

Cover page

Title: Pre-Surgical Trial of Letrozole in Post-Menopausal Patients with Operable Hormone-Sensitive Breast Cancer

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SCCC- 11118

Pre-Surgical Trial of Letrozole in Post-Menopausal Patients with Operable Hormone-Sensitive Breast Cancer

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UTSouthwestern
Harold C. Simmons
Comprehensive Cancer Center



Signature Page

**Protocol Version August 16, 2021 Version 5.0
SCCC-11118**

The signature below constitutes the approval of this protocol and the attachments, and provides the necessary assurances that this trial will be conducted according to all stipulations of the protocol, including all statements regarding confidentiality, and according to local legal and regulatory requirements and applicable U.S. federal regulations and ICH guidelines.

Protocol Title:

**Pre-Surgical Trial of Letrozole in Post-Menopausal Patients with
Operable Hormone-Sensitive Breast Cancer**

Principal Investigator (PI) Name: _____

PI Signature: _____

Date: _____

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LIST OF ABBREVIATIONS AE Adverse

Event	Absolute Lymphocyte Count
ALT	Alanine Aminotransferase
ALC	American Society of Clinical Oncology
AST	Aspartate Aminotransferase
ASCO	American Society of Clinical Oncology
BUN	Blood Urea Nitrogen
CBC	Complete Blood Count
CMP	Comprehensive Metabolic Panel
CR	Complete Response
CT	Computed Tomography
CTCAE	Common Terminology Criteria for Adverse Events
DLT	Dose Limiting Toxicity
DOT	Disease Oriented Team
DSMB	Data and Safety Monitoring Board
ECOG	Eastern Cooperative Oncology Group
FDA	Food and Drug Administration
GCP	Good Clinical Practice
H&P	History & Physical Exam
HRPP	Human Research Protections Program
IDE	Investigational Device Exemption
IHC	Immunohistochemistry
IND	Investigational New Drug
IV (or iv)	Intravenously
MRI	Magnetic Resonance Imaging
MTD	Maximum Tolerated Dose
NCI	National Cancer Institute
ORR	Overall Response Rate
OS	Overall Survival
PBMCs	Peripheral Blood Mononuclear Cells
pCR	Pathologic Complete Response
PD	Progressive Disease
PET	Positron Emission Tomography
PFS	Progression Free Survival
p.o.	per os/by mouth/orally
PR	Partial Response
RCB	Residual Cancer Burden
RECIST	Response Evaluation Criteria in Solid Tumors
SAE	Serious Adverse Event
SCCC	Simmons Comprehensive Cancer Center
SD	Stable Disease
SGOT	Serum Glutamic Oxaloacetic Transaminase
SPGT	Serum Glutamic Pyruvic Transaminase

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WBC

White Blood Cells

STUDY SCHEMA

**ER+/HER2-Negative Operable Breast Cancer, Stage I, II or III
in Post-menopausal Women**



**Paraffin block (preferably) or unstained slides from pre-treatment core biopsies for
IHC-based assays (Ki67, ER, PR, HER2)**



**Letrozole: 2.5 mg PO QD for 7-30 days
(last dose on the day before surgery)**



**Surgery
(Standard of Care: Mastectomy vs Lumpectomy)**

**Post-treatment frozen AND formalin fixed core biopsies (or surgical resection tissue when
intraoperative cores not feasible) for IHC-based assays (Ki67, ER, PR, HER2), and molecular
analysis**



**Follow up to assess disease status approximately every 6 months (+/- 1 month) for up to 10
years**

STUDY SUMMARY

Title	Pre-Surgical Trial of Letrozole in Post-Menopausal Patients with Operable Hormone-Sensitive Breast Cancer
Short Title	Letrozole in Post-Menopausal Patients with Operable Hormone-Sensitive Breast Cancer
Protocol Number	SCCC-11118
Phase	NA
Methodology	Exploratory investigational
Study Duration	15 years
Study Center(s)	UT Southwestern Medical Center and Parkland Health and Hospital System
Objectives	To determine gene expression and/ or mutational or proteomic signatures in breast tumors that continue to exhibit high proliferation (i.e., Ki67) upon hormone deprivation (with letrozole).
Number of Subjects	300
Diagnosis and Main Inclusion Criteria	Post-menopausal, ER-positive, HER2-negative stage I, II or III invasive mammary carcinoma
Study Product(s), Dose, Route, Regimen	Letrozole 2.5mg once daily.
Duration of administration	7-30 days, last dose on the day prior to surgical resection
Reference therapy	N/A
Statistical Methodology	For gene expression level and/or a mutational or proteomic signature parameters, we will use ANOVA to evaluate differences among three or more groups and unpaired two-sample t-test for differences between two groups stratified based on post-treatment Ki67 index. If underlying assumptions of these methods (e.g., normality and homoscedasticity) are not satisfied, we will use the nonparametric Kruskal-Wallis ANOVA for three or more group comparisons and the nonparametric Wilcoxon rank sum test for two group comparisons. Multiple comparisons will be adjusted. $P < 0.05$ will be considered statistically significant. We will split the data into training and validation datasets, and compute the prediction error to assess the model accuracy with cross validation.

1.0 Introduction

An estimated 175,000 new cases of breast cancer will be diagnosed this year in the United States and, despite adjuvant therapy with cytotoxic chemotherapy; many of these women will die of breast cancer. The heterogeneity of these tumors results in a highly varied response to current treatments. The developments of rationally-designed agents which target molecules differentially expressed in tumor have provided new prospects for the treatment breast cancer. These agents are in general tumor selective and therefore more clinically effective than chemotherapeutic drugs. Given that 75% of invasive breast cancers are either estrogen receptor (ER) or progesterone receptor (PR) positive, the most frequently utilized targeted therapies are those that modulate estrogen levels and/or ER function.

Estrogen receptor in breast cancer: Estrogen plays a critical role in the development and progression of breast cancers. The estrogen receptor (ER), a member of the nuclear receptor superfamily of transcription factors, mediates most of the actions of estrogen¹. There are two ERs, ER α and ER β . These are products of different genes and have similar but not identical structure. ER α is the most studied in breast cancer. Less is known about ER β and some data suggest it may opposing functions to those of ER α ^{2,3}. Estrogen signaling through ER occurs through distinct pathways. The classical mechanism of ER action involves estrogen binding to ER in the nucleus after which receptors dimerize and bind to specific estrogen-responsive elements (EREs) in the promoters of target genes⁴. A second non-classical mechanism involves ER-mediated gene expression without direct binding to DNA but through modulation of protein-protein interactions with other DNA-binding transcription factors⁵. Estrogen can also exert non-genomic actions which are too rapid to be accounted by gene transcription and RNA and protein synthesis. Reported nongenomic effects of 17 α -estradiol include direct or indirect activation of adenylate cyclase and production of cyclic AMP, mitogen activated protein kinase (MAPK), AKT, c-Src, Shc, the regulatory subunit of phosphoinositide 3-kinase (PI3K) p85, insulin-like growth factor I receptor (IGF-IR), epidermal growth factor receptors (EGFR), HER2 (ErbB2), and endothelial nitric oxide synthase (eNOS), among others⁶. Finally, amplified growth factor receptor signaling can post-translationally modify ERs and co-activators resulting in estrogen-independent transcriptional activity or ERs at EREs⁷.

Estrogen Modulation: The standard treatment for ER+ breast cancer includes therapies designed to block ER function. Selective ER modulators (SERMs), such as tamoxifen, block ligand binding to ER and partially block its activity. Ovarian ablation and luteinizing hormone-releasing hormone (LHRH) reduce the level of estrogen, thus inhibiting ligand-induced ER activation. Aromatase inhibitors (anastrozole, letrozole, and exemestane) do the same by blocking the main enzyme involved in the production of estrogen in post-menopausal women. Pure antiestrogens, such as fulvestrant bind ER and induce receptor degradation⁸. Several studies have suggested modest superiority of aromatase inhibitors over tamoxifen⁹⁻¹⁴.

Aromatase Inhibitors: Estrogen deprivation is a commonly utilized approach against hormone-dependent breast cancer. Aromatase inhibitors block the peripheral and intra-tumoral enzyme, p450 aromatase, which catalyzes the final step in estrogen synthesis¹⁵. These agents have the advantage of potentially blocking both genomic and non-genomic ER functions¹. Today, third-generation aromatase inhibitors, such as anastrozole (Arimidex), letrozole (Femara), and exemestane

(Aromasin) are currently the treatment of choice as first-line therapy of hormone-dependent metastatic disease in postmenopausal women and have been shown to be superior to tamoxifen in this setting¹⁰. In a pivotal double-blind phase III study, post-menopausal women with hormone receptor-positive or hormone receptor-unknown locally advanced or metastatic breast cancer received treatment with either letrozole or tamoxifen as first-line therapy. Letrozole not only showed

superior median time to progression compared to tamoxifen, but also superior time to treatment failure, overall response rate, and overall clinical benefit. There was a trend toward improved overall survival in the letrozole arm, but this was not significant. This study documented the superiority of letrozole over tamoxifen in first-line endocrine therapy in postmenopausal women with advanced breast cancer and was one of several trials demonstrating superiority of the aromatase inhibitors over tamoxifen in the first-line setting⁹.

The ATAC Study is a randomized trial compared anastrozole to tamoxifen and the combination of both as adjuvant therapy in over 9,000 post-menopausal women with early, ER-positive cancers. After a median follow-up of 33 months, there were fewer recurrences and fewer new primary breast cancers as well as a statistically better disease-free survival in the anastrozole arm compared to the other two treatments^{11, 12}. The MA.17 international Intergroup trial, initiated by the National Cancer Institute of Canada-Clinical Trials Group, was a double-blind, randomized, placebo-controlled trial performed in over 5,100 postmenopausal women with ER-positive or unknown primary breast cancer. It randomized patients who were disease-free after 5 years of adjuvant treatment with tamoxifen to an additional 5 years of treatment with letrozole or placebo. The planned duration of treatment for patients in the study was 5 years, but the trial was terminated early because of an interim analysis showing a favorable letrozole effect on time without recurrence or contralateral breast cancer¹³. An updated analysis was published more recently¹⁴. After a median follow-up of 30 months, women in the letrozole arm had statistically significantly better disease-free survival and distant disease-free survival than women in the placebo arm. Overall survival was the same in both arms. However, among lymph node-positive patients, overall survival was statistically significantly improved with letrozole¹⁴. Based on these studies, aromatase inhibitors have become the standard of care in post-menopausal women with hormone-dependent breast cancer.

Antiestrogen Resistance: While these therapies are effective initially in many patients, the overwhelming majority of ER+ breast cancers eventually become resistant to antiestrogens, ultimately resulting in disease progression and mortality. Several resistance mechanisms have been reported. These include the rare loss of ER by tumors as well as the increasingly frequent selection of cells with ESR1 activating mutations mainly within the ligand-binding domain (LBD). Ligand-independent ER-mediated transcription, and perturbation of the interaction ER and coactivators and corepressors of transcription¹ has been of particular interest. Alterations in the intracellular pharmacology including membrane-initiated steroid signaling (non-genomic) where cellular ER signaling occurs via direct interaction with growth factor signal transduction pathways. These can be activated by estrogen or SERMs such as Tamoxifen¹⁶. Activation of surface tyrosine kinase receptors such as IGF-IR, EGFR, and HER2 as well as kinases such as c-Src, PI3 kinase, MAPK, and AKT result in activation of ER or its coactivator proteins. This crosstalk between the ER receptor signaling pathway and growth factor receptor pathways can result in genomic activation of ER despite the presence of antiestrogens^{17,18}. SERMs may repress or activate transcription of estrogen-target genes depending on the absolute and relative levels of ER coregulator proteins^{19, 20}.

Upon the emergence of resistance, the agonistic effects of SERMs like tamoxifen can predominate, leading to breast tumor growth. However, very little clinical data exist to confirm these mechanisms in primary tumors.

Study Rationale: Pre-surgical and neoadjuvant studies with anti-cancer therapies have suggested that treatment-induced cellular or molecular endpoints in tumor tissues can correlate with clinical outcome. For example, treatment-induced tumor cell apoptosis, as measured by cleaved caspase-3

IHC, one week after administration of the HER2 antibody trastuzumab correlates with clinical response of HER2-overexpressing breast cancers ²¹. The neoadjuvant IMPACT trial compared the anastrozole, tamoxifen, and the combination. Drug-induced inhibition of tumor cell proliferation at 2 weeks, as measured by Ki67, was better in patients treated with anastrozole vs. the other two arms ^{22, 23}. This result parallels the result of the large ATAC trial where relapse-free survival was better in patients treated with adjuvant anastrozole compared to the other two arms ^{24, 25}. Taken together, these data suggest that the ‘intervened’ Ki67 index in ER+ surgically-removed breast cancers treated for a short time with tamoxifen or an aromatase inhibitor can be used to identify cancers that are highly sensitive to antiestrogens vs. those that are resistant or destined to recur faster (Fig 1). If so, tumors segregated as a function of a low or high Ki67 after a short pre-surgical period will harbor different somatic alterations, gene expression and/or protein signatures. In addition, we hypothesize that tumors with a high Ki67 after hormonal therapy will harbor genomic alterations, molecules or ‘pathways’ that are causally associated with antiestrogen resistance and/or a biomarker of such phenotype.

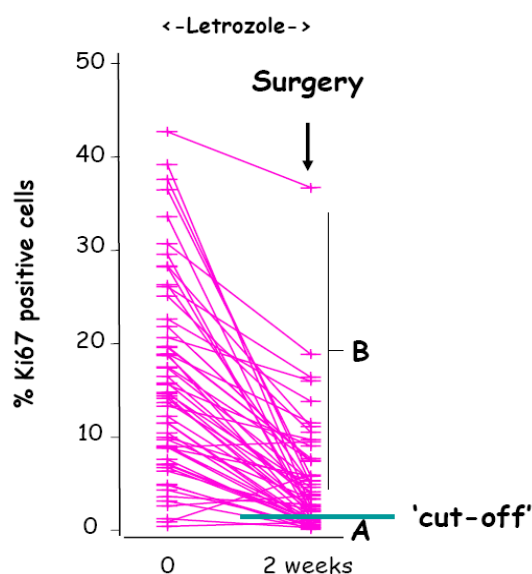


Figure 1. Clinical Trial Schema. Diagram shows the percentage of Ki67+ cells in pre- and post-anastrozole (2-week) tumor sections from biopsies of individual patients enrolled in the IMPACT trial (data provided by Mitch Dowsett). In this trial, patients continued therapy for a total of 3 months after a biopsy done at 2 weeks. In this application, we propose that letrozole will induce similar changes in tumor proliferation to those induced by anastrozole after a 2-week pre-surgical ‘window’. We hypothesize that 1) the Ki67 index measured in the surgical specimen will allow the segregation of highly hormone-dependent tumors vs. those that are not, and 2) tumors with high Ki67 in the surgical specimen will harbor genomic alterations, molecules or ‘pathways’ that are causally associated with antiestrogen resistance and/or a biomarker of such phenotype.

We propose a short pre-surgical non-therapeutic trial involving postmenopausal women with newly diagnosed ER+, HER2-Negative operable breast cancers. After undergoing a core needle biopsy

for tissue acquisition, study participants will take a 7 to 30-day course of letrozole in accordance with standard of care. They will then undergo definitive surgical resection of their primary tumor (mastectomy vs lumpectomy) as per standard of care guidelines. We should note that in all these patients, the standard of care will be at least 5 years of post-operative adjuvant letrozole – or another aromatase inhibitor. Pre and post treatment tumor tissue will be processed for routine histopathologic analysis and study analysis with:

1) Cell proliferation assay: Ki67 IHC.

In addition, post treatment tumor tissue will be processed for study analysis including:

1) DNA and RNA sequencing of tumor tissue

We hypothesize that:

1) The Ki67 index measured in the surgical specimen will allow the segregation of highly hormone-dependent tumors vs. those that are not. Molecular profiling of those tumors will identify genomic alterations and/or gene expression/protein alterations causally associated with endocrine resistance.

1.1 Letrozole

Femara (Letrozole) is a non-steroidal aromatase inhibitor (inhibitor of estrogen synthesis). It is an FDA approved agent from Novartis for the treatment of ER+ post-menopausal breast cancer.

1.11 Clinical Study Results

In a double-blind, phase III study, 907 post-menopausal women with hormone receptor-positive or hormone receptor-unknown locally advanced or metastatic breast cancer received treatment with either letrozole or tamoxifen as first-line therapy. Letrozole showed superior median time to progression (TTP) compared to tamoxifen (9.4 months vs. 6.0 months) ⁹. This trial was one of several supporting use of aromatase inhibitors over tamoxifen in post-menopausal patients in the metastatic setting.

The MA.17 International Intergroup Trial, initiated by the NCI of Canada-Clinical Trials Group, was a double-blind, randomized, placebo-controlled trial performed in over 5,100 postmenopausal women with ER+ or unknown primary breast cancer. This trial randomized patients who were disease-free after 5 years of adjuvant treatment with tamoxifen to an additional 5 years of treatment with letrozole or placebo. The planned duration of treatment for patients in the study was 5 years, but the trial was terminated early because of an interim analysis showing a favorable letrozole effect on time without recurrence or contralateral breast cancer¹³. An updated analysis was published more recently¹⁴. After a median follow-up of 30 months, women in the letrozole arm had statistically significantly better disease-free survival and distant disease-free survival than women in the placebo arm. Overall survival was the same in both arms. However, among lymph node-positive patients, overall survival was statistically significantly improved with letrozole. Letrozole is FDA approved for endocrine treatment of hormone-sensitive breast cancers in the adjuvant and metastatic settings.

Side effects encountered include hot flashes, anorexia, arthralgia, myalgia, and alopecia, which were all statistically significantly more common in those receiving letrozole; vaginal bleeding was statistically significantly more common in those receiving placebo. More patients receiving letrozole had a fracture, a new diagnosis of osteoporosis or cardiovascular disease on study, but

only the incidence of self-reported new osteoporosis was statistically significantly different between the two arms. Other side effects associated with the use of letrozole include, but are not limited to, nausea and vomiting, fatigue, headache, dizziness, swelling of the hands, feet, or lower legs, constipation, diarrhea, abdominal pain, and/or cough.

2.0 Objectives

2.0 Primary Objective

To determine that in breast tumors that continue to exhibit high proliferation (i.e., Ki67) upon hormone deprivation (with letrozole), their gene expression and/or a mutational or proteomic signatures will harbor molecules or ‘pathways’ that are biomarkers of resistance to endocrine therapy or a cause of it.

The ultimate goal of these aims is to identify clinically-targetable pathways which can

be exploited to enhance responses and survival in patients with ER+ breast cancer. This study will also provide a platform of ER+ tumors with pharmacodynamic information following estrogen suppression in vivo (Ki67) that can be used to segregate tumors into 'responders' and 'not responders' where we can focus the unbiased discovery of mechanisms of resistance to endocrine therapy

3.0 Eligibility Criteria

3.0 Eligibility waivers are not permitted. Subjects must meet all of the inclusion and exclusion criteria to be registered to the study. Study treatment may not begin until a subject is registered.

3.1 Patients must provide informed written consent

3.2 ECOG performance status 0-2.

3.3 Clinical stage operable I, II or III invasive mammary carcinoma, which is ER-positive by IHC and HER2-negative by Herceptest (0 or 1+) or not amplified by in situ hybridization as per routine clinical testing

3.3.1 Patients who have measurable residual tumor at the primary site

3.3.2 Patients who will undergo surgical treatment with either segmental resection or total mastectomy

3.4 Measurable tumor

Measurable disease: a mass that can be reproducibly measured by physical exam, mammogram or ultrasound and is at least 1 cm in size

3.5 Post-menopausal female subjects ≥ 18 years of age, as defined by any of the following:

- Subjects at least 55 years of age;
- Subjects under 55 years of age and amenorrhoeic for at least 12 months or follicle-stimulating hormone (FSH) values ≥ 40 IU/L and estradiol levels ≤ 40 pg/mL (140 pmol/L) or in postmenopausal ranges per local or institutional reference ranges;
- Prior bilateral oophorectomy or prior radiation castration with amenorrhea for at least 6 months.

(There is no upper age limit for enrollment to this study)

3.6 No prior chemotherapy for this primary breast cancer.

3.7 Patients with a prior history of contralateral breast cancer are eligible if they have no evidence of recurrence of their initial primary breast cancer.

3.8 Women may have been taking tamoxifen or raloxifene as a preventive agent prior to study entry but must have discontinued the drug for at least 14 days prior to study enrollment.

3.9 Subjects must have ended hormone replacement therapy (HRT) (e.g., conjugated estrogens tablets, USP, [Premarin]), at least 7 days prior to receiving the first dose of randomized therapy.

3.10 Patients must have adequate hepatic and renal function. All tests must be obtained less than 4 weeks from study entry. This includes:

Creatinine $\leq 2 \times$ upper limits of normal

3.11 Able to swallow and retain oral medication

4.0 Exclusion Criteria

Patients with locally advanced disease who are candidates for other preoperative chemotherapy at the time of initial evaluation. This may include patients with locally advanced disease such as:

4.0.1 Inflammatory breast cancer (T_{4d})

4.0.2 Fixed axillary lymph node metastases (N₂)

4.0.3 Metastasis to ipsilateral internal mammary node (N₃)

4.1 Locally recurrent breast cancer

4.2 Evidence of distant metastatic disease (i.e. lung, liver, bone, brain, etc.)

4.3 Serious medical illness that in the judgment of the treating physician places the patient at high risk of operative mortality.

4.4 Severe uncontrolled malabsorption condition or disease (i.e. grade II/III diarrhea, severe malnutrition, short gut syndrome)

4.5 Dementia, altered mental status, or any psychiatric condition that would prohibit the understanding or rendering of informed consent.

4.6 Use of an investigational drug within 30 days or 5 half-lives, whichever is longer, preceding the first dose of letrozole.

5.0 Registration Procedures

All subjects must be registered with the research office before enrollment to study. Prior to registration, eligibility criteria must be confirmed with the Study Coordinator. All subjects will be registered in the Velos Clinical Management System.

Upon confirmation of eligibility and enrollment as per the afore-mentioned instructions, the subject will be assigned a secondary number in the order of enrollment. All the subject numbers will end in “LETROZOLE”. For example, subject 001-LETROZOLE will become 001-01-LETROZOLE upon enrollment. If subject 002-LETROZOLE screen fails, and subject 003-LETROZOLE is the next subject enrolled, subject 003-LETROZOLE will become 003-02-LETROZOLE and so-on.

Each newly consented subject should be numbered using the schema provided above. Upon Registration in Velos, the study coordinator will assign the additional registration code according to the numbering schema outlined above and maintain the subject’s status throughout the life of the study.

The numbering schema should clearly identify the sequential number of the subject enrolled as well as the status of the subjects enrolled so that the number of subjects consented versus the number of subjects actually enrolled may be easily identified in the Velos system.

To be eligible for registration to the study, the participant must meet each inclusion and exclusion criterion listed in the eligibility checklist.

Note: All study documents should be in hand 24-48 hours prior to the patient's anticipated start date.

Upon satisfactory review of eligibility documents submitted, the Principal Investigator will approve enrollment. Once registration/enrollment is confirmed, proceed with protocol procedures.

Issues that would cause treatment delays should be discussed with the Protocol Chair. Any requests for eligibility exceptions and/or deviations must be approved in writing by the Principal Investigator, the Data and Safety Monitoring Committee, and the UT Southwestern IRB.

As is generally accepted, standard of care procedures performed prior to consent, but within the protocol defined screening window for each assessment, can be used for study purposes. All research-only procedures must be performed after patient consent.

6.0 Study Schedule of Events

Tests	Pre-Study	At the time of Surgery	Post - Op
History & Physical	X ¹		X ¹
Performance Status	X ¹		X ¹
CMP ²	X ¹		
Tumor Specimen	X ^{1,3}	X ^{1,4}	X ^{1,4}
(fixed, paraffin embedded)	X ^{1,3}	X ^{1,4}	X ^{1,4}
(frozen section)		X ^{1,4}	X ^{1,4}
Prescription Given Letrozole 2.5mg	X		
Pill Diary	X ⁶		X ⁶
Follow Up			X ⁷
Peripheral Blood Collection (Purple top) 10ml	X ⁵		

1. Performed as routine medical assessment if indicated
2. Comprehensive Metabolic Panel: Creatinine, glucose, total bilirubin, alkaline phosphatase, and SGOT/SGPT within 4 weeks.
3. We will need access to archived tumor sections from the diagnostic paraffin block (or 10 unstained slides) from the initial diagnostic core biopsy at some point during the study. These will be requested when appropriate and sent to:
Sunati Sahoo, M.D., Department of Pathology/UTSW, 5323 Harry Hines Blvd., Dallas, TX 75390-9072, Telephone: (214) 633-6361.
4. A tumor sample will be obtained from the surgical specimen at time of surgical resection, and flash frozen whenever possible. A companion formalin fixed paraffin embedded tissue sample should also be prepared. Both samples should be delivered (snap frozen tissue in liquid nitrogen) directly to: Saurabh Mendiratta, 6001 Harry Hines Blvd. NB5.112. Dallas, TX- 75390-8807
Phone: 214-648-6167.

5. One 10-ml purple top tube should be collected from all enrolled patients. This blood may be collected at any time during or after the study; however, ideally the specimen should be collected at the time of enrollment.
6. Will be given to patients along with medication and will be completed and returned at the post-operative visit.
7. Enrolled patients will be followed approximately every 6 months for up to 10 years. Follow up information may be collected from the patient's medical record if they are still coming to the respective clinic or they will be contacted by telephone. If during this follow up period, the patient experiences a recurrence of disease and undergoes a standard of care biopsy, a sample of this tumor

tissue may be obtained for additional research testing, pending there is leftover tumor tissue available and the patient agrees to provide the sample.

7.0 Treatment Plan

7.1 Letrozole Treatment

Patients will receive a prescription for letrozole to be taken orally at a dose of 2.5 mg/day for 7-30 days to allow for variations in surgical scheduling. Patients are to undergo surgical resection of their tumor the day after the last dose of letrozole.

7.2 Tissue Specimen Acquisition

Post-treatment tumor biopsies (a goal of 1-6 cores) will be obtained following 7-30 days of letrozole treatment on the day of surgery. Frozen AND formalin fixed core biopsies will be obtained whenever possible. The tissue will be used for study-specific assays as well as routine histopathology. Post-treatment core needle biopsy tissue will be obtained, whenever possible, by the surgeon intraoperatively at the time of routine surgical resection. A formalin-fixed paraffin embedded tumor block from the patient's surgical resection is also requested. This block will be used for a subset of the correlative studies if no tumor is obtained by the post treatment core biopsies. This block will be returned to pathology promptly after the correlative studies are completed.

*If post-treatment needle core biopsies cannot be performed, fresh tissue may be procured from the surgical specimen according to institutional guidelines. Tissue must be procured as rapidly as possible after the specimen is removed from the patient, preferably within 5-10 minutes. This option is suboptimal and discouraged as tissue degradation will affect the study specific assays. We realize; however, this may be unavoidable in some cases.

Instructions on fresh tissue specimen handling:

According to the following schedule, core biopsy specimens should be immediately flash frozen in liquid nitrogen* OR placed in 10% neutral buffered formalin immediately

following removal from the patient following procedures established by the Simmons Cancer Center Tissue Core.

- For the formalin-fixed cores, an approximately 20:1 volume of formalin to tissue should be used. Core biopsies placed in 10% neutral buffered formalin must fix at room temperature for at least 6 h and no more than 24 h. Core biopsies must be transferred from formalin to 70% ethanol after 6-24 h to prevent over fixation and placed in the refrigerator.

- Label ALL cryovials before freezing (see specific instructions #1 below).
- Send all samples to Saurabh Mendiratta at the address mentioned earlier.

# Cores Obtained	# cores flash frozen	# cores in 10% NBF
1	<u>1</u>	0
2	<u>2</u>	0
3	<u>2</u>	1
4	<u>3</u>	1
5	<u>3</u>	2
6	<u>4</u>	2

Tissue specimen labeling and documentation:

1. Label each cryovial and formalin-filled collection container with the patient name, Medical Record #, Patient study ID #, location of biopsy, collection date and time of collection using an ethanol resistant marker. Each set of patient samples should be accompanied by a Tissue Registration form.

7.3 Definitive Surgery

Surgical treatment (total mastectomy or segmental resection with lymph node evaluation if clinically indicated) will occur the day after completion of therapy.

The primary lesion obtained at the time of the definitive surgical procedure (partial or total mastectomy) will be sent for standard of care histopathologic analysis; wherever possible intra-operative cores from the central portion of the tumor will be obtained for study-specific assays. All specimens will be handled according to established institutional guidelines to maintain the accuracy of the analysis of tumor size and margin status.

7.4 Pathology

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Following standard of care histopathologic analysis, additional paraffin-embedded sections will be submitted at a later time to the Simmons Cancer Tissue Core to determine tumor proliferation with Ki67 (MIB1Ab, Dako Cytomation) IHC. These tests are of no clinical utility and will be done for research purposes only.

7.5 Further Testing

Frozen cores and/or peels from the formalin-fixed paraffin-embedded (FFPE) tumor blocks from the surgical specimen will be selected and/or macrodissected – when needed – based on $\geq 20\%$ tumor cellularity as assessed by Dr. Sahoo, expert breast pathologist in the trial. These will be sent to Dr. Carlos Arteaga's laboratory for further testing.

7.6 Blood and Negative Tissue Sample:

One 10 ml purple top tube should be collected from all enrolled patients. This blood may be collected at any time during or after the study; however, ideally the specimen should be collected before the patient receives any subsequent therapy. This blood is requested for the purpose of obtaining a buffy coat specimen to be banked for future nucleic acid extraction. This specimen will serve as a highly pure source of patient germline DNA for the correlative studies for this protocol.

The blood samples will be logged in, barcoded and processed by a dedicated research team member within 6 hours of collection.

All specimens will be stored in a locked freezer and subsequently distributed in a de-identified manner as needed for the correlative studies. Only the principal investigators and their designees will know the identity of these participants.

7.7 Follow Up Information

Patients that complete the course of letrozole and have their surgery will be followed for disease recurrence. This follow up will be approximately every six months from the patient's surgery date for up to ten years. The patient's disease status will be collected from their medical record, if available. In the event that follow up information is not available in the patient's medical record, a member of the research team will contact the patient.

8.0 Adverse Event Reporting

8.1 Adverse Event Monitoring

Adverse event data collection and reporting, which are required as part of every clinical trial, are done to ensure the safety of subjects enrolled in the studies as well as those who will enroll in future studies using similar agents. Adverse events are reported in a routine manner at scheduled

times during a trial. Additionally, certain adverse events must be reported in an expedited manner to allow for optimal monitoring of subject safety and care.

All subjects experiencing an adverse event, regardless of its relationship to study therapy, will be monitored until:

- the adverse event resolves or the symptoms or signs that constitute the adverse event return to baseline; any abnormal laboratory values have returned to baseline;
- there is a satisfactory explanation other than the study therapy for the changes
- observed; or death.

[Inserted from Adverse Event Definitions and Reporting Section – Appendix IB SCCC DSMC Plan]

8.1.1 Definition

The study will use the CTCAE version 5.0 for reporting. Adverse Events will be reported as

indicated by the appropriate following table (see below).

An adverse event is defined as any untoward or unfavorable medical occurrence in a human research study participant, including any abnormal sign (for example, abnormal physical exam or laboratory finding), symptom, clinical event, or disease, temporarily associated with the subject's participation in the research, whether or not it is considered related to the subject's participation in the research.

Adverse events encompass clinical, physical and psychological harms. Adverse events occur most commonly in the context of biomedical research, although on occasion, they can occur in the context of social and behavioral research. Adverse events may be expected or unexpected. Adverse event & Serious adverse event will only be collected till the last day of Letrozole dose taken.

Severity

Adverse events will be graded by a numerical score according to the defined NCI Common Terminology Criteria for Adverse Events (NCI CTCAE) and version number specified in the protocol. Adverse events not specifically defined in the NCI CTCAE will be scored on the Adverse Event log according to the general guidelines provided by the NCI CTCAE and as outlined below.

- Grade 1: Mild
- Grade 2: Moderate
- Grade 3: Severe or medically significant but not immediately life threatening
- Grade 4: Life threatening consequences
- Grade 5: Death related to the adverse event

Serious Adverse Events

ICH Guideline E2A and the UTSW IRB define serious adverse events as those events, occurring at any dose, which meets any of the following criteria:

- Results in death
- Immediately life-threatening
- Results in inpatient hospitalization or prolongation of existing hospitalization
- Results in persistent or significant disability/incapacity
- Results in a congenital anomaly/birth defect
- Based upon appropriate medical judgment, may jeopardize the subject's health and may require medical or surgical intervention to prevent one of the other outcomes listed in this definition.

Note: A "Serious adverse event" is by definition an event that meets any of the above criteria. Serious adverse events may or may not be related to the research project. A serious adverse event determination does not require the event to be related to the research. That is, both events completely unrelated to the condition under study and events that are expected in the context of the condition under study may be serious adverse events, independent of relatedness to the study itself. As examples, a car accident requiring overnight hospitalization would be a serious adverse event for any research participant; likewise, in a study investigating end-stage cancer care, any hospitalization or death which occurs during the protocol-specified period of monitoring for

adverse and serious adverse events would be a serious adverse event, even if the event observed is a primary clinical endpoint of the study.

8.1.2 Unanticipated Problems Involving Risks to Subjects or Others (UPIRSOs):

The term “unanticipated problem” is found, but not defined in the regulations for the Protection of Human Subjects at 45 CFR 46, and the FDA regulations at 21 CFR 56. Guidance from the regulatory agencies considers unanticipated problems to include any incident, experience, or outcome that meets each of the following criteria:

- Unexpected (in terms of nature, severity or frequency) AND
- Definitely or probably related to participation in the research AND
- Serious or a possible unexpected problem in that the research places subjects or others at greater risk of harm than was previously known or recognized. Note: Any serious adverse event would always suggest a greater risk of harm.

Follow-up

All adverse events will be followed up according to good medical practices.

8.1.3 Reporting

The UTSW IRB requires reporting of all UPIRSOs according to the guidance below. For participating centers other than UTSW, local IRB guidance should be followed for local reporting of serious adverse events. All SAEs occurring during the protocol-specified monitoring

period should be submitted to the UTSW study team within 2 business days of the center learning of the event.

UPIRSOs occurring on the study require expedited reporting, and are submitted to the UTSW IRB through the UTSW eIRB by the UTSW study team and to the SCCC DSMC Coordinator. Hardcopies or electronic versions of the eIRB report; FDA Form #3500A forms, or other sponsor forms, if applicable; and/or any other supporting documentation available should be submitted to the UTSW study team and will be forwarded to the DSMC Coordinator. The DSMC Coordinator forwards the information onto the DSMC Chairman who determines if immediate action is required. Follow-up eIRB reports, and all subsequent SAE documentation that is available are also submitted to the DSMC Chair who determines if further action is required. *(See Appendix IV of the SCCC DSMC Plan for a template Serious Adverse Event Form which may be utilized when a sponsor form is unavailable and SAE submission to the eIRB is not required).*

All serious adverse events which occur on research subjects on protocols for which the SCCC is the DSMC of record require reporting to the DSMC regardless of whether IRB reporting is required. Hardcopies or electronic versions of the FDA Form #3500A forms, or other sponsor forms, if applicable; and/or any other supporting documentation available should be forwarded to the DSMC Coordinator.

If the event occurs on a multi-institutional clinical trial coordinated by the UTSW Simmons Cancer Center, the DOT Manager or lead coordinator ensures that all participating sites are notified of the event and resulting action, according to FDA guidance for expedited reporting. DSMC Chairperson reviews all serious adverse events upon receipt from the DSMC

Coordinator. The DSMC Chairperson determines whether action is required and either takes action immediately, convenes a special DSMC session (physical or electronic), or defers the action until a regularly scheduled DSMC meeting.

Telephone reports to:

Investigator: Nisha Unni

Telephone Number: 214-648-4180

UTSW SCC Data Safety Monitoring Committee Coordinator (if fax report is not available) within 1 working day to 214-648-7097.

Written reports to:

Investigator: Nisha Unni

Email: nisha.unni@utsouthwestern.edu

UTSW SCC Data Safety Monitoring Committee Coordinator

Email: SCCDSMC@utsouthwestern.edu

Fax: 214-648-5949 or deliver to BLB.306

CC - STU2018-0015 Unni Form A3 - Prot Amend 3

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Study Number – SCCC-11118

UTSW Institutional Review Board (IRB)

Submit via eIRB with a copy of the final sponsor report as attached supporting documentation

1. SAEs

Serious adverse events (SAEs) for studies where the SCCC DSMC is the DSMC of record require reporting to the DSMC coordinator within 2 working days of PI awareness, or as described in the protocol.

2. Unanticipated Problems Involving Risks to Subjects or Others (UPIRSOs)

Local Serious Adverse Event UPIRSOs require reporting to the UTSW IRB within 48 hours of PI awareness of the event (life threatening or fatal events experienced by subjects enrolled by the investigator(s) under UTSW IRB jurisdiction).

Local UPIRSOs (non-serious events experienced by subjects enrolled by the investigator(s) under UTSW IRB jurisdiction) require reporting to the UTSW IRB within 5 business days of PI awareness of the event.

External UPIRSOs including those that occur as non-local events require reporting to the UTSW IRB within 10 working days of PI awareness of the event.

For further guidance for Investigators regarding safety reporting requirements for INDs and BA/BE studies, refer to FDA Draft Guidance document:

<http://www.fda.gov/downloads/Drugs/GuidanceComplianceRegulatoryInformation/Guidances/UCM227351.pdf>

8.2 Steps to Determine If an Adverse Event Requires Expedited Reporting

Step 1: Identify the type of adverse event using the NCI Common Terminology Criteria for Adverse Events (CTCAE v5).

Step 2: Grade the adverse event using the NCI CTCAE v5.

Step 3: Determine whether the adverse event is related to the protocol therapy

Attribution categories are as follows:

- Definite – The AE is clearly related to the study treatment.
- Probable – The AE is likely related to the study treatment.
- Possible – The AE may be related to the study treatment.
- Unrelated – The AE is clearly NOT related to the study treatment.

Note: This includes all events that occur within 30 days of the last dose of protocol treatment.

Any event that occurs more than 30 days after the last dose of treatment and is attributed (possibly, probably, or definitely) to the agent(s) must also be reported accordingly.

Step 4: Determine the prior experience of the adverse event.

Expected events are those that have been previously identified as resulting from administration of the agent. An adverse event is considered unexpected, for expedited reporting purposes only, when either the type of event or the severity of the event is not listed in:

- The current known adverse events listed in the Agent Information Section of this protocol;
- The drug package insert;
- The current investigator's Brochure

9.0 Dose Modifications and Management of Toxicity

Toxicity grading is based on the NCI-CTC Version 5.0. All NCI CTCAE grade 3 or 4 toxicities will result in Letrozole interruption.

9.1 Dose Reductions for Letrozole

There will be no dose reductions of letrozole. If a subject is required to stop treatment with letrozole for more than 3 days, the Principal Investigator must be consulted. Letrozole must be taken continuously for a minimum of 7-30 days.

9.2 Other

Other serious adverse events or grade 3 or 4 toxicity considered to be related to letrozole should be managed with symptomatic support and letrozole discontinued.

10. Drug Information

Other Names: Femara

Classification: Non-steroidal aromatase inhibitor (inhibitor of estrogen synthesis)

Mode of Action: Aromatase inhibitors inhibit the peripheral and intra-tumoral enzyme p450 aromatase, which catalyzes the final step in estrogen synthesis. Depriving ER of its ligand is an effective endocrine therapy. These agents have the advantage of potentially blocking genomic and non-genomic ER functions.

Storage and Stability: Letrozole is stored at room temperature.

Dose: 2.5 mg by mouth once a day for 7 – 30 days

Route of Administration: Oral. Tablets should be taken preferably in the morning with up to 200 ml of water and should be taken 1 hour before or 2 hours after meals. Patients who are unable to swallow tablets may dissolve the tablets in distilled water for administration.

Incompatibilities: None known.

Availability: Patients will be given a prescription for letrozole 2.5 mg to be taken by mouth daily

Interactions: Treatment of women with letrozole significantly lowers serum estrone, estradiol and estrone sulfate and has not been shown to significantly affect adrenal corticosteroid synthesis, aldosterone synthesis, or synthesis of thyroid hormones. Clinical interaction studies with cimetidine and warfarin indicated that the co-administration of letrozole with these drugs does not result in clinically-significant drug interactions.

11.0 Removal of Patients from Protocol Therapy

11.1 Disease Progression

Contra-lateral breast cancer is not considered disease progression. If the patient progresses during therapy with letrozole, she should proceed to surgery and be taken off letrozole and treated at the discretion of the treating physician. To our knowledge, this has never been reported to occur after such short treatment with an aromatase inhibitor like letrozole.

11.2 Extraordinary Medical Circumstances:

If at any time the constraints of this protocol are detrimental to the patient's health, letrozole should be discontinued. In this event:

- Notify the principal investigator in writing
- Document reason(s) for treatment termination on the flow sheets

11.3 If patient wishes to be taken off study

11.4 If the patient does not recover from toxicity in a timely fashion as outlined in section

11.5 Patient non-compliance with protocol

12.0 Statistical Considerations

The main objective of this Aim is to use the Ki67 index measured 7 – 30days after therapy with letrozole to segregate hormone receptor-positive breast cancers that are highly hormone-dependent vs. those that are not. For this analysis, we had access to the raw Ki67 data from both the IMPACT trial³⁶ (provided by Mitch Dowsett, Royal Marsden Hospital, London) and from the P024 trial²² (provided by Matt Ellis). With these data, we initially calculated that with 125 ER+ patients, 33% or 42 of them will exhibit a $\leq 1\%$ Ki67 after 2 weeks of treatment with the aromatase inhibitor. This will provide at least 90% power to detect a 2-fold difference in gene expression level between $\leq 1\%$ Ki67 (n=42) and $>1\%$ Ki67 (n=83) based on a conservative assumption that the standard deviation is 0.7 (base-two logarithmic scale) and the acceptable number of false positives is 0.01. In addition, 40 more patients will be enrolled to test the accuracy of the statistical model that will be developed based on the initial 125 patients. Therefore, a total sample size of 165 will not only yield an excellent power but also have the ability to examine the model accuracy.

Dr. Carlos L. Arteaga, conducted a similar study that so far has enrolled over 200 patients³⁴. Participation in this study has not resulted in any side effects and/or delays in surgery. Preliminary

analysis of the molecular data is already identifying novel potential mechanism of resistance to estrogen deprivation in ER+ breast cancer. We anticipate further discoveries with expansion of this trial as well as a source of tumors that we can use for validation of the initial findings.

Estimated Annual Accrual: Combined, UTSW Simmons Cancer Center and Parkland Hospital oncology clinic sees approximately 300-350 hormone-receptor positive breast cancer patients/year. This represents approximately 10% increase since 2004. We anticipate an annual growth rate of approximately 5%/year over the next 3 years. Eighty percent are patients with stage I or II breast

cancers. We estimate that 1-2 patients per week will be potential candidates for this trial. Patient accrual will be monitored closely by the Disease Oriented Team (DOT).

For gene expression level and/or a mutational or proteomic signature parameters, we will use ANOVA to evaluate differences among three or more groups and unpaired two-sample t-test for differences between two groups stratified based on post-treatment Ki67 index. If underlying assumptions of these methods (e.g., normality and homoscedasticity) are not satisfied, we will use the nonparametric Kruskal-Wallis ANOVA for three or more group comparisons and the nonparametric Wilcoxon rank sum test for two group comparisons. Multiple comparisons will be adjusted. $P < 0.05$ will be considered statistically significant. We will also split the data into training and validation datasets, and compute the prediction error to assess the model accuracy with cross validation.

13.0 Data Safety

This study will not provide direct clinical benefit to patient participants. Its purpose is to establish molecular profiles that will stratify ER positive breast cancers according to antiestrogen resistance and risk of recurrence. The data generated by this study will help the design of future clinical trials and may offer benefit to patients enrolled in those controlled trials. This is explained in the consent form. We should note that in all the patients enrolled in this study, the FDA-approved standard of care will be at least 5 years of post-operative adjuvant letrozole – or another aromatase inhibitor. Risks to subjects will be minimized by screening to assure appropriate selection of participants, strict inclusion and exclusion criteria, close monitoring, and safety monitoring and reporting. The procedures are consistent with sound research design.

We are proposing to use well-tolerated doses of letrozole in a very short time interval. Therefore, it is almost unheard of that we will encounter unacceptable toxicity from administration of this drug. We should emphasize that, based on current standards of care, all patients enrolled in this trial, will meet criteria for adjuvant letrozole for 5 years.

After signing consent, all patients must pass the screening procedure (detailed in the eligibility criteria in the protocol) to be eligible for the study. Every effort will be made to instruct the patient of the potential side effects of the treatment and of measures to mitigate them. Appropriate dose modifications of standard and study drugs secondary to toxicities are detailed in the protocol. The importance of notifying the principal investigator, the research nurse, or treating physician of ANY adverse event will be stressed. All serious adverse events will be promptly reported to all participating sites IRB and sponsoring institutions as outlined in the protocol.

The Principal Investigator or designee will inform the IRB of any serious adverse event in accordance with the institution's IRB policy. The investigator is responsible for the detection

and documentation of events meeting the criteria and definition of an AE or SAE, as provided in this protocol. During the study when there is a safety evaluation, the investigator or site staff will be responsible for detecting, documenting, and report AEs and SAEs, as detailed in the protocol. Furthermore, the investigator will be required to provide periodic safety updates on the conduct of the study at his/her site and notification of study closure to the participating sites IRB.

This study will be conducted in accordance with "good clinical practice" (GCP) and all applicable regulatory requirements, including, where applicable, the 1996 version of the Declaration of Helsinki. The investigator is responsible for ensuring that this protocol, the site's informed consent document, and any other information that will be presented to potential subjects (e.g., advertisements or information that supports or supplements the informed consent) are reviewed and approved by the appropriate IEC/IRB. The investigator agrees to allow the IEC/IRB direct access to all relevant documents. The IEC/IRB must be constituted in accordance with all applicable regulatory requirements.

14.0 Monitoring and Quality Assurance

Trial monitoring will be conducted no less than annually and refers to a regular interval review of trial related activity and documentation performed by the DOT, which includes but is not limited to accuracy of case report forms, protocol compliance, timeliness and accuracy of Velos entries and AE/SAE management and reporting. Documentation of trial monitoring will be maintained along with other protocol related documents and will be reviewed during internal audit.

The UTSW Simmons Cancer Center (SCC) Data Safety Monitoring Committee (DSMC) is responsible for monitoring data quality and patient safety for all UTSW SCC clinical trials. As part of that responsibility, the DSMC reviews all local serious adverse events and unanticipated problems in real time as they are reported and reviews adverse events on a quarterly basis. The quality assurance activity for the Clinical Research Office provides for periodic auditing of clinical research documents to ensure data integrity and regulatory compliance. A copy of the DSMC plan is available upon request.

The SCC DSMC meets quarterly and conducts annual comprehensive reviews of ongoing clinical trials, for which it serves as the DSMC of record. The QAC works as part of the DSMC to conduct regular audits based on the level of risk. Audit findings are reviewed at the next available DSMC meeting. In this way, frequency of DSMC monitoring is dependent upon the level of risk. Risk level is determined by the DSMC Chairman and a number of factors such as the phase of the study; the type of investigational agent, device or intervention being studied; and monitoring required to ensure the safety of study subjects based on the associated risks of the study. Protocol-specific DSMC plans must be consistent with these principles.

15.0 Changes in Protocol

Any change to the informed consent document must be reviewed and approved by the Principal

Investigator before being submitted to the Institutional Review Board/Independent Ethics Committee at participating institutions. Amendments should not be implemented until all necessary approvals have been obtained, except when necessary to eliminate an immediate hazard to study subjects.

16.0 Protocol Deviations

The Principal Investigator is responsible for implementing and maintaining quality assurance and quality control to ensure that studies are conducted according to the protocol, GCP, and all applicable regulatory requirements. A protocol deviation is any noncompliance with the protocol. Noncompliance can be on the part of the study participant, the Investigator, or the study site staff. Deviations to the protocol are not permitted except when necessary to eliminate an immediate hazard to study subjects.

17.0 Data Management

REDCap is the UTSW SCCC institutional choice for the electronic data capture of case report forms for this and all SCCC Investigator Initiated Trials. REDCap will be used for electronic case report forms in accordance with Simmons Comprehensive Cancer Center requirements.

Trial monitoring will be conducted no less than annually and refers to a regular interval review of trial related activity and documentation performed by the DOT, which includes but is not limited to accuracy of case report forms, protocol compliance, timeliness and accuracy of Velos entries and AE/SAE management and reporting. Documentation of trial monitoring will be maintained along with other protocol related documents and will be reviewed during internal audit.

The UTSW Simmons Comprehensive Cancer Center (SCCC) Data Safety Monitoring Committee (DSMC) is responsible for monitoring data quality and patient safety for all UTSW SCCC clinical trials. As part of that responsibility, the DSMC reviews all serious adverse events and UPIRSOs in real time as they are reported and reviews adverse events on a quarterly basis. The quality assurance activity for the Clinical Research Office provides for periodic auditing of clinical research documents to ensure data integrity and regulatory compliance. A copy of the DSMC plan is available upon request.

The SCCC DSMC meets quarterly and conducts annual comprehensive reviews of ongoing clinical trials, for which it serves as the DSMC of record. The Quality Assurance Coordinator (QAC) works as part of the DSMC to conduct regular audits based on the level of risk. Audit findings are reviewed at the next available DSMC meeting. In this way, frequency of DSMC monitoring is dependent upon the level of risk. Risk level is determined by the DSMC Chairman and a number of factors such as the phase of the study; the type of investigational agent, device or intervention being studied; and monitoring required to ensure the safety of study subjects based on the associated risks of the study. Protocol-specific DSMC plans must be consistent with these principles

18.0 Data Verification

The Principal Investigator is responsible for the conduct of the clinical trial at the site in accordance with Title 21 of the Code of Federal Regulations and/or the Declaration of Helsinki. The Principal Investigator is responsible for personally overseeing the treatment of all study patients.

The Principal Investigator must assure that all study site personnel, including sub-investigators and other study staff members, adhere to the study protocol and all FDA/GCP/NCI regulations and guidelines regarding clinical trials both during and after study completion.

The Principal Investigator at each institution or site will be responsible for assuring that all the required data will be collected and entered onto the Case Report Forms. Periodically, monitoring visits may be conducted and the Principal Investigator will provide access to his/her original

records to permit verification of proper entry of data. At the completion of the study, all case report forms will be reviewed by the Principal Investigator and will require his/her final signature to verify the accuracy of the data.

Data will be collected via paper CRFs and entered into the Redcap database. The study team will check data accuracy by performing source data verification. Source data verification is a direct comparison of the entries made on the CRFs against the appropriate source documentation. Discrepancies in the data will be brought to the attention of the Investigator and/or the Investigator's staff. Any necessary corrections will be made directly to the CRFs or via queries by the Investigator and/or the Investigator's staff.

19.0 Adherence to the Protocol

Except for an emergency situation, in which proper care for the protection, safety, and well-being of the study subject requires alternative treatment, the study shall be conducted exactly as described in the approved protocol.

Exceptions (also called single-subject exceptions or single-subject waivers): include any departure from IRB-approved research that is *not due to an emergency* and is:

- intentional on part of the investigator; or
- in the investigator's control; or
- not intended as a systemic change (e.g., single-subject exceptions to eligibility [inclusion/exclusion] criteria)

Reporting requirement*: Exceptions are non-emergency deviations that require *prospective* IRB approval before being implemented. Call the IRB if your request is urgent. If IRB approval is not obtained beforehand, this constitutes a major deviation. For eligibility waivers, studies which utilize the SCCC-DSMC as the DSMC of record must also obtain approval from the DSMC prior to submitting to IRB for approval.

Emergency Deviations: include any departure from IRB-approved research that is necessary to:

- avoid immediate apparent harm, or
- protect the life or physical well-being of subjects or others

Reporting requirement*: Emergency deviations must be promptly reported to the IRB within 5 working days of occurrence.

Serious Noncompliance (formerly called **major deviations** or **violations**): include any departure from IRB-approved research that:

- Increase risk of harm to subjects; and/or
- adversely affects the rights, safety, or welfare of subjects (any of which may also be an unanticipated problem); and/or
- adversely affects the integrity of the data and research (i.e., substantially compromises the integrity, reliability, or validity of the research)

Reporting requirement*: Serious Noncompliance must be promptly reported to the IRB within 5 working days of discovery.

Continuing Noncompliance: includes a pattern of repeated noncompliance (in one or more protocols simultaneously, or over a period of time) which continues **after** initial discovery, including inadequate efforts to take or implement corrective or preventive action within a reasonable time frame.

Reporting requirement*: Continuing Noncompliance must be promptly reported to the IRB within 5 working days of discovery.

Noncompliance (that is neither serious nor continuing; formerly called minor deviations) any departure from IRB-approved research that:

- Does not meet the definition of serious noncompliance or continuing noncompliance
- Reporting requirement***: Noncompliance that is neither serious nor continuing should be tracked and summarized at the next IRB continuing review, or the notice of study closure-whichever comes first..

*Reporting Requirements reflect UTSW HRPP/IRB guidelines; participating sites should follow the reporting guidelines for their IRB of record

20.0 Study Documentation

Pre-Study Documentation

- Prior to initiating the trial, the study team will collect all relevant regulatory documents, including but not limited to the following documents:
- A current *curriculum vitae* of the Principal Investigator and each sub-investigator listed on the FDA Form 1572
- A copy of the current medical licenses of the Principal Investigator and all sub-investigators listed on the FDA 1572
- A copy of the Protocol Acceptance Page signed and dated by the Principal Investigator
- A letter from the IRB stipulating approval of the protocol, the informed consent document and any other material provided to potential trial participants with information about the trial (e.g. advertisements)
- A copy of the informed consent document approved by both the sponsor and local IRB
- The current IRB Federalwide Assurance Letter
- Current laboratory certification for the reference laboratory and *curriculum vitae* of the laboratory director
- A list of current laboratory normal values for the reference laboratory
- A copy of the signed Delegation of Authority Log
- Protocol training documentation for study staff
- The drug destruction SOP

- A copy of the fully executed contract must be on record

Required regulatory documents include, but are not limited to the following: local IRB approvals (i.e., protocol, consent form, amendments, patient brochures, recruitment material, etc.), each participant's informed consent, enrollment form, eligibility checklist and tissue block registration, summary of unanticipated problems or protocol deviations, and documentation of expertise of the investigators.

21.0 Records Retention

Study documentation includes all Case Report Forms, data correction forms or queries, source documents, Sponsor-Investigator correspondence, monitoring logs/letters, and regulatory documents (e.g., protocol and amendments, IRB correspondence and approval, signed patient consent forms).

Source documents include all recordings of observations or notations of clinical activities and all reports and records necessary for the evaluation and reconstruction of the clinical research study.

Government agency regulations and directives require that the study investigator retain all study documentation pertaining to the conduct of a clinical trial. In the case of a study with a drug seeking regulatory approval and marketing, these documents shall be retained for at least two years after the last approval of marketing application in an International Conference on Harmonization (ICH) region. In all other cases, study documents should be kept on file until three years after the completion and final study report of this investigational study.

Following closure of the study, the site will maintain a copy of all site study records in a safe and secure location. Records may be destroyed according to SCCC and institutional policies.

22.0 Confidentiality

Names and records of patients taking part in this study will remain confidential. Members of the research team will comply with appropriate HIPAA guidelines. Records that contain patient identifiers will be available only to the physicians, nurses, and other health care personnel directly involved in the management of the patient. Data that include a unique patient identifier code, but not patient name or other information that could jeopardize patient privacy, will be available to research personnel involved in the study. Data will be stored in secured location in a password protected data base.

The investigator agrees to keep all information provided by this study in strict confidence and to request similar confidentiality from her staff and the IRB/IEC/REB. Study documents provided (protocols, investigators' brochures, CRFs and other material) will be stored appropriately to ensure their confidentiality.

Patient medical information obtained by this study is confidential, and disclosure to third parties other than those noted below is prohibited. Upon the patient's permission, medical information may be given to his or her personal physician or other appropriate medical personnel responsible for his or her welfare. This medical information must be made available to the IRB and DSMC, upon request, for source verification of study documentation. Data generated by this study must be available for inspection upon request by representatives of the U.S. FDA, local health authorities, Study Chair, principal Investigator and their authorized representative(s), collaborators and licensees, and the IRB for each study site, if appropriate.

23.0 Obligations of Investigators

The Principal Investigator is responsible for the conduct of the clinical trial at the site in accordance with Title 21 of the Code of Federal Regulations and/or the Declaration of Helsinki. The Principal Investigator is responsible for personally overseeing the treatment of all study patients. The Principal Investigator must assure that all study site personnel, including sub-investigators and other study staff members, adhere to the study protocol and all FDA/GCP/NCI regulations and guidelines regarding clinical trials both during and after study completion.

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Mayer EL, Christiansen J, Murphy D, Fitzgerald K, Wang K, Ross JS, Miller VA, Stephens PJ, Yelensky R, Garraway L, Meszoely I, Balko JM, **Arteaga CL**. Genomic profiling of ER+ breast cancers after short term estrogen suppression reveals alterations associated with endocrine resistance. *Sci. Transl. Med.* 2017 Aug 9; 9(402). pii: eaai7993. doi: 10.1126/scitranslmed.aai7993. PMID 28794284

Appendix I: Lab Manual

BLOOD SAMPLES:

1. 1 EDTA tube (10ml purple top tube)

Collection: Blood sample will be collected by the CRO staff.

Processing: Farjana Fattah lab group will process these samples for buffy coat. Processed within 6 hours of collection. SOP for buffy coat processing:

- Centrifuge the tube at 1100-1300g for 10 minutes at 4 degrees Celsius.
- Discard the plasma layer.
- Collect buffy coat into one labelled 2mL cryo tube and -80 degrees Celsius storage

Storage: Farjana's team will store these samples after processing and Saurabh Mendiratta will coordinate and collect from them from time to time.

TISSUE (Punch Biopsies):

1. 1-6 core biopsy OR 10 unstained slides

Collection: Cheryl Lewis group will collect the tissue sample.

- First four (1-4) cores would be completely flash frozen (no FFPE).
- If there is a fifth core, it should be formalin fixed. Sixth core, if available, should be flash frozen.

Instructions on fresh tissue specimen handling:

- According to the following schedule, core biopsy specimens should be immediately flash frozen in liquid nitrogen* OR placed in 10% neutral buffered formalin immediately following removal from the patient following procedures established by the Simmons Cancer Center Tissue Core.
- For the formalin-fixed cores, an approximately 20:1 volume of formalin to tissue should be used. Core biopsies placed in 10% neutral buffered formalin must fix at room temperature for at least 6 h and no more than 24 h. Core biopsies must be transferred from formalin to 70% ethanol after 6-24 h to prevent over fixation and placed in the refrigerator.
- Label ALL cryovials before freezing. Label each cryovial and formalin-filled collection container with the patient name, Medical Record #, Patient study ID #, location of biopsy, collection date and time of collection using an ethanol resistant marker. Each set of patient samples should be accompanied by a Tissue Registration form.

Storage:

Dr. Lewis group will store them and once these boxes will be full, all samples will be sent to Saurabh Mendiratta, 6001 Harry Hines Blvd. NB5.112. Dallas, TX- 75390-8807 Phone: 214-648-6167. It will be stored in Dr. Arteaga lab freezer.