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Phase IIB Randomized Trial of Oral Tamoxifen vs. Topical 4-hydroxytamoxifen gel vs. Control in Women with Atypical Hyperplasia, Lobular Carcinoma in Situ, or Increased Breast Cancer Risk

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Principal Investigator: Amy C. Degnim, M.D.
Professor, Department of Surgery
Mayo Clinic
200 First Street SW
Rochester, MN 55905
507-284-6357

IND Sponsor: Amy C. Degnim, M.D.
Professor, Department of Surgery
Mayo Clinic
200 First Street SW
Rochester, MN 55905
507-284-6357

Northwestern University Site PI
Seema A. Khan, MD

MD Anderson Cancer Center at Cooper Site PI
Catherine E. Loveland-Jones, MD, MS, FACS

Trial Supported by: National Cancer Institute

Drug Availability:

4hydroxytamoxifen gel and placebo available via drug company (BHIR)
Contact:

BESINS HEALTHCARE IRELAND Ltd. (BHIR)
16 Pembroke Street Upper
D02 HE63
Dublin 2, Ireland

Oral tamoxifen and placebo available via MRI Global. Contact:

Jonathan White, Ph.D.
MRI Global
Telephone: 816-326-5294
E-mail: jwhite@mriglobal.org

Site Investigators & Participating Sites:

Amy Degnim, MD
Mayo Clinic
2200 First Street SW
Rochester, MN 55905
507/284-6357

Seema A. Khan, MD
Northwestern University
250 E. Superior
Chicago, Ill 60611
312/503-4236

Catherine E. Loveland-Jones, MD, MS, FACS
MD Anderson Cancer Center at Cooper
Two Cooper Plaza
Camden, NJ 08103
855.632.2667

IND Sponsor:
Amy C. Degnim, MD
Mayo Clinic
200 First Street SW
Rochester, MN 55905
507/284-6357
IND #: 153332

Agent(s)/Supplier:
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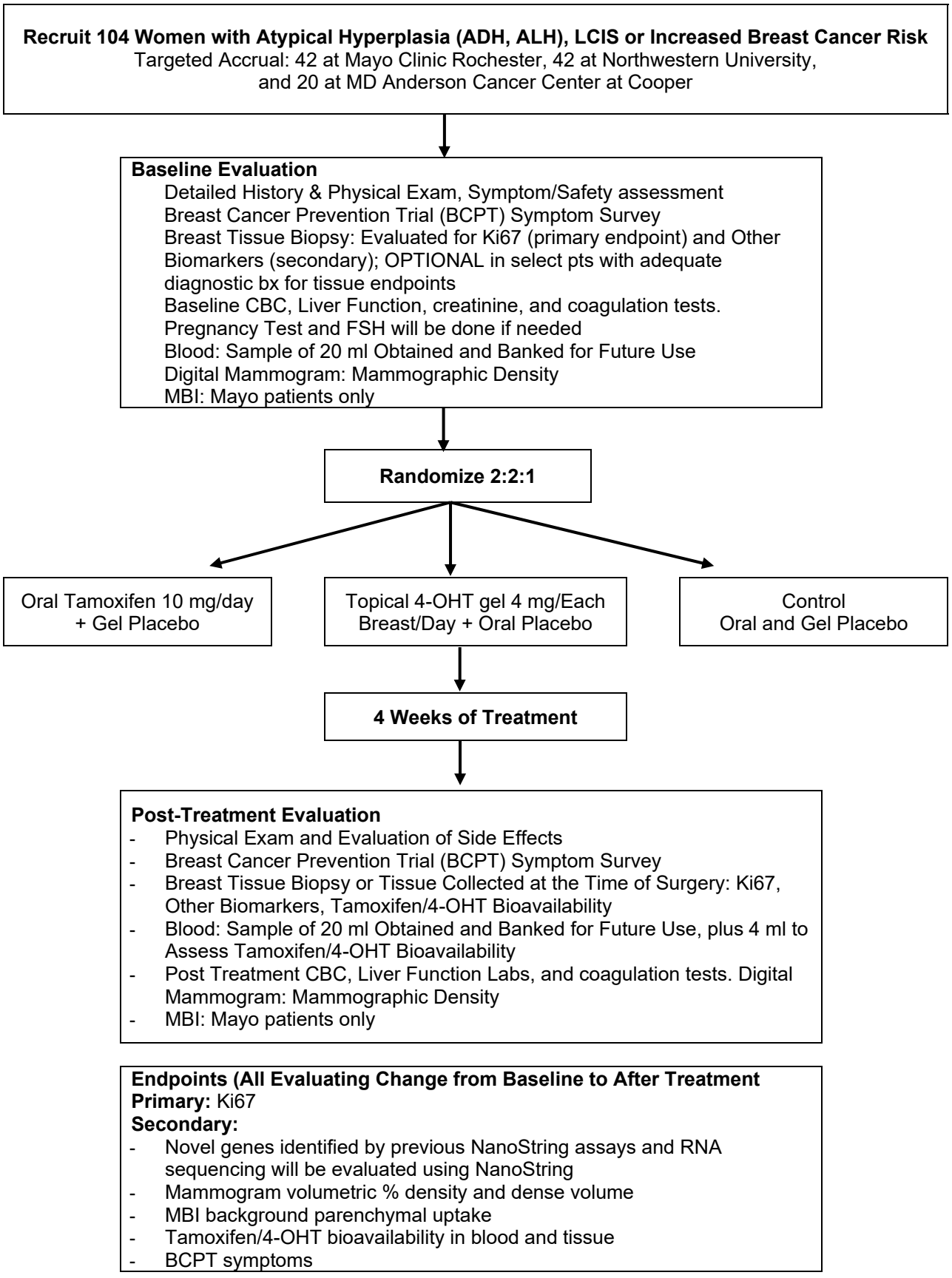
1.0 Schema


Recruit 104 Women with Atypical Hyperplasia (ADH, ALH), LCIS or Increased Breast Cancer Risk
Targeted Accrual: 42 at Mayo Clinic Rochester, 42 at Northwestern University, and 20 at MD Anderson Cancer Center at Cooper
Baseline Evaluation
Detailed History & Physical Exam, Symptom/Safety assessment
Breast Cancer Prevention Trial (BCPT) Symptom Survey
Breast Tissue Biopsy: Evaluated for Ki67 (primary endpoint) and Other Biomarkers (secondary); OPTIONAL in select pts with adequate diagnostic bx for tissue endpoints
Baseline CBC, Liver Function, creatinine, and coagulation tests.
Pregnancy Test and FSH will be done if needed
Blood: Sample of 20 ml Obtained and Banked for Future Use
Digital Mammogram: Mammographic Density
MBI: Mayo patients only
Randomize 2:2:1
Oral Tamoxifen 10 mg/day + Gel Placebo
Topical 4-OHT gel 4 mg/Each Breast/Day + Oral Placebo
Control Oral and Gel Placebo
4 Weeks of Treatment
Post-Treatment Evaluation
- Physical Exam and Evaluation of Side Effects
- Breast Cancer Prevention Trial (BCPT) Symptom Survey
- Breast Tissue Biopsy or Tissue Collected at the Time of Surgery: Ki67, Other Biomarkers, Tamoxifen/4-OHT Bioavailability
- Blood: Sample of 20 ml Obtained and Banked for Future Use, plus 4 ml to Assess Tamoxifen/4-OHT Bioavailability
- Post Treatment CBC, Liver Function Labs, and coagulation tests. Digital Mammogram: Mammographic Density
- MBI: Mayo patients only
Endpoints (All Evaluating Change from Baseline to After Treatment
Primary: Ki67
Secondary:
- Novel genes identified by previous NanoString assays and RNA sequencing will be evaluated using NanoString
- Mammogram volumetric % density and dense volume
- MBI background parenchymal uptake
- Tamoxifen/4-OHT bioavailability in blood and tissue
- BCPT symptoms

Definitions

Generic name: Tamoxifen Brand name(s): Nolvadex, Soltamox Availability: MRI Global	Generic name: Topical 4-OHT Brand name(s): NA Availability: BHIR Pharma, LLC

2.0 Background

We plan to prospectively study breast tissue changes after a short course of Tamoxifen (Tam). Women with atypical hyperplasia (AH) and lobular carcinoma in situ (LCIS) are at increased risk of breast cancer (BC) (~1-2 % per year). Over two decades ago, placebo-controlled randomized trials established that oral tamoxifen (20 mg/day) reduces breast cancer risk by 50% in generally defined high risk women, with ~70% reduction in women at high risk specifically due to atypical hyperplasia.[1] Years later, the side effects and toxicity of oral tamoxifen at 20 mg/day remain a significant barrier to its uptake and long-term compliance.[2, 3] To address the issue of toxicity, two main strategies have been pursued: 1) using a lower dose of oral tamoxifen, and 2) using a topical formulation of tamoxifen to avoid systemic side effects. Here we will perform a prospective study of women with AH, LCIS, or increased risk of BC based on risk model calculations concordant with current consensus guidelines (described in detail below) who will take a short course of prevention therapy; breast tissue samples will be evaluated pre- and post-therapy to identify and evaluate very early biomarkers of response. The overall goal of the study is to evaluate short- term changes in background breast tissue induced by either low-dose oral tamoxifen or topical 4-OHT gel in women with AH, LCIS, or increased risk of BC based on risk model calculations.

4hydroxytamoxifen (4-OHT) gel is a novel formulation of tamoxifen with local tissue absorption and less toxicity.

Recent experience with 4-hydroxytamoxifen gel (4-OHT) gel shows promise for improved local breast tissue absorption and efficacy, with low systemic toxicity. 4-OHT is an active tamoxifen metabolite. 4-OHT and another tamoxifen metabolite, endoxifen, are known to account for the anti-cancer activity of tamoxifen.[4] 4-OHT has greater affinity for the ERα receptor and better suppression of normal and BC cell growth *in vitro* than tamoxifen. [5-8] Transdermal 4-OHT is locally absorbed at effective doses. Human and animal studies show that transdermal delivery of 4-OHT via gel applied to the breast skin results in local absorption and concentration in breast tissue. [9-12] Compared to oral tamoxifen (20 mg dose), transdermal 4-OHT delivery achieves equivalent tissue doses with a simultaneous 80% reduction in plasma levels. [9, 13] In clinical trials, 4-OHT gel results in Ki67 reduction with less toxicity. In neoadjuvant window trials in DCIS and invasive BC, women treated with two to ten weeks of 4-OHT gel (2 mg/breast/day) versus oral tamoxifen (20 mg/day) show similar decreases in cellular proliferation (50-60%) assessed by Ki67.[9, 13] The elevation of serum coagulation proteins seen in patients taking oral tamoxifen does not occur in patients treated for the same time period with 4-OHT gel,[9] and patients report fewer vasomotor symptoms with 4-OHT.[14]

Low-dose (5 mg/day) oral tamoxifen shows similar efficacy to standard historical dose (20 mg/day) for breast cancer prevention, with few side effects.

Reducing the dose of oral tamoxifen is another alternate strategy to address toxicity and poor compliance with standard oral tamoxifen (20 mg/day), with a recently published trial by deCensi et al showing similar reductions in breast cancer risk with an approximate 5 mg/day dose (actually taken as 10 mg every other day or a split pill every day).[15] Minimum effective dose biomarker studies showed that 5 mg/day was not inferior to 20 mg/day in inhibiting breast cancer proliferation [16] and a prospective cohort study showed that 10 mg every other day reduced DCIS recurrence by 50%.[17] Based on these findings, a Phase III placebo-controlled randomized trial was undertaken to evaluate the efficacy of low-dose tamoxifen (~5 mg/day) for breast cancer prevention in women with breast intraepithelial neoplasia (AH, LCIS, or DCIS).[15] It is important to note that tamoxifen is not commercially available as a 5 mg pill, but rather only as a 20 mg or a 10

mg pill. Therefore, the women enrolled in this published study of low-dose tamoxifen vs placebo had the option to either take an entire pill every other day, or they could split their pills and take roughly a half of a pill every day.

Among the 500 enrolled women with median follow-up of 5.1 years, breast cancer events in either breast were reduced by approximately half (hazard ratio 0.48, p=0.02), with a 75% reduction of breast cancer events in the contralateral breast (i.e., “true” prevention; hazard ratio 0.25, p= 0.02). Patient reported toxicity for low-dose tamoxifen was similar to placebo in all symptom categories except for daily hot flashes, which were more slightly common in the tamoxifen group (2 per day) compared to the placebo group (1.5 per day). Thus, this trial of low- dose (~5 mg/day) oral tamoxifen showed a similar degree of breast cancer risk reduction, with lower toxicity, than seen in prior Phase III trials using the 20 mg/day dose of oral tamoxifen.[1] Based on the results of this recently published randomized trial of deCensi et al, low-dose tamoxifen is now recognized by ASCO as a valid alternate option for breast cancer risk reduction in women with atypical hyperplasia or LCIS.[18]

In this proposed trial we plan to utilize a reduced dose of tamoxifen (10 mg/day) as the comparator to the topical 4OHT gel intervention. There are several reasons for this approach. In the deCensi study, the ~5 mg/day oral tamoxifen resulted in breast cancer risk reduction similar to that seen in prior trials of 20 mg/day. Therefore, we expect that 10 mg/day oral tamoxifen would be at least as effective as the ~5 mg/day dose in terms of risk reduction, while likely also reducing side effects compared to the 20 mg/day dose. Given the very short (4 weeks) intervention timeframe, our primary goal is to evaluate biomarker changes. Additionally, other published studies have shown similar reductions in breast tissue proliferation markers whether the daily oral tamoxifen doses is 5 mg, 10 mg, or 20 mg, [19-21] including the biomarker Ki67 which is the primary endpoint of our proposed study. Based on the other studies described above, transdermal 4-OHT delivery should be able to achieve tissue concentrations at least equivalent to those resulting from 10 mg/day oral tamoxifen. Lastly, the study proposed here has a much shorter intervention (4 weeks), and thus compliance with dosing may have a greater effect on the planned primary endpoint (reduction in Ki67). Pill splitting would result in imprecise daily dosing and taking 10 mg every other day would increase the risk of missed doses due to a non-daily schedule. We have explored the cost of custom formulation of oral 5 mg pills and matching placebos but the cost is prohibitive ($250,000). Therefore, we plan for oral tamoxifen 10 mg/day as the active oral drug and dosage in the comparator arm versus the 4OHT gel. We anticipate that using a 10 mg/day dose of tamoxifen in the current clinical trial will also enhance patient accrual due to expected lower side effects compared to 20 mg/day. This approach has been discussed extensively with the supporting grant program officer at NCI (Dr. Brandy Heckman-Stoddard) and the investigational agent sponsor (Besins Healthcare), and this is judged to be the best approach, considering the many factors impacting this decision, including scientific justification, patient acceptance, feasibility, and cost.

The proliferation marker Ki67 is a promising biomarker of short-term response to tamoxifen.

There is a strong rationale and existing data to support Ki67 as a biomarker in BC prevention studies. In women with AH, Ki67 in the atypia lesion is a time-dependent marker of long-term BC risk, with higher 10-year BC risk in women with Ki67≥2%.[22] We have also reported that Ki67 in background normal lobules in SA is associated with long-term BC risk,[23] similar to recent findings reported in the Nurses Health Study in BBD tissues.[24] Furthermore, Ki67 is also reduced after short courses of tamoxifen. In several prospective window-of-opportunity trials of Tam (ranging from 3-8 weeks of treatment), Ki67 levels in background normal breast lobules show relative reductions of ~50% with oral tamoxifen doses of 5, 10, or 20 mg per day.[19-21] Recently, we have developed a method to quantitate Ki67 (as % cells positive) in benign breast tissues using digital image assessment with Aperio algorithms. Within 40 samples, the digital image measure of Ki67 % cells positive in background normal lobules correlated very strongly with a manual pathologist score (r = 0.95).

Reduction in mammographic density after tamoxifen use is associated with decreased BC risk.

Tam has consistently been shown to reduce mammographic density.[25-28] Our group recently demonstrated reductions in volumetric density in breast cancer patients after one year of treatment with Tam or aromatase inhibitors.[29] Importantly, these reductions in mammographic density after prevention therapy have been shown to be associated with decreased risk of later breast cancer in high risk women taking Tam for prevention,[30] as well as reduced recurrence and mortality in breast cancer patients taking Tam as adjuvant therapy.[25-28] Thus, in the planned study we will assess change in area and volumetric density measures from mammograms to determine if changes are detectable after only one month of treatment. Early imaging changes are a feasible potential endpoint, since short-term Tam use can induce detectable breast changes with sound speed ultrasound tomography after 1-3 months,[31] on MRI within 90 days,[32] and on molecular breast imaging (MBI) at 4 weeks (**Fig 1**).

Background Parenchymal Uptake (BPU) on MBI is a promising noninvasive imaging biomarker of response.

Molecular breast imaging (MBI) is a low-radiation dose nuclear medicine test that uses a high-resolution, semiconductor- based gamma camera to image the uptake of Tc-99m sestamibi in the breast.[33] As MBI is a functional imaging technique that relies on differences in preferential uptake of Tc- 99m sestamibi in metabolically-active cells, it is able to reveal cancers hidden among dense fibroglandular tissue on a mammogram (published incremental cancer detection of 7.5-16.5 per1000 women screened.[34, 35] In addition to detecting BC, MBI also depicts the level of Tc-99m sestamibi uptake in non-cancerous background fibroglandular tissue, termed background parenchymal uptake (BPU). BPU is subjectively assessed according to a validated lexicon as one of four categories (photopenic, minimal to mild, moderate, and marked), with high reader agreement (inter-reader κ=0.84, intra-reader κ=0.87) that describe the relative intensity of uptake in fibroglandular tissue.[36, 37] Importantly, BPU was recently shown to be associated with BC risk; women with moderate or marked BPU have 3.4 to 4.8-fold increased risk compared to women with photopenic or minimal-mild BPU, even when adjusted for percent mammographic density and hormonal influences. The exact mechanism of uptake of Tc-99m sestamibi in breast tissue is not entirely understood, but has been correlated with blood flow, mitochondrial activity, and mitotic activity in BCs.[38, 39] In a recent study led by Dr. Hruska, women with high BPU on screening MBI underwent 4 weeks of low-dose (5 mg or 10 mg) oral tamoxifen, with early decreases in BPU (**Fig 1**).[40] Therefore BPU is promising as a non-invasive measure of proliferative activity in the breast tissue.

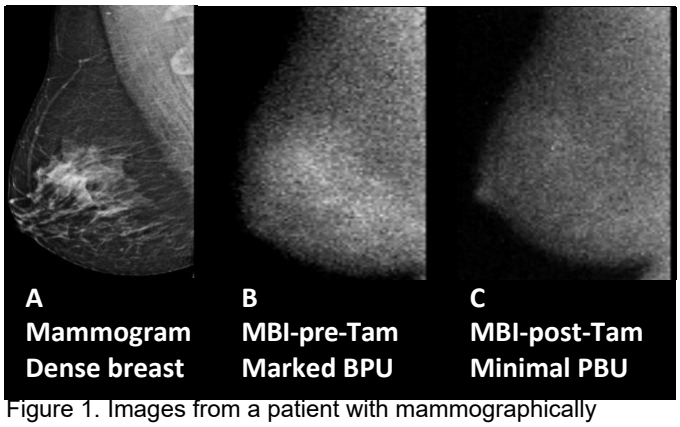


Figure 1. Images from a patient with mammographically dense breasts undergoing supplemental screening with MBI (A,B) and MBI again after 4 weeks low-dose (5 mg) oral tamoxifen (C).

Breast Cancer Prevention Trial (BCPT) Symptom Checklist

The BCPT Symptom Checklist was developed to assess side effects of medical interventions to prevent and treat breast cancer. Items were adapted from several existing questionnaires. The scale was then validated on women diagnosed with breast cancer receiving cancer treatment, women at risk for breast cancer receiving chemoprevention, menopausal women, and women undergoing normal aging. Factor analysis associations confirmed eight factors including hot flashes, nausea, bladder control, vaginal problems, musculoskeletal pain, cognitive problems, weight problems and arm problems and included 18 questions. [41, 42] Additional questions (N=7) have been added to

measure fatigue and sexual interest (Stanton, 2016 personal communications).

We plan to increase the dose of the investigational agent, 4hydroxytamoxifen (4OHT) gel, from the original dose of 4 mg/day (2 mg/breast/day) to 8 mg/day (4 mg/breast/day), for all remaining subjects accrued to the trial who are randomized to the 4OHT gel arm. Since the design is double-blinded, all participants who are not assigned to the 4OHT gel arm (i.e. the placebo and oral Tamoxifen arms) will also need to double the amount of placebo gel applied so that all participants are applying two pumps of gel per breast every day while on study intervention.

This modification is motivated by recent unpublished clinical trial findings showing lower than expected tissue concentrations with the 4 mg/day gel dose and lack of anticipated antiproliferative effect as measured by Ki67 in a study of women with DCIS. This recent multicenter presurgical phase 2 window trial was led by Dr. Seema Khan at Northwestern University, with 107 women accrued with ER+ DCIS who were randomized to oral tamoxifen "TAM" (20 mg/day + placebo gel) or 4OHT gel "4OHT" (2 mg/breast daily + oral placebo), for 4-10 weeks prior to surgery. 72 of 87 compliant women (39 oral tamoxifen and 33 4OHT gel) were evaluable for the primary endpoint, i.e. reduction in DCIS Ki67 labeling index.

Mean treatment duration was 47 days for TAM and 44 days for 4OHT gel (p=0.2). The median absolute decline in Ki67 (post-treatment compared to pre-treatment) was significant in the TAM (-3.7%, p<0.001) but not in 4OHT gel arm (-1.3%, p=0.2); between-arm p=0.002. Ki67 results following menopausal stratification also favored the TAM arm, especially in postmenopausal women: between-arm p=0.06 in 37 premenopausal women and p=0.02 in 35 postmenopausal women. Breast tissue 4OHT concentrations were lower (although not significantly) in the 4OHT arm in both superficial and deep tissue samples compared to the TAM arm (mean superficial tissue concentrations were 6.1 and 4.2 ng/g for TAM and 4OHT gel, respectively, p= 0.55; and in deep tissues were 5.7 and 3.8 ng/g, respectively, p= 0.06). Endoxifen (another active metabolite of tamoxifen) was abundant in plasma (11 ng/mL) and deep tissue (13 ng/g) of TAM arm, but present in only trace amounts in the 4OHT gel arm (undetectable in plasma and 0.31 ng/g in tissue), between-arm p <0.0001. Circulating TAM responsive markers (insulin like growth factor 1, sex hormone binding globulin, von Willebrand factor, and protein S total) and vasomotor symptoms were significantly and unfavorably modulated by TAM, but not by 4-OHT gel therapy.

In summary, these trial results showed that the anti-proliferative effect of 4 mg/day transdermal 4OHT gel was inferior to 20 mg oral TAM in DCIS. Tissue 4OHT concentration in gel treated subjects was lower, especially in deep tissues, although this did not quite reach significance (p=0.06). Endoxifen exposure was markedly higher with oral TAM therapy and is a likely explanation for the inferiority of 4-OHT gel. The study authors recommended that in future studies, use of higher 4OHT gel doses may overcome this difference.

Safety Data on 4OHT gel dose 8 mg/day. Safety data is available for the 8 mg/day dose from a BHR Pharma sponsored study of 4OHT gel for reduction in breast density (BHR-700-301, see attached Investigator's Brochure February 10, 2022, pages 59-60). In this trial 149 subjects with dense breasts (BI-RADS C/D) received 8 mg/day 4OHT gel and 73 subjects received placebo for up to 52 weeks.

Adverse Events for 8 mg/day dose. The 149 subjects in the 4OHT gel group experienced a total of 358 AEs, and the 73 subjects in the placebo group experienced a total of 178 AEs. Overall, the incidence of AEs and treatment-related AEs was similar in both treatment groups. With regards to the non-serious AEs reported during the clinical trial, approximately 70% of the AEs that occurred in subjects receiving 4OHT gel and 74% of those in subjects receiving placebo were deemed unrelated to study treatment. Only 5% of AEs reported by the 4OHT gel group and 2.8% of AEs in the placebo group were thought to be 'probably related' to study treatment. A summary of AEs by Medical

Dictionary for Regulatory Activities (MedDRA) body system is presented on pages 59-60 in the Investigators Brochure (updated February 10, 2022).

Serious Adverse Events with 8 mg/day dose. Five subjects, all of whom were treated with BHR-700 4-OHT gel, experienced 1 SAE each: invasive ductal breast carcinoma (N = 2), intraductal proliferative breast lesion (N = 1), endometrial adenocarcinoma (N = 1), and pulmonary embolism (N = 1). None of the SAEs had a fatal outcome. Of the 5 reported SAEs, 3 were deemed possibly related to study treatment (invasive ductal breast carcinoma, endometrial adenocarcinoma, and pulmonary embolism). However, they could not be determined as related as there were other factors that could also have been possible causes of these events. Concerning the reported SAEs, 5 reported SAEs for 222 subjects over the reporting period of the trial is extremely low and shows the safety and tolerability of 4OHT gel. Complete details on safety data are reported in the Investigators Brochure (updated February 10, 2022).

Planned implementation of increased 4OHT gel dose into ongoing clinical trial
As of 10/12/2022, 12 patients have already accrued to the trial and have been randomized to placebo, oral TAM, and 4OHT gel arms of the study. 10 have completed intervention and trial-related research studies, with 2 who have started and will complete the planned ongoing interventions. A temporary hold was placed on new enrollment on 10/12/22 so that all future patients enrolled may be treated with the higher 8 mg/day dose of 4OHT if randomized to that treatment arm.

Expansion of Eligibility Criteria April 2023
In order to expand the pool of eligible patients for the trial we will include patients without atypical hyperplasia or LCIS but who are at increased risk for breast cancer based on risk model calculations that are concordant with current consensus guidelines. For women at increased risk based on Gail / Breast Cancer Risk Assessment Tool 5-year risk 3%, or International Breast Intervention Study 10-year risk 5%, breast cancer prevention therapy is RECOMMENDED (Visvanathan Journal of Clinical Oncology 2019;37(33):3152-65, NCCN Guidelines v1.2023), and therefore these women are appropriate for inclusion in this trial of short-term oral versus 4OHT topical tamoxifen. In addition, we will also allow short periods of prior use of risk-reducing medications with corresponding periods without use prior to study participation (see Exclusion criteria).

Database
The study coordinators and stats team will use RAVE Medidata database for data entry. This secured database will only be accessed by study members. Each patient will be assigned a research ID for entry of data into this database. The research ID/participant name match will be kept in a secured file at each institution. Only the investigator, statisticians and study coordinators at the respective sites will have access to this file.

3.0 Goals

3.1 Primary Aim.
Change in Ki67 from pre-treatment to post-treatment

3.2 Secondary Aims.
Changes from pre-treatment to post-treatment in:

- Mammographic volumetric % density
- Mammographic dense volume
- Breast Cancer Prevention Trial Symptom Survey
- Molecular Breast Imaging background parenchymal uptake
- Changes in novel tissue biomarkers from our previous work; will be assessed with NanoString and immunohistochemistry
- 4-OHT and metabolite levels in blood and tissue
- Coagulation parameters

4.0 Patient Eligibility

4.1 Screening Inclusion Criteria

4.1.1 Willing to return to enrolling institution for follow-up.
4.1.2 Willing to complete required testing.
4.1.3 Ability to complete questionnaire.
4.1.4 Female (sex that was assigned at birth).
4.1.5 Age ≥ 18 years.
4.1.6 Ipsilateral intact breast with histology confirmation of atypical ductal or lobular hyperplasia, or LCIS, within the last 5 years, whether surgically excised or not; OR neither AH nor LCIS but increased breast cancer risk defined as either:
a) Gail model (Breast Cancer Risk Assessment Tool) 5 year breast cancer risk of ≥3%, or
b) International Breast Intervention Study model 10 year breast cancer risk of ≥5%.
4.1.7 ECOG performance status ≤1 (Karnofsky ≥70%; see Appendix A).
4.1.8 The effects of topical 4-OHT gel on the developing human fetus at the recommended therapeutic dose are unknown. However, oral tamoxifen is Pregnancy Category D—positive evidence of human fetal risk. For this reason, and because triphenylethylene antiestrogens, including tamoxifen, are known to be teratogenic, women of childbearing potential and their male partners must agree to use at least one effective form of birth control (abstinence is not an allowed method) prior to study entry and for the duration of study participation, and for 2 months following the last dose of study medications (participant can resume oral birth control pills for effective birth control measures after post-treatment biopsy is done). Effective birth control methods during treatment are copper and Mirena IUD [intrauterine device], diaphragm/cervical cap/shield, spermicide, contraceptive sponge, condoms. Women of childbearing potential must have a negative pregnancy test within five days before starting study medications. Tubal Ligation is also an acceptable form of birth control. Should a woman become pregnant or suspect she is pregnant while participating in this study, she should inform her study physician immediately.
4.1.9 Willingness to avoid exposing breast skin to natural or artificial sunlight (i.e., tanning beds) for the duration of the study.
4.1.10 Participants must have acceptable organ and marrow function as judged by treating physician's evaluation of baseline laboratory data.

4.2 Screening Exclusion Criteria

4.2.1 Clinically suspicious mass/lesions.
4.2.2 Breast cancer in the past 5 years.
4.2.3 Patients with any history of venous thromboembolic disease, regardless of timeframe (history of varicose veins and superficial phlebitis is allowed).
4.2.4 Current pregnancy or lactation.
4.2.5 History of other prior breast cancer-specific therapy within the previous 2 years (chemotherapy, anti-HER2 agents, endocrine agents, everolimus, CDK4-6 inhibitors).
4.2.6 Cytotoxic chemotherapy for any indication in last 2 years.
4.2.7 Prior use of SERMS or AIs including tamoxifen, raloxifene, anastrozole, letrozole, or exemestane for prevention or therapy within the past 5 years. UNLESS
a) Use was less than 6 months duration in the past 5 years and not used in the 1 year prior to enrollment, OR
b) Use was no greater than 2 months duration in the past 1 year and not used in the 6 months prior to enrollment.

4.2.8 Exogenous sex steroid, including oral contraceptive pill use within 1 month

prior to Pretreatment breast biopsy (). Use of vaginally administered estrogens and hormone coated IUD such as Mirena is permitted

4.2.9 History of any prior ipsilateral breast radiotherapy. Previous unilateral radiation of the contralateral side is allowed.

4.2.10 Skin lesions on the breast that disrupt the stratum corneum (e.g., eczema, ulceration).

4.2.11 History of endometrial neoplasia.

4.2.12 Current smoker. Cessation for at least 6 weeks.

4.2.13 Current users of potent inhibitors of tamoxifen metabolism. The potent inhibitors of tamoxifen metabolism are: bupropion, cinacalcet, fluoxetine, paroxetine, quinidine.

4.2.14 Participants may not be receiving any other investigational agents within 90 days of enrollment or during this study.

4.2.15 History of allergic reactions to tamoxifen.

4.2.16 Uncontrolled intercurrent illness that in the judgement of the treating physician would make them unsuitable for study participation.

4.2.17 Current use of anticoagulation medications.

4.2.18 Patients who are breastfeeding.

4.2.19 Participant will be excluded with any one or more of the following results within 30 days of randomization:

- Hemoglobin < 10 g/dL
- Leukocytes < 3,000/microliter
- Platelets < 100,000/microliter
- Total bilirubin > 1.5 x institutional upper limit of normal (ULN)
- AST (SGOT) > 1.5 x ULN
- ALT (SGPT) > 1.5 x ULN
- Alkaline phosphatase, S > 1.5 x ULN
- Albumin, S >1.5 x ULN
- Protein, total, S >1.5 x ULN
- Creatinine > 1.5 x ULN
- Antithrombin III <80% of normal
- Fibrinogen >1000 mg/dL

4.2.20 Patients who are taking any medications, herbal products, or OTC products that are moderate or strong CYP2D6 inhibitors or CYP3A inducers. Patients should also refrain from starting any drug or product with these properties during the study. This is to avoid any potential interactions with tamoxifen or 4-OHT. (Please see Appendix G.)

4.2.21 Clinically significant arrhythmia requiring ongoing medication for control / treatment, especially those with high risk of QT prolonging effects (Appendix H).

4.3 Enrollment – Inclusion Criteria

4.3.1 Negative pregnancy (serum or urine) test if of childbearing potential and/or FSH to verify menopausal status.

4.4 Enrollment – Exclusion Criteria

4.4.1 Identification of a clinically suspicious mass on examination

4.5 Identification of Potential Participants

Study coordinators will identify potential patients by reviewing breast clinic and breast surgeons' patient schedules for women diagnosed with ALH, ADH, LCIS or increased breast cancer risk as calculated by the breast clinic or other medical provider . Their

return appointment(s) schedule(s) will be reviewed for possible patient contact times. Patient charts will be reviewed to identify if they meet inclusion and exclusion criteria.

4.6 Patient Consent

The patients who meet study eligibility will be approached by the study coordinator prior to, during or after their clinical appointment to discuss the purpose of the study and what involvement in the study would entail. The consent form will be reviewed. If the patient agrees to be part of the study, they will be asked to sign the consent. A copy of the signed consent will be provided to the patient.

5.0 Test Schedule

Test & Procedures	**Baseline**	**End of Treatment (at the end of 4 weeks of treatment)[12]**
Pregnancy Test (serum or urine)	**X[1,2]**	
Follicle stimulating hormone (FSH)	**X[2,3]**	
Baseline labs [CBC and Liver Function testing (ALP, ALT, AST, ALB, TP, BILI), creatinine coagulation labs (fibrinogen, Antithrombin 3)]	**X[2]**	**X[2]**
Physical Examination	**X[2,4]**	**X[2]**
Two 10 ml amounts of blood (will be stored for future research)[5]	**X[2]**	**X[2]**
Blood Draw (4-OHT bioavailability)[6]		**X[2]**
Research Biopsy of Breast Tissue - **same quadrant as diagnostic biopsy[7] [Ki67, NanoString, 4-OHT bioavailability (post-treatment only);]** **Pretreatment diagnostic biopsy is OPTIONAL if meets requirements[8]**	**X[2,8]**	**X[2,9]**
Digital Screening Mammogram	**X[2,10]**	**X[2,10]**
MBI (Mayo Clinic site only)	**X[2,11]**	**X[2]**
BCPT Symptom Survey	**X[2]**	**X[2]**
Symptom /safety/AE assessment	**X[2]**	**X[2]**

1. For women of childbearing potential only.
2. Research Funded (Note: clinical surgical consult will not be research funded.)
3. For women with unknown menopausal status
4. Physical exam could be done within 3 months before treatment starts.

5. Blood for storage two 10 ml blood draws using purple top EDTA tubes – protect from light exposure (i.e., wrap in tin foil, use light sensitive brown bags). **First EDTA tube**- Aliquot (1) 4000uL blood pour for DNA extraction. Refrigerate, Centrifuge at lab standards. Process for three 1 ml aliquots of plasma into 3 cryovials 1 ml plasma each and 1ml WBC. Light Sensitive. Store in -80 freezer. **Second EDTA vial** is stored as whole blood.
6. Blood for 4-OHT bioavailability. 4 ml. green top heparin tube. Process for plasma. Protect from light exposure as light sensitive (i.e., wrap in tin foil, use light sensitive brown bags). Store in -80 freezer.
7. Baseline Pretreatment research tissue core biopsy information is located in Appendix F.
8. Pretreatment (baseline OPTIONAL) research tissue. This is optional if the subject has a pretreatment diagnostic breast biopsy that is 1) within 6 months of enrollment, 2) no exogenous sex steroids other than vaginal estrogen or hormone coated IUD within one month prior to the diagnostic biopsy, 3) has at least 4 normal TDLUs by pathologist review, and 4) if the FFPE tissue from the diagnostic biopsy will be available to the research study for primary (Ki67) and secondary tissue endpoints (RNA extraction). If Pretreatment research biopsy is performed, tissue will be collected by doing vacuum assist core biopsy, using a 9-11G needle. At Mayo Clinic, snap frozen tissues will be stored in BAP, and batch shipped to Dr. Radisky's lab in Jacksonville, FL; formalin fixed cores will be delivered to the Clinical Histology lab to create a single FFPE block if possible and a single H&E stain for each block. The H&E stained slide(s) will be delivered by the study coordinator to the breast pathologist, Dr. Solanki, or her designee, for core biopsy read within 21 days of biopsy. After the breast pathologist's review is complete, the FFPE blocks and H&E will be stored and batch shipped to Dr. Radisky's lab in Jacksonville, FL for Ki67 IHC, slide scanning at 40X, and Aperio quantitation. At Northwestern University and MD Anderson Cancer Center at Cooper, snap frozen cores will be stored at -80, and shipped to Dr. Radisky lab in Jacksonville; formalin fixed cores will be put up as a single FFPE block if possible (see details section 6.8.3). (Note, there will not be 4OHT testing on pretreatment breast tissue samples.)
9. The post-treatment research breast tissue sample is MANDATORY and can be obtained by either needle core approach (if no surgical excision is done for clinical care) or can be obtained by the surgical team intraoperatively after excision of the lesion and negative margins are obtained diagnostically. If core biopsy approach is used for the post-treatment sample, follow guidelines in #8 above. If surgical excisional biopsy is performed for clinical care, the post-treatment samples will be obtained by the surgical team. Two 1 cm^2 pieces of tissue will be excised by the surgeon after negative margins have been obtained diagnostically. One of these two pieces of tissue should be cut in half and placed into two separate sterile tubes for snap freezing-- one piece will go for 4-OHT to Dr. Reid's lab, and one for RNA extraction to Dr. Radisky's lab. The other 1 cm^2 piece of tissue will be put in formalin and processed in the Clinical Histology Lab for a single FFPE block and one single H&E stain. The H&E stain will be delivered by the study coordinator to the breast pathologist, Dr. Solanki, or her designee, for core biopsy read within 21 days of biopsy. After the breast pathologist's review is complete, the FFPE blocks and H&E will be stored and batch shipped to Dr. Radisky's lab in Jacksonville, FL for Ki67 IHC, slide scanning at 40X, and Aperio

quantitation.

10. Bilateral CC view (FFDM), 1 CC view of eachbreast.
11. MBI to be obtained at baseline. This will not be required if a current MBI was done within one month of starting treatment.

    Tests to be scheduled as described above, with the last dose of study drug within 2 days (preferably within 24 hours) prior to the post-treatment biopsy and blood draw. All other tests (mammogram, MBI) should be scheduled PRIOR to the post-treatment breast biopsy, but no more than 3 days prior.

    Participants will receive a total of $350.00 to $500.00 for completing the study. This will be paid as $350 upon completion of study intervention and post treatment tests and biopsy, with an additional $150 for subjects who undergo the pretreatment research biopsy (given at completion of pretreatment research biopsy). All participants will also receive a small gift of appreciation.

6.0 Stratification Factors

- Study site
- Diagnosis (ADH vs ALH/LCIS vs increased risk without AH or LCIS)
- Menopausal status (pre- vs. postmenopausal)

  For the purposes of this trial, post-menopausal is defined as:
  - A history of at least 12 months without spontaneous menstrual bleeding, or
  - A prior documented hysterectomy and bilateral salpingo-oophorectomy, or
  - A prior hysterectomy without oophorectomy or in whom the status of the ovaries is unknown, with a documented FSH level > or equal to 40, demonstrating confirmatory elevation in the postmenopausal range.

6.1 Randomization Procedures

6.1.1 After the patient has completed the informed consent process, confirmed eligibility and the values of the stratification factors are recorded in Medidata RAVE, the patient will be assigned a research ID, and will be randomly assigned to one of the treatment groups.
Randomization will be performed using dynamic allocation, implemented within the Medidata RAVE system. The Medidata RAVE system will randomize the patient after the study coordinators have recorded the stratification factors as part of the blinded data input. Pharmacy will be notified of the randomization via email to a pharmacy email account prompting the pharmacist to log in and view the unblinded treatment assignment.
Randomization groups:

- Tamoxifen tablet, placebo gel
- 4-OHT gel, placebo tablet
- Control (placebo tablet, placebo gel)

6.2 Verification of Materials

- Prior to randomization, verify the following:
- IRB approval at the registering institution
- Patient eligibility
- Existence of a signed consent form

6.3 Document of IRB Approval

This must be on file before registering any patients.

6.4 Correlative Research

6.4.1 Mandatory

A mandatory correlative research component is part of his study; the patient will be automatically registered onto this component.

6.5 Banking

At the time of informed consent, the following will be recorded:

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

6.6 Treatment on Protocol

Treatment on this protocol must commence at Mayo Clinic Rochester Northwestern University, or MD Anderson Cancer Center at Cooper under the supervision of Dr. Amy Degnim, Dr. Seema Khan, or Dr. Catherine Loveland-Jones respectively.

6.7 Treatment Start

After study randomization and all baseline evaluations are obtained, patients will begin treatment. Treatment must start within three months of baseline physical exam and labs, with Mammogram, MBI, and research core biopsy (if applicable) performed no longer than 7 days prior to starting treatment.

These studies should be coordinated with the date they can return for follow-up testing (or date of surgical excision if applicable).

6.8 Pretreatment

Pretreatment tests/procedures (see Section 5.0) must be completed within the guidelines specified on the test schedule.

6.8.1 Baseline Symptoms

In the medical records, verified by participant and/or identified on physical examination will be recorded.

6.8.2 Pretreatment Blood Samples

- Two 10 ml purple top EDTA vials of blood will be obtained at baseline. These will be used for banking purposes. These tubes should be protected from light exposure (i.e., wrap in tine foil, use light sensitive brown bags). At Mayo, these tubes will be delivered to the BAP lab. Northwestern and MD Anderson Cancer Center at Cooper will process the blood and store in -80 freezer and send in batches to Mayo Rochester.
- Specimen labels will be preprinted and should be used for the pretreatment blood and tissue specimens. Collection/companion form will be completed at time of collection and specimen information will be entered into RAVE database.

6.8.3 Pretreatment Tissue Samples

Pretreatment Research Biopsies

See Appendix F for details on breast tissue research biopsy.

The pretreatment cores will be divided as follows:
For 9-11 G vacuum assist biopsy cores, 4-6 cores will be collected:

- Two of the largest cores will be distributed between 2 separate tubes and snap frozen (1 core per tube).

- All remaining cores will be placed in formalin.

At Mayo Clinic, the snap frozen cores will be stored in BAP, and batch shipped to Dr. Radisky's lab in Jacksonville, FL; all remaining cores will be placed in formalin, delivered to the Clinical Histology lab to create a single FFPE block if possible, and a single H&E stain per block. The H&E stained slide(s) will be delivered bythe study coordinator to breast pathologist, Dr. Solanki, or her designee, for core biopsy read within 21 days of biopsy. If DCIS or invasive cancer is identified by the breast pathologist during the clinical read of the research biopsy H&E, then this information will be relayed to the assigned clinical care provider (surgeon if upcoming surgery or Breast Clinic physician if no surgery is planned).

After the breast pathologist's read is complete, the FFPE blocks and H&E will be stored by the study team and batch shipped to Dr. Radisky's lab in Jacksonville, FL for Ki67 IHC, slide scanning at 40X, and Aperio quantitation.

If research breast biopsy is not performed, then 6 unstained 5 micron and 3 unstained 10 micron FFPE sections from the pretreatment diagnostic breast biopsy will be stored by the study team and batch shipped to Dr. Radisky's lab in Jacksonville, FL for Ki67 IHC, slide scanning at 40X, and Aperio quantitation.

- At Northwestern University and MD Anderson Cancer Center at Cooper, the snap frozen cores will be stored at -80, and the remaining cores will be put up as an FFPE block (remaining cores in one block if possible) with a single H&E stain per block, to be reviewed by the designated study pathologist at Northwestern University and MD Anderson Cancer Center at Cooper for core biopsy read within 21 days of biopsy. If DCIS or invasive cancer is identified by the breast pathologist during the clinical read of the research biopsy H&E, then this information will be relayed to the site PI, Dr. Khan, or Dr. Catherine Loveland-Jones respectively. All frozen samples and tissue blocks will be shipped to Dr. Radisky's lab in Jacksonville.

- If pretreatment research breast biopsy is not performed, then 6 unstained 5 micron and 3 unstained 10 micron FFPE sections from the pretreatment diagnostic breast biopsy will be stored by the study team and batch shipped to Dr. Radisky's lab in Jacksonville, FL for Ki67 IHC, slide scanning at 40X, and Aperio quantitation.

- Batch shipments will occur after every 5 patients via shipping kit provided by Mayo Clinic Rochester BAP with ambient shipping supplies for the FFPE blocks, and frozen shipping supplies for the frozen cores and unstained slides.

    - Specimen labels will be preprinted and should be used for the pretreatment blood and tissue specimens. Collection/companion form will be completed at time of collection and specimen information will be entered into RAVE database.

    Specimens should be shipped to the following addresses:

    Post-treatment blood and tissue for tamoxifen/4-OHT levels
    Mayo Clinic-BAP LAB
    150 Third Street SW
    Stabile SL-16
    Rochester, MN 55905
    507-284-5777

    Frozen tissue for RNA extraction (pre- and post-treatment)
    and FFPE blocks/slides (pre- and post-treatment)

    Derek Radisky Laboratory
    Attn: Lab Personnel
    Mayo Clinic Jacksonville
    4500 San Pablo Road
    Griffin Building 3_331
    Jacksonville, FL 32224
    904-625-2844

    Blood specimens for banking (pre- and post-treatment)
    Mayo Clinic-BAP LAB
    150 Third Street SW
    Stabile SL-16
    Rochester, MN 55905
    507-284-5777

6.8.4 Digital Mammogram

Digital mammogram will be obtained AT BASELINE TO INCLUDE Bilateral craniocaudal (CC) view (full field digital mammogram=FFDM), i.e., 1 CC view of each breast.
Northwestern University and MD Anderson Cancer Center at Cooper will scrub patient identifiers from mammograms (retaining date of mammogram, laterality markers, study ID, and image acquisition parameters) and transfer them using a research code on a second digital file to the study team.

6.8.5 Patient Questionnaire Booklets

The Breast Cancer Prevention Trial (BCPT) Symptom Checklist will be administered to patients. This data will be entered into the study database (RAVE). Permission to use the BCPT was provided by Annette L. Stanton, PhD, Professor Departments of Psychology and Psychiatry and Biobehavioral

Sciences, UCLA (see Appendix B).

7.0 Treatment

7. 1 Study Drug

The research team will perform a placebo-controlled randomized study of four weeks (± 3 days) of oral tamoxifen vs. 4-OHT gel vs. Placebo (see schema). Oral and gel placebos will be used to create a double-blind study. Tablets will be in an opaque gelatin capsule. Tamoxifen will be mixed with microcrystalline cellulose powder. Placebo will be only microcrystalline cellulose powder in an identical opaque gelatin capsule.

Transdermal 4-OHT/placebo gel will come in a 1 mL canister pump and a protective cap. The pouch in the canister will contain 88 g of gel. A single pump contains 1 mL of gel at a 4-OHT concentration of 2 mg/mL. The canister will be labeled as “4-Hydroxytamoxifen 2 mg/mL or Placebo gel” and “Caution: New Drug-Limited by Federal law (US) to investigational use only”. Directions for gel application will also be included on the label. Patients will also be provided with more detailed directions, to apply 2 doses/pumps of gel to each breast every day (See Appendix D). BHIR Pharma will provide identically packaged 4-OHT gel and placebo gel that will be shipped to MRI Global.

MRI Global will provide oral tamoxifen (10 mg) and matching placebo. They will ship the oral and topical medications and matching placebos to the research pharmacist at each site, who will formulate kits for patient use. Each kit will include 2 canisters of 4- OHT/placebo gel and one bottle of tamoxifen/placebo capsules. Each kit will be labelled with kit number, protocol number and contents and storage temperature and the notice and “Caution: New Drug-Limited by Federal law (US) to investigational use only”.

MRI Global will perform dissolution, disintegration, and stability testing on the tamoxifen tablets and disintegration testing on the placebo. Additionally, weights are checked during manufacturing. Stability testing will be done on an annual basis.

Tamoxifen and placebo capsules will be packaged in bottles of 31 capsules. The bottle will have a label printed as “tamoxifen citrate 10 mg or placebo” with treatment kit number, protocol number, expiration date and directions to take one tablet daily. Additional details on a product insert will be provided (See Appendix C).

Pharmacy will remove the portion of the label indicating placebo or active drug when dispensing a kit. The pharmacy will remove the un-blinding label from the study drug container (and retain within the pharmacy), then attach the patient-specific research pharmacy label to each drug container. This label provides all relevant details including patient name, clinic number, IRB number, provider name, directions for use, name of drug, and quantity.

Patients will be asked to bring their kit back on their return visit. Any remaining pills will be counted, and gel dispensers will be weighed by research pharmacy. Along with the kit, a calendar will be provided where they mark each day the pill and gel were used, and any wasted pumps (see Appendix E). Non-compliance with study treatment will be considered for patients who have less than 80% compliance with daily drug use or have missed more than 3 doses in the final 14 days. All study persons will be evaluated for toxicities. Deviation(s) from the prescribed dosage regimen will be recorded and reported to the IRB at continuing review.

7.2 Participant Contact

Participants will be contacted 1 week (+/- 2 days) after study drug initiation. A phone call, portal message, or email at Week 1 will be to assess compliance, toxicity, and changes in medication and to address any questions by the participant.

7.3 Post-Treatment Biopsy

7.3.1 Core Biopsy

See Appendix F for details on post treatment core biopsy.

At Mayo Clinic, the two snap frozen cores will be delivered to BAP by the study coordinators. After accessioning, Dr. Joel Reid's lab will be paged to pick up and store tissue for tamoxifen/4-OHT levels; and the other snap frozen core will be stored and sent to Dr. Radisky's lab for RNA extraction; the remaining two cores will be put in formalin for a single FFPE block if possible. Dr. Reid's lab will additionally need to know the date and time of the last dose of tamoxifen/placebo and 4-OHT/placebo.

At Northwestern University and MD Anderson Cancer Center at Cooper, two of the largest cores will be distributed between 2 separate tubes and snap frozen (1 core per tube), one tube is designated for Dr. Reid's lab and the other is designated for Dr. Radisky's lab. These will be stored at -80 and will then be batch shipped to Mayo Clinic Rochester BAP and Dr. Radisky's laboratories respectively. The remaining cores will be put up as a single FFPE block (if possible) and shipped to Dr. Radisky's lab also in batches.

7.3.2 Surgical Biopsy

If the patient will undergo excisional biopsy, the post-treatment tissue samples can be taken at the time of surgery. Two 1 cm^2 pieces of tissue will be needed. One of these will be put in formalin for a single FFPE block; the other tissue piece should be cut in half and placed into two separate sterile tubes for snap freezing.

One of these two cut pieces will be snap frozen and designated for Dr. Joel Reid's lab for tissue tamoxifen/4-OHT levels), and the other will be snap frozen and designated for Dr. Radisky's lab for RNA extraction.

7.3.3 Specimen Handling

Preprinted labels will be provided and should be used for the post-treatment tissue specimens.
Collection/companion form will be completed at time of collection and specimen information will be entered into RAVE database.

Directions for Mayo Clinic: Collect tissue specimens. The snap frozen cores will be stored in BAP, and batch shipped to Dr. Radisky's lab in Jacksonville, FL; the remaining cores will be placed in formalin, delivered to the Clinical Histology lab to create a single FFPE block if possible and a single H&E stain per block. The H&E stain will be delivered by the study coordinator to breast pathologist, Dr. Solanki, or her designee, for core biopsy read within 21 days of biopsy. The FFPE blocks and H & E will be stored and batch shipped to Dr. Radisky's lab in Jacksonville, FL for Ki67 IHC, slide scanning at 40X, and Aperio quantitation after the breast pathologist's read is complete.

Northwestern University and MD Anderson Cancer Center at Cooper will snap freeze two of the largest cores, which will be distributed between 2 separate tubes and snap frozen (1 core per tube). and store in -80 freezer. One frozen core (or piece of tissue) will be designated for Mayo Clinic Rochester BAP and one for Dr. Radisky's lab. Northwestern University and MD Anderson Cancer Center at Cooper will process the remaining core samples for formalin (remaining cores or a single surgical tissue piece in a single FFPE block if possible) with research H&E stain cut for each block, to be reviewed by designated pathologist within 21 days of biopsy. Frozen samples and blocks will be shipped in batches to Dr. Radisky's lab.

Shipping Directions for Northwestern University and MD Anderson Cancer Center at Cooper: Specimens for Dr. Radisky's laboratory will be batch shipped to Jacksonville. Remaining specimens should be batch shipped to Mayo Clinic Rochester BAP (4OHT blood and frozen tissue),

Send an email to RSTBBD@mayo.edu prior to all tissue shipments and obtain confirmation that the receiving party is available to receive the shipment.

Use same shipping addresses as per section 6.8.3.

7.4 Post-Treatment Blood Samples

Post-treatment, two purple top 10 ml EDTA vials of blood will be obtained within 2 days of completing the study intervention. These will be used for banking purposes. At Mayo these tubes will be delivered to the BAP lab. Northwestern University and MD Anderson Cancer Center at Cooper will process the blood and store in -80 freezer and send in batches to Mayo Clinic Rochester BAP.

In addition, one 4 ml green top heparin tube will be used for tamoxifen/4-OHT and metabolite levels. The tube will need to be processed for plasma and stored in -80 freezer. Instructions at Mayo Clinic site will be to deliver to BAP, after accessioning and processing, Dr. Joel Reid's lab will be contacted to pick up and store for tamoxifen/4-OHT levels. Northwestern University and MD Anderson Cancer Center at Cooper will batch ship these specimens. The labels should include the patient research identifier, IRB # 19- 011444, the date the specimen was collected. Collection/companion form will be completed at time of collection and will additionally include the date and time the patient took their last dose of tamoxifen/4-OHT. Preprinted labels will be provided and should be used for post-treatment blood samples for banking. Collection/companion form will be completed at time of collection and specimen information will be entered into RAVE database.

8.0 Study Conduct

Mayo Clinic IRB approval of the protocol and any amendments will be required prior to implementation. Subject records will be stored in a secure location and subject confidentiality will be maintained. The investigator will be thoroughly familiar with the appropriate use of the study drug as described in the protocol. Master files of essential clinical documents will be maintained for the duration of the study.

During the patient's clinical visit to discuss findings of AH, LCIS, or increased breast cancer risk, the patient's physician will introduce the study to the patient. If the patient

agrees to participate in the study, a study coordinator will answer any additional study questions and start the informed consent process. The study coordinator will also provide, as indicated, appointments for a pregnancy test and FSH levels, baseline labs along with all other pre-treatment tests and procedures. The patient will be given a study kit by the RN study coordinator with directions for taking the pills and applying the gel. The study coordinator will also schedule a return visit appointment, in which the patient will complete the BCPT symptom questionnaire, along with post-treatment blood tests, study mammogram, breast biopsy (core or excisional), and study MBI (only at Mayo clinic) at the completion of treatment. Post-study breast biopsy and blood draw must be scheduled between days 25-31 of drug treatment with the last dose of study drug within 2 days (preferably within 24 hours) prior to the post-treatment biopsy and blood draw.

Other post-treatment tests will be scheduled PRIOR to the breast biopsy but not more than 3 days prior to the post-treatment breast biopsy. The RN coordinator will instruct patient that they need to avoid pregnancy during this treatment and 2 months after treatment and instruct: The effects of topical 4-OHT gel on the developing human fetus at the recommended therapeutic dose are unknown. However, oral tamoxifen is Pregnancy Category D—Positive evidence of human fetal risk. For this reason, and because triphenylethylene antiestrogens, including tamoxifen, are known to be teratogenic, women of childbearing potential and their male partners must agree to use at least one effective form of birth control (abstinence is not an allowed method) prior to study entry and for the duration of study participation, and for two months following the last dose of study medications (participant can resume oral birth control pills for effective birth control measures after post-treatment biopsy is done). Effective birth control methods are copper and Mirena IUD (intrauterine device), diaphragm/cervical cap/shield, spermicide, contraceptive sponge, condoms; these may be used during and after treatment. Oral contraceptives may only be used after the 4-week treatment is complete, and the post-treatment biopsy is obtained. Women of childbearing potential must have a negative pregnancy test (either serum or urine pregnancy test) within five days before starting study medications. Tubal Ligation is also an acceptable form of birth control. Should a woman become pregnant or suspect she is pregnant while participating in this study, she should inform her study physician immediately.

If DCIS or invasive cancer is identified by the breast pathologist during the clinical read of the research biopsy H&E, then this information will be relayed to the assigned clinical care provider (surgeon if upcoming surgery or Breast Clinic physician if no surgery is planned).

9.0 Protocol Treatment Table

Arm	Agent	Dose Level	Route	Day	Treatment

I	Oral Tamoxifen and placebo gel	10 mg oral tamoxifen; No active ingredient in gel	Oral and Topical	One daily dose orally and two topical doses to each breast	Pill - Tamoxifen or placebo. Take one tablet daily. Gel – 4-OHT or placebo. Apply two pumps to each breast, one pump at a time. Spread 1 pump of gel across entire breast. After 1st pump has dried apply 2nd pump. Caution: New Drug- Limited by Federal law (US) to investigational use.
2	Placebo tamoxifen and 4-OHT gel	No active oral ingredient; 2 ml of gel with 4OHT concentration of 2 mg/ml to each breast (total 4 mg per breast; 8 mg total for both breasts)	Oral and Topical	One daily dose orally and two topical doses to each breast	Pill - Tamoxifen or placebo. Take one tablet daily. Gel – 4-OHT or placebo. Apply two pumps of gel to each breast, one pump at a time. Spread 1 pump of gel across entire breast. After 1st pump has dried apply 2nd pump. Caution: New Drug- Limited by Federal law (US) to investigational use.
3	Placebo tamoxifen/placebo gel	No active ingredients orally nor in gel	Oral and Topical	One daily dose orally and two topical doses to each breast	Pill - Placebo. Take one tablet daily. Gel –placebo. Apply two pumps of gel to each breast, one pump at a time. Spread 1 pump of gel across entire breast. After 1st pump has dried apply 2nd pump. Caution: New Drug- Limited by Federal law (US) to investigational use.

9.1 Self-Administration Statement

Patients can be instructed in administration techniques, given written directions (see Appendix C and Appendix D), and granted treatment independence with coordinator approval**.** Patients will be instructed to apply gel and take their tablet about the same time every day. The gel should be applied after both breasts are washed, either by a shower or bath using a washcloth. Mornings are preferable time to use gel to minimize transferring to anyone while sleeping. Care needs to be taken to avoid flames or smoking while applying the gel due to alcohol content while in gel form. Once dry, this is no longer a problem. The treated breasts should be covered at all times to decrease the possible transfer to others and to protect from light. No washing or immersion of the area for a minimum of 4 hours after application.

9.2 Return to Consenting Institution

For this protocol, the patient must return to the consenting institution for evaluation at the end of treatment.

9.3 Data and Safety Monitoring Board (DSMB)

The Mayo Clinic Department of Surgery (DOS) Data and Safety Monitoring Board (DSMB) exists as a component of the Division of Surgery Research. The DOS DSMB is structured to serve clinical intervention trials. The function of a DSMB is to review data and endpoints on a timeline set forth by the Data Safety Monitoring Plan (DSMP) in the IRB approved protocol. The DSMB will evaluate Serious Adverse Events as reported. These events will also be reported to the IRN and NCI DCP. Membership of the DSMB includes the physician chair, physician representatives, a biostatistician, and an allied health program manager. The DSMB conducts an initial review of the protocol, informed consent, and the Data Safety Monitoring Plan (DSMP) prior to enrollment of subjects to determine the study's risks and benefits and safety of research subjects, suggest improving the study design, identify what data will be required for review and identify early stopping rules. The DOS DSMB meets quarterly reviewing each study at least bi-annually. Special meetings of the DSMB may be convened more often, as necessary, to address urgent concerns regarding patient safety and data integrity. Studies identified by the IRB for more intensive monitoring may be reviewed on a quarterly, rather than semiannual basis. Special sessions of the DSMB may be held to address protocol specific issues, as necessary if the events of that protocol require review prior to the next scheduled DSMB meeting. Such meetings may occur through an electronic format.

Evaluation of the study to help facilitate ongoing study management and compliance with applicable regulations, laws, and mandates will be conducted by the Mayo Clinic Office of Research Regulatory Support (ORRS) at the Mayo Clinic site and a to be named Mayo employee, will monitor Northwestern University and MD Anderson Cancer Center at Cooper. The study monitor will review processes after the first five subjects are entered into the database to ascertain all processes have been followed. After determining that the process is being followed, the monitor will assess at least annually.

9.3.1 Subject Safety

Study eligibility is confirmed by study coordinators. Breast Clinic physicians and Breast Surgeons will receive information when this study is activated through presentations by the study PI. The study coordinator will review medical records of patient with a diagnosis of ADH, ALH, LCIS, and/or increased breast cancer risk to confirm that they meet eligibility criteria. The patient's physician will be notified by the study coordinator of the intent to approach the patient for inclusion in this study.

There are no safety tests/questionnaires.

The patients will be informed that they are to contact the study coordinator if they encounter any problems with their treatment. The study coordinators will update the BBD study team of any anticipated adverse event at team meetings every other week (Dr. Khan's and Dr. Catherine Loveland-Jones' group will send an e-mail to the coordinator team at RSTBBD@mayo.edu who will bring this to the regular meeting and get back to Dr. Khan's or Dr. Catherine Loveland-Jones' team that the side effect was reviewed). If a serious adverse effect occurs, the study coordinator will contact the PI (Drs.

Degnim, Khan or Catherine Loveland-Jones), or her designee when PI is away from the clinical setting (Dr. Tina Hieken, Dr. Karthik Ghosh, Dr. Christine Klassen). Serious Adverse Events and contact processes are covered under 10.2.

9.3.2 Data Integrity

Data regarding study start and consent will be kept in PTRAX at Mayo. Northwestern University and MD Anderson Cancer Center at Cooper will follow their institution's tracking system. The following data will be recorded in the study database (RAVE) by the study coordinators:

- Study sample attainment dates
- Drug start and stop dates
- Deviation(s) from the prescribed dosage

regimen will be recorded.

- Missed drug dates or deviations for the prescribed dosage regimen reported by the patient will be reviewed with participant and documented in the study database. Participant diaries will be retained in the subject folder as source documents.
- Attribution to the study agent
- Adverse effects assessed according to CTAE terminology, along with start and stop dates; CTAE severity score (AEs without a CTCAE term will be graded according to general severity guidelines)
- SAEs, date and level, whether codes were broken to identify agent
- BCPT symptom survey results

9.3.3 Product Accountability

- The research pharmacy will store the drug kits in their area at each institution.
- When a patient has agreed to participate in the study, they will be randomized per the RAVE Medidata database. Once randomized, the research pharmacy will be notified of randomization results and will prepare kit for dispensing when the order and communication form is received from the study coordinators. The pharmacist will then dispense the kit to the study coordinator, who will deliver it to the patient, and provide instructions to the patient.
- The research pharmacy will be accountable for the drug kits.

9.3.4 Study Documentation

Study coordinators will maintain subject files. They are responsible for keeping these files up to date.

9.3.5 Study Coordinator

The BBD group works as a team and has study coordinators available to support the study. Funding for this study coordinator support is provided by the parent R01 grant funding the study. Team meetings will be held every other week. We will have monthly communications with Dr. Khan at Northwestern University and Dr. Catherine Loveland-Jones at MD Anderson Cancer Center at Cooper. A SAE will stimulate an immediate meeting with Dr. Degnim and the DSMB.

9.4 Breaking Codes in Double-Blinded Studies

A) **Situations requiring codes to be broken**: There are three distinct situations in which it is appropriate to break the codes for individual patients

enrolled in double-blind trials:

(1) In the event of an emergency or a serious adverse event for an individual patient.

(2) In the event that it would be helpful for the future clinical care of an individual patient after she/he has completed participation in the trial.

(3) When it is necessary to know the specific treatment assignment of each patient in the trial in order to manage them all appropriately after they have completed participation in the trial.

Study participants will receive study medications, blinded, from the investigational pharmacy. The blind will be maintained through the effort of the research pharmacist and the pharmacy.

In the event of an emergency or a serious adverse event, the PI (or their designee) will be notified to assess the medical necessity of unblinding. Emergency unblinding should only occur in medical events where not knowing the treatment assignment will affect ongoing treatment or significantly compromise the clinical management of the patient.

Unblinding due to a Serious and Unexpected Adverse Event will happen only with permission of Dr. Amy Degnim, or designated site PI in the event of emergency if Dr. Degnim cannot be reached.

Breaking the blind for serious and unexpected adverse events could occur when:
1. The blind should be broken for serious and unexpected adverse events that would meet the criteria for reporting as single occurrences or one or more occurrences. The blind would need to be broken to determine if there is a reasonable possibility that the drug caused the adverse event.
2. If the blind is broken and the subject was receiving **placebo**, the event should not be reported in an IND safety report because there is not a reasonable possibility that the study drug caused the adverse event.
3. If the blind is broken and the subject was receiving **drug treatment**, the suspected adverse reaction must be reported in an IND safety report. (Note: the case must meet the definitions of suspected adverse reaction, serious, and unexpected.)

If unblinding is necessary and after PI approval, the Mayo Clinic team designee will contact the site-specific pharmacy or statistical team / data manager to break the code while protecting the PI from the unblinded information, unless unblinding the PI is deemed necessary for either patient management or required event reporting. The reason and date of unblinding should be documented in Medidata RAVE by the statistical team / data manager.

Contact information for Dr. Degnim:
507-284-4499 (Medical Administrative Assistant)
For after hours or emergency notification, Dr. Degnim can be reached at:
Phone # 507-250-4149 or
Pager # 507-284-6357

Mayo Clinic Research Pharmacy:
Phone Number: 507-538-0008
Email: MCPHARMACYORSS@mayo.edu

Northwestern University Pharmacy:

Phone: 312-926-0747
Email: NMInvestigationalDrugService@nm.org
MD Anderson Cancer Center at Cooper Pharmacy:
Phone Number: 856-735-6231
Email: Pharmacy-MDA-IDS@CooperHealth.edu
If, in the judgment of the attending physician, it would be helpful for the future clinical care of the individual patient, the code may be broken if requested by the patient, and *after* the patient has discontinued any duration of the study drug interventions, and no sooner than one month AFTER discontinuation of the study drug interventions while protecting the PI from the unblinded information. That is, after the patient has been fully evaluated and all evaluation information has been recorded by the attending physician and the patient (if appropriate), the patient's clinical care team (but not the study PI) may request tinformation on which study therapy the patient was receiving and share this information with the patient. Information on the assigned treatment arm may be obtained per preferred institutional process, either via the Research Pharmacy or via a designated study team member other than the PI (Mayo Clinic).

9.5 Ancillary Treatment/Supportive Care

The duration of the intervention is short; thus, short-term toxicity is expected to be limited to only temporary vasomotor symptoms.

9.6 Dose Discontinuation Criteria

Any subject with an adverse event Grade 3 or higher (unless judged to be definitely unrelated to study agents) will be discontinued from both oral and topical interventions. These subjects may choose either to proceed with post-intervention blood and tissue samples, to continue participation with only clinical and symptom survey follow-up, or to withdraw from all further participation in the study.

9.7 Full Supportive Care

Patients should receive full supportive care while on this study. This includes blood product support, antibiotic treatment, and treatment of other newly diagnosed or concurrent medical conditions.

9.8 Definitions

Protocol Deviations
Noncompliance with the research protocol that does not increase risk or decrease benefit and/or affect the integrity of the data

Protocol Violation
Noncompliance with the IRB-approved protocol without prior sponsor and IRB approval. Violations generally increase risk or decrease benefit, affect the subject's rights, safety, or wellbeing, or impact the integrity of the data

Noncompliance
Defined as a failure to follow the regulations, applicable law, institutional policy, and deliberations of the IRB

Adverse Event
Any unfavorable symptom, sign, or disease (including an abnormal laboratory

finding) temporally associated with the use of a medical treatment or procedure, whether or not considered drug related.

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

Expedited Reporting
Expedited Reporting Requirements for IND/IDE Agents
Late Phase 2 and Phase 3 Studies: Expedited Reporting Requirements for Adverse Events that Occur on Studies under an IND/IDE within 30 Days of the Last Administration of the Investigational Agent/Intervention *Routine Reporting*
Events reported to sponsor via case report forms

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

9.9 Adverse Event Characteristics

Adverse events will be collected starting at the On Study visit and ending 30 days after the post-treatment biopsy is completed. Each participant will be asked about AEs at every study visit and participant contact. AEs will be documented and logged in the RAVE Database.

CTCAE term (AE description) and grade: The descriptions and grading scales found in the revised NCI Common Terminology Criteria for Adverse Events (CTCAE) version 5.0 will be utilized for AE reporting. All appropriate treatment areas should have access to a copy of the CTCAE version 5.0. A copy of the CTCAE version 5.0 can be downloaded from the CTEP web site: https://prevention.cancer.gov/clinical-trials/clinical-trials-management/cp-ctnet-instructions-forms

a. Identify the grade and severity of the event using the CTCAE version 5.0.
b. Determine whether the event is expected or unexpected (see Section 6.11).
c. Determine if the adverse event is related to the study intervention (agent, treatment, or procedure).
d. Determine whether the event must be reported as an expedited report. If yes, determine the timeframe/mechanism (see Section 10.3)
e. Determine if other reporting is required (see Section 10.0-10.2)
f. Note: All AEs reported via expedited mechanisms must also be reported via the routine data reporting mechanisms defined by the protocol.

Note: A severe AE is NOT the same as a serious AE, which is defined in Section 10.2

9.10 Expected vs. Unexpected Events

Expected events - are those described within the Section 9.10 of the protocol, the study specific consent form, package insert (if applicable), and/or the investigator brochure, (if an investigator brochure is not required, otherwise described in the general investigational plan).

Unexpected adverse events or suspected adverse reactions are those not listed in Section 10.2 of the protocol, the study specific consent form, package insert (if applicable), or in the investigator brochure (or are not listed at the specificity or severity that has been observed); if an investigator brochure is not required or

available, is not consistent with the risk information described in the general investigational plan.

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

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

9.11 Attribution to Agent(s) or Procedure

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

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

CTCAE[1] System Organ Class (SOC)	**Adverse event/ Symptoms**	**CTCAE Grade at which the event will not be reported in an expedited manner[2]**
Vascular Disorders	Hot flashes	≤Grade 3
Musculoskeletal and Connective Tissue Disorders	Generalized muscle weakness	≤Grade 3
Gastrointestinal Disorders	Nausea	≤Grade 3
Nervous System Disorders	Paresthesia	≤Grade 3

[1] Report any CTCAEs on https://prevention.cancer.gov/clinical-trials/clinical-trials-management/cp-ctnet-instructions-forms Additional instructions: The research coordinator will work with Site PI and complete IRB reporting to determine if FDA submission is needed.

Grades from aforementioned CTCAE site:
Grade refers to the severity of the AE. The CTCAE displays Grades 1 through 5 with unique clinical descriptions of severity for each AE based on this general guideline:
Grade 1 Mild; asymptomatic or mild symptoms; clinical or diagnostic observations only; intervention not indicated.
Grade 2 Moderate; minimal, local or noninvasive intervention indicated; limiting age-appropriate instrumental ADL*.
Grade 3 Severe or medically significant but not immediately life-threatening; hospitalization or prolongation of hospitalization indicated; disabling; limiting self-care ADL**.
Grade 4 Life-threatening consequences; urgent intervention indicated.
Grade 5 Death related to AE.

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

10.0 Other Required Reporting

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

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

10.1.1 Unexpected (in terms of nature, severity, or frequency) given (a) the research procedures that are described in the protocol-related documents, such as the IRB-approved research protocol and informed consent document; and (b) the characteristics of the subject population being studied;

10.1.2 Related or possibly related to participation in the research (in this guidance document, possibly related means there is a reasonable possibility that the incident, experience, or outcome may have been caused by the procedures involved in the research); and

10.1.3 Suggests that the research places subjects or others at a greater risk of harm (including physical, psychological, economic, or social harm) than was previously known or recognized.

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

10.2 Serious Adverse Event (SAE) Monitoring and Reporting

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

The site principal investigator is responsible for notifying FDA, Mayo Clinic Department of Surgery Data Safety Monitoring Board, and all participating investigators in a written safety report of any of the following:

- Any suspected adverse reaction that is both serious and unexpected.
- Any findings from epidemiological studies, pooled analysis of multiple studies, or clinical studies, whether or not conducted under an IND and whether or not conducted by the sponsor Amy Degnim, M.D., that suggest a significant risk in humans exposed to the drug.
- Any clinically important increase in the rate of a serious suspected adverse reaction over the rate stated in the protocol.
- Grade 4 AEs regardless of attribution to the study treatment or procedure.
- Grade 5 AEs (Deaths)
- Any death within 30 days of the patient's last study treatment or procedure regardless of attribution to the study treatment or procedure.
- Any death more than 30 days after the patient's last study treatment or procedure that is felt to be at least possibly treatment related must also be submitted as a Grade 5 AE, with a CTCAE type and attribution assigned.

SAE Reporting Summary

WHO	SAE	WHAT FORM	WHERE TO SEND
All sites	Pregnancy Reporting	https://prevention.cancer.gov/clinical-trials/clinical-trials-management/cp-ctnet-instructions-forms	RSTBBD@mayo.edu
		MedWatch 3500A: http://www.fda.gov/downloads/AboutFDA/ReportsManualsForms/Forms/UCM048334.pdf	The Mayo Clinic Office of Research and Regulatory Support will assist Mayo Clinic Rochester in filing the MedWatch 3500A form to the FDA. Please refer to section 10.3 for reporting timelines. Mayo Clinic Rochester will report the SAE to the following: DCP Regulatory Contractor, CCS Associates: safety@ccsainc.com
All sites	FDA Reporting	MedWatch 3500A: http://www.fda.gov/downloads/AboutFDA/ReportsManualsForms/Forms/UCM048334.pdf	RSTBBD@mayo.edu
All sites	SAEs	Mayo Clinic Department of Surgery Data and Safety Monitoring Board (DSMB)	Mayo Clinic Rochester will contact DSMB within 24 hours of notification of SAE

Mayo Clinic Rochester study team will contact the Mayo Clinic Department of Surgery (DOS) Data and Safety Monitoring Board (DSMB) within 24 hours of notification of SAE. The team will also report as indicated in section 10.2 SAE Reporting Summary.

Report the following SAE information when contacting the Mayo Clinic Department of Surgery (DOS) Data and Safety Monitoring Board (DSMB):

- Date and time of the SAE
- Date and time of the SAE report
- SAE description and attribution to drug
- Reporter name, email, and telephone number
- Affiliation/Institution where SAE occurred
- DCP and IRB protocol number
- Title of protocol

10.2.1 An initial assessment of the SAE will be made by the DSMB. The study team will submit to the Institutional Review Board who will determine which SAEs require FDA submission as IND safety reports as noted in section 10.2.

10.2.2 Follow-up of SAE

Mayo Clinic, Northwestern University and MD Anderson Cancer Center at Cooper will send requested follow-up reports to DCP as soon as possible. This information should be entered on the DCP SAE Report Form.

BHIR Besins Pharmacovigilance will not have any monitoring or reporting requirements. Besins will receive reports at the time of the annual Continuing Review.

10.3 Expedited Reporting Requirements for IND Agents

Late Phase 2 and Phase 3 Studies: Expedited Reporting Requirements for

Adverse Events that Occur on Studies under an IND/IDE within 30 days of the Last Administration of the Investigational Agent/Intervention.[1, 2]

FDA REPORTING REQUIREMENTS FOR SERIOUS ADVERSE EVENTS (21 CFR Part 312)

NOTE: Investigators **MUST** immediately report to the sponsor (NCI) **ANY** Serious Adverse Events, whether or not they are considered related to the investigational agent(s)/intervention (21 CFR 312.64)

An adverse event is considered serious if it results in **ANY** of the following outcomes:

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

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

Hospitalization	Grade 1 Timeframes	Grade 2 Timeframes	Grade 3 Timeframes	Grade 4 & 5 Timeframes
Resulting in Hospitalization ≥ 24 hrs.	10 Calendar Days			24-Hour 5 Calendar Days
Not resulting in Hospitalization ≥ 24 hrs.	Not required		10 Calendar Days	

NOTE: Protocol-specific exceptions to expedited reporting of serious adverse events are found in the Specific Protocol Exceptions to Expedited Reporting (SPEER) portion of the CAEPR

Expedited AE reporting timelines are defined as:

- "24-Hour; 5 Calendar Days" - The AE must initially be reported via AdEERS within 24 hours of learning of the AE, followed by a complete expedited report within 5 calendar days of the initial 24-hourreport.
- "10 Calendar Days" - A complete expedited report on the AE must be submitted within 10 calendar days of learning of the AE.

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

Expedited 24-hour notification followed by complete report within 5 calendar days for:

- All Grade 4, and Grade 5 AEs

Expedited 10 calendar day reports for:

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

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

Effective Date: May 5, 2011

10.4 Protocol Deviations

Minor protocol deviations must be documented and reported at the time of continuing review. Notifying the IRB through a reportable Event submission is required if a deviation that imposes a suspected increase in the risk of harm to participants or adversely affects the integrity of the data.

Study staff will refer to the protocol and study specific SOPs, institutional and departmental policies. All associated source documents will be collected, including all pertinent outside medical information, which will be reviewed and assessed by the PI or Co-investigator. The PI will determine whether event meets UPIRTSO or significant new information criteria and/or protocol violation/deviation definition.

Events will be reported per the Investigator's direction in alignment with the institutional policies to:
-Mayo IRB
-Data Safety Monitoring Board (DSMB)
-Food and Drug administration (FDA)- if Mayo sponsor/investigator holds the Investigational New Drug (IND) or Investigational Device Exemption, (IDE) contact Office of Research Regulatory Support (ORRS)
(See Section 10.0 Other Required Reporting)

Event will be documented in the Deviation Log at Mayo Clinic and all associated documentation will be retained. Northwestern University and MD Anderson Cancer Center at Cooper will follow their institutional policy for documentation.

11.0 Statistical Considerations

The primary hypothesis is that the reduction in Ki67 will not be inferior to that seen with oral tamoxifen. A sample size of 36 per treatment arm (36 receiving oral tamoxifen and 36 receiving 4OHT gel, with 18 placebo controls) will provide >80% power at α=0.05 to test this hypothesis. Volume density and MBI will be compared across study arms from pre-to post-treatment. Risk predictors identified from Nanostring-based mRNA expression assay (conducted under IRB 75-87) and biomarkers of tamoxifen response (conducted under IRB 15-000279) will also be tested.

Randomization and intervention. To achieve the planned sample size of N=90 women, we will over-accrue by ~15% to allow for cancellation, ineligibility, non-evaluable samples, or major treatment deviations and thus plan to enroll a total of N=104 at two study sites (42 at Mayo-Rochester, 42 at Northwestern University, 20 at MD Anderson Cancer Center at Cooper). Patients will be randomized 2:2:1 to oral Tam (10 mg/day), 4-OHT gel (4 mg/each breast/day), or placebo controls for four weeks. Estimated numbers of randomized subjects to each arm will be: 42 to oral Tam, 42 to 4OHT gel, and 21 double placebo, in order to arrive at final sample size of 36, 36, and 18. Treatment randomization will be stratified with respect to menopausal status (pre- vs. postmenopausal), diagnosis (ADH vs ALH/LCIS vs increased BC risk based on risk model calculations), and study site. Oral and gel placebos will be used to create a double-blinded study. After four weeks, blood draw, physical exam, BCPT symptom survey, digital mammogram (and MBI in Mayo subjects) will be repeated. A second breast tissue sample will be obtained from the same quadrant, either as a core biopsy or as tissue adjacent to the surgical excision site.

Feasibility. At Mayo Clinic in Rochester, MN, we see an average of 55 patients per year with ADH or ALH who would be eligible for this study. At Mayo Clinic, Drs. Degnim, Hieken, and Ghosh have successfully led accrual of over 500 women to prospective studies in the last six years, therefore successful accrual of women to this prospective study is feasible. At Northwestern University, there is a similar volume of women with diagnoses of ADH or ALH, and they have accrued ~400 women over five years to various studies requiring some form of intervention (either medication or biopsy). Accrual plan is for 42 subjects at Mayo Clinic, 42 at Northwestern University and 20 subjects at MD Anderson Cancer Center at Cooper. With an anticipated timeframe of 48 months to complete accrual, this averages to 1-2 patients per month per site. This study should be acceptable to patients as the duration of intervention is short and therefore short-term

toxicity is expected to be limited to only temporary vasomotor symptoms.

Translational studies. The primary endpoint will be change in Ki67 in background normal breast lobules (% cells positive by Aperio quantitation). Additional endpoints will include changes in volumetric density (% and dense volume) measured using VolparaTM both at baseline and after four weeks of treatment. In addition, promising genes from discovery work in Aim 2 will be evaluated using NanoString.

Changes in background parenchymal uptake (BPU) on MBI will also be employed as an exploratory endpoint. Blood samples and residual breast tissues collected pre/post Tam will be stored for future correlative studies.

Endpoints. The primary endpoint is change in Ki67 level pre/post treatment. The primary analysis for the primary endpoint will include all eligible patients who began study drug intervention and have available tissue measures of Ki67 levels at both timepoints (pre- and post-treatment).

A sensitivity analysis for the primary endpoint will also be performed. This sensitivity analysis will include eligible patients who have available tissue measures of Ki67 levels at both timepoints and were compliant with study treatment. Patients will be classified as non-compliant with the study treatment (and thus excluded from the sensitivity analysis) using criteria outlined above (patients who have less than 80% compliance with daily drug use or have missed more than 3 doses in the final 14 days).

As for the primary endpoint, primary analyses for secondary endpoints will include all eligible patients who began study drug and have available endpoint measurements at both timepoints; sensitivity analyses for each secondary endpoint will exclude non-compliant patients (as defined above). For analyses of adverse events, all patients whostarted the study treatment will be included, regardless of treatment compliance or availability of endpoint measurements at both timepoints.

Statistical analysis. The primary hypothesis is that the reduction seen with 4-OHT gel will be *not inferior* to that seen with oral tamoxifen. We also hypothesize that oral tamoxifen and 4-OHT gel will each be superior to placebo with respect to the percent change in Ki67 in background normal lobules between baseline and four weeks. Analyses will assess if significant changes in Ki67 are observed after four weeks of treatment by paired t-tests/Wilcoxon signed rank tests, stratified by treatment group. We will assess the non-inferiority of 4-OHT gel compared to oral tamoxifen by estimating a 95% confidence interval for the mean *difference* in percent change between the two agents after four weeks of treatment; if the lower limit of the confidence interval is less than the non- inferiority margin (described below), we will conclude that 4-OHT gel is non-inferior to oral tamoxifen. As exploratory analyses, we will assess pairwise differences between samples on active treatment (oral tamoxifen or 4-OHT gel, separately and combined) vs. placebo using two-sample t-tests/Wilcoxon rank sum tests. Similarly, we will assess changes from baseline in the expression of the candidate genes identified in Aim 2 (absolute log2 fold- change>2 and/or q-value<0.05). Volumetric density changes from baseline within treatment group will be assessed continuously, and evaluated using paired t-tests on the log-transformed density or Wilcoxon signed-rank tests; pairwise differences between treatment groups will be assessed with two-sample t-tests/Wilcoxon rank sum tests. BPU on MBI is measured as a four category ordinal scale; we will compare pre-to-post changes within group using Wilcoxon signed rank tests and will compare the percent of subjects decreasing BPU category by one or more level between groups using chi-square tests. We will assess the correlation between changes in volumetric density and changes in BPU, using Spearman correlation. Symptoms from the BCPT survey are measured on a five- level ordinal scale. We will compare differences in pre- to post-treatment symptom severity changes across

treatment arms using Wilcoxon rank sum tests.

Sample size/power. Prior studies suggest that oral tamoxifen will lead to a 50% mean relative reduction in Ki67 in normal lobules at four weeks compared to baseline.[19-21, 43] We assume that the standard deviation for the percent change in Ki67 is 30 percentage points, estimated based on a plausible range from published literature.[19-21, 43]] *A sample size of 36 per active treatment arm will provide >80% power at α=0.05 to test the hypothesis that 4-OHT gel is not inferior to oral tamoxifen with a non-inferiority margin of 20 percentage points (e.g., if oral tamoxifen results in a 50% mean reduction of Ki67, 4- OHT gel will be considered non-inferior if we can conclude that 4-OHT gel results in a mean reduction of Ki67 of at least 30%).* Further 18 subjects enrolled in a placebo arm will provide >90% power to detect a mean difference of 50% reduction for either treatment arm versus an assumed mean difference of 0% for placebo assuming a two-sided, two-sample t-test with α=0.05. Therefore n=90 subjects are required, but the study will over-accrue by 15% (n=104 patients) to account for cancellation, ineligibility, non-evaluable samples, or major treatment deviations.

Updates to Sample size/power with 4OHT dose modification 10/12/22.
We expect that the higher dose of 4OHT gel (8 mg/day) will be at least as effective as the original dose (4 mg/day), and therefore the primary endpoint, sample size, and power calculations remain the same and may even be conservative, assuming analysis including all subjects treated with 4OHT gel at either dose. We also plan to conduct sensitivity analyses for the primary endpoint excluding the 4mg/day group (this will be N=5 patients on the 4 mg/dose that would be excluded).

To date, 12 patients have been accrued (5 oral Tam, 5 4OHT gel 4 mg/day dose, and 2 controls). Subtracting these N=12 patients accrued from the total original planned sample size of N=104, there will be N=92 patients remaining to accrue who will be randomized after the 8 mg/day 4OHT gel dose is implemented, per existing 2:2:1 schema.

With anticipated sample size of 92 patients to be randomized after approval of the higher (8 mg/day) 4OHT gel dose, we estimate final total randomized numbers will be as follows: Total N=104, with 41 on oral Tam, 5 at the 4 mg/day dose of 4OHT gel, 36 at the 8 mg/day dose of 4OHT gel, and 22 dual-placebo controls.

***Anticipated power for the primary endpoint*.**
Primary endpoint is noninferiority of Ki67 change between 4OHT gel and oral Tam arms.

For a combined analysis including ALL subjects receiving 4OHT gel at EITHER dose, ie 41 oral Tam versus 41 with 4OHT gel (5 at 4mg/day dose plus 36 at 8 mg/day dose), keeping the original assumption of 15% attrition results in final sample of 36 per group and power of 80%.

For a *subgroup analysis* including ONLY the subjects receiving the higher 8 mg/day dose of 4OHT gel, the power ranges from 76% to 82%, depending on the amount of attrition (see Table below).

Table 1. Sample size and power considerations for primary endpoint, non-inferiority in Ki67 change between gel and oral

	Sample size & power ***assuming 15% attrition***	Sample size & power ***assuming 10% attrition***	Sample size & power ***assuming 5% attrition***	Sample size & power ***assuming no attrition***
Use entire sample,	36 per group (Power: 80%)	37 per group (Power 81%)	39 per group (Power 83%)	41 per group (Power: 85%)

including subjects with either 4 mg/day or 8 mg/day 4OHT gel				
Subgroup analysis including only those with 8 mg/day dose	36 oral Tam group, 31 in 8 mg/day 4OHT gel group (Power 76%)	37 oral Tam group, 32 in 8 mg/day 4OHT gel group (Power 78%)	39 oral Tam group, 34 in 8 mg/day 4OHT gel group (Power 80%)	41 oral Tam group, 36 in 8 mg/day 4OHT gel group (Power 82%)

12.0 Descriptive Factors

Examination of differences between groups on BCPT will occur across study arms on the 8 identified scales and on additional items measuring fatigue and sexual function.

13.0 Treatment/Follow-up Decision at Evaluation of Patient

Reason Off Treatment
Study Complete
Adverse Event
Death
Disease Progression
Lost to Follow-up
Non-Compliance
Participant Withdrawal
Participant Refused Follow-up
Physician Decision
Protocol Defined Follow-up Completed
Protocol Violation
Ineligible
Other

13.1 Ineligible

A patient is deemed *ineligible* if the patient did not satisfy each and every eligibility criteria for study entry. If the patient received treatment, the patient may continue treatment at the discretion of the physician as long as there are not safety concerns. The patient will continue in the Active Monitoring/Treatment phase of the study.

13.2 Major Violation

A patient is deemed a *major violation*, if protocol requirements regarding treatment are severely violated that evaluability for primary end point is questionable. If the patient received treatment, the patient may continue treatment at the discretion of the physician as long as there are not safety concerns. The patient will continue in the Active Monitoring/Treatment phase of the study.

13.3 Withdrawal / Screen Failure

A patient is deemed a *withdrawal* if she meets all eligibility criteria, consented to

participate, and is removed from the study or chooses not to participate for any reason. A patient is deemed a screen failure if she fails to meet all study inclusion / exclusion criteria, whether consented or not. Reasons for withdrawals and screen failures will be recorded in the study database.

Patients who withdraw from the study will be offered follow-up for resolution of adverse events, and this will consist of telephone or in-person visit evaluation as clinically needed and agreed upon by the patient.

If a patient withdraws from the study prior to initiating any of the study drug interventions, then she may be replaced with another patient toward the accrual total. If a patient withdraws from the study AFTER initiating any of the study drug interventions, then she will not be replaced with another patient toward the accrual total.

14.0 Body Fluid and Tissue Biospecimens

Body fluid and tissue biospecimens will be obtained both pre- and post-treatment.

Blood: 2 vials of whole blood, each 10 ml, drawn in purple top EDTA tubes (blood card specifies 2 - 10ml EDTA tubes). BAP will accession and label. Aliquot (1) 4000uL blood pour for DNA extraction. Refrigerate. Centrifuge at lab standards.
First EDTA vial should be processed for three 1 ml aliquots of plasma into 3 cryovials 1 ml plasma each and 1ml WBC. **Second EDTA vial** is stored as whole blood.
Freeze products from both vials. Post-treatment only-One 4 ml. vial of whole blood, drawn in green top heparin tube, processed for plasma. Stored at -80 freezer.

Tissue: Core biopsies or surgical tissue will be used to create two snap frozen samples and a single paraffin block (if applicable).

For more details, please see individual sections above as follows:
Pre-Treatment Blood Samples- section 6.8.2.
Pre-Treatment Tissue Samples- section 6.8.3.
Post-Treatment Biopsy- section 7.3.
Post-Treatment Blood Samples- section 7.4

15.0 Summary Table of Research Blood and Tissue Specimens to be Collected for this Protocol

Research	Specimen Purpose	Mandatory or Optional	Blood or Body Fluid being Collected	Type of Collection Tube (color of tube top)	Volume to collect per tube (# of tubes to be collected)	Pre-Intervention	Post-Intervention
Blood	Banking	Mandatory	Whole blood - Aliquot (1) 4000uL blood pour for DNA extraction. Refrigerate. Centrifuge at lab standards. **First EDTA vial** should be processed for three 1 ml aliquots of plasma into 3 cryovials 1 ml plasma each and 1ml WBC. **Second EDTA vial** is stored as whole blood Freeze. Light Sensitive	Purple/ EDTA	10 mL (2 tubes)	X	X
Blood	4-OHT levels	Mandatory	One 4 ml green top heparin tube will be used for 4-OHT levels. This tube is to be wrapped in tin foil due to light sensitivity. The tube will need to be processed for plasma and stored in - 80 freezer.	Green/ Heparin	4 mL		X
Breast Tissue Pre Treatment from Diagnostic Clinical Biopsy	Ki67 and RNA extraction	Mandatory if opting out of PreTreatment Research Biopsy	PreTreatment- 6 5 micron FFPE sections for H&E and Ki67, and to be determined IHC biomarker assays 3 10 micron FFPE sections for RNA extraction	FFPE	FFPE sections. See pathology manual for details of cores per tube, processing, and handling.	X	

Breast Tissue PreTreatment Research Biopsy	banking	Optional	4 vacuum assist cores- either 9 or 11 G For PreTreatment-Two cores snap frozen for RNA; two cores in formalin for single FFPE block The remaining cores/pieces placed in formalin and processed into 1 paraffin block if possible	Sterile	3 tubes-2 snap frozen, 1 in formalin. See pathology manual for details of cores per tube, processing, and handling.	X	
Breast Tissue Post Treatment	banking	Mandatory	4 vacuum assist cores- either 9 or 11 G For tissue taken during surgery two 1 cm^2 pieces. Post-Treatment- Two cores / tissue pieces snap frozen; one for 4OHT levels (see row below) and one for RNA extraction The remaining cores/pieces placed in formalin and processed into 1 paraffin block if possible	Sterile	3 tubes-2 snap frozen, 1 in formalin. See pathology manual for details of cores per tube, processing, and handling.		X
Research	**Specimen Purpose**	**Mandatory or Optional**	**Blood or Body Fluid being Collected**	**Type of Collection Tube (color of tube top)**	**Volume to collect per tube (# of tubes to be collected)**	**Pre-Intervention**	**Post-Intervention**

Breast Tissue	4-OHT levels	Mandatory	One core/piece snap frozen for 4-OHT levels	Sterile	1 tube-see pathology manual for details.		X

16.0 Remuneration

Participants will receive remuneration of $350-500. This will be prorated as follows: $150 upon completion of the optional baseline study biopsy, and $350 upon completion of study intervention and post-treatment biopsy. All Participants will also receive a small gift of appreciation.

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APPENDIX A

Performance Status Criteria

ECOG Performance Status Scale

Grade	Descriptions
0	Normal activity. Fully active, able to carry on all pre-disease performance without restriction.
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).
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.
3	In bed >50% of the time. Capable of only limited self-care, confined to bed or chair more than 50% of waking hours.
4	100% bedridden. Completely disabled. Cannot carry on any self-care. Totally confined to bed or chair.
5	Dead.

Karnofsky Performance Scale

Percent	Description
100	Normal, no complaints, no evidence of disease.
90	Able to carry on normal activity; minor signs or symptoms of disease.
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.

Percent	Description
60	Requires occasional assistance, but is able to care for most of his/her needs.
50	Requires considerable assistance and frequent medical care.
40	Disabled, requires special care and assistance.
30	Severely disabled, hospitalization indicated. Death not imminent.
20	Very sick, hospitalization indicated. Death not imminent.
10	Moribund, fatal processes progressing rapidly.
0	Dead.

Appendix B

Breast Cancer Prevention Trial Symptom Checklist

EVERYDAY PROBLEMS DURING THE PAST 4 WEEKS

We are interested in knowing how much you have been bothered by any of the following problems during the **PAST 4 WEEKS**. (*Circle one number on each line. If you do not have the problem, circle "not at all"*.)

During the past 4 weeks, how much were you bothered by:

	Not at All	**Slightly**	**Moderately**	**Quite a Bit**	**Extremely**
1. Hot flashes	0	1	2	3	4
2. Nausea	0	1	2	3	4
3. Vomiting	0	1	2	3	4
4. Difficulty with bladder control when laughing or crying	0	1	2	3	4
5. Difficulty with bladder control at other times	0	1	2	3	4
6. Vaginal dryness	0	1	2	3	4
7. Pain with intercourse	0	1	2	3	4
8. General aches and pains	0	1	2	3	4
9. Joint pains	0	1	2	3	4
10. Muscle stiffness	0	1	2	3	4
11. Weight gain	0	1	2	3	4
12. Unhappy with appearance of my body	0	1	2	3	4
13. Forgetfulness	0	1	2	3	4
14. Night sweats	0	1	2	3	4
15. Difficulty concentrating	0	1	2	3	4
16. Easily distracted	0	1	2	3	4
17. Arm swelling (lymphedema)	0	1	2	3	4
18. Decreased range of motion in arm on surgery side	0	1	2	3	4
19. Vaginal Discharge	0	1	2	3	4
20. Vaginal bleeding or spotting	0	1	2	3	4
21. Genital itching/irritation	0	1	2	3	4
22. Lack of energy	0	1	2	3	4
23. Tiredness	0	1	2	3	4
24. Lack of interest in sex	0	1	2	3	4
25. Low sexual enjoyment	0	1	2	3	4

Appendix C
Instructions Oral Tamoxifen

1. Take your pill at the same time every day.
2. If you miss a dose, take the dose as soon as you remember unless it is almost time for your next dose. If it is almost time for your next dose, wait until then and take a regular dose. Mark the missed dose on the calendar provided. Do not take a double dose.
3. You can take the pill with or without food. There are no specific foods to avoid.
4. If you start any new medication while you are on study, please call your study coordinator at 1-877-588-9301 to let them know. She will check with the study doctor and your personal physician so that possible interactions are managed safely.
5. If you develop hot flashes, some suggestions for managing them:
    - Dress in layers, avoid turtlenecks, wool and synthetic material.
    - Keep your bedroom cool.
    - Avoid alcohol, caffeine, hot or spicy foods.
    - Avoid hot tubs, hot showers and saunas.
    - Engage in physical activity such as walks.

Appendix D
Instructions 4-OHT Gel Application

1. The gel is flammable while in gel form: do not apply near fire, flame, heat or smoke. Once dry, the gel is not flammable.
2. Prior to applying gel, shower or wash breasts with a washcloth and dry thoroughly.
3. Remove the cap, point the canister toward the sink or a wastebasket, prime the canister by pushing on the pump all the way several times until gel is dispensed. Do not use the first dose as it may not be a full dose.
4. If you don't push the pump all the way or you discard more than one dose, discard the pumped doses and record this in your diary.
5. Pump one dose into the palm of your hand and make sure to release pump completely between doses.
6. Spread the gel evenly across your entire breast, without rubbing.
7. Repeat for the other breast.
8. After the gel is dry, apply a second pump of gel to each breast.
9. Wash your hands immediately with soap and water.
10. Replace the cap on the canister.
11. Allow the gel to air dry for 2 minutes and then cover immediately with clothing. The gel is colorless and will not stain clothing.
12. Do not wash your breasts or immerse in water for a minimum of 4 hours after application. If this is not possible, delay application until after immersion, such as after you swim.
13. If you miss a dose, do not double the dose. If your next dose is within 12 hours, skip it, note it on the provided calendar and just resume the gel at the next scheduled time. If your next dose is more than 12 hours later, apply the missed dose and resume your normal dose at the next scheduled time.
14. Mark on each canister the date and the time of your first dose.
15. Keep the canisters at room temperature and keep out of the reach of children.
16. Do not expose treated area to sunlight at any time.
17. For the duration of the study, do not apply any other cream, lotion, or moisturizer to your breasts at any time.
18. Avoid contact between the application area and the skin of another person. Skin contact is allowable after area is washed. However, don't wash this area for at least 4 hours after application.
19. At the end of the study bring the canisters back with you to your follow-up visit with the study coordinator.

Appendix E
Patient Diary

Protocol Number Start Date:_______________Stop Date:________________
Name
Subject ID
Site

Thank you for our participation. Please complete this diary every day by circling the correct answer and bring it with you for your follow-up visit.

You will need to apply the gel to both breasts and take your capsule every morning.

DAY	MONDAY	TUESDAY	WEDNESDAY	THURSDAY	FRIDAY	SATURDAY	SUNDAY
DATE							
Did you apply your gel? Wasted pumps/ discarded gel	Right- No / yes Left- No / yes No / yes If yes, how many? __________	Right- No / yes Left- No / yes No / yes If yes, how many? __________	Right- No / yes Left- No / yes No / yes If yes, how many? __________	Right- No / yes Left- No / yes No / yes If yes, how many? __________	Right- No / yes Left- No / yes No / yes If yes, how many? __________	Right- No / yes Left- No / yes No / yes If yes, how many? __________	Right- No / yes Left- No / yes No / yes If yes, how many? __________
Did you take your capsule	Yes No	Yes No	Yes No	Yes No	Yes No	Yes No	Yes No
How many hot flashes did you have in the past 24 hours?	0 1-5 6-10 11-15	0 1-5 6-10 11-15	0 1-5 6-10 11-15	0 1-5 6-10 11-15	0 1-5 6-10 11-15	0 1-5 6-10 11-15	0 1-5 6-10 11-15
Did you have night sweats in the past 24 hours?	Yes No	Yes No	Yes No	Yes No	Yes No	Yes No	Yes No
Did you have any muscle weakness in the past 24 hours?	Yes No	Yes No	Yes No	Yes No	Yes No	Yes No	Yes No
Did you experience any nausea in the past 24 hours?	Yes No	Yes No	Yes No	Yes No	Yes No	Yes No	Yes No
Did you experience any numbness or tingling in your hands or feet in the past 24 hours?	Yes No	Yes No	Yes No	Yes No	Yes No	Yes No	Yes No

Last dose of Tamoxifen capsule: Date___________Time_________
Last dose of gel application to breast: Date_________Time_______

Appendix F Radiology Guide

Percutaneous needle cores will be taken in radiology with imaging guidance.

1. Baseline/pretreatment research biopsies:
    - Performed by needle core approach using a 9-11G vacuum assist needle, with guidance.
    - The biopsy should target a dense area of tissue; the goal is to obtain more glandular rather than adipose tissue.
        - For patients enrolled after biopsy of ADH/ALH/LCIS, the research biopsy should consistently be in the same quadrant, to the patient's lateral and/or above the diagnostic biopsy site in the same breast targeting 1 cm from the previous clip (if clip at biopsy site).

          If clip is >2 cm away from the biopsy site, then the research biopsy should be obtained at a location estimated to be 1 cm lateral or above from the biopsy site, maintaining adequate distance from the clip so that it is not removed with the tissue sampling. The radiologist will save detailed information on the location of biopsy. If the biopsy is performed using stereotactic or tomosynthesis guidance, recording the following two metrics is recommended: (1) compression thickness, and (2) depth of the biopsy target. If the biopsy is performed by ultrasound, capturing post-biopsy cine clips of the biopsy site using virtual convex imaging (when using a linear array transducer) in the transverse, longitudinal, radial, and antiradial directions is recommended. Document in the biopsy note the location of the research biopsy in relationship to the diagnostic biopsy site. Clip placement at the site of the research biopsy is favored but at the discretion of the radiologist performing the research biopsy. A new clip should be placed at the research biopsy site if the original diagnostic biopsy clip had migrated >2 cm and was not in good position.

        - For patients enrolled by high risk criteria (no recent biopsy of high risk lesion), the pretreatment research biopsy may be obtained from either breast. The goal is to obtain more glandular rather than adipose tissue, so targeting a denser area of tissue is desired. The research biopsy location is at the discretion of the radiology team at each institution. The radiologist will save detailed information on the location of biopsy. Clip placement at the site of the research biopsy is favored but at the discretion of the radiologist performing the research biopsy. If no clip is placed and the research biopsy demonstrates malignant disease, then the patient should return as soon as possible for clip placement while the biopsy site can be discerned by imaging.

    For 9-11G vacuum assist cores, 4-6 cores will be collected:
    - Two of the cores will be placed in 2 separate tubes and snap frozen
    - The remaining cores will be placed in formalin

Study coordinators will provide collection tubes, labels, Dewar with liquid nitrogen and specimen collection/companion form prior to procedure and will pick up samples after collection is complete.

- Preprinted labels will be provided and should be used for pretreatment tissue samples. (Provided by Study Coordinator).
- Collection/companion form containing additional specimen collection details will be completed at time of collection

2. Post-treatment research biopsies:
   - When performed by needle core approach using a 9-11G needle
   - The biopsy should target a dense area of tissue and should consistently be in the same quadrant, medial or below the diagnostic biopsy site in the same breast targeting 1 cm from the previous clip. Again, the goal is to obtain more glandular rather than adipose tissue.

If clip is >2 cm away from ~~not located~~ the biopsy site, then the research biopsy should be obtained at a location estimated to be 1 cm medial or below from the biopsy site, maintaining adequate distance from the clip so that it is not removed with the tissue sampling. The radiologist will save detailed information on the location of biopsy. If the biopsy is performed using stereotactic or tomosynthesis guidance, recording the following two metrics is recommended: (1) compression thickness, and (2) depth of the biopsy target. If the biopsy is performed by ultrasound, capturing post-biopsy cine clips of the biopsy site using virtual convex imaging (when using a linear array transducer) in the transverse, longitudinal, radial, and antiradial directions is recommended. Document in the biopsy note the location of the research biopsy in relationship to the diagnostic biopsy site. Clip placement at the site of the research biopsy is favored but at the discretion of the radiologist performing the research biopsy. A new clip should be placed at the research biopsy site if the original diagnostic biopsy clip had migrated >2 cm and was not in good position.

- For patients enrolled by high risk criteria (no recent biopsy of high risk lesion), the pretreatment research biopsy may be obtained from either breast. The goal is to obtain more glandular rather than adipose tissue, so targeting a denser area of tissue is desired. The research biopsy location is at the discretion of the radiology team at each institution. The radiologist will save detailed information on the location of biopsy. Clip placement at the site of the research biopsy is favored but at the discretion of the radiologist performing the research biopsy. If no clip is placed and the research biopsy demonstrates malignant disease, then the patient should return as soon as possible for clip placement while the biopsy site can be discerned by imaging.

For 9-11G vacuum assist cores, 4-6 cores will be collected:

- Two of the cores will be placed in 2 separate tubes and snap frozen
- The remaining cores will be placed in formalin

Study coordinators will provide collection tubes, labels, Dewar with liquid nitrogen and specimen collection/companion form prior to procedure and will pick up samples after collection is complete.

- Preprinted labels will be provided and should be used for post-treatment tissue samples. (Provided by Study Coordinator).
- Collection/companion form containing additional specimen collection details will be completed at time of collection

Post-treatment research breast tissue sample can be obtained by either needle core approach (if no surgical excision is done for clinical care) or can be obtained by the surgical team intraoperatively after excision of the lesion.

If patient has bilateral or multi-centric atypia/LCIS, at Mayo Dr. Degnim will review and recommend one side for research biopsy. At Northwestern University, Dr. Seema Khan will review and make recommendation. At MD Anderson Cancer Center at Cooper, Dr. Catherine Loveland-Jones will review and make recommendations.

Appendix G
Medications with potential interactions with tamoxifen or 4hydroxytamoxifen

Inhibitors of Cytochrome P450 (CYP2D6) Enzymes

Strong Inhibitors:	***Moderate Inhibitors:***
Quinidine	Terbanafine
Cinacalcet	Duloxetine
Bupropion	Sertraline
Fluoxetine	
Paroxetine	

Inducers of Cytochrome P450 (CYP3A4/5) Enzymes

Glucocorticoids	Pioglitazone
Efavirenz	Fosamprenavir
Nevirapine	Rifampin
Carbamazepine	Eslicarbazepine
Oxcarbazepine	Phenobarbital
Phenytoin	St. John's Wort
Modafinil	

Legend: The extent of inhibition may not be well defined and/or the degree of inhibition may vary for the drugs listed. Note that if a drug inhibits CYP3A4, it is expected to induce CYP3A5, although literature proving this for each drug is not available. All information from Pharmacogenomic Associations Tables, Mayo Clinic: Mayo Medical Laboratories. Retrieved from http://intranet.mayo.edu/charlie/center-individualized-medicine/files/2017/05/Pharmacogenomic-Inhibitor-Inducer-and-Substrates-table.pdf

Appendix H
Drug-Induced Prolongation of the QT Interval
http://online.factsandcomparisons.com/lco/action/doc/retrieve/docid/1081/5909323

Highest Risk		
Adagrasib	Dofetilide	Procainamide
Ajmaline*	Dronedarone	QuiNIDine
Amiodarone	Iboga	QuiNINE
Arsenic Trioxide	Ibutilide	Selpercatinib
Astemizole*	Ivosidenib	Sertindole
Bedaquiline	Lenvatinib	Sotalol
ChlorproMAZINE	Levoketoconazole	Terfenadine*
Cisapride	Methadone	Vandetanib
Delamanid*	Mobocertinib	Vernakalant*
Disopyramide	Papaverine	Ziprasidone

Moderate Risk		
Amisulpride (Oral)*	Etecalcetide	Oxytocin
Azithromycin (Systemic)	Fexinidazole	Osimertinib
Capecitabine	Flecainide	PAZOPanib
Carbetocin	Floxuridine	Pentamidine (IV only)
Ceritinib	Fluconazole	Pilsicainide*
Chloroquine	Fluorouracil (Systemic)	Pimozide
Citalopram	Flupentixol*	Piperaquine*
Clarithromycin	Gadobenate Dimeglumine	Probucol*
Clofazimine	Gemifloxacin	Propafenone
ClomiPRAMINE	Gilteritinib	Propofol
CloZAPine	Halofantrine*	QUEtiapine
Crizotinib	Haloperidol	Ribociclib
Dabrafenib	Imipramine	RisperiDONE
Dasatinib	Inotuzumab Ozogamicin	Saquinavir
Desflurane	Isoflurane	Sevoflurane
Domperidone*	LevoFLOXacin (Oral Inhalation)*	Sparfloxacin*
Doxepin (Systemic)	LevoFLOXacin (Systemic)	SUNItinib
Doxepin (Topical)	Lofexidine	Tegafur
Doxifluridine	Meglumine Antimoniate	Thioridazine
Droperidol	Midostaurin	Toremifene
Encorafenib	Moxifloxacin (Systemic)	Vemurafenib
Entrectinib	Nilotinib	Voriconazole
Erythromycin (Systemic)	OLANZapine	
Escitalopram	Ondansetron (IV only)	

***Drugs not available in the United States**