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A Trial of Endocrine Response in Women with Invasive Lobular Breast Cancer

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Protocol Chair
Priscilla McAuliffe, M.D.
UPMC Hillman Cancer Center
Magee-Womens Hospital of UPMC
300 Halket Street, Suite 2601
Pittsburgh, PA 15213
Phone: 412-641-4274
Fax: 412-641-6461
Email: mcauliffepf@mwri.magee.edu

Protocol Co-Chair
Rachel Jankowitz, MD

Co-Investigators
Shannon Puhalla, M.D.
Adam Brufsky, M.D., Ph.D.
Priya Rastogi, M.D.
Vikram Gorantla, MD
Leisha Emens, MD
Gijsberta van Londen, MD
Ronald Johnson, M.D.
Emilia Diego, M.D.
Jennifer Steiman, M.D.
Atilla Soran, MD
Joanna Lee, MD

Breast Pathology:
Beth Clark, M.D.

Laboratory Investigators:
Steffi Oesterreich, Ph.D.
Jennifer Xavier, Ph.D.

Department of Pharmacology & Chemical Biology; \ Co-Authors
UPMC Hillman Cancer Center
Magee-Womens Research Institute; Pittsburgh, PA

Statisticians:
Ying Ding, Ph.D.
UPMC Hillman Cancer Center; Biostatistics Facility

Coordinating Center: UPMC Hillman Cancer Center
Magee-Womens Hospital of UPMC
300 Halket Street, Suite C-
310Pittsburgh, PA 15213

Study Coordinator: Lucia Borrasso, CCRC
Research Coordinator Magee-Womens
Hospital
300 Halket St., Suite
C-310
Phone: 412-641-3304
Fax: 412-641-3456
Email: borrlm@upmc.edu

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1.0 PROTOCOL SUMMARY

Postmenopausal women with hormone receptor-positive (HR-positive) and HER2-negative invasive lobular carcinoma histologically confirmed on a diagnostic core biopsy and with adequate organ function who are awaiting a definitive surgical procedure or initiation of neoadjuvant systemic therapy are eligible. Once they have given consent and have been registered, they will be randomized to receive one of three open-label treatments: fulvestrant (500 mg, administered as two 250 mg IM injections, given on days 1 and 15), anastrozole (1mg given orally daily), or tamoxifen (20 mg given orally daily). Surrogate biomarkers of response will be assessed on a baseline research core biopsy (preferred), or material from the archival clinical diagnostic core biopsy, of the breast tumor and on post-treatment biopsy. For patients going to definitive surgery, the operation will be scheduled between 21 to 27 days after study drug administration. The post-treatment biopsy will be done on surgically excised breast tissue. However, if surgery is delayed or if the patient is not scheduled for surgery, a post-treatment repeat research core biopsy will be collected. For patients in whom neoadjuvant treatment is planned, the study treatment will be administered for 21 to 27 days prior to initiation of other treatment.

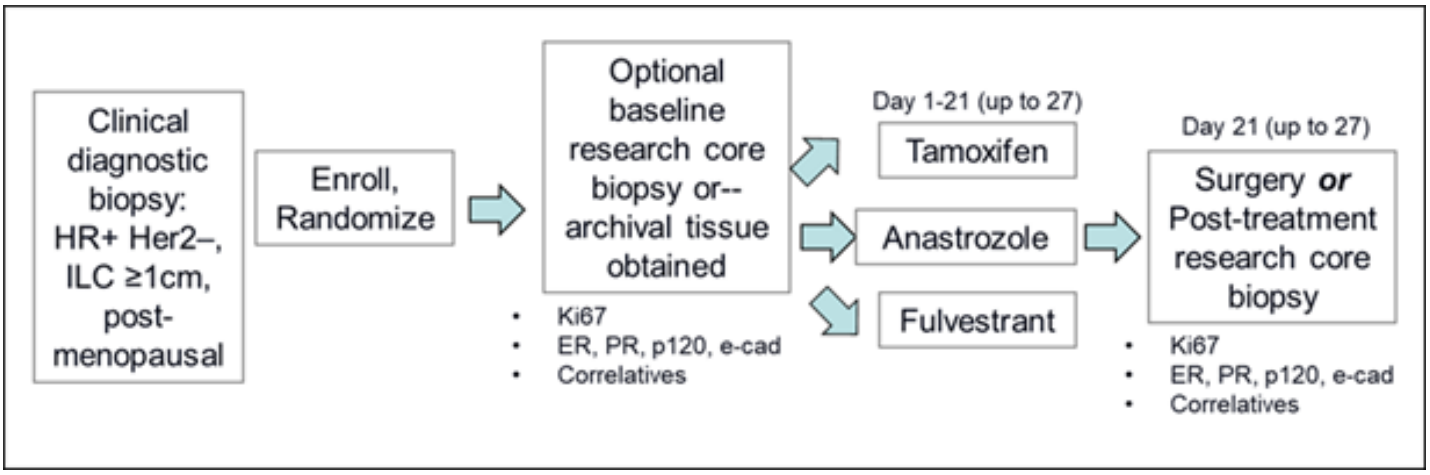
We hypothesize that fulvestrant is more effective than anastrozole or tamoxifen in reducing Ki67 in ILC, and that Ki67 reduction will correlate with alterations in expression of ER and ER-regulated genes. Differential Ki67 effect in this study will serve as a surrogate for outcome of ILC patients on endocrinetherapy.

We will evaluate ER and PR protein expression, as well as ER-related and ILC-specific candidate gene mRNA expression in ILC tissues, at baseline and following neoadjuvant endocrine therapy, in an effort to identify biomarkers of endocrine response and putative drivers of endocrine resistance in ILC. Finally, we will evaluate associations between changes in Ki67 in ILC tissues following neoadjuvant endocrine therapy with ER protein expression, or ER and candidate gene mRNA expression at baseline and post-treatment.

Our exploratory objectives will include an examination of associations between germline and somatic DNA sequence variants with changes in Ki67 in ILC tissues following neoadjuvant endocrine therapy. We will collect one blood sample per patient for DNA analysis.

This planned study will use a randomized design. We will accrue 67participants with ILC tumors to each of the three treatment arms (tamoxifen, anastrozole or fulvestrant). Study investigators and personnel will meet monthly to review study accrual, procedures, and safety. A 21-day intervention period is chosen because ample data are available regarding Ki67 response with tamoxifen, anastrozole, and fulvestrant alone 14-21 days after initiation of treatment that can provide historical control and information for statistical considerations. Specifically, proliferation 14 days following hormonal treatment can be correlated with long term outcome. Fulvestrant has been shown to modulate Ki67 14-21 days after its administration in HR+ breast cancer patients in the neoadjuvant setting after even one single dose. In addition, this intervention interval is not likely to delay scheduled surgery. Of note, to accommodate weekends, holidays, and minor scheduling conflicts, up to 27 days of treatment will bepermitted.

2.0 SCHEMA



3.0 HYPOTHESES

We hypothesize that fulvestrant is more effective than anastrozole or tamoxifen in reducing Ki67 in ILC and that Ki67 reduction will correlate with alterations in expression of ER and ER-regulated genes. Differential Ki67 effect in this study will serve as a surrogate for outcome of ILC patients on endocrine therapy.

4.0 OBJECTIVES

4.1 Primary Objective

To determine the change from baseline to post-treatment Ki67 values in HR-positive, HER2-negative ILC tissue derived from postmenopausal women awaiting definitive surgery or further neoadjuvant treatment who are randomized to 21-27 days of neoadjuvant endocrine treatments with fulvestrant (two 250 mg IM injections given on day 1 and 15), anastrozole (1mg given orally daily), or tamoxifen (20mg given orally daily).

4.2 Secondary Objectives

4.2.1 To compare between arms Ki67 values following neoadjuvant therapy.

4.2.2 To evaluate ER protein expression in ILC tissues at baseline and following neoadjuvant endocrine therapy.

4.2.3 To evaluate PR protein expression in ILC tissues at baseline and following neo-adjuvant endocrine therapy.

4.2.4 To evaluate ER-related and ILC-specific candidate gene mRNA expression in ILC tissues at baseline and following neoadjuvant endocrine therapy in an effort to identify biomarkers of endocrine response and putative drivers of endocrine resistance in ILC.

4.2.5 To evaluate associations between changes in Ki67 in ILC tissues following neoadjuvant endocrine therapy with ER and PR protein expression, or ER and candidate gene mRNA expression at baseline and post-treatment.

4.3 Exploratory Objectives

4.3.1 To evaluate DNA methylation in ILC tissues at baseline and following neoadjuvant endocrine therapy.

4.3.2 To evaluate associations between germline and somatic DNA sequence variants with changes in Ki67 in ILC tissues following neoadjuvant endocrine therapy.

4.3.3 To evaluate the activity of signaling pathways in ILC tissues by immunohistochemical or other protein analyses, such as histone modifications, at baseline and following neoadjuvant endocrine therapy.

5.0 BACKGROUND AND RATIONALE

5.1 Current Treatment Approaches in Breast Cancer

Among women in the United States, breast cancer will be diagnosed in 1 in 8 individuals, and it is the second most common cause of cancer-related death. While most women with breast cancer survive their disease, others suffer recurrences and death. Currently, most women with breast cancer, even stage I disease, will be offered systemic therapy such as endocrine therapy and/or chemotherapy. Combination chemotherapy reduces risk of recurrence by up to 35% and risk of mortality by up to 27%.[1] However, a proportion of hormone receptor positive (HR-positive) patients do not seem to benefit from adjuvant chemotherapy in the node-negative[2], and perhaps in the node-positive[3], setting.

Endocrine therapies targeting the action or production of estrogens are often the most important agents in the treatment of HR-positive breast cancer. Despite the success of endocrine therapies in reducing disease recurrence and increasing overall survival, 20-25% of patients receiving adjuvant tamoxifen or aromatase inhibitors will experience disease relapse within 10 years of treatment initiation[4]. Additionally, late recurrence, or recurrence after 5 years of therapy, can be particularly problematic in HR-positive patients[5, 6]. Recently, it has become apparent that extended adjuvant hormonal therapy is beneficial to both node-negative and node-positive patients[4, 7]. Unfortunately, adjuvant endocrine therapies significantly affect quality of life in many women, leading to notoriously low compliance and adherence rates[8]. Furthermore, biomarkers that are predictive of response/resistance to tamoxifen and AIs in *individual* patients or breast cancer *subsets* are lacking. It is critical to define mechanisms of endocrine resistance and to identify subgroups of patients who need more, less, or better tailored, endocrine therapies.

5.2 Endocrine Therapy Modalities

Endocrine therapies can be used to inhibit estrogen receptor activity in patients with HR-positive disease. Tamoxifen and the aromatase inhibitors (letrozole, anastrozole, and exemestane) are currently utilized as adjuvant setting, and increasingly in the neoadjuvant setting. Additionally, fulvestrant is available for the treatment of patients who have progressed or recurred following frontline endocrine therapy. Importantly, each of these therapies has a unique mechanism of action that may prove to have differing efficacies in individual breast tumors. However, there are currently no biomarkers or strategies for identifying patients who may respond best to a specific endocrine therapy.

Tamoxifen is a selective estrogen receptor modulator (SERM). In HR-positive patients, tamoxifen reduces the risk of breast cancer recurrence and mortality by 47% and 26%, respectively.[1] The recent analysis of the international Adjuvant Tamoxifen, Longer Against Shorter (ATLAS) study showed evidence of benefit with prolonged tamoxifen use for 10 years improved outcomes over 5 years of therapy.[4] However, tamoxifen is extensively metabolized and its metabolites have mixed agonist/antagonist activity in target tissues, including breast cancer. In laboratory models of tamoxifen resistance, breast cancer cells can proliferate in the presence of tamoxifen compounds by converting to recognize tamoxifen as an estrogen[9, 10].

Third generation aromatase inhibitors (letrozole, anastrozole, and exemestane) are currently utilized as the primary adjuvant treatment modality in post-menopausal women with HR-positive disease based on improved efficacy and safety in comparison to tamoxifen[4, 11]. These therapies block the peripheral conversion of androgens to estrogen via inhibition of CYP19A1 aromatase, thus depleting estrogen and preventing estrogen receptor activation. For instance, letrozole has been demonstrated to reduce circulating estradiol concentrations by >99%[12]. Models of aromatase inhibitor resistance have implicated up-regulated growth factor signaling and estrogen-independent ER activity as potential resistance mechanisms[13-17].

Fulvestrant is a selective estrogen receptor downregulator (SERD) indicated for the treatment of HR-positive metastatic breast cancer in postmenopausal women with disease progression following endocrine therapy[8]. Fulvestrant is not officially indicated for the treatment of breast cancer in the adjuvant/neoadjuvant setting. Whereas tamoxifen binding to ER actually stabilizes ER, fulvestrant induces rapid ER degradation, blocking ER activity by eliminating the receptor[18, 19]. Though resistance mechanisms are poorly understood, recent results from the phase 3 CONFIRM trial demonstrated improved efficacy of higher fulvestrant dosing (500 mg vs. 250 mg)[20].

5.3 Invasive Lobular Carcinoma

Though the majority of newly diagnosed breast tumors are invasive ductal carcinomas (IDC), 5-15% of tumors are of the invasive lobular carcinoma (ILC) subtype[21-25]. Thus, ILC accounts for roughly 25,000-30,000 new breast cancer cases in the United States annually, with ILC incidence significantly increasing in the last two decades[26, 27]. If considered an independent cancer type, ILC would rank as the 6th most common cancer in women, occurring with similar frequency as non-Hodgkin's lymphoma and melanoma[28].

ILC is pathologically, clinically and biologically unique among breast tumors. ILC is more common among older patients, who generally present with larger tumors than IDC patients[21, 29, 30], owing in part to the unique growth pattern of ILC tumors. ILC grows linearly along mammary ducts creating long thin masses characterized by a single-file pattern of cells invading surrounding tissue[31, 32]. This growth pattern makes ILC difficult to detect by mammography and to surgically resect[33]. Further, ILC has an atypical pattern of metastatic growth. Although both ILC and IDC most commonly spread to the bone, ILC metastasizes to lung and CNS less frequently, and instead targets the ovaries and gastrointestinal tract[21].

Compared to IDC, ILC is more often ER-positive and progesterone receptor-positive (PR-positive), and displays lower proliferation markers; ILC is more often diploid, of lower histological grade, and HER2-negative[21, 26, 30]. In fact, the vast majority of ILC tumors are ER-positive; a number of studies including queries of the NCI SEER database report >90% ER-positivity among ILC[21, 22, 26, 30, 34].

5.4 Endocrine Therapy in ILC

The combination of favorable biomarkers and patient/tumor characteristics typical of ILC makes adjuvant endocrine therapy an attractive treatment option for ILC patients. In addition to >90% of ILCs being ER-positive, only 5-14% of ILC are clinically HER2-positive (versus ~25% of IDCs)[21, 34-38]; the majority of HER2-positive ILC represent the aggressive pleomorphic variant[34, 38]. Based on these observations, the majority of ILC patients could be expected to have very favorable outcomes when treated with tamoxifen or aromatase inhibitors in comparison to breast cancer patients as a whole. However, a disconnect between ILC biomarkers and response to endocrine therapy has been observed for over 25 years[39], and minimal data has actually been reported regarding the response of ILC patients to endocrine therapy. The existing data, recently reviewed by our group[16], suggests that overall response to endocrine therapy in ILC is not consistent with its favorable biomarkers.

Two recent reports provide compelling evidence that endocrine response in ILC merits additional study. Colleoni and colleagues specifically examined outcomes of special histological types of luminal cancer, according to the ER or PR positivity of the tumors[40]. A total of 6558 ILC and IDC patients were included, with 99.6% of tumors considered ER-positive (24 IDC and 2 ILC were ER-negative/PR-positive). All patients received adjuvant endocrine therapy, including tamoxifen and/or aromatase inhibitors. Multivariate Cox proportional regression demonstrated that ILC patients had shorter disease-free survival (HR 1.27), distant metastasis-free survival (HR 1.48) and overall survival (HR 1.34) compared to IDC patients. The biggest difference in outcome was observed in distant metastasis-free survival in Grade I ILC versus IDC (HR 2.83).

Most recently, Metzger-Filho and colleagues presented a retrospective analysis of the BIG 1-98 trial (comparing 5 years daily monotherapy with tamoxifen versus the aromatase inhibitor letrozole) investigating outcomes in IDC versus ILC patients[41]. The magnitude of benefit of adjuvant letrozole significantly varied by histology in that the degree of benefit with letrozole vs. tamoxifen in women with ILC was more pronounced than in women with IDC. Amongst IDC patients (n=2599), the 8-year DFS was 82% for letrozole-treated patients and 75% for tamoxifen treated patients (HR 0.8), confirming the increased benefit for letrozole versus tamoxifen as previously reported. However, over the same period, DFS was 82% versus 66% for ILC patients treated with letrozole or tamoxifen, respectively (HR 0.48). The inferior outcome of ILC patients on tamoxifen was particularly seen in the immunohistochemically-defined luminal A subgroup. These data bring into question whether certain ILC patients receive benefit from adjuvant tamoxifen monotherapy, and highlight the need for new investigation in to this breast cancer subtype.

5.5 Rationale for Neoadjuvant Fulvestrant in Breast Cancer

Fulvestrant is not officially indicated for the treatment of breast cancer in the neoadjuvant setting. The Phase II, randomized, open-label, multicenter NEWEST trial compared the biological and clinical activity of fulvestrant 500 mg versus fulvestrant 250 mg in the neoadjuvant breast cancer setting in postmenopausal women with newly diagnosed, ER positive, locally advanced breast cancer who had not received any prior breast cancer treatments. After 4 weeks of treatment, fulvestrant 500 mg reduced mean K-i67 labeling index significantly more than fulvestrant 250 mg, with the fulvestrant 500 mg dose reducing mean Ki67 by 78.8% reduction from baseline.[42]

Moreover, a Phase II, double-blind, randomized, multicenter trial to compare the biological activity of fulvestrant with and without anastrozole or anastrozole alone as neoadjuvant treatment in 121 postmenopausal women with ER positive primary breast cancer showed significant reduction from baseline in mean ER, PgR, and Ki67 index with all treatment groups.[43] Again, with just a 14-21 day exposure, a

single 500mg dose of fulvestrant yielded a reduction in mean Ki67 index of 85%. This was comparable to that seen in the anastrozole alone arm in which the mean reduction in Ki67 from baseline was 89%.

5.6 Rationale for Neoadjuvant Fulvestrant in ILC

As described above, limited data are available regarding clinical response to endocrine therapies for ILC patients. The Oesterreich laboratory has begun to characterize endocrine response in these cell lines as model systems for ILC. Using the only two existing estrogen receptor (ER)-positive ILC cell lines, MDA MB 134VI and SUM44PE (described by us in [44]), our team has shown a different response of ILC to endocrine therapies compared to the invasive ductal carcinoma (IDC) cell line MCF-7. In MDA MB 134VI fulvestrant can completely block estrogen-induced growth while tamoxifen can only inhibit ~75% of growth. In SUM44PE cells fulvestrant blocks growth, and while tamoxifen can also inhibit growth, the effect is less compared to fulvestrant. Additionally, SUM44PE cells appear to be less sensitive to estrogen deprivation (conditions that mimic aromatase inhibitor therapy). Of note although these ILC cell lines present with varying degrees of resistance to tamoxifen and/or aromatase inhibitors, *both cell lines remain responsive to fulvestrant*. Furthermore, estrogen-mediated degradation of the estrogen receptor (ER) – a well described response in IDC – is attenuated in ILC cells. However, ER remains sensitive to degradation in ILC cells by fulvestrant. These observations suggest that endocrine resistance in ILC cells may reflect estrogen-independent ER activity, rendering them resistant to tamoxifen or AI but sensitive to receptor degradation by fulvestrant. Based on these preclinical observations in ILC cell lines, we hypothesize that fulvestrant will produce improved inhibition of breast tumor growth when used in ILC patients compared with tamoxifen or aromatase inhibitors.

In addition to Ki67, other biomarkers, such as expression of ER protein and mRNA and ER target genes may serve as effective measures of tumor response to endocrine therapy as demonstrated in several neoadjuvant studies[8, 9,10]. The Oesterreich lab has identified ER target genes likely to be related to endocrine response specifically in ILC patients using their ILC cell line models. We will evaluate this initial panel of genes (~150 candidates) in this trial as markers of ER activity and potential determinants of endocrine resistance.

These *in vitro* findings are consistent with the recent clinical reports described above suggesting decreased benefit from endocrine therapy in ILC patients, in particular tamoxifen. We hypothesize that fulvestrant will produce improved inhibition of growth when used to treat ILC patients, versus tamoxifen or aromatase inhibitors, based on the *de novo* endocrine resistance we have observed in ILC model systems. However, as fulvestrant is not currently approved in the neoadjuvant/adjuvant setting, a neoadjuvant window trial affords us an ideal opportunity to examine the surrogate biomarkers of clinical efficacy of each mode of endocrine therapy specifically in ILC patients.

The proposed study is a hypothesis-driven clinical trial that could significantly improve our understanding of ILC. Identification of a biological signal of tamoxifen or AI-resistance and/or fulvestrant-sensitivity in ILC patients would have dramatic implications for the future management of this breast cancer subtype. Currently, endocrine therapy after an early-stage breast cancer diagnosis often follows a “one-size-fits- all” approach, with most premenopausal women receiving tamoxifen, and most postmenopausal receiving aromatase inhibitor therapy. If our trial indicates that these treatments were less effective in comparison to fulvestrant at decreasing the growth and proliferation of ILC tumors, it would warrant a much larger trial in ILC patients to confirm these findings. As fulvestrant is already approved for treatment of stage IV breast cancer, this trial could be rapidly mounted, and a positive result could lead to more effective treatment for the 25,000-30,000 new patients diagnosed with ILC in the United States annually.

5.7 Ki67 as a Surrogate Biomarker of Therapeutic Efficacy

A number of recent studies have investigated and validated immunohistochemical analyses of the proliferation marker Ki67 as a short-term biomarker of long-term outcomes in breast cancer. In the IMPACT neoadjuvant study, investigators assessed Ki67 positivity in tumors at baseline and following 2 and 12 weeks of neoadjuvant tamoxifen or anastrozole. Anastrozole was found to more strongly suppress Ki67 than tamoxifen at both timepoints after initiation of therapy. This results was consistent with the improved efficacy of anastrozole over tamoxifen observed in the larger ATAC trial[45]. Furthermore, high Ki67 positivity following two weeks of therapy was associated with inferior DFS[46]; multivariate analyses suggested that the 2-week post-treatment Ki67 value and the associated change in Ki67 during treatment were associated with outcomes. Similar results were observed in the P024 trial, which reported that increased post-treatment (16 weeks of neoadjuvant letrozole) Ki67 positivity was associated with reduced DFS[47]. Moreover, results from the Z1031 trial demonstrated that each of the third generation AIs produce similar decreases in Ki67 positivity and that baseline Ki67 was predictive of clinical benefit, as measured by reduction in tumor size[48]. Recognizing the usefulness of Ki67 as a surrogate marker, an international working group convened to standardize assessment of Ki67[47]. Large scale evaluation of the utility of pre- and post-treatment Ki67 is underway with the POETIC trial[49]. Importantly, none of the above studies has evaluated tumor histology as part of accrual, and the proportion of ILC patients on trial has not been reported. However, these trials demonstrate that Ki67 is a useful short-term surrogate for long-term outcomes of breast cancer patients on endocrine therapy.

5.8 Expression of ER and Target Genes as Biomarkers of ER Activity in ILC

In addition to Ki67, other biomarkers, such as expression of ER protein and mRNA, as well as the expression of ER target genes, may serve as effective measures of ER activity and tumor response to endocrine therapy. Expression of ESR1, the gene encoding ER, at baseline has been examined in several studies and has been found to be associated with response to endocrine therapy[48, 50]. Though protein expression of ER itself is the key predictor of response to endocrine therapy, the dynamics of ER expression following endocrine therapy in patients is poorly understood. Laboratory studies in IDC cell lines (e.g. MCF-7) have demonstrated that estrogen induces moderate ER degradation; tamoxifen instead stabilizes ER, whereas fulvestrant induces rapid and complete degradation[18, 19]. In preliminary studies using ILC cells, the Oesterreich laboratory has observed differential regulation of ER by estrogen in IDC vs. ILC cells. Whereas estrogen induces the degradation of ER in IDC cells (MCF-7, T47D), ER is largely unchanged in ILC cells (MDA MB 134VI, SUM44PE). Consistent with this, ILC cell lines express extremely high levels of ER in standard conditions relative to IDC cells. Based on these observations, we hypothesize that ER protein or mRNA at baseline or following therapy may identify patients that do or do not respond to endocrine therapy.

In addition to ER itself, a number of reports in the literature have examined the expression of ER target genes in patient samples prior to adjuvant endocrine therapy, as well as pre- and post-therapy in the neoadjuvant setting. Many of these studies have been focused on identifying gene expression signatures of response or resistance specifically in pre-treatment samples[51-53]; these are often hindered by limited sample size and there is consequently little overlap in signatures between studies. Several neoadjuvant studies have examined ER activity by monitoring the expression of canonical ER target genes and correlating expression with clinical response to therapy[54-57]. However, the signatures often overlap with proliferation-related genes, making the signatures minimally informative versus standard Ki67 analyses.

We have taken the approach of first identifying ILC-specific ER target genes in model systems and measuring the expression of validated target genes in the neoadjuvant setting. Importantly, the selection of

a limited subset of genes (versus genome-wide expression) allows us to leverage the utility of the Nanostring Counter platform in analyzing FFPE tissues. Additionally, these genes are likely to be specifically related to endocrine response in ILC based on their identification in ILC model systems. We identified an initial panel of genes (~150 candidates) using the ILC cell lines described above. Initial validation is being performed using xenografts. Genes that are consistently regulated by estrogen in all three ILC models will be further evaluated in this trial as markers of ER activity as well as putative drivers of endocrine resistance.

5.9 Summary

Although ILC patients present with biomarkers consistent with good outcomes, recent reports suggest that ILC patients do not receive the same benefit from endocrine therapies as IDC patients. Response and resistance to endocrine therapy is poorly understood in this population. We propose to undertake a randomized neoadjuvant window trial comparing the efficacy of three endocrine treatments – tamoxifen, anstrozole or fulvestrant – in post-menopausal ILC patients. We will use pre- and post-treatment Ki67 as a surrogate marker for response to therapy. Based on our observations in pre-clinical models of ILC, we hypothesize that while all three therapies will decrease Ki67, fulvestrant will produce the greatest reduction in Ki67, reflecting *de novo* endocrine resistance observed in model systems. Additionally, we will measure expression of estrogen receptor, progesterone receptor and ILC-specific target genes to identify biomarkers of endocrine response and putative drivers of endocrine resistance in ILC.

A 21-day intervention period is chosen because ample data are available regarding surrogate markers and changes in hormone receptor status with tamoxifen, anastrozole, and fulvestrant 14-21 days after initiation of treatment that can provide historical control and information for statistical considerations. Importantly, proliferation determined 14 days following hormonal treatment can be correlated with long term outcome. In addition, a 21-day intervention interval is not likely to delay a scheduled surgery. Our trial will hopefully provide the rationale for the differential treatment of patients with ILC tumors as opposed to other HR-positive breast cancers.

6.0 PATIENT POPULATION

6.1 Inclusion Criteria

6.1.1 Histologically confirmed invasive lobular breast cancer, that is hormone receptor-positive and HER2-negative, measuring at least 1 centimeter (cm) radiographically or clinically, clinical stages I-III. (Note: See Appendix A for staging criteria.) Invasive lobular histology will be diagnosed at the enrolling institution for purposes of study participation. Subsequently, invasive lobular histology will be confirmed by central pathology review, but this central review will not be required prior to patient enrollment.

6.1.2 Prior to initiation of study agents, study participants will be highly encouraged to undergo a baseline research core biopsy of their breast tumor. If this is not possible or the patient refuses, the pre-treatment tumor sample must be obtained from their archival diagnostic core biopsy. If definitive surgery is not performed at day 21-27 after study treatment, a second post-treatment research core biopsy will need to be obtained from their breast tumor. For patients undergoing surgery, the second biopsy will be removed from the breast tumor tissue excised during their operation. Note: In the event that the baseline breast tumor biopsy performed for research purposes does not yield adequate tumor tissue for analysis of the primary and secondary endpoints, tissue will be requested from the patient's archival clinical diagnostic core biopsy if it is available.

The patient will still remain on study and complete protocol therapy as planned in this unlikely event.

6.1.3 Hormone receptor (HR) status of the invasive component must be documented before trial enrollment. The tumor must be HR-positive. HR will be considered positive if staining is 1% or greater for ER and/or PR. This will be determined at the enrolling institution for purposes of study participation and enrollment onto the trial. Subsequently, HR status will be confirmed by central pathology review, but this central review will not be required prior to enrolling the patient. HER2 status will be determined locally only, based upon current ASCO/CAP guidelines.

6.1.4 Patients must be female.

6.1.5 Participants must be fully postmenopausal (see Appendix B).

6.1.6 ECOG performance status of 0, 1 or 2 (see Appendix C).

6.1.7 Adequate organ and marrow function as defined by a history and physical exam that rules out co-morbidities that would be exclusions to participation in the study (see exclusion criteria) and clinical laboratory parameters as deemed clinically appropriate by the treating physician.

6.1.8 Prior use of hormone contraceptives and replacement therapy is allowed (e.g., estrogen and/or progestin), but must have been discontinued at least 30 days prior to the study enrollment. Vaginal preparations (e.g., Vagifem® or Estring®) are allowed.

6.1.9 Participant must be aware of the nature of her malignancy, understand the study requirements and risks and be able and willing to sign a written informed consent document.

6.2 Exclusion Criteria

6.2.1 Prior or concurrent use of hormonal therapy, chemotherapy, radiation therapy, or novel therapyto treat the current breast cancer, including any history of prior irradiation to the ipsilateral breast. Additionally, the patient must not have had hormonal therapy for breast cancer treatment or for breast cancer prevention within 2 years prior to study enrollment. (Note: Synchronous breast, cancer (including bilateral breast cancer) at separate sites is permissible, provided the patient does not receive medical treatments for breast cancer or radiation therapy to the ipsilateral breast during the 21-day study intervention period.

6.2.2 Concurrent use of any other investigational agents.

6.2.3 History of allergic reactions/hypersensitivity attributed to compounds of similar chemical or biologic composition to tamoxifen, anastrozole, or fulvestrant or any of theiringredients.

6.2.4 History of thromboembolic disease or uterine cancer that is considered a contraindicationto tamoxifen.

6.2.5 Active hepatitis viral infections or a known history of liver disease, especially moderate (Child-Pugh Class B) to severe (Child-Pugh Class C) hepatic impairment.

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

6.2.7 HER-2 positivity.

6.2.8 Increased Risk of bleeding: including a history of a bleeding diathesis and/or known history of severe thrombocytopenia. NOTE: Anticoagulant use is not a contraindication to fulvestrant, but caution is advised in administration in patients on anticoagulation. Patients on anticoagulation who will receive fulvestrant will have PT and aPTT/INR assessed at baseline (see Study Calendar).

6.3 Inclusion of Women and Minorities

Individuals of all races and ethnic groups are eligible for this trial. There is no bias towards age or race in the clinical trial outlined. This trial is open to the accrual of women only.

7.0 INVESTIGATIONAL PLAN

7.1 Recruitment

Patients will be recruited through the breast cancer clinics at each of the participating centers.

7.2 Determination of Eligibility

After eligibility is established at the participating institution, participants will be registered by the study staff and assigned a *study number*.

Study treatment cannot begin until the patient is registered.

Subjects who sign a consent form, but do not initiate protocol treatment for any reason (i.e., subjects who are screen failures) will be replaced and not be counted towards our accrual goal.

7.3 Drug Administration

Subjects will be provided with a 21-day supply of tamoxifen or anastrozole tablets or will receive IM fulvestrant on days 1 and 15. Tablets are to be taken at the same time daily for 21-24 days. Patients will take their last doses of study drug on the day of surgery of their operation or their post-treatment research core biopsy (day 21-24). Patients randomized to fulvestrant will receive 500 mg (administered as two 250 mg IM injections) at the infusion center on day 1 and 15.

No premedication regimens are planned.

There will be no dose reductions for tamoxifen, anastrozole or fulvestrant. If felt to be in the best interests of the participant, the study drug may be discontinued.

This is an open-label, unblinded study. Patients will be randomized between the three treatment arms.

7.4 Dosing Delays and Modifications

Toxicity assessments will be done using NCI Common Terminology Criteria for Adverse Events Version 4.0 which is available at http://ctep.cancer.gov/reporting/ctc.html.

Missed doses should not be replaced; the planned treatment schedule should be maintained.

To assess for tolerability, the research coordinator(s) will call the patient 7 to 10 days after initiation of on-study treatment. The coordinator will also assess toxicities at the time of surgery or post-treatment research core biopsy. Patients will be instructed to call the site-specified urgent contact for any issues or questions. Additional evaluations will occur, as necessary, to evaluate and treat observed toxicity.

7.4.1 Hematologic Toxicity

It is not expected that hematologic toxicity will be identified in this study; therefore, laboratory results are planned only at baseline as necessary for routine pre-surgical evaluation. However, in the event that unscheduled laboratories are obtained for any reason, the study drug will be discontinued if any of the following hematologic toxicities are identified: an ANC ≤1,500, platelet count ≤100,000, and hemoglobin ≤8 g/dL.

7.4.2 Non-Hematologic Toxicity

Due to the short treatment duration in a relatively healthy population of women, we do not anticipate toxicities requiring delay in surgical management or other primary treatment. However, if a woman experiences grade 3 or 4 non-hematologic, treatment-related toxicity(s), the study drug should be discontinued until toxicity improves to grade ≤1 (or to baseline if initially > grade 1). If toxicity improves or resolves, subsequent doses of study drug may be resumed at the discretion of the treating investigator.

7.4.3 Additional Information

Side effects will be managed symptomatically. In the unlikely case of toxicity, appropriate medical treatment should be used (including anti-emetics for nausea/vomiting, anti-diarrheals for diarrhea as above, etc.)

Any change to treatment schedule must be approved in advance by the Protocol Chair.

7.5 Supportive Care and Concomitant Therapy

The concurrent administration of any other therapies intended to treat the primary condition including chemotherapy and biologic agents is not permitted. Similarly, the use of other concurrent investigational drugs is not allowed.

In general, concomitant medications and therapies deemed necessary for the supportive care and safety of the subject are allowed, provided their use is documented in the medical records.

7.6 Additional Treatment

Additional treatment – chemotherapy, radiation therapy, and /or endocrine manipulations for women with an ER and/or PR positive tumor – may not be administered during the 21-27 day treatment part of this study.

7.7 Duration of Study Participation and Duration of Therapy

In the absence of adverse event(s), treatment will continue for a total of 21-27 days (this includes 6 additional days of treatment to account for holidays, weekends and minor scheduling conflicts) immediately prior to the patient's scheduled surgery or post-treatment research core biopsy, or until one of the following criteria applies:

- Concurrent illness that prevents further administration of treatment
- Pregnancy
- Unacceptable adverse event(s)
- Patient decides to withdraw from the study
- General or specific changes in the patient's condition that renders the patient unacceptable for further treatment in the judgment of the investigator

7.8 Duration of Follow-Up

Patients continuing to experience adverse events attributable to the study drug at the end of study visit will be followed until resolution or stabilization of the adverse event(s).

7.9 Additional Information

No subject remuneration is planned.

8.0 STUDY CALENDAR

Parameter	Baseline[1]	Day 1-21 (up to Day 27)	Day 7-10 from Day 1	Day of Surgery (Day 21-27)
History and physical	X			
Vital signs and weight	X			
Height	X			
Performance status	X			
Clinical laboratory assessments[2]	(X)			
PT/INR/aPTT [3]	(X)			
Study Blood Sample [4]	X			
Study Tissue Samples [5] Research Biopsy Fresh tumor sample	 X X			 X X
Study drug administration		X[6]		X[6]
Adverse Events			X	X[7]
Drug Diary[8]			X	X

(X) = Optional

Note: Additional tests may be performed at the discretion of the treating investigator as clinically indicated.

1. Within 6 weeks prior to screening, unless otherwise noted. (Note: Baseline height can be outside of this window without needing approval from the Protocol Chair.)
2. Clinical laboratories (e.g., CBC with differential/platelets or chemistries at the discretion of the treating physician to determine appropriateness for therapy).
3. PT/INR/aPTT is required within 28 days only if on anticoagulation.
4. One 10mL tube of blood will be drawn for research purposes at any time prior to surgery or post-treatment research biopsy.
5. Tissue from an optional but highly encouraged baseline research biopsy will be collected after the patient is enrolled on study, and from the definitive post-treatment research biopsy or surgical specimen. If the patient declines the baseline research biopsy, the pre-treatment tumor tissue must be obtained from the diagnostic core biopsy. Note: If research biopsy fails to yield adequate tumor tissue for analysis patient will remain on study and complete study therapy as planned.
6. A study-specific supply of tamoxifen, anastrozole, or fulvestrant will be supplied. Dosing will begin with the morning (am) dose on Day 1 with the final dose to be taken on the day of surgery or their post treatment research biopsy if they do not go to surgery by day 27. The surgery or post treatment biopsy must be scheduled between day 21-27. Fulvestrant will be given in the outpatient clinic on Days 1 and 15.
7. Subjects will be instructed to call the research staff with any new complaints after initiating study drugs. Coordinator will assess patient's adverse events at any time during days 7 through 10 and at time of surgery or post-treatment research biopsy.
8. The coordinator will give the patient a drug diary on day 1 for completion and pick up the diary on day of surgery or post-treatment research biopsy.

9.0 MEASUREMENT OF EFFECT

The purpose of this study is to evaluate biomarker modulation following a short term administration of tamoxifen, anastrozole, or fulvestrant in women with primary operable ILC. A change in tumor measurements is not expected and is not an endpoint in this study.

10.0 PHARMACEUTICAL INFORMATION

10.1 Tamoxifen

Note: Please refer to package insert for complete prescribing and toxicity information.

10.1.1 Other Names

Nolvadex, tamoxifen citrate, NSC# 180973

10.1.2 Classification

Hormone antagonist (antiestrogen).

10.1.3 Mode of Action

Tamoxifen and its metabolites possess antiestrogenic activity due to their ability to compete with estradiol for binding to receptors in the cells of tumors that contain high amounts of estrogen receptors (such as breast cancer). The tamoxifen estrogen receptor complex is translocated from the cytoplasm of cancer cells to the nucleus where it reduces DNA synthesis and cellular responses to estrogen. Tamoxifen also displays mild estrogenic activity and induces secretion of transforming growth factor beta (TGF-beta), which has inhibitory effects on many types of epithelial cells.

10.1.4 Storage and Stability

Tamoxifen is stored at room temperature protected from light.

10.1.5 Administration

Tamoxifen is administered orally, at a dose of 20 mg.

10.1.6 Supply

Tamoxifen will be provided free of charge to subjects for the duration of participation in this study.

10.1.7 Incompatibilities

Tamoxifen is a potent inhibitor of hepatic cytochrome P450 mixed function oxidases (MFO). The effect of tamoxifen on the metabolism and excretion of other drugs requiring MFO for activation is unknown.

Phenobarbital decreased tamoxifen serum level significantly in one patient. Concomitant bromocriptine therapy has been shown to elevate serum tamoxifen levels. Tamoxifen may potentiate the anticoagulant effects of warfarin.10.1.8 Adverse Effects

Please see the Tamoxifen Package Insert for more details on the known precautions, warnings, and adverse reactions

10.2 Anastrozole

Note: Please refer to package insert for complete prescribing and toxicity information.

10.2.1 Other Names

Arimidex

10.2.2 Classification

Non-steroidal competitive inhibitor of the aromatase enzyme system.

10.2.3 Mode of Action

In post-menopausal women, estrogens are derived from the action of the aromatase enzyme which converts adrenal androgens to estrone and estradiol. Anastrozole is a nonsteroidal aromatase inhibitor. Anastrozole is highly potent and specific for aromatase, and represents the fourth generation of aromatase inhibitors.

10.2.4 Storage and Stability

Anastrozole is stored at controlled room temperature at 20 - 25°C.

10.2.5 Administration

Anastrozole is administered orally, at a dose of 1 mg, once daily, without regard to meals.

10.2.6 Supply

Anastrozole will be provided free of charge by AstraZeneca to subjects for the duration of participation in this study.

10.2.7 Incompatibilities

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

10.2.8 Adverse Effects

Please see the Anastrozole Package Insert for more details on the known precautions, warnings, and adverse reactions.

10.3 Fulvestrant

Note: Please refer to package insert for complete prescribing and toxicity information.

10.3.1 Other Names

ICI-182, 780; Faslodex

10.3.2 Classification

Fulvestrant is an estrogen receptor downregulator. One of its main differences from tamoxifen and the other SERMs is its lack of agonistic activity. It represents a new class of drug that binds, blocks and degrades the ER.

10.3.3 Mode of Action

Estrogen Receptor Antagonist.

10.3.4 Storage and Stability

Refrigerate, 2°-8°C (36°-46°F). To protect from light, store in the original carton until time of use.

10.3.5 Administration

Fulvestrant 500mg should be administered intramuscularly into the buttocks slowly as two 5mL injections, one in each buttock. A dose of 250mg is recommended in patients with moderate hepatic impairment.

10.3.6 Supply

Fulvestrant will be provided free of charge and is supplied as 50mg/ml injection in 5-mL syringes for intramuscular administration.

10.3.7 Incompatibilities

No known drug-drug interactions.

10.3.8 Adverse Effects

Please see the Fulvestrant Package Insert for more details on the known precautions, warnings, and adverse reactions

10.3 Drug Accountability

The research pharmacy at each institution will keep records for medication receipts, dispensation, and destruction. These procedures, including drug destruction and disposition at the completion of the study, will be pursuant to ICH/GCP guidelines and institutional policies. A drug diary will be given to the patients for completion and collected for submission to the Coordinating Center.

11.0 CORRELATIVE STUDIES

11.1 Tissue Samples

11.1.1 Tissue Sample Collection

Representative tumor samples will be collected from all patients as outlined below. Importantly, all tissue fixations will be performed using neutral buffered formalin only, to ensure compliance with Ki67 measurement guidelines (described below).

Pre-treatment research samples: ILC histology and HR status confirmation will occur at the local sites to determine patient eligibility based on their diagnostic clinical breast biopsy. After the patient is enrolled, they will undergo an optional but highly encouraged baseline research biopsy of their breast tumor for collection of paraffin and fresh frozen tumor tissue. If the patient declines the biopsy or it is not feasible, or if it is attempted but unsuccessful, then baseline tumor tissue will be collected from their archival clinical paraffin core biopsy. All study specimens will be transferred to the study team at Magee-Womens Hospital (MWH) to be reviewed by the central pathologist for determination of quantitative ER and PR, Ki67, E cadherin, and p120 staining and other planned analyses. Central pathology review is not necessary for eligibility/enrollment.

Post-treatment research samples: Following 21-27 days of neoadjuvant endocrine therapy, a post-treatment research core biopsy will be obtained from either the surgically excised breast tumor or from a second post-treatment research biopsy of the breast before any other neoadjuvant treatment is given. Samples will be formalin-fixed and paraffin-embedded using standard methods, and frozen tissue will be stored. As with the baseline research biopsy, the tissue from the post-treatment research core biopsy will also be analyzed centrally for the primary and secondary end points.

Note: Tissue samples will be collected regardless of delays or change in the schedule of study medication except for patients who withdraw consent; it will not be considered a protocol deviation if the preferred timelines for tissue collection above are not met. Also, radiologic guidance (e.g., ultrasound) may be used per institutional preference for collection of the core breast tumor biopsies. If any research specimen fails to yield adequate tumor tissue for analysis, samples will be requested from patient archival tissue

11.1.2 Tissue Sample Analyses

11.1.2.1 Immunohistochemistry (IHC)

Slides will be stained using commercially available monoclonal antibodies under the direction of local lead study pathologists for standard diagnostic markers including ER, PR, and HER2, which are done routinely. Confirmation of ILC histology for study enrollment will be performed locally.

After enrollment, diagnostic specimens will be dual-stained centrally for E-cadherin/p120. Ki67 and other biomarkers for primary and secondary end points will be performed centrally.

For the purposes of the study for enrollment, HR status of the invasive component must be documented on the diagnostic core biopsy locally, and HR assays will be considered positive if there are at least 1% positive tumor nuclei in the sample for ER and/or PR on testing in the presence of expected reactivity of internal (normal epithelial elements) and external controls as per

recent ASCO/ACP guidelines. Also for the purposes of study enrollment, ILC histology will be considered positive according to local enrolling site IHC assessment.

ER and PR IHC will be evaluated centrally by the H-score method. These markers will be assessed centrally at MWH for all study specimens (on both the mandatory baseline and post-treatment research biopsies).

Recently, the International Ki67 in Breast Cancer Working Group published guidelines for both performing and interpreting Ki67 analyses, in particular with regard to neoadjuvant treatment evaluation[47]. We will strictly adhere to these guidelines to assure that our measurements are analytically valid and that our results will be able to come assessed in the context of other recent and future breast cancer trials. These guidelines recommend specific fixation conditions, sample processing, antibody handling, and scoring methods. As such, all samples will be re-assessed centrally to ensure consistency.

Slides from baseline and post-treatment research samples may undergo additional immunohistochemical or protein analyses for targets of interest in future exploratory studies.

Note: Central pathology review is not necessary for eligibility/enrollment; these analyses will be performed on the research biopsy tissue for study purposes only.

11.1.2.2 Candidate Gene Expression Analysis

We will extract RNA from a 5-micron section of baseline and surgical FFPE tissue research tissue samples to measure the mRNA expression of ER (ESR1) and other candidate genes using the Nanostring nCounter platform[58]. This platform captures and individually counts mRNA transcripts using a color-coded hybridization strategy. Importantly, as this technology is amplification-independent, it is free of enzyme-derived biases and is highly amenable to the small nucleic acid fragment sizes typically obtained from FFPE tissues. Further, these assays are able to measure the expression of hundreds of genes using minimal input sample (~100ng RNA), preserving these valuable specimens while yielding high-quality data. In addition to ESR1, we will measure the expression of ~150 genes that have been identified using pre-clinical models to be estrogen-regulated genes in ILC that represent putative markers of endocrine response or resistance.

11.1.2.2 DNA methylation analysis

We will extract genomic DNA from a 5-micron section of baseline and surgical research FFPE tissue samples to evaluate DNA methylation status either as specific candidate genes (ESR1, etc) or using genome wide approaches. We will describe the baseline and change in ILC tissue and correlate these data with changes in Ki67.

11.1.2.3 DNA sequence variants analyses

We will extract genomic DNA from a 5-micron section of baseline and surgical research FFPE tissue samples to be assessed for DNA sequence variants (DSVs) associated with post-therapy changes in Ki67 in ILC. We will use state-of-the-art systems, such as Ion Torrent and exome/whole genome next-generation sequencing. To differentiate somatic from germine DSVs, we will compare identified variants from tumor tissue with matched blood specimens, as described below.

11.2 Blood Samples

11.2.1 Blood Sample Collection

A single 10 ml tube of blood will be collected from all patients on study for research purposes. As these samples will be used to assess germline DNA sequence variants, there will be no effect of treatment, and they may be collected at baseline or any time prior to the day of surgery or post-treatment research biopsy. We anticipate that blood samples will also allow for use in future exploratory analyses.

11.2.2 Blood Sample Analyses

Blood samples will be stored for future correlative analyses.

11.2.3 Assessing germline DNA sequence variation

Determining the role of DNA sequence variants (DSVs), including mutations, deletions, copy number aberration, etc., from malignant tissues requires 'normal', diploid reference genomes in order to exclude the effects of genetic polymorphisms during analyses. Blood collected during this study will provide genomic DNA to serve as matched normal samples for each ILC tissue sample. We will isolate DNA from buffy coat using standard methods. Isolated DNA is stable for long-term storage for future analyses. As any studies in to the role of DSVs in ILC tissues from this study will be limited by techniques amenable to FFPE tissue samples, the high-quality germline DNA isolated from blood samples will be adequate as matched controls for any future studies.

11.3 Leftover Samples

Any leftover study blood and tissue samples will be stored at **Magee-Womens Hospital of UPMC** or **the Magee- Womens Research Institute.**

11.4 Additional Information

The study coordinator will keep a log (separate logs will be kept for the blood and tissue samples) that includes the study number, a specimen serial number, the patient's name, time point in therapy, and the date and time that the sample was drawn. The sample will be labeled with a serial number only. The laboratory technician will keep a log with the specimen number, conditions, processing and storage information.

The laboratory investigators will be blinded to the subject identifiers and clinical data while generating the research data; additionally, the reported results will not disclose any unique patient identifiers.

Note: The correlative sample collection schedules outlined above are based on an ideal subject. The sample schedule should be followed as closely as is realistically possible; however, the schedule may be modified due to problems such as scheduling delays or conflicts (e.g., clinic closure, poor weather conditions, vacations, etc.).

11.5 Future Research

The study PI and collaborators have approval by the TBCRC to use all research bio-specimens collected during the conduct of this trial to address the research questions described in the protocol document. All

future use of residual or repository specimens collected in this trial for purposes not prospectively defined will require review and approval by the TBCRC according to its established policies, whether the specimens are stored in a central site or at a local institution in a virtualrepository.

Secondary use of bio-specimens for new endpoints must be submitted to the TBCRC Central Office for possible review by the TBCRC Correlative Science Review Committee.

12.0 ADVERSE EVENTS

12.1 General

This study will use the descriptions and grading scales found in the revised National Cancer Institute Common Terminology Criteria for Adverse Events (CTCAE) Version 4.0 for adverse event reporting that can be found at http://ctep.cancer.gov/reporting/ctc.html.

Information about all grade 3 or 4 adverse events (according to CTCAE Verions 4.0), whether volunteered by the subject, discovered by investigator questioning, or detected through physical examination, laboratory test or other means, will be collected, recorded, and followed as appropriate.

All adverse events experienced by subjects will be collected from the time of first dose of study medication, throughout the study and until the final study visit. Subjects continuing to experience toxicity after discontinuation of the study drug may be contacted every month for additional assessments until the toxicity has resolved or is deemed irreversible. Any adverse event experienced during or after the surgical procedure that the investigator feels is related to preoperative treatment will be captured.

12.2 Definitions

12.2.1 Adverse event (AE): Any undesirable sign, symptom or medical condition occurring after starting study drug (or therapy) even if the event is not considered to be related to thestudy.

Medical conditions/diseases present before starting study treatment are only considered adverse events if they worsen after starting study treatment (any procedures specified in the protocol). Adverse events occurring before starting study treatment but after signing the informed consent form will be recorded. Abnormal laboratory values or test results constitute adverse events only if they induce clinical signs or symptoms or require therapy.

12.2.2 Serious adverse event or reaction: Any untoward medical occurrence that at any dose: results in death, is immediately life-threatening, requires inpatient hospitalization or prolongation of existing hospitalization, results in persistent or significant disability/incapacity, is a congenital anomaly/birth defect, cancer, or overdose.

The definition of serious adverse event (experience) also includes *important medical event*. Medical and scientific judgment should be exercised in deciding whether expedited reporting is appropriate in other situations, such as important medical events that may not be immediately life-threatening or result in death or hospitalization but may jeopardize the patient or may require intervention to prevent one of the other outcomes listed in the definition above. These should also usually be considered serious. Examples of such events are intensive treatment in an emergency room or at

home for allergic bronchospasm; blood dyscrasias or convulsions that do not result in hospitalization; or development of drug dependency or drug abuse.

A hospitalization planned before the start of the study agent(s) and/or for a preexisting condition that has not worsened does not constitute a serious adverse event (e.g., elective hospitalization for breast reconstruction of the ipsilateral breast). A hospitalization for a social reason in the absence of an adverse event also does not meet the criteria for a serious adverse event.

Note: Any occurrence of pregnancy must also be reported in the same time frame as a serious adverse event.

12.2.3 Unexpected adverse event: An adverse event, which varies in nature, intensity or frequency from information on the investigational drug/agent provided in the Investigator's Brochure, package insert or safety reports. Any adverse event that is not included in the informed consent is considered "unexpected".

12.2.4 Expected (known) adverse event: An adverse event, which has been reported in the Investigator's Brochure. An adverse event is considered "expected", only if it is included in the informed consent document as a risk.

12.3 Relationship

The relationship of all adverse events and serious adverse events to study medication will be assessed by an investigator and assigned as follows:

12.3.1 Definitely: An adverse event that has a timely relationship to the administration of the investigational drug/agent, follows a known pattern of response, for which no alternative cause is present.

12.3.2 Probably: An adverse event, that has a timely relationship to the administration of the investigational drug/agent, follows a known pattern of response, but for which a potential alternative cause may be present.

12.3.3 Possibly: An adverse event, that has a timely relationship to the administration of the investigational drug/agent, follows no known pattern of response, but a potential alternative cause does not exist.

12.3.4 Unlikely: An adverse event that does not have a timely relationship to the administration of the investigational drug/agent, follows no known pattern of response, does not reappear or worsen after re-administration of the investigational drug/agent (if applicable), and for which there is evidence that it is related to a cause other than the investigational drug/agent.

12.3.5 Unrelated: An adverse event, for which there is evidence that it is definitely related to a cause other than the investigational drug/agent. In general, there is no timely relationship to the administration of the investigational drug/agent, or if there is a timely relationship, the event does not follow a known pattern of response, and there is an alternative cause.

12.4 Reporting Procedures

12.4.1 General

All adverse events will be captured on the appropriate study-specific case report forms (CRFs).

12.4.2 Serious Adverse Events

All serious adverse events, regardless of causality to study drug, will be reported to the Principal Investigator and/or the Study Coordinator at each institution, and also to the Coordinating Center.

All serious adverse events must be reported to the Coordinating Center within 1 business day after the investigator becomes aware of the event. Events should be reported using a MedWatch form (3500) as available on the FDA website (see link below).

Follow-up information must also be reported within 1 business day of receipt of the information by the investigator.

The Coordinating Center will disseminate information regarding serious adverse events to the participating sites within 5 days of review of the information by the Protocol Chair (or her designee in the event of extended absence) only in the case that the event(s) is believed to be related (i.e., possibly, probably, or definitely) related to the study medication. The Coordinating Center will be responsible for reporting of events to the FDA and supporters, as appropriate (outlined below).

12.4.3 Institutional Review Board

All adverse events (AEs) and serious adverse events (SAEs) will be reported to the IRB per current institutional standards. If an adverse event requires modification of the informed consent, these modifications will be provided to the IRB with the report of the adverse event. If an adverse event requires modification to the study protocol, these modifications will be provided to the IRB as soon as is possible.

12.4.4 Food and Drug Administration (FDA)

In this trial, unexpected AEs believed to be definitely, probably, or possibly related to the medications will be reported to the Food and Drug Administration. The coordinating center will be responsible for submitting AEs to the FDA for all participating sites via MedWatch (using the online form available athttps://www.accessdata.fda.gov/scripts/medwatch/ or by phone 1-800- FDA-1088.

12.4.5 Astra Zeneca

Astra Zeneca, Inc. will be provided with copies of all serious and/or unexpected adverse experiences, within two working days by the Coordinating Center. The FDA will also be provided with information, inclusive of above, of: (1) all serious, unexpected, adverse events, (2) any significant increase in the frequency of serious expected adverse events, and, (3) any significant increase in the frequency of therapeutic failures.

Any new information regarding any adverse experience must also be submitted to WorldwideProduct Safety within two working days by the Coordinating Center.

13.0 DATA AND SAFETY MONITORING

13.1 Data Management

All information will be collected on study-specific case report forms by the study staff at each institution. The necessary forms will be provided to each site by the Coordinating Center.

The completed forms will be forwarded to the Coordinating Center for central review and inclusion in the study dataset with relevant source documentation as outlined in the case report forms. The data submission schedule is as follows:

At the time of registration:

- Registration Forms
- Informed Consent Form (signed by the subject)
- Eligibility Checklist
- Source documents related to eligibility and randomization
- Research tissue samples with documentation

At time of surgery/post-treatment research biopsy:

- Adverse Forms
- Drug diary
- Study blood along with documentation
- Pertinent source documents

Final Assessment:

- AE form if patient has continuing AE after surgery
- Pertinent source documents

All study data will be reviewed for completeness and accuracy by the Protocol Chair or his/her designee. The Principal Investigator (or his/her designee) at each respective institution is responsible for review, and ensuring the completeness and accuracy, of the data generated by his/her institution.

13.2 Meetings

The Coordinating Center will schedule teleconferences to take place approximately monthly and will include the Protocol Chairs and Principal Investigators from each site. The following study team members involved with the conduct of the trial will be included as appropriate: study coordinators, data managers, research nurses, sub-investigators, collaborators (if applicable), and statistician.

During these meetings matters related to the following will be discussed: enrollment rate relative to expectation, characteristics of participants, retention of participants, adherence to protocol (potential or real protocol violations), validity and integrity of the data, shipment of serum and tissue samples, and progress of data for objectives.

A summary of the items discussed at each teleconference will be maintained by the Coordinating Center.

13.3 Monitoring

As above, all data will be sent to the Coordinating Center for central collation and review. Subject cases from sites can be chosen for a thorough review; in the event that a case is chosen for central review by the Coordinating Center, the participating site will be asked to provide copies of all source documents and to ensure that all data is current. There are no formal on-site evaluations planned by the Coordinating Center.

Investigator/Sub-investigators, regulatory, CRS management, clinical research coordinators, clinical research associates, data managers, and clinic staff meet monthly in the Breast Center Data Safety Monitoring Board (DSMB) to review and discuss study data to include, but not limited to, the following:

- serious adverse events
- subject safety issues
- recruitment issues
- accrual
- protocol deviations
- breaches of confidentiality

All toxicities encountered during the study will be evaluated on an ongoing basis according to the NCI Common Toxicity Criteria version 4.0. All study treatment associated adverse events that are both serious and unexpected will be reported to the IRB. Any modifications necessary to ensure subject safety and decisions to continue, or close, the trial to accrual are also discussed during these meetings. If any literature becomes available which changes the risk/benefit ratio or suggests that conducting the trial is no longer ethical, the IRB will be notified in the form of an Unanticipated Problem submission and the study may be terminated. The Breast Center DSMB also reviews protocol deviations and unanticipated problems.

All study data reviewed and discussed during these meetings will be kept confidential. Any breach in subject confidentiality will be reported to the IRB in the form of an Unanticipated Problem submission. The summaries of these meetings are forwarded to the UPCI DSMC which also meets monthly following a designated format.

For all research protocols, there will be a commitment to comply with the IRB's policies for reporting unanticipated problems involving risk to subjects or others (including adverse events). DSMC progress reports, to include a summary of all serious adverse events and modifications, and approval will be submitted to the IRB at the time of renewal.

Both the UPCI DSMC as well as the individual disease center DSMB have the authority to suspend accrual or further investigate treatment on any trial based on information discussed at these meetings.

14.0 ADMINISTRATIVE PROCEDURES

14.1 Protocol Amendments

Any changes to the protocol will be made in the form of an amendment and must be approved by the IRB before implementation. The Protocol Chair (or her designee) is responsible for the coordination and development of all protocol amendments and will disseminate this information to the participating centers. Participating site may not make any changes to the protocol without approval of the Coordinating Center and the Coordinating Center's IRB approval.

14.2 Informed Consent

An investigator will explain to each subject the nature of the study, its purpose, procedures involved, expected duration, potential risks and benefits. Each subject will be informed that participation in the study is voluntary and that she may withdraw from the study at any time, and that withdrawal of consent will not affect her subsequent medical treatment. This informed consent will be given by means of a standard written statement and will be submitted for IRB approval prior to use. No patient will enter the study before her informed consent has been obtained. In accordance with the Health Information Portability and Accountability Act (HIPAA), the written informed consent document (or a separate document to be given in conjunction with the consent document) will include a subject authorization to release medical information to the study sponsor and supporting agencies and/or allow these bodies, a regulatory authority, or Institutional Review Board access to subjects' medical information that includes all hospital records relevant to the study, including subjects' medical history.

14.3 Ethics and Good Clinical Practice

This study must be carried out in compliance with the protocol and Good Clinical Practice, as described in:

1. ICH Harmonized Tripartite Guidelines for Good Clinical Practice 1996.
2. US 21 Code of Federal Regulations dealing with clinical studies (including parts 50 and 56 concerning informed consent and IRB regulations).
3. Declaration of Helsinki, concerning medical research in humans (Recommendations Guiding Physicians in Biomedical Research Involving Human Subjects, Helsinki 1964, amended Tokyo 1975, Venice 1983, Hong Kong 1989, Somerset West 1996).

The investigator agrees to adhere to the instructions and procedures described in it and thereby to adhere to the principles of Good Clinical Practice that it conforms to.

14.4 Regulatory Authorities

14.4.1 Institutional Review Board

Information regarding study conduct and progress will be reported to the Institutional Review Board (IRB) per the current institutional standards of each participating center.

14.4.2 Food and Drug Administration (FDA)

This trial does not involve an Investigational New Drug (IND) application and no reporting is required with regards to the clinical trial outlined at this time.

15.0 COORDINATING CENTER & SITE REPONSIBILITIES

15.1 Protocol Chair

The Protocol Chair is responsible for performing the following tasks:

- Coordinating, developing, submitting, and obtaining approval for the protocol as well as its subsequent amendments.
- The protocol chair (or a designee in their absence) will be responsible for a final review of eligibility of subjects to be registered

- Assuring that all participating institutions are using the correct version of the protocol.
- Taking responsibility for the overall conduct of the study at all participating institutions andfor monitoring the progress of the study.
- Reviewing and ensuring reporting of Serious Adverse Events (SAE).
- Reviewing data from all sites.

15.2 Coordinating Center

The Coordinating Center is responsible for performing the following tasks:

- Ensuring that IRB approval has been obtained at each participating site prior to the first patient registration at that site, and maintaining copies of IRB approvals from each site.
- Managing central patient registration.
- Collecting and compiling data from each site.
- Establishing procedures for documentation, reporting, and submitting of AE's and SAE's to the Protocol Chair, and all applicable parties.
- Facilitating audits by securing selected source documents and research records from participating sites for audit.

15.3 Participating Sites

Participating sites are responsible for performing the following tasks:

- Following the protocol as written, and the guidelines of Good Clinical Practice (GCP).
- Submitting data to the Coordinating Center.
- Registering all patients with the Coordinating Center by submitting patient registration form, and signed informed consent promptly.
- Providing sufficient experienced clinical and administrative staff and adequate facilities and equipment to conduct a collaborative trial according to the protocol.
- Maintaining regulatory binders on site and providing copies of all required documents to the Coordinating Center.
- Collecting and submitting data according to the schedule specified by the protocol.

Additional information for participating sites:

15.3.1 Staffing

The participating sites will provide experienced staff, and adequate equipment and facilities to support this clinical trial. The participating sites will also be responsible for research staff training in computer applications, human subjects research, and HIPAA compliance, as well as the continuing education in these areas as required by local institutional standards.

15.3.2 Documentation

Each participating site is responsible for submitting copies of all relevant regulatory documentation to the Coordinating Center. The required documents include, but are not limited to the following: local IRB approvals (i.e., protocol, consent form, amendments, patient brochures and recruitment material, etc.), IRB membership rosters, summary of unanticipated problems or protocol deviations, and documentation of expertise of the investigators. The Coordinating Center will provide each participating site with a comprehensive list of the necessary documents. It is the responsibility of the participating sites to maintain copies of all documentation submitted to the Coordinating Center.

15.3.3 Confidentiality

All unpublished information that the Coordinating Center gives to the investigator shall be kept confidential and shall not be published or disclosed to a third party without the prior written consent of the Protocol Chair (or her designee).

15.3.4 Record Retention

Following closure of the study, each participating center will maintain a copy of all site study records in a safe and secure location. The Coordinating Center will inform the investigator at each site at such time that the records may be destroyed.

15.3.5 Publication

It is understood that any manuscript or releases resulting from the collaborative research will be circulated to all participating sites prior to submission for publication or presentation. The Protocol Chair will be the final arbiter of the manuscript content.

15.3.6 Additional Information

Each participating site is responsible for submitting additional information as requested by the Protocol Chair (or her designee). The Coordinating Center may terminate the study at a participating site in the event that these conditions are not followed.

16.0 Statistical Considerations

16.1 Analysis of Primary Endpoint

Ki67 proliferative index is measured as the percent of positively staining cells. Given the log-normal distribution of the Ki67 measurements generally observed in this population[59], values will be log-transformed for statistical analysis. Therefore, the primary endpoint will be the change in Ki67 at Day 21-24 compared to baseline, given by log ($Ki67_{Day\ 21}/Ki67_{BL}$). For descriptive purposes, summary statistics and

corresponding 95% confidence intervals for BL, Day 21 and percent change from baseline will be reported within study arm on the original scale with appropriate 95% confidence intervals.

Comparisons across study arms will be made using a general linear model adjusting for institutional effect. Pairwise comparisons between treatment arms will use the Tukey-Kramer test to control for multiple comparisons. If distributional assumptions are violated, non-parametric methods will be used. All tests will be two-sided with a nominal 0.05 significance level. Estimates and corresponding 95% confidence intervals will be reported for all summary statistics.

The primary analysis will be conducted using available biomarker values, with multiple imputation considered if >20% of data are missing at any timepoint for any treatment group. Data will be missing if samples are not collected or if samples are not analyzable. If patients discontinue study therapy but do not withdraw consent from study follow-up, post-treatment research biopsy tissue may still be collected.

16.2 Analysis of Secondary Endpoints:

16.2.1 Ki67 (log2-transformed) following endocrine therapy will be compared between treatment arms, using the same analysis approach as for the primary endpoint.

16.2.2 and 16.2.3 ER and PR protein expression will be measured by IHC using a semi-quantitative H-Score[60]. Summary statistics and corresponding 95% confidence intervals will be reported within each treatment arm. Change from baseline comparisons (e.g., $\log_2(ER_{Day\ 21}/ER_{BL})$) across treatment arms will be made using a general linear model adjusting for institutional effect.

16.2.4 mRNA expression of ER and other candidate genes will be measured as counts using NanoString nCounter technology. Raw counts will be normalized first to the positive control set, followed by adjustment to the background controls and then normalized to the reference set of genes. Quality control diagnostics regarding sample content and normalization parameters will be performed and statistical methods will be applied to the log-transformed data. Summary statistics will be reported within each treatment arm and changes from baseline will be compared across treatment arms for each candidate gene controlling the false discovery rate[61].

16.2.5 Associations between changes in Ki67 and protein and mRNA expression will be made onthe quantitative measurements using nonparametric correlation estimates.

16.3 Analysis of Exploratory Endpoints

Descriptive statistics with appropriate confidence intervals will be reported for exploratory objectives regarding DNA methylation, sequence variations and signaling pathways.

16.4 Sample Size and Accrual Rate:

Preliminary data are not available to estimate treatment effects specifically in ILC tumors, but published estimates from similar populations (i.e. postmenopausal patients with ER-positive disease) will be used to aid in sample size calculations. The IMPACT trial reported mean percent reduction in Ki67 of 60% (95% CI: 48%, 68%) and 76% (95% CI: 68%, 82%) in the tamoxifen and anastozole arms, respectively, at 2 weeks compared to baseline[45]. Data obtained from Clarke et. al[62], demonstrated a similar reduction in Ki67 in the tamoxifen treated arm for ILC, ER+ subset of subjects, although results were not published for this subset of subjects. Results from two Phase II multicenter randomized neoadjuvant trials of Fulvestrant

(500mg) demonstrate a mean reduction in Ki67 of 75% after 2 weeks of treatment [43] and a mean reduction of 79% (95% CI: 71%, 85%) after 4 weeks of treatment [63] in ER positive invasive breast carcinomas in postmenopausal women. However, based on preclinical observations by the Oesterreich laboratory, described in Section 5, we hypothesize that fulvestrant will produce greater decreases in Ki67 than tamoxifen or an aromatase inhibitor in ILC tumors.

A simulation study was performed using an estimated mean (16.3) and standard deviation (19.3) of Ki67 at time of diagnosis from a random sample (n=32) of hormone receptor positive, ILC specific tumors from women at least 50 years of age at our institution. For each treatment arm, Baseline and Day 21 Ki67 values were randomly generated from a truncated multivariate normal distribution to obtain correlated Ki67 values between 0 and 100. The observed Ki67 estimates above were used for the baseline mean and standard deviation parameters for all treatment arms and effect sizes of 60%, 75% and 85% reduction of mean and standard deviation were fixed for the Week 21 parameters in the tamoxifen, anastrozole and fulvestrant arms, respectively. Simulated Ki67 values were then log-transformed and differences were compared across treatment arms using the Kruskal-Wallis test for the global null hypothesis followed by pairwise Wilcoxon Rank Sum tests to compare treatment arms. A non-parametric test was used to compare the endpoint log ($Ki67_{Day\ 21}/Ki67_{BL}$) because small values for $Ki67_{BL}$ occasionally produced observations in the tail of the distribution. A Type I Error rate of 0.05 was verified simulating under the null hypothesis of no change from baseline. Simulating under the alternative hypothesis, sample sizes of n=45 per arm provided at least 80% power to reject the global null hypothesis of no change and at least 80% power to reject each pairwise comparison. Therefore, groups of size n=67 will provide sufficient power allowing for 33% attrition. Actual standard deviations and within subject correlation will effect power, however, parameter estimates in the simulations were based on observed data when possible.

A total of 201 subjects will be randomized to one of three treatment arms.

16.5 Randomization:

Subjects will be randomized to treatment arm, stratified by institution, in random blocks of size 6 with an allocation ratio of 1:1:1, using the password-protected, web-based University of Pittsburgh Cancer Institute Treatment Randomization System (Randomizer). Prior to site initiation, the site coordinator will inform the Study Coordinator of the names and e-mail addresses of the site personnel responsible for randomization and the site pharmacist. The Study Coordinator will enter these personnel and their roles in Randomizer and reply with instructions for the use of the system.

16.6 Reporting and Exclusions

16.5.1 Evaluation of toxicity

All patients will be evaluable for toxicity from the time of their first dose of tamoxifen, anastrozole, or fulvestrant. As noted in section 12, grade 3 or higher adverse events (CTCAE Version 4.0) will be recorded. The type and severity of adverse events will be summarized for all patients and stratified by treatment arm.

16.5.2 Evaluation of response

The primary analysis will be conducted on all available paired samples for enrolled patients. Secondary analyses may include analysis of subsets (e.g., excluding patients who did not comply with treatment) or sensitivity analyses accounting for missing biomarker data.

16.5.3 Discontinuation and withdrawal of subjects

- All patients who initiate protocol treatment will be included in the overall evaluation of response (intent-to-treat analysis). All reasons for discontinuation of treatment should be documented clearly in the medical record. Note: Patients who sign a consent form, but do not initiate study drug will be replaced.

- Non-compliance with the study protocol; including, but not limited to not attending the majority of scheduled visits. The PI will determine when non-compliance should lead to removal from study. Note: The patients will still be included in the overall evaluation of response (intent-to-treatanalysis).

- Unacceptable major toxicity. Note: The patients will still be included in the overall evaluation of response (intent-to-treat analysis).

- At patient's own request. Note: The reason for discontinuation from the study must be documented. The patients will be included in the overall evaluation of response (intent- to-treat analysis) if any protocol therapy was administered prior to withdrawal.

- The study is closed for any reason (e.g. new information shows that the patient's welfare would be at risk if she continued study treatment).

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APPENDIX A: American Joint Committee on Cancer Staging

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

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

APPENDIX A (CONTINUED): American Joint Committee on Cancer Staging

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

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

APPENDIX A (CONTINUED): American Joint Committee on Cancer Staging

Stage Grouping	**T**	**N**	**M**
0	Tis	N0	M0
I	T1	N0	M0
IIA	mT0	N1	M0
	T1	N1	M0
	T2	N0	M0
IIB	T2	N1	M0
	T3	N0	M0
IIIA	T0	N2	M0
	T1	N2	M0
	T2	N2	M0
	T3	N1	M0
	T3	N2	M0
IIIB	T4	N0	M0
	T4	N1	M0
	T4	N2	M0
IIIC	Any T	N3	M0
IV	Any T	Any N	M1

APPENDIX B: MENOPAUSAL STATUS

The following criteria will be used for this protocol to define *postmenopausal:*

- Age ≥56 or older with no spontaneous menses for at least 12 months prior to study entry; or
- Age <56 or younger with no spontaneous menses for at least 12 months prior to study entry (e.g., spontaneous or secondary to hysterectomy) and with a documented estradiol level in the postmenopausal range according to local institutional/laboratory standard; or
- Documented bilateral oophorectomy.

Women failing to meet one of these criteria will be classified as *pre-menopausal*.

APPENDIX C: Performance Status Criteria

Score	Definition	Karnofsky Equivalent
0	Asymptomatic	100
1	Symptomatic, fully ambulatory	80 – 90
2	Symptomatic, in bed less than 50% of day	60 – 70
3	Symptomatic, in bed more than 50% of day, but not bedridden	40 – 50
4	Bedridden	20 – 30

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APPENDIX D: DRUG DIARY

Patient Name:			Patient Number:
Drug Name:			Drug Dosage:
Day	Date: Month/Day/Yr	Time of dose	Use the space below to make notes about things you would like to tell your doctor (examples: missing a dose, unusual symptoms, etc).
1	/ /20____		
2	/ /20____		
3	/ /20____		
4	/ /20____		
5	/ /20____		
6	/ /20____		
7	/ /20____		
8	/ /20____		
9	/ /20____		
10	/ /20____		
11	/ /20____		
12	/ /20____		
13	/ /20____		
14	/ /20____		
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24	/ /20____		