Protocol Title: An Exploratory Study of Neoadjuvant Endocrine Therapy in Hormone Receptor-Positive HER2-Negative Node-Negative Breast Cancer Patients to Assess Responses and Mechanisms of Endocrine Resistance

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MEDICAL COLLEGE OF WISCONSIN
CANCER CENTER

CLINICAL STUDY PROTOCOL

An Exploratory Study of Neoadjuvant Endocrine Therapy in Hormone Receptor-Positive HER2-Negative Node-Negative Breast Cancer Patients to Assess Responses and Mechanisms of Endocrine Resistance

Lubna Chaudhary, MD, MS

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Version 2 – 10/21/16

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PROTOCOL SUMMARY

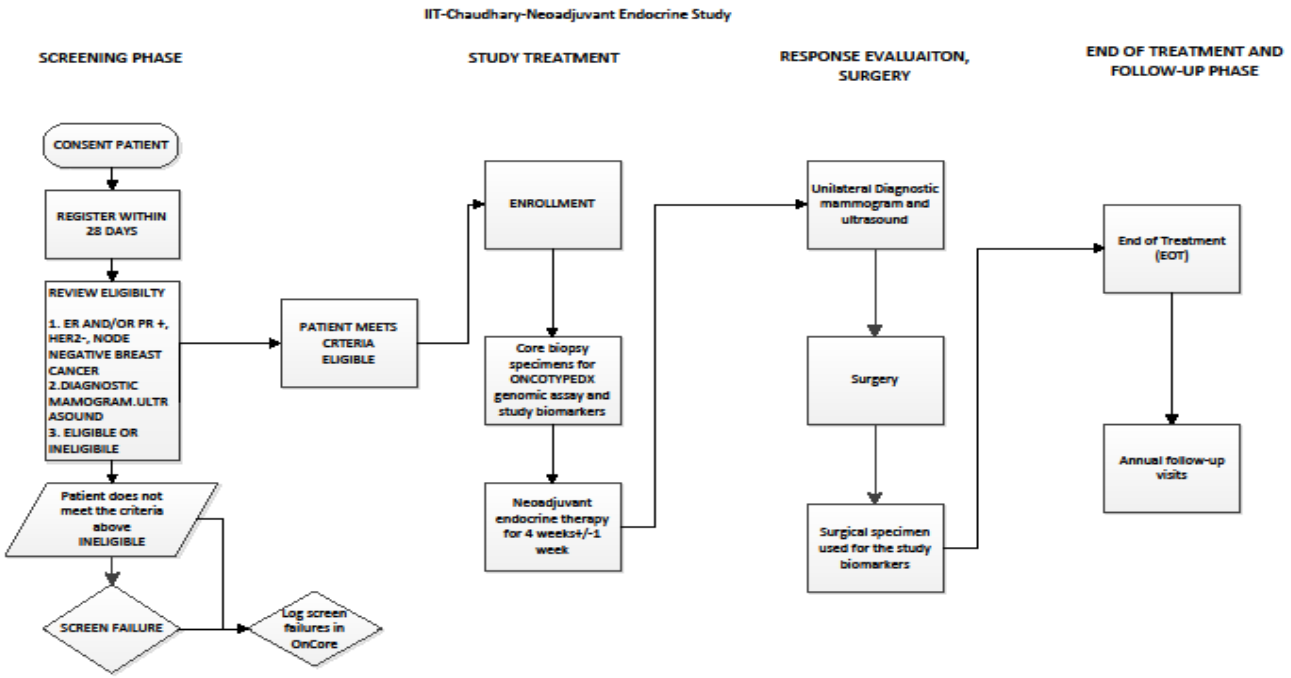
Title	An Exploratory Study of Neoadjuvant Endocrine Therapy in Hormone Receptor-Positive HER2-negative Node-Negative Breast Cancer Patients to Assess Responses and Mechanisms of Endocrine Resistance
Protocol Number	FP00011093; PRO 30178.
IND Sponsor	N/A
Principal Investigator	Lubna Chaudhary, MD, MS
Study Sites	St. Joseph's Hospital, West Bend Community Memorial Hospital, Dr. Chaudhary Froedtert Memorial Lutheran Hospital
Clinical Trial Phase	Exploratory
Study Disease	Hormone receptor-positive (HR+), HER2-negative (Her2-), node-negative pre and postmenopausal breast cancer patients.
Study Rationale	Patients with HR+ HER2- node-negative breast cancers generally undergo surgical resection upfront, followed by adjuvant chemotherapy, if needed, in addition to adjuvant endocrine therapy. Because endocrine therapy is primarily delivered in the postoperative setting, the ability to assess the tumor response to this treatment modality is lost and very difficult to assess. This study offers the unique opportunity to assess the responsiveness of breast tumors to endocrine therapy while the tumors are still *in vivo* by treating patients with endocrine therapy before surgery and assessing molecular changes with treatment. By comparing pre- and post-treatment levels of molecular markers in individual tumors, we expect to identify predictors of responsiveness to existing agents and identify new candidate therapeutic targets.
Primary Objectives	Our primary objective is to determine the frequency of increased **HER family of receptor tyrosine kinases** protein expression in tumors, following treatment with neoadjuvant endocrine therapy and their correlation with Ki-67 tumor responses. We will measure cancer cell protein levels of growth factor receptors of the HER family before and after neoadjuvant endocrine therapy. The data will be used to inform a future randomized trial of combined endocrine and the most promising anti-HER targeted therapy.
Secondary Objectives	- To determine changes in additional selected molecular markers (CK5+/ER- progenitor cells, PD-L1, PD-L2, BTK, iron-related proteins) and correlate with KI-67 responsiveness to endocrine therapy in our pre- and post-treatment tumor specimens.

	- Assess changes in tumor size on radiographic images with neoadjuvant endocrine therapy and correlate with changes in biomarkers and Ki-67.
Endpoints	Primary endpoints are: - Change in cancer cell protein levels of HER family members **(HER1-4)** with neoadjuvant endocrine therapy and association with Ki-67. We hypothesize that the expression of one or more HER proteins (HER1-4) will be upregulated in at least 50% of tumors after four weeks of neoadjuvant endocrine therapy and this upregulation will correlate with Ki-67 expressed in more than 10% of cancer cells. Secondary end points are: - Determine whether an increased proportion of **therapy-resistant CK5+ progenitor cells** are negatively correlated with Ki-67 response and tumor volume reduction, in response to neoadjuvant endocrine therapy. - Assess changes in the **% expression of ER and PR** in pre- and post-treatment breast cancer tumor specimens and their correlations with Ki-67 and radiographic responses. - Assess changes in **BTK protein expression** with neoadjuvant endocrine therapy and correlation with responses. - Changes in **iron-related protein expression, i.e., transferrin, ferritin, ferroportin and ribonucleotide reductase** with neoadjuvant endocrine therapy and correlation with responses. - Changes in **PD-L1 and PD-L2 protein expression** with neoadjuvant endocrine therapy and correlation with responses. - Assess **changes in tumor size on radiographic images** with neoadjuvant endocrine therapy and correlate with changes in biomarkers and Ki-67. Chart review planned at about five years from completion of study to assess patient outcomes, i.e., ipsilateral, contralateral or distant recurrences.
Study Design	Exploratory study.
Study Agent/ Intervention Description	This is an exploratory interventional study that initiates standard-of-care anti-estrogen treatment preoperatively for four weeks, +/- 1 week.
Number of Subjects	37
Subject Participation Duration	Patients to be treated C1D1 for four weeks +/- 1 week.

Duration of Follow up	Within 30 days after completion of study treatment
Long term Follow up	As standard of care, patients will be followed by oncology provider(s) at least annually from surgery (+/- 3 months). Cancer outcomes up to five years post-treatment will be collected by chart review.
Estimated Time to Complete Enrollment:	2 years
Statistical Methodology:	We hypothesize that expression of one or more HER proteins (HER1-4) will be upregulated in at least 50% of tumors after four weeks of neoadjuvant endocrine therapy and this upregulation will correlate with Ki-67 expressed in more than 10% of cancer cells, as compared to upregulation in 30% or less of tumors without the therapy. The change in Ki-67 expression will be dichotomized, as a categorical variable with ≥10% increase coded as high and <10% coded as low. - For the primary outcome: An exact binomial one sample test of proportions will be used for to test the hypothesis that proportion of upregulated HER proteins will be at least 50% after therapy versus the null hypothesis that the proportion of upregulated HER proteins is not greater than 30%. - For analyzing - the differences in proportion of upregulated HER proteins between the high difference in Ki-67 and low difference in Ki-67 groups, two sample tests of proportions will be used. - Similarly, appropriate two sample tests will be used to test differences in biomarkers between the high difference in Ki-67 and low difference in Ki-67 groups. We will use the appropriate kind of regression instead of two sample tests, to test differences in biomarkers between the high difference in Ki-67 and low difference in Ki-67groups if we need to adjust for covariates. - Additionally, regression analysis will also be done to evaluate the changes in the biomarkers and their association with patient specific covariates, such as age, race and other relevant predictors of interest. Radiographic responses will be assessed by WHO criteria. We will use logistic regression (and other generalized linear models as appropriate) to measure association between the response rates and patient specific covariates such as age, race and other relevant predictors of interest.

	To achieve at least 80% power at significance level of 0.05, when testing the one sided one sample hypothesis that the proportion of tumors with HER protein upregulation with neoadjuvant endocrine therapy is at least 50% versus the null hypothesis that the proportion is no larger than 30%, **the minimum number of patients required is 37.**
Safety Assessments	- Given the short nature of the treatment regimen (3-5 weeks), and expected low grades of toxicity, it is considered highly unlikely that a large percentage of patients will experience adverse events (AEs) from the endocrine treatment. - Patients would be assessed at beginning and end of treatment with neoadjuvant endocrine therapy at their follow-up visit within 30 days after completion of study treatment. - This study will be reviewed by the Medical College of Wisconsin Cancer Center Data and Safety Monitoring Committee (MCWCC DSMC).

SCHEMA


IIT-Chaudhary-Neoadjuvant Endocrine Study
SCREENING PHASE
STUDY TREATMENT
RESPONSE EVALUAITON, SURGERY
END OF TREATMENT AND FOLLOW-UP PHASE
CONSENT PATIENT
REGISTER WITHIN 28 DAYS
REVIEW ELIGIBILTY
1. ER AND/OR PR +, HER2-, NODE NEGATIVE BREAST CANCER
2.DIAGNOSTIC MAMOGRAM.ULTRASOUND
3. ELIGIBLE OR INELIGIBILE
PATIENT MEETS CRTERIA ELIGIBLE
ENROLLMENT
Core biopsy specimens for ONCOTYPEDX genomic assay and study biomarkers
Neoadjuvant endocrine therapy for 4 weeks+/-1 week
Unilateral Diagnostic mammogram and ultrasound
Surgery
Surgical specimen used for the study biomarkers
End of Treatment (EOT)
Annual follow-up visits
Patient does not meet the criteria above INELIGIBLE
SCREEN FAILURE
Log screen failures in OnCore

STUDY CALENDAR

Period / Procedure	Screening[1,2]	Neoadjuvant Endocrine Therapy Treatment[9]				Surgery	End of treatment visit[10]	Annual Follow up[11]
Study Day/ Visit Day		**Week 1[2]**	**Week 2**	**Week 3**	**Week 4**			
Informed Consent	X							
Adverse Event Assessment	X						X	
Current Medications	X						X	
Treatment / Drug Administration								
Aromatase Inhibitor OR Tamoxifen		X	X	X	X			
Calendar Collection							X	
Imaging								
Unilateral diagnostic mammogram and ultrasound[3]	X					X		
Clinical Procedures								
Physical Exam	X	X					X	
Vital Signs	X	X					X	
Medical History	X	X					X	
Performance Status	X	X					X	
Recurrence Status								X
Laboratory Procedures								
CBC w/ Diff[4]	X							
Blood Chemistry[5]	X							
Fasting Lipid Panel[6]	X							
Pregnancy Test (HCG)[7]	X							
Study Biomarkers[8]	X					X		

1. A patient can be screened and have status of week one on the same day.
2. Screening procedures and drug administration to be done within 28 days of enrollment.

3. Unilateral diagnostic mammogram and ultrasound to be done within 60 days prior to enrollment and within 5 days prior to surgery.
4. Including CBC with differential and platelet count.
5. Including alkaline phosphatase, ALT/AST, total bilirubin, calcium, phosphorus, creatinine, potassium, sodium, chloride, and bicarbonate.
6. Fasting lipid panel to be done once at screening; lipid panel may be performed within the last 12 months.
    - This project does not ask Froedtert to bill routine care costs to patient insurance but does ask Froedtert to invoice MCW for the following services:
        - Lipid panel if it needs to be repeated and not obtained as SOC prior to study participation consideration
7. For premenopausal women only.
8. Study biomarkers: to be collected on initial core biopsy and on surgical tumor specimens, as described in **Table 1**. Details on handling and preparation of tissue specimen and slides for study evaluations are explained in detail below in section **Pathologic Evaluation and Handling of Tissue Specimens**.
9. The patient would be treated with endocrine therapy in a neoadjuvant setting for four weeks (+/- 1 week), with dosing continuing until surgery (+/- 2 days).
10. End of treatment visit to be completed within 30 days of surgery.
11. Long term follow ups are to be done annually (+/- 3 months) from surgery up to 5 years.

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I. BACKGROUND:

Luminal breast cancers, as defined by estrogen receptor (ER) and/or progesterone receptor (PR) expression, account for 75% of all newly diagnosed breast cancers (BC). Deprivation of estrogen signaling through endocrine-targeted therapy has become the mainstay of treatment in hormone receptor (HR)-positive disease. Anti-estrogen therapy, also known as endocrine therapy, provides significant benefit to patients in terms of decreasing the risk of recurrence, as well as improving survival. Endocrine therapy is also well tolerated with relatively low toxicity. Unfortunately, metastatic recurrences occur in about 20–25% of patients and remain a major cause of BC mortality, representing a significant issue for optimal clinical management.[1] Resistance to endocrine therapy is a serious concern and it is extremely important to further understand and characterize the underlying molecular features of resistance for more accurate response prediction and for selecting the optimal clinical management. Several mechanisms and pathways have been explored in the recent years in an attempt to understand the complexity of development of endocrine resistance, however, it is unclear if altered levels and changes in tumor biomarkers are predictive of aggressiveness of HR+ breast cancers and development of endocrine resistance.

Patients with **HR+ HER2-negative node-negative breast cancer** generally undergo surgical resection upfront, followed by adjuvant chemotherapy, if indicated, in addition to adjuvant endocrine therapy. The introduction of gene-expression profiling technologies has been an important step toward understanding the distinct molecular subtypes of breast cancer and their prognostic importance.[2] It is unclear whether gene-expression profiling tests currently approved to predict the benefit of chemotherapy may be of use to determine if patients more likely to experience resistance to endocrine therapy. OncotypeDx assay is a validated genomic assay routinely used in clinical practice to assess risk of recurrence and benefit of adjuvant chemotherapy.

Endocrine therapy is mostly delivered in the adjuvant setting. However, in some cases, endocrine therapy is given in a neoadjuvant setting (treatment before surgery) to reduce tumor size and simplify surgery. Investigations involving adjuvant treatment can be challenging because the primary tumor has been surgically removed and is thus not available for on-treatment molecular analysis. Furthermore, long-term follow-up is required for survival and disease recurrence outcomes, which can be difficult to interpret, as recurrence may be the result of the inherent cancer aggressiveness, rather than acquired resistance to therapy. Additionally, a large sample size is required for such studies to produce statistically meaningful results and data collection on relevant outcomes takes considerable time and effort to acquire.[1]

Advantages of the Neoadjuvant Treatment Approach

The neoadjuvant setting presents a number of advantages for investigating the characteristics of tumor responsiveness and resistance. The primary tumor remains in place during treatment and clinical response can be determined by physical exam, as well as with imaging techniques. Furthermore, tumors can be sampled before and after neoadjuvant therapy, allowing for assessment of changes in protein or gene transcript

levels with treatment. These data can be then be correlated to clinical response, allowing for a dynamic comparison of clinical and molecular responses in both responsive and resistant tumors.[1,3,4,5]

Neoadjuvant endocrine therapy is a safe and effective strategy for treatment and response assessment in both pre- and postmenopausal women with HR+ HER2 negative disease. Most of the randomized clinical trials comparing different endocrine agents in neoadjuvant setting have involved patients with locally advanced breast cancer (LABC) and the duration of treatment has ranged anywhere from two weeks to four months, with responses as early as 10 to 14 days. Aromatase inhibitors have shown increased efficacy compared with tamoxifen in postmenopausal breast cancer patients, whereas tamoxifen alone or with ovarian function suppression are standard treatment options for premenopausal women with HR+ breast cancer.[6] The P024 trial showed that neoadjuvant treatment with letrozole is superior to tamoxifen in terms of clinical response rate (55% versus 36%, P = 0.001) and breast conservation rate (45% versus 35%, P = 0.022) in postmenopausal women with LABC.[7] The IMPACT trial treated 330 postmenopausal women with LABC with anastrozole, tamoxifen or a combination of tamoxifen and anastrozole for 12 weeks before breast surgery and found no significant differences in objective response rates between the three arms (37%, 36%, and 39% respectively, p=0.23).[8] The STAGE trial showed improved objective response rates, in addition to increased rates of breast conservation surgery in premenopausal women with 24 weeks of neoadjuvant anastrozole, combined with goserelin, as compared with tamoxifen combined with goserelin.[9] ACOSOG-Z1031 showed no significant difference in clinical responses or surgical outcomes between the three commonly used aromatase inhibitors, exemestane, letrozole and anastrozole, in postmenopausal women.[10]

Given the challenges involved in understanding molecular changes predictive of tumor responsiveness and development of endocrine resistance with adjuvant endocrine treatment in **HR+ HER2-negative breast cancer** patients, **neoadjuvant endocrine therapy** in this patient population is a safe and innovative approach to assess changes in therapy-relevant tumor biomarkers with treatment and correlated those with changed with tumor proliferation and responses. Emerging analytical platforms now become available for previously unachievable evaluations of expression patterns in cancer cells. Novel marker data can then be correlated with clinical responses, allowing for a dynamic comparison of basal pretreatment levels and post-treatment molecular changes in both responsive and resistant tumors. We believe that this may result in powerful biomarkers of responses to endocrine therapy.

We propose an exploratory interventional study to investigate changes in HER-protein expression along with other biomarkers of interest and correlations with tumor cell proliferation and responses in patients with HR+ HER2 negative early stage breast cancer treated with neoadjuvant endocrine therapy. By comparing pre- and post-treatment levels of molecular markers in individual tumors, we expect to identify predictors of responsiveness to existing agents, in addition to new candidate therapeutic targets. Insight gained from this exploratory study is expected to form the basis for a

phase II randomized trial of a combination of endocrine therapy with a second targeted drug for selected patients.

Mechanisms of Endocrine Resistance and Biomarkers of Interest

Endocrine resistance involves both ERα-independent[11,12] and ERα-dependent mechanisms[13,14] that remain to be fully resolved. These mechanisms may differ between breast cancer subtypes. Tumor marker-based classification of HR+ breast cancers into therapy-relevant subtypes is an ongoing effort.

HER2/EGFR Family of Receptor Tyrosine Kinases (RTKs) Overexpression

Resistance to endocrine therapy is common in HR+ breast cancers that **overexpress HER2.**[15] Many studies have reported cross-talk between ER and receptor tyrosine kinases (RTKs), such as HER1 and HER2, which are members of the human epidermal growth factor receptor (HER/EGFR/ERBB) family.[16,17] Overexpression of these receptors in a subset of ER+ breast cancers suggests that tyrosine kinase signaling drives proliferation and evasion of apoptosis in these cancers, representing either a primary mechanism in the case of innate resistant tumors or a switch in driving mechanisms to evade the action of endocrine therapy in tumors with acquired resistance.[18,19] Turnbull et al.[20] studied gene expression changes in breast cancer tumors treated with neoadjuvant aromatase inhibitors to assess association of proliferation genes with clinical and molecular responses. Proliferation-associated genes were significantly downregulated in tumors responding to endocrine therapy and upregulated in nonresponders by as early as two weeks. We recently reanalyzed this data **(Rui H et al; in preparation)** to study 53 RTKs and to assess changes in mRNA levels at two weeks and three months of neoadjuvant endocrine therapy. In many tumors, mRNA encoding EGFR or HER2 increased fourfold or more (note log scale; Figure 1).

However, it is unknown to what extent such increase occurs in patient tumors at the level of HER-protein expression. This is important since HER-targeted agents work on proteins.

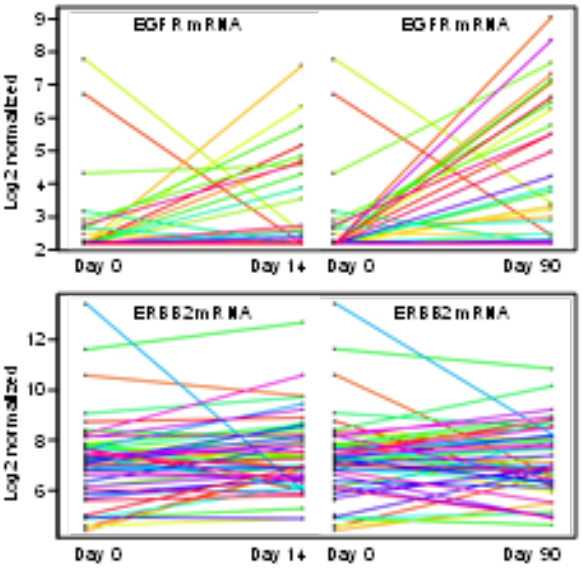

Figure 1. Changes in mRNA levels in EGFR and HER2 at Day 14 and Day 90 of endocrine treatment.

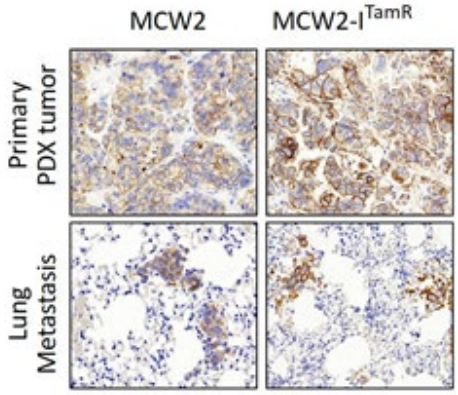

Figure 2. Patient-derived Luminal B MCW2 human breast cancer shows upregulation of HER2 protein in Tam-resistant line, including lung metastases.

Intriguingly, tamoxifen treatment induces increased HER2 protein in cancer cells of a patient-derived breast cancer model analyzed *in vivo* (Figure 2). Furthermore, while parental PDX model is unresponsive to trastuzumab (anti-HER2 antibody), the tamoxifen-resistant tumors (TamR) with elevated HER2 expression were highly responsive to trastuzumab when tested in mice (Figure 3). **Our study will evaluate if treatment-associated changes in protein expressions in HER family of RTKs correlate with tumor responses.**

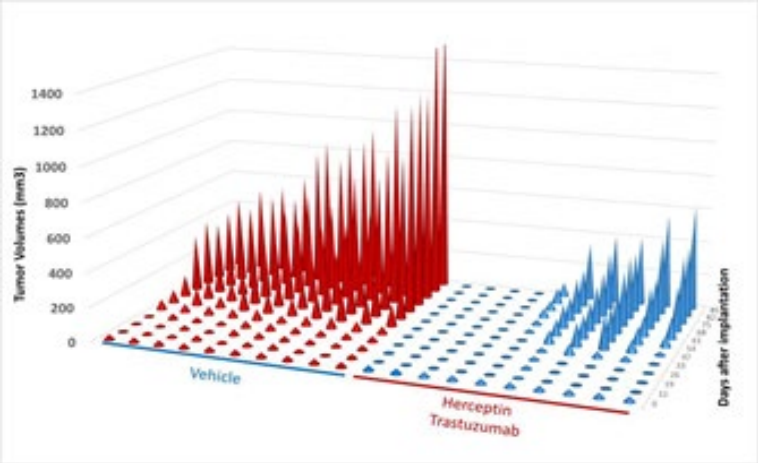

Figure 3. Tamoxifen-resistant MCW2-I PDX model with upregulated HER2 becomes sensitive to trastuzumab. Note that four of 10 trastuzumab-treated mice did not have tumor 'recurrence' within 90-day treatment period.

Reduces Ki-67 is considered a good surrogate of response to treatment. Correlating changed in HER protein expression with Ki-67 would be an **innovative approach** to assess the association of HER protein upregulation and tumor responses to endocrine therapy.

Cyclin-CDK-RB Pathway

Another potential pathway of interest in HR+ breast cancers is **the cyclin-CDK-RB pathway** (cyclin dependent kinase-retinoblastoma) that can be altered in luminal breast cancer and appears to be associated with endocrine resistance.[21] High expression of cyclin-D1 is associated with activation of cyclin-dependent kinases CDK4 and CDK6 and progression to the S-phase of cell cycle. Studies have linked high expression of cyclin-D1 to tamoxifen resistance and high expression of cyclin-E1 to letrozole resistance.[22,23] **Dr. Hallgeir Rui and colleagues at MCW** have extensive laboratory experience with breast cancer xenograft models and have shown that additional mechanisms of resistant ER+ breast cancer may be via the emergence of **basal cytokeratin-5 positive (CK5+) progenitor cells**, which display resistance to chemotherapy and tamoxifen.[24] **We now want to assess CK5+ progenitor cells in breast cancer tumors and determine if an increased proportion of therapy-resistant CK5+ progenitor cells is negatively correlated with tumor proliferation reduction in response to neoadjuvant endocrine therapy.** Patients with ER+/CK5+ breast cancer may benefit from emerging BCL6-inhibitors and may be harmed by prolactin receptor antagonists.[24]

Bruton's Tyrosine Kinase (BTK) Expression

Bruton's tyrosine kinase (BTK) belongs to Tec family tyrosine kinases and causes activation of multiple downstream pathways of cell survival and proliferation.[25] Recently, an alternate isoform of BTK, BTK-C, was identified as a novel survival factor for breast

cancer cells in a large-scale, loss-of-function analysis of human tyrosine kinases, using an RNA interference library by Eifert et al.[26] That study showed that, although BTK-C is expressed at relatively low levels in several human breast cancer cell lines and tumor tissues, BTK-C provides an essential function by protecting breast cancer cells from apoptosis. **BTK expression, in association with endocrine therapy, has not been explored. Emergence of BTK expression in tumors after treatment with neoadjuvant endocrine therapy may be indicative of development of resistance in future.** Patients with BTK-expressing breast cancer may benefit from BTK inhibitors, such as ibrutinib.

Iron-Related Proteins:

Iron homeostasis deregulation and the role of **iron-related proteins** in breast cancer tumorigenesis and in association with clinicopathological features are another area of growing interest. **Transferrin receptor (CD71)** is involved in the cellular uptake of iron and is expressed on proliferating cells. It may be implicated in promoting the growth of endocrine-resistant phenotypes within HR+ breast cancers. Habashy et al.[27] studied CD71 protein expression in patients with breast cancer, as well as *in vitro* cell models of acquired resistance to endocrine therapy. There was significant elevation of transferrin receptor in all cell models of acquired resistance. CD71 was found to be an independent prognostic factor in the ER+ cohort of patients and was associated with increased tumor proliferation, basal CKs, EGFR/HER2 expression and shortened breast cancer-specific survival. Changes in other iron-related protein expression, i.e., ferritin, ferroportin and ribonucleotide reductase with endocrine therapy, and possible clinical correlation with tumor proliferation and responses have not been explored. **Our study plans to measure changes in iron-related proteins and their correlation with tumor responses. Patients with high ribonucleotide reductase expressions may be an indication of development of resistance and these patients may benefit from ribonucleotide reductase inhibitors.**

Programmed Death Receptor 1 (PD-1) Axis

Programmed death receptor 1 (PD-1) is a member of the immunoglobulin superfamily and is expressed on activated T cells, B cells, natural killer cells and myeloid cells.[28] PD-1 conveys an inhibitory signal to T cells, and thus, impedes immune responses.[29] PD-1 and its ligands, PD-L1 and PD-L2, interact to downregulate the activation of T cells in cancer, as well as in autoimmune conditions and infections. Recently, immune checkpoint blockades targeting PD-1 and PD-1 ligands have shown promising therapeutic efficacy in several tumor types.[30] In breast cancer, the reported frequency of PD-L1 expression by carcinoma cells varies considerably between studies (1.7%–58%).[31-35] A recent meta-analysis showed high PD-L1 protein expression to be a negative prognostic factor in breast cancer and was associated with high tumor grade, negative ER and PR status, positive HER2 status and high Ki-67.[36] Although high PD-1 and its ligands expression are primarily seen in triple negative and HER2-positive breast cancer subtypes, it is certainly seen in some luminal breast cancers, as well. Whether there is an increase or emergence of PD-1 protein expression in HR+ breast tumors after treatment with neoadjuvant

endocrine therapy has not been explored. **This would be an important pathway to assess in correlation with tumor responses due to the availability and potential benefit from PD-1inhibitors (nivolumab and pembrolizumab).**

Ki-67 Expression

Expression of the protein Ki-67 is considered a surrogate of cellular proliferation.[37] Iinternational consensus statements, such as those provided by the European Society of Medical Oncology and Saint Gallen International Breast Cancer Conference, do support the role of Ki-67 in distinguishing between the two intrinsic subtypes of HR+ BC, namely luminal A and B subtypes.[38,39] Assuming that the main mechanism of action for endocrine therapy in BC is mainly through inducing cell-cycle arrest, **an on-treatment Ki-67 read out can be considered a surrogate for response to endocrine therapy**. Data from the STAGE trial support the role of on-treatment Ki-67 measurement for predicting clinical responses.[9,40] In addition, in ACOSOG-Z1031, on-treatment Ki-67 levels predicted clinical responses.[10] In the IMPACT trial, Ki-67 was measured as early as two weeks and the superiority of anastrozole over tamoxifen in postmenopausal women was confirmed, based on a greater reduction in Ki-67 levels after two and 12 week.[41] **Quantifying levels of Ki-67 expression (% positive cells) pre- and post-treatment along with radiographic responses to neoadjuvant endocrine therapy will provide measures of responses and allow correlation with our study biomarkers.**

Future Directions

This project is significant and has the potential to have a strong impact because it poses clinically relevant questions. It aims to assess molecular changes with endocrine treatment, facilitating recognition of potential targetable resistance mechanisms and pathways**.** Our expectations are that the proposed studies will reveal mechanistic and preclinical insight to support novel strategies for improved clinical management of HR+ breast cancer patients. Given the small sample size and the exploratory nature of the study, we may not have enough responses to reach a statistically significant result. However, we expect to determine how frequently the protein levels of HER family of RTKs are increased following endocrine therapy. This information would be useful in selecting patients for a follow-up trial with targeted HER/EGFR therapy. Likewise, we expect to determine the frequency of upregulation in CK5+/ER-negative subpopulation of cancer cells, as well as BTK expression in HR+ breast cancer. This information may be used to select patients to a follow-up trial with BCL6 inhibitor and BTK inhibitor, respectively. Patients with changes in iron-related proteins, such as high ribonucleotide reductase expressions, may benefit from ribonucleotide reductase inhibitors and those with increased PD-1 expression may benefit from PD-1 inhibitors. Lack of a significant radiographic response or change in Ki-67 would potentially be an important prognostic indicator of development of early endocrine resistance.

Taken together, these research aims will characterize the changes in tumor microenvironment and association with tumor responses to endocrine therapy. The knowledge from these studies will help define signaling pathways and

predictive biomarkers of short and long-term responses to endocrine therapy and potential emergence of endocrine resistance in low responding tumors. The results from this project would be critical in providing preliminary data for support for a follow-up phase II randomized study of endocrine therapy, combined with other targeted therapy based on molecular tumor profile.

II. HYPOTHESIS AND OBJECTIVES

Hypothesis

We hypothesize that in HR+ HER2 negative tumors of early stage breast cancer patients, expression of one or more HER proteins (HER1-4) will be upregulated in at least 50% of tumors after four weeks of neoadjuvant endocrine therapy and this upregulation will correlate with Ki-67 expressed in more than 10% of cancer cells.

The following objectives are designed to test these hypotheses:

Primary Objective

Determine the frequency of increased **HER-protein expression** in tumors following treatment with neoadjuvant endocrine therapy and their association with tumor Ki-67 expression. We will measure cancer cell protein levels of growth factor receptors of the HER family before and after neoadjuvant endocrine therapy. The data will be used to inform a future randomized trial utilizing a combination of endocrine treatment and the most promising anti-HER targeted therapy.

Secondary Objectives and Associative Studies

Our secondary objectives are focused on other biomarkers and pathways of interest. Our goal is to determine changes in gene expression and/or protein levels of the following biomarkers with neoadjuvant endocrine therapy and correlation with tumor responses.

1. Determine whether an increased proportion of **therapy-resistant CK5+ progenitor cells** are negatively correlated with a response in Ki-67 and tumor volume reduction, in response to neoadjuvant endocrine therapy.
2. Assess changes in the **% expression of ER and PR** in pre- and post-treatment breast cancer tumor specimens and their correlations with Ki-67 and radiographic responses.
3. Assess changes in **BTK protein expression** with neoadjuvant endocrine therapy and correlation with responses.
4. Changes in **iron-related protein expression, i.e., transferrin, ferritin, ferroportin and ribonucleotide reductase** with neoadjuvant endocrine therapy and correlation with responses.
5. Changes in PD-L1 and PD-L2 protein expression with neoadjuvant endocrine therapy.

6. Assess changes in tumor size on radiographic images with neoadjuvant endocrine therapy and correlate with changes in biomarkers and Ki-67.
7. Chart review planned at about five years from completion of study to assess patient outcomes, i.e., ipsilateral, contralateral or distant recurrences.

III. ELIGIBILITY CRITERIA

Inclusion Criteria

1. Unilateral diagnostic breast mammogram and ultrasound within 60 days of enrollment.
2. Pathologically proven diagnosis of invasive breast cancer, clinical stage I or II.
3. Patients must be clinically lymph node negative. Lymph node negativity must be confirmed by clinical exam and/or ultrasound imaging.
4. The patient must be female.
5. Age ≥18 years.
6. Estrogen and/or progesterone receptor positive tumor defined ≥1% positively staining cells by immunohistochemistry, according to the current ASCO/CAP guidelines.
7. HER2/neu must be negative by immunohistochemistry (IHC) or fluorescence *in situ* hybridization (FISH).
8. ECOG performance status 0 to 2.
9. Bilateral breast cancer and/or multifocal, multicentric disease is allowed if all disease sites are biopsied.
10. Appropriate pretreatment evaluations for protocol entry, including no clinical evidence for distant metastases, based upon the following minimum diagnostic workup: history/physical examination, including breast exam (inspection and palpation of the breasts), clinically negative axillary lymph nodes, **within 28 days prior to study entry.**
11. The patient must qualify for anti-endocrine treatment (treatment of choice), per the treating medical oncologist.
12. The patient must provide study-specific informed consent prior to study entry.
13. Patients with a prior history of breast cancer will be considered eligible, if they have completed all treatment (including endocrine therapy) more than 2 years prior to registration.
14. Patients must not have had a prior treatment for this breast cancer or for any malignancy diagnosed or treated within the past 2 years, with the exception of non-melanomatous skin cancer, carcinoma *in situ* of the cervix..
15. Women of child bearing age will be advised to use adequate methods of contraception. Adequate methods of contraception for premenopausal women include barrier methods and/or non-hormonal methods (Intrauterine devices etc.).
16. Negative pregnancy test. (if applicable)

17. Strong CYP2D6 inhibitors will be prohibited with tamoxifen, as it can decrease the efficacy of tamoxifen. There are no known strong interactions with aromatase inhibitors.
18. Adequate organ function with baseline lab values.
    - Absolute neutrophil count (ANC) ≥ 1500/μL
    - Hemoglobin (Hb) ≥ 9g/dL
    - Platelet count ≥ 100,000/μL
    - Alanine aminotransferase (ALT) and aspartate aminotransferase (AST) ≤ 3x upper limit of normal (ULN)
    - Serum bilirubin within ≤ 1.5 x ULN

Exclusion Criteria

1. AJCC clinical T4, N1-3 or M1, breast cancer.
2. Synchronous non-breast malignancy (exceptions include non-melanomatous skin cancer, carcinoma *in situ* of the cervix).
3. Purely noninvasive breast cancer (i.e., ductal carcinoma in situ, lobular carcinoma in situ).
4. Men with breast cancer. Male breast cancer is a rare event and it is unclear if neoadjuvant endocrine treatment approach is safe in men.
5. Medical, psychiatric or other condition that would prevent the patient from receiving the protocol therapy or providing informed consent.
6. Pregnant or lactating women are ineligible.

IV. STUDY DESIGN AND TREATMENT PLAN

General description: This is an exploratory interventional study to investigate changes in HER-protein expression along with other biomarkers of interest and correlations with tumor cell proliferation and responses in patients with HR+ HER2 negative early stage breast cancer treated with neoadjuvant endocrine therapy.

Number of subjects: 37

Primary End point: Determine the frequency of increased protein expression of **HER family of receptor tyrosine kinases** in tumors, following treatment with neoadjuvant endocrine therapy and their correlation with Ki-67 tumor responses.

Secondary End points:

- To determine changes in additional selected molecular markers (CK5+/ER- progenitor cells, PD-L1, PD-L2, BTK, iron-related proteins) and correlate with Ki-

67 responsiveness to endocrine therapy in our pre- and post-treatment tumor specimens.

- Assess changes in tumor size on radiographic images with neoadjuvant endocrine therapy and correlate with changes in biomarkers and Ki-67.

Study Timeline:

Primary completion: We expect that the study will reach primary completion 24 months from the time the study opens to accrual.

Study completion: We expect that the study will reach study completion 36 months from the time the study opens to accrual.

Treatment Plan

1. At MCW, all patients undergo diagnostic mammogram and ultrasound of the breast (standard of care) to further evaluate an abnormal breast finding and to obtain core biopsies for diagnostic purposes. If the tumor is visualized on both imaging modalities, at least the largest bidimensional measurements will be recorded. If possible, three-dimensional measurements of tumors will be obtained.
2. Patients with a diagnosis of ER and/or PR positive, HER2 negative node negative invasive breast cancer would be screened for this study (refer to the schema).
3. Node negativity will be established by clinical exam and/or ultrasound
4. After the informed consent is obtained with the patient's signature, the eligibility process begins. If the patient is deemed eligible, then the patient can participate in any study related procedures.
5. Interested patients must meet with a medical oncologist prior to study entry to determine if Oncotype Dx testing is recommended. If recommended and the patient is amenable to the possibility of receiving chemotherapy, there must be adequate biopsy tissue for testing. If adequate tissue is not available for the Oncotype Dx testing, patients may undergo additional biopsies. If a patient plans to decline chemotherapy, regardless of a high Oncotype Dx results, and elects to forgo the test, they will still be eligible for enrollment. If the patient elects to undergo an additional biopsy, but no residual tumor is found, then, the patient is not eligible for the study.
6. The Oncotype Dx assay is obtained initially in order to know which patient would benefit from the addition of adjuvant chemotherapy after her surgery, so that all patients undergo the appropriate standard-of-care postsurgical treatment. Treatment with neoadjuvant endocrine therapy is likely to induce changes within tumor biology and Oncotype Dx assay done after surgery in this setting may not provide reliable results, and thus, will not be performed after surgery.
7. Once enrolled, patients would be treated with the current standard-of-care endocrine therapy. Choice of endocrine therapy (aromatase inhibitors or tamoxifen) would be decided by medical oncologist, following a review of the patient's medical history and menstrual status. The patient would be treated with

endocrine therapy in a neoadjuvant setting for four weeks (+/- 1 week), with dosing continuing until surgery (+/- 2 days).

8. Patient compliance would be monitored by providing a calendar sheet to the patients to log in their daily use of prescribed endocrine therapy (Appendix.1). This calendar sheet will be returned to our clinical trial staff upon completion of the four-week (+/- 1 week) period of neoadjuvant endocrine treatment.
9. Core biopsy specimens from the initial biopsy at diagnosis would be used for OncotypeDx assay, as well as for study biomarkers assessments. One hematoxylin and eosin slide and up to 15 to 20 unstained charged slides cut at 4 microns thick would be obtained from pathology blocks with residual tumor. These specimens will be reserved for study labs/biomarkers for pre- treatment after the Oncotype has been completed (if applicable). Biomarkers to be assessed pre- and post-treatment are shown in **Table 1.** Details on handling and preparation of tissue specimen and slides for study evaluations are explained in detail below in section **Pathologic Evaluation and Handling of Tissue Specimens.**
10. If adequate tissue is not available for all study biomarkers to be done, additional biopsies will be requested. However, if the patient declines an additional biopsy and enough tissue is available to obtain some but not all study biomarkers, the patient would still qualify and be enrolled to the study.
11. Patients would undergo unilateral diagnostic mammogram and ultrasound within five days prior to surgery to assess radiographic responses to the pretreatment imaging obtained at diagnosis. The CRC/CRN should notify Dr. Gonyo in Radiology prior to surgery. Diagnostic mammogram and the US are to be assessed using WHO criteria for response.
12. Patients would undergo surgery as planned at the end of the four-week (+/- 1 week) period of neoadjuvant endocrine treatment.
13. Surgical specimen would be use for post-treatment assessments. Changes in proliferation marker Ki-67 between pre- and post-treatment tumor specimens would be assessed by our breast pathologists. One hematoxylin and eosin slide and up to 15 to 20 unstained charged slides cut at 4 microns thick would be obtained from pathology blocks with residual tumor. These specimens will be reserved for study labs/biomarkers for post-treatment. Biomarkers to be assessed pre- and post-treatment are shown in **Table 1.** Details on handling and preparation of tissue specimen and slides for study evaluations are explained in detail below in section **Pathologic Evaluation and Handling of Tissue Specimens.**
14. Any remaining tumor tissue would be banked in MCW Tissue Bank according to the MCW Tissue Bank quality control measures. Specimens would be deidentified. A separate consent form would be used for tissue banking. Patients who do not participate in the Tissue Banking are still eligible.
15. Patients would be treated per standard-of-care guidelines following surgery. The initial Oncotype Dx assay, if done, would be used by the treating medical oncologist to decide if adjuvant chemotherapy is indicated.
16. A follow-up visit would be scheduled within 30 days from surgery. Further follow-up visits would be per the discretion of the patient's treating physicians, who will follow NCCN guidelines.

17. As standard-of-care treatment, patients will be followed by oncology provider(s) at least annually (+/- 3 months). Cancer outcomes up to five years post-treatment will be collected annually (+/- 3 months) by chart review.
18. Given the short course of study treatment (~3-5 weeks) and none-few low-grade toxicities expected, we expect most of our study patients to complete the 4-week study treatment. Patients should be treated for at least 2 weeks with neoadjuvant endocrine therapy to be considered evaluable for the study end points. All dosed patients with at least 2 weeks of neoadjuvant endocrine therapy received would be included in the analyses.
19. All adverse events grade 3 and above, regardless of attribution, will be collected and reviewed at the required timepoints (screening and/or week 1, and end of treatment visit).

Table 1. Biomarkers to be assess pre- and post-treatment with neoadjuvant endocrine therapy

Biomarkers	**End Points**
Ki-67	Assess change in proliferation with treatment.
HER tyrosine kinase receptor expression	Assess frequency of increased protein levels of HER1-4 and correlation with response to treatment.
ER% / PR%	Assessment of pre- and post-treatment changes and response to treatment.
CK5+/ER- progenitor cells	Assess proportion of CK5+/ER- cancer cells and correlation with response to treatment.
Iron related proteins	Assess changes in protein expression and correlation with responses.
BTK expression	Assess changes in protein expression and correlation with responses.
PD-L1/PD-L2	Assess changes or emergence of protein expression with treatment and correlation with responses.

Pathologic Evaluation and Handling of Tissue Specimens

1. The surgical specimens will be subject to routine pathological analysis. Routine hematoxylin and eosin (H&E) stain will be used for morphological analysis and for the determination of carcinoma diagnosis. The samples will be assessed by our breast pathologists.
2. One H&E slide and up to 15 to 20 unstained slides charged and cut at 4 microns thick will be reserved for study labs/biomarkers for pre and post-treatment assessments. These will be sent to **Dr. Hallgeir Rui's** laboratory (suite C4980) at the **Medical College of Wisconsin Translational and Biomedical Research Center (TBRC).**
3. Protein expression will be quantified on slides, using optimized immunohistochemical protocols for planned study biomarkers.

4. Ki-67 assessment on pre- and post-treatment specimens would include assessment by manual counting of stained tumor cells by our pathologists (dual stain CD45/Ki-67 to differentiate between lymphocytes and tumor cells and allow accurate counting of stained tumor cells), as well as quantitative immunohistochemistry.
5. Any residual tissue from pretreatment biopsy specimens and post-treatment surgical specimens would be stored at MCW Tissue Bank according to the MCW Tissue Bank quality control measures. The stored specimens would be deidentified.

Study Drugs

- Aromatase inhibitors: Anastrozole, Letrozole, Exemestane
- Tamoxifen

All agents are FDA approved for use in breast cancer patients and are similarly priced. Commercial stock will be used for this exploratory study.

Anastrozole
Anastrozole is a potent and selective nonsteroidal aromatase inhibitor.
Route of administration: Oral
Dose: 1 mg once daily. May be administered with or without food.
Dose adjustments: No dosage adjustment necessary in any degree of renal or hepatic impairment.

Letrozole

Letrozole is a potent and selective nonsteroidal aromatase inhibitor.
Route of administration: Oral
Dose: 2.5mg once daily. May be administered with or without food.
Dose adjustments:

- Renal impairment: No dosage adjustment necessary.
- Hepatic impairment:
  - Mild to moderate impairment (Child-Pugh class A or B): No dosage adjustment necessary.
  - Severe impairment (Child-Pugh class C) and cirrhosis: 2.5 mg every other day
  - Non-cirrhotic patients with elevated bilirubin: There are no dosage adjustments provided in the manufacturer's labeling (effect has not been determined).

Exemestane
Exemestane is an irreversible, steroidal aromatase inhibitor.
Route of administration: Oral
Dose: 25 mg once daily. Administer after a meal.

Dose adjustments: No dosage adjustment necessary in any degree of renal or hepatic impairment.

Tamoxifen

Tamoxifen is a selective estrogen receptor modulator. Tamoxifen competitively binds to estrogen receptors on tumors and other tissue targets, producing a nuclear complex that decreases DNA synthesis and inhibits estrogen effects producing cytostatic effects.
Route of administration: Oral
Dose: 20 mg once daily. May be administered with or without food.
Dose adjustments: No dosage adjustment necessary in any degree of renal or hepatic impairment.
Food interaction: Grapefruit juice may decrease the metabolism of tamoxifen. Avoid grapefruit juice.

Prohibited Medications

The following agents must be stopped at least one week prior to registration and must not be administered during the study intervention.

- Any agent with estrogenic properties, including herbal preparations. This also includes hormone replacement therapy of any type, or raloxifene.
- Strong CYP2D6 inhibitors will be prohibited with tamoxifen, as it can decrease the efficacy of tamoxifen.
- Any other anti-neoplastic approach, such as chemotherapy or radiation, must not be administered while the patient is receiving study treatment.

Concomitant Medications

All three aromatase inhibitors and tamoxifen are generally safe to administer with other medicines. Concomitant use of agents and herbal products that alter ER function are specifically not allowed, as mentioned in the prohibited medications.

Definitions of Radiographic Responses

If a tumor is visualized on both imaging modalities, at least the largest bidimensional measurements will be recorded. If possible, three-dimensional measurements of tumors will be obtained. If a tumor is visualized on only one imaging modality (only a mammogram or only an ultrasound), at least the largest bidimensional measurements will be recorded. If possible, three dimensional measurements of tumors will be obtained.

The ultrasound images will be acquired such that the largest dimensions of the tumor in at least two of four planes (radial, anti-radial, sagittal, or transverse) are recorded.

- **WHO criteria** will be used to assess tumor response to drug therapy. **Complete Response (CR):** The disappearance of all known disease, based on a comparison between the measurements at baseline and after four weeks of treatment with neoadjuvant therapy.
- **Partial Response (PR):** A 50% or greater decrease in the product of the bidimensional measurements of the lesion (total tumor size), based on a comparison between the measurements at baseline and after four weeks of treatment with neoadjuvant therapy.
- **No Change (NC):** A 50% decrease in total tumor size cannot be established nor has a 25% increase in the size of the lesion been demonstrated.
- **Progressive Disease (PD):** A 25% or greater increase in the total tumor size of the measurable lesions (calculated on the smallest diameter recorded over time).

V. STUDY ENTRY AND WITHDRAWAL; STUDY PROCEDURES

Required Preregistration Screening Tests and Procedures

The study-specific assessments are detailed in this section and are outlined in the study calendar. Screening assessments must be performed within 28 days prior to enrollment. Any results falling outside of the reference ranges may be repeated at the investigator's discretion. Treatment or visit delays for public holidays or weather conditions do not constitute a protocol violation.

A written, signed informed consent form (ICF) and a Health Insurance Portability and Accountability Act (HIPAA) authorization must be obtained before any study-specific assessments are initiated. A separate consent form for tissue banking of residual tumor specimens would be used. Signed copies of the study consent forms would be given to the subject. and a copy will be filed in the medical record. The original study consent will be kept on file with the study records.

All patients who are consented will be registered in OnCore®, the MCW Cancer Center clinical trial management system. The system is password protected and meets HIPAA requirements.

Registration Procedures

Registration and Monitoring of Patients

1. Patients will be screened for eligibility and registered by a certified research associate/certified research coordinator (CRC/CRA) in the Clinical Trials Office of the Froedtert & the Medical College of Wisconsin Clinical Cancer Center.

2. Patients will be registered in the institutional OnCore® web-based system under the CRA console.

3. Registration of patients and information regarding consenting, eligibility review, on-study status, treatment status and follow-up will be monitored, using the OnCore® calendar system.

4. Serious adverse events (SAEs) and protocol deviations will be reported through the OnCore® reporting system.

5. Questions regarding OnCore systems and data reporting should be reported to OnCore® data management.

6. Questions regarding patient eligibility and registration procedures should be directed to the principal investigator, Lubna Chaudhary, MD, MS (lchaudhary@mcw.edu, pager 414-318-8288, office 414-805-4600).

7. Subject Withdrawal. Given the short nature of the treatment regimen (three to five weeks), and none or few low-grade toxicities are expected, it is not considered likely that a large percentage of patients will withdraw from the study, once endocrine treatment has begun. However, subjects have the right to withdraw from the study at any time. In the rare case that a patient withdraws from the protocol after the initiation of treatment, only the data collected up until the date of withdrawal will be used in analysis.

8. A subject may be withdrawn from the study, if she wishes to decline treatment on the study. Subjects may also be withdrawn if there is a violation of the protocol inclusion and exclusion criteria, as deemed relevant by the treating physician and the principal investigator. As these patients would be withdrawn prior to delivery of study-related treatment, they will then be replaced on the study and not counted toward the total accrual goal of 39 patients. Similarly, patients withdrawing for any other reason prior to delivery of treatment will be replaced on the study and not counted toward the total accrual goal.

9. Patients who withdraw from the study should be treated in accordance with normal standard of care. Follow-up will be per the discretion of the patient's treating physicians, but at a minimum be in accordance with NCCN guidelines.

Pretreatment Period

Screening Assessments

Once the informed consent is signed, proceed with the screening procedures and assessments which must be completed within 28 days of prior to enrollment.

- Physical examination
- Vital signs
- Complete medical history
- Performance status (ECOG, KPS, etc.) (Appendix.2)

- Adverse event assessment
- Current medications
- Blood chemistry assessment, including
  - Complete blood count (CBC) with differential and platelet count
  - Alkaline phosphatase, aspartate aminotransferase/alanine aminotransferase - (ALT/AST)
  - total bilirubin
  - calcium
  - phosphorus,
  - creatinine,
  - potassium,
  - sodium,
  - chloride,
  - bicarbonate,
  - fasting lipid panel (low-density lipoprotein [LDL], total cholesterol, triglycerides) - within the past 12 months.
- Serum or urine pregnancy test for premenopausal women
- Specimen collection for Oncotype Dx and study biomarkers once enrolled.
- Unilateral diagnostic breast mammogram and ultrasound within 60 days of enrollment

Study Procedures during Treatment

Patients must have met eligibility criteria on Day 1 to be treated. The clinic visit can be the same for screening, eligibility assessment and if enrolled, to start treatment with endocrine therapy for week 1. Patient will get treatment for 4 weeks (+/-1 week) before their surgery. No additional clinic follow ups are planned during this period, however can be scheduled per discretion of treating physician.

Surgery

- Diagnostic mammogram and ultrasound are required within 5 days prior to surgery
- Surgical specimen collection for study biomarkers

End of Treatment Visit

The end of treatment visit would be scheduled within 30 days from surgery.

- Physical examination
- Vital signs

- Complete medical history
- Performance status (ECOG, KPS, etc.)
- Current medications
- Adverse event assessment

Long-term/Outcomes Follow-Up Procedures

As standard of care, patients will be followed by oncology provider(s) at least annually after surgery (+/- 3 months). Cancer recurrence outcomes up to five years post-treatment will be collected by chart review annually.

VI. ADVERSE EVENTS: DEFINITIONS AND REPORTING REQUIREMENTS

Adverse Event (AE) and Serious Adverse Events (SAE)
The investigator and team will follow the Medical College of Wisconsin policies related to adverse event reporting. Common Terminology Criteria for Adverse Events (CTCAE) version 5 would be used. This information may be found on the Human Research Protection Program website.

Serious AE (SAE) means any untoward medical occurrence:

- **Death**. Results in death.
- **Life threatening**. Is life threatening (refers to an AE in which the patient was at risk of death at the time of the event; it does not refer to an event which hypothetically might have caused death if it were more severe).
- **Hospitalization.** Requires inpatient hospitalization or prolongation of an existing hospitalization
- **Disability/incapacity**. Results in persistent or significant disability or incapacity. (Disability is defined as a substantial disruption of a person's ability to conduct normal life functions).
- **Medically important event.** This refers to an AE that may not result in death, be immediately life threatening, or require hospitalization, but may be considered serious when, based on appropriate medical judgment, may jeopardize the patient, or require medical or surgical intervention to prevent one of the outcomes listed above.

Unanticipated Problem Involving Risk to Subject or Other (UPIRSO)

The investigator and team will follow the Medical College of Wisconsin policies related to unanticipated problems involving risks to subjects or others. This information may be found on the Human Research Protection Program website.

AE Attribution and Grading

Table 2. Adverse Event Grading

Grades	Description
0	No AE (or within normal limits)
1	Mild; asymptomatic or mild symptoms; clinical or diagnostic observations only; intervention not indicated.
2	Moderate; minimal, local or noninvasive intervention; limiting age-appropriate instrumental ADL*
3	Severe or medically significant but not immediately life-threatening; hospitalization or prolongation of hospitalization indicated; disabling; limiting self-care ADL*
4	Life-threatening consequences; urgent intervention indicated
5	Death related to AE

NCI CTCAE: National Cancer Institute Common Terminology Criteria for Adverse Events; ADL: activities of daily living.
* Instrumental ADLs include preparing meals, shopping, using the telephone, managing money. Self-care ADLs include bathing, dressing, using the toilet, taking medications.

Adverse Event Attribution

Attribution is an assessment of the relationship between the AE and the medical intervention

Table 3. Adverse Event Attribution

Relationship	Attribution	Description
Unrelated to study drug/intervention	Unrelated	The AE *is clearly* ***NOT*** *related* to the intervention
	Unlikely	The AE *is* ***doubtfully related*** to the intervention
Related to study drug/intervention	Possible	The AE ***may be related*** to the intervention
	Probable	The AE ***is likely related*** to the intervention
	Definite	The AE ***is clearly related*** to the intervention

Relationship Assessment: In-Depth Definitions

For all collected AEs, the clinician who examines and evaluates the subject will determine the adverse event's causality, based on temporal relationship and his/her clinical judgment. The degree of certainty about causality will be graded using the categories below:

Definitely Related: There is clear evidence to suggest a causal relationship, and other possible contributing factors can be ruled out. The clinical event, including an abnormal laboratory test result, occurs in a plausible time relationship to drug administration and cannot be explained by concurrent disease or other drugs or chemicals. The response to withdrawal of the drug (dechallenge) should be clinically plausible. The event must be pharmacologically definitive, with use of a satisfactory rechallenge procedure, if necessary.

Probably Related: There is evidence to suggest a causal relationship, and the influence of other factors is unlikely. The clinical event, including an abnormal laboratory test result, occurs within a reasonable time sequence to administration of the drug, is unlikely to be attributed to concurrent disease or other drugs or chemicals, and follows a clinically reasonable response on withdrawal (dechallenge). Rechallenge information is not required to fulfill this definition.

Possibly Related: There is some evidence to suggest a causal relationship (e.g., the event occurred within a reasonable time after administration of the trial medication). However, the influence of other factors may have contributed to the event (e.g., the subject's clinical condition, other concomitant events). Although an adverse drug event may rate only as "possibly related" soon after discovery, it can be flagged as requiring more information and later be upgraded to "probably related" or "definitely related", as appropriate.

Unlikely: A clinical event, including an abnormal laboratory test result, whose temporal relationship to drug administration makes a causal relationship improbable (e.g., the event did not occur within a reasonable time after administration of the trial medication) and in which other drugs or chemicals or underlying disease provides plausible explanations (e.g., the subject's clinical condition, other concomitant treatments).

Unrelated: The AE is completely independent of study drug administration, and/or evidence exists that the event is definitely related to another etiology. There must be an alternative, definitive etiology documented by the clinician.

Unexpected Adverse Events
Review all unexpected grade 3, and all grade 4, and 5 adverse events, as well as any others requiring expedited reporting as defined in this protocol. (Grades 4 and 5 events

must be reported to the DSMB within five calendar days of study staff's knowledge.)

The drugs used in this study are FDA approved for the use of women with hormone receptive positive breast cancer. The importance of monitoring these drugs used prior to surgery is vital therefore any adverse events that are grade 3, 4, or 5 along with causality of possibly, probable or definitely should be reported to the institutions IRB as per policy.

Expected Adverse Events

The following tables provides the expected adverse events for aromatase inhibitors: Anastrozole, letrozole and exemestane and tamoxifen.

Table 4. Expected Adverse Events for Aromatase inhibitors

Likely	Less Likely	Rare
Fatigue	Hypertension	Heart disease
Joint musculoskeletal pain	Constipation	Chest pain
Loss of bone density over time	Nausea/Vomiting	
	Weight gain	
	Sweating/Hot flashes	
	Mood alteration (depression, anxiety)	
	Hair thinning	
	Headaches	

Table 5. Expected Adverse Events from Tamoxifen

Likely	Less Likely	Rare
Fatigue	Difficulty with sleep	Ischemic cardiovascular event
Hot flashes/Sweating	Constipation	Thromboembolic event
Decrease in libido	Nausea/Vomiting	Uterine cancer
	Weight gain	
	Mood alteration (depression, anxiety)	
	Hair thinning	
	Headache	
	Hypertension	

Patient Assessments

1. As noted, given the short nature of the treatment regimen (three to five weeks), and expected low grades of toxicity, it is considered highly unlikely that a large percentage of patients will experience adverse events (AEs) from the endocrine treatment.

2. All grade 3 and above AEs would be recorded regardless of attributions.
3. Patients would be assessed at beginning and end of treatment with neoadjuvant endocrine therapy as well as at their follow up visit within 30 days after completion of study treatment.
4. Expected side effects are minimal. The most commonly reported symptom is fatigue. Fatigue is characterized by a state of generalized weakness with a pronounced inability to summon sufficient energy to accomplish daily activities.
5. Other less commonly reported side effects are anti-estrogen symptoms, i.e., hot flashes, mood changes and weight changes. Some of the rare but serious side effects, such as ischemic cardiovascular event, thromboembolic event or uterine cancer with tamoxifen are long-term side effects and are not expected to occur during this short course of neoadjuvant treatment. Similarly, decrease in bone density with aromatase inhibitors is a slow and long-term side effect, not expected to be seen during the four-week study period.
6. There are no dose modifications or reductions recommended with endocrine treatment.

VII. STATISTICAL CONSIDERATIONS

Statistical analyses:

Demographic and baseline characteristics, such as age, race, weight, values of all biomarkers pre and post-treatment, response measures will be summarized, using means, standard deviations, medians, ranges for continuous variables, and proportions for categorical variables.

This is an exploratory study to gather information on how frequently protein levels of HER growth factor receptors (HER1-4) are upregulated along with changes in other study biomarkers and correlation with Ki-67 in response to short-term neoadjuvant endocrine in early stage HR+ breast cancer. We hypothesize that expression of one or more HER proteins (HER1-4) will be upregulated in at least 50% of tumors after four weeks of neoadjuvant endocrine therapy and this upregulation will correlate with Ki-67 expressed in more than 10% of cancer cells, as compared to upregulation in 30% or less of tumors without the therapy. The change in Ki-67 expression will be dichotomized, as a categorical variable with ≥10% increase coded as high and <10% coded as low.

a) For the primary outcome: An exact binomial one sample test of proportions will be used for to test the hypothesis that proportion of upregulated HER proteins will be at least 50% after therapy versus the null hypothesis that the proportion of upregulated HER proteins is not greater than 30%.
b) For analyzing - the differences in proportion of upregulated HER proteins between the high difference in Ki-67 and low difference in Ki-67 groups, two sample tests of proportions will be used.
c) Similarly, appropriate two sample tests will be used to test differences in biomarkers between the high difference in Ki-67 and low difference in Ki-67 groups. We will use the appropriate kind of regression instead of two sample tests, to test differences in biomarkers between the high difference in Ki-67 and low

difference in Ki-67 groups if we need to adjust for covariates.

d) Additionally, regression analysis will also be done to evaluate the changes in the biomarkers and their association with patient specific covariates, such as age, race and other relevant predictors of interest.

Radiographic responses will be assessed by WHO criteria. Radiographic response rates will be measured as the rate of complete response (CR) or partial response (PR) in patients treated with neoadjuvant endocrine therapy. Complete response is defined as the resolution of tumor on post-treatment imaging as compared with pretreatment imaging, while partial response is defined as 50% reduction in tumor size on post-treatment imaging, as compared with pretreatment imaging. We will estimate the rates of response with 95% confidence intervals. We will use logistic regression (and other generalized linear models as appropriate) to measure association between the response rates and patient specific covariates such as age, race and other relevant predictors of interest.

We will use a family type I error rate of 0.05 significance throughout with Bonferroni adjustments for multiple testing wherever appropriate, so that the overall family type I error rate is maintained.

Sample Size and Power Calculation

To achieve at least 80% power at significance level of 0.05, when testing the one sided one sample hypothesis that the proportion of tumors with HER protein upregulation with neoadjuvant endocrine therapy is at least 50% versus the null hypothesis that the proportion is no larger than 30%, **the minimum number of patients required is 37.**

Early Stopping for Futility

Early stopping for futility and interim analysis in clinical trials are done to ensure safety of the patients and prevent any unnecessary intervention. This is an exploratory study to understand changes in tumor biology with neoadjuvant endocrine treatment. All study biomarkers including changes in HER family of RTKs are being studied to assess responses and development of endocrine resistance. In this study, patients are being treated with endocrine therapy in the neoadjuvant setting for a short duration of time (4 weeks +/- 1 week) without causing any significant delay in their surgery. They will be treated with the same endocrine therapy in the adjuvant setting for at least 5 years which is standard of care. These drugs are routinely used in neoadjuvant setting in patients deemed to be poor surgical candidates or requiring a delay in surgery for variety of reasons and is considered a safe approach with minimal risk to the patients particularly when used for a short duration as done in this study.

The primary objective of this study is to assess changes/upregulation of one or more HER proteins (HER1-4) after neoadjuvant endocrine treatment. Given the exploratory nature and small sample size of this study, we may not see enough upregulation of HER

proteins to meet statistical significance. Up regulation or lack thereof of HER proteins is not a known prognostic factor for patient outcomes at this time and will not impact the safety or clinical management of these patients. Interim analysis and stopping for futility is not indicated in this setting of an exploratory study where the goal is to understand changes in tumor microenvironment to learn more about tumor responses or lack thereof to endocrine treatment. We expect to get important preliminary data from this exploratory study for future follow up clinical trials.

Missing Data management: Observed data will be included in listings, summary tables and statistical analysis, with counts and proportions of missing data, if any. The only likely source of missing data in our study is the absence of some biomarker information due to lack of tissue specimens. As already mentioned, we will attempt to obtain additional biopsy tissue to complete the information, but in case this is not feasible, the evaluation of these biomarkers will be done dropping the missing samples from the analysis. This missed data is likely to be at random and there will be no imputation of missing data.

VIII. PATIENT SAFETY MONITORING AND CONFIDENTIALITY

This study will be reviewed by the Medical College of Wisconsin Cancer Center Data and Safety Monitoring Committee (MCWCC DSMC). A summary of the MCWCC DSMC activities are as follows:

- Review the clinical trial for data integrity and safety
- Review all grade 3 and higher adverse events, as well as any others requiring expedited reporting as defined in this protocol. (Grades 4 and 5 events must be reported to the DSMC within five calendar days of study staff's knowledge.)
- Review all Data and Safety Monitoring reports.
- Submit a summary of any recommendations related to study content.
- Terminate the study if deemed unsafe for patients.

1. A copy of the MCWCC Data and Safety Monitoring Plan and membership roster will be maintained in the study research file and updated as membership changes. The committee will review reports from the study PI twice annually (or more frequently if needed) and provide recommendations on trial continuation, suspension or termination as necessary.
2. Any available DSMC letters will be submitted to the IRB of record as required.
3. Data on patient information and follow-up information will be collected and entered into the password protected OnCore® system. Data will be accessible only by the research team and DSMC.
4. Hard copy forms will be kept in a locked file in offices of the CTO. The forms will be linked to patients by a unique identifier and patient initials in the event that the

identifier is transcribed incorrectly. Access to these forms will be limited to the CRA and PI only as the data collected will be entered in to OnCore®.

IX. QUALITY ASSURANCE

This trial has been determined to be an intermediate risk trial by the MCW Cancer Center Clinical Trials Office Quality Assurance Team. This will be reviewed by the MCW CC CTO Quality Assurance team as follows

- Intermediate risk trials are reviewed every year.
- 20% of subject files will be selected randomly for review (a maximum of 10 subjects at each monitoring time point). Consent/eligibility and objective-based data will be reviewed for those files selected.
- One file will be selected randomly for a comprehensive review at each monitoring time point.
- Regulatory documents (IRB submissions, reportable events, etc.) will be reviewed at each monitoring time point.

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Appendix 1. IIT-Chaudhary-Endocrine: Monthly Intake Diary

Institution: _____ **Physician:** _____ **Study ID#:** _____ **Initials (L, FM):** _____

Instructions for the participant: This is a monthly calendar on which you are to record the daily anti-estrogen pill you take each day for the 4-week period. If you develop any side effects from the pill, describe side effect on the day that it happened on the calendar below. If you ever miss taking a pill, write missed on the date. Please bring your calendars with you to your follow up visit after you've completed the 4-week period. If you take over the counter medications, for example cold & allergies, please write on the day what medication you took.

Special Instructions: Please **take 1 anti-estrogen pill, ONCE each day, preferably in the morning with food & a glass of water**. Tablets MUST be swallowed whole, do NOT crush or chew. **AVOID grapefruit & products containing grapefruit juice while you are taking tamoxifen.**

If you have any questions, please contact: _____ **Phone:** _____

Month:			Year:			
Sunday	**Monday**	**Tuesday**	**Wednesday**	**Thursday**	**Friday**	**Saturday**

Patient Name: _____ **Date:** _____

Surgeon: _____ **Surgery Date:** _____

Appendix 2. Performance Status criteria

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

Appendix 3. IIT-Chaudhary-Endocrine: Specimen Submission Form

An Exploratory Study of Neoadjuvant Endocrine Therapy in Hormone Receptor-Positive HER2-Negative Node-Negative Breast Cancer Patients to Assess Responses and Mechanisms of Endocrine Resistance

PI: Lubna Chaudhary, MD, MS

For Clinical Trials Office to complete: Please send pathology for pre- and post- treatment, One H&E slide and 15 to 20 unstained charged slides cut at 4 microns thick, to Dr. Hallgeir Rui's laboratory (suite C4980) at the Medical College of Wisconsin Translational and Biomedical Research Center (TBRC).

Date of Specimen Collection: _____ (Date found on surgical path report) Date of Shipment: _____

Study Identification Number: _____

Shipped From:

_____

_____

Contact Name: _____ Contact Number: _____

Contact Fax: _____ Contact Email: _____

For Dr. Hallgeir Rui's laboratory to complete: Please note any residual tissue from pretreatment biopsy specimens and post-treatment surgical specimens are to be stored at MCW Tissue Bank. Please submit accordingly and notify Clinical Research team of submission. Please sign and send this form to the clinical trials office by fax or email.

Time Point	Date Received	Any Residual Specimen?	Residual specimen sent to MCW Tissue Bank?	Comments:
Pre - treatment specimen (biopsy)				
Post - treatment specimen (definitive surgery)				

_____ _____

Signature of Person Receiving Specimens ***Date***