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Alternative Dosing of Exemestane in Postmenopausal Women with Stage 0-II ER-Positive Breast Cancer: a Randomized Presurgical Trial

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SCHEMA

Alternative Dosing of Exemestane in Postmenopausal Women with Stage 0-II ER-Positive Breast Cancer: a Randomized Presurgical Trial

Screening visit (Italian sites only)
For women with prior histopathologic biopsy diagnosis of in-situ or invasive carcinoma without ER assessment, consent to obtain material for ER staining to determining eligibility
For women without a prior histopathologic biopsy, obtain consent to perform core needle biopsy with requisite guidance (palpation or imaging) to assess eligibility criteria based on a diagnosis of ER-positive in-situ or invasive breast carcinoma.

↓

Baseline clinic visit (within 14 days prior to randomization**)***
Postmenopausal women with cT_{0-2}, cN_{0-1}, M_x, histologically confirmed ER-positive primary breast cancer or women with larger tumors who refuse neo-adjuvant therapy before surgery. No previous treatment for breast cancer. Baseline medical history, physical exam, concomitant medication, baseline symptoms, tobacco/alcohol use assessment and blood collection for clinical lab tests and circulating biomarkers

↓

Randomization (day 0)
Eligibility confirmation, randomization (stratification by center and by BMI <25 versus ≥25 kg/m^2)

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Intervention (N=180)

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For at least four up to six weeks
Arm 1: Exemestane 25 mg/daily
Arm 2: Exemestane 25 mg/ 3 times a week (3 active tablets and 4 placebo tablets)
Arm 3: Exemestane 25 mg/once a week (1 active tablet and 6 placebo tablets)

↓

Treatment start (day 1)
The participant starts treatment, a telephone contact will be performed only if different from the randomization day

↓

Telephone contact (a week before surgery +3 days)
Review of self-reported compliance, concomitant medications, toxicity assessment.

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Final clinical visit (the day of surgery or the day before)
Physical exam, toxicity assessment, concomitant medications, blood collection for clinical lab tests and circulating biomarkers, compliance/review pill diary. The blood withdrawn for biomarkers will be at 12 hours, 1.5-2.5 days and 5.5-6.5 days from last active dose respectively for arm 1, 2 and 3 depending on scheduled final visit. Participants continue intervention until the night before surgery

↓

Surgery (4 to 6 weeks; ideally at day 29)
Surgery performed; Surgical specimens collected

***** After identifying the pool of 180 potentially eligible women with ER-positive in-situ or invasive breast cancer, women in Italy and the U.S. undergo identical procedures. Specifically, consent for blood draw, laboratory testing, completion of questionnaire, etc. -as outlined under baseline clinic visit- will be performed.

Endpoints
Primary endpoint: Percentage change in time of serum estradiol concentration at alternative dosing schedules compared to daily dose.
Secondary endpoints: Safety and toxicity, change in Ki-67 and PgR expression, tissue estradiol, exemestane and 17-dihydroxyexemestane concentration.
Circulating estrogens (including ultrasensitive estradiol measurement), androgens and sex hormone binding globulin, exemestane and 17-dihydroxyexemestane, adipokines, glucose, insulin and lipid profile. Further secondary endpoints will be proteomics and UGT2B17 genotype and measurement of crown-like structures in non-cancerous breast fat tissue.

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1. OBJECTIVES

1.1 Primary Objective

It has been shown that exemestane reduces estradiol levels both at the standard dose (25 mg/day) and at lower doses. We expect to achieve with 25 mg three times a week and 25 mg/week similar effects in estradiol reductions to the standard dose of 25 mg daily.
Non-inferiority of percent change in time of serum estradiol levels, adjusted for baseline levels, following four up to six weeks of exemestane 25 mg given three times per week or one time per week compared with exemestane 25 mg daily dosing.

1.2 Secondary Objectives

Secondary objectives are:

- To assess safety and toxicity.
- To support the preventive activity of exemestane we will investigate the change in Ki-67 and PgR levels in tumor cells and the adjacent intraepithelial neoplasia or benign histologic structures.
- To assess possible association of estradiol level with tissue and circulating biomarkers.
- To investigate possible pharmacogenetic markers.
- To assess drug levels on tissue samples.
- To investigate tissue and circulating proteomics profiling.

2. BACKGROUND

2.1 Breast Cancer

Breast cancer is the most common cancer in women and its incidence is rapidly increasing, largely because of an ageing population and several lifestyle changes, such as decreases in physical activity, increase in obesity, later age at first childbirth, and reductions in breastfeeding. Estrogen is a key factor in breast cancer carcinogenesis, and reductions in its synthesis can decrease breast cancer risk. Estrogen production is driven by the aromatase enzyme, which converts androgens to estrogens. Trials in the adjuvant setting have shown that aromatase inhibitors effectively prevent breast cancer recurrence (1) and breast cancer events in at-risk women (2,3).

The pre-surgical - window of opportunity - trial design has been successfully used to streamline drug development, check tumor sensitivity to a given drug and shape up new preventive strategies (4). The change in Ki-67 labeling index is considered an appropriate endpoint biomarker for preoperative studies (5,6). The post-treatment tumor expression of Ki-67 has been shown to be correlated with disease-free survival and overall survival (7). In addition the Ki-67 analysis on adjacent IEN or atypical hyperplasia can assess the potential preventive activity of the tested agent (8,9).

2.2 Exemestane

Exemestane is an irreversible aromatase inhibitor. This drug is highly specific and inhibits peripheral conversion of androstenedione to estrogen down to 98%. The drug was recently approved for the adjuvant setting, and the placebo–controlled phase III MAP.3 chemoprevention trial showed an overall 65% reduction in breast cancers and a specific ER-positive breast cancer reduction of 73% in the intervention arm compared to the placebo group (3).

Exemestane is in fact a steroidal aromatase inactivator with a long-lasting effect attributable to a tight covalent binding to the enzyme. The estradiol suppression persists for at least 5 days after administration of a single dose (10). This suggests, especially in a prevention setting, the possibility to reduce the dose and change the time schedule, while still maintaining a significant estradiol reduction. A study of 10 patients found that women who took a daily dose of 10 mg of exemestane achieved equivalent estradiol suppression as women who took a dose of 25 mg daily (11).
The exemestane steroidal structure suggests it may have androgenic properties that could potentially counteract any anti-estrogenic properties in bone and possibly sexual function and menopausal symptoms. The favorable profile on bone may represent an advantage of this agent in prevention settings in unaffected women at increased risk for breast cancer.

2.3 Rationale

Despite the strong positive findings in breast cancer prevention with tamoxifen, the attitude towards chemoprevention remains ambivalent, and the associated side effects hamper the drug uptake by high-risk women who could benefit from its preventive effects.
Among the strategies to overcome tamoxifen side effects, we have intensively investigated alternative low doses of tamoxifen in several phase II studies (12-14), and in two phase III studies (15,16).
More recently AIs have proven their capability to lower cancer incidence in high risk women. The Mammary Protocol 3 (MAP.3) trial (3) was a randomized, placebo-controlled, double-blind trial of exemestane 25 mg/day administered to postmenopausal women 35 years or older with at least one of the following risk factors: 60 years or older; Gail 5-year risk score greater than 1.66%; prior atypical ductal or lobular hyperplasia or LCIS; or DCIS with mastectomy. A total of 4,560 women (median age 62.5 years, median Gail risk score 2.3%) were randomly assigned to either exemestane or placebo. At a median follow-up of 35 months, 11 invasive breast cancers were detected in those given exemestane and 32 in those given placebo, with a 65% relative reduction in the annual incidence of invasive breast cancer (0.19% vs 0.55%; HR 0.35, 95% CI 0.18-0.70; p = 0.002).
The IBIS II Prevention trial included 1,920 women randomly assigned to receive anastrozole 1 mg daily and 1,944 assigned to placebo. After a median follow-up of 5 years, 2% of women in the anastrozole group vs. 4% in the placebo group have developed breast cancer (HR 0.47 95% CI 0.32, 0.68; $p < 0.0001$). The predicted cumulative incidence of all breast cancers after 7 years has been 5.6% in the placebo group and 2.8% in the anastrozole group (2). Musculoskeletal adverse events were common in the anastrozole group, but mostly of moderate severity. Vasomotor symptoms were common in both groups but the incidence was higher in women on anastrozole. No increases in fractures, myocardial infarction, or cardiac failure were observed between the groups.
However we might expect that an AI would achieve low acceptability similar to the SERMs by high risk women for the undesirable symptoms, bone density loss, musculoskeletal ache, climacteric syndrome, and ultimate possible impact on QoL, significantly compromising their motivation and adherence to a preventive treatment. Therefore, a significant step forward to promote breast cancer prevention in postmenopausal women is to ameliorate QoL issues by seeking for the minimally active AI dose and an intermittent schedule. A lighter schedule, compared to the standard regimen, may be much more appealing to a healthy at-risk woman.

For this purpose we are proposing a pre-surgical phase IIb, randomized, double-blind, multi-center study for postmenopausal women with histologically confirmed ER-positive breast cancer (Stage 0-II) comparing the exemestane standard daily dose regimen versus two alternative, less frequent dose regimens.

Rationale for Biomarkers
Estradiol: Overall, a strong positive association between breast cancer risk and circulating levels of

estrogens is now well confirmed among postmenopausal women; the top 20% hormone levels versus bottom 20% have a two- to three-fold higher risk of breast cancer (17). Interestingly, circulating estradiol levels can predict risk of hormone receptor positive breast cancer for up to 16-20 years. Top versus bottom quartile contrast RR was 2.0 (95% CI: 1.4-2.7; p trend<0.001) for <10 years, 2.0 (95%CI: 1.3-3.1; p trend=0.002) for >10-20 years before diagnosis (18).
The direct consequence of AIs in postmenopausal women is a steep decrease in circulating estrogens and their metabolites. For the assessment of exemestane activity at different doses and schedules the most appropriate biomarker appears to be circulating estradiol concentrations (19-21). In postmenopausal women, estradiol levels are low and their assessment is technically challenging as routine labs generally lack standardized sensitive methods. During estrogen suppression with AIs for breast cancer treatment and prevention, the accuracy of estradiol assay becomes of paramount importance as plasma levels are very low (<5 pmol/L). Liquid chromatography coupled with tandem mass spectrometry (LC-MS/MS) provides greater sensitivity and specificity than radio immunoassays (RIA).

Ki-67: Several studies indicate that staining with the Ki-67 antibody may be useful in monitoring response to anti-estrogen therapy. We previously conducted a presurgical trial assessing tamoxifen dose escalation. Our study showed that the effects of tamoxifen on breast cancer cell proliferation, as assessed by the change in the percentage of cells expressing Ki-67, was comparable among women who received a daily tamoxifen dose of 1 mg, 5 mg, or 20 mg administered for 4 weeks (22). These results led us to focus on low dose tamoxifen in several chemoprevention trials. Ki-67 proliferation index has hence increasingly been used as a surrogate endpoint. Additional evidences from neoadjuvant trials pointed to a prognostic value of Ki-67. Measurements of tumor Ki-67 level after only 2 weeks treatment with either tamoxifen or anastrozole or their combination improved the prediction of recurrence-free survival over baseline Ki-67 after a median of 3 years in postmenopausal women (6,23).
Our findings on standard and low dose tamoxifen confirmed this observation, Ki-67 expression after 4 weeks of tamoxifen predicted recurrence-free and overall survival significantly better than baseline Ki-67. Post-tamoxifen Ki-67 point increase predicted a 5% significant increased risk of breast cancer recurrence. Likewise, overall mortality, mostly due to breast cancer, was better predicted by post-tamoxifen Ki-67 (7).
More recently, we conducted a WOP trial in postmenopausal women with exemestane (25 mg/day) demonstrating a significant reduction in Ki-67 (a 10 point decrease) without any significant toxicity(24). To support the hypothesis of an alternative dose , the Ki-67 was reduced at the same extent irrespective of time since last drug intake (≤24 hours or >24 hours) and serum concentrations of exemestane (10 nM vs 2 nM), according to time since last drug intake (25).

Progesterone Receptor (PgR): Compared to Ki-67 the role of PgR is less clear, at least with tamoxifen treatment (26). Its modulation by tamoxifen is controversial, some studies showed no significant effect on PgR expression. In a low percentage of participants a decreased expression after short tamoxifen treatment has been observed, and these participants showed a worse response to the treatment, but the data were more informative if both pre and post treatment PgR expression was taken into account (27). Moreover, this receptor may change in time also with no treatment. Similarly to Ki-67 exemestane can significantly reduce PgR expression after 6 weeks of treatment (24). It will be useful to compare the modulating effect by exemestane dose and eventually correlate it to Ki-67 changes.

Sex Hormones: Similarly to estradiol, other circulating sex hormones are involved in breast carcinogenesis, and their analysis can contribute to a better understanding of their role in the tumor pathogenesis and drug efficacy through their modulation. Among postmenopausal women, higher levels of endogenous sex hormones and lower levels of sex hormone binding globulin (SHBG) are associated with increased risk of breast cancer. Literature data clearly show the AIs activity on these hormones is due to the direct enzyme inactivation. We have recently published data in premenopausal women that

showed in a multivariable analysis that SHBG had an HR = 2.26 (95 %CI 1.04-4.89) for the lowest versus the highest tertile (28). Endocrine effects of exemestane standard treatment in postmenopausal breast cancer patients result in suppression of estrone, estradiol and estrone sulfate and lowering of SHBG levels, and it increases testosterone, DHEAS and FSH levels. In this study it will be useful to compare the modulation of such hormones to evaluate the drug activity at different schedules. Their evaluation may help to understand the activity of the different proposed doses, and eventually provide an indication to response prediction (19,29,30).

Insulin – Insulin resistance: There is evidence of an association of androgen excess and insulin resistance, glucose intolerance and dyslipidemia in animal models as well as in women. Insulin resistance is considered an important feature of the metabolic syndrome, and is a risk factor for cardiovascular diseases, type 2 diabetes and cancer (31,32).
In aromatase deficient mice, in association with the increased levels of androgens, increased abdominal adiposity and higher insulin levels has been described. Several polymorphisms of the aromatase gene CYP19 (rs1008805, rs2446405 and rs2414096) are associated with increased risk for insulin sensitivity and diabetes in African Americans, Caucasians and Japanese(33). Based on these observations, we will measure insulin, glucose levels and insulin sensitivity (HOMA) to assess whether there is a different effect among the three exemestane doses.

Lipid profile: A concern with the use of aromatase inhibitors relates to an increased risk of cardiovascular events. It has been reported that, in postmenopausal women, the increase in cardiovascular risk may be ascribed to changes in lipid metabolism and, above all, to the elevation of low-density lipoprotein (LDL) cholesterol. Amongst aromatase inhibitors, exemestane may potentially have a more neutral effect on circulating lipid changes compared to letrozole and anastrozole. After 6 weeks treatment of exemestane (24), total and HDL cholesterol decreased by -10 mg/dL (IQR -21 to –2) and _-7 mg/dL, (IQR -14 to -2), respectively. Triglycerides were reduced by -8 mg/dL (IQR -28 to –9). Similar results were previously described, however associated with an increase in LDL-cholesterol(34,35). Based on these observations, we will measure total, LDL,HDL cholesterol and triglyceride levels to assess whether there is a different effect among the three exemestane doses.

Adipokines: In the global picture of breast carcinogenesis, adipose tissue has been shown to play an important role. In particular, two adipokines, leptin and adiponectin, have been studied as risk biomarkers for breast cancer associated with obesity in postmenopausal breast cancer patients. Leptin may influence breast cancer through enhancing expression of aromatase providing a link between body weight (adipose tissue), estrogen levels and the risk for breast cancer development as well as poor prognosis (36). Adiponectin appears to have anti-inflammatory, antiatherogenic, anti-angiogenic and anti-diabetic properties. Serum adiponectin levels are reduced in obese women and they increase after relevant weight loss. Low levels of adiponectin are also closely linked to insulin resistance and hyperinsulinemia, which were demonstrated to be positively associated with breast cancer risk (37).
In a few subjects, it has been reported that exemestane can reduce leptin plasma level (38); if this effect can be confirmed on a larger population it can be considered an additional activity and potential effect for cancer treatment as well as prevention.

Proteomics: Proteomic analyses have been studied, with varying success, to identify on breast cancer tissue an expression profiling in order to discover protein biomarkers/signatures suitable for: characterization and subtyping of tumors, early diagnosis, and both prognosis and prediction of outcome (39). This dataset will allow us to run spectra network analysis for protein identification and comprehensive characterization of post-translational modifications, in addition to the traditional database search approach. It will also allow us to further develop the spectra network analysis algorithm for higher

charged peptides using multiple fragmentation capability of the new Fusion Tribrid mass spectrometer in Dr. Chen's Proteomics Shared Resource for the Herbert Irving Comprehensive Cancer Center (HICCC) at CUMC (40,41).

UGT2B17: Exemestane and its major metabolite 17-dihydroexemestane are metabolized by UGT enzyme. UGT genetic variations play a role in altered 17-dihydroexemestane glucuronidation and overall exemestane metabolism. The prevalence of the polymorphic UGT2B17 whole-gene deletion is approximately 30% in Caucasians. In vitro studies UGT2B17 gene deletion correlates with a significant decrease of 17-dihydroexemestane excretion and consequently increasing circulating levels (42). This could reflect an increased exemestane activity. We have data from a pre-surgical study, consistent with these observations, showing a significant association of the UGT2B17 deletion with increased serum concentration of 17-dihydroexemestane (25). Interestingly, Ki-67 decreased equally irrespective of time since last drug intake supporting the rationale of testing differential drug schedules.

Crown-like structures: The potential links between obesity, inflammation, and aromatase expression are still unclear. In both dietary and genetic models of obesity, necrotic adipocytes surrounded by macrophages forming crown-like structures (CLS) in the mammary glands and visceral fat have been observed. The presence of CLS was associated with activation of NF-κB and increased levels of proinflammatory mediators (TNF-α, IL-1β, Cox-2), which were paralleled by elevated levels of aromatase expression and activity in the mammary gland and visceral fat of obese mice (43). These preclinical findings were further assessed in 30 women undergoing breast surgery (44). CLS of the breast (CLS-B) was found in nearly 50% (14 of 30) of patient samples. The severity of breast inflammation, defined as the CLS-B index, correlated with both body mass index ($P < 0.001$) and adipocyte size ($P = 0.01$). Increased NF-κB binding activity and elevated aromatase expression and activity were found in the inflamed breast tissue of overweight and obese women.

3. SUMMARY OF STUDY PLAN

We will conduct a multicenter, pre-surgical double-blind non-inferiority phase IIb study in which participants will be randomized to receive either exemestane 25 mg/day or 25 mg/ three times a week or a single dose of 25 mg/week for a minimum of 4 up to 6 weeks.

A total of 180 post-menopausal women will be accrued; 60 per arm, enrolled in 35 months and treated for a minimum of 4 up to 6 weeks.

Participants will be histologically-confirmed ER-positive (ER $\geq$10%) primary breast cancer patients who are candidates for breast surgery. Postmenopausal women younger than 76 years of age with cT_{0-2}, cN_{0-1}, M_x or women with larger tumors who refuse neo-adjuvant therapy before surgery are eligible. No previous treatment for breast cancer is allowed.

Complete physical exam and safety lab tests will be performed at baseline and at end of treatment (28+1, 35+1, 42+1 days). Phone contact will occur at day 1 and a week before surgery (+3 days).

Biomarkers: blood samples will be collected at baseline and the end of treatment (fasting blood for biomarkers will be collected prior to randomization and either on the day of surgery or the day before; fasting is strongly recommended but is not mandated), tissue samples will be collected from the diagnostic or research biopsy and at the time of surgery.

Duration of study will be four years: 35 months for accrual, 4-6 weeks of treatment, and one year for biomarkers measurement.

4. PARTICIPANT SELECTION

4.1 Inclusion Criteria

4.1.1 Postmenopausal women (* postmenopausal: age ≥60 years, or amenorrhea ≥ 12 months, or bilateral oophorectomy, or - in women with hysterectomy only - FSH in the menopausal levels as per local institutional guidelines if < 60 years old) with histologically-confirmed ER-positive (≥ 10%) primary breast cancer stage cT_{0-2}, cN_{0-1}, M_x. Women with larger tumors who refuse chemo and/or endocrine neoadjuvant therapy can be eligible.

4.1.2 ECOG performance status ≤1 (Karnofsky ≥70%; see Appendix A).

4.1.3 Participants must have normal organ and marrow function as defined below:

Leukocytes	≥3,000/microliter
Absolute neutrophil count	≥1,500/microliter
Platelets	≥100,000/microliter
Total bilirubin	≤ 2 x institutional ULN
AST (SGOT)/ALT (SGPT)	≤1.5 × institutional ULN
Serum creatinine	≤ 1.5 times institutional ULN

4.1.4 Ability to understand and the willingness to sign a written informed consent document.

4.2 Exclusion Criteria

4.2.1 BMI < 18.5 Kg/m^2.

4.2.2 Previous treatment for breast cancer including chemotherapy, endocrine therapy and radiotherapy. Women with prior DCIS who were treated with surgery only and whose treatment ended ≥2 years prior to enrollment are eligible for the trial.

4.2.3 Women who are planned to receive neoadjuvant therapy.

4.2.4 Participants may not be receiving investigational agents.

4.2.5 History of allergic reactions attributed to compounds of similar chemical or biologic composition to exemestane.

4.2.6 Uncontrolled intercurrent illness including, but not limited to, ongoing or active infection, symptomatic congestive heart failure, unstable angina pectoris, cardiac arrhythmia, or psychiatric illness/social situations that would limit compliance with study requirements.

4.2.7 Other co-existing invasive malignancies (with the exclusion of basal cell carcinoma or skin squamous cell carcinoma) diagnosed during the last 2 years before randomization.

4.2.8 History of severe osteoporosis (T score ≤ -4 either spine or hip), or presence of vertebral fracture.

4.2.9 Use of systemic Hormone Replacement Therapy (HRT) in the last 30 days prior to the randomization. The use of non-systemic estrogen (such as vaginal estrogen use) is allowed.

4.2.10 Use of any chemopreventive agents (SERM) in the last 3 months.

4.2.11 Concomitant use of CYP3A4 inducer medication (rifampicin, phenytonin, carbamazepine, phenobarbital, and St. John's wort).

4.3 Inclusion of Women and Minorities

Participants will be adult women of all races and ethnic groups. Breast cancer is extremely rare in men and men have limited amounts of normal breast tissue; therefore, male participants will not be recruited to this trial. Children will not be recruited to the trial because breast cancer is not relevant to the child population. Every attempt will be made to recruit minority women at the Italian sites. However, given that the distribution of the Italian population mainly consists of white Caucasians (about 93%), we expect very few participants of different ethnic background to be enrolled at the Italian sites. At the US sites efforts will be made to enroll women from a diversity of ethnic and socio-economic backgrounds.

4.4 Recruitment and Retention Plan

This multicenter protocol will be conducted at the following five sites: the European Institute of Oncology (IEO), Milan, Italy; the E.O. Galliera, Genoa, Italy; the Columbia University Medical Center (CUMC), New York, NY; the UT MD Anderson Cancer Center, Houston, TX; and the Moffitt Cancer Center, University of South Florida, Tampa, FL. The IEO will be the Lead Site. The high rate of breast cancer surgeries at these sites will allow the recruitment of the required samples size. Efforts will be made to enroll women from a diversity of ethnic and socio-economic backgrounds. Recruitment and retention effort will be evaluated routinely by the site coordinator and the study staff. The study recruitment and retention plan will be modified as necessary to promote rapid accrual and to assure 100% recruitment rate of participants. Please refer to the study-specific recruitment and retention plan for more details.

5. AGENT ADMINISTRATION

Intervention will be distributed on an outpatient basis. Reported adverse events (AEs) and potential risks are described in Section 6.2.

5.1 Dose Regimen and Dose Groups

Arm 1
Agent: Exemestane
Doses: one dose of 25 mg once a day
Duration: 4 to 6 weeks

Arm 2
Agent: Exemestane
Dose: 25 mg three times a week
Duration: 4 to 6 weeks

Arm 3
Agent: Exemestane
Doses: 25 mg once a week
Duration: 4 to 6 weeks

5.2 Exemestane Administration

Investigational drug pharmacy will be responsible for drug distribution. Dr. Bonanni (PI) and the local Site Investigators or their designees will be responsible for drug administration. They can delegate to other study investigators (MDs and research nurses) to distribute the agent on their behalf.

Participants will receive a 6-week supply (2 wallets containing 3 blisters each) of the drug/placebo as outlined in the clinical procedure schema (see Section 7.1 for a detailed description). Each wallet will consist of 3 blisters, each blister for 7 days of treatment. Each blister will have 7 tablets numbered from 1 to 7 to clearly mark the order to be taken by the participants. Based on the arm, the blister will contain 7 active tablets 1 through 7, or three active tablets corresponding to days 1, 3 and 5 and four placebo tablets at days 2, 4, 6 and 7, or one active tablet placed at day 1 and 6 placebo in the remaining 6 days. The participant will be advised to take one tablet per day after dinner, always at approximately 8 PM, starting from day 1 till the night before surgery. If for any reason they will skip a tablet they will be instructed to leave it behind and take the one for the corresponding day. Participants will be asked to contact the study coordinator the first time they miss a dose.

5.3 Run-in Procedures

No run-in procedure will take place in the present protocol.

5.4 Contraindications

Eligible participants should not have specific contraindication to exemestane, considering also the short period of treatment. Any pre-existing musculoskeletal disorder should be carefully evaluated before enrolling the participants. Furthermore, medications that may impact participant safety or scientific integrity of the study are non-allowed, such as the following: strong CYP 3A4 inducers (*e.g.*, rifampicin, phenytoin, carbamazepine, phenobarbital, or St. John's wort).

5.5 Concomitant Medications

All medications (prescription and over-the-counter), vitamin and mineral supplements, and/or herbs taken by the participant will be documented on the concomitant medication CRF and will include: 1) start and stop date, dose and route of administration, and indication. Medications taken for a procedure (*e.g.*, biopsy) should also be included.

5.6 Dose Modification

Participants will be asked to maintain the full dose throughout the treatment period. Due to the short time of treatment no dose modification will be applied.

Toxicity will be evaluated using the NCI terminology criteria (CTCAE version 4.0.3, published 06/14/2010).

If grade 1 or 2 toxicity occurs, the participant will be maintained on treatment irrespective of attribution to

the study drug. In case of grade 3 toxicity unrelated or unlikely to be related to study treatment, participant may remain on treatment as per physician judgment, women who experience other grade 3 or more severe adverse events will be removed from the study.

Toxicity	Attribution to study drug				
Grade	Unrelated	Unlikely	Possible	Probable	Definite
1	C	C	C	C	C
2	C	C	C	C	C
3	C^1	C^1	W	W	W
4	W	W	W	W	W

C = Continue drug

C^1 = Continue drug as per physician judgment

W = Withdrawal

5.7 Adherence/Compliance

5.7.1
There is no "gold standard" yet on how to measure participant compliance. Recent data from ongoing chemoprevention studies have estimated compliance to be between 60% and 100%, and this disparity can be attributed to adherence methodology assessment.
Compliance estimates will be limited to pills that fall within the required dosing period (depending on total number of days between first dose and surgery). To be compliant, a participant has to take ≥ 80% of the active scheduled pills overall and also specifically, ≥ 80% compliant in the last 7 days before surgery. We propose the use of multiple methods of adherence monitoring: subject self-reporting, pill diary and tablets count. Drug and its metabolite will be also measured, but due to different schedules and the half-life of exemestane, these measurements may not be indicative of compliance for arms 2 and 3.

5.7.2
Pill diary completion: Each participant will receive a 42 days diary to facilitate tracking and recording of pill intake (Appendix B). Each participant will be asked to write Yes/No in the corresponding day of the diary and to register the time of drug intake. Participants are asked to fill the diary and some additional space will be left for participant's notes. Participants will be asked to contact the study coordinator the first time they miss a dose. Each pill diary will be returned at the final scheduled visit. The amount of pills and the pill diary are for the total admitted period of treatment.

Dose count: Each participant will receive a 42 day supply of the drug / placebo and will be asked to return both wallets with full and empty blisters. Overall compliance will be measured by blinded staff as follows:
number of tablets taken (i.e., number of tablets given-number of tablets returned)/number of tablets that should have been taken during that period of time.
Any missed pill in each blister will be recorded in order to collect the compliance of active pills at a later stage (unblinded statistician).

In cases where the tablets are not returned, the compliance will be calculated only from the pill diary.

Drug/metabolite plasma levels: Circulating levels of exemestane and 17-dihydroxyexemestane will be

measured using LC-MS/MS technology.

6. PHARMACEUTICAL INFORMATION

6.1 Exemestane (IND# 52662, IND Sponsor: NCI, DCP)

Exemestane is a steroidal, suicide inhibitor of aromatase, the principal enzyme that converts androgens to estrogens. Exemestane is approved for adjuvant treatment of postmenopausal women with estrogen receptor-positive early breast cancer after receiving 2−3 years of prior tamoxifen therapy and for treatment of advanced breast cancer in postmenopausal women whose disease has progressed following tamoxifen therapy. The usual dose is 25 mg once daily after a meal since drug bioavailability is increased 40% with food. Though not approved for breast cancer prevention, exemestane administered to postmenopausal women with a moderately increased risk of breast cancer (≥60 years; Gail five-year risk score >1.66%; prior atypical ductal or lobular hyperplasia or lobular carcinoma *in situ*; or ductal carcinoma *in situ* with mastectomy) resulted in a 65% relative risk reduction of invasive breast cancer compared with placebo treatment after three years of follow-up in the MAP.3 trial (3). NCI, DCP is continuing to evaluate exemestane for breast cancer prevention.

Film-coated 25 mg exemestane tablets are provided as white, round, lenticular tablets with uniform appearance and intact edges for once daily oral administration after a meal (Actavis Group PTC ehf, initial United Kingdom (UK) approval in 2010. Full prescribing information is in the summary of product characteristics (SPC) available at: www.medicines.org.uk/emc/medicine/24704). Each tablet contains 25 mg exemestane and the following excipients in its core: povidone K30, maize starch (bleached), starch (partially pregelatinized), sodium starch glycolate type A, cellulose microcrystalline type 101, talc, silica (colloidal anhydrous), magnesium stearate, and polysorbate 80. The tablet film coating contains: partly hydrolyzed polyvinyl alcohol (PVA), titanium dioxide (E171), macrogol 3350, and talc.

Matching placebo tablets are round, lenticular, film-coated tablets containing partially pregelatinized maize starch (Starch 1500), microcrystalline cellulose (Avicel PH102), sodium starch glycolate (Explotab), colloidal silicon dioxide (Aerosil 200), and magnesium stearate (Ligamed MF-2-V). The film coating is Opadry II white, which is PVA based.

Blinded blister packages of study medication (exemestane and placebo tablets) for the three different dosing regimens will be provided: Arm 1) 25 mg exemestane qd (once daily, seven exemestane tablets per week); Arm 2), 25 mg exemestane 3 times a week (three exemestane tablets and four placebo tablets per week); and Arm 3), 25 mg exemestane qw (once weekly, one exemestane tablet and six placebo tablets per week).

6.2 Reported Adverse Events and Potential Risks

According to the UK SPC for 25 mg exemestane film-coated tablets from Actavis UK Ltd., the most commonly reported adverse events (AEs) by early breast cancer patients treated with exemestane were (in order of frequency): hot flushes (22%), arthralgia (18%) and fatigue (16%). AEs similarly or less commonly reported by early breast cancer patients treated with exemestane compared to tamoxifen were headache (14%), insomnia (13%), sweating increased (12%), gynecological disorders (11%), and dizziness (10%). Similar to early breast cancer, in advanced BC hot flushes were the most common AE (14%), followed by nausea (12%). Other common AEs (≥1% to <10%) seen in clinical studies and postmarketing reports were: anorexia, alopecia, carpal tunnel syndrome, constipation, depression, diarrhea, dyspepsia, fracture, osteoporosis, other primary cancer, pain, paresthesia, peripheral edema,

pruritis, reduction in bone mineral density, rash, urticaria, vaginal haemorrhage, visual disturbance, and vomiting. Uncommon AEs (≥0.1% to <1%) were: acute generalized exanthematous pustulosis, asthenia, gastric ulcer, hepatitis (including cholestatic hepatitis, hepatic enzyme increased, blood bilirubin increased, and blood alkaline phosphatase increased), leucopenia, lymphocyte count decreased, somnolence, thrombocytopenia, and thromboembolism. Though rare, ischemic cardiac events were not significantly different with exemestane compared to tamoxifen (4.5% *vs*. 4.2%), including individual AEs hypertension (9.9% *vs*. 8.4%), myocardial infarction (0.6% *vs*. 0.2%), and cardiac failure (1.1% *vs*. 0.7%) (Full prescribing information in the SPC is available at: www.medicines.org.uk/emc/medicine/24704).

6.3 Availability

Exemestane and matching placebo tablets in blister packages will be supplied by NCI, DCP.

6.4 Agent Distribution

The European Institute of Oncology will be responsible for the distribution to the Italian sites. Exemestane will only be released by NCI, DCP after documentation of IRB approval of the DCP-approved protocol and consent is provided to DCP and the collection of all Essential Documents is complete (see DCP website for description of Essential Documents).

NCI, DCP-supplied agents may be requested by the Investigator (or their authorized designees) at each Organization. DCP guidelines require that the agent be shipped directly to the institution or site where the agent will be prepared and administered. DCP does not permit the transfer of agents between institutions (unless prior approval from DCP is obtained). DCP does not automatically ship agents; the site must make a request. Agents are requested by completing the DCP Clinical Drug Request form (NIH-986) (to include complete shipping contact information) and faxing or emailing the form to the DCP agent repository contractor:

John Cookinham
MRIGlobal
DCP Repository
1222 Ozark Street
North Kansas City, MO 64116
Phone: (816) 360-3805
FAX: (816) 753-5359
Emergency Telephone: (816) 360-3800
Email: NCI.DCP@mriglobal.org

6.5 Agent Accountability

The Investigator, or a responsible party designated by the Investigator, must maintain a careful record of the inventory and disposition of all agents received from DCP using the NCI Drug Accountability Record Form (DARF) or an institutionally-approved accountability system. The Investigator is required to maintain adequate records of receipt, dispensing and final disposition of study agent. This responsibility has been delegated to the local pharmacist. Include on receipt record from whom the agent was received and to whom study agent was shipped, date, quantity and batch or lot number. On dispensing record, note quantities and dates study agent was dispensed to and returned by each participant.

6.6 Packaging and Labeling

Exemestane and matching placebo tablets for the three dosing arms will be packaged in blinded blister packages and distributed by NCI, DCP or its authorized designee.

Tablets will be packaged in blister card packaging. Each blister card will contain 7 tablets. Participants will receive two wallets of 3 blister cards each (6 blister cards for up to 6 weeks of treatment).

A three-part label will be used for blinding provided by the NCI DCP Repository.

The fixed part of the label will remain attached to the blister cards wallet and will identify the following:

> Exemestane 25 mg or Placebo 21 Tablets
> Expiration Date: XX-XXXX
> Protocol: MDA2014-04-01
> Patient ID: _____
> Rand #: _____
> Dispensed Date: _____
>
> Take one tablet once a day at the same time each day.
> Store at Controlled Room temperature 59°F - 77°F (15°C - 25°C)
> Keep Out of Reach of Children
>
> Distributed by MRIGlobal for NCI
> 1222 Ozark Street
> North Kansas City, MO 64116
>
> CAUTION: NEW DRUG – LIMITED BY FEDERAL LAW TO INVESTIGATIONAL USE ONLY

The first tear-off portion of the label will be removed from the wallet at the time of dispensing and will be affixed to the Compliance Drug Labels form (Appendix C). This portion of the label will be identical to the first portion of the label, except that it will contain a scratch off area with the unblinding information should the PI need to be unblinded in case of emergency (See Section 6.9).

The second tear-off portion of the label will be removed from the wallet by the pharmacist at the time of dispensing. This portion of the label will contain unblinded information and will be maintained by the pharmacist following institutional guidelines or maybe destroyed if this portion of the label is not needed for drug accountability purposes.

6.7 Storage

Study drug will be stored in a secure location at controlled room temperature, with excursions permitted between 15°-25°C (59°-77°F).

6.8 Registration/Randomization

Screening and Registration into the DMI Database:

Once informed consent has been signed, participants will be registered into the DMI database. The DMI database will assign a participant's PID upon completion of the registration process.

Randomization:
Participants will be assigned a randomization number once the following has been accomplished: eligibility has been verified at the site level, eligibility has been confirmed by the site PI, eligibility CRF has been entered into the DMI web application and the randomization process has been completed by CLO staff. Once eligibility has been verified and confirmed by the CLO staff, the randomization number will be generated by the database and assigned to the participant. Refer to Section 13.2 for details of randomization.

Screening/Registration/Randomization into site-specific databases:
The DMI is the database of record for the study. Registration and randomization should occur per the procedures outlined above. If the site staff need to enter study data into site-specific electronic databases per their institutional requirements, they should do so in accordance with their institutional policies and procedures.

Appropriate CRFs must be completed for any participant who signs an informed consent. If a consented participant is a screen failure and deemed ineligible, the following CRFs must be completed: 1) the Registration CRF; 2) the Randomization CRF with the eligibility box checked "no", 3) the Inclusion and Exclusion CRFs showing why the participant is ineligible, 4) the Off-Study CRF, 5) the Adverse Event CRF, 6) the Concomitant Medication CRF and 7) the Verification CRF. If no Adverse Event and/or Concomitant Medications were assessed by the time the participant is deemed ineligible, the "NONE" box will be checked to complete both CRFs. All participants who sign an informed consent must formally go off study. All participant registration information will be entered into DMI. If a participant experiences a serious adverse event during the screening process, an SAE form must be completed.

6.9 Blinding and Unblinding Methods

- Participants will be blinded to exemestane schedule.
- The Statistician and the Site Study Pharmacist will not be blinded to exemestane schedule.
- All other Investigators will be blinded to exemestane schedule.
- All participants will take 1 tablet per day.
- Study assignments will be unblinded to the Study Investigators and Site Coordinators after all of the data are collected and the study database has been locked. Unblinding will also occur if the participant's physician deems that unblinding is necessary, such as in the case of unacceptable toxicity thought to be related to the study agent or progressive disease.
- The Data and Safety Monitoring Board will also be blinded unless unblinding is warranted (if the participant's physician deems that unblinding is necessary, if the participant becomes pregnant, or after all of the data are collected and the study database has been locked).
- Unblinding will only take place after consultation with the NCI, DCP Task Order Monitor (Medical Monitor). Unblinding will be conducted as follows:

1) The Site Investigator contacts the Protocol Principal Investigator and requests the participant's treatment status be unblinded.

2) The Protocol Principal Investigator contacts the NCI, DCP Task Order Monitor (Medical Monitor) and requests the participant's treatment status be unblinded. The Protocol Principal Investigator then conveys the Task Order Monitor's decision to the Site Investigator. The Site Investigator then proceeds with unblinding as written out below.

3) If the NCI Task Order Monitor cannot be reached and the participant requires emergency care, the Protocol Principal Investigator may authorize the Site Investigator to break the blind.

4) If the Site Investigator is unable to reach the Protocol Principal Investigator and the participant requires emergency care, then the Site Investigator must proceed with unblinding as written out below.

5) The Site Investigator requests the participant's treatment status be unblinded by the research pharmacist (or designated individual responsible for dispensing drug).

6) The Site Investigator officially takes the participant off-study.

7) The date and reason for breaking the blind must be submitted by the Site Investigator to the Protocol Principal Investigator, Bernardo Bonanni, MD, as soon as possible.

8) It is the responsibility of the Protocol Principal Investigator to report the date and reason for breaking the blind to the **NCI Medical Monitor, Eva Szabo, MD,** via email to szaboe@mail.nih.gov, as soon as possible after receiving this information from the Site Investigator.

9) The date and reason for breaking the blind must be submitted by the Protocol Principal Investigator to the MD Anderson Consortium Principal Investigator, Powel H. Brown, MD, PhD, or designee as soon as possible via email to phbrown@mdanderson.org or lavornik@mdanderson.org or phone at (713) 792-4509.

10) The date and the reason for breaking the blind will be reported by the MD Anderson Consortium Principal Investigator or designee to the MD Anderson DSMB as soon as possible.

6.10 Agent Destruction/Disposal

At the completion of investigation, all unused study agent will be returned to NCI, DCP Repository according to the DCP "Guidelines for AGENT RETURNS" and using the DCP form "Return Drug List".

The guidelines and the form are available on the DCP website.

7. CLINICAL EVALUATIONS AND PROCEDURES

7.1 Schedule of Events

The possibility of taking part in a pre-surgical trial will be presented to incoming postmenopausal women with a highly suspicious palpable lesion or a newly diagnosed ER-positive DCIS or breast cancer. The baseline visit will be scheduled for interested potential participants after histological confirmation.

SCHEDULE OF EVENTS

Evaluation/ Procedure	Screening Visit[1]	Baseline Visit/ Registration (within 14 days prior to randomization)	Randomization	Phone call (Day 1)	Phone call (1 week +3 days prior to surgery)[2]	Final Visit (Days 28+1, 35+1, or 42+1)[3]	Surgery (Days 29, 36 or 43) [12]
Screening Informed Consent	X						
Tru-cut biopsy (Eligibility ER + breast cancer)	X						
Informed Consent		X					
Assess eligibility		X					
Medical history		X					
Physical exam/ height/weight/vital signs		X				X[9,10]	
Tobacco / alcohol use assessment		X					
Fasting blood lab tests[4]		X				X	
Blood lab tests that may not be fasting[5]		X				X[10]	
Fasting blood for biomarkers [6]		X				X	
Tissue for biomarkers (FFPE blocks or slides)		X					X
Frozen tissue for biomarkers							X
Frozen tissue for proteomics[11]							X[11]
Concomitant medications		X			X	X	
Baseline symptoms		X					
Confirm eligibility		(X)	X				
Randomization			X				
Dispense study agent[13]			X[13]				
Treatment initiation				X [7]			
Collect study agent						X	X
Compliance					X	X	
Review agent diary/record						X	
Adverse events					X	X	
QoL questionnaire[8]		X				X	

[1] For the Italian sites only
[2] If the participant is seen on site for the physical exam and blood safety tests on Day 7 prior to surgery, perform all procedures in person rather than as a phone call. In all other cases call the participant on the phone 1 week +3 days prior to surgery
[3] Based on institutional procedure, the final visit may occur on the same day as surgery or the day before. The Final Visit must be

conducted on Days 28+1, 35+1, or 42+1.
[4] **Fasting** blood lab tests (local lab): Glucose, total cholesterol, HDL cholesterol and triglycerides. Fasting is strongly recommended, but is not mandated. Every effort should be taken to draw these labs fasting.
[5] Blood tests for safety that do not require fasting: complete blood count (CBC), AST, ALT, Na, K, BUN, total bilirubin, Cl, Ca, alkaline phosphatase, creatinine, and FSH if needed
[6] **Fasting** blood collection for biomarkers: estradiol, exemestane, 17-dihydroxyexemestane, estrone, estrone-sulphate, androstenedione, testosterone, SHBG, insulin, leptin, adiponectin. Fasting is strongly recommended, but is not mandated. Every effort should be taken to draw these labs fasting.
[7] The first phone call is not performed if the day of call coincides with the day of the baseline visit
[8] Quality of Life questionnaire
[9] Do not repeat the height assessment at the Final Visit. Height measurement will be used from the baseline visit
[10] May be done within 7 days prior to surgery or the day of surgery
[11] US Sites Only
[12] Surgery must occur on Days 29, 36 or 43. For this study coordinators are advised to count back from the day of surgery so that participants can either have 28, 35 or 42 days of study agent (ending on the 7th day of blister pack). Study agent can be dispensed in advance of starting agent on Day 1, However, coordinators must remind the participants not to start the study agent until the agent start date is provided to them by the coordinating center, in most cases via a telephone call from the coordinating site to the participant, indicating when to start the agent.
[13] In person or by mail.

Unresolved AEs with a "possibly", "probably" or "definitely" attribution to study agent at final visit will be monitored via telephone call at 20-30 days after surgery. AEs occurred after surgery will not be recorded.

7.2 Pre-study Testing at Italian Sites

At the Italian sites, women who wish to consider participating in the trial but have not received a confirmed diagnosis of ER-positive in-situ or invasive breast cancer will require consent for additional evaluations to determine eligibility. Specifically, women who have undergone a histopathologic biopsy diagnosed as in-situ or invasive cancer that has not been stained for ER will be consented to provide access to archival tissue for immunohistochemistry. This may require access to samples from non-study sites. Among women who have not undergone a histopathologic biopsy, consent will be sought to perform core needle biopsies (guided by palpation or imaging) to establish a diagnosis of ER-positive in-situ or invasive cancer.

7.3 Baseline Testing/Pre-study Evaluation

Pre-study evaluation is:

- ER positive (ER $\geq$10%) breast cancer histologically confirmed

Baseline exams are:

- **Fasting** blood lab tests that are performed at the local lab (glucose, total cholesterol, HDL cholesterol and triglycerides). Fasting is strongly recommended, but is not mandated. Every effort should be taken to draw these labs fasting.

- Blood lab tests for safety that do not require fasting (complete blood count (CBC), AST, ALT, Na, K, BUN, total bilirubin, Cl, Ca, alkaline phosphatase, creatinine, and FSH if needed)

- Fasting blood sample collection for biomarkers. Fasting is strongly recommended, but is not mandated. Every effort should be taken to draw these labs fasting.

- Physical exam and medical history, vital signs, alcohol and tobacco assessment, anthropometric measurement (height, weight, waist measurement, hip measurement) and quality of life assessment; for the purpose of the present study, minimal requirement for the physical exam visit include the following body/system sites: breasts/ cardiovascular/ gastrointestinal/ lymphnodes

For any participant who is deemed as screen failure, blood samples collected at Baseline are to be destroyed locally at the respective Participating Organization (PO). A Note to File detailing the destruction procedure and associated information is to be generated and documented in the participant's research folder or electronic health record.

At the baseline clinic visit, participants will be instructed on the importance of drug compliance. Participants will be provided and instructed on the use of a pill diary to be completed daily, signed and dated by the participant. Participants will be informed that a pill count will be conducted on their study medication use so that their study medication blisters with remaining tablets must accompany them to the subsequent clinic visit.

Plan the beginning of drug administration according to the day fixed for surgery. Surgery must occur on Days 29, 36 or 43. For this study coordinators are advised to count back from the day of surgery so that participants can either have 28, 35 or 42 days of study agent (ending on the 7th day of blister pack). Study agent can be dispensed in advance of starting agent on Day 1, However, coordinators must remind the participants not to start the study agent until a specific day set for the start of study drug.

7.4 Evaluation During Study Intervention

A phone contact on day one for treatment initiation will occur. If day one is on weekend or holiday the participants will be contacted the previous working day, if randomization and day one coincide, no phone call will be performed.

A phone call a week (+3 days) before surgery to monitor safety and compliance will occur. If the participant is seen on site for the physical exam and non-fasting blood safety tests on Day 7 prior to surgery, perform all procedures in person rather than as a phone call. In all other cases call the participant on the phone 1 week +3 days prior to surgery.

7.5 Evaluation at Completion of Study Intervention

At the final visit, participants will undergo physical exam, vital signs, and anthropometric measurements (weight, waist measurement, hip measurement). These procedures may be done **within 7 days prior to surgery or on the day of surgery**. Do not repeat the height assessment at the Final Visit. Height measurement will be used from the baseline visit.

Blood tests for safety that do not require fasting may be done **within 7 days prior to surgery or on the day of surgery**:

- complete blood count (CBC), AST, ALT, Na, K, BUN, total bilirubin, Cl, Ca, alkaline phosphatase, and creatinine

All other procedures are performed the day of surgery or the day before surgery:

Fasting blood lab tests that are performed at the local lab (glucose, total cholesterol, HDL cholesterol and triglycerides). Fasting is strongly recommended, but is not mandated. Every effort should be taken to draw these labs fasting.

Fasting blood for biomarkers will be collected. Fasting is strongly recommended, but is not mandated. Every effort should be taken to draw these labs fasting.

The date and time of blood draw, the date and time of surgery, and the date and time of last pill intake will be recorded.

The use of concomitant medications, adverse events, compliance, and quality of life assessment will be assessed. Study agent will be collected. A review of agent diary will be performed.

Any kind of surgery will be allowed from simple lumpectomy to total mastectomy. Tissue samples will be collected from the surgical specimen and processed according to Section 10. A detailed Manual of Operations and Procedures will be implemented with the specific procedures for each specimen.

7.6 Post-intervention Follow-up Period

- The follow up visit is a telephone contact to follow up on previously reported unresolved AEs with a "possibly", "probably" or "definitely" attribution to study agent.
- Only those participants who have reported unresolved AEs with a "possibly", "probably" or "definitely" attribution to study agent at the previous visit need to be contacted.
- Telephone call should occur at 20-30 days after Surgery. Any AE occurring between surgery and the phone call will not be reported
- The study coordinator will attempt to contact the participant as many times as needed to reach the participant during the "20-30 days after Surgery" window, but a minimum of two attempts must be made. If the coordinator is not able to reach the participant by phone within the study visit window, a protocol deviation is filed.

7.7 Methods for Clinical Procedures

Assessment of ER positive breast cancer (Italian sites only)
A tru-cut diagnostic biopsy of the primary tumor will be performed percutaneously with a 14 gauge needle after the administration of local anaesthetic. A single skin incision serving both biopsies with the tip of an n.11 scalpel will be performed. Among women who require a biopsy to determined eligibility, the method used to guide the biopsy (guidance with palpation or radiological imaging) will vary with clinical presentation.
The biopsy will be fixed in 10% neutral-buffered formalin for 6-8 hours before being embedded in paraffin. Sections (4-micron thick) are cut, de-waxed and stained with hematoxylin and eosin for conventional histopathological examination (presence and prevalence of normal, hyperplastic, in situ and cancer tissue, grading of the tumor and occurrence of peritumoral vascular invasion) and IHC for the assessment of the ER (mandatory), PgR (optional), Ki-67 labeling index (optional), HER-2 expression (optional).

IEO centralized evaluation of immunohistochemical (IHC) biomarker analyses (All Sites)
Pathology reports of breast biopsy and definitive surgery will be collected by each site.

Tissue samples will be required from both the initial diagnostic breast biopsy (US sites) and the

subsequent surgical excisional procedure. A formalin-fixed and paraffin-embedded (FFPE) block or 6 unstained slides from each time point will be stored locally before shipping to IEO at the end of the study (or more frequently in the case of unstained slides – please refer to the Manual of Operations and Procedures for complete instructions).

It is advised that whenever possible FFPE blocks should be stored locally, and slides to be used for IHC biomarker analyses should be prepared not earlier than one week before shipping in order to avoid antigen decay potentially affecting the IHC procedures. If that is not possible, slides may be cut per institutional practice and stored. Six 5-micron thick sections will be cut from FFPE block and mounted on charged slides, kept in the dark (i.e., in a covered box) and kept at room temperature (18-22°C, 64-71°F) till shipment.

At IEO, every set of slides will be stained by H&E and evaluated for expression of ER, PgR, Her-2 and Ki-67. The IHC analysis will run by using an automated immunostainer (Dako) and the FDA-approved kits Pharm-DX (for ER and PgR) and Herceptest (both by Dako). For Ki-67, we will use the MIB-1 antibody from Dako. The extent of immunostaining will be evaluated strictly following the ASCO-CAP guidelines and the recommendations of the Ki-67 working group (45).

Procedures at the time of surgery (all sites)
At the time of surgery a small but representative sample of the excised cancerous tissue will be obtained, as well as a specimen of adjacent and distant grossly benign tissue for study biomarkers.

8. CRITERIA FOR EVALUATION AND ENDPOINT DEFINITION

8.1 Primary Endpoint

For the purpose of the main endpoint, percent change in serum estradiol concentrations, we will refer to an outsource laboratory (Quest Diagnostics, San Juan Capistrano, CA), that has obtained a CLIA certificate for their high sensitivity estradiol test and will perform estradiol measurements. The method applied is based on LC-MS/MS technology, able to measure down to 2 pg/ml.
We expect to achieve with 25 mg three times a week and 25 mg once a week similar effects in estradiol reductions to the standard dose of 25 mg daily.

8.2 Secondary Endpoints

We will investigate the effects of alternative dosing of exemestane on the following secondary endpoints: (see Section 13.5 for a detailed statistical analysis):

- Exemestane safety and toxicity will be evaluated at the clinic visit according to the Common Terminology Criteria for Adverse Events v4.0 (CTCAE) and by a self-administered Quality of Life questionnaire (MENQOL).
- Change in Ki-67 expression comparing pre-treatment versus post-treatment specimen to compare the antiproliferative effect among the different doses.
- Serum drug measurements of exemestane and 17-dihydroxyexemestane at the end of treatment.
- Additional validated method of estradiol measurement. This method has a lower detection limit (1 pg/ml) than the CLIA certified estradiol test (Quest) and will serve as a quality control since it has proven to more effectively detect estradiol concentrations at the very low level, which is characteristic of older postmenopausal women.

- Serum concentrations of estrone, estrone-sulfate, will be measured by LC-MS/MS while androstenedione and testosterone will be measured by RIA. Sex hormone binding globulin serum levels will be measured by a chemiluminescent microparticle immunoassay (CMIA) on the ARCHITECT *i* System (Abbott Laboratories, Weisbaden, Germany).
- Insulin concentrations will be measured with the Architect Immunoassay analyzer (Abbott Laboratories, Abbott Park, IL, US). Glucose will be measured at each local lab
- Adipokines: change in leptin and adiponectin serum concentrations will be analyzed and compared among the different treatments arms. These measurements will be performed by the use of commercially available enzyme linked immunoassays purchased from R&D systems (SPACE Import-Export Srl, Milan, Italy).
- Lipid profile (total cholesterol, HDL and triglycerides) will be determined locally at baseline and before surgery.Measurement of breast tissue estradiol concentration in tumor and normal breast at time of surgery.
- Centralized evaluation of ER, PgR, Her2 and Ki67 expression in tumor comparing pre-treatment levels (tru-cut biopsy) to post-treatment level expression (surgical specimen).
- Centralized evaluation of Ki-67 in adjacent intraepithelial neoplasia and or grossly benign tissue.
- Drug measurements of exemestane and 17-dihydroxyexemestane on frozen tissue samples, when available.
- Proteomic analysis will be performed with the new Fusion Tribrid mass spectrometer in Dr. Chen's Proteomics Shared Resource for the Herbert Irving Comprehensive Cancer Center (HICCC) at CUMC.
- To analyze the UGT2B17 gene we will use Taqman copy number variation assay (Life Technologies, Monza, Italy).
- Crown like structures in mammary fat tissue by IHC (CD68), comparing pre-treatment (tru-cut biopsy) versus post-treatment (surgical specimens).

8.3 Off-Agent Criteria

Participants may stop taking study agent for the following reasons: completed the protocol-prescribed intervention, adverse event or serious adverse event, inadequate agent supply, non-compliance, concomitant medications, medical contraindications. Participants will continue to be followed, if possible, for safety reasons and in order to collect endpoint data according to the schedule of events. Participants will not be replaced.

8.4 Off-Study Criteria

Participants may go 'off-study' for the following reasons: the protocol intervention and any protocol-required follow-up period is completed, adverse event/serious adverse event, lost to follow-up, non-compliance, concomitant medication, medical contraindication, withdraw consent, death, determination of ineligibility (including screen failure).

8.5 Study Termination

NCI, DCP as the study sponsor has the right to discontinue the study at any time.

9. CORRELATIVE/SPECIAL STUDIES

We will collect and process flash frozen tissue, paraffin-embedded tissue, serum and whole blood for evaluation of biomarkers.

9.1 Rationale for Methodology Selection

Estradiol

Serum estradiol concentrations will be determined by use of a CLIA certified test, according to MD Anderson policies for primary endpoint analyses. This test is based on a non-derivatized LC-MS/MS technology that has a detection limit of 2 pg/ml, a limit that may not be sufficient to detect estradiol levels in all postmenopausal women . Any samples that have estradiol results below the detection limit will be assigned to have a concentration of ½ the value of the detection limit.

Because of the critical issue of sensitivity of the method, we have decided to include an additional validated bioanalytical method performed in a CLIA certified laboratory for steroid testing. Estradiol will be measured by a two-dimensional liquid chromatography with mass spectrometry detection LC-MS/MS) after liquid-liquid extraction. Duplicate standard curves, water blanks and four assay control pools are processed with samples to assess accuracy and precision of the assay. This method has a lower detection limit (1 pg/ml) than the CLIA certified estradiol test (Quest) and will serve as a quality control since it has proven to more effectively detect estradiol concentrations at the very low level, characteristic of older postmenopausal women. The functional sensitivity of the test is 1 pg/ml and the within assay precision of two control samples were 13% at 1.0 pg/mL and 8.8% at 5 pg/mL, respectively.

Ki-67

Ki-67 labeling index modulation in preoperative studies has proven to be an appropriate surrogate marker for outcome in participants who are administered antiestrogen therapies.

The Division of Pathology at IEO is in strict compliance with the recommendations of the International Working Group for Ki-67 assessment in breast cancer (45) and performs central revision of Ki67 within the International Breast Cancer Study Group. Ki-67 labeling index expression will be evaluated by IHC, as previously described (46). Specifically, Ki-67 is assessed by IHC according to recent international recommendations (45) using the Mib-1 monoclonal antibody (1:50 dilution; Dako, Denmark), using an automated immunostainer (Dako). We will evaluate all the cells in the diagnostic biopsies, and 2000 cells from three high power (×400) microscopic fields randomly selected at the periphery of the tumor in surgical samples, as previously reported (46). The Ki-67 labeling index is calculated as the percentage of Ki-67 immunoreactive cells over the total number of counted cells.

ER, PgR and HER2

Centralized analyses of ER, PgR (PharmDX) and Her2 (herceptest) expression will be determined by IHC on tumor sections from biopsy and surgical samples, as previously described(45).

Estrone, estrone-sulfate

Estrone will be measured by a two-dimensional liquid chromatography with mass spectrometry detection LC-MS/MS) after liquid-liquid extraction. Duplicate standard curves (2.5-00 pg/mL), water blanks and four assay control pools are processed with samples to assess accuracy and precision of the assay. The detection limit of the assay is 2.5 pg/mL and the intra and inter-assay coefficient of variation of a pooled human serum sample of 21 pg/mL were 4.8% and 9.0%, respectively.

Estrone-sulfate will be measured by a two-dimensional liquid chromatography (HPLC) with triple quadrupole mass spectrometry detection. Estrone-sulfate from serum is first partially purified by protein precipitation, and then further purified by gradient reverse phase HPLC. Duplicate standard curves (10-1000 ng/dL), water blanks and four assay control pools are processed with samples to assess accuracy and

precision of the assay. The detection limit of the assay is 10 ng/dL and the intra and inter-assay coefficient of variation of a pooled human serum sample of 25 ng/dL were 3.6% and 9.3%, respectively.

Androstenedione

Serum concentrations of androstenedione will be measured by a radioimmunoassay kit (DSL-3800 ACTIVE, Beckman Coulter S.r.l., Rome, Italy), contining androstenedione coated tubes. The procedure follows the basic principle of radioimmunoassays where there is competition between a radioactive and a non-radioactive antigen for a fixed number of antibody binding sites. The amount of I^{125}-labeled androstenedione bound to the antibody is inversely proportional to the concentration of androstenedione present in the sample. The separation of free and bound antigen is achieved by decanting the antibody-coated tubes. The sensitivity of the assay is 0.03 ng/mL and the intra and inter-assay coefficients of variation of a control provided with the kit (0.5 ng/mL) were 10% and 14% respectively. Interassay coefficients of variation of our in-house prepared serum pool (1.6 ng/mL) was 14% for androstenedione.

Testosterone

Serum testosterone will be determined by an electrochemiluminescent immunometric assay (Roche Diagnostics S.p.A., Monza, Italy) designed for the Cobas e411 automated analyzer. The sensitivity of the assays was 2.5 ng/dL and the inter-assay coefficient of variation of our in-housed pooled serum sample (18 ng/dL) was 4.6.

Sex hormone binding globulin (SHBG)

Serum concentrations of SHBG will be determined by a chemiluminescent microparticle immunoassay (CMIA) on the ARCHITECT *i* System (Abbott Laboratories, Weisbaden, Germany). This assay shows very good agreement with our previously adopted platforms and the assay performance in terms of reproducibility were improved. Sensitivity of the method is $\leq$ 0.01nmol/L and intra and inter-assay coefficient of variation were below 5.5% at 3 different control levels (low, median, high).

Insulin

Serum concentrations of insulin will be determined by a chemiluminescent microparticle immunoassay (CMIA) on the ARCHITECT *i* System (Abbott Laboratories, Weisbaden, Germany). This assay shows very good agreement with our previously adopted platforms and the assay performance in terms of reproducibility were improved. The sensitivity of the method is $\leq$ 1.0 µU/mL, and inter-assay coefficient of variation are below 2% at 3 different control levels (low, median, high) produced by Abbott. Interassay coefficient of variation of our in-house prepared serum pool (mean: 4.9 µU/mL) is 3.9%.

Adiponectin and leptin

Serum adiponectin and leptin will be measured using a commercial enzyme-linked immunosorbent assay kit (R&D Systems, Minneapolis, MN). Serum samples should not undergo freeze and thaw cycles before adiponectin and leptin measurements. Particularly, leptin is very sensitive to freeze-thaw cycles and will be determined after first thaw.

The minimum detectable dose of adiponectin is 0.25 ng/mL and the inter-assay coefficient of variation of our in-house prepared serum pool (mean: 12.5 µg/mL) was 6.9%. The minimum detectable dose of leptin is 7.8 pg/mL. and the inter-assay coefficient of variation of our in-house prepared serum pool (mean: 9.7 ng/mL) was 6.6%.

Pharmocokinetics

EXE and 17-dihydroEXE concentrations will be determined by mass spectrometry (MS) using Waters® Xevo™ TQ MS in electrospray positive ionization mode (Waters, Manchester, UK), optimized by multiple reaction monitoring mode. We used Zorbax Eclipse Plus C18 columns and mobile phase

MeOH/H_2O with 0.1% formic acid. EXE pure substance was provided by Pfizer Inc., 17β-hydroxy EXE and EXE-19-d3 were purchased from Toronto Research Chemicals (Toronto, Ontario, Canada).

Proteomic Analysis
Fresh frozen tumor tissues will be homogenized and quantified using the standard operating protocols established in HICCC Proteomics Shared Resource. Exosomes will be isolated from serum using SBI's ExoQuick exosome kit (47-49), and the enrichment of exosomes will be verified by Western blot. 100 μg of exosomes from each time point of the same participant will be proteolytically cleaved with trypsin and then chemically labeled with mass spectrometer detectable quantification reagent, TMT isobaric mass tags (TMT126, 127, 128, 129)(50,51) separately for quantification. 10 μg from each TMT labeled sample (4plex) will be mixed per LC-MS/MS run. Three technical replications will be performed per sample. For the LC-MS/MS analysis, 40 μg of TMT-labeled peptide mixture will be separated by nano-HPLC and analyzed by the new Orbitrap Fusion Tribrid mass spectrometer. The proposed proteomic studies will take advantage of its vastly improved sensitivity, as well as other features that can be optimized for complex samples. For example, parallelization of the newly designed instrument maximizes the amount of high-quality data acquired by synchronizing operations of several different mass analyzers. Dynamic scan management enables selection, sorting, and routing of precursors to different fragmentation modes, enabling us to optimize the instrument for PTM characterization. Also, synchronous MS3 precursor selection significantly increases the number of peptides accurately quantified in isobaric mass tagging experiments. The new Orbitrap Fusion Tribrid mass spectrometer has increased sensitivity for detecting lower abundant proteins, which allows us to use less starting material and increase the number of biological replications for analysis.

Pharmacogenetics
DNA will be extracted from whole blood EDTA treated samples (Qiagen, Italy). We will use Taqman copy number variation assay (Life Technologies, Monza, Italy) for the UGT2B17 genotyping. The expected minor allele frequency (UGT2B17 deletion) in the Caucasian population is 0.26. We recently demonstrated a statistically significant association of the UGT2B17 gene deletion with increased serum concentrations of 17-dihydroxyEXE (Johansson et al. SABCS 2014) in a pre-surgical trial involving 47 participants randomized to exemestane 25 mg/daily for six weeks, exemestane levels were also higher, although the difference was not statistically significant between groups (homozygous wt *versus* heterozygous and homozygous UGT2B17 deleted).

Crown like structures (CLS)
We will use CD68 (mouse monoclonal KP1 antibody; Dako; dilution 1:4,000) to identify CLS in mammary fat tissue in FFPE sections stained by H&E. All cases will be reviewed by the same breast histopathologist. Light microscopy will be used to assess for evidence of CLS.

Biorepository
Leftover specimens will be stored for future unspecified analyses. Leftover samples will be retained at IEO Central Biobank if informed consent for biobanking is signed.

Biologic specimens collected during the conduct of the trial that are not used during the course of the study will be considered deliverables under the contract and thus the property of the NCI. At study completion, NCI reserves the option to either retain or relinquish ownership of the unused biologic specimens. If NCI retains ownership of specimens, the Contractor shall collect, verify and transfer the requested biologic specimens from the site to a NCI-specified repository or laboratory at NCI's expense.

9.2 Comparable Methods

We will use established and validated assays for determination of changes in biomarkers expression in this study. We plan to evaluate biomarkers in serum, tissue and DNA for secondary endpoints evaluation.

As to the primary endpoint, the method has been validated and already described in peer-reviewed publications (52).

For secondary serum biomarkers we will utilize standardized protocols of commercially available kits already described in peer-reviewed publications. Standard control samples as well as an in-house pooled serum sample will be analyzed in each run to monitor for inter- and intra assay coefficient of variation. We will pair samples obtained from the same participant (pre and post-treatment) and run samples in batches to reduce analytical variability. As to SHBG (28,53), HOMA index (53,54) and adipokines (37,55) our group has already published several papers involving these biomarkers.

10. SPECIMEN MANAGEMENT

10.1 Laboratories

Planned analysis of biomarkers will be centralized to specialized laboratories as outlined below.

10.1.1 Blood samples

Specimen (amount)	**Laboratory**
Serum 1 aliquot (1 mL) Estradiol, Estrone and estrone-sulfate levels	Nigel Clarke, PhD Quest Diagnostics Nichols Institute
Serum 1 aliquot (1 mL) Ultra sensitive estradiol levels	inVentiv Health, Princeton, NJ
Serum, 6 aliquots (1 mL each) Androstenedione, Testosterone, SHBG levels and Insulin levels Adiponectin and Leptin levels	Harriet Johansson, MSc, PhD Division of Cancer Prevention and Genetics European Institute of Oncology
Serum 1 aliquot (1 mL) EXE and 17-dihydroEXE Levels	Prof. Gunnar Mellgren, MD, PhD Director, Laboratory Medicine and Pathology Haukeland University Hospital and University of Bergen
Serum 1 aliquot (1 mL) Proteomic Biomarker Discovery	Emily Chen, PhD Proteomics Shared Resource Herbert Irving Comprehensive Cancer Center Columbia University Medical Center
Whole Blood EDTA-treated, 2 aliquots (1 mL each) DNA extraction and UGT2B17 copy number variation analysis	Harriet Johansson, MSc, PhD Division of Cancer Prevention and Genetics European Institute of Oncology

10.1.2 Tissue samples

Specimen	**Laboratory**
Tru-cut Biopsy 1 (FFPE) – Italian Sites Histological evaluation prior to randomization	Pathology staff at institution where diagnosis was performed
Tru-cut FFPE sections from Biopsy 1 – Italian Sites Six consecutive 5-micron thick sections from FFPE tru-cut biopsy for centralized analyses of Ki67 (and ER, PGR HER2)	Prof. Giuseppe Viale, MD Division of Pathology European Institute of Oncology
FFPE block or 6 unstained slides from the initial diagnostic biopsy – US Sites	Prof. Giuseppe Viale, MD Division of Pathology European Institute of Oncology
Surgical specimen 1: Breast Tumor Tissue (FFPE) Histological evaluation of surgical specimen	Pathology staff at each institution where definitive diagnosis was performed
Surgical specimen 1: FFPE sections from breast tumor Six consecutive 5-micron thick sections from each FFPE block mounted on charged slides [preferably within 7 days prior to shipping]	Prof. Giuseppe Viale, MD Division of Pathology European Institute of Oncology
Surgical specimen 2: Flash Frozen Breast Tumor (minimal wet weight: 200 mg) Estradiol/exemestane measurements **Surgical specimen 3: Flash Frozen Normal Breast (**minimal wet weight: 200 mg) Estradiol/exemestane measurements	Prof. Gunnar Mellgren, MD, PhD Director, Laboratory Medicine and Pathology Haukeland University Hospital and University of Bergen
Surgical specimen 4: Flash Frozen Breast Tumor (minimal wet weight: 50 mg) Proteomic Biomarker Discovery **US Sites only**	Emily Chen, PhD Proteomics Shared Resource Herbert Irving Comprehensive Cancer Center Columbia University Medical Center
Surgical specimen 5: FFPE sections from normal breast fat Crown like structures (IHC staining)	Potential Anxillary Study Investigator to be defined

The analysis of serum concentrations of estradiol, estrone and estrone-sulfate will be performed at the Quest Diagnostics Nichols Institute, San Juan Capistrano, CA, US under the supervision of Nigel Clarke.

The analysis of serum concentrations of ultrasensitive estradiol will be performed at inVentiv Health lab, Princeton, NJ, USA.

The analysis of serum concentrations of exemestane and its main metabolite 17-dihydroxy-exemestane will be performed by the Hormone Laboratory, Haukeland University, Bergen, Norway under the supervision of Prof. Gunnar Mellgren MD, PhD.

The analysis of serum concentrations of androstenedione, testosterone, SHBG, insulin, adiponectin and leptin will be performed at the IEO Cancer Prevention Lab, Milan, Italy under the supervision of Harriet Johansson, MSC, PhD. The IEO Cancer Prevention Lab is also responsible DNA extraction, UGT2B17 copy number variation analysis, and for Centralized Biobanking of leftover specimens.

The evaluation of IHC biomarker analyses will be centralized to the Division of Pathology, IEO, Milan, Italy under the supervision of Professor Giuseppe Viale.

Proteomic profiling will be centralized to Dr. Chen's Proteomics Shared Resource for the Herbert Irving Comprehensive Cancer Center (HICCC) at Columbia University Medical Center (CUMC).

10.2 Collection and Handling Procedures

Specific instructions about specimen handling and storage will be provided in a separate Manual of Operations and Procedures.

10.2.1 Blood

It is strongly recommended for all blood samples for biomarkers to be drawn under fasting condition (at least 6 hours) preferably between 8 a.m. and 10 a.m. at baseline and the day of surgery (or the day before, depending on the local institutional procedures).

A total of **10 ml of blood** will be withdrawn for **safety lab exams and lipid profile** at baseline and before surgery.

At baseline and before surgery a total of **20 ml of blood** will be collected into vacuum blood collection tubes containing beads coated with clotting activator for **serum** separation to be employed for circulating **biomarker analysis.** Accordingly with specific tubes adopted at your hospital, please refer to the manufacture instructions. Allow the blood to clot at room temperature for 30 minutes. Then, spin in centrifuge for 10 minutes. Sarstedt Serum Monovettes tubes require 10 min centrifugation at 2000 x g at room temperature, while BD Vacutainer® SST™ and PST™ gel tubes should be spun at a speed of 1300 x g. Swing-out buckets best option for both systems. . After centrifugation, the yellow top layer, which corresponds to serum, is pipetted using the disposable transfer pipettes in 10 even aliquots (about 1.0 mL each) into the polypropylene cryotubes (Thermo Fisher Scientific), specifically labeled according to the Instructions in the Manual of Operations and Procedures. Tubes are tightly capped and stored in a dedicated – 80°C freezer equipped with a temperature control and temperature log chart or an alarm monitoring system per institutional standards.

At baseline and before surgery a total **3 ml whole blood** will be collected in tubes containing EDTA-K2 as anti-coagulant agent. The tube is gently mixed and blood is pipetted using disposable transfer pipettes in 2 even aliquots (about 1.5 mL each) into the polypropylene cryotubes (Thermo Fisher Scientific), specifically labeled according to the Instructions in the Manual of Operations and Procedures . Tubes are tightly capped and stored in a dedicated –80°C freezer equipped with a temperature control and

temperature log chart or an alarm monitoring system per institutional standards.

Specimen collection kits for storage of serum and blood samples will be provided by the Central Laboratory, at the Division of Cancer Prevention and Genetics, European Institute of Oncology in Milan, Italy (See the Instructions in the Manual of Operations and Procedures for requesting kits). Briefly, they will provide labels and polypropylene cryotubes and rack (Thermo Fisher Scientific) and instructions for specimen handling, processing, labeling, tracking and storage.

Each local site must be equipped with a -80°C (range -70 °C to -80 °C) freezer provided with temperature control 24 hours a day, 7 days a week and temperature log charts or an alarm monitoring system, better if electronic. The Center should also be equipped with a back-up freezer.

10.2.2 Tissue

Breast tissue fixation with neutral buffered formalin for 4–48 hours has been shown to be adequate. Thus, when tissue is fixed in neutral buffered formalin, IHC for Ki-67 is robust across a wide range of fixation times. Tissue handling guidelines that are already in place for ER (8–72 hours of neutral buffered formalin fixation) are therefore more than adequate for Ki-67. Once tissue is properly fixed and embedded in paraffin, antigenicity is well preserved, potentially for decades.

FFPE sections from breast tumor (baseline and surgical specimens) will be cut locally by each site and shipped for **centralized evaluation of immunohistochemical biomarker analyses** at IEO. Six consecutive 5-micron thick sections will be cut from each FFPE block and mounted on charged slides, preferably within 7 days prior to shipping.

Flash frozen specimen from surgical resections **(**minimal wet weight: 200 mg) both from the tumor and the macroscopically negative normal tissue will be collected within 20-30 minutes, frozen on liquid nitrogen and stored in freezer at –80°C.

There could be some cases of insufficient tumor tissue available to be snap frozen (in situ histology, small size of invasive cancer, etc). However, any effort should be made to collect and freeze normal breast tissue for secondary endpoint measurements.

Note for the US Sites: Additional **Flash Frozen Breast Tumor tissue from the surgical specimen (**minimal wet weight: 50 mg) will be collected at the US Sites only for Proteomic Biomarker Discovery at the Proteomics Shared Resource, Herbert Irving Comprehensive Cancer Center, Columbia University Medical Center. This specimen is only collected by the US sites, stored in local freezers at –80°C until the end of the study, and shipped to CUMC per the shipping instructions in the Manual of Operations and Procedures.

10.3 Shipping Instructions

In order to keep shipping costs at an affordable level, Participating Centers are requested to store blood samples at -80°C until pick-up by a courier will be arranged by and to the Central Laboratory at the Division of Cancer Prevention and Genetics, European Institute of Oncology in Milan, Italy. Samples will be shipped on dry ice in compliance with the International Air Transport Association (IATA) Dangerous Goods Regulations. Specific instructions about specimen shipping will be provided in a dedicated Manual of Operations and Procedures.

Tissue specimens will be stored locally until shipment the Central Laboratory at the Division of Cancer

Prevention and Genetics, European Institute of Oncology in Milan, Italy. Storage and shipment instructions are outlined in the Manual of Operations and Procedures.

Note to the US Sites: Tissue collected for Proteomic Biomarker Discovery will be stored locally and shipped to the Proteomics Shared Resource, Herbert Irving Comprehensive Cancer Center, Columbia University Medical Center. Storage and shipment instructions are outlined in the Manual of Operations and Procedures.

10.4 Specimen Banking

Leftover specimens will be stored at IEO Central Biobank for optional studies.

Biologic specimens collected during the conduct of the trial that are not used during the course of the study will be considered deliverables under the contract and thus the property of the NCI. At study completion, NCI reserves the option to either retain or relinquish ownership of the unused biologic specimens. If NCI retains ownership of specimens, the Contractor shall collect, verify and transfer the requested biologic specimens from the site to a NCI-specified repository or laboratory at NCI's expense.

11. REPORTING ADVERSE EVENTS

DEFINITION: AE means any untoward medical occurrence associated with the use of a drug in humans, whether or not considered drug related. An AE can therefore be any unfavorable and unintended sign, symptom, or disease temporally associated with participation in a study, whether or not related to that participation. This includes all deaths that occur while a participant is on a study.

Please note that all abnormal clinical laboratory values that are determined to be of clinical significance based on a physician's assessment are to be reported as AEs. Those labs determined to be of no clinical significance or of unknown clinical significance (per the physician's assessment) should not be reported as AEs. Any lab value of unknown clinical significance should continue to be investigated/followed-up further for a final determination, if possible.

A list of AEs that have occurred or might occur (Reported Adverse Events and Potential Risks) can be found in §6.2, Pharmaceutical Information, as well as the Investigator Brochure or package insert.

11.1 Adverse Events

11.1.1 Reportable AEs

All AEs that occur after the informed consent is signed and baseline assessments are completed must be recorded on the AE CRF (paper and/or electronic) whether or not related to study agent.

11.1.2 AE Data Elements:

The following data elements are required for adverse event reporting.

- AE verbatim term
- NCI Common Terminology Criteria for Adverse Events version 4.0 (CTCAE v4.0) AE term (MedDRA lowest level term)
- CTCAE (MedDRA) System Organ Class (SOC)
- Event onset date and event ended date

- Treatment assignment code (TAC) at time of AE onset
- Severity grade
- Attribution to study agent (relatedness)
- Whether or not the event was reported as a serious adverse event (SAE)
- Whether or not the subject dropped due to the event
- Outcome of the event

11.1.3 Severity of AEs

11.1.3.1 Identify the AE using the CTCAE version 4.0. The CTCAE provides descriptive terminology (MedDRA lowest level term) and a grading scale for each AE listed. A copy of the CTCAE can be found at http://ctep.cancer.gov/protocolDevelopment/electronic_applications/ctc.htm

AEs will be assessed according to the grade associated with the CTCAE term. AEs that do not have a corresponding CTCAE term will be assessed according to the general guidelines for grading used in the CTCAE v4.0 as stated below.

CTCAE v4.0 general severity guidelines:

Grade	Severity	Description
1	Mild	Mild; asymptomatic or mild symptoms; clinical or diagnostic observations only; intervention not indicated.
2	Moderate	Moderate; minimal, local or noninvasive intervention indicated; limiting age-appropriate instrumental activities of daily living (ADL)*.
3	Severe	Severe or medically significant but not immediately life-threatening; hospitalization or prolongation of hospitalization indicated; disabling; limiting self-care ADL**.
4	Life-threatening	Life-threatening consequences; urgent intervention indicated.
5	Fatal	Death related to AE.

ADL

*Instrumental ADL refers to preparing meals, shopping for groceries or clothes, using the telephone, managing money, *etc*.

**Self-care ADL refers to bathing, dressing and undressing, feeding self, using the toilet, taking medications, and not bedridden.

11.1.4 Assessment of relationship of AE to treatment

The possibility that the adverse event is related to study agent will be classified as one of the following: not related, unlikely, possible, probable, definite.

11.1.5 Follow-up of AEs

All AEs, including lab abnormalities that in the opinion of the investigator are clinically significant, will be followed according to good medical practices and documented as such. AEs with a “possibly”, “probably” or “definitely” attribution to study agent will be monitored up to 20-30 days after surgeryvia phone call.

11.2 Serious Adverse Events

11.2.1 DEFINITION: Regulations at 21 CFR §312.32 (revised April 1, 2014) defines an SAE as any untoward medical occurrence that at any dose has one or more of the following outcomes:

- Death
- A life-threatening AE
- Inpatient hospitalization or prolongation of existing hospitalization
- A persistent or significant incapacity or substantial disruption of the ability to conduct normal life functions
- A congenital anomaly or birth defect
- Important medical events that may not be immediately life-threatening or result in death or hospitalization should also be considered serious when, based upon appropriate medical judgment, they may jeopardize the patient and may require medical or surgical intervention to prevent one of the other outcomes.

11.2.2 Reporting SAEs to DCP

The organization that experiences the serious adverse event (SAE) should report the SAE to the following 3 entities: 1) NCI DCP, 2) DCP's regulatory contractor CCSA, and 3) MDACC, the CLO. Detailed reporting instructions are provided below. In addition, all participating organizations will follow their IRB requirements for SAE reporting.

11.2.2.1 The Lead Organization and all Participating Organizations will report SAEs on the DCP SAE Report Form found at http://prevention.cancer.gov/clinical-trials/clinical-trials-management/protocol-information-office/pio-instructions-and-tools/2012-consortia.

11.2.2.2 Reporting within 24 hours of knowledge of the event.

11.2.2.2(A) Report to the NCI DCP Medical Monitor within 24 hours:
Contact the DCP Medical Monitor by phone within 24 hours of knowledge of the event.

Eva Szabo, MD
NCI/Division of Cancer Prevention
9609 Medical Center Drive, Rm 5E-102
Bethesda, MD 20892 (For FEdEX, Rockville, MD 20850)
Phone: (240) 276-7011
Fax: (240) 276-7848
Email: szaboe@mail.nih.gov

Include the following information when calling the Medical Monitor:

- Date and time of the SAE
- Date and time of the SAE report
- Name of reporter
- Call back phone number
- Affiliation/Institution conducting the study
- DCP protocol number
- Title of protocol
- Description of the SAE, including attribution to drug and expectedness

11.2.2.2(B) Report to the Consortium Lead Organization (CLO) PI (Dr. Powel Brown) within 24 hours of knowledge of the event:
Report all SAEs to the Consortium Lead Organization PI (Dr. Powel Brown) within 24 hours of knowledge of the event. The same information reported to the DCP Medical Monitor should be provided to the CLO Coordinator via email, phone or fax within 24 hours of knowledge of the event.

11.2.2.3 Reporting within 48 hours of knowledge of the event:

11.2.2.3 (A) Email the written SAE reports to the DCP Medical Monitor within 48 hours of learning of the event using the paper SAE form. The SAE forms should be obtained at http://prevention.cancer.gov/clinical-trials/clinical-trials-management/protocol-information-office/pio-instructions-and-tools/2012-consortia.

11.2.2.3 (B) The written SAE reports will also be emailed to DCP's Regulatory Contractor, CCS Associates, at safety@ccsainc.com.

11.2.2.3 (C) The written SAE report will also be faxed to the Consortium Lead Organization PI (Dr. Powel Brown), at (713) 792-4003 or emailed at PHBrown@mdanderson.org.

It is the responsibility of the CLO to inform the Lead Protocol PI upon receipt of the report from the organization experiencing the event.

11.2.2.4 The DCP Medical Monitor and regulatory staff will determine which SAEs require FDA submission.

11.2.2.5 The Lead Organization and all Participating Organizations will comply with applicable regulatory requirements related to reporting SAEs to the IRB/IEC.

11.2.2.6 Follow-up of SAE
Site staff should send follow-up reports as requested when additional information is available. Additional information should be entered on the DCP SAE form in the appropriate format. Follow-up information should be sent to DCP as soon as available. SAEs will be monitored up to 30 days after post-treatment with a visit or a phone call based on the severity of the SAE

12. STUDY MONITORING

12.1 Data Management

This study will report clinical data using the Data Management Initiative (DMI) web-based application managed by the Consortium Biostatistics and Data Management Core. Data Management Initiative (DMI) infrastructure has been developed in the Division of Quantitative Sciences (DQS), MD Anderson Cancer Center. This infrastructure supplies integrated database and software services for web-based data collection, randomized treatment assignment, reporting, query, data download, and data quality management. The DMI will be the database of record for the protocol and subject to NCI and FDA audit. All DMI users will be trained to use the DMI system and will comply with the instructions in the protocol-specific "DMI User Manual" as well as applicable regulatory requirements such as 21 CFR; Part 11. Data management procedures for this protocol will adhere to the Data Management Plan (DMP) on file at the DCP for contract HHSN261201200034I.

12.2 Case Report Forms

Participant data will be collected using protocol-specific case report forms (CRF) developed from the standard set of DCP Chemoprevention CRF Templates and utilizing NCI-approved Common Data Elements (CDEs). The approved CRFs will be used to create the electronic CRF (e-CRF) screens in the DMI application. Site staff will enter data into the e-CRF. Amended CRFs will be submitted to the DCP Protocol Information Office for review and approval. Approved changes will be programmed into the DMI database by the Consortium Biostatistics and Data Management Core.

12.3 Source Documents

Source documentation will include only those documents containing original forms of data, including clinic charts, shadow files, hospital charts, and physician notes. Data recorded directly on the CRFs designated as source documents (i.e., no prior written or electronic record of data) will be considered source data. All other data recorded on the CRFs will not be considered source documentation.

12.4 Data and Safety Monitoring Plan

The Data and Safety Monitoring Plan for the MD Anderson Consortium is on file at the DCP. This study will be monitored by the MDACC Data and Safety Monitoring Board, the data and safety monitoring board of record for this study. The Data Safety and Monitoring Board (DSMB) reports to the President, or his designee, as the on-campus representative of The University of Texas Board of Regents. It oversees the data and patient safety issues for randomized clinical trials that originate at MD Anderson; that are coordinated or analyzed by MD Anderson and are not being monitored by any other DSMB; or have been designated as requiring DSMB monitoring at the request of the IRB, the CRC, or institution. The primary objectives of the DSMB are to ensure that patients' rights pertaining to participation in a research study are protected, and that patients' interests are prioritized over the interests of the scientific investigation. Responsibilities include:

(a) Review interim analyses of outcome data (prepared by the study statistician or other responsible person at the time points defined in the study) approved by the IRB and additional time points as determined by the DSMB, and to recommend, if necessary, whether the study needs to be changed or terminated based on these analyses;
(b) Determine whether, and to whom, outcome results should be released prior to the reporting of study results;
(c) Review interim toxicity data and efficacy of treatment;
(d) Review major research modifications proposed by the investigator or appropriate study committee prior to implementation (e.g., termination, dropping an arm based on toxicity results from the study or results of other studies, increasing target sample size).

Refer to the Data and Safety Monitoring Plan for the MD Anderson Consortium on file at the DCP for further details.

12.5 Sponsor or FDA Monitoring

The NCI, DCP (or their designee), pharmaceutical collaborator (or their designee), or FDA may monitor/audit various aspects of the study. These monitors will be given access to facilities, databases, supplies and records to review and verify data pertinent to the study.

12.6 Record Retention

Clinical records for all participants, including CRFs, all source documentation (containing evidence to study eligibility, history and physical findings, laboratory data, results of consultations, *etc*.), as well as IRB records and other regulatory documentation will be retained by the Investigator in a secure storage facility in compliance with Health Insurance Portability and Accountability Act (HIPAA), Office of Human Research Protections (OHRP), Food and Drug Administration (FDA) regulations and guidances, and NCI/DCP requirements, unless the standard at the site is more stringent. For NCI/DCP, records will be retained for at least three years after the completion of the research. NCI will be notified prior to the planned destruction of any materials. The records should be accessible for inspection and copying by authorized persons of the Food and Drug Administration, the Italian Ministry of Health representative and Italian Agency of Drug (AIFA). If the study is done outside of the United States, applicable regulatory requirements for the specific country participating in the study also apply.

12.7 Cooperative Research and Development Agreement (CRADA)/Clinical Trials Agreement (CTA)

N/A

13. STATISTICAL CONSIDERATIONS

13.1 Study Design/Description

The trial is a randomized, double-blind, non-inferiority phase IIb study. Participants will be randomized to receive exemestane as one dose of 25 mg once a day; or 25 mg three times a week; or 25 mg once a week for at least four up to six weeks. Analysis will be performed according to intention to treat principle and then according to efficacy.

13.2 Randomization/Stratification

In order to keep at minimum the imbalance in treatments a stratified blocked randomization strategy will be used considering only the relevant prognostic factors. Participants will be stratified according to center and BMI (<25 kg/m^2 versus ≥ 25 kg/m^2). Subjects with a BMI <25 kg/m^2 will be limited to 50% per site. A fixed block randomization will be carried out with a block size of 6 patients.

13.3 Accrual and Feasibility

We expect to screen 300 potential participants to randomize 180 women, 60 per arm. We assume the drop-out rate to be 10%. Our goal is to achieve a total sample size of 162 evaluable participants (54 participants per arm).

13.4 Primary Objective, Endpoint(s), Analysis Plan

Primary objective and primary endpoint
Since the primary objective of this study is to assess if the reduction in estradiol with the standard dose is comparable with the other lower doses, we will measure the estradiol before and after treatment. Thus, the primary endpoint is the percentage change of serum estradiol concentration from baseline and we will compare the median change and percentage changes among arms.

We choose the percentage change as the primary endpoint because it will improve generalization of results, given the wide variability among different assays, and because we predict that a large percentage of participants will have the final values of estradiol below the detectable level.

Analysis plan
We will present full distribution and median values of estradiol at baseline (before treatment), at post (after treatment), and changes and percentage changes (with interquartile ranges) of estradiol level, from baseline to post, by arms. Differences by arms of % change, change and final values will be tested considering t-test and ANCOVA models. Estradiol at baseline will be included as explanatory variable together with other possible confounders such as BMI, age and time since last dose. Normal distribution of residuals from full model will be checked and, if needed, a transformation will be considered.

We will also compare percentages of participants with final values of estradiol below the detectable level by arms with Chi-square tests and logistic models.

We will carry out the two comparisons with the standard dose considering alfa=0.025.

Power analysis
Given the expected reduction of at least 80% change with exemestane at 25 mg daily (56) considered as reference group (control arm) against 25 mg 3 times a week, or 25 mg weekly, for the power calculation we assume a non-inferiority difference of 6% in percentage changes of estradiol after treatment from baseline, using a one-sided, two-sample t-test. A total sample size of 162 participants (54 participants per arm), enables us to achieve 80% power with a margin of equivalence of -6% in the mean percentage changes from baseline at the lower dose compared to the standard dose. The true difference between the means percentage changes is assumed to be 0.0%.

The significance level of the test is 0.025 to take into account of multiple comparisons (we will compare the changes at the two low doses with the standard dose)(57).

The data are drawn from populations with common standard deviations of 11% (58).

Assuming a 10% drop-out rate while on study, we would need to randomize 180 participants (60 participants per arm) to achieve the total sample size of 162 evaluable participants (54 participants per arm).

13.5 Secondary Objectives, Endpoints, Analysis Plans

We will attempt to understand the mechanism of action of exemestane on the primary endpoint of the study. More specifically, we will assess the effects of exemestane on the secondary endpoint biomarkers to see whether the effects of different doses on the primary endpoint is through the secondary endpoint biomarkers or independent of them. This will entail exploratory analysis of the primary endpoint by the secondary endpoint biomarkers while accounting for the effect of treatment.

Secondary objectives:

- To assess safety and toxicity.
- To support the preventive activity of exemestane we will investigate the change in Ki-67 and PgR level on the tumor adjacent intraepithelial neoplasia and/or grossly benign tissue.
- To assess possible association of estradiol level with tissue and circulating biomarkers.
- To investigate possible pharmacogenetic markers.
- To assess drug levels on tissue samples.
- To investigate tissue and circulating proteomics profiling.

Secondary endpoints:

- Exemestane safety and toxicity will be evaluated at the clinic visit according to the Common Terminology Criteria for Adverse Events v4.0 (CTCAE) and by a self-administered Quality of Life questionnaire (MENQOL).
- Change in Ki-67 expression comparing pre-treatment versus post-treatment specimen to compare the antiproliferative effect among the different dosages.
- Serum drug measurements of exemestane and 17-dihydroxyexemestane at the end of treatment.
- Additional validated method of estradiol measurement. This method has a lower detection limit (1 pg/ml) than the CLIA certified estradiol test (Quest) and will serve as a quality control since it has proven to more effectively detect estradiol concentrations at the very low level, which is characteristic of older postmenopausal women.
- Serum concentrations of estrone, estrone-sulfate, will be measured by LC-MS/MS while androstenedione and testosterone will be measured at baseline and at surgery. Sex hormone binding globulin serum levels will be measured by a chemiluminescent microparticle immunoassay (CMIA) on the ARCHITECT *i* System (Abbott Laboratories, Weisbaden, Germany).
- Change in concentrations of insulin, glucose (HOMA index) and lipid profile (total cholesterol, HDL cholesterol and triglycerides will be evaluated.
- Adipokines: change in leptin and adiponectin serum concentrations will be analyzed and compared among the different treatments arms. These measurements will be performed by the use of commercially available enzyme linked immunoassays purchased from R&D systems (SPACE Import-Export Srl, Milan, Italy).
- Measurement of breast estradiol concentration in tumor and normal breast tissue at time of surgery.
- Centralized evaluation of ER, PgR, Her2 expression in tumor comparing pre-treatment levels (tru-cut biopsy) to post-treatment level expression (surgical specimen). Centralized evaluation of Ki-67 in adjacent intraepithelial neoplasia and or grossly benign tissue.
- Drug measurements of exemestane and 17-dihydroxyexemestane on frozen tissue samples, when available.
- Proteomic analysis will be performed with the new Fusion Tribrid mass spectrometer in Dr. Chen's Proteomics Shared Resource for the Herbert Irving Comprehensive Cancer Center (HICCC) at CUMC.
- To analyze the UGT2B17 gene we will use Taqman copy number variation assay (Life Technologies, Monza, Italy).
- Crown like structures in mammary fat tissue by IHC (CD68), comparing pre-treatment (tru-cut biopsy) versus post-treatment (surgical specimens).

Analysis plan

We will present full distributions and median values of circulating biomarkers, Ki-67, exemestane and 17-dihydroxyexemestane in tissue at baseline, after treatment and also of changes and percentage changes (with interquartile ranges) of all continuous variable, by arms. ANCOVA models will evaluate the associations of post values (after treatment) and changes from baseline by study arms adjusting for baseline values, explanatory variables and possible confounders (such as age and BMI). Normal distribution of residuals from full models will be checked and, if needed, a transformation will be considered.

For binary variables, when we need to assess differences in frequencies, Chi-squares tests and Odds ratios (ORs) will be calculated. Multivariate logistic models will be considered to assess differences between

arms, adjusting for possible confounders. We will report nominal P-values and we will highlight associations that meet a false discovery rate adjusted P less than or equal to .05 by the Benjamini and Hochberg method.

Analyses on secondary endpoints will be considered as exploratory since we will not have enough power to draw definitive conclusions.

13.6 Reporting and Exclusions

The primary analysis will employ an intention-to-treat approach, which includes all women irrespective of compliance. An according-to-protocol subgroup analysis will be restricted to women who are "compliant", such as participants who have taken ≥ 80% of the active scheduled pills, and notably, due to the different arm schedule, ≥ 80% of the active pills has to be reached also in the last 7 days before surgery.

Due to limitations of estradiol analysis procedures, small concentrations cannot be precisely measured. These concentrations are said to be below the limit of detection (LOD). We will consider a statistical method that uses the characteristics of the distribution of the values above the LOD to estimate the values below the LOD. This can be done with an extrapolation technique or maximum likelihood estimation that has smaller error rates than all the standard replacement techniques (Methods of Dealing with Values Below the Limit of Detection using SAS. Croghan et al.).

Patterns of observations for covariates will be classified as complete cases (no missingness), terminal (monotone) dropouts, intermittent (non-monotone) dropouts, mixed and no-data. Kendall's τ_b will be computed in order to test the correlation between a dichotomous indicator of missing observations and treatment assigned as well as any possible baseline covariates.

13.7 Evaluation of Toxicity

All participants will be evaluable for toxicity from the time of their first dose of exemestane.

13.8 Evaluation of Response

All participants included in the study who have taken at least one dose of study agent will be assessed for response to study treatment even if there are major protocol deviations or if they are ineligible. All of the participants who met the eligibility criteria (with the possible exception of those who did not receive study agent) will be included in the main analysis. All conclusions regarding efficacy will be based on all eligible participants.

All randomized participants who received at least one dose will be included in the study analysis with an intent-to-treat approach. A secondary analysis will also be carried out after excluding non-compliers and participants who will drop-out and who will refuse treatment after randomization. Using a more conservative approach, participants who drop-out will be considered in the failure group. Both the intent-to-treat and efficacy analyses will be conducted on a per-participant basis.

Reasons for excluding participants from the analysis will be clearly reported and sub analyses may not serve as the basis for drawing conclusions concerning efficacy. For all measurements of response, the 95% confidence intervals will also be provided.

13.9 Interim Analysis

There is no interim analysis planned.

13.10 Ancillary Studies

No ancillary studies are planned.

14. ETHICAL AND REGULATORY CONSIDERATIONS

14.1 Form FDA 1572

Prior to initiating this study, the Protocol Lead Investigator at the Lead or Participating Organization(s) will provide a signed Form FDA 1572 stating that the study will be conducted in compliance with regulations for clinical investigations and listing the investigators, at each site that will participate in the protocol. All personnel directly involved in the performance of procedures required by the protocol and the collection of data should be listed on Form FDA 1572.

14.2 Other Required Documents

14.2.1 Current (within two years) CV or biosketch for all study personnel listed on the Form FDA 1572 and Delegation of Tasks form for the Lead Organization and all Participating Organizations.

14.2.2 Current medical licenses (where applicable) for all study personnel listed on Form FDA 1572 and Delegation of Tasks form for the Lead Organization and all Participating Organizations.

14.2.3 Lab certification (*e.g.*, CLIA, CAP) and lab normal ranges for all labs listed on Form FDA 1572 for the Lead Organization and all Participating Organizations.

14.2.4 Documentation of training in "Protection of Human Research Subjects" for all study personnel listed on the FDA Form 1572 and Delegation of Tasks form for the Lead Organization and all Participating Organizations.

14.2.5 Documentation of Federalwide Assurance (FWA) number for the Lead Organization and all Participating Organizations.

14.2.6 Signed Investigator's Brochure/Package Insert acknowledgement form.

14.2.7 Delegation of Tasks form for the Lead Organization and all Participating Organizations signed by the Principal Investigator for each site and initialed by all study personnel listed on the form

14.2.8 Signed and dated NCI, DCP Financial Disclosure Form for all study personnel listed on Form FDA 1572 for the Lead Organization and all Participating Organizations

14.3 Institutional Review Board Approval

Prior to initiating the study and receiving agent, the Investigators at the Lead Organization and the Participating Organization(s) must obtain written approval to conduct the study from the appropriate IRB. Should changes to the study become necessary, protocol amendments will be submitted to the DCP PIO

according to DCP Amendment Guidelines. The DCP-approved amended protocol must be approved by the IRB prior to implementation

14.4 Informed Consent

All potential study participants will be given a copy of the IRB-approved Informed Consent to review. At the Italian sites, potentially eligible women who would like to participate in the trial will sign an IRB-approved screening Informed Consent. If they are eligible based on the ER status of their breast cancer, the Italian women will then sign the main study Informed Consent. The investigator will explain all aspects of the study in lay language and answer all questions regarding the study. If the participant decides to participate in the study, she will be asked to sign and date the Informed Consent document. The study agent(s) will not be released to a participant who has not signed the Informed Consent document.

Those who refuse to participate or who withdraw from the study will be treated without prejudice.

Participants must be provided the option to allow the use of blood samples, other body fluids, and tissues obtained during testing, operative procedures, or other standard medical practices for further research purposes. If applicable, statement of this option is included within the informed consent documents.

Prior to study initiation, the informed consent document must be reviewed and approved by NCI, DCP, the Consortium Lead Organization, and the Central IRB for the US sites and the Ethics Committees in Italy. Any subsequent changes to the informed consent must be approved by NCI, DCP, the Central IRB, and then submitted to each organization's IRB for approval prior to initiation."

14.5 Submission of Regulatory Documents

All regulatory documents are collected by the Consortia Lead Organization and reviewed for completeness and accuracy. Once the Consortia Lead Organization has received complete and accurate documents from a participating organization, the Consortium Lead Organization will forward the regulatory documents to DCP's Regulatory Contractor:

Paper Document/CD-ROM Submissions:
Regulatory Affairs Department
CCS Associates, Inc.
2001 Gateway Place, Suite 350 West
San Jose, CA 95110
Phone: 650-691-4400

E-mail Submissions:
regulatory@ccsainc.com

Regulatory documents that do not require an original signature may be sent electronically to the Consortium Lead Organization for review, which will then be electronically forwarded to the DCP Regulatory Contractor.

14.6 Other

This trial will be conducted in compliance with the protocol, Good Clinical Practice (GCP), and the applicable regulatory requirements.

15. FINANCING, EXPENSES, AND/OR INSURANCE

Participants will not be responsible for the costs of this study. Study agent will be provided at no cost to the participants. If, as a result of participation in this study, an individual experiences injury from known or unknown risks of the research procedures as described in the informed consent, immediate medical care and treatment, including hospitalization, if necessary, will be available. No monetary compensation is available for the costs of medical treatment for an injury, thus, the participant will be responsible for the costs of such medical treatment, either directly or through their medical insurance and/or other forms of medical coverage.

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APPENDIX A

Performance Status Criteria

ECOG Performance Status Scale		**Karnofsky Performance Scale**	
Grade	**Descriptions**	**Percent**	**Description**
0	Normal activity. Fully active, able to carry on all pre-disease performance without restriction.	100	Normal, no complaints, no evidence of disease.
		90	Able to carry on normal activity; minor signs or symptoms of disease.
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).	80	Normal activity with effort; some signs or symptoms of disease.
		70	Cares for self, unable to carry on normal activity or to do active 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.	60	Requires occasional assistance, but is able to care for most of his/her needs.
		50	Requires considerable assistance and frequent medical care.
3	In bed >50% of the time. Capable of only limited self-care, confined to bed or chair more than 50% of waking hours.	40	Disabled, requires special care and assistance.
		30	Severely disabled, hospitalization indicated. Death not imminent.
4	100% bedridden. Completely disabled. Cannot carry on any self-care. Totally confined to bed or chair.	20	Very sick, hospitalization indicated. Death not imminent.
		10	Moribund, fatal processes progressing rapidly.
5	Dead.	0	Dead.

Appendix B

Exemestane Pill Diary

PID: __ __ __ __ __ __ __ __ __ __ **Site: __ __ __ __ __** **Visit#: _____**

Number of Pills Given: _____ **Agent Returned: ☐ Yes ☐ No**

Total Daily Dose: 25 mg **Number of Pills Returned: _____**

1. Please complete daily. You should take 1 tablet once a day, preferably at dinner.
2. Please do not take an extra dose to "make up" for the missed dose. Take your next dose as scheduled. The first time you miss a dose, please contact the study coordinator at:_____
3. DRUG INTERACTION Please report as soon as possible to the study doctor if you will be prescribe on of these medicaments: rifampicin, phenytonin, carbamazepine, phenobarbital, and St. John's wort

Day		Date	Took 1 tablet – Yet/No and Time	Notes
Wallet 1				
Blister 1	1			
	2			
	3			
	4			
	5			
	6			
	7			
Blister 2	8			
	9			
	10			
	11			
	12			
	13			
	14			

Blister 3	15			
	16			
	17			
	18			
	19			
	20			
	21			
Day		**Date**	**Took 1 tablet –** **Yet/No and Time**	**Notes**
Wallet 2				
Blister 1	22			
	23			
	24			
	25			
	26			
	27			
	28			
Blister 2	29			
	30			
	31			
	32			
	33			
	34			
	35			

Blister 3	36			
	37			
	38			
	39			
	40			
	41			
	42			

Patient's Signature: _____ Date: _____

Reviewer's Signature:_____ Date: _____

APPENDIX C

COMPLIANCE DRUG LABELS

(Used for Randomization)

INSTITUTION CODE	PARTICIPANT ID	VISIT TYPE	VISIT DATE *(MM/DD/YYYY)*
_____	_____	_____	__ __ / __ __ / __ __ __ __

Affix Labels Here:

Were Labels Affixed? ☐ Yes ☐ No

Investigator's Signature: _____ Date of Investigator's Signature: _ _ / _ _ / _ _ _ _
(MM/DD/YYYY)

Investigator's Name (Please Print): _____