Mayo Clinic Cancer Center

MC1931-Pharmacodynamic Study of Estrogen Suppression Threshold-Directed Therapy (ESTDT) of Anastrozole as Adjuvant Therapy for Early Stage Breast Cancer

Sponsor/Principal Investigator: [redacted]

Co-Investigators: [redacted]

Statistician: [redacted]

Patient Advocate: [redacted]

√Study contributor(s) not responsible for patient care

FDA IND #: 146718

Trial Supported by:
Funding: Mayo Clinic Breast Cancer Specialized Program of Research Excellence (SPORE)

Drug Availability:

Commercial Agents: anastrozole (1mg), letrozole
Supplied Agents: anastrozole (10mg)

Site Investigators & Participating sites:

[redacted]

[redacted]

Document History	**(Effective Date)**
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MCCC Amendment 2	pending

Protocol Resources

Questions:	Contact Name:
Patient eligibility*, test schedule, treatment delays/interruptions/adjustments, dose modifications, adverse events, forms completion and submission	[redacted]
Drug administration, infusion pumps, nursing guidelines	[redacted]
Clinical Research Coordinator (lead site)	[redacted]
Clinical Data Coordinator (lead site)	[redacted]
Breast Program Coordinator	[redacted]
Protocol document, consent form, regulatory issues	[redacted]
Laboratory contact	[redacted]
Serious Adverse Event Reporting	[redacted]

*No waivers of eligibility allowed

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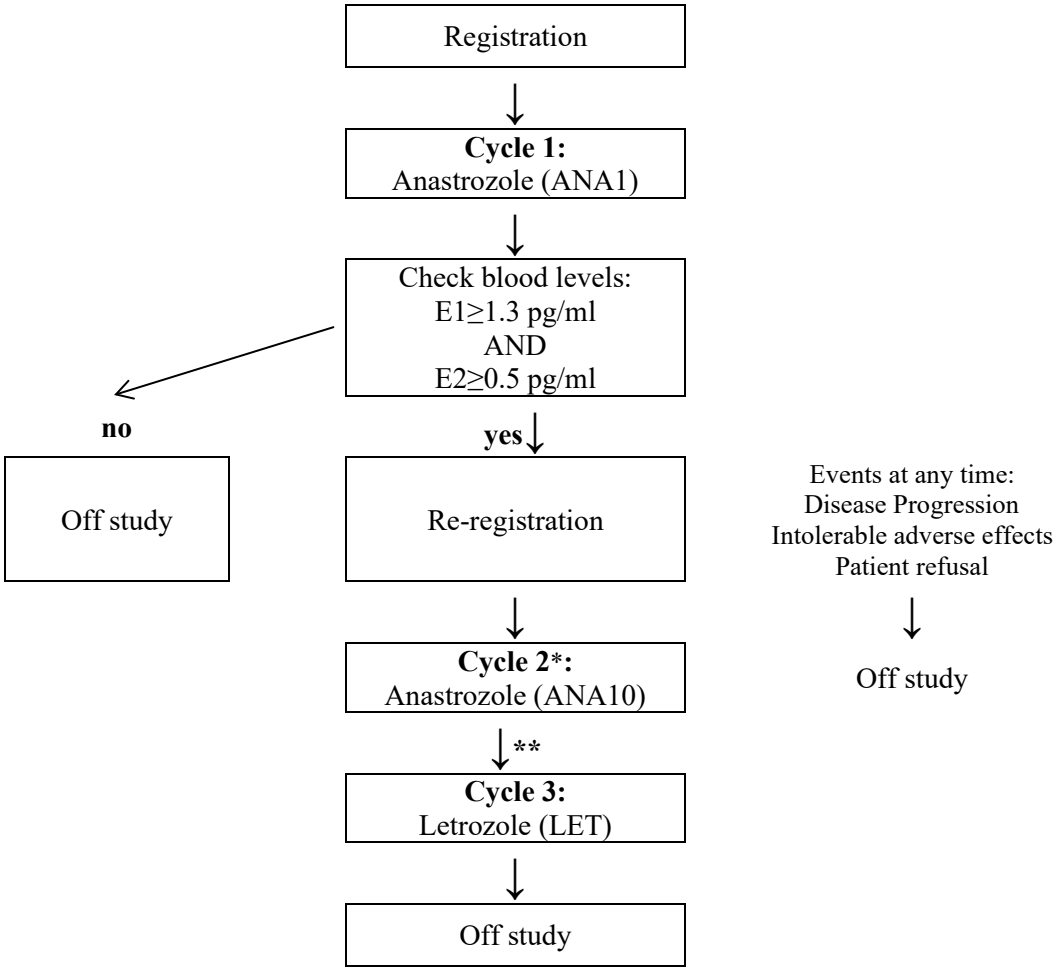
Schema



*Enrollment will be temporarily suspended after 6 patients have been re-registered and started ANA10

**Adverse event assessment will be carried out for all patients who begin ANA10, 28-35 days after the completion of ANA10 (regardless of whether the patient continued on to letrozole)

Cycle = 56-70 days (8-10 weeks)

ANA1 = Anastrozole 1 mg ANA10 = Anastrozole 10 mg LET = Letrozole

Generic name: anastrozole Brand name(s): Arimidex® Availability: Commercial (ANA1) Provided by study (ANA10)	Generic name: letrozole Brand name(s): Femara® Availability: Commercial

1.0 Background

1.1 Rationale for selected approach

The third-generation aromatase inhibitors (AIs) anastrozole, exemestane, and letrozole, all play a major role in the adjuvant therapy of postmenopausal women with early stage estrogen receptor α (ERα)-positive breast cancer[1,2]. The assumption is that for all three AIs the mechanism of action in inhibiting tumor growth is only through decreasing estrogen production from androgenic precursors, by inhibiting aromatase[3], which is encoded by the CYP19A1 gene[4-6]. Whereas all three AIs are potent in terms of inhibition of *in vivo* aromatization[7-9], letrozole has been found to be the most potent[10]. However, large phase III adjuvant clinical trials do not indicate any difference in efficacy between the three AIs. Specifically, the MA.27 trial showed no significant difference between anastrozole and exemestane[11] in terms of DFS, and the FACE trial showed no difference between anastrozole and letrozole in terms of both DFS and OS[12].

We previously performed a pharmacokinetic and pharmacodynamic study in 649 patients receiving anastrozole adjuvant therapy[13] that showed 21% and 30%, respectively, had estradiol (E2) and estrone (E1) concentrations above the lower limit of quantitation (LLQ), with a broad range of detectable concentrations.

Until recently there were no clinical studies which systematically tested whether the degree of estrogen suppression is associated with AI efficacy in early stage ERα-positive breast cancer. To address this unmet need, matched case-control studies[14] were performed utilizing data from the MA.27 and PreFace trials. MA.27 enrolled postmenopausal women with resected stage I-III ERα-positive breast cancer who were randomized to either anastrozole or exemestane for five years. PreFace evaluated adjuvant letrozole in a single-arm phase IV trial. A breast event was considered to be any of the following: local-regional breast cancer recurrence [including ipsilateral DCIS], distant breast cancer recurrence, contralateral breast cancer [invasive or DCIS], or death with or from breast cancer without prior recurrence.

Due to the lack of an independent validation cohort, a bootstrap re-sampling approach was undertaken to determine 'best' cut points for the biomarkers, E1 and E2. Specifically, 500 bootstrap samples were constructed by sampling with replacement using 222 matched case-control sets from the 247 matched case-control sets. The set of potential cut-points assessed for each biomarker included values from its lower limit of quantification to its 85th percentile value (across all women). The 'best' cut point for a given biomarker was chosen using the maximum concordance approach of Liu.[15] Specifically, for each potential cut point y_i, an indicator variable x_i was constructed where x_i=1 if the patient's biomarker value was at or above y_i; x_i=0 otherwise. Then, for each bootstrap sample, j, and each cut point, i, a concordance statistic C_{ij} was generated from fitting a stratified Cox model with case-control set as the strata, time set to the constant of 1, and the indicator variable for the cut point was fit to the data.[16] The cut point where the maximum value of the concordance statistic, max C_j, occurred was determined for each bootstrap sample j. The cut point most often found to be the max C_j cut point across the 500 samples was chosen for further evaluation. Having established cut points for E1 and E2, multivariate conditional logistic regression modeling using all 247 matched pairs was used to refine the model of risk. A secondary exploratory analysis was carried out using the refined model to obtain an estimate of the odds of EBCE for each treatment cohort separately. Statistical analyses were carried out using SAS 9.4 and the survConcordance() function in the survival package of R software.

The pre-AI E1 and E2 concentrations were similar among the 3 treatment cohorts, and were highly correlated within each treatment cohort with Spearman rank coefficients (ρ) of 0.84 for anastrozole; 0.89 for exemestane; and 0.90 for letrozole. After six months of AI therapy, E1 and E2 concentrations were below the LLQ in 41.3% and 11.9%, respectively, for anastrozole; 63.7% and 35.4%, respectively, for exemestane, and 79.3 % and 48.7%, respectively, for letrozole. The correlation between E1 and E2 concentrations after six months of AI therapy was moderate for anastrozole (ρ= 0.54) and exemestane (ρ=0.52) but weak for letrozole (ρ= 0.15). There was a very weak correlation between both E1 and E2 with AI concentrations after 6 months of treatment (ρ=-0.20 and -0.12 for anastrozole; -0.18 and -0.12 for exemestane, and -0.11 and -0.09 for letrozole, respectively). Six-month plasma concentrations of each of the AIs showed substantial inter-patient variability.

1.2 Estrone and Estradiol Suppression and Risk of an Early Breast Cancer Event

A potential cut point for E1 after six months of treatment was searched for between its lower limit of quantification (1.0 pg/mL) and the 85th percentile value of its distribution (1.7 pg/mL). For each cut point, a stratified Cox model was fit to the first of 500 bootstrap samples, and an estimate of the odds of EBCE and the concordance statistic was determined. This process was repeated for the remaining 499 bootstrap samples. From the 500 estimates of the odds ratio for a given cut point, the median and 2.5th and 97.5th percentile value of its distribution was determined and presented in Figure 1 for E1 and in Figure 2 for E2. The value of E1 most often having the maximum concordance value was 1.3 pg/mL and as such is the threshold for E1 (Figure 1). The process was repeated for E2 searching between the lower limit of quantification (0.3 pg/mL) and 85th percentile value of its pre-treatment distribution (0.7 pg/mL). The value of E2 most often having the maximum concordance value among the 500 bootstrap samples was 0.5pg/mL (Figure 2)**.**

Using the cut point values identified for E1 and E2, multivariate conditional logistic modeling was used to assess whether or not the risk of EBCE differed with respect to whether E1 and E2 remain at or above its threshold after 6 months of treatment. Patients with E1 ≥1.3 pg/mL and E2 ≥0.5 pg/mL were found to have a 2.2 fold (95% CI: 1.42-3.47) increase in risk of an EBCE relative to patients with E1, E2, or both below these thresholds.

The question as to whether this relationship holds for each treatment group was determined. **The risk of an EBCE was increased 3.0 fold (sets=91: 95% CI: 1.56-5.76, p=0.001) for those with E1 and E2 values at or above their threshold after 6 months of anastrozole**; but not in those treated with exemestane (sets=95: 95% OR=1.66; CI: 0.82-3.33, p=0.16) or letrozole (sets=59: 95% OR=1.62; CI: 0.39-6.82, p=0.51).

In the case of anastrozole, an odds ratio of three means that a patient with an E2 and E1 at or above the thresholds had a three-fold risk of recurrence compared with a patient with the same matching factors (in this case: age, disease stage, BMI category, and adjuvant chemotherapy status) who had an E2 or E1 below the threshold.

Figure 1

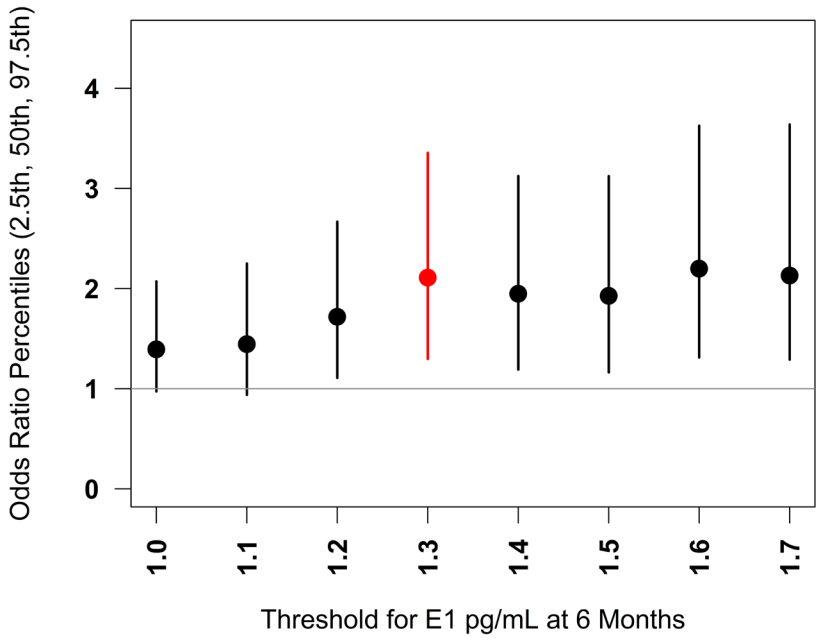


Figure 1 Legend. Bootstrap Resampling for E1 Odds Ratio Distributions from (all 247 Case-Control Sets). E1≥1.3 vs. <1.3 Odds Ratio (95% CI): 1.75 (1.19, 2.55), p=0.004

Figure 2

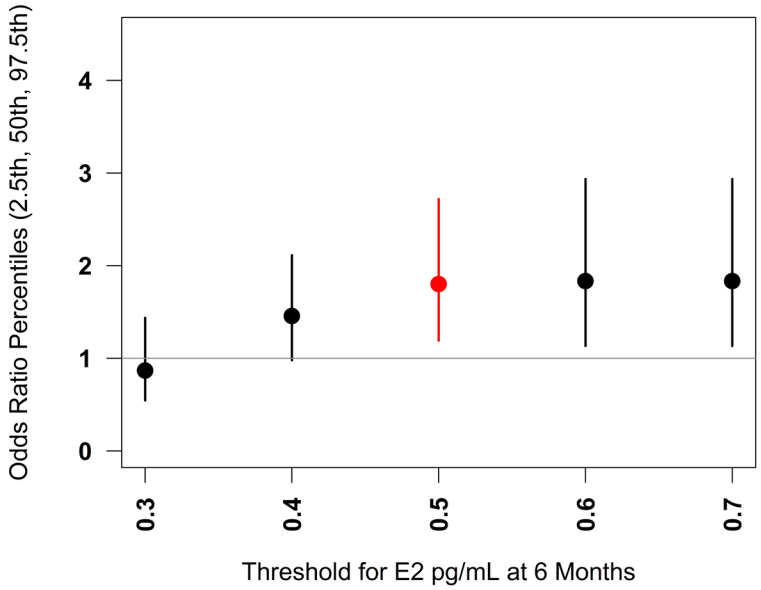


Figure 2 Legend. Bootstrap Resampling for E2 Odds Ratio Distributions from (all 247 Case-Control Sets). E2≥0.5 vs. <0.5 Odds Ratio (95% CI): 1.44 (1.02, 2.03), p=0.04

1.3 Preclinical laboratory studies supporting a difference between anastrozole and exemestane and letrozole

Our preclinical data indicated that anastrozole, but not letrozole or exemestane, behaved in a fashion similar to E2. Anastrozole could activate ERα-dependent transcription (Figure 3), but the effect decreased with increasing concentrations. Additionally, in the presence of 100 nM anastrozole, we also observed time-dependent ERα degradation. Given that these data indicated that anastrozole but not exemestane or letrozole, had a mechanism of action in addition to inhibition of aromatase, and was a ligand for ERα, we performed radioligand binding assays (Figure 4), surface plasmon resonance (Figure 5), and nuclear magnetic resonance studies (Figure 6) that confirmed that, indeed anastrozole was a ligand for ERα.

Figure 3.

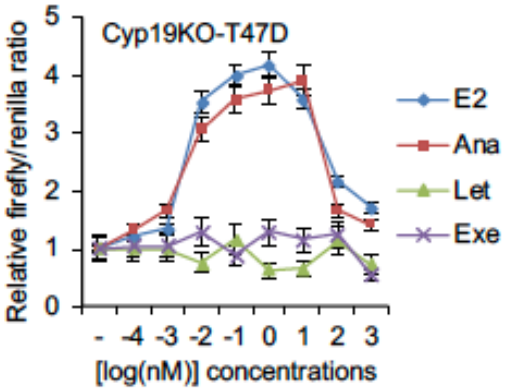


Figure 3 Legend: Estrogen response element-dependent luciferase assay in CYP19A1 CRISPR KO T47D cells treated with indicated concentrations of E2, anastrozole (Ana), letrozole (Let) or exemestane (Exe).

Figure 4.

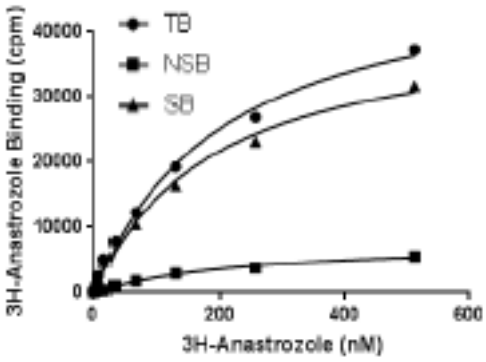


Figure 4 Legend: Radioligand binding assays. [H3]-anastrozole binding with ERα protein. TB: total binding, NSB: non-specific binding, SB: specific binding.

Figure 5.

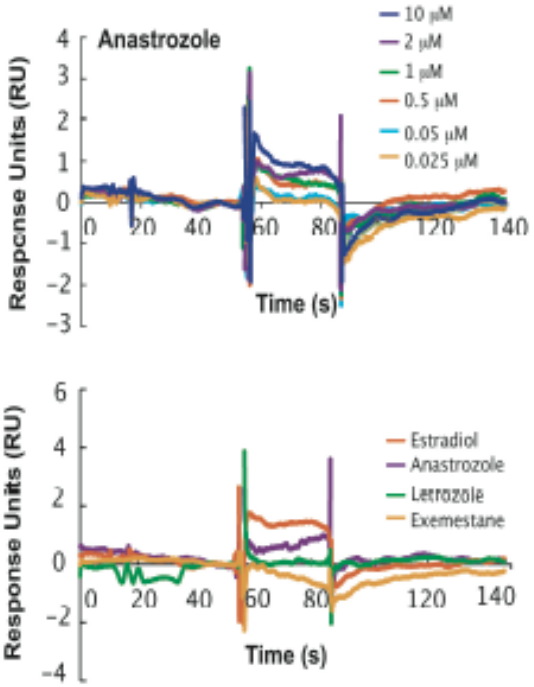


Figure 5 Legend: ERα binding assays of anastrozole at different concentrations as indicated using a surface plasmon resonance biosensor. Comparisons among all four compounds at 2 μM.

Figure 6.

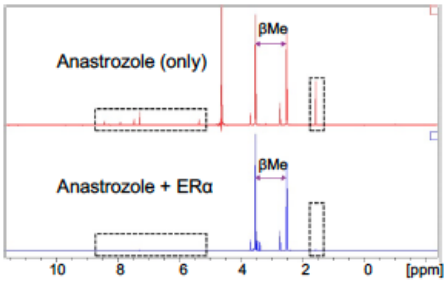


Figure 6 Legend: Anastrozole binding of ERα detected by nuclear magnetic resonance (NMR) spectroscopy. One-dimensional proton Carr-Purcel-Meiboom-Gill NMR spectroscopy of 120 μM anastrozole in the absence (top, red) and presence (bottom, blue) of 3 μM ERα protein. Dashed boxes showed decreased NMR signals. Spectra were normalized to each other using β-mercaptoethanol (βMe) signal shown in purple.

We then tested the effect of anastrozole on cell proliferation in the presence of estrogen levels below, at, and above the thresholds identified in the clinical studies described above. In vitro, E1 and E2 below 1.3 pg/mL and 0.5 pg/mL, respectively, had little effect on CYP19A1 KO T47D cell proliferation. Anastrozole, but neither letrozole nor exemestane, potentiated estrogen effects on cell proliferation when estrogen levels were above the thresholds, compared to estrogen alone, especially when both E1 and E2 were above the thresholds (Figure 7 A-C). Anastrozole, but neither letrozole nor exemestane, potentiated estrogen-induced ERE luciferase activity when estrogen levels were above the thresholds (Figure 7 D-F).These phenomena were also observed in an MCF7 cell line.

Figure 7.

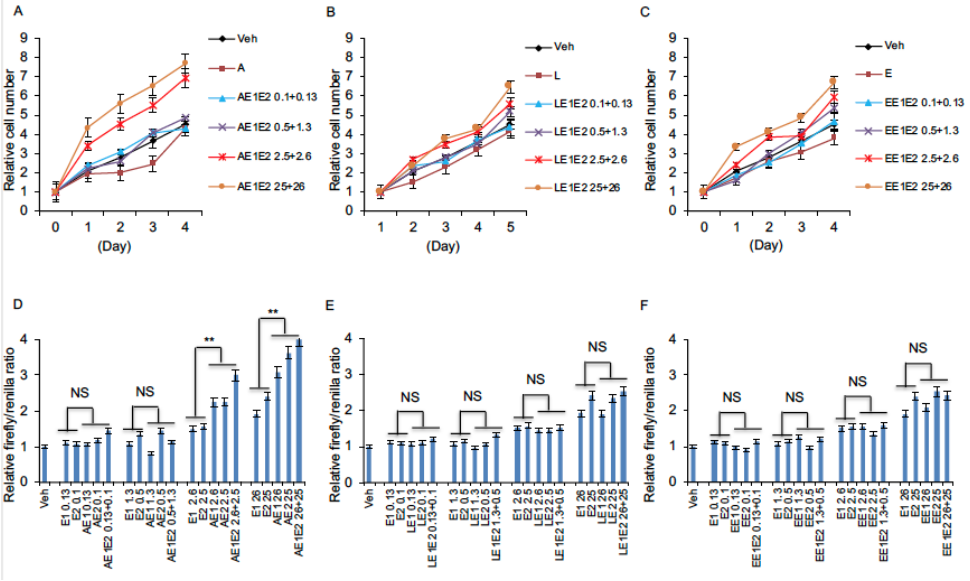


Figure 7 Legend: Anastrozole potentiates estrogen's effects when estrogen levels are above the thresholds. A. Cell growth of CYP19A1 KO T47D cells in the presence of anastrozole (A) (27 ng/ml) plus indicated concentrations of estrone (E1) (0.13, 1.3, 2.6, and 26 pg/mL) and estradiol (E2) (0.1, 0.5, 2.5, and 25 pg/mL). B. Cell growth of CYP19A1 CRISPR KO T47D cells in the presence of letrozole (L) (215 ng/mL) plus estrone (E1) and estradiol (E2). C. Cell proliferation of CYP19A1 CRISPR KO T47D cells in the presence of exemestane (E) (10 ng/mL) plus estrone (E1) and estradiol (E2). D-F. Estrogen response element-dependent luciferase assay in CYP19A1 CRISPR KO T47D cells treated with indicated concentrations of E1 (0.13, 1.3, 2.6, and 26 pg/mL), E2 (0.1, 0.5, 2.5, and 25 pg/mL), or in combination with anastrozole (A) (27 ng/mL), letrozole (L) (215 ng/mL), and exemestane (E) (10 ng/mL). Error bars represent the SEM of 3 independent experiments. **p<0.01.

1.4 Trial Design

To evaluate the clinical impact of these findings, this clinical trial is designed to evaluate anastrozole in the adjuvant setting for patients with early stage ERα-positive breast cancer. Specifically, E1 and E2 levels will be assessed in patients who receive standard dose adjuvant anastrozole (1mg once daily). For patients who have inadequate suppression of E1 and E2 after 2 months of treatment, with levels above the thresholds of 1.3 pg/ml and 0.5 pg/ml respectively, the dose of anastrozole will be escalated to 10 mg once daily. E1 and E2 levels will be re-assessed after 2 months of treatment on 10 mg of anastrozole. E1 and E2 will be measured by a CLIA-approved liquid chromatography with tandem mass spectrometry (LC/MS/MS) in the Immunochemical Core Laboratory at Mayo Clinic. Details of the methodology have been published[17-19]. In addition, anastrozole and letrozole concentrations will be measured by Zeruesenay Desta, PhD, at Indiana University, a long-time collaborator, who has performed these assays in past studies[20]. The main purpose of performing the AI assays is to ensure that the patients are taking the drug, but we are also interested in the steady state concentrations of anastrozole in the women who take the 10 mg daily dose.

The 10 mg dose of anastrozole has been previously compared with standard 1 mg dosing in patients with metastatic breast cancer[21]. There was no difference in clinical efficacy and toxicities were comparable. Regarding the cost of anastrozole therapy, the 1 mg dose

costs about $2/day and the 10 mg dose costs roughly $20/day, which translates to $60/month and $600/month, respectively. For patients who receive anastrozole at 10 mg per day for 8-10 weeks, patients will be advised to take letrozole, an FDA-approved agent, to complete their course of adjuvant endocrine therapy. After 8-10 weeks of letrozole, patients will have blood samples obtained for E1, E2, and letrozole concentrations. The rationale for this approach is that further research will be required before it is known if continuing anastrozole at 10 mg per day will provide long term benefit to patients.

1.41 Safety Run-in

At the request of the FDA, re-registration to the ANA10 treatment portion of the trial will be initially limited to a maximum of 6 patients. After 6 patients have re-registered, enrollment will temporarily halt. If two or more of these 6 patients develop a DLT (as specified in Section 7.0 of the protocol) within 28-35 days of starting ANA10, the study will remain closed to enrollment to allow the study team to review toxicity data thoroughly and make decisions regarding changes to the protocol. If at most one of these 6 patients develops a DLT within 28-35 days of starting ANA10, the FDA will be notified and re-registration will be reopened.

1.42 Adjustments to Eligibility in Response to the COVID-19 Pandemic

Due to the COVID-19 pandemic and late 2020 surge in cases, some patients have experienced delays in their surgical procedures and have been started on endocrine therapy preoperatively to bridge to the operating room. Typically the time on treamtment is several weeks or up to 4 months. In response to this, the protocol has been amended to adapt to these circumstances, and allow patients who have received preoperative endocrine therapy to participate. Specifically, the half-life of anastrozole and letrozole is approximately 50 hours and exemestane is 24 hours; as such, these agents are cleared from the system after 5 half-lives. To this end, patients who received preoperative endocrine therapy with an aromatase inhibitor can participate if they have been off treatment for at least 4 weeks prior to registration. This will allow adequate elimination of the drug and baseline steady-state prior to study participation. Tamoxifen is much less commonly used in postmenopausal women as preoperative therapy. Due to its longer half-life of 5-7 days, patients who received preoperative tamoxifen can participate if they have been off treatment for at least 12 weeks prior to registration.

In alignment with these changes, prior receipt of an aromatase inhibitor or SERM (tamoxifen or raloxifene) for the prevention of breast cancer is allowed. In this setting, given these agents are typically administered for up to 5 years, a longer wash out of 6 months is preferred.

Additionally, there have been delays for post-operative Medical Oncology consultation; as such, the requirement to complete all cancer treatment within 60 days prior to registration has been removed. Specifically, patients must have completed treatment, but any time between that and registration is permissible.

2.0 Goals

2.1 Primary Goal

To estimate the proportion of women who have adequate estrone (E1) and estradiol (E2) suppression after 8-10 weeks of adjuvant anastrozole 10 mg once daily (ANA10) having had inadequate E1 and E2 suppression after 8-10 weeks of standard dose anastrozole 1 mg once daily (ANA1).

2.2 Secondary Goals

2.21 To estimate the proportion of women with elevated E1 and E2 levels after 8-10 weeks of adjuvant ANA1.

2.22 To estimate the proportion of women with elevated E1 and E2 levels after 8-10 weeks of adjuvant ANA1 whose E1 and E2 levels remain elevated after 8-10 weeks of adjuvant ANA10.

2.23 To examine the toxicity profile of ANA1 over the 8-10 weeks of treatment, ANA10 over the 8-10 weeks of treatment, and letrozole over the 8-10 weeks of treatment.

2.24 To examine concentrations of anastrozole at both the ANA1 and ANA10 dose levels.

2.25 To examine E1 and E2 concentrations, as well as letrozole drug levels, in patients receiving letrozole (following ANA10).

2.26 To bank DNA for examination of SNP-set(s) determined in the ongoing Mayo Clinic Breast Cancer SPORE Project 4

2.3 Exploratory Objectives

To examine the association between clinical variables such as age, age at menopause, BMI, receipt of chemotherapy, chemotherapy regimen, and dose on E1 and E2 levels after 8-10 weeks of ANA10.

3.0 Registration Patient Eligibility

3.1 Registration - Inclusion Criteria

3.11 Women of age ≥18 years.

3.12 Disease characteristics:

3.121 Histological confirmation of invasive breast carcinoma.

3.122 Stage I-III breast cancer

3.123 Estrogen receptor (ER) positive disease according to ASCO/CAP guidelines as ER≥1% positive nuclear staining.

3.13 Completion of all planned cancer treatments prior to registration:
1) surgical resection of breast and nodal surgery;
(NOTE: Reconstructive surgery does not have to be completed)
2) adjuvant radiation therapy, if needed;
3) and neoadjuvant and/or adjuvant chemotherapy, if needed.

3.14 Post-menopausal defined as

- Age ≥60 and amenorrhea >12 consecutive months
  OR
- Previous bilateral oophorectomy
  OR
- Age <60 and amenorrhea >12 consecutive months and documented follicle stimulating hormone (FSH) level within post-menopausal range according to institutional standard

NOTE: Patients who did not meet these criteria at time of diagnosis and received pre-operative (neoadjuvant) or post-operative (adjuvant) chemotherapy will not be allowed to participate.

3.15 ECOG Performance Status (PS) 0, 1, or 2 (Appendix I).

3.16 The following laboratory values obtained ≤14 days prior to registration:

- Hemoglobin ≥8.0 g/dL
- Absolute neutrophil count (ANC) ≥1500/mm^3
- Platelet count ≥70,000/mm^3
- Total bilirubin ≤1.5 x ULN
- Alanine aminotransferase (ALT) and aspartate transaminase (AST) ≤3 x ULN

3.17 Ability to swallow oral medication

3.18 Provide written informed consent.

3.19a Willingness to provide mandatory blood specimens for correlative research (see Section 14.0).

3.19b Willing to return to enrolling institution for follow-up (during the Active Monitoring Phase of the study).

3.2 Registration - Exclusion Criteria

3.21 Pre-menopausal women receiving ovarian function suppression (goserelin, leuprolide, etc.)

3.22 Stage IV (metastatic) breast cancer.

3.23 HER2 positive breast cancer as defined by
- HER2 IHC ≥3+
- HER2/CEP17 ≥2.0
- HER2/CEP17 <2.0 and average HER2 copy number of ≥6.0 signals/cell

3.24 Prior endocrine therapy for this breast cancer.
Exceptions:
1) Pre-operative aromatase therapy (anastrozole, letrozole, or exemestane) and last treatment was ≥4 weeks prior to registration
OR
2) Pre-operative tamoxifen therapy and last treatment was ≥12 weeks prior to registration

3.25 Currently receiving any of the following cancer-directed therapies:
- Radiation therapy
- Systemic therapy such as chemotherapy (standard or investigational)

3.26 Bisphosphonate therapy started <4 weeks prior to registration.

NOTE: If patient is currently on bisphosphonate therapy she must be on stable dose for ≥4weeks prior to registration.

Patients not currently taking bisphosphonates will be allowed to start bisphosphonate therapy after completion of anastrozole (1mg and 10 mg daily (if given)). Information regarding bisphosphonate therapy will be collected.

3.27 Current use of systemic or topical exogenous estrogen or progesterone (menopausal hormone replacement therapy [HRT]).

3.28 Prior ovarian function suppression (leuprolide, goserelin, etc).

3.29a Inability to provide informed consent.

3.29b History of contralateral DCIS or invasive breast cancer.

NOTE: Exception allowed if

1. Patient did not receive adjuvant endocrine therapy

OR

2. Patient received adjuvant endocrine therapy but has been off treatment for at least 6 months prior to registration

3.29c Concurrent active malignancy or history of malignancy ≤3 years prior to registration.
NOTE: Exceptions allowed for successfully treated cervical carcinoma in situ, lobular carcinoma in situ of the breast, papillary thyroid cancer, or non-melanoma skin cancer.

3.29d Prior prevention therapy with an aromatase inhibitor or a SERM.
Exception: Therapy with a SERM (tamoxifen or raloxifene) is allowed if patient has been off treatment for ≥6 months prior to registration.

3.3 Re-Registration – Inclusion Criteria

3.31 Confirmation that baseline blood sample was drawn and submitted.

3.32 Blood estrogen levels after Cycle 1anastrozole (ANA1) must meet the following criteria:
E1≥1.3 pg/ml, AND
E2≥0.5 pg/ml

4.0 Test Schedule

4.1 Test schedule for breast cancer

	Active Monitoring Phase								
Tests and procedures	≤14 days prior to registration	At end of Cycle 1[1]	Re-registration	After ReReg and prior to Tx on C2D1[2]	Nurse call 2-4 weeks after starting ANA10	First 6 pts: once during Cycle 2 Days 28-35[3]	At end of Cycle 2[4]	30 days after last dose of ANA10	At end of Cycle 3
Window		±7 days			±7 days		±7 days	±3 days	+14 days
History and exam[5]	X	X		X[6]					X
Height and weight[7]	X	X					X^R		X
Adverse event assessment, vital signs (BP, pulse), ECOG PS	X	X		X	X[8]	X^R	X^R	X^R	X
Hematology: CBC/differential	X	X^R				X^R	X^R	X^R	
Chemistries: alanine transaminase (ALT), aspartate transaminase (AST), total bilirubin	X	X^R				X^R	X^R	X^R	
Research blood specimens (see Section 14.0)[,R]	X^R	X^{R9}					X^R		X^R
Medication Diary		X					X		X

Cycle = 56-70 days (8-10 weeks), R= Research funded

[1] Approximately 9 weeks after start of ANA1 ±7 days; or at discontinuation of ANA1 if that event occurs earlier than 8-10 weeks.
[2] Visit to obtain prescription after blood results are received.
[3] One-time safety assessment for first six patients who re-register to ANA10
[4] Approximately 9 weeks after start of ANA10 ±7 days; or at discontinuation of ANA10 if that event occurs earlier than 8-10 weeks. Must be prior to starting letrozole.
[5] Physical exam is required at baseline. After baseline, physical exam may be performed at any time as clinically indicated, but is not required for protocol.
[6] Nurse visit for ANA10 education (prior to starting ANA10)
[7] Only weight needed after baseline
[8] Call to assess/collect adverse events and verify patient is taking ANA10 and wants to continue (no vital signs or PS); Refill ANA10 by mail if needed
[9] E1 and E2 results required prior to re-registration.

4.2 Survival Follow-up

This study does not follow patient survival. Once patients complete protocol treatment and procedures they are off study.

5.0 Stratification Factors: None

6.0 Registration/Randomization Procedures

6.1 Registration (Step 1)

To register a patient, access the Mayo Clinic Cancer Center (MCCC) web page and enter the registration/randomization application. The registration/ randomization application is available 24 hours a day, 7 days a week. Back up and/or system support contact information is available on the website. If unable to access the website, call the MCCC Registration Office at [redacted] between the hours of 8 a.m. and 4:30 p.m. Central Time (Monday through Friday).

The instructions for the registration/randomization application are available on the MCCC web page [redacted] and detail the process for completing and confirming patient registration. Prior to initiation of protocol treatment, this process must be completed in its entirety and an MCCC subject ID number must be available as noted in the instructions. It is the responsibility of the individual registering the patient to confirm the process has been successfully completed prior to release of the study agent. Patient registration via the registration/randomization application can be confirmed in any of the following ways:

- Contact the MCCC Registration Office [redacted] If the patient was fully registered, the MCCC Registration Office staff can access the information from the centralized database and confirm the registration.
- Refer to “Instructions for Remote Registration” in section “Finding/Displaying Information about A Registered Subject.”

6.2 Verification of materials

Prior to accepting the registration, registration/randomization application will verify the following:

- IRB approval at the registering institution
- Patient eligibility
- Existence of a signed consent form
- Existence of a signed authorization for use and disclosure of protected health information

6.3 Documentation of IRB approval

Documentation of IRB approval must be on file in the Registration Office before an investigator may register any patients.

In addition to submitting initial IRB approval documents, ongoing IRB approval documentation must be on file (no less than annually) at the Registration Office [redacted] If the necessary documentation is not submitted in advance of attempting patient registration, the registration will not be accepted and the patient may not be enrolled in the protocol until the situation is resolved.

When the study has been permanently closed to patient enrollment, submission of annual IRB approvals to the Registration Office is no longer necessary.

6.4 Correlative Research

6.41 Mandatory

A mandatory correlative research component is part of this study, the patient will be automatically registered onto this component (see Sections 3.19a and 14.0*)*.

6.42 Banking

At the time of registration, the following will be recorded:

- Patient has/has not given permission to store and use her/his data and sample(s) for future research on cancer at Mayo.
- Patient has/has not given permission to store and use her/his data and sample(s) for future research to learn, prevent, or treat other health problems.
- Patient has/has not given permission for MCCC to give her/his data and sample(s) to researchers at other institutions.

6.5 Treatment on protocol

Treatment on this protocol must commence at Mayo Clinic under the supervision of a medical oncologist.

6.6 Treatment start

Treatment cannot begin prior to registration and must begin ≤21 days after registration.

6.7 Pretreatment

Pretreatment tests/procedures (see Section 4.0) must be completed within the guidelines specified on the test schedule.

6.8 Baseline symptoms

All required baseline symptoms (see Section 10.6) must be documented and graded.

6.9a Study drug

Study drug is available on site.

6.9b Blood draw kits

Blood draw kit is available on site for sites outside of Rochester, Minnesota.

6.9c Study Conduct

The clinical trial will be conducted in compliance with regulations (21 CFR 312, 50, and 56), guidelines for Good Clinical Practice (ICH Guidance E6), and in accordance with general ethical principles outlined in the Declaration of Helsinki; informed consent will be obtained from all participating patients; the protocol and any amendments will be subject to approval by the designated IRB prior to implementation, in accordance with 21 CFR 56.103(a); and subject records will be stored in a secure location and subject confidentiality will be maintained. The investigator will be thoroughly familiar with the appropriate use of the study drug as described in the protocol and Investigator's Brochure. Essential clinical documents will be maintained to demonstrate the validity of the study and the integrity of the data collected. Master files should be established at the beginning of the study, maintained for the duration of the study and retained according to the appropriate regulations.

6.9d Re-registration (Step 2)

6.9d1 To re-register a patient, access the Mayo Clinic Cancer Center (MCCC) web page and enter the registration/randomization application. The registration/ randomization application is available 24 hours a day, 7 days a week. Back up and/or system support contact information is available on the website. If unable to access the website, call the MCCC Registration Office at [redacted] between the hours of 8 a.m. and 4:30 p.m. Central Time (Monday through Friday).

NOTE: Re-registration to Cycle 2 will be temporarily halted once 6 patients have been re-registered for a safety run-in.

6.9d2 Treatment cannot begin prior to re-registering to the second phase and will begin ≤14 days after registration to the second phase.

7.0 Protocol Treatment

7.1 Treatment Schedule

7.11 Treatment medication table

Cycle	Agent	Dose Level	Route	Day	Duration
1	Anastrozole	1 mg	oral	daily	8-10 weeks
2	Anastrozole	10 mg	oral	daily	8-10 weeks
3	Letrozole	2.5 mg	oral	daily	8-10 weeks

7.2 Agents

Anastrozole and letrozole are oral agents commonly used as adjuvant treatment for ER-positive breast cancer to reduce the risk of recurrence.

Protocol treatment should be taken at the same time every day (morning preferred) and may be taken without regard for food. EXCEPTION: On the day of the end of cycle blood draw, the treatment should be taken **after** the blood draw is completed.

7.3 Cycle 1 Anastrozole (ANA1)

7.31 Basics

A research blood sample must be drawn at baseline prior to treatment or patient is unevaluable and must be replaced.

All patients will be prescribed the FDA-approved dose of anastrozole at 1mg daily (standard adjuvant dose) for the first 8-10 weeks.

At the end of the first cycle, blood will be drawn for drug levels and E1 and E2 levels.

Patients should be encouraged to take their anastrozole in the morning each day EXCEPT the day of the blood draw when they should take their anastrozole after this sample has been drawn.

7.32 Blood draw at end of cycle

Patients will have blood drawn for estrone and estradiol concentrations and all samples for the week will be batched for processing in the Immunochemical Core Laboratory the following Monday.

Results will be reported the following day (usually Tuesday).

Patients who do not meet the following criteria will be given a recommendation for 5 years of anastrozole and go off study.

E1≥1.3 pg/ml
AND
E2≥0.5 pg/ml

Patients whose estrogen levels meet these criteria and are willing to continue will proceed to Cycle 2.

NOTE: Patients unwilling to continue will go off study and be replaced.

7.33 Logistics

Study team will contact patients by telephone and tell the patients to either:

1) Continue anastrozole 1mg and go off study.

OR

2) Come to the clinic for an appointment in the next couple weeks (prior to running out of ANA1) to re-register and obtain the new prescription.

Re-registration is required for these patients prior to Cycle 2.

7.4 Cycle 2 Anastrozole (ANA10)

7.41 Safety run-in period (first six patients re-registered to ANA10)

Re-registration will temporarily halt after maximum of 6 patients have been re-registered to the study.

Re-registered patients will be given a 4-5 week supply of anastrozole 10 mg (ANA10) daily.

A study nurse will call patients after 2-3 weeks to review AEs and determine whether patients are willing to continue ANA10.

Also, after 28-35 days of ANA10 treatment, first six patients will return to registering instituion for adverse event monitoring. A patient is considered to have developed a DLT if any of the following serious adverse event definitely, probably, or possibly attributed to anastrozole 10 mg occurs within 28-35 days of starting ANA10.

CTCAE SOC	DLT Definition
Cytopenias (including Blood and lymphatic system disorders and Investigations)	Grade 4 Neutrophil count decreased lasting >7 days ≥Grade 3 Platelet count decreased with clinically significant bleeding Febrile neutropenia
Hepatobiliary disorders	≥Grade 3 Hepatic failure: Any drug-induced liver injury (DILI) (AKA Hy's Law cases)

CTCAE SOC	DLT Definition
Unspecified	Any ≥Grade 3 adverse event per NCI Common Terminology Criteria for Adverse Events v5.0, excluding the following: Any ≥Grade 3 electrolyte alteration* lasting ≤72 hours, is not clinically complicated, and resolves spontaneously or responds to conventional medical interventions Any ≥Grade 3 serum amylase increased or lipase increased not associated with symptoms or clinical manifestations of pancreatitis Grade 3 nausea, vomiting, or diarrhea (lasting ≤72 hours) which responds to maximal supportive treatment(s) Grade 3 fatigue (even if it lasts >1 week)
Death	Any death occurring in these patients during treatment or during 30 days after last dose

*Electrolyte alterations may include the following events in SOC "Metabolism and nutrition disorders"

Hypercalcemia or Hypocalcemia
Hyperglycemia or Hypoglycemia
Hyperkalemia or Hypokalemia
Hypermagnesemia or Hypomagnesemia
Hypernatremia or Hyponatremia
Hyperphosphatemia or Hypophosphatemia

If 1 of the first 6 patients enrolled in the safety run-in experience any dose limiting toxicities (DLTs) (as defined in Section7.4.1 above), attributed (definitely, probably, or possibly) to anastrozole 10mg the FDA must be notified immediately.

If two or more of thse 6 patients develops a DLT (as specified in Section 7.0 of the protocol) within 28-35 days of starting ANA10, the study will remain closed to enrollment to allow the study team to review toxicity data thoroughly and make decisions regarding changes to the protocol. If at most one of these 6 patients develops a within 28-35 days of starting ANA10, re-registered will reopen.

Patients who develop a DLT will have a research blood draw and go to the extended follow-up phase where an adverse event assessment will be undertaken 28-35 days after treatment discontinuation.

Patients who do not develop a DLT and are willing to continue, will have the remaining ANA10 supply (5-6 weeks) shipped to the patient by FedEx.

At the end of the second cycle, or at discontinuation of ANA10, blood will be drawn for drug levels and E1 and E2 levels.

Patients should be encouraged to take their anastrozole in the morning each day EXCEPT the day of the blood draw when they should take their anastrozole after this sample has been drawn.

Patients who complete Cycle 2 will proceed to Cycle 3.

Patients unable to complete Cycle 2 will have research blood draw and then go to extended follow-up where an adverse event assessment will be undertaken 28-35 days after treatment discontinuation.

7.42 After safety run-in period

Re-registered patients will be given a 4 week supply of anastrozole 10 mg (ANA10) daily.

Study nurse will call patients after 2-3 weeks to review AEs and determine whether patients are willing to continue ANA10.

If patients are willing to continue, then the remaining ANA10 supply (6 weeks) will be shipped to the patient by FedEx.

At the end of the second cycle, or at discontinuation of ANA10, blood will be drawn for drug levels and E1 and E2 levels.

Patients should be encouraged to take their anastrozole in the morning each day EXCEPT the day of the blood draw when they should take their anastrozole after this sample has been drawn.

Patients who complete Cycle 2 will proceed to Cycle 3.

Patients unable to complete Cycle 2 will have research blood draw and then go to extended follow-up, where an adverse event assessment will be undertaken 28-35 days after treatment discontinuation.

7.5 Cycle 3 Letrozole

Patients will be prescribed the FDA-approved dose of letrozole at 2.5 mg daily for 8-10 weeks.

At the end of the third cycle, blood will be drawn for drug levels and E1 and E2 levels. Patients should be encouraged to take their letrozole in the morning each day EXCEPT the day of the blood draw when they should take their letrozole after this sample has been drawn.

Patients will be given a recommendation for 5 years of letrozole and go off study.

7.6 Return to consenting institution

For this protocol, the patient must return to the consenting institution as indicated in the Test Schedule Section 4.0 (Active Monitoring Phase).

7.7 Treatment by local medical doctor (LMD)

Treatment by a local medical doctor (LMD) for routine clinical and emergent care is allowed.

8.0 Dosage Modification Based on Adverse Events

→ ***ALERT:*** ***ADR reporting may be required for some adverse events (See Section 10.0)*** ←

8.1 Dose Levels (Based on Adverse Events in Tables 8.*2* and 8.3)

Dose Level	Anastrozole (ANA1)	Anastrozole (ANA10)	Letrozole (LET)
1*	1 mg daily	10 mg daily	2.5 mg daily

*Dose level 1 refers to the starting dose.

NOTE: There are no dose modifications for anastrozole or letrozole.

→ → ***Use the NCI Common Terminology Criteria for Adverse Events (CTCAE) current version (v5.0)* unless otherwise specified*** ← ←

* Located at [redacted]

8.2 Anastrozole

There will be no anastrozole dose reductions.

If any Grade 3 or 4 adverse event is encountered that is considered at least possibly related to anastrozole, anastrozole therapy will be discontinued. For unrelated or unlikely related events, hold anastrozole until the AE has resolved. Once resolved, resume anastrozole at the previous dose. If anastrozole is held for >3 weeks, permanently discontinue anastrozole therapy.

Single missed doses of anastrozole are not made up.

Any treatment interruption of 3-7 days should be made up at the end of the cycle.

Any treatment interruption greater than 7 days and patient will go off study and will be replaced.

9.0 Ancillary Treatment/Supportive Care

9.1 Full supportive care

Patients should receive full supportive care while on this study. This includes blood product support, antibiotic treatment, and treatment of other newly diagnosed or concurrent medical conditions. All blood products and concomitant medications such as antidiarrheals, analgesics, and/or antiemetics received from the first day of study treatment administration until 30 days after the final dose will be recorded in the medical records.

9.2 Blood products and growth factors

Blood products and growth factors should be utilized as clinically warranted and following institutional policies and recommendations. The use of growth factors should follow published guidelines of the Journal of Clinical Oncology, Volume 33, No 28 (October 1), 2015: pp. 3199-3212 (WBC growth factors) AND Journal of Clinical Oncology, Volume 28, No 33 (November 20), 2010: pp. 4955-5010 (darbepoetin/epoetin).

9.3 Antiemetics

Antiemetics may be used at the discretion of the attending physician.

9.4 Contraindicated agents

9.51 Any agent with estrogenic or putatively estrogenic properties. NOTE: This includes menopausal hormone therapy of any type, including systemic and topical therapies. This also includes agents such as megestrol acetate or SERMS (raloxifene or tamoxifen)

9.52 Over-the-counter products and supplements considered to have an estrogenic effect such as: ginseng, gingko biloba, black cohosh, dong quai or fortified soy supplements/phytoestrogen preparations.

9.53 Any other cancer directed treatment approach, such as chemotherapy or radiation, must not be administered while the patient is taking study drug.

9.54 Bisphosphonates are not allowed to be initiated during anastrozole therapy. Initiation of bisphosphonate therapy will be allowed after completion of anastrozole therapy (ANA10 or ANA1 if patient is not going on to ANA 10).

NOTE: Patients on stable dose of bisphosphonates for ≥4 weeks at the time of registration are allowed to continue.

10.0 Adverse Event (AE) Monitoring and Reporting

The site principal investigator is responsible for reporting any/all serious adverse events to the sponsor as described within the protocol, regardless of attribution to study agent or treatment procedure.

The sponsor-investigator is responsible for notifying FDA and all participating investigators in a written safety report of any of the following:

- Any suspected adverse reaction that is both serious and unexpected.
- Any findings from laboratory animal or *in vitro* testing that suggest a significant risk for human subjects, including reports of mutagenicity, teratogenicity, or carcinogenicity.
- Any findings from epidemiological studies, pooled analysis of multiple studies, or clinical studies, whether or not conducted under an IND and whether or not conducted by the sponsor, that suggest a significant risk in humans exposed to the drug
- Any clinically important increase in the rate of a serious suspected adverse reaction over the rate stated in the protocol or Investigator's Brochure (IB).

Summary of SAE Reporting for this study
(please read entire section for specific instructions):

WHO:	WHAT form:	WHERE to send:
All sites	Pregnancy Reporting [redacted]	Mayo Sites – attach to MCCC Electronic SAE Reporting Form [redacted]
Mayo Clinic Sites	Mayo Clinic Cancer Center SAE Reporting Form: [redacted]	Will automatically be sent to [redacted]

Definitions

Adverse Event
Any untoward medical occurrence associated with the use of a drug in humans, whether or not considered drug related.

Suspected Adverse Reaction
Any adverse event for which there is a reasonable possibility that the drug caused the adverse event.

Expedited Reporting
Events reported to sponsor within 24 hours, 5 days or 10 days of study team becoming aware of the event.

Routine Reporting
Events reported to sponsor via case report forms

Events of Interest
Events that would not typically be considered to meet the criteria for expedited reporting, but that for a specific protocol are being reported via expedited means in order to facilitate the review of safety data (may be requested by the FDA or the sponsor).

10.1 Adverse Event Characteristics

CTCAE term (AE description) and grade: The descriptions and grading scales found in the revised NCI Common Terminology Criteria for Adverse Events (CTCAE) version 5.0 will be utilized for AE reporting. All appropriate treatment areas should have access to a copy of the CTCAE version 5.0 A copy of the CTCAE version 5.0 can be downloaded from the CTEP web site:
[redacted]

a. Identify the grade and severity of the event using the CTCAE version 5.0.
b. Determine whether the event is expected or unexpected (see Section 10.2).
c. Determine if the adverse event is related to the study intervention (agent, treatment or procedure) (see Section 10.3).
d. Determine whether the event must be reported as an expedited report. If yes, determine the timeframe/mechanism (see Section 10.4).
e. Determine if other reporting is required (see Section 10.5).
f. Note: All AEs reported via expedited mechanisms must also be reported via the routine data reporting mechanisms defined by the protocol (see Sections 10.6 and 18.0).

NOTE: A severe AE is NOT the same as a serious AE, which is defined in Section 10.4.

10.2 Expected vs. Unexpected Events

Expected events - are those described within the Section 15.0 of the protocol, the study specific consent form, package insert (if applicable), and/or the investigator brochure, (if an investigator brochure is not required, otherwise described in the general investigational plan).

Unexpected adverse events or suspected adverse reactions are those not listed in Section 15.0 of the protocol, the study specific consent form, package insert (if applicable), or in the investigator brochure (or are not listed at the specificity or severity that has been observed); if an investigator brochure is not required or available, is not consistent with the risk information described in the general investigational plan.

Unexpected also refers to adverse events or suspected adverse reactions that are mentioned in the investigator brochure as occurring with a class of drugs but have not been observed with the drug under investigation.

An investigational agent/intervention might exacerbate the expected AEs associated with a commercial agent. Therefore, if an expected AE (for the commercial agent) occurs with a higher degree of severity or specificity, expedited reporting is required.

NOTE: *The consent form may contain study specific information at the discretion of the Principal Investigator; it is possible that this information may NOT be included in the protocol or the investigator brochure. Refer to protocol or IB for reporting needs.

10.3 Attribution to agent(s) or procedure

When assessing whether an adverse event (AE) is related to a medical agent(s) medical or procedure, the following attribution categories are utilized:

Definite - The AE *is clearly related* to the agent(s)/procedure.
Probable - The AE *is likely related* to the agent(s)/procedure.
Possible - The AE *may be related* to the agent(s)/procedure.
Unlikely - The AE *is doubtfully related* to the agent(s)/procedure.
Unrelated - The AE *is clearly NOT related* to the agent(s)/procedure.

10.31 AEs Experienced Utilizing Investigational Agent(s) and Commercial Agent(s) on SEPARATE Arms

- An AE that occurs on an arm using an investigational agent /intervention under an IND must be assessed in accordance with the guidelines for investigational agents.
- An AE that occurs on an arm using a commercial agent on a separate treatment arm must be assessed as specified in the protocol**. (**Refer to the **Commercial** Reporting Table in Section 10.4).

  Commercial agent expedited reports must be submitted to the FDA via MedWatch 3500A

  [redacted]

10.32 EXPECTED Serious Adverse Events: Protocol Specific Exceptions to Expedited Reporting

For this protocol only, the following Adverse Events/Grades are expected to occur within this population and do not require Expedited Reporting. These events must still be reported via Routine Reporting (see Section 10.6).*

*Report any clinically important increase in the rate of a serious suspected adverse reaction (at your study site) over that which is listed in the protocol or investigator brochure as an expedited event.

*Report an expected event that is greater in severity or specificity than expected as an expedited event.

*Specific protocol exceptions to expedited reporting should be reported expeditiously by investigators **ONLY** if they exceed the expected grade of the event.

CTCAE System Organ Class (SOC)	Adverse event/ Symptoms	CTCAE Grade at which the event will not be reported in an expedited manner[1]
General disorders and administrations site conditions	Fatigue	≤Grade 3
Investigations	Alanine aminotransferase increased	≤Grade 3
	Aspartate aminotransferase increased	≤Grade 3
	Blood bilirubin increased	≤Grade 3
Musculoskeletal and connective tissue disorders	Arthralgia	≤Grade 3

[1] These exceptions only apply if the adverse event does not result in hospitalization. If the adverse event results in hospitalization, then the standard expedited adverse events reporting requirements must be followed.

The following hospitalizations are not considered to be SAEs because there is no "adverse event" (*i.e.*, there is no untoward medical occurrence) associated with the hospitalization:

- Hospitalizations for respite care
- Planned hospitalizations required by the protocol
- Hospitalization planned before informed consent (where the condition requiring the hospitalization has not changed post study drug administration)
- Hospitalization for elective procedures unrelated to the current disease and/or treatment on this trial
- Hospitalization for administration of study drug or insertion of access for administration of study drug
- Hospitalization for routine maintenance of a device (*e.g.*, battery replacement) that was in place before study entry
- Hospitalization, or other serious outcomes for signs and symptoms of progression of the cancer.

10.4 Expedited Reporting Requirements for Commercial or Commercial Imaging Agents (Non-IND) Agent(s) ONLY:

10.41 Expedited Reporting Requirements for Adverse Events that Occur in a Non-IND trial within 30 Days of the Last Administration of a Commercial Agent[1, 2]

FDA REPORTING REQUIREMENTS FOR SERIOUS ADVERSE EVENTS (21 CFR Part 312)
NOTE: Investigators **MUST** immediately report to the sponsor **ANY** Serious Adverse Events, whether or not they are considered related to the investigational agent(s)/intervention (21 CFR 312.64)
An adverse event is considered serious if it results in **ANY** of the following outcomes:

1) Death
2) A life-threatening adverse event
3) An adverse event that results in inpatient hospitalization or prolongation of existing hospitalization for ≥ 24 hours
4) A persistent or significant incapacity or substantial disruption of the ability to conduct normal life functions
5) A congenital anomaly/birth defect.
6) Important Medical Events (IME) that may not result in death, be life threatening, or require hospitalization may be considered serious when, based upon medical judgment, they may jeopardize the patient or subject and may require medical or surgical intervention to prevent one of the outcomes listed in this definition. (FDA, 21 CFR 312.32; ICH E2A and ICH E6).

ALL SERIOUS adverse events that meet the above criteria **MUST** be immediately reported to the sponsor within the timeframes detailed in the table below.

Hospitalization	Grade 1 Timeframes	Grade 2 Timeframes	Grade 3 Timeframes	Grade 4 & 5 Timeframes
Resulting in Hospitalization ≥24 hrs	7 Calendar Days			24-Hour 3 Calendar Days
Not resulting in Hospitalization ≥24 hrs	Not required		7 Calendar Days	

Expedited AE reporting timelines are defined as:

- "24-Hour; 3 Calendar Days" - The AE must initially be reported within 24 hours of learning of the AE, followed by a complete expedited report within 3 calendar days of the initial 24-hour report.
- "7 Calendar Days" - A complete expedited report on the AE must be submitted within 7 calendar days of learning of the AE.

[1]Serious adverse events that occur more than 30 days after the last administration of investigational agent/intervention and have an attribution of possible, probable, or definite require reporting as follows:

Expedited 24-hour notification followed by complete report within 3 calendar days for:

- All Grade 4, and Grade 5 AEs

Expedited 7 calendar day reports for:

- Grade 2 adverse events resulting in hospitalization or prolongation of hospitalization
- Grade 3 adverse events

[2] For studies using PET or SPECT IND agents, the AE reporting period is limited to 10 radioactive half-lives, rounded UP to the nearest whole day, after the agent/intervention was last administered. Footnote "1" above applies after this reporting period.

Effective Date: May 5, 2011

NOTE: Refer to Section 10.32 for exceptions to Expedited Reporting

10.42 General reporting instructions

The Mayo IND and/or MCCC Compliance will assist the sponsor-investigator in the processing of expedited adverse events and forwarding of suspected unexpected serious adverse reactions (SUSARs) to the FDA and IRB.

Use Mayo Expedited Event Report form [redacted] or investigational agents or commercial/investigational agents on the same arm.

For commercial agents (for commercial agent(s) on its own arm):

Attach the MedWatch 3500A form to the Mayo Expedited Event Report form [redacted]

10.43 Reporting of re-occurring SAEs

ALL SERIOUS adverse events that meet the criteria outlined in table10.41 MUST be immediately reported to the sponsor within the timeframes detailed in the corresponding table. This reporting includes, but is not limited to SAEs that re-occur again after resolution.

10.5 Other Required Reporting

10.51 Unanticipated Problems Involving Risks to Subjects or Others (UPIRTSOS)

Unanticipated Problems Involving Risks to Subjects or Others (UPIRTSOS) in general, include any incident, experience, or outcome that meets **all** of the following criteria:

1. Unexpected (in terms of nature, severity, or frequency) given (a) the research procedures that are described in the protocol-related documents, such as the IRB-approved research protocol and informed consent document; and (b) the characteristics of the subject population being studied;
2. Related or possibly related to participation in the research (in this guidance document, possibly related means there is a reasonable possibility that the incident, experience, or outcome may have been caused by the procedures involved in the research); and
3. Suggests that the research places subjects or others at a greater risk of harm (including physical, psychological, economic, or social harm) than was previously known or recognized.

Some unanticipated problems involve social or economic harm instead of the physical or psychological harm associated with adverse events. In other cases, unanticipated problems place subjects or others at increased *risk* of harm, but no harm occurs.

Note: If there is no language in the protocol indicating that pregnancy is not considered an adverse experience for this trial, and if the consent form does not indicate that subjects should not get pregnant/impregnate others, then any pregnancy in a subject/patient or a male patient's partner (spontaneously reported) which occurs during the study or within 120 days of completing the study should be reported as a UPIRTSO.

Mayo Clinic Cancer Center (MCCC) Institutions:

If the event meets the criteria for IRB submission as a Reportable Event/UPIRTSO, provide the appropriate documentation and use the Mayo Clinic Cancer Center Expedited Event Report form [redacted] he Mayo Clinic Compliance Unit will review and process the submission to the Mayo Clinic IRB and work with the IND Coordinator for submission to FDA.

10.52 Death

Note: A death on study requires both routine and expedited reporting regardless of causality, unless as noted below. Attribution to treatment or other cause must be provided.

Any death occurring within 30 days of the last dose, regardless of attribution to an agent/intervention under an IND requires expedited reporting within 24-hours.

Any death occurring greater than 30 days with an attribution of possible, probable, or definite to an agent/intervention under an IND requires expedited reporting within 24-hours.

Reportable categories of Death

- Death attributable to a CTCAE term.
- Death Neonatal: A disorder characterized by cessation of life during the first 28 days of life.
- Death NOS: A cessation of life that cannot be attributed to a CTCAE term associated with Grade 5.
- Sudden death NOS: A sudden (defined as instant or within one hour of the onset of symptoms) or an unobserved cessation of life that cannot be attributed to a CTCAE term associated with Grade 5.
- Death due to progressive disease that cannot be attributed to a CTCAE term associated with Grade 5 should be reported as **Grade 5 "Disease progression"** under the system organ class (SOC) of General disorders and administration site conditions. Evidence that the death was a manifestation of underlying disease (e.g., radiological changes suggesting tumor growth or progression: clinical deterioration associated with a disease process) should be submitted.

10.53 Secondary Malignancy

- A ***secondary malignancy*** is a cancer caused by treatment for a previous malignancy (e.g., treatment with investigational agent/intervention, radiation or chemotherapy). A secondary malignancy is not considered a metastasis of the initial neoplasm.
- All secondary malignancies that occur following treatment with an agent under an IND will be reported. Three options are available to describe the event:
  - Leukemia secondary to oncology chemotherapy (e.g., Acute Myeloctyic Leukemia [AML])
  - Myelodysplastic syndrome (MDS)
  - Treatment-related secondary malignancy
- Any malignancy possibly related to cancer treatment (including AML/MDS) should also be reported via the routine reporting mechanisms outlined in each protocol.

10.54 Second Malignancy

A second malignancy is one unrelated to the treatment of a prior malignancy (and is NOT a metastasis from the initial malignancy). Second malignancies require ONLY routine reporting unless otherwise specified.

10.55 Pregnancy, Fetal Death, and Death Neonatal

If a female subject (or female partner of a male subject) taking investigational product becomes pregnant, the subject taking should notify the Investigator, and the pregnant female should be advised to call her healthcare provider immediately. The patient should have appropriate follow-up as deemed necessary by her physician. If the baby is born with a birth defect or anomaly, a second expedited report is required.

Prior to obtaining private information about a pregnant woman and her infant, the investigator must obtain consent from the pregnant woman and the newborn infant's parent or legal guardian before any data collection can occur. A consent

form will need to be submitted to the IRB for these subjects if a pregnancy occurs. If informed consent is not obtained, no information may be collected.

In cases of fetal death, miscarriage or abortion, the mother is the patient. In cases where the child/fetus experiences a serious adverse event other than fetal death, the child/fetus is the patient.

NOTE: When submitting Mayo Expedited Adverse Event Report reports for "Pregnancy", "Pregnancy loss", or "Neonatal loss", the potential risk of exposure of the fetus to the investigational agent(s) or chemotherapy agent(s) should be documented in the "Description of Event" section. Include any available medical documentation. Include this form:

[redacted]

10.551 Pregnancy

Pregnancy should be reported in an expedited manner as **Grade 3 "Pregnancy, puerperium and perinatal conditions - Other (pregnancy)"** under the Pregnancy, puerperium and perinatal conditions SOC. Pregnancy should be followed until the outcome is known.

10.552 Fetal Death

Fetal death is defined in CTCAE as "A disorder characterized by death in utero; failure of the product of conception to show evidence of respiration, heartbeat, or definite movement of a voluntary muscle after expulsion from the uterus, without possibility of resuscitation."

Fetal loss at any gestational age should be reported expeditiously, as **Grade 4 "Pregnancy loss" under the SOC of "Pregnancy, puerperium and perinatal conditions""** under the Pregnancy, puerperium and perinatal conditions SOC.

10.553 Death Neonatal

Neonatal death, defined in CTCAE as "Newborn death occurring during the first 28 days after birth" that is felt by the investigator to be at least possibly due to the investigational agent/intervention.

A neonatal death should be reported expeditiously as **Grade 4 "Death neonatal" under the SOC of General disorders and administration site conditions**.

10.6 Required Routine Reporting

10.61 Baseline and Adverse Events Evaluations

Pretreatment symptoms/conditions to be graded at baseline and adverse events to be graded at each evaluation.
Grading is per CTCAE v5.0 **unless** alternate grading is indicated in the table below:

CTCAE System/Organ/Class (SOC)	Adverse event/Symptoms	Baseline	End of C1/C2 only	Each evaluation
Blood and lymphatic disorders	Anemia	X	X	
General disorders and administration site conditions	Fatigue	X		X
Investigations	Alanine aminotransferase increased	X	X	
	Aspartate aminotransferase increased	X	X	
	Blood bilirubin increased	X	X	
	White blood cell decreased	X	X	
Musculoskeletal and connective tissue disorders	Arthralgia	X		X
Vascular disorders	Hot flashes	X		X

10.62 All other AEs

Submit via appropriate MCCC Case Report Forms (i.e., paper or electronic, as applicable) the following AEs experienced by a patient and not specified in Section 10.6:

10.621 Grade 2 AEs deemed *possibly, probably, or definitely* related to the study treatment or procedure.

10.622 Grade 3 and 4 AEs regardless of attribution to the study treatment or procedure.

10.623 Grade 5 AEs (Deaths)

10.6231 Any death within 30 days of the patient's last study treatment or procedure regardless of attribution to the study treatment or procedure.

10.6232 Any death more than 30 days after the patient's last study treatment or procedure that is felt to be at least possibly treatment related must also be submitted as a Grade 5 AE, with a CTCAE type and attribution assigned.

10.7 Late Occurring Adverse Events

Refer to the instructions in the Forms Packet (or electronic data entry screens, as applicable) regarding the submission of late occurring AEs following completion of the Active Monitoring Phase (i.e., compliance with Test Schedule in Section 4.0).

11.0 Treatment Evaluation/Measurement of Effect

Plasma concentration levels of E1 and E2 will be obtained prior to start of protocol therapy, after 8-10 weeks of anastrozole 1 mg daily (Cycle 1), after 8-10 weeks of anastrozole 10 mg daily (Cycle 2), and after 8-10 weeks of letrozole 2.5 mg daily (Cycle 3).

12.0 Descriptive Factors: None

13.0 Treatment/Follow–up Decision at Evaluation of Patient

13.1 Blood specimens

13.11 Patients for whom the baseline blood sample (prior to treatment on this study) is NOT obtained and submitted, will go off study and be replaced.

13.12 Patients for whom the blood sample prior to ANA10 is NOT obtained will go off study and be replaced.

13.2 Anastrozole 1 mg daily for 8-10 weeks (ANA1):

13.21 Patients who are able to tolerate anastrozole treatment have no signs of disease recurrence may continue treatment with anastrozole for a maximum of 10 weeks.

13.22 Patients who are not able to tolerate anastrozole treatment or develop disease recurrence will discontinue protocol treatment and will go off study where further treatment is at the discretion of her medical team.

13.23 Patients who register but then refuse to begin anastrozole treatment will go off study where further treatment is at the discretion of her medical team. Baseline and on study case report forms are to be submitted. On-study material and the End of Active Treatment/Cancel Notification Form must be submitted. No further data submission is necessary.

13.3 At completion of 8-10 weeks of anastrozole 1 mg daily for 8-10 weeks

13.31 Patients who have completed a minimum of 8 weeks and maximum of 10 weeks of anastrozole 1 mg daily and whose plasma concentrations of E1 and E2 meet one of the following criteria:

(a) E1>1.3 pg/ml and E2≤ 0.5 pg/ml

(b) E1≤ 1.3 pg/ml and E2> 0.5 pg/ml

(c) E1≤ 1.3 pg/ml and E2≤ 0.5 pg/ml

will go off study where further treatment is at the discretion of her medical team.

13.32 Patients who have completed a minimum of 8 weeks and maximum of 10 weeks of anastrozole 1 mg daily and whose plasma concentrations of E1 and E2 are such that:

E1 ≥1.3 pg/ml and E2≥0.5 pg/ml

may re-register and begin treatment with anastrozole 10 mg daily for 8-10 weeks. Patients who choose not to re-register will go off study where further treatment is at the discretion of her medical team.

13.4 Anastrozole 10 mg daily for 8-10 weeks (ANA10):

13.41 Patients who are able to tolerate anastrozole treatment and have no signs of disease recurrence may continue treatment with anastrozole at 10 mg daily dose for a maximum of 10 weeks.

13.42 Patients who are not able to tolerate anastrozole treatment or develop disease recurrence will discontinue protocol treatment. A blood specimen is to be collected for E1 and E2 determination and then the patient will go off study where further treatment is at the discretion of her medical team.

13.43 Patients who re-register but then refuse to begin anastrozole treatment will go off study where further treatment is at the discretion of her medical team.

13.5 At completion of 8-10 weeks of anastrozole 10 mg daily for 8-10 weeks

Patients who have completed a minimum of 8 weeks and maximum of 10 weeks of anastrozole 10 mg daily and have a blood draw for E1 and E2 determinations may begin treatment with letrozole 2.5 mg daily for 8-10 weeks.

If the patient chooses not to begin treatment with letrozole, a blood specimen is to be collected for E1 and E2 determination. The patient will then go off study where further treatment is at the discretion of her medical team.

13.6 Letrozole 2.5 mg daily for 8-10 weeks

13.61 Patients who are able to tolerate letrozole treatment and have no signs of disease recurrence may continue treatment with letrozole 2.5 mg daily dose for a maximum of 10 weeks. After the completion of a minimum of 8 weeks and maximum of 10 weeks of letrozole, a blood specimen is to be collected for E1 and E2 determination. The patient will then go off study where further treatment is at the discretion of her medical team.

13.62 Patients who are not able to tolerate letrozole treatment or develop disease recurrence will discontinue protocol treatment. A blood specimen is to be collected for E1 and E2 determination and then the patient will go off study where further treatment is at the discretion of her medical team.

13.7 Ineligible

A patient is deemed *ineligible* if after registration, it is determined that at the time of registration, the patient did not satisfy each and every eligibility criteria for study entry. The patient will go off study where futher treatment is at the discretion of her medical team. On study and end of treatment case reports are to be submitted.

13.8 Cancel

A patient is deemed a *cancel* if he/she is removed from the study for any reason before any study treatment is given. On-study material and the End of Active Treatment/Cancel Notification Form must be submitted. No further data submission is necessary.

14.0 Body Fluid Biospecimens

14.1 Summary Table of Research Blood and Body Fluid Specimens to be Collected for this Protocol

Research (Section for more information)	Specimen Purpose (check all that apply)	Mandatory or Optional	Blood or Body Fluid being Collected	Type of Collection Tube (color of tube top)	Volume to collect per tube (# of tubes to be collected)	Baseline Prior to C1D1	End of Cycle 1	End of Cycle 2	End of Cycle 3	Process at site? (Yes or No)	Temperature Conditions for Storage /Shipping
DNA	☒ Correlative ☒ Eligibility Confirmation ☒ Banking ☐ Other (specify)	Mandatory	Whole Blood	EDTA (purple)	10 mL (2)	X				No	Ambient
E1 and E2 assays	☒ Correlative ☒ Banking	Mandatory	Serum	Serum (red)	10 mL (1)	X	X	X	X	Yes	Frozen
Plasma for anastrozole	☒ Correlative ☒ Banking	Mandatory	Plasma	NaHep (green)	10 mL (1)		X	X		Yes	Frozen
Plasma for letrozole	☒ Correlative ☒ Banking	Mandatory	Plasma	NaHep (green)	10 mL (1)				X	Yes	Frozen

14.2 Collection and Processing

14.21 Blood for DNA

Draw two 10 ml EDTA tubes (for DNA extraction). No processing necessary.

Deliver unrefrigerated to:
Biospecimen Accessioning and Processing Lab (BAP) at each site.

BAP will perform DNA extraction from one tube. Extract buffy coat and plasma from 2nd tube and store at <-65ºC.

AZ and FL will batch ship to BAP in Rochester, MN monthly.

14.22 Blood for E1/E2 Assays

14.221 Baseline
One 10 mL serum (red top) tube (for E1 and E2 assays). Serum will be divided into two aliquots with one aliquot to be sent directly to Mayo Clinic Immunochemical Core Laboratory.

Second aliquot will be stored for future use.

14.222 End of Cycles 1-3

NOTE: Blood needs to be drawn immediately before the next dose of anastrozole/letrozole to reflect steady state. Patients should be encouraged to take their anastrozole/letrozole in the morning each day EXCEPT the day of the blood draw when they should take their anastrozole after this sample has been drawn.

One 10 mL serum (red top) tube (for E1 and E2 assays). Serum will be divided into two aliquots with one aliquot to be sent directly to Mayo Clinic Immunochemical Core Laboratory.

Second aliquot will be stored for future use.

14.23 Blood for anastrozole and/or letrozole levels

One 10 mL sodium heparin tube for aromatase inhibitor level measurement by [redacted]

Centrifuge within 1 hour of draw at 3000 rpm for 10 minutes.

Harvest plasma and store in 3 aliquots of approximately 1.5 mL in cryo-freezer tubes and frozen immediately at <-65ºC (-80ºC freezer).

Biospecimens Accessioning and Processing (BAP) will store frozen, and ship one 1.5mL aliquot from each sample drawn as a batch at the completion of the study.

The other two aliquots from each draw will be stored frozen for future use.

14.3 Shipping and Handling

14.31 Florida and Arizona only: Kits will be used for this study.

14.311 Kits will be supplied by the Biospecimen Accessioning and Processing Shared Resource (BAP).

14.312 The kit contains supplies and instructions for collecting, processing and shipping specimens.

14.313 Participating institutions may obtain kits by faxing the Supply Order Form to the number listed on the form. Because we are charged for all outgoing kits, a small, but sufficient, supply of the specimen collection kits should be ordered prior to patient entry. **Supply Order Forms must be filled in completely and legibly for quick processing.**

14.314 Kits will be sent via Fed Ex® Ground at no additional cost to the participating institutions. **Allow at least two weeks to receive the kits.**

14.315 Kits will not be sent via rush delivery service unless the participating institution provides their own Fed Ex® account number or alternate billing number for express mail. **Cost for rush delivery of kits will not be covered by the study.**

14.316 All specimens must be collected and shipped Monday – Thursday ONLY

For Mayo Clinic in Rochester, MN samples may be collected MON-FRI (as there is no shipping required)

14.32 Shipping Specimens

14.321 Shipping from Arizona and Florida

Samples should be spun down, aliquoted, and frozen prior to shipping to BAP at Mayo Clinic in Rochester, MN

Ship one aliquot frozen directly to Immunochemical Core Lab in Rochester, MN on day of collection.

Remaining samples should be shipped to BAP at Mayo Clinic in Rochester, MN on day of collection.

14.322 END OF STUDY ONLY: Shipping for anastrozole/letrozole levels

Indiana University will process the samples for drug level measurements.

Ship one 1.5 mL frozen samples per patient at end of study by overnight express service on dry ice to:

Indiana University School of Medicine
Division of Clinical Pharmacology
[redacted]

14.4 Background and Methodology

14.41 Estrone and estradiol assays

Pre- and post-AI treatment E1 and E2 levels will be measured by CLIA-approved liquid chromatography with tandem mass spectrometry (LC/MS/MS) assays in the Immunochemical Core Laboratory at Mayo Clinic. Details of the methodology have been published.[13,22] The assay involves liquid extraction of 0.5 ML sample (plasma or serum depending on study) with methylene chloride

followed by derivatization with dansyl chloride, separation using a two – dimensional ultra-performance liquid chromatography system and analysis on an Agilent 6490 mass spectrometer using electrospray ionization on a positive mode. The combination of derivatization and two-dimensional chromatography (separation on two columns [Loading: Agilent Zorbax XDB-C18: 2.1 × 30 mm 3.5 micron; Analytical: Agilent Poroshell 120 EC-C18: 2.1 × 50 mm 2.7 micron]) afforded improved sensitivity and precision. Intra-assay CVs for E1 are 17.8%, 7.5%, and 6.1% at 0.30, 0.50, and 0.84 pg/mL, respectively. Intra-assay coefficients of variation (CV) for E2 are 11.8%, 7.3%, and 6.0% at 0.25, 0.51, and 0.85 pg/mL, respectively. Inter-assay CVs for E2 are 10.8%, 8.5%, and 6.9% at 0.29, 0.50, and 0.77 pg/mL, respectively.

14.42 Aromatase Inhibitor Assays

14.421 Quantification of drugs and metabolites

Anastrozole and letrozole and relevant metabolites will be measured in the laboratory of Zeruesenay Desta, Ph.D., at Indiana University School of Medicine, using liquid chromatography-tandem mass spectrometry (LC/MS/MS) assays. All chemicals (anastrozole and letrozole) and internal standards (anastrozole and letrozole) will be purchased from Toronto Research Chemicals (North York, Ontario, Canada). LC-MS/MS grade water, methanol, acetonitrile, and formic acid were obtained from Fisher Scientific (Waltham, MA). Although relevant metabolites of anastrozole may be measured, these metabolites are pharmacologically inactive and their concentrations are much lower than the parent compounds.

14.422 Sample preparation

Plasma or serum sample aliquots (50 µL) will be spiked with 30 µL of an internal standard solution in methanol containing anastrozole (50 nM) for assay of letrozole samples and letrozole (50 nM) for assay of anastrozole samples. LC-MS/MS grade methanol (220 µL) was added to precipitate proteins followed by vortex mixing (90 seconds, 2,000 rpm), and centrifugation (20 minutes, 3,000 rpm, 4°C). The resulting supernatant (150 µL) will be transferred to clean plates for analysis via LC-MS/MS.

14.423 LC-MS/MS Method

Chromatographic separation for anastrozole, letrozole, and respective metabolites will be achieved using an Acquity BEH C18 column (1.7 µM, 2.1 x 100 mm) with a Critical Clean precolumn filter (2.1 x 0.2 µM) (Waters) heated to 35°C and a binary gradient at a flow rate of 0.4 ml/min. The gradient elution will be held at 65:35 mobile phase A (5 mM ammonium formate with 0.1% formic acid in water): mobile phase B (acetonitrile with 0.1% formic acid) for 1 minute. Then this will be increased linearly to 52% mobile phase B over 30 seconds, held at 52% for 2.5 minutes followed by increase to 100% mobile phase B over 30 seconds, and then held at 100% for 30 seconds before returning to initial conditions over 0.5 minutes. The total run time is expected to be six minutes. Samples will be analyzed (2 µL injection volume) using the QTRAP 6500+ UHPLC-MS/MS system (AB Sciex) with the turbo electrospray source operated in positive ion mode.

Anastrozole (294-225), hydroxy-anastrozole (31o-241), anastrozole-N-glucuronide (470-294), anastrozole-O-glucuronide 1 (486- 310), anastrozole-O-glucuronide 2 (486+310), and letrozole (286-217) m/z transitions will be monitored in multiple reaction monitoring mode. Anastrozole-O- glucuronide 1 and 2 will be denoted based on differences in retention time.

15.0 Drug Information

15.1 Anastrozole (Arimidex®)

15.11 Background

Anastrozole is a nonsteroidal, competitive inhibitor of the aromatase enzyme system which binds to the heme group of aromatase, a cytochrome P450 enzyme that catalyzes the conversion of androgens to estrogens.

15.12 Formulation and storage

Anastrozole is commercially available as 1 mg tablets. Store at controlled room temperature 20-25˚C (68-77˚F) [see USP Controlled Room Temperature].

15.13 Administration

Administer orally with or without food. Refer to the treatment section for specific administration instructions.

15.14 Pharmacokinetic information

Absorption: Absorption of anastrozole is rapid and maximum plasma concentrations typically occur within 2 hours of dosing under fasted conditions.

Food reduces the rate but not the overall extent of anastrozole absorption.

Distribution: Plasma concentrations approach steady-state levels at about 7 days of once daily dosing. Anastrozole is 40% bound to plasma proteins in the therapeutic range.

Metabolism: Metabolism of anastrozole occurs by N-dealkylation, hydroxylation and glucuronidation.

Excretion: Eighty-five percent of radiolabeled anastrozole was recovered in feces and urine. Hepatic metabolism accounts for approximately 85% of anastrozole elimination. Renal elimination accounts for approximately 10% of total clearance. The mean elimination half-life of anastrozole is 50 hours.

15.15 Potential drug interactions

Cytochrome P450: None

15.16 Known potential adverse events

Consult the package insert for the most current and complete information. Common adverse reactions include hot flashes, asthenia, arthritis, pain, arthralgia, pharyngitis, hypertension, depression, nausea and vomiting, rash, osteoporosis, fractures, back pain, insomnia, headache, bone pain, peripheral edema, increased cough, dyspnea and lymphedema.

15.17 Drug procurement

Anastrozole 1 mg is commercially available: Patients will be given a prescription for anastrozole to be filled at the pharmacy of their choice.

Anastrozole 10 mg: Study medication will be provided by the Mayo Clinic Breast Cancer SPORE.

15.18 Nursing guidelines

15.181 May take with food if needed for nausea. Instruct patient to report unrelieved nausea or vomiting.

15.182 Instruct patient that hot flashes may occur. Manage hot flashes with non-hormonal interventions (e.g. venlafaxine XR 75 mg daily).

15.183 Assess for changes in bowel patterns. Manage diarrhea with non-prescription drugs. Instruct patient to report unrelieved diarrhea.

15.184 Headache may occur. Can be managed with non-prescription analgesics. Instruct patient to report headaches that are not relieved.

15.185 Vaginal dryness may occur. Instruct patient in the use of lubricating agents.

15.186 Mild swelling may occur in the arms and legs. Instruct patient to elevate extremities when at rest to relieve the swelling.

15.187 While thrombophlebitis is rare, instruct patient to report any pain, redness, marked swelling in the arms and/or legs, dizziness, or shortness of breath to their health care provider immediately or seek medical attention in an emergency room.

15.2 Letrozole (Femara®)

15.21 Background

Letrozole is a nonsteroidal, competitive inhibitor of the aromatase enzyme system which binds to the heme group of aromatase, a cytochrome P450 enzyme that catalyzes the conversion of androgens to estrogens.

15.22 Formulation and storage

Letrozole is commercially available as 2.5 mg tablets. Store at 77°F (25°C); excursions permitted to 59°F to 86°F (15°C to 30°C) [see USP Controlled Room Temperature].

15.23 Administration

Administer orally with or without food.
Refer to the treatment section for specific administration instructions.

15.24 Pharmacokinetic information

Distribution: V_d: ~1.9 L/kg
Protein binding: weak
Metabolism: Hepatic via CYP3A4 and CYP2A6 into an inactive carbinol metabolite
Half-life elimination: Terminal: ~ 2 days
Excretion: Urine (90%; 6% as unchanged drug, 75% as glucuronide carbinol metabolite, 9% as unidentified metabolites)

15.25 Potential Drug Interactions

Cytochrome P450: Substrate of CY2A6 (minor), CYP3A4 (minor).
Inhibitor of CYP2A6 (strong), CYP2C19 (moderate)

15.26 Known potential adverse events:

Consult the package insert for the most current and complete information.

Common adverse events include cataracts, eye irritation, palpitations, cardiac failure, tachycardia, dysaesthesia, arterial thrombosis, memory impairment, irritability, nervousness, urticaria, increased urinary

frequency, leukopenia, stomatitis, pyrexia, vaginal discharge, appetite increase, dryness of skin and mucosa and disturbances in taste and thirst.

15.27 Drug procurement

Commercial supplies. Patients will be given a prescription for letrozole to be filled at the pharmacy of their choice.

15.28 Nursing Guidelines

15.281 Manage hot flashes with non-hormonal interventions (e.g., venlafaxine XR 75 mg daily).

15.282 Manage pain (arthralgias). Instruct patient to report unrelieved pain.

15.283 May take with food if needed for nausea. Instruct patient to report unrelieved nausea or vomiting.

15.284 Assess for changes in bowel patterns. Manage diarrhea or constipation with non-prescription drugs. Tell patients to report unrelieved diarrhea or constipation.

15.285 If patient experiences difficulty breathing or sudden onset chest pain, instruct them to seek emergency medical attention immediately.

15.286 Monitor for signs of edema, instruct patient to report any swelling in legs, feet, or hands.

16.0 Statistical Considerations and Methodology

16.1 Study Design

This goal of this study is to assess whether women with inadequate estrogen suppression after 8-10 weeks of adjuvant anastrozole 1 mg daily (ANA1) would benefit from increasing their dose of anastrozole to 10 mg daily (ANA10). Two analytic approaches will be taken to address this objective. The first approach focuses on the set of patients who are adherent to treatment schedule, that is, if at least 80% of the planned dose is taken will adequate estrogen suppression be achieved. The second approach focuses on intentions, that is, among those patients who start an 8-10 week course of anastrozole 10 mg daily what proportion will have achieved adequate estrogen suppression at treatment discontinuation. Patients who fail to provide a blood sample after discontinuing treatment will be considered not to have achieved adequate estrogen suppression.

The study was designed to assess in the adherent cohort whether the estrogen suppression rate with anastrozole 10 mg daily for 8-10 weeks (ES-ANA10) after inadequate estrogen suppression of 8-10 weeks of adjuvant anastrozole 1 mg daily is at most 25% against the alternative that it is at least 50%.

Inadequate estrogen suppression is defined as E1>1.3 pg/ml and E2>0.5 pg/ml after 8-10 weeks of treatment with an aromatase inhibitor.

Based on preliminary findings from MA27, we expect approximately 20% of women with ER+ primary breast cancer to have inadequate estrogen suppression with anastrozole 1 mg daily for 8-10 weeks. Thus, if 160 patients were enrolled then we would expect 32 of them to have IES-ANA1. There may be a few patients who refuse to switch to anastrozole 10 mg daily for 8-10 weeks followed by a blood draw for E1 and E2 testing.

Assuming at most 3 women will refuse to continue or fail to adhere to treatment, 29 patients who are adherent to treatment will be available to evaluate the primary aim.

With a sample size of 29 patients in the adherence cohort, a one-sided binomial test of proportions with a significance level of 0.05 will have a 87% chance of rejecting the null hypothesis that the estrogen suppression rate with anastrozole 10 mg daily for 8-10 weeks is at most 25% when the true estrogen suppression rate with anastrozole 10 mg daily for 8-10 weeks is at least 50%. Specifically, the null hypothesis is rejected if the number of women who are ES-ANA10 is 12 or more among these 29 patients.

16.2 Analyses

16.21 Primary Analysis

The primary analysis will include all patients with inadequate estrogen suppression after 8-10 weeks of adjuvant anastrozole 1 mg daily who provide a blood sample after completing 8-10 weeks of anastrozole 10 mg daily will be included in the analysis of the primary endpoint. Patients who fail to provide a blood sample after discontinuing anastrozole 10 mg daily for 8-10 weeks

Adverse events will be graded and their attributions assigned using NCI CTCAE v5.0.

For each cohort, point and interval estimates for the estrogen suppression rate with anastrozole 10 mg daily for 8-10 weeks will be constructed using the properties of the binomial distribution.

16.3 Accrual rate and enrollment

Accrual Rate: MCR – anticipate 300 patients per year meeting eligibility criteria and 100 patients per year agreeing to participate in the clinical trial.

Total Expected Accrual: 160 minimum to obtain 32 patients who reach Cycle 3 with 29 patients evaluable for study endpoints

16.4 Stopping rules

16.41 Feasbility stopping rule

The percentage of patients who have inadequate estrogen suppression (E1>1.3 pg/ml and E2>0.5 pg/ml) will be examined after every 25th patients has completed 8-10 weeks of anastrozole at the 1 mg daily dose. If this percentage is 10% or less among these 25 patients and all the previously enrolled patients is 10% or less, the trial will temporarily halt for the study team to review study data to make a trial recommendation to FDA and IRB.

16.42 Safety Stopping rule:

Adverse events will be examined at the completion of each treatment. Enrollment to the trial will be temporarily halted if:

(a) During or at completion of treatment Cycle 1 with anastrozole 1 mg daily, 2 or more of the first 6 patients or 30% or more of the patients treated with the agent have developed a Grade 4 cytopenia or some other Grade 3-4 adverse event during or at completion of that agent

(b) During or at completion of treatment Cycle 2 with anastrozole 10 mg daily, if 2 or more of the first 6 patients develops a DLT (as specified in Section 7.0 of the protocol) within 28-35 days of starting ANA10. The study will remain closed to enrollment to allow the study team to review toxicity data thoroughly and make decisions regarding changes to the protocol. If at most one of these 6 patients develops a DLT within 28-35 days of starting ANA10, re-registration will reopen.

After at least 7 patients have been re-registered and have begun ANA10, 30% or more of the patients treated with the agent have developed a Grade 4 cytopenia or some other Grade 3-4 adverse event during or at completion of that agent

(c) During or at completion of treatment Cycle 3 with letrozole 2.5 mg daily, 2 or more of the first 6 patients or 30% or more of the patients treated with the agent have developed a Grade 4 cytopenia or some other Grade 3-4 adverse event during or at completion of that agent

(d) A patient death on protocol treatment or within 30 days of discontinuing all protocol treatment that is considered possibly, probably or definitely related to treatment.

The study team will review study data and then make a trial recommendation to FDA and IRB.

16.5 Analysis Plan for Secondary Outcome and Translational Endpoint

Descriptive statistics will be used to describe the distribution of E1 and E2 concentrations following ANA10 and letrozole. The percent change in E1 and E2 concentrations from pre-AI levels will be determined and graphically depicted using spider plots.

16.6 Data & Safety Monitoring

16.61 Safety review

The principal investigator(s) and the study statistician will review the study monthly to identify accrual, adverse event, and any endpoint problems that might be developing.

The Mayo Clinic Cancer Center (MCCC) Data Safety Monitoring Board (DSMB) is responsible for reviewing accrual and safety data for this trial at least biannually, based on reports provided by the MCCC Statistical Office.

16.7 Subset Analyses for Minorities

16.71 Study availability

This study will be available to all eligible female patients, regardless of race or ethnic origin.

16.72 Statistical analysis by subset

There is no information currently available regarding differential effects of this regimen in subsets defined by race, gender, or ethnicity, and there is no reason to expect such differences to exist. Therefore, although the planned analyses will look for differences in treatment effect based on racial groupings, the sample size is not increased in order to provide additional power for subset analyses.

16.73 Regional population

The geographical region served by MCCC has a population which includes approximately 3% minorities. Expected sizes of racial by gender subsets are shown in the following table:

Accrual Targets			
	Sex/Gender		
Ethnic Category	**Females**	**Males**	**Total**
Hispanic or Latino	5		5
Not Hispanic or Latino	155		155
Ethnic Category: Total of all subjects	160	**0**	160
Racial Category			
American Indian or Alaskan Native			
Asian	7		7
Black or African American	6		6
Native Hawaiian or other Pacific Islander			
White	147		147
Racial Category: Total of all subjects	160	**0**	160

Ethnic Categories:

Hispanic or Latino – a person of Cuban, Mexican, Puerto Rican, South or Central American, or other Spanish culture or origin, regardless of race. The term "Spanish origin" can also be used in addition to "Hispanic or Latino."

Not Hispanic or Latino

Racial Categories:

American Indian or Alaskan Native – a person having origins in any of the original peoples of North, Central, or South America, and who maintains tribal affiliations or community attachment.

Asian – a person having origins in any of the original peoples of the Far East, Southeast Asia, or the Indian subcontinent including, for example, Cambodia, China, India, Japan, Korea, Malaysia, Pakistan, the Philippine Islands, Thailand, and Vietnam. (Note: Individuals from the Philippine Islands have been recorded as Pacific Islanders in previous data collection strategies.)

Black or African American – a person having origins in any of the black racial groups of Africa. Terms such as "Haitian" or "Negro" can be used in addition to "Black or African American."

Native Hawaiian or other Pacific Islander – a person having origins in any of the original peoples of Hawaii, Guam, Samoa, or other Pacific Islands.

White – a person having origins in any of the original peoples of Europe, the Middle East, or North Africa.

17.0 Pathology Considerations/Tissue Biospecimens: None

18.0 Records and Data Collection Procedures

18.1 Submission Timetable

Data submission instructions for this study can be found in the Data Submission Schedule.

18.2 Survival Follow-up -- None

See Section 4.

18.3 CRF completion

This study will use Medidata Rave® for remote data capture (rdc) of all study data. Data collection for this study will be done exclusively through the Medidata Rave® clinical data management system. Access to the trial in Rave is granted through the iMedidata application to all persons with the appropriate roles assigned in Regulatory Support System (RSS). To access Rave via iMedidata, the site user must have an active account and the appropriate Rave role (Rave CRA, Read-Only, Site Investigator) on the organization roster at the enrolling site.

18.4 Site responsibilities

Each site will be responsible for insuring that all materials contain the patient's initials, MCCC registration number, and MCCC protocol number. Patient's name must be removed.

18.5 Supporting documentation

This study requires supporting documentation for diagnosis prior to study entry. These documents should be submitted within 14 days of registration. Patient medication diaries will be uploaded to Medidata Rave.

18.6 Overdue lists

A list of overdue forms and outstanding queries will be available in Rave through the Rave Task Summary. In addition to this, the Overdue Materials report is available on the Cancer Center Systems homepage.

19.0 Budget

19.1 Costs charged to patient

- Routine clinical care
- Anastrozole 1mg and letrozole

19.2 Tests to be research funded

- Routine blood testing prior to ANA10
- Safety assessment visits for ANA10: 1) for first 6 patients; 2) 30 days after last dose
- Nurse visit and routine blood testing prior to Cycle 3
- Research testing on blood samples

19.3 Other budget concerns

Mayo Clinic Breast Cancer SPORE will provide funding to support the costs of running this study.

Mayo Clinic Breast Cancer SPORE will provide anastrozole 10 mg (ANA10) for use in this study.

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Appendix I ECOG Performance Status

ECOG PERFORMANCE STATUS*	
Grade	**ECOG**
0	Fully active, able to carry on all pre-disease performance without restriction
1	Restricted in physically strenuous activity but ambulatory and able to carry out work of a light or sedentary nature, e.g., light house work, office work
2	Ambulatory and capable of all selfcare but unable to carry out any work activities. Up and about more than 50% of waking hours
3	Capable of only limited selfcare, confined to bed or chair more than 50% of waking hours.
4	Completely disabled. Cannot carry on any selfcare. Totally confined to bed or chair.
5	Dead

*As published in Am. J. Clin. Oncol.:
Oken, M.M., Creech, R.H., Tormey, D.C., Horton, J., Davis, T.E., McFadden, E.T., Carbone, P.P.: Toxicity And Response Criteria Of The Eastern Cooperative Oncology Group. Am J Clin Oncol 5:649-655, 1982.

The ECOG Performance Status is in the public domain therefore available for public use. To duplicate the scale, please cite the reference above and credit the Eastern Cooperative Oncology Group, Robert Comis M.D., Group Chair.

From http://www.ecog.org/general/perf_stat.html

Appendix II Patient Medication Diary

Anastrozole – Cycle 1

Patient initials (optional)_____ Study ID Number_____

Please complete this diary on a daily basis. Record the date & time that you took your anastrozole in the appropriate "Day" box.

On the days that you do not take any study drug, please write in "0". If you forget to take your daily dose, please write in "0", but remember to take your prescribed dose at the next regularly scheduled time.

Take anastrozole in the morning. On the days you are scheduled to have your blood drawn do not take your dose until after your study visit & instructed by the study team.

Week 1) Week of: _____

Anastrozole 1 mg	***Day 1***	***Day 2***	***Day 3***	***Day 4***	***Day 5***	***Day 6***	***Day 7***
MM/DD/YY							
Time							

Week 2) Week of: _____

Anastrozole 1 mg	***Day 8***	***Day 9***	***Day 10***	***Day 11***	***Day 12***	***Day 13***	***Day 14***
MM/DD/YY							
Time							

Week 3) Week of: _____

Anastrozole 1 mg	***Day 15***	***Day 16***	***Day 17***	***Day 18***	***Day 19***	***Day 20***	***Day 21***
MM/DD/YY							
Time							

Week 4) Week of: _____

Anastrozole 1 mg	***Day 22***	***Day 23***	***Day 24***	***Day 25***	***Day 26***	***Day 27***	***Day 28***
MM/DD/YY							
Time							

Week 5) Week of: _____

Anastrozole 1 mg	***Day 1***	***Day 2***	***Day 3***	***Day 4***	***Day 5***	***Day 6***	***Day 7***
MM/DD/YY							
Time							

Week 6) Week of: _____

Anastrozole 1 mg	***Day 8***	***Day 9***	***Day 10***	***Day 11***	***Day 12***	***Day 13***	***Day 14***
MM/DD/YY							
Time							

Week 7) Week of: _____

Anastrozole 1 mg	***Day 15***	***Day 16***	***Day 17***	***Day 18***	***Day 19***	***Day 20***	***Day 21***
MM/DD/YY							
Time							

Week 8 Week of: _____

Anastrozole 1 mg	***Day 22***	***Day 23***	***Day 24***	***Day 25***	***Day 26***	***Day 27***	***Day 28***
MM/DD/YY							
Time							

Week 9) Week of: _____

Anastrozole 1 mg	***Day 1***	***Day 2***	***Day 3***	***Day 4***	***Day 5***	***Day 6***	***Day 7***
MM/DD/YY							
Time							

Week 10) Week of: _____

Anastrozole 1 mg	***Day 8***	***Day 9***	***Day 10***	***Day 11***	***Day 12***	***Day 13***	***Day 14***
MM/DD/YY							
Time							

Patient signature: _____ Date: _____

My next scheduled visit is: _____

If you have any questions, please contact: _____

Telephone #: _____

Study Coordinator Use Only

Number of pills returned _____ Number of Bottles returned: _____

Discrepancy Yes____/No____ Verified by _____ Date_____

Anastrozole –Cycle 2

Patient initials (optional) **Study ID Number**

Please complete this diary on a daily basis. Record the date & time that you took your anastrozole in the appropriate "Day" box.

On the days that you do not take any study drug, please write in "0". If you forget to take your daily dose, please write in "0", but remember to take your prescribed dose at the next regularly scheduled time.

Take anastrozole in the morning. On the days you are scheduled to have your blood drawn do not take your dose until after your study visit & instructed by the study team.

Week 1) Week of: _____

Anastrozole 10 mg	***Day 1***	***Day 2***	***Day 3***	***Day 4***	***Day 5***	***Day 6***	***Day 7***
MM/DD/YY							
Time							

Week 2) Week of: _____

Anastrozole 10 mg	***Day 8***	***Day 9***	***Day 10***	***Day 11***	***Day 12***	***Day 13***	***Day 14***
MM/DD/YY							
Time							

Week 3) Week of: _____

Anastrozole 10 mg	***Day 15***	***Day 16***	***Day 17***	***Day 18***	***Day 19***	***Day 20***	***Day 21***
MM/DD/YY							
Time							

Week 4) Week of: _____

Anastrozole 10 mg	***Day 22***	***Day 23***	***Day 24***	***Day 25***	***Day 26***	***Day 27***	***Day 28***
MM/DD/YY							
Time							

Week 5) Week of: _____

Anastrozole 10 mg	***Day 1***	***Day 2***	***Day 3***	***Day 4***	***Day 5***	***Day 6***	***Day 7***
MM/DD/YY							
Time							

Week 6) Week of: _____

Anastrozole 10 mg	***Day 8***	***Day 9***	***Day 10***	***Day 11***	***Day 12***	***Day 13***	***Day 14***
MM/DD/YY							
Time							

Week 7) Week of: _____

Anastrozole 10 mg	***Day 15***	***Day 16***	***Day 17***	***Day 18***	***Day 19***	***Day 20***	***Day 21***
MM/DD/YY							
Time							

Week 8 Week of: _____

Anastrozole 10 mg	***Day 22***	***Day 23***	***Day 24***	***Day 25***	***Day 26***	***Day 27***	***Day 28***
MM/DD/YY							
Time							

Week 9) Week of: _____

Anastrozole 10 mg	***Day 1***	***Day 2***	***Day 3***	***Day 4***	***Day 5***	***Day 6***	***Day 7***
MM/DD/YY							
Time							

Week 10) Week of: _____

Anastrozole 10 mg	***Day 8***	***Day 9***	***Day 10***	***Day 11***	***Day 12***	***Day 13***	***Day 14***
MM/DD/YY							
Time							

Patient signature: _____ Date: _____

My next scheduled visit is: _____

If you have any questions, please contact: _____

Telephone #: _____

Study Coordinator Use Only

Number of pills returned _____ Number of Bottles returned: _____

Discrepancy Yes____/No____ Verified by _____ Date_____

Letrozole – Cycle 3

Patient initials (optional) **Study ID Number**

Please complete this diary on a daily basis. Write in the amount of the dose of letrozole that you took in the appropriate "Day" box.

On the days that you do not take any study drug, please write in "0". If you forget to take your daily dose, please write in "0", but remember to take your prescribed dose at the next regularly scheduled time.

Take letrozole in the morning. On the days you are scheduled to have your blood drawn do not take your dose until after you have had your blood drawn.

Week of: _____

Letrozole	***Day 1***	***Day 2***	***Day 3***	***Day 4***	***Day 5***	***Day 6***	***Day 7***
MM/DD/YY							
Time							

Week of: _____

Letrozole	***Day 8***	***Day 9***	***Day 10***	***Day 11***	***Day 12***	***Day 13***	***Day 14***
MM/DD/YY							
Time							

Week of: _____

Letrozole	***Day 15***	***Day 16***	***Day 17***	***Day 18***	***Day 19***	***Day 20***	***Day 21***
MM/DD/YY							
Time							

Week of: _____

Letrozole	***Day 22***	***Day 23***	***Day 24***	***Day 25***	***Day 26***	***Day 27***	***Day 28***
MM/DD/YY							
Time							

Week of: _____

Letrozole	***Day 1***	***Day 2***	***Day 3***	***Day 4***	***Day 5***	***Day 6***	***Day 7***
MM/DD/YY							
Time							

Week of: _____

Letrozole	***Day 8***	***Day 9***	***Day 10***	***Day 11***	***Day 12***	***Day 13***	***Day 14***
MM/DD/YY							
Time							

Week of: _____

Letrozole	***Day 15***	***Day 16***	***Day 17***	***Day 18***	***Day 19***	***Day 20***	***Day 21***
MM/DD/YY							
Time							

Week of: _____

Letrozole	***Day 22***	***Day 23***	***Day 24***	***Day 25***	***Day 26***	***Day 27***	***Day 28***
MM/DD/YY							
Time							

Week of: _____

Letrozole	***Day 1***	***Day 2***	***Day 3***	***Day 4***	***Day 5***	***Day 6***	***Day 7***
MM/DD/YY							
Time							

Week of: _____

Letrozole	***Day 8***	***Day 9***	***Day 10***	***Day 11***	***Day 12***	***Day 13***	***Day 14***
MM/DD/YY							
Time							

Patient signature: _____ Date: _____

My next scheduled visit is: _____

If you have any questions, please contact: _____

Telephone #: _____

Study Coordinator Use Only

Number of pills returned _____ Number of bottles returned: _____

Discrepancy Yes____/No____ Verified by _____ Date_____