GCC1366- A PROSPECTIVE STUDY OF NEOADJUVANT NON-STEROIDAL AROMATASE INHIBITORS IN POSTMENOPAUSAL WOMEN WITH OPERABLE HORMONE RECEPTOR-POSITIVE BREAST CANCER TO EVALUATE THE ANTI-PROLIFERATIVE RESPONSE IN OBESE AND OVERWEIGHT PATIENTS

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SCHEMA

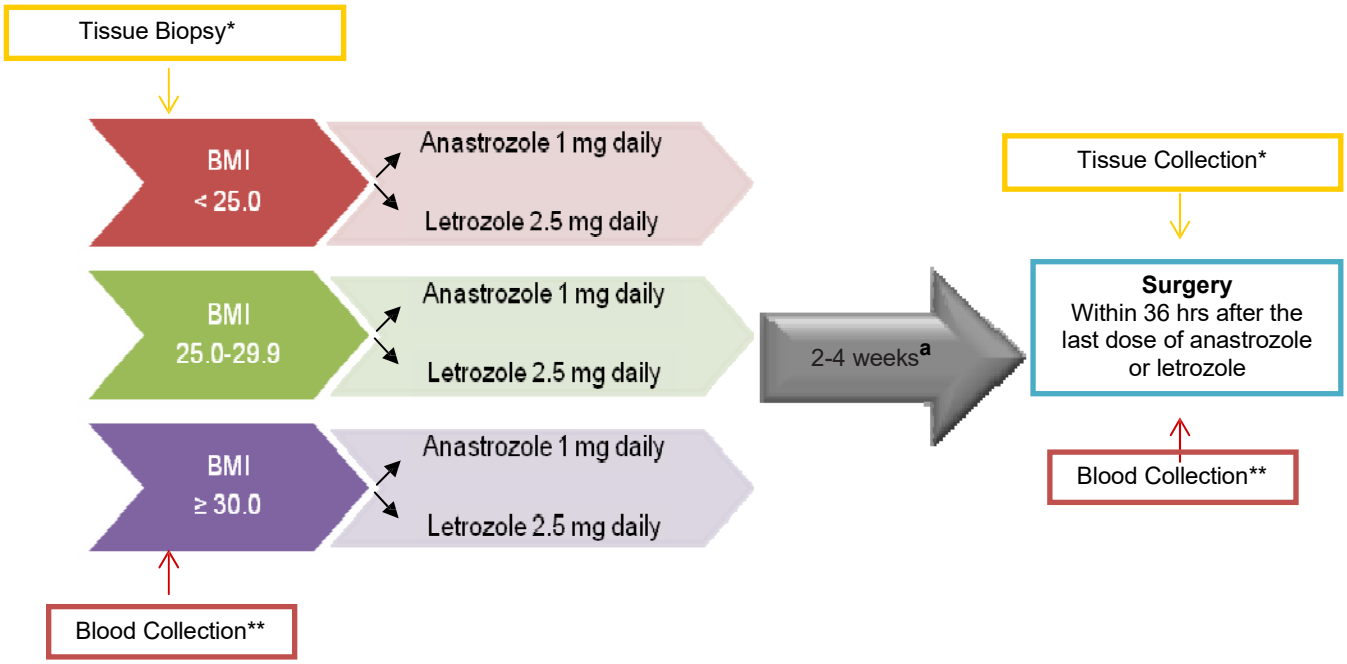
GCC1366 - A PROSPECTIVE STUDY OF NEOADJUVANT NON-STEROIDAL AROMATASE INHIBITORS IN POSTMENOPAUSAL WOMEN WITH OPERABLE HORMONE RECEPTOR-POSITIVE BREAST CANCER TO EVALUATE THE ANTI-PROLIFERATIVE RESPONSE IN OBESE AND OVERWEIGHT PATIENTS

Patient population: Postmenopausal women with operable stage I, II, III hormone receptor-positive breast cancer

- Cohort 1: Patients with BMI < 25.0 kg/m^2 treating with anastrozole
- Cohort 2: Patients with BMI ≥ 25.0-29.9 kg/m^2 treating with anastrozole
- Cohort 3: Patients with BMI ≥ 30 kg/m^2 treating with anastrozole
- Cohort 4: Patients with BMI < 25.0 kg/m^2 treating with letrozole
- Cohort 5: Patients with BMI ≥ 25.0-29.9 kg/m^2 treating with letrozole
- Cohort 6: Patients with BMI ≥ 30 kg/m^2 treating with letrozole

Sample size: 15 patients in each cohort (total of 90 patients)

Schema:



[a]A patient may continue anastrozole or letrozole beyond 4 weeks prior to surgery, if in the opinion of the treating physician, the patient will benefit from extended endocrine therapy. These patients will have a core biopsy performed at 2-4 weeks after initiation of therapy to assess cellular response by Ki-67 measurement to determine if they are responding to endocrine therapy. Biopsies obtained for this purpose will be used in the analysis for primary endpoint.

Diagnostic (DS) & Correlative (CS) studies:

*Tissue biopsy/collection

1. ER, PR, HER2, and Ki67 expression (DS)
2. Oncotype Dx in pre-treatment samples (DS)
3. GP88 (CS)

Optional if feasible

4. Infiltrating immunoregulatory cells (IRC): Myeloid suppressor cells (MDSC), T cells, NK cells, and NK-T cells (CS)
5. Local pro-inflammatory cytokines; Leptin, IL-6, IL-8, TNF-α, VEGF (CS)

** Blood collection

1. Estradiol level, FSH (DS)
2. Serum GP88, Estradiol level (sensitive measure) (CS)

Optional if feasible

3. Metabolomic profiling (CS)
4. Circulating pro-inflammatory cytokines: Leptin, IL-6, IL-8, TNF-α, VEGF, IFNγ, IL-4, GM-CSF, IL-10, IL-17A (CS)
5. Circulating immunoregulatory cells (IRC): Myeloid suppressor cells (MDSC), T cells, NK cells, and NK-T cells (CS)

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1. OBJECTIVES

1.1. Primary Objectives

1. To evaluate the percent change in proliferative index (Ki67) in the primary breast tumor after neoadjuvant treatment with standard dose anastrozole or letrozole in normal, overweight, and obese patients with ER positive breast cancer (T1-3, N0/1, M0).

1.2. Secondary Objectives

1. To assess estradiol levels at baseline and after treatment with standard dose anastrozole or letrozole in normal, overweight, and obese patients.
2. To evaluate differences in baseline GP88 level and Oncotype Dx® assay in primary ER positive breast tumors from normal, overweight, and obese patients.
3. To evaluate the association of AI-induced Ki67 response with baseline and posttreatment GP88 level and baseline Oncotype Dx® assay in normal, overweight, and obese patients.

1.3. Exploratory Objectives

1. To assess circulating and infiltrating immunoregulatory cells (IRC) at baseline and after treatment with standard dose anastrozole or letrozole in normal, overweight, and obese patients. (optional when feasible)
2. To evaluate local and circulating pro-inflammatory cytokines at baseline and after treatment with standard dose anastrozole or letrozole in normal, overweight, and obese patients. (optional when feasible)

2. BACKGROUND

Breast cancer is the second leading cause of cancer-related deaths among women worldwide. Approximately three-quarters of all breast cancer patients present with hormone receptor-positive disease. Endocrine therapies, particularly tamoxifen and aromatase inhibitors (AIs), are one of the mainstays of treatment for patients with hormone receptor-positive breast cancer. Due to their superior efficacy and favorable side effect profile, AIs have become the standard treatment for postmenopausal women with hormone receptor-positive breast cancer.[1]

2.1 Neoadjuvant Endocrine Therapy for Breast Cancer

In patients with hormone receptor-positive tumors, endocrine therapy has also been evaluated in the neoadjuvant setting in three large phase III randomized trials. These trials include the P024 trial, the Immediate Preoperative Anastrozole, Tamoxifen or Combined with Tamoxifen (IMPACT) trial, and the Preoperative "Arimidex" compared to Tamoxifen (PROACT) trial.[2-4] Letrozole, a non-steroidal AI, was studied in comparison to tamoxifen in the P024 trial.[2] This trial was a randomized, double blind, multi-center study, in which three hundred and thirty-seven postmenopausal women with ER and/or PR-positive (ER/PR expression > 10%) breast cancer were treated with either tamoxifen or letrozole for 4 months prior to surgery. The study met its primary endpoint and demonstrated that letrozole was superior to tamoxifen with a clinical response rate of 55% vs. 36% ($p < 0.001$). The rates of breast conserving surgery (BCS) were also higher in the letrozole arm compared to tamoxifen (45% vs. 35%; $p = 0.022$). In contrast to tamoxifen, the overall response rate with letrozole was comparable in both EGFR/HER2-positive and negative subsets in the subsequent biomarker analysis. Letrozole was also more effective in suppressing the proliferative index, Ki67, than tamoxifen ($p=0.0009$). Despite the impressive response rates with letrozole in the P024 trial, anastrozole, another non-steroidal AI, failed to demonstrate superior response rates compared to tamoxifen in both the IMPACT and PROACT trials.[3,4] The IMPACT trial enrolled 330 postmenopausal women with ER-positive (ER expression > 1%) breast cancer who were randomly assigned to either anastrozole (A), tamoxifen (T), or the combination (C) for 3 months prior to surgery.[3] There was no statistically significant difference in response rates measured by both clinical exam (A 37%, T 36%, and C 39%) and ultrasound (A 24%, T 20%, and C 28%). However, there was a trend toward improvement in BCS with anastrozole in 124 women who were not initially deemed to be candidates for BCS (44% vs. 31%; $p = 0.23$). Among HER2-positive breast cancer, the response rate also appeared to be higher in the anastrozole group but this was not statistically significant (58% vs. 22%; $p = 0.18$). The PROACT trial included 451 postmenopausal women with ER/PR-positive breast cancer who were randomly assigned to anastrozole or tamoxifen for 12 weeks.[4] In contrast to the IMPACT trial, concurrent chemotherapy was allowed in this trial. The objective responses were similar in both the anastrozole and tamoxifen groups measured by both clinical exam (50% vs. 46.2%) and ultrasound (39.5% vs. 35.4%). However, in patients who received endocrine therapy alone, there was a statistically significant improvement in BCS rate in the anastrozole group (43% vs. 30.8%; $p = 0.04$). Data are more limited with exemestane which is a steroidal AI. A single phase II trial with 115 postmenopausal women with ER-positive breast cancer demonstrated an impressive clinical response rate of 76.3% with exemestane compared to 40% with tamoxifen ($p=0.05$). The rate of BCS was also significantly higher in the exemestane group

(36.8% vs. 20%; p = 0.05).[5] Meta-analysis of these four clinical trials (P024, IMPACT, PROACT, and exemestane trials) confirmed the benefit of AIs over tamoxifen in all aspects including objective response rate by clinical exam (relative risk (RR), 1.29; 95% CI, 1.14–1.47; p < 0.001), objective response rate by ultrasound (RR, 1.29; 95% CI, 1.10–1.51; p = 0.002), and BCS rate (RR, 1.36; 95% CI, 1.16–1.59; p < 0.001). Toxicities seem to be comparable between AIs and tamoxifen, particularly hot flashes, nausea, and fatigue. The only significant difference was more headaches in the AI group (p = 0.011) but this toxicity was easy to manage. The ACOSOG Z1031 trial, which compared letrozole, anastrozole, and exemestane in the neoadjuvant setting, also demonstrated significant improvements in surgical outcomes.[6] Over 50% of patients who were eligible only for mastectomy at the start of treatment were able to undergo BCS, and 83% of patients who were marginal candidates for BCS at the start of treatment were able to have BCS. Therefore, treatment of hormone receptor-positive patients with neoadjuvant AI therapy appears to significantly increase patient eligibility for BCS. In addition, the overall BCS rate of 68% in the ACOSOG Z1031 trial is similar to that observed in other trials using neoadjuvant chemotherapy.[7,8]

2.2 Biomarkers of response to neoadjuvant AI therapy

ER/PR: Estrogen receptor (ER) and progesterone receptor (PgR) are hormone receptors that are expressed in approximately 75% of breast cancers in postmenopausal women. ER and/or PR expression have traditionally been considered the most important biomarkers in the management of breast cancer patients and have been utilized to determine the use of adjuvant endocrine therapy. Estrogen receptor expression is the most important predictor of response to endocrine therapy.[9,10] It has been shown that patients with ER and PgR negative breast cancers derive no benefit from adjuvant endocrine therapy[11], although there may be a small benefit in patients with ER negative and PgR positive breast cancers.[9] In patients with higher quantitative levels of ER expression, the benefit of endocrine therapy in the neoadjuvant, adjuvant and metastatic setting also appears to be greater.[12,13] Progesterone receptor expression, on the other hand, is primarily prognostic and not predictive.[9,11] Patients with ER positive, PgR negative tumors have a worse prognosis than those with ER and PgR positive tumors, however, both groups of patients appear to derive a similar benefit from endocrine therapy.

Ki67: Ki67 protein is a cellular marker for proliferation. Ki67 labeling has been reported to be a good prognostic marker and to correlate well with the S phase fraction[14] and mitotic index.[14,15] A change in expression of Ki67 after short-term exposure to an investigational agent is frequently used to determine the efficacy, particularly in the neoadjuvant setting. For neoadjuvant chemotherapy, two previous studies[16,17] showed a significant correlation between a reduction in Ki67 of more than 25% and a longer disease-free survival. However, subsequent studies[18-20] on the reproducibility of Ki67 measurements in the core needle biopsy suggested that a change in Ki67 score of at least 32-50% between two determinations is required to be considered statistically significant and attributable to treatment effect for an individual patient. For neoadjuvant endocrine therapy, Dowsett et al.[21] reported that a reduction in Ki67 could be observed 2 weeks after initiation of neoadjuvant endocrine therapy and was largely maintained after 12 weeks of treatment in the majority of patients in the Immediate Preoperative "Arimidex" (anastrozole), Tamoxifen, or Arimidex Combined with Tamoxifen (IMPACT) trial. In this study, postmenopausal women with previously untreated ER-positive breast cancer were randomized to receive daily treatment with anastrozole, tamoxifen, or the combination of anastrozole and tamoxifen for 12 weeks before surgery. Biomarkers were measured in the core-cut biopsied specimens taken before and after 2 weeks of treatment, as well as in surgical specimens after 12 weeks of treatment. The geometric means of reduction in Ki67 at 2 and 12 weeks for anastrozole were 76% and 82% respectively. In their subsequent publication[22], Ki67 expression after 2 weeks of treatment, depending on the tertiles of tumor Ki67 expression, was more strongly associated with recurrence free survival (log-rank P = .008) than tumor Ki67 expression at baseline (log-rank P = .07). However, there was no correlation made between the percentage change in the Ki67 expression and recurrence free survival. A drop in Ki67 levels after 2 weeks of neoadjuvant AI treatment has been shown to be indicative of response to neoadjuvant AI therapy in several clinical trials. Therefore, biopsy and evaluation of Ki67 levels in tumors after 2 weeks of neoadjuvant endocrine therapy is considered a standard approach for assessing therapeutic response and guiding a decision for continuing neoadjuvant therapy or proceeding to immediate surgery.[23]

2.3 Obesity and Breast Cancer

Obesity

Obesity is an emerging global health problem. According to the National Institute of Health (NIH) and World Health Organization (WHO) classification, normal weight is defined as body mass index (BMI) ≥18.5 to 24.9 kg/m^2, overweight as BMI ≥25.0 to 29.9 kg/m^2 and obesity as BMI ≥30 kg/m^2.[24] The WHO estimates that 1.5 billion people are overweight and more than 500 million are obese worldwide.[25] The incidence of overweight and obese adults and children has been steadily increasing during the last two decades, and it is estimated that one third of US adults are obese.[26] Several studies demonstrate a strong correlation between obesity and increased incidence of and/or mortality from several types of cancer.[27,28] In the US, up to 20% of all cancer deaths in women and 14% in men are attributable to obesity.[27]

Purpose for Current Study: Assessment of Aromatase Inhibitors in Overweight and Obese Patients

It has been long established that obesity is a risk factor for the development of breast cancer in postmenopausal

women.[29-31] While the relationship between obesity and breast cancer is intricate, a major factor is the increased production of estrogen in excess adipose tissue in obese women after menopause.[31] Aromatase or CYP19 is a member of the cytochrome P450 superfamily that is involved in the conversion of androgens to estrogens via aromatization.[32] In postmenopausal women, the sole source of estrogens comes from this peripheral conversion of androgens by aromatase. This enzyme is present in multiple organs including adipose tissue, brain, blood vessels, skin, bone, endometrium, and breast tissue.[32] Given the fact that one of the main sources of aromatase is from adipose tissue, it is hypothesized that overweight and obese women have higher levels of aromatase and therefore, may have poorer outcomes when using the standard dose of AI. It has been previously observed that obese patients have increased risk of recurrence and mortality, including breast cancer-specific mortality, compared to normal weight women.[33,34] More recent studies also support this observation that obese patients have poor outcomes, particularly with AI treatment. Two adjuvant clinical trials in hormone receptor-positive breast cancer comparing AI and tamoxifen demonstrated reduced efficacy of AIs in women with higher BMI compared to normal weight patients.[35,36] However, this difference in outcome between normal and obese patients was not apparent with tamoxifen. The first clinical trial was the Arimidex, Tamoxifen, Alone or in Combination (ATAC) trial. This trial included a total of 5,172 postmenopausal hormone receptor–positive women who were randomly assigned to receive either anastrozole, tamoxifen, or the combination.[35] In this study, women with high BMI (> 35 kg/m^2) at baseline had more recurrences compared to women with low BMI (< 23 kg/m^2) with an adjusted HR of 1.39 (p 0.03), and significantly more distant recurrences (HR 1.46; p 0.01). The second study was the Austrian Breast and Colorectal Cancer Study Group trial 12 (ABCSG-12).[36] This trial compared the outcomes of anastrozole and tamoxifen in premenopausal women undergoing ovarian suppression with the gonadotropin-releasing hormone agonist goserelin. Similar to the ATAC trial, the ABCSG-12 trial also demonstrated a significant increase in the risk of disease recurrence in overweight patients (HR 1.60; p 0.02) and more than a doubling in the risk of death (HR 2.14; p 0.01) compared to normal weight patients. More intriguing, the outcome was even worse when comparing treatment within the overweight group alone; patients treated with anastrozole had almost a 50% increase in risk of disease recurrence (HR, 1.49; p 0.08) and a three-fold increase in risk of death (HR, 3.03; p 0.004) compared to patients treated with tamoxifen. Taken together, these studies suggest that higher levels of peripheral aromatase in overweight and obese women might reduce the efficacy of the standard dose of anastrozole and that higher doses or more complete inhibition of aromatase might be more effective in such women. In line with prior studies, a recent clinical trial also demonstrated that overweight and obese patients have higher levels of both estradiol and estrone at both baseline and after AI treatment. When comparing letrozole and anastrozole, letrozole appeared to provide greater suppression of both estrogen types. Furthermore, unlike anastrozole, there was no difference in the benefit of letrozole observed among obese vs. normal weight patients in the subset analysis of the breast international group 1-98 (BIG 1-98) trial.[37] Nevertheless, it remains unclear whether this small difference in the estrogen levels in overweight /obese and normal weight patients will result in less anti-tumor activity that can explain the poor outcomes previously observed in the ATAC and ABCSG-12 trials.

Therefore, this protocol addresses a significant unanswered question in the management of breast cancer patients, whether specific aromatase inhibitors work as effectively in overweight and obese individuals as they do in normal weight patients. The information that is obtained from this study has the potential to change the management of breast cancer patients and may lead to improvements in outcomes.

2.4 Correlative Study Background

GP88 or PC cell-derived growth factor (PCDFG): GP88 is a novel biomarker that was discovered by Dr. Ginette Serrero. GP88 has been shown to be associated with resistance to tamoxifen and aromatase inhibitors.[38] Expression of GP88 is also associated with invasiveness and VEGF expression.[39] More recently, GP88 has been shown to confer resistance to anti-HER2 therapy in HER2-overexpressing breast cancer cells.[40]

Oncotype Dx®: Oncotype Dx® or the 21-gene RT-PCR assay is another gene based approach that quantifies the likelihood of distant metastasis in patients with early-stage ER-positive HER2-negative breast cancer.[41] This test has also been shown to have predictive value to assess the likely benefit from adjuvant chemotherapy.[42] Currently, this test is recommended for routine standard practice by NCCN guideline version 3.2013 to assist in estimating likelihood of recurrence and benefit from adjuvant chemotherapy in patients with ER-positive, HER2-negative breast cancer (category 2A).

Optional: Immunoregulatory Cells: In the past few decades the importance of immune surveillance and cancer development has been increasingly recognized. Multiple types of patrolling cells of the immune system provide continuous surveillance and eliminate cells that undergo malignant transformation. Some tumors may escape the immune defense system by masking their tumor antigens. Alternatively, tumors may survive by promoting the production of suppressor cells which block effector cells that would normally attack it.

- Myeloid-derived suppressor cells (MDSCs): Multiple studies have established that a group of cells derived from the bone marrow termed myeloid-derived suppressor cells are directly involved in the suppression of immune responses in cancer.[43,44] These cells normally express CD33, CD11b, and CD15 but lack expression of monocytic differentiation (CD14), dendritic cells (CD11c), and macrophage (CD68). MDSCs exert their immunosuppressive effect by both an antigen-specific and nonspecific manner depending on their location and tumor type. At the tumor site, the immunosuppressive effect of MDSCs is mediated mainly by the production of nitric oxide and high arginase activity. This combination results in not only induction of apoptosis in T cells but increased production of reactive oxygen species that suppress T cell function irrespective of the nature of the antigens.[45] In breast cancer, the adoptive transfer of ex vivo expanded HER2-specific T cells can only induce tumor regression when combined with the depletion of MDSCs.[46]
- Regulatory T cells (Treg cells): Treg cells are a specialized subpopulation of T cells that suppress activation of the immune system to maintain the homeostasis and tolerance to self-antigens. This subpopulation of T cells is an important "self-check" of the immune system to prevent autoimmunity but is also associated with tolerance

of the immune system in cancer. In general, naturally occurring Treg cells are $CD4^+CD25^+$ cells and are generated in the thymus as part of the normal peripheral T-cell repertoire. Treg can be distinguished from activated T cells based on the high expression of CD25, cytotoxic T-lymphocyte antigen 4 (CTLA4), and the transcription factor forkhead box P3 (FoxP3).[47]

- Natural Killer cells (NK cells): NK cells are a subtype of cytotoxic lymphocytes that are a major component of the innate immune system. NK cells play a critical role in rejecting cancer cells as well as virally infected cells. NK cells are defined as large granular lymphocytes that do not express T-cell markers (T-cell receptor antigen or TCR and pan T cell marker CD3) or B cell surface immunoglobulin (Ig) but usually express the surface markers CD16 (FcγRIII) and CD56 in humans. In addition, up to 80% of NK cells also express CD8.[48]

Optional: Pro-inflammatory Cytokines: High pro-inflammatory cytokine levels are associated with poor outcomes in breast cancer patients. White adipose tissue (WAT) in obese patients is a source of local and circulating pro-inflammatory cytokines.[49,50] These cytokines also attract and promote differentiation of monocytes to macrophages which results in the secretion of additional pro-inflammatory cytokines and pro-angiogenic factors. The result is a chronic inflammatory microenvironment which favors tumor cell motility and invasion, promotes epithelial-mesenchymal transition, and enhances survival, proliferation and renewal of cancer stem cells (CSCs). These pro-inflammatory cytokines can also activate many cancer-related signaling pathways and may increase aromatase expression in mammary tissue, both which may lead to progression of breast cancers.

Optional: Metabolomics profiling: Metabolomics is an emerging field and one of the core areas of system biology research that focus on the holistic study of low-molecular-weight organic and inorganic (typically < 1,500 Da) metabolites. These metabolites are the building blocks for many biological components and are central in intermediary metabolism. They also have an active role in regulating several cellular functions and are involved in cell signaling.[51] Metabolomics profiling allows unbiased global assessment of metabolites with a small amount of blood. Currently, there are limited data on the changes that occur in the whole metabolomics profiling from aromatase inhibitors, particularly in correlation with the body mass index.

3. PATIENT SELECTION

3.1 Inclusion Criteria

1. Females greater than or equal to 18 years.
2. Postmenopausal status, defined as no menstrual cycle for 12 months or surgical removal of ovaries.
3. Histologically confirmed adenocarcinoma of the breast.
4. Evidence of hormone sensitive, ER rich primary tumor defined by an Allred score of ≥6.
5. HER2 negative in the primary tumor as defined by:
   - Grade 0 or 1+ staining intensity (on a scale of 0 to 3) by means of IHC analysis OR
   - Grade 2+ staining intensity by means of IHC analysis with gene amplification on fluorescence in situ hybridization (FISH) < 2.0 OR
   - Gene amplification on fluorescence in situ hybridization (FISH) < 2.0.
6. Eastern Cooperative Oncology Group (ECOG) performance status < 3 (Appendix A).
7. Unresected operable breast cancer stage I, II, III with primary tumor ≥ 1.0 cm (Appendix B).
8. Ability to understand and willingness to sign a written informed consent document.
9. Patients must not have received any prior chemotherapy, radiation therapy, or endocrine therapy for their current breast cancer. Patients who received tamoxifen or raloxifene or another agent for prevention of breast cancer may be included as long as the patient has discontinued the treatment at least one month prior to baseline study biopsy.
10. Patients must have an adequate tumor tissue sample prior to enrollment available for correlative studies as defined below: Core needle biopsy or incisional biopsy samples that can provide ≥ 5 unstained sections of 5 micron thickness. Fine needle aspiration (FNA) sample alone is not sufficient.
11. Patients must have adequate organ function as defined below:
    - Total bilirubin within normal institutional limits
    - AST(SGOT)/ALT(SGPT) < 2.5 x institutional upper limit of normal
    - Creatinine clearance ≥ 10 mL/min/1.73 m^2

3.2 Exclusion Criteria

1. Previous or current systemic malignancy within the past 3 years other than breast cancer or adequately treated cervical carcinoma in situ or basal/squamous carcinoma of the skin.
2. Patients may not be receiving any other investigational agent.
3. History of allergic reactions or hypersensitivity to compounds of similar chemical or biologic composition to anastrozole or letrozole.

4. REGISTRATION PROCEDURES

4.1 General Guidelines

Upon signing consent, sites will sequentially assign a three digit screening number with an "S" prefix. University of Maryland Greenebaum Cancer Center will be identified as site #1, therefore their screening numbers will begin with "S101" followed by "S102" etc. The Cancer Institute at St. Joseph Medical Center will be identified as site #2, therefore their screening numbers will begin with "S201" followed by "S202" etc. These screening numbers will be used on all samples as described in section 8 of this protocol. Eligible patients will be registered onto the study by the lead site study coordinator. Following registration, patients should begin protocol treatment within 5 business days. All of the patients' documents will be kept confidential in a secured area.

4.2 Registration Process

To register a patient, the following documents should be completed by a research team member and sent to study coordinators, Nancy Tait or Cheryl Young, via fax, (410) 328-1741, or e-mail, ntait@umm.edu or Cheryl.Young@umm.edu

- Copy of required laboratory tests
- Signed patient consent form
- HIPAA authorization form
- BMI determination
- Other appropriate forms (e.g., Eligibility Screening Worksheet, Documentation of Pathology and Diagnosis)

To complete the registration process, the Coordinator will

- Assign the patient a four digit study number according to the Registration Worksheet (Appendix F). NOTE: this number is different than the assigned site screening number.
- Register the patient into the appropriate cohort as determined by BMI
- Communicate the cohort assignment back to the requestor using email

4.3 Pretrial Screening

Patients will be screened before entry to establish eligibility. The following assessments must be obtained within 3 weeks of trial entry:

- Medical history including all significant conditions.
- Concomitant therapy before entry documented to establish eligibility.
- ECOG performance status.
- Physical examination including vital signs, height, weight and BMI determination.
- Clinical tumor assessment by breast examination for palpable tumors including clinical tumor size, character, mobility, and location of the breast mass. For non-palpable tumors, clinical tumor size and location will be assessed by imaging studies.
- Laboratory studies: CBC with diff; chemistries including sodium, potassium, chloride, bicarbonate ($HCO3^-$ or $CO2$), BUN, creatinine, fasting blood glucose, total bilirubin, calcium, total protein, albumin, AST (SGOT), ALT (SGPT), alkaline phosphatase, LDH, phosphorus, magnesium, estradiol level, FSH, PT/INR, and PTT.

The following baseline breast imaging can be obtained within 8 weeks of trial entry:

- Baseline mammogram, breast ultrasound, and breast magnetic resonance imaging (MRI) as clinically indicated. All imaging should be performed as per standard of care. When feasible, breast MRI should also be performed in addition to mammogram and breast ultrasound.

5. TREATMENT PLAN

5.1 Agent Administration

Treatment will be administered on an outpatient basis. No investigational or commercial agents or therapies other than anastrozole or letrozole may be administered with the intent to treat the patient's malignancy.

- A total of 90 patients will be enrolled with 15 patients in each cohort below.

- Cohort 1: Patients with BMI < 25.0 kg/m^2 treating with anastrozole
- Cohort 2: Patients with BMI ≥ 25.0-29.9 kg/m^2 treating with anastrozole
- Cohort 3: Patients with BMI ≥ 30 kg/m^2 treating with anastrozole
- Cohort 4: Patients with BMI < 25.0 kg/m^2 treating with letrozole
- Cohort 5: Patients with BMI ≥ 25.0-29.9 kg/m^2 treating with letrozole
- Cohort 6: Patients with BMI ≥ 30 kg/m^2 treating with letrozole

- For each BMI category, the first 15 patients will be treated with anastrozole (cohorts 1, 2 and 3), and the following 15 patients will be treated with letrozole (cohorts 4, 5, and 6). Appendix F will be used by the study coordinators outlined in section 4.2 to ensure stratification to cohorts is managed appropriately.
- Treatment with anastrozole or letrozole will be administered and continued for a minimum of 14 days and a maximum of 28 days (2-4 weeks). Surgery will be performed between weeks 2-3 of treatment unless there are compelling medical or personal reasons that prevent a patient from having surgery during this time. In those cases, patients may continue anastrozole or letrozole up to 4 weeks before surgery.
- Anastrozole will be taken by mouth once daily on a continuous basis at the fixed dose of 1 mg per day in cohorts 1, 2, 3 for 2-4 weeks. Surgery should be performed within 36 hours of the last dose of anastrozole.
- Letrozole will be taken by mouth once daily on a continuous basis at the fixed dose of 2.5 mg per day in cohorts 4, 5, 6 for 2-4 weeks. Surgery should be performed within 36 hours of the last dose of letrozole.
- A patient may be continued on anastrozole or letrozole beyond 4 weeks (up to 18 weeks) if in the opinion of the treating physician, the patient will benefit from extended endocrine therapy. In this context, patients will have a core needle biopsy performed at 2-4 weeks after treatment to assess Ki67 response to AI therapy. Patients with an appropriate response to treatment as determined by a decrease in Ki67 levels (≤10%) will be continued on AI treatment. Patients without an appropriate decrease in Ki67 levels will be recommended to have immediate surgery or a switch to neoadjuvant chemotherapy if desired by the patient and treating physician. This has become a standard approach to assessing response to neoadjuvant AI therapy and guiding treatment decisions.[23] This is currently the treatment approach that is used in the University of Maryland Medical System for patients with ER positive breast cancer receiving neoadjuvant endocrine therapy. (personal communication, Dr. Michael Schultz, UMMS, St. Joseph Medical Center, Towson, MD)

5.2 Study Plan

- A total of 90 patients will be enrolled with 15 patients in each cohort below.
  - Cohort 1: Patients with BMI < 25.0 kg/m^2 treating with anastrozole
  - Cohort 2: Patients with BMI ≥ 25.0-29.9 kg/m^2 treating with anastrozole
  - Cohort 3: Patients with BMI ≥ 30 kg/m^2 treating with anastrozole
  - Cohort 4: Patients with BMI < 25.0 kg/m^2 treating with letrozole
  - Cohort 5: Patients with BMI ≥ 25.0-29.9 kg/m^2 treating with letrozole
  - Cohort 6: Patients with BMI ≥ 30 kg/m^2 treating with letrozole
- Based on the patients' calculated BMI, patients in each BMI category (normal, overweight, and obese) will be enrolled in the different cohorts as described above. The first 15 patients in each BMI category will be treated with anastrozole. After completion of enrollment in cohorts 1, 2, and 3, subsequent patients will be treated with letrozole in cohorts 4, 5, and 6.
- Anastrozole 1 mg or Letrozole 2.5 mg oral daily will be administered and continued for a minimum of 14 days and a maximum of 28 days (2-4 weeks). Surgery will be performed between weeks 2-3 of treatment unless there are compelling medical or personal reasons that prevent a patient from having surgery during this time. In those cases, patients may continue anastrozole or letrozole up to 4 weeks before surgery. Surgery should be performed within 36 hours of the last dose of anastrozole or letrozole.
- Tumor tissue that is obtained for diagnosis or to assess response to initial AI therapy will be utilized for correlative studies.
- A patient may be continued on anastrozole or letrozole beyond 4 weeks (up to 18 weeks) if in the opinion of the treating physician, the patient will benefit from extended endocrine therapy. In this context, patients will have a core needle biopsy performed at 2-4 weeks after treatment to assess Ki67 response to AI therapy. Patients with an appropriate response to treatment as determined by a decrease in Ki67 levels (≤10%) will be continued on AI treatment. Patients without an appropriate decrease in Ki67 levels will be recommended to have immediate surgery or a switch to neoadjuvant chemotherapy if desired by the patient and treating physician. This has become a standard approach to assessing response to neoadjuvant AI therapy and guiding treatment decisions.[23] This is currently the treatment approach that is used in the University of Maryland Medical System for patients with ER positive breast cancer receiving neoadjuvant endocrine therapy. (personal communication, Dr. Michael Schultz, UMMS, St. Joseph Medical Center, Towson, MD)
- Patients on extended AI neoadjuvant treatment having core biopsy at 2-4 weeks for Ki67 determination for clinical

decision making will be approached and consented for additional research tissues to be taken at the same time as the biopsy for Ki67 determination. These additional passes (2-4, 14 gauge) are considered minimal risk and have been performed in the context of several neoadjuvant AI clinical trials[6,52] without adverse outcome reported. (personal communication, Dr. John Olson, UMMC, Baltimore, MD)

- Blood samples will be collected prior to starting treatment and within 3 days or on the day of surgery. Additional blood samples will be obtained as clinically necessary.
- Clinical assessment will be performed prior to enrollment and within 3 days or on the day of surgery. For patients who continue neoadjuvant endocrine therapy past 4 weeks, clinical assessment will be performed at the time of clinical biopsy and every 4-6 weeks thereafter. For clinical evidence of progression, patients will be offered immediate surgery or switch to neoadjuvant chemotherapy at the discretion of the treating physician.
- Radiological assessment including mammogram, ultrasound, and breast MRI will be performed as per standard of care.

5.3 General Concomitant Medication

5.3.1 Permitted

Thyroid replacement drugs are permitted. Vitamin D, calcium, and bisphosphonates are permitted for patients on this study at investigator discretion.

5.3.2 Not Permitted

Any use of hormone replacement therapy drugs is not permitted (example megace). Please note that raloxifene and idoxifene are to be considered as hormone replacement therapy due to their mechanism of action being an estrogen like agent.

Topical use of vaginal estrogens is not permitted.

Aromatase inhibitors other than anastrozole or letrozole are not permitted. Tamoxifen and fulvestrant are not permitted.

Concurrent chemotherapy is not permitted.

5.4 Duration of Therapy

For most patients, treatment will be administered for a minimum of 14 days and a maximum of 28 days (2-4 weeks) prior to definitive surgery. Surgery will be performed between weeks 2-3 of treatment unless there are compelling medical or personal reasons that prevent a patient from having surgery during this time. In those cases, patients may continue anastrozole or letrozole up to 4 weeks before surgery.

A patient may be continued on anastrozole or letrozole beyond 4 weeks (up to 18 weeks) if in the opinion of the treating physician, the patient will benefit from extended endocrine therapy. In this context, patients will have a core needle biopsy performed at 2-4 weeks after treatment to assess Ki67 response to AI therapy. Patients with an appropriate response to treatment as determined by a decrease in Ki67 levels (≤10%) will be continued on AI treatment. Patients without an appropriate decrease in Ki67 levels will be recommended to have immediate surgery or a switch to neoadjuvant chemotherapy if desired by the patient and treating physician. This has become a standard approach to assessing response to neoadjuvant AI therapy and guiding treatment decisions.[23] This is currently the treatment approach that is used in the University of Maryland Medical System for patients with ER positive breast cancer receiving neoadjuvant endocrine therapy. (personal communication, Dr. Michael Schultz, UMMS, St. Joseph Medical Center, Towson, MD)

Surgery should be performed within 36 hours of the last dose of anastrozole or letrozole. The treatment should be continued until the time of the surgery or until one of the criteria in section 5.8 applies.

5.5 Surgical Considerations

5.5.1 Marker Placement Prior to Initiation of Therapy

A titanium marker or “clip” will be placed at the tumor site(s) prior to starting treatment at the discretion of the treating surgeon in women who may be candidates for breast conservation surgery. This marker will identify the tumor location(s) for surgical resection.

5.5.2 Breast Surgery and Axillary Staging

Following preoperative treatment, women will undergo breast conserving surgery or mastectomy as well as axillary staging at the discretion of the treating surgeon. It is recommended that the definitive surgery take place within 36 hours of the

last dose of anastrozole or letrozole.

5.6 Postoperative Treatments

Postoperative treatments including adjuvant chemotherapy, radiation, and endocrine therapy will be at the discretion of the treating physicians.

5.7 Duration of Follow-up

Patients will be followed for 30 days on study after the last dose of anastrozole or letrozole prior to surgery. For patients who receive extended neoadjuvant therapy with anastrozole or letrozole and for patients who receive other primary treatment after anastrozole or letrozole administration prior to surgery, patients will be followed for 30 days on study after the last dose of anastrozole or letrozole. Patients continuing to experience adverse events attributable to anastrozole or letrozole will be followed as needed until resolution or stabilization of the adverse events. Patients who are either found to be ineligible or refuse to start treatment after consenting will not be followed and will be replaced. Their information will not be collected. After 30 days after the last dose of anastrozole or letrozole, if there are no continuing adverse events attributable to treatment, patients will be off study.

5.8 Criteria for Removal from Study

Patients will be removed from study when any of the criteria listed below applies. The reason for study removal and the date the patient was removed must be documented in the case report form.

- Evidence of disease progression during treatment at the discretion of the treating investigator.
- Non-compliance with the study protocol; including, but not limited to not attending the majority of scheduled visits. The Protocol Chair will determine when non-compliance should lead to removal from study.
- Unacceptable major toxicity defined as ≥ grade 3 toxicity related to anastrozole/letrozole.

Note: Toxicity will be graded according to the National Cancer Institute (NCI)-Common Terminology Criteria for Adverse Events (CTCAE) version 4.0.

- Concurrent illness or condition that would, in the judgment of the treating investigator, affect assessment of clinical status to a significant degree or require discontinuation of study treatment.
- At subject's own request.

Note: The reason for discontinuation from the study must be documented.

- Study is closed for any reason.
- Subject withdraws consent for follow-up.
- Subject is lost to follow-up.

Patients who are removed from the study before completing at least 2 weeks of anastrozole or letrozole will not be counted toward accrual and will be replaced with new patients. However, tissue samples from the surgical procedure will be collected unless patients withdraw consent.

6. DOSING DELAYS

Anastrozole and letrozole are generally well tolerated. The previous study with anastrozole and letrozole in the preoperative setting demonstrated only 3.8% grade 3-4 toxicities.[53] The following rules will be followed for the management of adverse events that are due to anastrozole or letrozole. Toxicity grades are according to the NCI CTCAE version 4.0.

There will be no dose reductions in anastrozole or letrozole.

Supportive care measures should be implemented first. Dose interruption should only be implemented when all supportive care measures have been exhausted without an improvement of patient status. For unresolved grade 3 or 4 adverse events or any clinically significant, lower-grade adverse event despite supportive care measures, treatment with anastrozole or letrozole can be interrupted for a maximum of **5 days** until the patient recovers completely or the adverse event reverts to NCI-CTCAE Grade 1 or to baseline grade.

If recurrence of the adverse event after drug holiday or interruptions is observed and patients are unable to resume the treatment after 5 days, patients will be removed from the study and should proceed with surgery or other treatment. Patients who are removed from the study before completing at least 14 days of anastrozole or letrozole will not be counted toward accrual and will be replaced with new patients. However, tissue samples from the surgical procedure will be collected unless patients withdraw consent.

7. PHARMACEUTICAL INFORMATION

7.1 Anastrozole

7.1.1 Description

Anastrozole or Arimidex® is a non-steroidal aromatase inhibitor. It is chemically described as 1,3-Benzenediacetonitrile, a, a, a', a'-tetramethyl 5-(1H-1,2,3-triazol-1-ylmethyl) and the molecular formula C17H19N5, with a molecular weight of 293.4.

7.1.2 Availability

Anastrozole will be prescribed by the treating physician and covered by the patients insurance. Both generic anastrozole and Arimidex® are permitted but when possible Arimidex® is preferred.

These tablets should be stored at controlled room temperature 20-25°C (68-77°F).

Additional information regarding the formulation as well as storage and handling instructions can be found in the approved package insert.

7.2 Letrozole

7.2.1 Description

Letrozole or Femara® is a non-steroidal aromatase inhibitor. It is chemically described as 4,4'-(1H-1,2,4-triazol-1- ylmethylene) dibenzonitrileand the molecular formula C17H11N5, with a molecular weight of 285.302.

7.2.2 Availability

Letrozole will be prescribed by the treating physician and covered by the patients insurance. Both generic letrozole and Femara® are permitted but when possible Femara® is preferred.

These tablets should be stored at controlled room temperature 20-25°C (68-77°F).

Additional information regarding the formulation as well as storage and handling instructions can be found in the approved package insert.

8. CORRELATIVE STUDIES

Both blood samples and tissue samples will be collected. All samples will be identified using the subject Screening Number - "S101" etc.

8.1 Tumor Tissue Samples

8.1.1 Collections

Tumor tissue samples from the following time points will be obtained:

1. Prior to starting treatment; tissue sample from the original diagnostic procedure. (T0)
2. At the time of definitive surgery (i.e., mastectomy or lumpectomy). A separate tumor biopsy is not needed on the day of surgery; tissue will be taken from the surgical specimen. (T1)
3. Patients on extended AI therapy will have a core biopsy at 2-4 weeks for Ki67 determination. (T2)
4. Patients on extended AI neoadjuvant treatment having core biopsy at 2-4 weeks for Ki67 determination for clinical decision making will be approached and consented for additional research tissues to be taken at the same time as the biopsy for Ki67 determination. These additional passes (2-4, 14 gauge) are considered minimal risk and have been performed in the context of several neoadjuvant AI clinical trials[6,52], without adverse outcome reported. (personal communication, Dr. John Olson, UMMC, Baltimore, MD)
5. Hematoxylin and eosin stained sections will be examined by a local pathologist to establish a diagnosis and confirm the presence of breast cancer.

The tissue acquisition form in the appendix D should be filled out for each patient sample being shipped.

8.1.2 Tissue Sample Processing

8.1.2.1 Immunohistochemistry

Immunohistochemistry (IHC) will be used to determine the pre-treatment and post-treatment expression of ER, PR, HER2, Ki67 and GP88 and when feasible IRC (MDSC, CD4/CD8 T cells, NK cells, and NK-T cells), and pro-inflammatory cytokines (Leptin, IL-6, IL-8, TNF-α, VEGF). The expression of these biomarkers will be quantified by Assisted Quantitative Image Analysis. Studies for ER, PR, HER2 and Ki67 will be performed by immunohistochemistry technicians in the pathology department at the University of Maryland or affiliated study sites. Studies for GP88 and when possible, IRC and pro-inflammatory cytokines will be performed by immunohistochemistry technicians in the pathology department at the University of Maryland. The interpretation of IHC studies will be performed by Olga Ioffe, M.D., breast pathologist in the Department of Anatomic Pathology, University of Maryland. The University of Maryland laboratory is certified by both Clinical Laboratory Improvement Amendments (CLIA) and College of American Pathologists (CAP).

Five-micron sections of formalin-fixed, paraffin-embedded tissue will be tested. Buffer will replace primary antibody for negative controls. All attempts should be made to process tissue specimens within 1 hour of surgery.

ONLY baseline or pretreatment samples will be sent for Oncotype Dx®.

Oncotype Dx® will be requested and sent to Genomic Health as per routine clinical practice. This test is considered standard of care in these patients according to the current NCCN guidelines.

8.2 Blood Samples

8.2.1 Collections

Blood samples from the following time points will be obtained. These blood samples will be drawn at 2-3 time points including:

1. Prior to starting anastrozole or letrozole (B0)
2. On the day of surgery or within 3 days of surgery (B1)
3. In patients receiving extended neoadjuvant endocrine therapy beyond 4 weeks, blood samples will be obtained 2-4 weeks after initiation of AI therapy (B2) and within 3 days or on the day of surgery (B1).

CBC, CMP, phosphorus, magnesium, PT/INR/PTT, estradiol level, and FSH will be processed per sites usual standard of care practices. For research purposes samples for GP88 and estradiol levels (sensitive measurements) will be collected.

When feasible, samples for IRC, pro-inflammatory cytokines, and metabolomics profiling should be collected at these time points.

For **research blood samples**, the following venipuncture tubes should be used:

Test	*Amount (ml)*	*BD Vacutainer Tubes*
Serum GP88	6 x 2	Red top & Na Heparin
Immunoregulatory cells (IRC)	8	Green-red top (CPT™ Ficoll Tube)
Pro-inflammatory cytokines	6	Lavender top (EDTA anti-coagulant)
Metabolomic profiling	10	Uncoated glass red top
Estradiol level	10	Red top

8.2.2 Blood Sample Processing and Storage

8.2.2.1 Serum GP88

Samples will be labeled with the subject's screening ID and time point (B0, B1, B2), frozen within 2 hours after collection and stored at the research site. Samples will be batched and subsequently shipped to Dr. Ginette Serrero. Dr. Serrero can be contacted via email at gserrero@agpharma.com.

8.2.2.2 Immunoregulatory Cells (IRC)

IRC sample will be labeled with the subjects screening ID and time point (B0, B1, B2) and collected in 1 tube of 8 ml green-red top (CPT™ Ficoll Tube).

Please notify contact persons via email and/or telephone **PRIOR** to sample collection. Transportation arrangements will be made via email. Samples must be transported on the day of collection under ambient conditions Monday through Friday.

Tonya Webb, PhD
University of Maryland School of
Medicine 685 West Baltimore
Street HSF-I Room 380
Baltimore, MD 21201
Telephone: (410) 706-4109
Email: TWebb@som.umaryland.edu

8.2.2.3 Pro-inflammatory Cytokines

Circulating pro-inflammatory cytokine analysis including Leptin, IL-4, IL-6, IL-8, IL-10, IL-17A, IFNγ, VEGF, TNFα, and GM-CSF will be performed at the University of Maryland Cytokine Core Laboratory. Blood samples should be collected in an EDTA tube, inverted 8-10 times after collection, centrifuged for 10 minutes at 1000xg within 30 minutes of collection. Plasma should be transferred to 2ml aliquots that are labeled with the subjects screening ID and time point (B0, B1, B2) and frozen at -80°C.

All attempts should be made to process all specimens within **30 minutes** after collection. Samples will be batched and subsequently sent to:

Lisa Hester
Cytokine Core Lab
University of Maryland, Baltimore
Bressler Research Bldg
655 W. Baltimore St.
7th Floor, Room 07-010, Bay F
Baltimore, MD 21201
Telephone: (410) 706-1508
Email: lhester@medicine.umaryland.edu

8.2.2.4 Estradiol levels

Blood for sensitive measurement of estradiol levels should be collected in a 10 ml red top tube, inverted 8-10 times after collection, centrifuged for 10 minutes at 1000xg within 30 minutes of collection. Plasma should be transferred to 2ml aliquots that are labeled with the subjects screening ID and time point (B0, B1, B2) and frozen at -80°C.

All attempts should be made to process all specimens within **30 minutes** after collection. Samples will be sent to an outside laboratory for processing. Details to be provided to the sites prior to shipment.

8.2.2.5 Metabolomic Profiling

Metabolomic profiling will be performed by Dr. Joann Dorgan. Blood samples should be labeled with the subjects screening ID and time point (B0, B1, B2), centrifuged at 1500xg for 20 minutes at 4°C. Plasma should be transferred into 2ml aliquots that are labeled with the subjects screening ID and the timepoint (B0, B1, B2) and frozen at - 80°C.

All attempts should be made to process all specimens within **60 minutes** after collection. Samples will be batched and subsequently sent to:

Rena Lapidus, PhD
Director, UMGCC Translational Laboratory
Bressler Research Building, BRB9-037
Baltimore, Maryland 21201
410-328-8092
Email: rlapidus@som.umaryland.edu

A specimen log for IRC, pro-inflammatory cytokines, estradiol levels and Metabolic Profiling (appendix E) should be filled out for each patient and retained on site as part of the subject study records. A copy should be provided to each lab at the time of sample shipment.

9. STUDY CALENDAR AND VISIT SCHEDULE

Baseline evaluations are to be conducted within 3 weeks prior to start of protocol therapy. However, baseline breast imaging may be obtained within 8 weeks of trial entry.

Patients receiving 2-4 weeks of neoadjuvant endocrine therapy.

Procedure	Pre-study[e]	Week 1-4	Surgery[a,f]	Off Treatment[f]
Treatment:				
Anastrozole 1 mg daily		A		
Letrozole 2.5 mg daily		A		
Clinical assessment:				
Informed consent	X			
Demographics	X			
Medical history	X			
Current medications	X		X	X
Physical exam	X		X	X
Vital signs	X		X	X
Height	X		X	
Weight	X		X	X
BMI	X		X	
Performance status	X		X	
Adverse event evaluation			X	X
Tumor measurement[b]	X		X	
Laboratory test:				
CBC w/diff, plts	X		*	*
Chemistries[c,]	X		*	*
PT/INR/PTT	X			
Estradiol level, FSH	X			
Correlative studies (Research procedures):				
Blood samples				
Serum GP88		X[f]	X[g]	
Estradiol level (sensitive measurement)		X[f]	X[g]	
IRC[d]		X[f]	X[g]	
Pro-inflammatory cytokines[d]		X[f]	X[g]	
Metabolomic profiling[d]		X[f]	X[g]	
Tumor Tissue samples			X	

A: Anastrozole 1 mg **OR** Letrozole 2.5 mg PO daily according to cohort of enrollment (see treatment plan section 5). Treatment will be started on day 1 and continue until the day of definitive surgery (lumpectomy or mastectomy). Treatment will be continued for a minimum of 14 days and a maximum of 28 days (2-4 weeks). Surgery will be performed between weeks 2-3 of treatment unless there are compelling medical or personal reasons that prevent a patient from having surgery during this time. In those cases, patients may continue anastrozole or letrozole up to 4 weeks before surgery.

a: Surgery should be performed within 36 hours of the last dose of anastrozole or letrozole. Clinical assessments, laboratory tests, and correlative studies can be performed on the day of surgery or within 3 days prior to surgery.

b: Tumor measurement: clinical tumor size, character, mobility, and location of the breast mass if palpable. Tumor size will also be assessed from surgical specimen. For non-palpable tumors, clinical tumor size and location will be assessed by imaging studies. Imaging studies include mammogram, breast ultrasound, and breast magnetic resonance imaging (MRI) as clinically indicated. When feasible, breast MRI should also be performed in addition to mammogram and breast ultrasound.

c: Chemistries: sodium, potassium, chloride, bicarbonate, BUN, creatinine, fasting blood glucose, total bilirubin, calcium, total protein, albumin, AST (SGOT), ALT (SGPT) , alkaline phosphatase, LDH, phosphorus, and magnesium. Patients need to be fasting at least 9 hours before the test.

d: When feasible blood will be collected for IRC, pro-inflammatory cytokines and metabolomic profiling

e: Visits **are applicable to ALL subjects** enrolled, regardless of duration of neoadjuvant endocrine therapy

f. Collected within 3 days prior to study drug initiation.

g. Samples may be collected on the day of surgery or within 3 days of surgery.

*Additional blood will be drawn as clinically indicated.

Patients receiving extended neoadjuvant endocrine therapy, > 4 weeks.

Procedure	Week 2-4 bx to assess response[a]	Week 5-18 q4-6 wks
Anastrozole 1 mg daily	A	A
Letrozole 2.5 mg daily	A	A
Current medications	X	X
Physical exam	X	X
Vital signs	X	X
Height	X	
Weight	X	X
BMI	X	X
Performance status	X	X
Adverse event evaluation	X	X
Tumor measurement[b]	X	X
CBC w/diff, plts	*	*
Chemistries[c]	*	*
Serum GP88	X	
Estradiol level (sensitive measurement)	X	
IRC[d]	X	
Pro-inflammatory cytokines[d]	X	
Metabolomic profiling[d]	X	
Tumor Tissue samples	X	

A: Anastrozole 1 mg **OR** Letrozole 2.5 mg PO daily according to cohort of enrollment (see treatment plan section 5). Treatment will be started on day 1 and continue until the day of definitive surgery (lumpectomy or mastectomy). Treatment will be continued for a minimum of 14 days.

a: A biopsy to assess response to treatment and blood samples will be obtained 2-4 weeks after initiation of treatment.
b: Tumor measurement: clinical tumor size, character, mobility, and location of the breast mass for palpable tumors. Tumor size will also be assessed from surgical specimen. For non-palpable tumors, clinical tumor size and location will be assessed by imaging studies. Imaging studies include mammogram, breast ultrasound, and breast magnetic resonance imaging (MRI) as clinically indicated. When feasible, breast MRI should also be performed in addition to mammogram and breast ultrasound.
c: Chemistries: sodium, potassium, chloride, bicarbonate, BUN, creatinine, fasting blood glucose, total bilirubin, calcium, total protein, albumin, AST (SGOT), ALT (SGPT) , alkaline phosphatase, LDH, phosphorus, and magnesium. Patients need to be fasting at least 9 hours before the test.
d: When feasible blood will be collected for IRC, pro-inflammatory cytokines and metabolomic profiling.

*Additional blood will be drawn as clinically indicated.

VISIT SCHEDULE

The study consists of a maximum 21-day (3 weeks) screening phase prior to registration, start of study drug administration within 5 business days of registration. The follow up will last 30 days after the last dose of anastrozole or letrozole prior to surgery.

9.1 Registration

Registration will take place once the consented patient has completed the necessary screening procedures and is deemed eligible for study entry by the investigator or designee. Each registered patient will be assigned a unique identification number. Treatment should begin within 5 business days after registration. Please see registration process in more detail in section 4.2.

9.2 Pre-treatment

These following procedures and tests are to be conducted within 3 weeks prior to starting treatment except baseline imaging studies can be performed within 10 weeks.

- Informed consent (signed and dated by patient)
- Medical history and demographics
- Inclusion/Exclusion Criteria
- Concomitant medications and treatments will be recorded from 1 month prior to the start of study

treatment.

- Physical examination including examination of major body systems, height, body weight, BMI, ECOG performance status, and vital signs (temperature, blood pressure, heart rate, respiratory rate)
- Clinical tumor assessment by breast examination for palpable tumors including clinical tumor size, character, mobility, and location of the breast mass. The patient will be examined by one of the treating investigators or his/her designee at each specified time point and after the final dose of treatment.
- Laboratory studies: CBC with diff; chemistries including sodium, potassium, chloride, bicarbonate ($HCO3^-$ or CO_2), BUN, creatinine, fasting blood glucose, total bilirubin, calcium, total protein, albumin, AST (SGOT), ALT (SGPT), alkaline phosphatase, LDH, phosphorus, magnesium, estradiol level, FSH, and PT/INR/PTT.
- Baseline mammogram, breast ultrasound, or breast magnetic resonance imaging (MRI) as indicated.
- Collection of research blood samples: Serum GP88 and sensitive estradiol measurements and when feasible IRC, pro-inflammatory cytokines, and metabolomics profiling
- Collection of tumor sample: Paraffin blocks and/or unstained slides

9.3 Study Days

9.3.1 Patients receiving 2-4 weeks of neoadjuvant endocrine therapy.

The period called “Study Days” begins when the patient receives the initial dose of anastrozole or letrozole (Week 1 Day 1). Assessments are scheduled as defined in the study calendar. However, assessments may be repeated more often, as clinically indicated. A maximum 5-day delay for resolution of study drug-related toxicities is allowed. A patient should be discontinued from the study if a greater than 5-day delay occurs.

9.3.2 Patients receiving extended neoadjuvant endocrine therapy > 4 weeks.

The period called “Study Days” begins when the patient receives the initial dose of anastrozole or letrozole (Week 1 Day 1). Assessments are scheduled as defined in the study calendar. However, these assessments may be repeated more often, as clinically indicated. A maximum 5-day delay for resolution of study drug-related toxicities is allowed. A patient should be discontinued from the study if a greater than 5-day delay occurs.

9.4 End of Study (Treatment) / Withdrawal

All patients must continue to be observed for 30 days after the last dose of anastrozole or letrozole. At the end of the study treatment, the following procedures should be performed within 4 weeks after surgery.

- Assessment of Concomitant Treatments including medication use
- Physical examination including examination of major body systems, body weight, ECOG performance status, and vital signs (temperature, blood pressure, heart rate, respiratory rate)
- Adverse event assessment

10. MEASUREMENT OF EFFECT

For the purposes of this study, patients should be evaluated at the start of treatment and at the time of surgery. Patients on extended neoadjuvant endocrine therapy will be reevaluated every 4-6 weeks until surgery. Measurements of effect are not in the specific objectives of this study and not part of the formal statistical plan; however, we will collect these data for descriptive purposes.

10.1 Pathologic Response Determination

Pathological response in the breast, and when appropriate, in the axilla will be assessed.

- Pathologic Complete Response (pCR): No viable invasive cancer in pathologic specimen, as determined by histological examination. We will report whether in situ disease only was present in the specimen versus no disease. We will also report for each woman whether she had a pCR in the breast only, in the lymph nodes only, or in both.
- Nodal Disease: We will report the number of women who had positive lymph node prior to starting study drugs, or at the time of surgery: 0, 1-3, 4-9, or ≥10 lymph nodes.

10.2 Clinical Response Determination

Clinical response in the breast will be evaluated, and when appropriate, in the axilla, infraclavicular, supraclavicular

regions, and skin. At the time of study enrollment, patients will have a breast examination. Physical examination findings will be documented including clinical tumor size, character, mobility, and location of the breast mass for palpable tumors. The patient will be examined by one of the treating investigators or his/her designee at each specified time point and at the time of the surgery.

Endpoint Definitions for Clinical Response (UICC criteria)

- Complete response (cCR) in the breast on physical exam will be defined as the absence of any palpable abnormality: i.e., no skin or breast thickening, mass, or associated skin or nipple changes. CR will be recorded separately for the breast and axilla.
- Partial response (cPR) in the breast will be defined as a 50% or greater decrease in the product of biperpendicular diameters as measured with a ruler, compared with the pretreatment measurement.
- Stable disease (cSD) in the breast will be a defined as palpable disease which does not fit the definition of PR or PD.
- Progressive disease (cPD) will be defined as an increase in the product of biperpendicular diameters of 25% or greater compared to the original measurement.
- Inevaluable disease (cID) is defined as breast cancer that is not palpable in two dimensions.

11. STATISTICAL CONSIDERATIONS

11.1 Study Design/Endpoints

Patients will enter the study according to their BMI. There are a total of 6 cohorts in this study as described below:

- Cohort 1: Patients with BMI < 25.0 kg/m^2 treating with anastrozole
- Cohort 2: Patients with BMI ≥ 25.0-29.9 kg/m^2 treating with anastrozole
- Cohort 3: Patients with BMI ≥ 30 kg/m^2 treating with anastrozole
- Cohort 4: Patients with BMI < 25.0 kg/m^2 treating with letrozole
- Cohort 5: Patients with BMI ≥ 25.0-29.9 kg/m^2 treating with letrozole
- Cohort 6: Patients with BMI ≥ 30 kg/m^2 treating with letrozole

The first 15 patients in each BMI category will be treated with anastrozole. After completion of the enrollment in cohort 1, 2, and 3, the following patients will be treated with letrozole in cohort 4, 5, and 6. The primary endpoint that will be used to assess treatment efficacy is percent reduction in Ki67. Sample size calculations are based on the power of the rank test under Lehmann's alternative hypothesis using a Monte Carlo resampling procedure.[54]

We expect a sufficiently higher percent reduction in Ki67 in the control, i.e., normal weight group compared to overweight and obese patients. With a sample size of 15 patients per group, and odds parameters of 2 and 3, respectively, indicating that the odds are 2:1 and 3:1 that a patient in overweight and obese groups, respectively, will have lower Ki67 percent reduction than a patient in a control group. There will be above 80% power of the rank sum statistic under the Lehmann alternative. Testing will be done at the 0.05 one-sided level of significance, using the Jonckheere-Terpstra test statistic. (Please note: if odds parameter is 3, it indicates that the odds are 3:1 that a patient from the control group (normal BMI) has the greater percent reduction in Ki67 level than a patient in obese group.) We used the Jonckheere-Terpstra statistic because it provides more power than the Kruskal-Wallis statistic under the ordered alternatives. We stated the hypothesis that the odds of Ki76 reduction between patients' group is proportional to their BMI.

Secondary objectives: Secondary objectives include a number of correlative studies. The expression of ER, PR, HER2, GP88 as well as the levels of local pro-inflammatory cytokines (when possible) and the percentage of infiltrating IRC (when possible), will be assessed in tumor samples and compared between three cohorts of patients, including normal weight, overweight, and obese patients as well as between patients taking anastrozole vs. letrozole. We will also evaluate and compare estradiol, serum GP88, circulating pro-inflammatory cytokines (when possible), circulating IRC (when possible) and metabolomics profiling (when possible) in peripheral blood among normal weight, overweight, and obese cohorts as well as between patients taking anastrozole vs. letrozole. Comparing between pre-treatment and post-treatment specimens, effects of anastrozole or letrozole treatment in these parameters in tumor tissue and peripheral blood will be estimated. All patients' characteristics will be compared between patients' cohort using BMI. We expect this aspect of the study to be exploratory and hypotheses generating. Statistical analysis will be done in S-plus (v. 8.0, TIBCO) and in R using bioconductor package. All required analyses will be conducted by the GCC Biostatistic Shared Service.

11.2 Sample Size/Accrual Rate

This trial will enroll a total of **90 patients**. At an estimated accrual rate of 2-3 patients per month, we expect that this trial will need to accrue patients for anywhere from 30-45 months.

12. ADVERSE EVENT REPORTING, DATA SAFETY AND MONITORING PLAN

12.1 Adverse Events (AEs)

An Adverse Event is any untoward occurrence reflecting a change from baseline state after signing informed consent and up to 30 days from last administration of the study medication. A Serious Adverse Event (SAE) is any AE that is 1) Fatal, 2) Life Threatening 3) Requires or prolongs hospital stay, 4) Results in a persistent or significant disability or incapacity. 5) Results in a congenital anomaly or birth defect 6) Is considered by the Investigator as an important medical event.

12.1.1 The descriptions and grading scales found in the revised NCI Common Terminology Criteria for Adverse Events (CTCAE) version 4.0 (Available through http://ctep.cancer.gov) will be utilized for AE reporting. AEs of any grade will be documented in the case report form.

12.1.2 AEs can be "Expected" if described in the Informed Consent or "Unexpected". The attribution of the AE is Definite if the AE is clearly related to study treatment; Probable if it is likely related to the study treatment; Possible if it is equally likely related to study treatment or to some other circumstance, and Unlikely if most probably some other event was responsible for the adverse event, and Unrelated if the Adverse Event is entirely explained by circumstances not caused by the study medication.

12.1.3 Data Safety Monitoring Board (DSMB). DSMB oversight will be conducted in accordance with the Greenebaum Cancer Center DSMB standard operating procedures. It is the responsibility of the Principal Investigator to oversee the safety of the study. This safety monitoring will include careful assessment and appropriate reporting of adverse events as noted above.

This study will be monitored by the UMGCC Data and Safety Monitoring and Quality Assurance Committee and will follow the Data Safety and Monitoring Plan as outlined in the Clinical Investigator Handbook of the UMGCC Clinical Research Office. Monitoring will be conducted as per the plan on file with the University of Maryland IRB (also available in the UMGCC Clinical Investigator Handbook found at http://www.umgcc.org/research/clinical_research.htm).

12.1.4 Clinical Trial Monitoring. Data safety and verification monitoring will be conducted in accordance with the Greenebaum Cancer Center Sponsor-Investigator Monitoring Standard Operating Procedure (SOP), the Code of Federal Regulations (CFR), and FDA and International Counsel on Harmonization (ICH) E6 Guidelines.

12.2 Expedited reporting of serious adverse events (SAEs)

The University of Maryland IRB requires the expeditious reporting (within 5 business days) of SAEs that are unexpected and probably related to the study intervention (See reportable new information policy available from http://www.hrpo.umaryland.edu/default.asp). All other SAEs are collected in the UMGCC clinical research database (ONCORE) and are reviewed by the UMGCC DSM/QAC as per their SOP and summarized for the IRB in the annual renewal.

For those events that require a change to the informed consent, the data entry staff who input these data will immediately notify the appropriate research coordinator and regulatory coordinator of this change.

In the event of overdoses and secondary malignancies, the reporting requirements will be the same as for serious adverse event.

12.3 Data Management

Clinical data will be entered into the OnCore® database by the designated data manager at the University of Maryland Marlene and Stewart Greenebaum Cancer Center (UMGCC). Oncore® is 21CRF11.10 (electronic medical records) compliant and is equipped for HIPAA-compliant internet-based entry of protocol tracking and review information.
All study data will be collected by the research team at each and every study visit and recorded in the research record. This data will then be entered in to the OnCore® study database.

Remote VPN access will be provided for each participating site along with Oncore® training. This access and training will occur prior to enrollment of the first patient at the site, to ensure staff are adequately trained and able to enter data within 5 days of the visit occurring. All study data will be collected by the research team at each and every study visit and recorded in the research record. This data will then be entered in to the OnCore® study database.

All source documents will be obtained and retained along with any study forms, and placed into the patient's research folder according to the policies around record retention in place at each site.

12.4 Compliance with Laws and Guidance's

This study will be conducted in accordance with current U.S. Food and Drug Administration (FDA) Regulations, Good Clinical Practices (GCPs) and International Counsel on Harmonization (ICH) E6 Guidelines.

12.5 Institutional Review Board (IRB)

This protocol will be submitted to the University of Maryland IRB. Study procedures will begin once local IRB approval is secured. All amendments, instances of reportable new information (i.e. unanticipated problems, data breaches, etc.) and continuing review reports will be submitted to the University of Maryland IRB.

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APPENDIX A: ECOG Performance Status Scale

Grade	***Descriptions***
0	Normal activity. Fully active, able to carry on all pre-disease performance without restriction.
1	Symptoms, but ambulatory. Restricted in physically strenuous activity, but ambulatory and able to carry out work of a light or sedentary nature (e.g., light housework, office work).
2	In bed <50% of the time. Ambulatory and capable of all self-care, but unable to carry out any work activities. Up and about more than 50% of waking hours.
3	In bed >50% of the time. Capable of only limited self-care, confined to bed or chair more than 50% of waking hours.
4	100% bedridden. Completely disabled. Cannot carry on any self-care. Totally confined to bed or chair.
5	Dead.

APPENDIX B: American Joint Committee on Cancer Staging (AJCC)

T – Primary Tumor	
TX	Primary tumor cannot be assessed
T0	No evidence of primary tumor
Tis	Carcinoma in situ
Tis (DCIS)	Ductal carcinoma in situ
Tis (LCIS)	Lobular carcinoma in situ
Tis (Paget)	Paget's disease of the nipple with no tumor Note: Paget's disease associated with a tumor is classified according to the size of the tumor.
T1	Tumor ≤ 2 cm in greatest dimension
T1mic	Microinvasion ≤ 0.1 cm in greatest dimension
T1a	Tumor > 0.1 cm but not > 0.5 cm in greatest dimension
T1b	Tumor > 0.5 cm but not > 1 cm in greatest dimension
T1c	Tumor > 1 cm but not > 2 cm in greatest dimension
T2	Tumor > 2 cm but not > 5 cm in greatest dimension
T3	Tumor > 5 cm in greatest dimension
T4	Tumor of any size with direct extension to (a) chest wall or (b) skin, only as described below
T4a	Extension to chest wall, not including pectoralis muscle
T4b	Edema (including peau d'orange" or ulceration of the skin of the breast, or satellite skin nodules confined to the same breast
T4c	Both T4a and T4b
T4d	Inflammatory carcinoma

N – Regional lymph nodes	
NX	Regional lymph nodes cannot be assessed (e.g., previously removed)
N0	No regional lymph node metastasis
N1	Metastasis in movable ipsilateral axillary lymph node(s)
N2	Metastases in ipsilateral axillary lymph nodes fixed or matted, or in clinically apparent ipsilateral internal mammary nodes in the absence of clinically evident axillary lymph node metastasis
N2a	Metastasis in ipsilateral axillary lumph nodes fixed to one another (matted) or to other structures
N2b	Metastasis only in clinically apparent ipsilateral internal mammary nodes and in the absence of clinically evident axillary lymph node metastasis
N3	Metastasis in ipsilateral infraclavicular lymph node(s), or in clinically apparent ipsilateral internal mammary lymph node(s) and in the presence of clinically evident axillary lymph node metastasis; or metastasis in ipsilateral supraclavicular lymph node(s) with or without axillary or internal mammary lymph node involvement
N3a	Metastasis in ipsilateral infraclavicular lymph node(s) and axillary lymph node(s)
N3b	Metastasis in ipsilateral internal mammary lymph node(s) and axillary lymph node(s)
N3c	Metastasis in ipsilateral supraclavicular lymph node(s)

APPENDIX B (Continued)

pN – Regional lymph nodes	
pNX	Regional lymph nodes cannot be assessed (e.g., previously removed or not removed for pathologic study)
pN0	No regional lymph node metastasis histologically, no additional examination for isolated tumor cells
pN0(i-)	No regional lymph node metastasis histologically, negative IHC
pN0(i+)	No regional lymph node metastasis histologically, positive IHC, no IHC cluster > 0.2 mm
pN0(mol-)	No regional lymph node metastasis histologically, negative molecular findings (RT-PCR)
pN0(mol+)	No regional lymph node metastasis histologically, positive molecular findings (RT-PCR)
pN1mi	Micrometastasis (> 0.2 mm, none > 2.0 mm)
pN1	Mestastasis in one to three axillary lymph nodes and/or in internal mammary nodes with microscopic disease detected by sentinel lymph node dissection but not clinically apparent
pN1a	Mestastasis in one to three axillary lymph nodes
pN1b	Metastasis in internal mammary nodes with microscopic disease detected by sentinel lymph node dissection but not clinically apparent
pN1c	Mestastasis in one to three axillary lymph nodes and in internal mammary lymph nodes with microscopic disease detected by sentinel lymph node dissection but not clinically apparent
pN2	Metastasis in four to nine axillary lymph nodes, or in clinically apparent internal mammary lymph nodes in the absence of axillary lymph node metastasis
pN2a	Metastasis in four to nine axillary lymph nodes (at least one tumor deposit > 2.0 mm)
pN2b	Metastasis in clinically apparent internal mammary lymph nodes in the absence of axillary lymph node metastasis
pN3	Metastasis in 10 or more axillary lymph nodes, or in infraclavicular lymph nodes, or in clinically apparent ipsilateral internal mammary lymph nodes in the presence of one or more positive axillary lymph nodes; or in more than three axillary lymph nodes with clinically negative microscopic metastasis in internal mammary lymph nodes; or in ipsilateral supraclavicular lymph nodes
pN3a	Metastasis in 10 or more axillary lymph nodes (at least one tumor deposit > 2.0 mm), or metastasis to the infraclavicular lymph nodes
pN3b	Metastasis in clinically apparent ipsilateral internal mammary lymph nodes in the presence of one or more positive axillary lymph nodes; or in more than three axillary lymph nodes and in internal mammary lymph nodes with microscopic disease detected by sentinel lymph node dissection but not clinically apparent
pN3c	Metastasis in ipsilateral supraclavicular lymph nodes

M – Distant metastasis	
MX	Distant metastasis cannot be assessed
M0	No distant metastasis
M1	Distant metastasis

APPENDIX C: Study Drug Diary

☐ Anastrozole

☐ Letrozole

Please complete this diary every time you take your anastrozole or letrozole. You should also use this diary to record any side effects that you experience and medications that you take other than your anastrozole or letrozole. Please be sure to bring this diary with you to your next doctor's visit.

You will take one tablet of anastrozole or letrozole by mouth once a day. You should continue anastrozole or letrozole until you have your surgery (i.e. lumpectomy or mastectomy). Anastrozole or letrozole is a tablet that can be taken with or without food. You should take anastrozole or letrozole at around the same time every day. Please contact your study doctor or nurse with any new complaints or symptoms that you have after starting to take anastrozole or letrozole. If you have severe symptoms, you may be told to stop taking anastrozole or letrozole. Please do not make any changes in your anastrozole or letrozole dose without speaking with your study doctor or nurse.

Dose #	**Date Taken**	**Time Taken**	**Comments** (Side effects, other medications)

Dose #	Date Taken	Time Taken	Comments (Side effects, other medications)

Note: For any time that you did not take your anastrozole or letrozole (Example: You forgot to take it), write the reason that you did not take it in the "Comments" field.

Completed by:_____ Date: _____ Signature of Patient

Reviewed by: _____ Date: _____ Signature of Study Staff

APPENDIX D: Tissue Acquisition Form

Protocol Title: GCC1366 - A PROSPECTIVE STUDY OF NEOADJUVANT NON-STEROIDAL AROMATASE INHIBITORS IN POSTMENOPAUSAL WOMEN WITH OPERABLE HORMONE RECEPTOR-POSITIVE BREAST CANCER TO EVALUATE THE ANTI-PROLIFERATIVE RESPONSE IN OBESE AND OVERWEIGHT PATIENTS

Study Coordinator/Research Nurse: Please fill out and send form as instructed in section 8 of protocol.

Patient Initials _____ Screening ID#: S_____ Institution:_____ Attending physician: _____

Date consented for clinical protocol:_____Diagnosis: _____

Consent to Fresh Surgical Core that is frozen for left over tissue storage for future research? ___Y ___N

Origin of Tissue:_____Date Tissue Obtained: _____

Type of specimens: _____

Please mark:

☐ **Prior to starting treatment or original diagnosis procedure (T0)**

☐ **Clinical biopsy week 2-4 (T2)**

☐ **At the time of definitive surgery (T1)**

Please mark: ☐ **Anastrozole** ☐ **Letrozole**

Cohort: ☐ **1** ☐ **2** ☐ **3** ☐ **4** ☐ **5** ☐ **6**

Researcher: Please fill out

Date Samples Received:_____Data entered into database: Yes / No

Pathologist: Please fill out

Diagnosis:_____

Results of IHC staining:

ER_____% PR_____% Ki67_____% HER2_____by IHC

HER2 Ratio_____by FISH

Pathologist Name:_____ Date:_____

Completed form must be returned to:_____ Fax _____

APPENDIX E: Pro-inflammatory Cytokines, IRC, Estradiol levels and Metabolomic Profiling Specimen Log

Patient Initials:___ ___ ___ **Patient Screening ID #: S ___ ___ ___**
Institution:_____ **Attending physician: _____**

Please mark: ☐ Anastrozole ☐ Letrozole

Cohort: ☐ 1 ☐ 2 ☐ 3 ☐ 4 ☐ 5 ☐ 6

Sample	Date	Time drawn	Time processed	Comment
Prior to starting anastrozole or letrozole (B0)				
On the day of biopsy to assess clinical response (B2)				
On the day of surgery or within 3 days of surgery (B1)				

APPENDIX F: Registration Worksheet

Cohort 1 BMI<25.0 Anastrozole – Subject #	Subject Initials and Screening # (S101 etc.)	Confirmed BMI	Site
1001			
1002			
1003			
1004			
1005			
1006			
1007			
1008			
1009			
1010			
1011			
1012			
1013			
1014			
1015			
1016 (replacement only)			
1017 (replacement only)			
1018 (replacement only)			
Cohort 2 BMI ≥25.0-29.9 Anastrazole – Subject #			
2001			
2002			
2003			
2004			
2005			
2006			
2007			
2008			
2009			
2010			
2011			
2012			
2013			
2014			
2015			
2016 (replacement only)			
2017 (replacement only)			
2018 (replacement only)			
Cohort 3 BMI ≥30 Anastrazole – Subject #			
3001			
3002			
3003			
3004			

3005			
3006			
3007			
3008			
3009			
3010			
3011			
3012			
3013			
3014			
3015			
3016 (replacement only)			
3017 (replacement only)			
3018 (replacement only)			
Cohort 4 BMI<25.0 Letrozole – Subject #			
4001			
4002			
4003			
4004			
4005			
4006			
4007			
4008			
4009			
4010			
4011			
4012			
4013			
4014			
4015			
4016 (replacement only)			
4017 (replacement only)			
4018 (replacement only)			
Cohort 5 BMI ≥25.0-29.9 Letrozole – Subject #			
5001			
5002			
5003			
5004			
5005			
5006			
5007			
5008			
5009			
5010			
5011			
5012			

5013			
5014			
5015			
5016 (replacement only)			
5017 (replacement only)			
5018 (replacement only)			
Cohort 6 BMI ≥30.0 Letrazole – Subject #			
6001			
6002			
6003			
6004			
6005			
6006			
6007			
6008			
6009			
6010			
6011			
6012			
6013			
6014			
6015			
6016 (replacement only)			
6017 (replacement only)			
6018 (replacement only)			