermediate vessel counts will be run in parallel with each series of slides to insure appropriate CD31 staining. An invasive breast carcinoma specimen with high microvascular staining will be used for the positive control, and the nonstaining areas in the same tumor tissue will be used for the negative control. Light microscopy will be used to identify three regions within or immediately adjacent to the tumor that contained the greatest microvessel density ("hotspots"). Microvessel counts were then performed using a x200 field within the designated hotspot. Of the three areas where the highest number of discreet microvessels are stained, the area of greatest counts will be chosen for scoring. Any immunoreactive endothelial cell that is separate from adjacent microvessels is considered a "countable" vessel.

Image Analysis.

On the basis of the intracellular and extracellular localizations of TSP-1, tumor expression levels of TSP-1 were measured by image analysis as described previously to integrate overall tissue expression levels. Briefly, we used a CAS 2000 two-color system (Becton Dickinson, San Jose, CA) that used a light microscope attached to an interactive microcomputer capable of high-speed digital image processing for cell measurements. Image channels were matched to two-component immunohistochemical staining to enhance the image of one stain in each channel. One channel was used to identify all components in the tissue counterstained with methyl green (*i.e.,* all nuclear components), and the other channel was used to calculate the density of brown stain (diaminobenzidine) per tissue area to identify the proportion of cells stained with antibodies to TSP-1. Image analysis-based units of staining are reported as absorbance (*A*). A negative control

accompanying each specimen was used to set the antibody threshold such that nonspecific background staining was eliminated from the study measurement. A minimum of 10 fields with varying intensities were examined for all radical prostatectomy, transurethral prostatectomy, or biopsy specimens.

3.0 DRUG INFORMATION

Anastrozole:

a. DESCRIPTION

Anastrozole is a nonsteroidal aromatase inhibitor. Anastrozole is highly potent and specific for aromatase, and represents the fourth generation of aromatase inhibitors. Unlike aminoglutethimide, an early aromatase inhibitor, anastrozole does not inhibit adrenal steroid synthesis. Patients taking anastrozole, therefore, do not require glucocorticoid or mineralocorticoid replacement therapy. Anastrozole has a significant effect on suppression of serum estradiol levels, and it offers an alternative to tamoxifen in antagonizing estrogen. Anastrozole causes less weight gain than megestrol and may offer a survival advantage over megestrol in women with advanced breast carcinoma. Anastrozole was FDA-approved for the treatment of advanced breast cancer in postmenopausal women whose disease has progressed during tamoxifen therapy in December 1995. In September 2000, the FDA approved anastrozole for the first-line treatment of postmenopausal women with advanced or metastatic breast cancer Mechanism of Action: Anastrozole inhibits aromatase, the enzyme that catalyzes the final step in estrogen production. Anastrozole is an oral, competitive, non-steroidal inhibitor of aromatase and is less likely to exhibit agonist or antagonist steroidal properties. The formation of adrenal corticosteroids or aldosterone is not affected by anastrozole; only serum estradiol concentrations are affected by anastrozole. In postmenopausal women, the principal source of circulating estrogens is from the conversion of adrenal and ovarian androgens (androstenedione and testosterone) to estrogens (estrone and estradiol) by aromatase in peripheral tissues. Inhibition of aromatase may result in a more complete estrogen block than surgical ablation. Extraglandular sites are more amenable to aromatase inhibition by anastrozole than are premenopausal ovaries. Inhibiting the biosynthesis of estrogens is one way to deprive the tumor of estrogens and to restrict tumor growth. Estradiolplasma concentrations decrease about 80% from the baseline with continued dosing of anastrozole. Aromatase inhibitors might also inhibit estrogen production at the tumor cell. However, tumor production of estradiol may be insignificant because aromatase activity appears to be low. Anastrozole has little or no effect on CNS, autonomic, or neuromuscular function

b. TOXICOLOGY

Human Toxicity: Hot flashes (26.5%) are the most commonly reported adverse reaction to anastrozole during first-line therapy for breast cancer. In patients receiving anastrozole for progressive breast cancer, hot flashes occurred in 12.6% of patients. Vaginal irritation (i.e., dryness) and vaginal bleeding have also been reported with anastrozole. Vaginal bleeding occurs primarily during the first few weeks after changing from existing hormonal therapy to treatment with anastrozole. If bleeding persists, further evaluation should be considered. Tumor flare with associated bone pain has been reported during first-line anastrozole therapy for breast cancer.

GI adverse reactions occurred in up to one-third of patients receiving anastrozole. These reactions included, anorexia, constipation, abdominal pain, diarrhea, nausea/vomiting, and xerostomia (dry mouth). Weight gain has been reported in 2% of patients taking anastrozole, but occurs less frequently than with megestrol. Elevated hepatic enzymes, primarily serum gamma glutamyl transferase (GGT), have been observed in patients with liver metastases receiving anastrozole or megestrol. These changes were likely due to the progression of liver disease in these patients, but other contributing factors cannot be ruled out. Nervous system adverse reactions to anastrozole include anxiety, confusion, depression, dizziness, headache, hypertonia, insomnia, and paresthesias. Thromboembolism has been reported in patients treated with anastrozole (3.5%). The incidence of thrombosis is less than that reported with tamoxifen (6.5%) or megestrol (4.7%) during clinical trials. Specific cases included angina, cerebrovascular accident, cerebral ischemia, cerebral infarct, myocardial infarction, myocardial ischemia, pulmonary embolism, retinal vein thrombosis, and thrombophlebitis.

Other reported adverse events to anastrozole regardless of causality are arthralgia, asthenia, back pain, chest pain (unspecified), diaphoresis, dyspnea, fever, flu-syndrome, hypertension, increased cough, lethargy, pain (unspecified), myalgia, pelvic pain, peripheral edema, pharyngitis, pruritus, rash (unspecified), and vasodilation. Infections including bronchitis, rhinitis, sinusitis, and urinary tract infections have been reported in 2—5% of patients receiving anastrozole.

Drug Interactions: Anastrozole should not be given concurrently with any estrogens or estrogen-containing products, including oral contraceptives and some OTC hormonal preparations (e.g., prasterone or androsteindione, dehydroepiandrosterone, DHEA), as these could interfere with the pharmacologic action of anastrozole

c. PHARMACOLOGY

Kinetics: Anastrozole is well absorbed and distributed throughout the systemic circulation (85% bioavailability). Maximum plasma concentrations occur within 2 hours. Pharmacokinetics are linear, even with repeated dosing. Plasma concentrations approach steady-state levels by about the seventh day of once-daily dosing. Hepatic metabolism accounts for approximately 85% of elimination of anastrozole. Within 72 hours, about 60% of a dose is excreted in the urine as metabolites and only 10% as unchanged drug. Three metabolites have been identified in plasma and urine, and there are several unidentified minor metabolites. No pharmacological activity has been attributed to triazole, the main circulating metabolite. The other known metabolites are a glucuronide conjugate of hydroxy-anastrozole and a glucuronide conjugate of anastrozole. Although hepatic cirrhosis reduces apparent oral clearance of anastrozole, no dosage adjustments are needed because plasma concentrations remain within the same range as for patients without hepatic disease. Anastrozole has a terminal elimination half-life of about 50 hours. Renal clearance of anastrozole does decrease proportionally with creatinine clearance, but overall this has very little effect on total body clearance. No dosage adjustments are therefore necessary for patients with impaired renal function.

Formulation: Anastrozole is supplied in 1mg tablet

Administration of anatrozole: 1mg po once a day

Storage and stability: Store at controlled room temperature (59˚F to 86˚F [15˚C to 30˚C])

Supplier: Anastrozole is commercially available for purchase by the third party

Mechanism of Drug Destruction: Documentation indicating study drug was Destroyed will be sent to AstraZeneca.

4.0 STAGING CRITERIA

The staging criteria for this study will be the FIGO Classification, 2003 Clinical Staging – Carcinoma of the breast. In order to be eligible for this study, patients must have been diagnosed with DCIS or invasive breast cancer on core biopsy.

5.0 ELIGIBILITY CRITERIA

Each of the criteria in the following section must be met in order for a patient to be considered eligible for registration.

5.1 Patients must have suspicion of DCIS or early invasive breast cancer on mammography.

5.2 Patients must have histologically confirmed diagnosis of DCIS or early invasive breast cancer on core biopsy for final registration.

5.4 Patients must be over 18 years of age

5.5 Patients must be postmenopausal as defined by one of the following criteria:

a. Prior bilateral oophorectomy OR

b. > 12 months since LMP with no prior hysterectomy OR

c. a & b not applicable AND age >=50

5.6 Patients must be positive for either ER or PR or both

5.7 Patients must be informed of the investigational nature of this study and must sign and give written informed consent in accordance with institutional and federal guidelines.

6. STRATIFICATION FACTORS: No stratification is planned

7.0 TREATMENT PLAN

7.1. Patients entered into this trial will be treated with the following regiment.

a. Anastrozole 1 mg by mouth for 2 - 4 weeks. The study doctor or a member of the study team will document the exact start time of treatment.

7.2 Good Medical Practice

The following pre-study tests should be obtained within 28 days prior to beginning treatment in accordance with good medical practice. Results of these tests do not determine eligibility and minor deviations would be acceptable if they do not impact on patient safety in the clinical judgement of the treating physician. The Study Coordinator must be contacted if there are significant deviations in the values of these tests.

WBC $\geq$ 2,000/µl, ANC >1,000/mm^3 and platelets > 100,000/ mm^3.

No serious illness or condition that would compromise ability to comply with protocol treatment and follow up.

7.4 Criteria for removal from protocol treatment

a. Progression of disease.

b. Unacceptable toxicity

c. Delay in treatment due to toxicity > 14 days beyond scheduled treatment time.

d. The patient may withdraw from the study at any time for any reason.

e. Completion of treatment.

7.3. All reasons for discontinuation of treatment must be documented in the Flow sheet.

7.5 All patients will be followed until death or a maximum of 7 years.

8.0. TOXICITY TO BE MONITORED AND DOSAGE MODIFICATION

8.1 Acute morbidity potentially associated with hormonal therapy will be monitored and recorded on the Therapy and Toxicity Summary Form (Form #xxxxx) for all patients during therapy. Any chronic toxicities will be recorded on the Follow-Up Form (Form #xxxxx) at each follow-up visit. This will include hot flushes (as defined in Section 10.5), vaginitis , vaginal bleeding, tumor flare, nausea, vomiting, constipation, diarrhea, abdominal pain, xerostomia. All toxicities will be assessed based on the NCI Common Toxicity Criteria. Others include wt gain, anxiety, confusion, dizziness, insomnia, paresthesia, thromboembolism, fatigue,UTI, artralgia and injection site reaction.

Hot Flashes: Avoid hormone treatment for hot flushes. Use hormone alternatives, example; clonidine, Beralgan, prozac or effexor while continuing with treatmentprotocol treatment.

8.2 Unexpected or fatal toxicities (including suspected reactions) must be reported to the to the Study Coordinator

9.0 STUDY PARAMETERS

Test/Observation	Prior to Study	Post Anastrozole	Post Lumpectomy
History	X		
Physical Examination	X		
Mammographic findings (suspicious for DCIS or early invasive breast cancer)	X		
MRI findings	X	X	
Ki-67	X (on core biopsy)		X
Confirm DCIS or early invasive breast cancer on pathology	X (on core biopsy)		Final pathological diagnosis
Assessment of Tumor Grade and Histologic Type	X(on core biopsy)		Final pathological diagnosis
ER/PR status	X(on core biopsy)		Confirm ER/PR status
IHC scores of markers of angiogenesis	X(on core biopsy)		IHC scores of markers of angiogenesis

10.0 CRITERIA FOR EVALUATION AND ENDPOINT DEFINITIONS

10.1 Absolute percent Ki-67 change pre anastrozole and post anastrozole as well as percentage of patients achieving less Ki-67 less than 10%

10.2 Histopathological response.

A complete response (CR) indicating the disappearance of the primary tumour; a partial response (PR) indicating a reduction of $\geqslant$50%; stable disease (SD) indicating a reduction of <50% or increase in size of <25%; progressive disease (PD) indicating an increase in size of $\geqslant$25%.

The pathological CR will be evaluated by tumour excision and axillary node resection (in case of invasive breast cancer only). Two grading classifications will be utilised to evaluate the tumour response after chemotherapy. In the Chevallier classification, the absence of invasive tumour cells, or persistence of *in situ* disease, and negative axillary lymph nodes define a pathological CR. In the Sataloff classification, we have similar criteria for a pathological CR, but also observe the persistence of small clusters of widely dispersed cells in these cases.

We will correlate patients with degree of drop of Ki-67 with degree of pathological response

10.3 MRI is obtained at the base line and 4 weeks after anastrozole.

MRI characteristics pre and post anastrozole

10.4 IHC determination of markers of angiogenesis and ER, PR and pre anastrozole and post anastrozole expressed as IHC scores and percentage.

11.0 STATISTICAL CONSIDERATIONS

Statistical Power: In this non-randomized trial, the primary surrogate marker will be Ki-67. Individuals who exhibit at least 40% reduction in Ki-67 from baseline will be categorized as responders. For p= probability of response, the null hypothesis is specified as p_0, and the alternative as p_1. Our null hypothesis is that after 4 weeks of therapy, no more than 5% of the subjects will exhibit at least 40% decrease in Ki-67 levels. Table 1 gives the number of subjects necessary to detect differences of 25% to 45% from the null proportion of 5%, using the binomial test with a significance level of 0.05. Thus, for 90% power, 24 evaluable subjects are necessary to detect a difference of 25% from the null. Because of the necessity of working with patients with a small tumor size all patients accrued may not be evaluable. We anticipate a non-evaluability rate of 50% as quoted by experienced investigators. Hence, we will accrue 42 patients on this trial.

In this non-randomized trial, the primary surrogate marker will be Ki-67 level.

Table 1. Number of subjects, $p_0 = 0.05$

p_1	80% Power	90% Power
0.30	18	24
0.35	12	18
0.40	10	12
0.45	9	10
0.50	5	9

All of the quantitative results generated by IHC (percentage of eg. Ki67) will be given as their arithmetric mean levels. The comparison of changes in proliferation markers and receptor levels between responders and nonresponders will be performed using the Mann-Whitney *U* test.

The Primary outcome is response to treatment. For each participant, the post-treatment level of Ki-67 will be compared to the pre-treatment level ok ki-67. If at least a 40%

decrease from the pretreatment level achieved, the participant will be classified as a responder. The proportion responding and the 95% confidence interal for this proportion will be calculated. The 2-sided binomial test with $\alpha = 0.05$ will be preformed to test $p_0 = 0.05$ vs. $p_0 \neq 0.05$.

The secondary aims of the study are to evaluate change from baseline in the MRI parameters, vascular volume (V_b) and K_2. The distribution in changes from baseline in tumor estrogen, V_b, and K_2 will be examined using probability plots. The Kolmogorov-Smirnov goodness-of-fit test for normality will be performed. If indicated, normalizing transformations will be applied. Ninety-five percent confidence intervals for mean change from baseline and mean percent change from baseline will be computed.

Immunohistochemistry of p53, TSP-1, CD31, CD105, and VEGF will be analyzed as markers of angiogenesis. We expect to observe a greater anti-angiogenic effect in the tumor with the higher angiogenic activity (i.e. with a higher CD31 and CD106 vessel density or a higher VEGF before treatment). According to pre-treatment CD31, CD105, or VEGF, the tumors will be categorized into 3 groups with high, medium, and low angiogenic activities. Normalizing transformations for each variable will be considered. We will evaluate these parameters using analysis of variance to test for reduction from baseline, followed by the sequentially rejective Bonferroni procedure for multiple comparisons to maintain an overall significance level of 0.05. Changes in p53 and TSP-1 will evaluated similarly.

Data Safety and Monitoring Committee (DSMB)

This study will be monitored throughout accrual and follow-up periods by the UCI Data Safety and Monitoring Board (DSMB). There is a single DSMB to monitor all UCI therapeutic trials. This committee is responsible for reviewing interim analyses prepared by the study statistician and for recommending whether the study needs to be modified or terminated based on these analyses. This committee also determines when the study results will be submitted for publication or otherwise released to the public. It will review any major modifications to the study proposed by the Study Committee. The Study Committee is responsible for monitoring the data from the study for toxicity, feasibility, and accrual. The study committee also initiates minor changes in the study such as clarification of eligibility criteria.

12.0 DISCIPLINE REVIEW

There will be no discipline review for this study.

13.0 REGISTRATION GUIDELINES

13.1 Patients must be registered prior to initiation of treatment
13.2 For either method of registration, the individual registering the patient must have completed the appropriate Registration Form.

Patients will not be registered if the IRB approval date expired.

14.0 ETHICAL AND REGULATORY CONSIDERATIONS

The following will be observed to comply with Food and Drug Administration regulations for the conduct and monitoring of clinical investigations; they also represent sound research practice:

Informed Consent
The principles of informed consent are described by Federal Regulatory Guidelines (Federal Register Vol. 46, No. 17, January 27, 1981, part 50) and the Office for Protection from Research Risks Reports: Protection of Human Subjects (Code of Federal Regulations 45 CFR 46). They must be followed to comply with FDA regulations for the conduct and monitoring of clinical investigations.

Institutional Review
This study must be approved by an appropriate institutional review committee as defined by Federal Regulatory Guidelines (Ref. Federal Register Vol. 46, No. 17, January 27, 1981, part 56) and the Office for Protection from Research Risks Reports: Protection of Human Subjects (Code of Federal Regulations 45 CFR 46).

14.1 Ethics review

The investigator must submit written approval to AstraZeneca before he or she can enroll any subject into the study.

If any modifications are made to the protocol or informed consent documents, the new versions will be submitted to AstraZeneca for approval.

14.2 Audits and inspections

Authorized representative of AstraZeneca, a regulatory authority, and Independent Ethics Committee (IEC) or an Institutional Review Board (IRB) may visit the centre to perform audits or inspections, including source data verification. The purpose of an AstraZeneca audit or inspection is to systematically and independently examine all study-related activities and documents to determine whether these activities were conducted, and data were recorded, analyzed and accurately reported according to the protocol, Good Clinical Practice (GCP), guidelines of the International Conference on Harmonization (ICH), and any applicable regulatory requirements. The investigator should contact AstraZeneca immediately if contacted by a regulatory agency about an inspection at his or her centre.

14.3 Subject data protection

In accordance with the Health Information Portability and Accountability Act (HIPAA), the Written Informed Consent Form must include language that informs the subject that authorized representatives at AstraZeneca will have access to subject's medical information that includes all hospital records relevant to the study, including subject's medical history for research purposes.

15.0 ADVERSE EVENT REPORTING

15.1 An **adverse event** is defined as any untoward medical occurrence that occurs from the first dose of study medication until 30 days after the final dose, regardless of whether it is considered related to a medication.

In addition, any known untoward event that occurs subsequent to the adverse event reporting period that the investigator assesses as possibly related to the investigational medication should also be considered an adverse event.

15.2 A **preexisting condition** should **not** be reported as an adverse event unless the condition worsens or episodes increase in frequency during the adverse event reporting period.

15.3 Symptoms of the targeted cancer should not be reported as adverse events.

15.4 A **serious** adverse event is one that is fatal or life-threatening (i.e., results in an immediate risk of death), is permanently or substantially disabling, requires or prolongs hospitalization (only if related to an unexpected complication), or is a congenital anomaly, new cancer or medication overdose. This category also includes any other event the investigator judges to be serious or which would suggest a significant hazard, contraindication, side effect or precaution.

15.5 An **unexpected** event is one that is **not** listed as a known toxicity of the investigational drug in the protocol, the consent form, the package insert, or the investigator brochure.

15.6 **Serious, unexpected, drug-related** adverse events must be reported to the FDA and to IRB within 10 working days (report deaths within 3 days to the FDA). Please report these events to the FDA on the **MEDWATCH form** and ensure "causality" is assigned.

A cover page should accompany the Medwatch form indicating the following:

- Arimidex Investigator Sponsored Study (ISS)
- The investigator IND number assigned by the FDA
- The investigator's name and address
- The trial name and AstraZeneca Reference number

Send by way of fax to: (302) 886-4620, Attention Arimidex ISS Safety Representative

If a non-serious AE becomes serious, this and other relevant follow-up information must also be provided to AstraZeneca and the FDA.

10.6.1 Address for submitting **MEDWATCH forms** to the **FDA**:

MEDWATCH
5600 Fishers Lane
Rockville, MD 20852-9787
FAX #: 1-800-FDA-0178

MASTER FORMS SET

This section includes copies of all data forms which must be completed for this study. These include:

a. Registration Form (Form #1)

b. **DCIS** and Early Invasive Breast Cancer Prestudy Form (Form #2)

c. Baseline Tumor Assessment Form (Form #3)

h. Treatment and Toxicity Form

i. Follow-Up Tumor Assessment Form (Form #4)

l. Follow-Up Form (Form #5)

m. Off Treatment Notice (Form #6)

n. Notice of Death (Form #7)

o. Specimen submission form

For IRB use only, not to be included in patient information.

UNIVERSITY OF CALIFORNIA
CONSENT TO ACT AS A HUMAN RESEARCH SUBJECT

TITLE: **UCI 03-16:** Phase II Chemoprevention Trial – Anastrozole in the DCIS and Early Invasive Breast Cancer in Postmenopausal Women

INVESTIGATORS: Rita Mehta, MD
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Division of Surgery/Oncology (714) 456-8030
Orhan Nalcioglu, MD, Professor
Min-Ying Su, Assistant Professor
Radiological Sciences,
Research Imaging Center (949) 824-6001

NAME OF SUBJECT:

PURPOSE OF STUDY: I have been invited to participate in this study because I have breast cancer that is limited to the breast ducts or showing early evidence of spread beyond the breast duct. The purpose of this study is to find out what effects (good or bad) the hormonal blocking agent anastrozole has on me or my breast cancer. As part of this study, the study team would also like to do laboratory testing on tissue samples to find out as much as possible about breast cancer and how this treatment might affect the disease. They would also like to use the tissue to find out about other cancers or other diseases. At the end of this form, I will be given the option to allow some excess tissue from my tumor to be kept and used for research purposes.

This study will include approximately 24 subjects being enrolled at the University of California, Irvine.

PROCEDURES:
I will receive 1 mg. of anastrozole by mouth every day for 2 - 4 weeks. I will also have an MRI performed prior to and after treatment with anastrozole. I will still be seen by my doctor at regular intervals for checkups.

If I take part in this study, I will have the following tests and procedures:

Procedures that are part of regular cancer care and may be done even if I do not join the study:

- Physical Exam
- Blood Counts (approximately 3 teaspoons per draw)
- Scans (MRI)

I will undergo preoperative history and physical (H&P) and routine labs approximately within 2-8 weeks of surgery.
There will be no additional MRI apart from the research related pre and post anastrozole scan.
Standard procedures being done because I am in this study:

- Monitoring for side effects
- MR imaging pre and post anastrozole treatment

Procedures that are being tested in this study:

- Anastrozole as treatment of in situ and early invasive breast cancer and possible prevention of progression into invasive cancer

I will be in this study until my disease gets worse or the side effects become too severe. My disease status will be followed for up to 7 years.

I understand that the investigator can remove me from the study without my consent for, but not limited to, the following reasons: if it is judged to be in my best interest in an effort to improve my medical care; if I am unable to follow the instructions given to me and/or adhere to the study schedule; if I experience an injury or illness, or if the study is stopped. I may withdraw from this study at any time for any reason. My decision to withdraw will not affect my relationship with or medical care at UCIMC. My doctor would like to continue to follow me when I have withdrawn from the study without collecting data. My doctor will schedule these follow-up visits. My doctor will also discuss any medications that I may still have whether for treatment or side effects and will advise me if they should be continued in the case of side effects medications or returned in the case of treatment medications.

RISKS:
While on the study, I am at risk for the side effects described below. My doctor will discuss these risks with me prior to starting the study. There also may be other side effects that the study team cannot predict and are unforeseeable. Care will be taken during the anastrozole therapy to make side effects less serious and uncomfortable. Many side effects go away shortly after the hormone therapy is stopped, but in some cases side effects can be serious or long-lasting or permanent.

Very Likely
Fatigue and tiredness
Headache
Pain
Back pain
Abdominal pain

Injection site pain
Pelvic Pain
Chest pain
Flu like symptoms
Fever
Accidental injury
Hot flushes
Nausea
Vomiting
Constipation
Diarrhea
Reduced appetite
Lowered red cells
Fluid retention in legs
Bone pain
Arthritis
Dizziness
Joint pain
Numbness and tingling in hands and feet
Depression
Anxiety
Sore throat
Difficulty breathing
Cough increased
Rash
Sweating
Urinary tract infection

Less Likely, but Serious
Thromboembolic phenomena, muscle pain, dizziness, and lowered white cells.

Vaginal bleeding has been reported infrequently (<1%), If bleeding persists, further evaluation should be considered. I will undergo vaginal ultrasound and biopsy if necessary.
It is possible that I could become claustrophobic (very upset and afraid of being in a small space) during the MRI. Although this does not happen very often, it might be uncomfortable for me. If this happens, I can choose to stop any of the scanning procedures at any time.

No short-term ill effects have been reported for MRI scans to date. Longer-term risks are being studied at this time. The effects of MRI on pregnant women and children under age two are not known. The loud, pounding sound made by the MRI can be uncomfortable. I will be offered the option of wearing earplugs to decrease the noise.

Removal of blood by a needle may produce pain, bruising, bleeding, swelling, dizziness, or rarely fainting or infection.

BENEFITS: The study team cannot and does not guarantee that I will benefit if I take part in this study. The treatment I receive may even be harmful. My doctors feel that my participation in this study will give me at least as good a chance as I might expect from other treatments. The researchers hope the information learned from this study will benefit other patients with invasive breast cancer in the future.

The benefit of the study to society may be the use of information gained on the toxicity and effectiveness of this therapy for breast cancer in the future.

ALTERNATIVE TREATMENTS:

Instead of being in this study, I have these options:
I may receive this hormone blocker therapy or other hormone blocker therapy without participating in this study.

I can get treatment for in situ and early invasive breast cancer without being on this study. The treatment on this study may be available at this center or at other locations.

My doctor will discuss these alternatives with me prior to starting the study. I am encouraged to discuss these options with my friends and families.

COSTS/COMPENSATION:

I will receive no payment for taking part in this study. All of the medical examinations, diagnostic tests and laboratory fees associated with this study are considered standard of care for the treatment of this disease. All of these costs will be billed to my third party payer. In the event a standard of care procedure is not covered my insurer/third party payer will be responsible for these costs.
Any tests, examinations, or other procedures that are performed solely for research purposes will be discussed and will not be billed to me or my insurer/third party payer. In this study, the research related items are the study drug, a urine/serum pregnancy test, the MRI before and after treatment with Anastrozole, and the collection of data.

I understand that if I am injured as a result of my participation in this study, I will be provided reasonable and necessary medical care to treat the illness or injury at no cost to me or to my insurer/third party payer. The University of California does not provide any other form of compensation for injury. I understand that I must report any suspected study-related illness or injury to the study investigator immediately.

CONFIDENTIALITY: I understand that my study records will be kept confidential in a locked office. My participation will be kept confidential by the study team and by any other University personnel authorized to have access to study records. The research team, authorized representatives of the University of California, Irvine, the Food and Drug Administration, National Cancer Institute, and other regulatory entities may have access to my study records to protect my safety and welfare. In addition, authorized representatives from AstraZeneca (the funding entity) will have access to my study records for examination of all study-related activities to determine whether certain activities were conducted, and data were recorded according to protocol, Good Clinical Practice (GCP). I understand that any information derived

from this research project that personally identifies me will not be voluntarily released or disclosed by these entities without my separate consent, except as specifically required by law. Any data used for presentation or publication will be anonymous and/or disguised so that I cannot be personally identified.

I have been told that the study team understands the HIPAA obligations associated with this study and they have attached a separate form called "Authorization for Release of Personal Health Information (Protected Health Information) for Research Purposes" to this consent for me to sign. The reasons why I will be asked to sign that Authorization is explained in that form.

OTHER CONSIDERATIONS:

If I Agree to Allow the Use of My Specimen(s) for Future Research:
I understand that any specimen(s) (e.g., tissue, blood, urine) obtained for the purposes of this study will become the property of the University of California, Irvine (UCI) and that once I have provided the specimens I will not have access to them. I understand that the specimen(s) will be used for research and such use may result in inventions or discoveries that could become the basis for new products or diagnostic or therapeutic agents. In some instances, these inventions and discoveries may be of potential commercial value and may be patented and licensed by the University. I understand that I will not receive any money or other benefits derived from any commercial or other products that may be developed from use of the specimens.

If I Do Not Agree to Allow the Use of My Specimen(s) for Future Research:
I understand that my specimen(s) (e.g., tissue, blood, urine) obtained for the purposes of this study will become the property of the University of California, Irvine (UCI) and that once I have provided the specimen(s) I will not have access to them. I understand that the specimen(s) will be discarded or destroyed once they have been used for the purposes described in the protocol. They will not be used for future research.
I agree to the use of my specimens for research and teaching purposes related to my cancer.

_____ Yes _____ No _____ Initial

I agree to be contacted in the future to discuss whether I will give permission for my specimens to be used for genetic research.

_____ Yes _____ No _____ Initial

I agree to allow my specimens to be used for research unrelated to my cancer.

_____ Yes _____ No _____ Initial

I agree to allow my specimens to be used for future research.

_____ Yes _____ No _____ Initial

I understand that the study team has no significant financial interest in the outcome of this study.

NEW FINDINGS: If, during the course of this study, significant new information becomes available that may relate to my willingness to continue to participate, this information will be provided to me by the investigator.

IF I HAVE QUESTIONS: If I have questions about the study, I can contact a member of the study team listed on the front of this consent form. If I have any comments or questions regarding the conduct of this research and my rights as a research subject, I will contact the Office of Research Administration by phone, (949) 824-6068 or (949) 824-2125, or at 5171 California Avenue, Irvine, CA. 92697-7600.

I may also get more information about clinical trials by calling the NCI's Cancer Information Service at 1-800-4-CANCER (1-800-422-6237) or TTY: 1-800-332-8615. I may also visit the NCI's Web sites for comprehensive clinical trials information at: http://cancertrials.nci.nih.gov or http://cancernet.nci.nih.gov.

VOLUNTARY PARTICIPATION: I have read the attached "Experimental Subject's Bill of Rights" and have been given a copy of it and this consent form to keep. I understand that participation in this study is voluntary. I may refuse to answer any question or discontinue my involvement at any time without penalty or loss of benefits to which I might otherwise be entitled. My decision will not affect my future relationship with UCI or quality of care at UCI Medical Center.

My signature below indicates that I have read the information in this consent form and have had a chance to ask any questions I have about the study. I consent to participate.

Signature of Subject	Date
Signature of Investigator	Date
Signature of Witness	Date
Print Name of Witness	

19.0 APPENDIX

19.1 This study will utilize the CTC (NCI Common Toxicity Criteria) version 2.X for toxicity and Adverse Event reporting. A copy of the CTC version 2.X can be downloaded from the CTEP home page (http://ctep.info.nih.gov). **All appropriate treatment areas should have access to a copy of the CTC version 2.X.**