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A Randomized, Double-blind, Placebo-controlled Study of 4-hydroxytamoxifen Topical Gel in Women with Mammographically Dense Breast

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Protocol Revision or Amendment # Version 20

SCHEMA

A Randomized, Double-blind, Placebo-controlled Study of 4-hydroxytamoxifen Topical Gel in Women with Mammographically Dense Breast

Women age 40-69 years, or less than 40 years if Gail risk is greater than 1.66%.
All participants will have mammographically dense breast (heterogeneously dense or extremely dense based on ACR BIRADS 4th or 5th edition classification)

↓

Baseline measures: Mammographic density and eligibility labs, BESS[2] questionnaire, 4-OHT[1] metabolites, research labs: estradiol, IGF[3] related markers, CRP[4], SHBG[5], vWB[6] Factor, Factor VIII, lipid profile
Optional breast biopsy: Tissue biomarkers: 4-OHT[1] metabolites, TDLU[7] involution, collagen structural changes, estrogen related transcription (SET index), Ki-67, COX-2, p16, CD68

↓

Randomization 1:1
Stratified by study site and baseline breast density (heterogeneously dense (C) or extremely dense (D))

↓

Intervention: 2mg per breast topical 4-OHT gel versus topical placebo gel applied to both breasts per day for 12 months

Phone call at Day 7-14 and at Month 3;
Clinic visit at 6 months (May be a telephone visit for participants affected by COVID-19 closures);
Phone call at Month 9

↓

Final clinic visit at 12 months
(For participants affected by COVID-19 closures - Month 12 visit will be conducted by telephone. Participants will be reconsented for extended gel application with mammogram performed within 6 months)

Optional breast biopsy (For participants affected by COVID-19 closures can occur at the time of the final mammogram if gel application was extended)

↓

Post-intervention follow-up at 24 months (up to 32 months for participants affected by COVID-19 closures): standard of care bilateral mammogram

Primary endpoint: To estimate and compare the percent change in mammographic breast density (using Cumulus software) from baseline to Month 12 [or Month 13-18 under the COVID-19 Contingency Plan] in women applying 2mg 4-OHT gel per breast versus placebo based on tomosynthesis imaging using the 2D synthetic image.

Secondary endpoints:
1. To estimate and compare the percent change in mammographic breast density (using Volpara) from Baseline to Month 12 in women applying 2mg 4-OHT gel per breast versus placebo.
2. To estimate and compare the percent change in mammographic breast density (using LIBRA) from Baseline to Month 12 in women applying 2mg 4-OHT gel per breast versus placebo.
3. To compare the percentage of women who underwent a change in BIRADS category, comparing pre-and post- treatment measurements, for recipients of active agent versus placebo.
4. To conduct subgroup analyses including BIRAD category, menopausal status, cup size, and total breast volume.
5. To estimate percentage of women with ≥ 10% absolute decrease in quantitative mammographic density percentage between baseline and 12 months, comparing between treated group 2mg per breast 4-OHT gel to placebo based on Cumulus method.
6. To describe symptoms assessed by BCPT Eight Symptom Scale (BESS) questionnaire and laboratory toxicity assessment (Factor VIII (F VIII), Von Willebrand (vWB) factor, Sex Hormone-Binding Globulin (SHBG), lipid profile).
7. To evaluate plasma measurements of 4-OHT and related metabolite levels and factors related to tamoxifen exposure.
8. To evaluate tissue measurements of 4-OHT and related metabolite levels and factors related to tamoxifen exposure.

Exploratory endpoints:

1. To compare the Cumulus vs. Volpara vs. LIBRA breast density measurement methods to estimate percent change in mammographic breast density from baseline to Month 12 in women applying 2mg of 4-OHT gel per breast vs. placebo.
2. To perform correlation of drug concentrations in the plasma and the tissue.
3. To evaluate plasma measurements of Insulin-like growth factor (IGF) pathway members, C-reactive protein (CRP), and estradiol.
4. To compare the 2D natural vs. 2D synthetic breast density measurement using the Cumulus method to estimate percent change in mammographic breast density from baseline to Month 12 in women applying 2mg of 4-OHT gel per breast vs. placebo.
5. To evaluate tissue biomarkers (among women undergoing optional pre- and post-treatment biopsies):
   Terminal duct lobular unit (TDLU) Involution
   Collagen structural changes
   $SET_{ER/PR}$ index: estrogen related transcription
   Ki-67, COX-2, p16, CD68.
6. To examine whether any reductions in mammographic density seen after 1 year of 4-OHT vs. placebo gel application persist at 24 months (or longer under the COVID-19 Contingency Plan), one year after gel application has stopped.

[1] 4-hydroxytamoxifen (4-OHT), [2] BCPT Eight Symptom Scale (BESS), [3]Insulin-like growth factor (IGF), [4] C-reactive protein (CRP+), [5] Sex hormone-binding globulin (SHBG), [6] Von Willebrand factor (vWB), [7] Terminal duct lobular unit (TDLU)

TABLE OF CONTENTS

1. OBJECTIVES

The overall objective is to evaluate whether daily application of 2mg (2mL) per breast of 4-hydroxytamoxifen (4-OHT) topical gel versus placebo lowers breast density in women with mammographically dense breast, based on the intent-to-treat principle, i.e., all participants who have MD measurements from both baseline and 52 weeks, regardless of compliance, will be included in the primary analysis.

1.1 Primary Objectives

To estimate and compare the percent change in mammographic breast density (using Cumulus software) from baseline to Month 12 [or Month 13-18 under the COVID-19 Contingency Plan] in women applying 2mg 4-OHT gel per breast versus placebo based on tomosynthesis imaging using the 2D synthetic image.

1.2 Secondary Objectives

Note: data from Month 13-18 visit instead of Month 12 will be used if participants were seen under the COVID-19 Contingency Plan.

1. To estimate and compare the percent change in mammographic breast density (using Volpara) from Baseline to Month 12 in women applying 2mg 4-OHT gel per breast versus placebo.
2. To estimate and compare the percent change in mammographic breast density (using LIBRA) from Baseline to Month 12 in women applying 2mg 4-OHT gel per breast versus placebo.
3. To compare the percentage of women who underwent a change in BIRADS category, comparing pre-and post- treatment measurements, for recipients of active agent versus placebo.
4. To conduct subgroup analyses including BIRAD category, menopausal status, cup size, and total breast volume.
5. To estimate percentage of women with ≥ 10% absolute decrease in quantitative mammographic density percentage between baseline and 12 months, comparing between treated group 2mg per breast 4-OHT gel to placebo based on Cumulus method.
6. To describe symptoms assessed by BCPT Eight Symptom Scale (BESS) questionnaire and laboratory toxicity assessment (Factor VIII (F VIII), Von Willebrand (vWB) factor, Sex Hormone-Binding Globulin (SHBG), lipid profile).
7. To evaluate plasma measurements of 4-OHT and related metabolite levels and factors related to tamoxifen exposure.
8. To evaluate tissue measurements of 4-OHT and related metabolite levels and factors related to tamoxifen exposure.

1.3 Exploratory Objectives

1. To compare the Cumulus vs. Volpara vs. LIBRA breast density measurement methods to estimate percent change in mammographic breast density from baseline to Month 12 in women applying 2mg of 4-OHT gel per breast vs. placebo.
2. To perform correlation of drug concentrations in the plasma and the tissue.
3. To evaluate plasma measurements of Insulin-like growth factor (IGF) pathway members, C-reactive protein (CRP), and estradiol.
4. To compare the 2D natural vs. 2D synthetic breast density measurement using the Cumulus method to estimate percent change in mammographic breast density from baseline to Month 12 in women applying 2mg of 4-OHT gel per breast vs. placebo.

5. To evaluate tissue biomarkers (among women undergoing optional pre- and post-treatment biopsies):
   Terminal duct lobular unit (TDLU) Involution
   Collagen structural changes
   $SET_{ER/PR}$ index: estrogen related transcription
   Ki-67, COX-2, p16, CD68.
6. To examine whether any reductions in mammographic density seen after 1 year of 4-OHT vs. placebo gel application persist at 24 months (or longer under the COVID-19 Contingency Plan), one year after gel application has stopped.

2. BACKGROUND

2.1 Breast Cancer Risk Reduction

Tamoxifen oral tablet at 20 mg/day for 5 years is currently FDA approved for the use of breast cancer risk reduction. However, despite the known fact that it can reduce breast cancer risk by 50%, less than 20% of high risk women choose to take Tamoxifen for risk reduction; mostly because of the low, but still slight increased risk of endometrial cancer, thromboembolic events (TEE), arthralgia and menopausal side effects (hot flushes, weight gain, hair thinning). These systemic side effects unfortunately cause underutilization of this effective breast cancer chemoprevention agent. One approach to improve the benefit/ risk ratio might be to consider an innovative way to administer the agent where the preventive effects remains, but systemic side effects are not present (or minimal).

2.2 Study Agent

4- hydroxytamoxifen topical gel (4-OHT gel) is a local (breast) transdermal administered agent and has been initially evaluated in clinical trials for cyclical breast pain and has been shown to be well tolerated [1] Further, 4-OHT and oral tamoxifen was studied in the neoadjuvant setting in 55 patients with invasive breast cancer; Ki-67 decreased with both applications (gel and oral) [2]. In a very recent study, funded through the NCI-DCP-Prevention Consortium; a regimen of 6-10 weeks of 4-OHT gel at 4mg/day was compared to 20 mg/day oral tamoxifen in patients with ductal carcinoma *in situ* [3]. Compared with oral tamoxifen, a recent DCP sponsored study found that 4-OHT gel did not result in statistically significantly lower breast parenchymal drug concentrations or reductions in Ki-67. Furthermore, risk factors for TEE increased in the oral Tamoxifen group, but not in the 4-OHT gel group (factor VIII, von Willebrand factor and SHBG). While the toxicity profile is safer, it will be also important to show that the gel form achieves the ultimate desired endpoint; reduction in breast cancer incidence. Currently, there are no prospective studies evaluating the preventive effect of 4-OHT gel in high risk women. Therefore, a pilot study evaluating 4-OHTs effect in breast tissue of high risk women is needed. However, such a study would ideally require a validated risk marker.

2.3 Rationale for Study Agent and Primary Endpoint Marker

Mammographic density (MD) is one of the strongest independent predictors of breast cancer risk among women, apart from older age and *BRCA1/2* mutation [4]. It is estimated that women with the highest MD have a two- to sixfold increased risk of developing breast cancer [5]. MD is determined by the differential ability of X-rays to permeate the different cell types and components of human breast tissue. In this regard, there are two main components of the breast: fibroglandular tissue and adipose tissue. Mammographically

dense breast tissue represents fibroglandular breast tissue, which includes the epithelial ducts and glands as well as interlobular connective tissue. In contrast, adipose tissue easily allows passage of x-rays, yielding low density on a mammogram MD is heavily influenced and inversely proportional to age, reflecting the relative decrease in glandular breast tissue and increase in fatty breast tissue associated with aging [6]. A recent meta-analysis of 42 studies evaluating MD and breast cancer risk showed no differences observed by age/menopausal status at mammography or by ethnicity, however significantly and progressively increasing risk parallel to increasing MD among women undergoing mammographic screening [7].

Studies of tamoxifen in the adjuvant and preventive setting have demonstrated that a decline in MD of approximately 10% is consistently associated with better outcomes [8, 9]. In a case-control analysis nested within the IBIS-I breast cancer prevention trial, Cuzick *et al.*, observed that approximately 46% of tamoxifen-treated women experienced a ≥10% reduction in MD at 12−18 months and these women had a 63% reduction in breast cancer risk (odds ratio [OR] =0.37, 95% confidence interval [CI] =0.20–0.69, p=0.002) as compared with all women on placebo (regardless of MD) [8]. In contrast, tamoxifen-treated women with <10% reduction in MD did not experience risk reduction compared to all women on placebo (OR=1.13, 95% CI 0.72–1.77, p=0.60). Further, several other retrospective analyses have reported that among women with ER-positive breast cancer treated with tamoxifen, those whose MD declined in the unaffected breast [10, 11, 12], had better outcomes (reduced risk of recurrence [10, 13] or death from breast cancer [11, 12]). In contrast, other agents that have shown chemopreventive efficacy, including raloxifene and exemestane, have not been reported to consistently reduce MD [14].

The original corporate sponsor completed a 6-month study of 4-OHT gel for breast density reduction using 0.05% (w/v) 4-OHT, a less concentrated gel than currently available (0.2% (w/v) gel). Though overall results of this study were nonsignificant, post-hoc analysis of patients with baseline breast density ≥80% (n=19; 4-OHT=9; placebo=10) revealed a −4% *vs*. a +0.7% (p=0.024) change in baseline density following 6 months of treatment for 4-OHT gel 1mg/d per breast *vs.* placebo, respectively (source NCI TORF FF-2015 document).

Taken together, changes in MD would be an ideal "biomarker" to be evaluated as the primary endpoint in this short-term prevention using 4-OHT gel in high risk women. The duration of 12 months was chosen because most studies referenced above reported mammographic data at least 12 months.

Preliminary information gained from this study will enable us to plan a prospective, large, national study using 4-OHT gel for breast cancer risk reduction that can be easily performed not only at academic but also non-academic institutions. Therefore, it is very important that we choose endpoints that are validated, easily measurable and do not require special and expensive technology. This future possibility was already discussed with and is supported and welcomed by the SWOG Cancer Prevention and Epidemiology Group.

Potential Surrogate Biomarkers

This study will also enable us to evaluate other potential surrogate biomarkers in the context of early phase prevention studies. <u>As an exploratory endpoint,</u> our proposed laboratory correlates and biomarkers are discussed below:

<u>Tissue Biomarkers</u>

Overview: Tissue biomarkers have been selected to interrogate the following targets that represent:

a) Structure-function activity of terminal ductal lobular units (TLDUs) by counting acinar density
b) Breast tissue density by assessing the patterns of collagen matrix
c) Hormone-mediated cellular activity by measuring the transcriptional expression levels of ER-alpha and ER-associated genes ($SET_{ER/PR}$ index)

d) Pharmacogenomic response to endocrine therapy by measuring Ki67 protein by immunohistochemistry and aurora kinase-alpha gene expression
e) Inflammatory response to 4-OHT therapy by measuring COX2 and p16 proteins, and recruitment of CD68 monocytes.

All biomarkers will be compared in biopsies obtained before and after exposure to the 4-OHT gel, in order to explore pharmacodynamic responses of interest. That said, the baseline measures will also be evaluated, particularly for items a-c (above) that might be less likely to change in response to short-term exposure, but combine to provide the cellular, matrix and targeted molecular context of the tissues in each participant prior to exposure to treatment.

a) TDLU Involution: TDLU involution is characterized by a reduction in the number and size of TDLUs and their secretory acinar units [15]. Reduced involution in TDLUs has been associated with increased risk of breast cancer in several studies of women with benign breast disease [16], [17]. Furthermore, a recent study showed that, especially amongst premenopausal women, reduced TDLU involution was associated with higher area and volumetric MD; indicating that TDLU involution and MD are correlated markers of breast cancer risk. In continuation of this, there is also data suggesting that increased MD and reduced TDLU involution are actually independent risk factors for breast cancer [18]. Taken all these together, in our proposal, as an exploratory endpoint, our aim is to assess TDLU involution, correlate with MD and evaluate whether baseline TDLU involution correlates/predicts change in MD induced by 4-OHT gel. The TDLU density (acini per TDLU) is readily determined from pathologic review of the same routine H&E stained tissue sections that are usually used for diagnosis using image analysis tools for area-based quantitation.

b) Collagen structural changes: Increased breast density is associated with an increase in the deposition of extracellular matrix (ECM) components, especially collagen. Several studies have shown the link between breast cancer, breast density and increased deposition of stromal collagen [19, 20]. Interestingly, Keely and co-workers have shown in an in vitro model that increasing the density of collagen in the matrix is sufficient to disrupt breast epithelial differentiation, suggesting that matrix density is itself an important regulator of cellular behavior [21, 22, 23]. Keely et al found evidence for a collagen "signature" of parallel fibril alignment that is present even before a tumor is palpable. This signature could be potentially used to identify high risk areas in the breast tissue. These investigators [21] first introduced the tumor associated collagen signature (TACS) nomenclature to describe collagen alignment patterns. The TACS phenotypes are currently classified into three groups. TACS-1 describes the standard desmoplastic response of increased collagen deposition surrounding initiating tumor cells. TACS-2 is observed as straightened fibers aligned tangentially around developing tumors, while TAC-3 is seen as radially aligned fibers that facilitate local invasion [24]. The assessment is based on pattern, rather than intensity, and the authors qualitatively searched for these patterns in a tissue microarray of samples from human breast cancers and this exploratory analysis suggested that the presence of the TACS-3 collagen alignment pattern in invasive breast cancers may be a risk factor or disease free and disease specific survival (DFS and DSS respectively) for patients who had ER-positive breast cancer that measured more than 1.35 cm in diameter [25]. Clearly, there is risk of statistical over fitting to the outcomes, but the observations are intriguing because of the novelty of collagen pattern assessments and the known relevance of mammographic breast density to risk of developing ER+ breast cancer.

While increased deposition of stromal collagen is associated with breast density and breast cancer risk, there is no data related to collagen structural changes as a predictor to changes in MD induced by 4- OHT gel, and it is not known whether the time it takes to remodel collagen fibers (weeks to months in a healing wound) would be relevant to a short duration of exposure to the transdermal agent, or whether the existing patterns of collagen deposition influence the susceptibility of breast epithelial and stromal cells to this agent.

However, an important issue is the method used to evaluate collagen deposition patterns in the biopsies of benign breast tissues. Indeed, the high-energy photon transmission that was used in the research literature describing TACS collagen patterns is too complicated and distant from routine diagnostic practice to be broadly applicable. But the Masson's trichrome stain is routine, cheap, ubiquitous in pathology labs (for example, we use it to assess patterns of early fibrosis in liver biopsies), and can be combined with polarization light microscopy to clearly identify the birefringence of aligned collagen fibers (as we frequently observe when looking for amyloid proteins). Masson's trichrome staining of collagen fibers would be readily generalizable to diagnostic practice.

c) $SET_{ER/PR}$ index: We have previously published a gene expression index for estrogen receptor-related gene expression that was theragnostic for prognosis following endocrine therapy for early (node-negative), and following chemo-endocrine therapy for higher-risk (node-positive) hormone receptor-positive and HER2-negative HR+/HER2- breast cancers.[26] However, it is only recently that we have been able to measure the SET index from formalin-fixed and paraffin-embedded tumor tissues from residual disease. This $SET_{ER/PR}$ index measures 18 transcripts that are strongly correlated with both ER and PR expression, and are not related to proliferation. The $SET_{ER/PR}$ index compares the expression of those 18 transcripts to 10 reference transcripts within the assay, and also includes probes to measure the expression levels of *ESR1*, *PGR*, *ERBB2*, and *AURKA* (aurora kinase A) transcripts. The reproducibility of the $SET_{ER/PR}$ index exceeds 99% across batches and technicians, and the assay is performed on a single 10µm tissue section from FFPE blocks of biopsies from breast cancers. We expect that the less cellular samples from benign breast tissues would require at least 10 TDLUs (usually obtained from 3 x 10µm sections from core biopsies). Furthermore, the $SET_{ER/PR}$ index is independently prognostic following endocrine therapy in metastatic breast cancer samples, and in primary tumor samples from node-positive or node-negative breast cancers. In an exploratory in silico analysis, we observed no change in SET index values measured from sequential core biopsies of invasive HR+/HER2- cancers taken before and after a 2-week exposure to neoadjuvant endocrine therapy. That result was different from the pharmacodynamic reduction in the proliferation-related signature of genomic grade index that was reported in the same samples. However, the observed stability of the SET index during short-term endocrine therapy might be consistent with a long-term treatment that continues to have efficacy over a long time. Otherwise, rapid suppression would probably render the treatment ineffective over the longer term. We do not know whether this will also be the case with non-malignant tissues – perhaps shorter-term suppression of endocrine capacity may be possible.

The $SET_{ER/PR}$ index assay is technically feasible using sections from 20-gauge core biopsy samples from metastatic sites (2-3 cores), whereas many mammotome core biopsy samples of proliferative benign histologic lesions are of 11-gauge or 9-gauge and contain considerably more epithelial tissue. In our limiting dilution study using purified RNA, this assay provided accurate measurement down to 30 ng of RNA, and that is considerably less than we typically obtain from core biopsy sections. This project will be the first to evaluate the $SET_{ER/PR}$ index in the setting of endocrine therapy in benign tissues (and is therefore innovative) so the rationale is based on the index as a measure of ER-related transcription, a biological process that is known to be important in benign breast disease, and is central to the pharmacology of the prevention strategy to be tested. We will also analyze the ratio of fatty/non-fatty tissue. Our analysis will explore two hypotheses: i) whether pharmacodynamic responses to exposure of tissues to 4-OHT (e.g. reduction in proliferation or TLDU density) will be greater when the tissues have higher $SET_{ER/PR}$ index; and ii) whether exposure to 4-OH decreases the $SET_{ER/PR}$ index.

d*) Markers of proliferation and inflammation:* Ki-67 has been widely evaluated in previous phase I and II prevention trials that have revealed conflicting data [27], [28], [29], [30]. In one study, a reduction in Ki-67 was seen with 6 months of letrozole therapy; whereas another study did not show any changes with 6 months of letrozole therapy[28], [27]. In another study of arzoxifene, no changes in Ki-67 were seen [31]. A separate study was done in premenopausal women using 12 months of lignans and a reduction in Ki-67 was observed [30].

It is possible that the mechanism of action for Ki-67 reduction is different for tamoxifen and indeed a reduction in Ki-67 in patients with DCIS has been shown with oral as well as 4-OHT [3]. However, the effect in breast tissue of high risk women is not known. Therefore, we would like to evaluate changes in Ki-67 induced by 4-OHT in our study.

As other exploratory endpoints, we will evaluate changes in COX-2, p16, and CD68 with 4-OHT treatment. The inducible *COX-2* gene is the master switch that activates the inflammatory response. Induction of COX-2 by any inflammatory stimulus (*e.g.*, tobacco, alcohol, ischemia, trauma, pressure, foreign bodies, toxins, bacteria, viruses, lipopolysaccharides, *etc*.) quickly results in the biosynthesis of prostaglandins of the E-series, particularly prostaglandin E2 (PGE-2), and these prostaglandins in turn on the inflammatory response [32] . Molecular studies show the role of COX-2 in the progression of breast cancer [33], and meta-analysis of epidemiologic studies show impact of selective and non-selective agents that reduce breast cancer risk by inhibition of COX-2 [34]. Currently there is no data evaluating 4-OHT effect on COX-2 expression in high risk women.

Among cell cycle check points, p16 serves as a negative regulator of cell cycle [35]. An alteration in the cell cycle check points is one of the most common abnormalities encountered in the molecular basis of human cancer and unrestricted cellular proliferation due to deregulation of cell cycle is the key feature of malignancy. Altered expressions of p16 and the correlation with clinical and pathologic factors in breast cancer have been reported, mostly showing correlation with poor prognostic features [36]. Given its important role in breast carcinogenesis, our aim is to evaluate if 4-OHT can reduce expression of p16 in high risk breast tissue and whether p16 could serve as a potential surrogate biomarker in future phase I, II prevention trials.

CD68 (marker of Tumor Associated Macrophages: Tumor-associated macrophage (TAM) represent the major inflammatory component of the stroma of many tumors, including breast cancer that can express several protumoral functions, including secretion of growth factors and matrix-proteases, promotion of angiogenesis and suppression of adaptive immunity[37]. The protumoral role of TAM in cancer is further supported by clinical studies that found a correlation between the high macrophage content of tumors and poor patient prognosis and also by evidence showing that long-term use of non-steroidal anti-inflammatory drugs reduces the risk of several cancers [37]. Currently, there is no data related to the role of TAM related inflammation and its reversal with a preventive agent. Therefore, in our current study we will evaluate CD68 (marker of TAM) in high risk breast tissue of women treated with 4-OHT.

<u>Plasma Biomarkers: IGF Pathway:</u>
Studies have shown that dysregulation of the IGF pathway and an increase in insulin-like growth factor 1 (IGF-1) can result in increased risk of breast cancer[38]. A previous study has shown that 2 months use tamoxifen can reduce IGF-1/IGFBP-3 [39] and another study showed the same with raloxifene [40] . We have previously shown an increase in IGFBP-1 with 6 months use of anastrozole [41]. Currently, there is no data related to the modulation of the IGF pathway, therefore we aim to study modulation of this pathway induced by 4-OHT treatment.

Rationale for including Alcohol and Tobacco Assessment Questionnaires
Increasing evidence suggests that tobacco and alcohol use are risk factors in the development of intraepithelial neoplasia and cancer. In addition, tobacco and alcohol use may adversely affect agent intervention, for example by altering the safety profile or metabolism of a drug. Standardized assessments of tobacco and alcohol use during clinical trials will aid in understanding the potential relationship between the use of these products and clinical endpoints or cancer prevention biomarkers. Therefore, NCI, DCP is including assessment of tobacco and alcohol use at baseline and Month 12 to determine the potential impact

of tobacco and alcohol use on 1) treatment toxicity and symptom burden, and 2) the efficacy of treatment intervention.

In summary, the overall objective is to evaluate whether daily application of 4 mg of 4-OHT topical gel (2 mg per breast) versus placebo lowers breast density in women with mammographically dense breast.

3. SUMMARY OF STUDY PLAN

We will conduct a prospective, randomized, double-blind, placebo-controlled phase II study of 4-hydroxytamoxifen topical gel in women with mammographically dense breast.

Women age 40-69 years, or less than 40 years if Gail risk is greater than 1.66% will be eligible. All participants will have mammographically dense breast (heterogeneously dense or extremely dense based on ACR BIRADS 4th or 5th edition classification).

Women with prior annual mammograms showing heterogeneously dense or extremely dense breasts will be approached for enrollment into the study prior to their next annual mammogram. Informed consent must be obtained prior to starting any further study procedures. Consenting should be done prior to next annual mammogram (the baseline study mammogram). Once the participant has signed consent, the mammography center should be alerted not to delete the raw images. In centers where raw images are stored, consent can occur after the annual mammogram that will serve as the baseline mammogram for the study. The mammogram must be within 3 months of randomization. Raw images must be obtained from the Baseline, 12-Months and 24-Months mammograms. Mammography reports from the previous annual mammography visit should also be obtained. If it is not possible to see a participant in person for the Month 12 visit during the COVID-19 pandemic, the visit will be rescheduled per the COVID-19 Contingency Plan (see Section 7 for details) and mammograms will be rescheduled accordingly and corresponding raw images from that mammogram will be obtained.

158 women with confirmed eligibility will be randomized 1:1 to 2 arms. Participants in Arm A will receive 4-OHT, participants in Arm B will receive placebo. The daily dose of gel application is 4 mg (2 mg per breast). The 4-OHT gel or placebo will be applied topically in 1 pump actuation to each breast. Women will undergo baseline toxicity assessment, blood draw and instructions on how to apply the gel and an optional breast core biopsy. While women will not be stratified by menstrual cycle (as there is minimal, if any, effect of the menstrual cycle on our primary endpoint of mammographic density), their menstrual cycle will be recorded. Randomization will be performed stratified by study site and baseline breast density category (heterogeneously dense (C) or extremely dense (D)). The research nurse/coordinator will call the participants at Day 7-14 to verify the start of study agent, and at months 3 and 9 for toxicity assessment. The participants will return to the clinic at 6 months for toxicity assessments, physical exam and vital signs, lab tests and plasma biomarker blood draws, collection of unused study agent, and to receive additional study agent. At 12 months, the participants will return to clinic for toxicity assessment, collection of unused study agent, collection of blood for plasma biomarkers, standard of care mammogram, and the optional breast core biopsy, if she opted to participate in this part of the study. At 24 months the research staff will collect the standard of care bilateral mammogram images; the participants will not be approached. For the period of the COVID-19 pandemic, if participants cannot be seen in person at Months 6 and/or 12, the COVID-19 contingency plan will be followed (see Section 7 for details).

4. PARTICIPANT SELECTION

4.1 Inclusion Criteria

4.1.1 Women age 40-69 years, or less than 40 years if 5-year breast cancer Gail risk is ≥ 1.66%.

4.1.2 Mammographically dense breast (heterogeneously dense (C) or extremely dense (D), based on ACR BIRADS© fifth edition classification or heterogeneously dense (3) or extremely dense (4), based on ACR BIRADS© fourth edition classification) in either breast.

4.1.3 ECOG performance status ≤1 (Karnofsky ≥70%; see Appendix A)

4.1.4 Participants must have normal organ and marrow function defined as ALL of the below:

White Blood Cells	≥3,000/microliter
Platelets	≥100,000/microliter
Total bilirubin	≤1.5 × institutional upper limit of normal (ULN)
AST (SGOT)/ALT (SGPT)	≤1.5 × institutional upper limit of normal (ULN)

4.1.5 Participant must have a gynecology examination within the last 5 years (gynecology examination is not required if participant had a hysterectomy).

4.1.6 Premenopausal women using a hormonal or non-hormonal Intra-Uterine Device (IUD) birth control method will be eligible, if they have been on the same IUD for at least 3 months prior to enrollment and plan to continue using the same method throughout the study.

4.1.7 Women who are using postmenopausal hormones, and are planning to continue the same regimen through the study intervention are eligible to participate.

4.1.8 Willingness to avoid exposing breast skin to natural or artificial sunlight (i.e. tanning beds) for the duration of the study.

4.1.9 Ability to understand and the willingness to sign a written informed consent document.

4.2 Exclusion Criteria

4.2.1 History of allergic reactions attributed to compounds of similar chemical or biologic composition to 4-OHT gel.

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

4.2.3 Pregnant, unwilling to use adequate contraception during study treatment duration, had given birth, or nursed at any time during the last 12 months (see footnote 1 below).

4.2.4 Women with a previous history of invasive breast cancer or bilateral DCIS or current untreated DCIS. Women with a history of cancer within the last 3 years, except for non-melanoma skin cancer. Women with unilateral DCIS (with or without radiation therapy) are eligible as long as they have an unaffected breast.

4.2.5 Prior bilateral breast surgery (mastectomy, segmental mastectomy, or breast augmentation surgery including breast implants or breast reductions).

4.2.6 Women with "mosaic mammographic screening views", i.e., whose larger breast size precludes being imaged within a single mammographic screening view.

4.2.7 Women with active liver disease.

4.2.8 Women with a uterus and abnormal uterine bleeding, or prior diagnosis of endometrial hyperplasia, endometrial polyps, or endometrial cancer.

4.2.9 Prior use of SERMS and AIs, except for a maximum of 3 months and at least 12 months prior.

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

4.2.11 Treatment with any investigational drug or investigational biologic within 30 days of initiating study treatment or during the study.

[1] The effects of 4-OHT gel on the developing human fetus at the recommended dose are unknown. For this reason and because tamoxifen is known to be teratogenic, all heterosexually active women who may become pregnant must agree to use a reliable non-hormonal contraceptive method or a hormonal IUD during the study and for 2 months after completing study medications. Reliable nonhormonal methods of contraception include barrier contraception and an Intra-Uterine Device (IUD). Hormonal IUDs are also allowable methods of birth control. [Note: Women who had tubal ligation or had a partner who had undergone a vasectomy (and are monogamous) are eligible for the study and are not required to use barrier contraception.

4.3 Inclusion of Women and Minorities

Men and women less than 40, unless they have sufficient breast cancer risk that they would be undergoing mammography screening, are excluded because these populations are not routinely screened using mammography. Women of all races and ethnic groups are eligible for this trial.

4.4 Recruitment and Retention Plan

Participants will be recruited for enrollment on this trial from mammographic screening units and breast clinics at participating institutions, with particular attention to enhancing racial, ethnic and socioeconomic diversity. Prior to enrollment, the study will be discussed in detail with the participant, and possible toxicities will be presented. The informed consent document will be reviewed with the participant by the study physician or other medical staff authorized by the participating institution to obtain Informed Consent. To facilitate accrual, information about the trial will be advertised, for example, on the websites of participating institutions.

Refer to the study-specific Recruitment and Retention Plan for more details.

5. AGENT ADMINISTRATION

Intervention will be administered on an outpatient basis. Reported AEs and potential risks are described in Section 6.2.

5.1 Dose Regimen and Dose Groups/Gel Application

Participants will self-administer the 4-OHT/ placebo gel. The participants will be instructed to apply the gel daily to both breasts, freshly washed, either by washcloth, shower, or bath, preferably in the morning (in order to minimize potential transfer to the partner at night). If morning shower is not possible, the participant will wash the breasts with a washcloth before gel application, to remove the prior day's dose. If a morning swim is planned, application should be after the swim. No washing or immersion in water should occur for at least 4 hours following gel application. The gel should not be used near fire, flame or while smoking since it is flammable due to alcohol. Once dry, the gel is no longer flammable. Detailed instructions regarding application are in Appendix B and a demonstration of gel application will occur during the Randomization visit. A model bust may be used to demonstrate to the patient how to apply the gel if such a model is available. The treated breasts should be covered at all times to avoid transfer to other people and protect from natural or artificial sunlight; contact is permitted after the treated area has been washed with soap and water and washing is allowable after a minimum of 4 hours post-application.

Each 4-OHT/placebo gel canister will contain ~100 mL gel product and will be metered to dispense 2 mg of 4-OHT/placebo gel per pump. There will be 200 mg of 4-OHT in 100 mL of 4-OHT gel. Because there are 80 reliable doses per canister, each canister will provide 40 days of therapy at 2 mg/day/per breast. Daily dose of 4-OHT/placebo gel application is 4 mg (2 mg per breast). Five gel canisters will be given to the participant at the time of randomization and should be returned at Month 6 visit. One (1) ml (one actuation of the pump) should be applied each day per breast. At Month 6 visit five additional canisters will be provided to the participant and the containers provided at the time of randomization will be collected. All containers will be collected at the Month 12 visit.

COVID-19 Contingency Plan:
During the COVID-19 pandemic, if a participant is not able come for an in person visit at Month 6, instruct the participant during the phone visit to keep all used canisters and to bring the canisters to the next in person clinic visit. Five additional canisters will be provided to the participant (via mail or picked up in person). At Month 12, participants will be telephone consented to extend the gel application and instructed to keep using the gel until their mammogram can be rescheduled up to 18 Months. In addition they will be provided five additional canisters (via mail or picked up in person) and all canisters will be collected at the mammogram visit.

5.2 Run-in Procedures

N/A

5.3 Contraindications

Participants are to avoid exposure of the treated breast skin to natural or artificial sunlight. This includes sunbathing or the use of tanning beds with the breasts exposed. Also, women who have dermatologic conditions causing the breakdown of skin in the area of gel application should not use 4-OHT gel.

5.4 Concomitant Medications

All medications (prescription and over-the-counter), vitamin and mineral supplements, and/or herbs taken by the participant will be documented on the concomitant medication CRF and will include: 1) start and stop date, if known; if unknown, mark "unknown" in the database, 2) dose and route of administration, and 3) indication.

5.5 Dose Modification

For grade 1-2 toxicities, no dose modifications will be made. If a grade 2 toxicity possibly, probably, or definitely related to study drug re-occurs, the gel application will be stopped until the adverse event resolves, then full dose will be reintroduced. If the toxicity re-occurs for a 3rd time, the participant will be taken off study drug. For grade 3 toxicity possibly, probably, or definitely related to study drug, gel application will be stopped until the adverse event resolves, then full dose will be reintroduced. If the adverse event re-occurs, the participant will be taken off study drug. Participants experiencing Grade 4 toxicities of any attribution will be taken off study drug.

5.6 Adherence/Compliance

5.6.1 The interval from initiation of drug to end of treatment and final mammogram may vary. If a participant has used the gel at least 9 months and 75% of days during that interval, she will be considered compliant.

5.6.2 The following method will be used to monitor each participant's agent compliance:
1) Participants will be given a 4-OHT diary and instructed to complete an entry each time a dose is taken (see Appendix D, 4-OHT diary).
2) Canisters will be weighed when released and also upon return.

If there are discrepancies between the two measures of compliance, the lower compliance value will be used as a conservative estimate of compliance. If the canister weight is the lower of the two compliance values any discarded doses will be considered in the calculation of compliance.

6. PHARMACEUTICAL INFORMATION

6.1 Study Agent (IND # *59,081*, BHR Pharma, LLC)

The study agent, 4-OHT, is being provided as a topical hydroalcoholic gel for transdermal administration to the breasts for localized treatment. 4-OHT is a highly active metabolite of tamoxifen, a selective estrogen-receptor modulator (SERM) which is approved as an oral tablet for the prevention and treatment of estrogen receptor positive breast cancer. 4-OHT and its related metabolite, endoxifen, are thought to provide the bulk of the pharmacological activity of tamoxifen since they are ~100 times more active than tamoxifen, which is now viewed as a prodrug.
4-OHT (Z:E 1:1 racemic mixture) is provided as a 0.2% (w/v, 200 mg/mL) hydroalcoholic gel in a pump. Each pump action expels 1 mL of gel containing 2 mg of 4-OHT. Participants are to be advised to pump one time to expel 1 mL of gel for application to each breast for a total daily dose of 4 mg 4-OHT. Gel excipients are alcohol (~75%), isopropyl myristate, hydroxypropyl cellulose, and potassium phosphate

buffer pH 7. Matching placebo gel is the same composition without 4-OHT. Due to the high concentration of alcohol (~75%), participants are advised to not apply the gel while near fire, flame, heat, or while smoking; the gel is not flammable once it has dried.

6.2 Reported Adverse Events and Potential Risks

4-OHT has undergone preclinical animal testing to support human clinical studies. In pharmacokinetic (PK) and toxicokinetic studies, dermal (topical, rats) and subcutanous (injection, rats and dogs) dosing of 4-OHT resulted in dose-proportional increases in drug bioavailability with elimination primarily by the fecal route. 4-OHT has been evaluated in toxicology studies in rats (dermal, subcutaneous) and dogs (subcutaneous). Topical 4-OHT up to 200 μg/kg/day was generally well tolerated by rats, while subcutaneous doses of up to 150 μg/kg/day were well tolerated by dogs. There were no clinical signs of toxicity; however, decreases in body weight, reduced uterus and ovary size, and changes in the genital tract were observed. Dermal irritation studies in rabbits of 4-OHT *vs*. placebo gel induced comparable skin reactions, consisting of none or slight to moderate edema or erythema, on scarified and non-scarified flanks.

4-OHT was not mutagenic or genotoxic in the Ames test (with and without metabolic activation), the HPRT gene mutation assay in Chinese hamster cells, and the *in vivo* mouse micronucleus test. Additionally, no chromosomal abnormalities were seen in human lymphocytes treated with 4-OHT, with or without metabolic activation. Consistent with these results, topical 4-OHT was not carcinogenic in rats treated with up to 1000 μg/kg/day for 101 weeks. Finally, the reproductive toxicity of 4-OHT was evaluated in seven studies with rats and three studies with rabbits. Repeated doses of up to 200 μg/kg/day of dermal or subcutaneous administration of 4-OHT were well tolerated by rats and rabbits. High doses of topical 4-OHT led to impaired female fertility and retardation in fetal body weight, increased incidence of resorptions, and wavy ribs in some fetuses.

Topical 4-OHT in alcohol solution or gel has been administered to >450 pre- and postmenopausal women. In phase 1 safety and PK studies, topical 4-OHT was provided to women at doses ranging from 0.25–1 mg/breast/day for three weeks up to three months. Topical 4-OHT was well tolerated with only drug-related mild pruritus being observed in a few patients. Additionally, topical 4-OHT resulted in substantial breast tissue uptake with minimal metabolism and very low systemic plasma concentrations.

Multiple phase 2 pharmacodynamic studies have been completed in pre- and postmenopausal women with treatment durations of up to six months and doses up to 4 mg/day. Topical 4-OHT has been shown to be well-tolerated, absorbed, and pharmacologically active in breasts for reducing breast pain (mastalgia), breast density, and breast cancer cell proliferation in women with ductal carcinoma *in situ* (DCIS) and locally advanced breast cancer. In a placebo-controlled, six-month phase 2 study of 4-OHT topical gel in premenopausal women with cyclical breast pain, the most common AEs seen in 4-OHT- *vs*. placebo-treated women (4 mg/day) were: headache (20% *vs*. 10%), nasopharyngitis (11% *vs*. 12%), application site reaction (4% *vs*. 0%), and pharyngitis (4% *vs*. 0%)[1]. These events were all mild to moderate and some headaches and application site reactions (application site burning, itching) were deemed drug-related. Additional drug-related AEs seen in 4-OHT-treated women were decreased appetite, exfoliative dermatitis, and hypotension. No significant changes in hematologic parameters, liver function, renal function, electrolytes, or serum glucose were observed.

In a recent NCI, DCP-sponsored presurgical study of women with DCIS, 6–10 weeks of topical 4-OHT gel at 4 mg/day (2 mg to each breast) or oral tamoxifen at 20 mg/day resulted in similar 4-OHT breast levels and similar reductions in breast tumor cell proliferation[3]. Systemic levels of 4-OHT were markedly lower (five-fold) with topical 4-OHT gel compared with oral tamoxifen. 4-OHT topical gel was well-tolerated

and the most common AEs that were either similar or less frequent in 4-OHT- *vs*. tamoxifen-treated women were: hot flush (50% *vs*. 50%), breast pain (42% *vs*. 64%), fatigue (33% *vs*. 29%), and hyperhydrosis (25% *vs*. 43%). Only three AEs were elevated in 4-OHT-treated women compared with tamoxifen-treated women: pruritis (17% *vs*. 0%), vulvovaginal dryness (17% *vs*. 0%), and weight increased (17% *vs*. 0%).

6.3 Availability

4-OHT and matching placebo gel are provided by the NCI, DCP Repository. Each gel canister will contain ~100 mL gel product (200 mg of 4-OHT in 100 mL of gel) and will be metered to dispense 2 mg of active agent per pump. Because there are 80 reliable doses per canister, each canister will provide 40 days of therapy at 2 mg/day/per breast. Placebo canisters will contain an equal weight of placebo gel.

6.4 Agent Distribution

Agents will only be released by NCI, DCP after documentation of IRB approval of the DCP-approved protocol and consent is provided to DCP and the collection of all Essential Documents is complete (see DCP website for description of Essential Documents).

NCI, DCP-supplied agents may be requested by the Investigator (or their authorized designees) at each Organization. DCP guidelines require that the agent be shipped directly to the institution or site where the agent will be prepared and administered. DCP does not permit the transfer of agents between institutions (unless prior approval from DCP is obtained). DCP does not automatically ship agents; the site must make a request. Agents are requested by completing the DCP Clinical Drug Request form (NIH-986) (to include complete shipping contact information) and faxing or mailing the form to the DCP agent repository contractor:

John Cookinham
MRIGlobal
DCP Repository
1222 Ozark Street
North Kansas City, MO 64116
Phone: (816) 360-3805
FAX: (816) 753-5359
Emergency Telephone: (816) 360-3800

6.5 Agent Accountability

The Investigator, or a responsible party designated by the Investigator, must maintain a careful record of the inventory and disposition of all agents received from DCP using the NCI Drug Accountability Record Form (DARF) or an institutionally-approved accountability system. The Investigator is required to maintain adequate records of receipt, dispensing and final disposition of study agent. This responsibility may be delegated to the site coordinator, institutional pharmacist or their designees. Include on receipt record from whom the agent was received and to whom study agent was shipped, date, quantity and batch or lot number. On dispensing record, note quantities and dates study agent was dispensed to and returned by each participant. Drs. Arun, Garber, Khan, Kumar, Chalasani, Beckwith and Wilke or their representatives will be responsible for study agent accountability for participants at UT MD Anderson Cancer Center, Dana Farber Cancer Institute, Northwestern University and Moffitt Cancer Center, University of Arizona Cancer

Center, University of Minnesota, and University of Wisconsin, respectively. DCP requirements for agent accountability and the required forms are available on the DCP website.

6.6 Packaging and Labeling

4-OHT will be packaged by the Besins Healthcare, Inc. Labeling and distribution to the sites will be done by NCI, DCP or its authorized designee.

Transdermal 4-OHT or placebo gel will be packaged into canisters. A dispensing pump system that allows the administration of the correct amount of topical gel will be used. Each canister will have a label printed with the treatment kit number, volume and concentration of gel, lot number, protocol number and "placebo or 4-OHT gel" along with the statement "Caution: New Drug- Limited by Federal law (US) to investigational use only". Detailed instruction for gel application will be provided. Each participant will be provided with 5 canisters at initial dispensing, and another 5 canisters at Month 6 Visit. Under the COVID-19 Contingency Plan 5 additional canisters will be provided at Month 12 (see Section 5.1 for more details).

Canisters will be packaged into kits, 5 canisters per kit. A three-part label will be attached to each kit. The fixed part of the label will remain attached to the kit. The first tear-off portion of the label (the middle portion) will be removed from the kit at the time of dispensing and will be affixed to the Compliance Drug Labels form (Appendix C). This portion of the label will be identical to the first portion of the label, except that it will contain a scratch off area with the unblinding information should the PI need to be unblinded in case of emergency (See Section 6.9). The second tear-off portion of the label will be removed from the kit by the pharmacist at the time of dispensing. This portion of the label will contain unblinded information and will be maintained by the pharmacist following institutional guidelines or maybe discarded if this portion of the label is not needed for drug accountability purposes due to the use of electronic capture.

Placebo gel will be packaged into canisters for the purpose of demonstration. These canisters are labeled "For demonstration purposes only" and provided to study coordinators to show consented participants how to pump the canister and what the gel looks like." Participants are allowed to touch the gel and are asked to wash their hands after the demonstration.

6.7 Storage

All study drug must be stored in a secure limited-access area, at controlled room temperature (20 – 25ºC [68° to 77°F]; excursions are permitted to 15° to 30°C [59° to 86°F]) in accordance with labeled storage requirements. Participants will be instructed to store the study drug at home at room temperature, and to avoid extreme heat or cold during transportation from the clinic to home. Investigational labeling will include instructions to keep the product out of the reach of children.

6.8 Registration/Randomization

Screening and Registration into the DMI Database:
Once informed consent has been signed, participants will be registered into the DMI database. The DMI database will assign a participant's PID upon completion of the registration process.

Randomization:
Participants will be assigned a randomization number once the following has been accomplished: eligibility

has been verified at the site level, eligibility has been confirmed by the site PI, and eligibility CRF has been entered into the DMI web application. The randomization number will be generated by the database and assigned to the participant. Randomization will be performed stratified by study site and baseline breast density category (heterogeneously dense (C) or extremely dense (D)). Refer to **Section 13.2** for details of randomization.

Screening/Registration/Randomization into site-specific databases:
The DMI is the database of record for the study. Registration and randomization should occur per the procedures outlined above. If the site staff needs to enter study data into site-specific electronic databases per their institutional requirements, they should do so in accordance with their institutional policies and procedures.

Appropriate CRFs must be completed for any participant who signs an informed consent. If a consented participant is a screen failure and deemed ineligible, the following CRFs must be completed: 1) the Registration CRF; 2) the Randomization CRF with the eligibility box checked "no", 3) the Inclusion and Exclusion CRFs showing why the participant is ineligible, 4) the Off-Study CRF, 5) the Adverse Event CRF, 6) the Concomitant Medication CRF and 7) the Verification CRF. If no Adverse Event and/or Concomitant Medications were assessed by the time the participant is deemed ineligible, check the appropriate boxes on the Off Study eCRF. All participants who sign an informed consent must formally go off study. All participant registration information will be entered into DMI. If a participant experiences a serious Adverse Event during the screening process, a Serious Adverse Event (SAE) form must be completed.

6.9 Blinding and Unblinding Methods

- Participants will be blinded to 4-OHT topical gel or placebo.
- The Statistician and the Study Pharmacist will not be blinded to 4-OHT topical gel or placebo.
- All other Investigators will be blinded to 4-OHT topical gel or placebo.
- The MDACC Data and Safety Monitoring Board will also be blinded unless unblinding is warranted (the reasons for unblinding are outlined below).
- All participants will self-administer the gel by pump actuation. Participants in the placebo and treatment group will administer daily 1 pump worth of gel to each breast.
- Study assignments will be unblinded to the Study Investigators and Site Coordinators after all of the data are collected and the study database has been locked. Unblinding will also occur if the participant's physician deems that unblinding is necessary, such as in the case of unacceptable toxicity thought to be related to the study agent or progressive disease, or if the participant becomes pregnant.
- Unblinding will only take place after consultation with the NCI, DCP Medical Monitor Marjorie Perloff, MD.
- If unblinding is indicated prior to the completion of the study, it will be conducted as follows:
    - The Site Investigator contacts the Protocol Principal Investigator (Dr. Arun) and requests the participant's treatment status be unblinded.
    - The Protocol Principal Investigator (Dr. Arun) contacts the NCI, DCP Medical Monitor and requests the participant's treatment status be unblinded. The Protocol Principal Investigator then conveys the Medical Monitor's decision to the Site Investigator. The Site Investigator or their designee then proceeds with unblinding as written out below.

- If the NCI, DCP Medical Monitor cannot be reached and the participant requires emergency care, the Protocol Principal Investigator may authorize the site Investigator to break the blind.
- If the Site Investigator is unable to reach the Protocol Principal Investigator and the participant requires emergency care, then the Site Investigator must proceed with unblinding as written out below.
- The Site Investigator requests the participant's treatment status be unblinded by the research pharmacist (or designated individual responsible for dispensing drug).
- The Site Investigator officially takes the participant off-study.
- The date and reason for breaking the blind must be submitted by the Site Investigator to the Protocol Principal Investigator as soon as possible.
- It is the responsibility of the Study Principal Investigator to report the date and reason for breaking the blind to the **NCI, DCP Medical Monitor, Marjorie Perloff, MD,** as soon as possible.
- The date and reason for breaking the blind must be submitted by the Study Principal Investigator to the MD Anderson Consortium Principal Investigator, Powel H. Brown, MD, PhD, or designee as soon as possible via email to phbrown@mdanderson.org or phone at (713) 792-4509.
- The date and the reason for breaking the blind will be reported by the MD Anderson Consortium Principal Investigator or designee to the MD Anderson DSMB as soon as possible.

6.10 Agent Destruction/Disposal

At the completion of investigation, all undispensed study agent will be returned to NCI, DCP Repository according to the DCP "Guidelines for AGENT RETURNS" and using the DCP form "Return Drug List". The Guidelines and form are available on the DCP website. Unused drug, that has been dispensed to participants, but that is returned unused by the participants, will be disposed of by each institution following institutional guidelines after canisters have been weighed and the weights have been recorded. Record the weight to the nearest hundredth.

7. CLINICAL EVALUATIONS AND PROCEDURES

7.1.1 Schedule of Events

Evaluation/ Procedure	Baseline Testing/ Prestudy Evaluation/ Randomization [a]	Breast core biopsy (Optional)	Start of Intervention	Day 7-14[c]	Month 3[c]	Month 6	Month 9[c] or Early Termination	Month 12	Month 24
	≤60 days of Day 1		Day 1[b]		Day 90 ± 14 days	Day 180 ± 14 days	Day 270 ± 14 days	Day 365 ± 30 days	Day 730 ± 30 days
Informed Consent	X								
Assess Eligibility	X								
Confirm Eligibility	X								
Registration	X								
Randomization	X								
Medical History	X								
Physical Exam	X[d]					X[d]		X[d]	
Vital Signs/ Height and Weight [e]	X					X[e]		X[e]	
Laboratory Tests [f]	X[f]							X[f]	
Pregnancy Test	X[g]	X[g]				X		X	
Mammogram	X[h]							X	X
Breast core biopsy (Optional)[i]		X[i]						X	
Blood for Biomarkers [j]	X					X		X	
Concomitant Medications	X			X	X	X	X	X	
Adverse Events				X	X	X	X	X	
Dispense Study Agent	X*					X			
Collect Unused Study Agent						X		X	
Demonstration of gel application	X*								
Start Study Agent			X						
Review Agent Diary [l] / Drug compliance	X*			X[k]	X	X	X	X	
Telephone Contact				X[k]	X		X		
BESS Questionnaire [m]	X					X		X	
Tobacco and Alcohol Use Assessment [n,o]	X [n]							X[o]	

[a] Baseline Testing/ Prestudy Evaluation may be done on the same day as Randomization.

[b] Participants will start treatment within 7 days of dispensing drug.
[c] Telephone contact. The telephone call can be replaced with alternate forms of communication preferred by the participant – text, email, health portal message or other where local policy allows, as long as all required information is collected.
[d] At Baseline the physical exam should include a clinical breast exam, nodal, if not performed within the last 12 months. At Months 6 and 12 the physical exam should include a breast exam.
[e] Do not repeat height measurement after Baseline Testing.
[f] CBC (hemoglobin, hematocrit, RBC, WBC, platelet count), total bilirubin, AST (SGOT)/ALT (SGPT). In premenopausal women: FSH, LH, estradiol. H&H are not part of the eligibility criteria. Results from outside (i.e., non-study site) CLIA approved laboratories drawn $\leq$ 60 days of Day 1 may be used to confirm eligibility. The outside labs must show normal values for each result.
[g] A urine or serum pregnancy test $\leq$ 14 days before starting study agent for women of child-bearing potential. Results must be known prior to randomization and drug dispensing. If the pregnancy test result is positive at Baseline (any time before randomization), the participant is a Screen Failure. For participants who agree to the optional biopsy the pregnancy test will be repeated if needed to satisfy the requirement of having a negative pregnancy test within 14 days of starting study agent.
[h] Obtain previous annual mammography report. Once the participant has signed consent, the mammography center should be alerted not to delete the raw images. In centers where raw images are stored, consent can occur after the annual mammogram that will serve as the baseline mammogram for the study. The mammogram must be within 3 months of randomization.
[i] The optional biopsy will be done after randomization (may be done on the day of randomization, but before start of intervention).
[j] Refer to sections 8.2 and 10.1 for a description of blood biomarkers.
[k] Confirm start of gel application.
[l] Refer to Appendix D.
[m] Refer to Appendix E.
[n] See Appendix F "Alcohol and Tobacco Use Assessment Questionnaires – Baseline"
[o] See Appendix G "Alcohol and Tobacco Use Assessment Questionnaires – Month 12". Assessed in person or on the phone.

* Dispense study drug after randomization. Dispensing of study agent does not have to be on the same day as randomization. Prescriptions should be written/signed after the participant is randomized. Randomization number must be confirmed prior to dispensing. For women who agree to the optional biopsy – dispensing study agent, demonstrating of gel application, and reviewing the agent diary and drug compliance will occur on the day of the biopsy.

7.1.2 Schedule of Events

COVID-19 Contingency Plan: assumes that the participants have to miss in person visits at either Mo 6 or 12, or both

Evaluation/ Procedure	Baseline Testing/ Prestudy Evaluation/ Randomization[a]	Breast core biopsy (Optional)	Start of Intervention	Day 7-14[c]	Month 3[c]	Month 6	Month 9[c] or Early Termination	Month 12	Month 13-18	Month 24 + 8 Months
	≤60 days of Day 1		Day 1[b]		Day 90 ± 14 days	Day 180 ± 14 days	Day 270 ± 14 days	Day 365 ± 30 days		
Informed Consent	X								X (Note 5)	
Assess Eligibility	X									
Confirm Eligibility	X									
Registration	X									
Randomization	X									
Medical History	X									
Physical Exam	X[d]								X[d]	
Vital Signs/ Height and Weight [e]	X								X[e]	
Laboratory Tests [f]	X[f]								X[f]	
Pregnancy Test	X[g]	X[g]				(Note 1)		(Note 1)	X	
Mammogram	X[h]								X	X
Breast core biopsy (Optional)[i]		X[i]							X	
Blood for Biomarkers [j]	X								X	
Concomitant Medications	X			X	X	X	X	X	X	
Adverse Events				X	X	X	X	X	X	
Dispense Study Agent	X*					X (Note 2)		X (Note 2)		
Collect Unused Study Agent						(Note 3)		(Note 3)	X	
Demonstration of gel application	X*									
Start Study Agent			X							
Review Agent Diary [l] / Drug compliance	X*			X[k]	X	X	X	X	X	
Telephone Contact				X[k]	X	X	X	X		
BESS Questionnaire [m]	X					X (Note 4)		X (Note 4)	X	
Tobacco and Alcohol Use Assessment [n,o]	X [n]								X[o] (Note 6)	

[a] Baseline Testing/ Prestudy Evaluation may be done on the same day as Randomization.
[b] Participants will start treatment within 7 days of dispensing drug.
[c] Telephone contact. The telephone call can be replaced with alternate forms of communication preferred by the participant – text, email, health portal message or other where local policy allows, as long as all required information is collected.
[d] At Baseline the physical exam should include a clinical breast exam, nodal, if not performed within the last 12 months. At Month 13-18 the physical exam should include a breast exam.
[e] Do not repeat height measurement after Baseline Testing.
[f] CBC (hemoglobin, hematocrit, RBC, WBC, platelet count), total bilirubin, AST (SGOT)/ALT (SGPT). In premenopausal women: FSH, LH, estradiol. H&H are not part of the eligibility criteria. Results from outside (i.e., non-study site) CLIA approved

laboratories drawn ≤ 60 days of Day 1 may be used to confirm eligibility. The outside labs must show normal values for each result.

[g] A urine or serum pregnancy test ≤ 14 days before starting study agent for women of child-bearing potential. Results must be known prior to randomization and drug dispensing. If the pregnancy test result is positive at Baseline (any time before randomization), the participant is a Screen Failure. For participants who agree to the optional biopsy the pregnancy test will be repeated if needed to satisfy the requirement of having a negative pregnancy test within 14 days of starting study agent.

[h] Obtain previous annual mammography report. Once the participant has signed consent, the mammography center should be alerted not to delete the raw images. In centers where raw images are stored, consent can occur after the annual mammogram that will serve as the baseline mammogram for the study. The mammogram must be within 3 months of randomization.

[i] The optional biopsy will be done after randomization (may be done on the day of randomization, but before start of intervention).

[j] Refer to sections 8.2 and 10.1 for a description of blood biomarkers.

[k] Confirm start of gel application.

[l] Refer to Appendix D.

[m] Refer to Appendix E.

[n] See Appendix F "Alcohol and Tobacco Use Assessment Questionnaires – Baseline"

[o] See Appendix G "Alcohol and Tobacco Use Assessment Questionnaires – Month 12". Assessed in person or on the phone.

***** Dispense study drug after randomization. Dispensing of study agent does not have to be on the same day as randomization. Prescriptions should be written/signed after the participant is randomized. Randomization number must be confirmed prior to dispensing. For women who agree to the optional biopsy – dispensing study agent, demonstrating of gel application, and reviewing the agent diary and drug compliance will occur on the day of the biopsy.

Note 1: Premenopausal women should be reminded that they are strongly advised not to become pregnant while on study and should continue using appropriate contraceptive methods.

Note 2: Mail or email the **4-OHT Diary** to the participant. Ship a new 6-month supply of the study agent to the participant, if you can do so. Ask the participant to record their daily use of agent on the 4-OHT Diary; using a new Diary for each Canister. **Instruct the participant to bring their completed Diaries to the next in person clinic visit.** ***Instruct the participant to keep all used canisters and to bring the canisters with ALL of their completed Diaries to the next in person clinic visit.*** Follow other Contingency Plan instructions from Section 7.2 below.

Note 3: Instruct the participant to keep all used canisters and to bring the canisters with ALL of their completed Diaries to the next in person clinic visit.

Note 4: Mail or email the **BESS QUESTIONNAIRE** to the participant ahead of the telephone visit, if possible. Ask the participant to fill it out ahead of the visit, to sign and date the form at the time of completion, and keep it. Collect the information during the visit, if allowed by the institution and if the participant feels comfortable to discuss the responses over the phone. Participants should return the **BESS QUESTIONNAIRE** at their next in person visit. If you were not able to mail/email the **BESS QUESTIONNAIRE** ahead of the telephone visit, collect the information over the phone, if possible. Record the answers.

Note 5: Participants that are asked to extend their gel application will be reconsented following the CIRB remote consent procedure for the extended gel application.

Note 6: **ALCOHOL and TOBACCO ASSESSMENTs** will be done in clinic when participants return for their mammogram.

7.2 Baseline Testing/Prestudy Evaluation

Baseline Testing/Prestudy Evaluation/Randomization (≤ 60 days of Day 1)

Baseline Testing/Prestudy Evaluation interventions will be conducted ≤ 60 days of Day 1, may require multiple visits or may be done on the day of randomization and will consist of the following procedures:

- Women with prior annual mammograms showing heterogeneously dense or dense breast/s will be approached for enrollment into the study prior to their next annual mammogram. Informed consent must be obtained prior to starting any further study procedures. Consenting should be done prior to next annual mammogram, that will serve as the baseline study mammogram. Once the participant has signed consent, the mammography center should be alerted not to delete the raw images. Raw images must be obtained for study purposes (see instructions in Section 8.1). In centers where raw images are stored, consent can occur after the annual mammogram (the baseline mammogram for this study). The mammogram must be within 3 months of randomization. Mammography report from the previous annual visit should also be obtained.

- Registration: Once informed consent has been signed, participants will be registered into the DMI database. The DMI database will assign a participant's PID upon completion of the registration process. Participants will also be registered into site-specific registry databases as applicable.

- Data collection including race, height, weight, cancer family history, reproductive history, previous breast biopsy results (if applicable), hormonal medication use (HRT, OCP, SERM, aromatase inhibitors), BRCA status (if available), cup size, BIRADs for each site and medication assessment for drugs known to inhibit 4-OHT gel *or* tamoxifen action.

- Limited physical examination must be done ≤ 60 days of Day 1. IInclude a clinical breast exam if not performed within the last 12 months. If a breast mass is found on initial examination, the participant will receive standard evaluation and will not participate until the mass has been classified as benign.

- Laboratory tests will include the following:
  - CBC (hemoglobin, hematocrit, RBC, WBC, platelet count)
  - Total bilirubin
  - AST (SGOT)/ALT (SGPT)

- Staging of reproductive age. All women will be asked for the date of their last menstrual cycle. In premenopausal women: FSH, LH, estradiol.

- A urine or serum pregnancy test within 14 days of starting study agent for women of child-bearing potential. Results must be known prior to randomization and drug dispensing. The urine pregnancy test must be done in the clinic. The pregnancy test will be repeated at 6 months and 12 months visits.
  - If the pregnancy test result is positive at Baseline (any time before randomization), the participant is a Screen Failure.
  - For participants with the optional biopsy the pregnancy test will be repeated if needed to satisfy the requirement of having a negative pregnancy test within 14 days of starting study agent.

- Concomitant medications will be documented.

Once registration is complete, eligibility has been confirmed and eligibility CRF is entered into the web application, participants will be assigned a randomization number pre-generated by the database.

- Randomization will be performed stratified by study site and baseline breast density (heterogeneously dense (C) or extremely dense (D)).

- Dispense study drug after randomization. Dispensing of study agent does not have to be on the same day as randomization. Prescriptions should be written/signed after the participant is randomized. Randomization number must be confirmed prior to dispensing.

- Participants will be provided with the gel kit with a 6 months' supply and an agent diary (see Appendix D). The agent diary will be reviewed with the participant. Demonstration of gel application will be done. Instructions on how to apply gel will be provided. See Appendix B. Participants who agree to the option biopsy will receive their gel kit on the day of the biopsy.

- Blood draw for research biomarkers.

- BESS Questionnaire (refer to Appendix E).

- Tobacco and Alcohol Use Assessment, using the Baseline questionnaires. See Appendix F "Alcohol and Tobacco Use Assessment Questionnaires – Baseline". Refer to Appendix H for resources for alcohol and tobacco quitting. These resources can be given to individuals if there is concern about alcohol or tobacco dependence. It is not expected that investigators will refer individuals for assistance or that they will undertake the care of individuals for alcohol or tobacco dependence.

Optional Breast Core Biopsy

- Breast core biopsy for participants who agreed to the optional procedure. The optional biopsy will be done after randomization. It may be done on the day of randomization, but before start of intervention. If the participant is on aspirin, non-steroidal anti-inflammatory drugs or Vitamin E, she will be instructed to discontinue these and return in 7-10 days for the biopsy. The participant will start to apply the gel at least 24 hours after the biopsy. If the biopsy has been performed in the morning, the participant can start applying the gel the next morning, after 24 hours. If the biopsy was performed in the afternoon, and the participant wishes to apply the gel in the mornings for the duration of the study, then the participant can start applying the get on the morning of the 2nd day after the biopsy. Remind the participant to start treatment within 7 days of dispensing drug.

- For women who agree to the optional biopsy – dispensing study agent, demonstrating of gel application, and reviewing the agent diary and drug compliance will occur on the day of the biopsy. See instructions above.

Start of Intervention (Day 1)

- Participants will start treatment within 7 days of dispensing drug.

- A telephone contact is scheduled for 7-14 days post Day 1.

7.3 Evaluation During Study Intervention

Day 7-14: Telephone Contact
The following will be performed:

- Verification of the start date of gel application.
- Concomitant medications.
- Adverse events.
- Participants will be reminded of the need to complete the agent diary daily.

The telephone call can be replaced with alternate forms of communication preferred by the participant – text, email, health portal message or other where local policy allows, as long as all required information is collected.

Month 3 [Day 90 ± 14 days]: Telephone Contact
The following will be performed:

- Adverse events.
- Concomitant medications.
- Drug compliance and agent diary review.

The telephone call can be replaced with alternate forms of communication preferred by the participant – text, email, health portal message or other where local policy allows, as long as all required information is collected.

Month 6 [Day 180 ± 14 days]
At month 6 the participant will return to clinic as per standard of care breast high risk screening follow-up.
The following will be performed:

- Physical exam, including a breast examination.
- Vital signs, weight.
- A urine or serum pregnancy test will be performed. The urine pregnancy test must be done in the clinic. If the pregnancy test result is positive at the 6 Months visit, do not dispense study agent, follow the pregnancy to term and complete the Outcome of Pregnancy CRF. Take the participant off study at the completion of the pregnancy when the outcome of pregnancy is known.
- Blood draw for research biomarkers.
- Concomitant medication.
- Adverse events.
- Collect unused study agent.
- Compliance check will be performed by evaluating the participant's agent diary.
- Participant will be provided with a 6 months' supply of study agent (gel kit) and a new agent diary.
- BESS Questionnaire (refer to Appendix E).

Month 9 [Day 270 ± 14 days]: Telephone Contact

The following will be performed:

- Adverse events.
- Concomitant medications.
- Drug compliance and agent diary review.

The telephone call can be replaced with alternate forms of communication preferred by the participant – text, email, health portal message or other where local policy allows, as long as all required information is collected.

7.4 Evaluation at Completion of Study Intervention

Month 12 [Day 365 ± 30 days]

Month 12 clinic visit is a standard high risk evaluation.
The following will be performed:

- Physical exam, including a breast examination.
- Vital signs, weight.
- Laboratory tests will include the following:
  - CBC (hemoglobin, hematocrit, RBC, WBC, platelet count)
  - Total bilirubin
  - AST (SGOT)/ALT (SGPT)
- In women who were considered premenopausal at baseline: FSH, LH, estradiol.
- A urine or serum pregnancy test will be performed. The urine pregnancy test must be done in the clinic. If the pregnancy test result is positive at the 12 Months visit, follow the pregnancy to term and complete the Outcome of Pregnancy CRF. Take the participant off study at the completion of the pregnancy when the outcome of pregnancy is known.
- Bilateral mammogram. The raw images must be obtained (see instructions in Section 8.1) for study purposes.
- For participants who underwent the baseline breast biopsy, the biopsy will be repeated.
- Blood draw for research biomarkers
- Adverse events.
- Concomitant medication.
- Collect unused study agent.
- Compliance check will be performed by evaluating the participant's agent diary.
- BESS Questionnaire (refer to Appendix E).
- Tobacco and Alcohol use assessment will be performed at Month 12, using the Month 12 questionnaires (see Appendix G "Alcohol and Tobacco Use Assessment Questionnaires – Month 12").
- Participants will be reminded of the need to return for their Month 24 visit, which is their standard clinical high risk follow-up visit.

7.5 Post-intervention Follow-up Period

Month 24 [Day 730 ± 30 days]

- Standard of care bilateral mammogram. Raw images must be obtained for study purposes (see instructions in Section 8.1).

COVID-19 Contingency Plan

The Contingency assumes that the participants have to miss in person visits at Mo 6 and/or. If the participants are able to come in person for Mo 6 and 12 Visits, proceed per protocol during the pandemic.

Month 6 [Day 180 ± 14 days]: Telephone Contact
The following will be performed:

- Concomitant medication.
- Adverse events.
- Premenopausal women should be reminded that they are strongly advised not to become pregnant while on study and should continue using appropriate contraceptive methods.
- Mail or email the 4-OHT Diary to the participant. Ask the participant to record their daily use of agent on the 4-OHT Diary; using a new Diary for each Canister.
- Ship or have participant pickup a new 6-month supply of the study agent.
- Instruct the participant to keep all used canisters and to bring the canisters with all of their completed Diaries to the next in person clinic visit.
- Drug compliance and agent diary review.
- Mail or email the **BESS QUESTIONNAIRE** to the participant ahead of the telephone visit, if possible. Ask the participant to fill it out ahead of the visit and collect the information during the visit, if allowed by the institution and if the participant feels comfortable to discuss the responses over the phone. Participants should return the **BESS QUESTIONNAIRE**, at their next in person visit. If you were not able to mail/email the **BESS QUESTIONNAIRE** ahead of the telephone visit, collect the information over the phone, if possible. Record the answers.

Month 9 [Day 270 ± 14 days]: Telephone Contact

The following will be performed:

- Adverse events.
- Concomitant medications.
- Drug compliance and agent diary review.

The telephone call can be replaced with alternate forms of communication preferred by the participant – text, email, health portal message or other where local policy allows, as long as all required information is collected.

Month 12 [Day 365 ± 30 days]: Telephone Contact
The following will be performed:

- Adverse events.
- Concomitant medication.
- Premenopausal women should be reminded that they are strongly advised not to become pregnant while on study and should continue using appropriate contraceptive methods.
- Mail or email the 4-OHT Diary to the participant. Ask the participant to record their daily use of agent on the 4-OHT Diary; using a new Diary for each Canister.
- Participants will be asked to extend their gel application and will be reconsented following the CIRB remote consent procedure for the extended gel application.
- Ship or have the participant pickup a new 6-month supply of the study agent.
- Instruct the participant to keep all used canisters and to bring the canisters with all of their completed Diaries to the next in person clinic visit.
- Drug compliance and agent diary review.

- Mail or email the **BESS QUESTIONNAIRE** to the participant ahead of the telephone visit, if possible. Ask the participant to fill it out ahead of the visit, to sign and date the form at the time of completion, and keep it. Collect the information during the visit, if allowed by the institution and if the participant feels comfortable to discuss the responses over the phone. Participants should return the **BESS QUESTIONNAIRE** at their next in person visit. If you were not able to mail/email the **BESS QUESTIONNAIRE** ahead of the telephone visit, collect the information over the phone, if possible. Record the answers.
- Participants will be reminded of the need to reschedule their mammogram within the next 6 months.
- Participants that do not want to extend their gel application should be taken off agent and asked to allow the collection of their next available mammogram for study purposes.

7.4 Evaluation at Completion of Study Intervention

Month 13-18

The following will be performed:

- Physical exam, including a breast examination.
- Vital signs, weight.
- Laboratory tests will include the following:
  - CBC (hemoglobin, hematocrit, RBC, WBC, platelet count)
  - Total bilirubin
  - AST (SGOT)/ALT (SGPT)
- In women who were considered premenopausal at baseline: FSH, LH, estradiol.
- A urine or serum pregnancy test will be performed. The urine pregnancy test must be done in the clinic. If the pregnancy test result is positive at the 13-18 Month visit, follow the pregnancy to term and complete the Outcome of Pregnancy CRF. Do not take the participant off study until the completion of the pregnancy when the outcome of pregnancy is known.
- Bilateral mammogram. The raw images must be obtained (see instructions in Section 8.1) for study purposes.
- For participants who underwent the baseline breast biopsy, the biopsy will be repeated if participant extended their gel application.
- Blood draw for research biomarkers
- Adverse events.
- Concomitant medication.
- Collect unused study agent.
- Compliance check will be performed by evaluating the participant's agent diary.
- BESS Questionnaire (refer to Appendix E).
- Tobacco and Alcohol use assessment will be performed at Month 12, using the Month 12 questionnaires (see Appendix G "Alcohol and Tobacco Use Assessment Questionnaires – Month 12").
- Participants will be reminded of the need to return for their next annual mammogram (Month 24 + 8 Months), which is their standard clinical high risk follow-up visit.

7.5 Post-intervention Follow-up Period

Month 24 + 8 Months

- Collection of the standard of care bilateral mammogram images. Raw images must be obtained for study purposes (see instructions in Section 8.1).

7.6 Methods for Clinical Procedures

Breast core biopsy (see also Section 10.2):
The breast core biopsy (image guided) is optional. A 14-gauge needle will be used. Every effort will be made to collect 4 cores of tissue, with the minimum of 2 cores per participant.

Tissue will be considered adequate for research assays if the H&E stained slide from the block demonstrates at least one TDLU in every tissue core. One H&E slide will be prepared for adequacy and histological assessment. Usually each terminal duct lobular unit contains 200 to 2500 cells. If no terminal duct lobular units are seen, deeper sections will be obtained. Subsequent slides will be used for biomarker analysis. Refer to Section 10.2 for tissue collection and handling procedures.

8. CRITERIA FOR EVALUATION AND ENDPOINT DEFINITION

8.1 Primary Endpoint

The primary objective of this study is to evaluate mammographic density (MD) changes induced by 4-OHT gel versus placebo in women with mammographically dense breast. Our hypothesis is that 4-OHT will reduce MD. Baseline and 12 months mammogram [or mammograms from Month 13-18 under the COVID-19 contingency plan] will be evaluated for 4-OHT effect using Cumulus software for the primary endpoint evaluation. Analysis will be based on tomosynthesis imaging using the 2D synthetic image.

MD will be classified using BIRADS descriptions on mammographic reports (i.e: predominantly adipose tissue, scattered fibroglandular densities, heterogeneously dense or extremely dense). BIRADS MD assessment is a consistently utilized measure of breast fibroglandular tissue volume, which can be universally followed and reproduced. BIRADS is a scheme for putting the findings from mammogram screening into a small number of well-defined categories. In addition to BIRADS, baseline and follow up MD will be quantified and recorded using semi-automated software, Cumulus, which has been utilized in earlier density assessment studies [10], [11], [12]. Cumulus reading will be centralized with the reader blinded to the timepoint and treatment group.

For the primary endpoint, the densest breast will be used.

At each institution mammographic images will be collected by a study specific data coordinator who will be overseen by the site Investigator. This data coordinator will also ensure that images are collected in usable format with appropriate de-identification. Follow the instructions in Section 10.2 for Collection and Handling of images.

In summary, the effect of utilized software on the density percent assessment is estimated to be minimal. Based on this, we will use:

a) Downgrading of the baseline reported BIRADS breast density.
b) Decrease in breast fibroglandular density by 10% or more as assessed by semi- and automated Breast Density software.

8.2 Secondary Endpoints

Note: data from Month 13-18 visit instead of Month 12 visit will be used if participants were seen under the COVID-19 Contingency Plan. If data were not collected at Month 6 under the COVID-19, such

participants will have missing data.

Breast biopsy for secondary and exploratory endpoints will be performed in the opposite (non-target) breast.

8.2.1 We will estimate and compare the percent change in mammographic breast density (using Volpara) from Baseline to Month 12 in women applying 2mg 4-OHT gel per breast versus placebo.

8.2.2 We will estimate and compare the percent change in mammographic breast density (using LIBRA) from Baseline to Month 12 in women applying 2mg 4-OHT gel per breast versus placebo.

8.2.3 We will compare the percentage of women who underwent a change in BIRADS category, comparing pre-and post- treatment measurements, for recipients of 4-OHT gel versus placebo.

8.2.4 We will conduct subgroup analyses including BIRAD category, menopausal status, cup size, and total breast volume.

8.2.5 We will estimate the percentage of women with ≥ 10% absolute decrease in quantitative mammographic density percentage between baseline and 12 months, comparing between treated group 2mg per breast 4-OHT gel versus placebo based on Cumulus method.

8.2.6 Markers of Toxicity: Baseline, 6 and 12 months BESS questionnaire will be used. Further blood based toxicity markers will be also evaluated at baseline and again at months 6 and 12 (F VIII, vWB factor, SHBG, lipid profile).

8.2.7 Markers of Tamoxifen exposure (in plasma): Plasma measurements of 4-OHT and related metabolite levels and factors related to tamoxifen exposures will be measured at baseline and again at months 6 and 12. (If baseline drug metabolite levels are low; other plasma biomarkers will not be measured at baseline and month 12. Further, the second analysis of 4-OHT and metabolites at 6 and 12 months will also not be performed).

8.2.8 Markers of Tamoxifen exposure (in tissue): Tissue measurements of 4-OHT and related metabolite levels and factors related to tamoxifen exposures will be measured at baseline and again at month 12. (If baseline drug metabolite levels are low; other tissue biomarkers will not be measured at baseline and month 12. Further, the second analysis of 4-OHT and metabolites at 12 months will also not be performed).

8.3 Exploratory Endpoints

8.3.1 To compare the Cumulus vs. Volpara vs. LIBRA breast density measurement methods to estimate percent change in mammographic breast density from baseline to Month 12 in women applying 2mg of 4-OHT gel per breast vs. placebo.

8.3.2 To perform correlation of drug concentrations in the plasma and the tissue.

8.3.3 To evaluate plasma measurements of Insulin-like growth factor (IGF) pathway members, C-reactive protein (CRP), and estradiol.

8.3.4 To compare the 2D natural vs. 2D synthetic breast density measurement using the Cumulus method to estimate percent change in mammographic breast density from baseline to Month 12 in women applying 2mg of 4-OHT gel per breast vs. placebo.

8.3.5 To evaluate tissue biomarkers (among women undergoing optional pre- and post-treatment biopsies):

- Structure-function activity of TLDUs will be evaluated by counting acinar density.
- Breast tissue density will be evaluated by assessing the patterns of collagen matrix.
- Hormone-mediated cellular activity will be evaluated by measuring the transcriptional expression levels of ER-alpha and ER-associated genes ($SET_{ER/PR}$ index).
- Pharmacogenomic response to 4-OHT will be evaluated by measuring Ki67 protein by immunohistochemistry and aurora kinase-alpha gene expression.
- Inflammatory response to 4-OHT therapy will be evaluated by measuring COX2 and p16 proteins, and recruitment of CD68 monocytes by immunohistochemistry.

8.3.6 To examine whether any reductions in mammographic density seen after 1 year of 4-OHT vs. placebo gel application persist at 24 months (or longer under the COVID-19 Contingency Plan), one year after gel application has stopped.

8.4 Off-Agent Criteria

Participants may stop applying the study gel for the following reasons: completed the protocol-prescribed intervention, adverse event or serious adverse event, inadequate agent supply, noncompliance, concomitant medications, medical contraindication, or pregnancy. Participants will continue to be followed, if possible, for safety reasons and in order to collect endpoint data according to the schedule of events. We plan to accrue 7 more participants per arm to account for women who are not evaluable because they discontinued participation prior to meeting criteria for contributing evaluable results. Women who discontinue the agent for any reason should continue on study if possible to allow for collection of their routine mammograms.

8.5 Off-Study Criteria

Participants may go 'off-study' for the following reasons: the protocol intervention and any protocol-required follow-up period is completed, adverse event/serious adverse event, lost to follow-up, non-compliance, concomitant medication, medical contraindication, withdraw consent, or death. Participants found to be ineligible during the screening phase and after signing the Informed Consent document and assigning the PID number, will be considered "screen failures". Such participants will be taken off study and the appropriate end of study CRFs will be completed for these participants.

8.6 Study Termination

NCI, DCP as the study sponsor has the right to discontinue the study at any time.

9. CORRELATIVE/SPECIAL STUDIES

9.1 Rationale for Methodology Selection

Section 8 outlines our primary and secondary endpoint biomarkers. The primary endpoint measurement will be done by assessing MD using standard clinical methods. For secondary endpoints, again, we choose methods that have been previously published, either by our group or other investigators, that are widely available, practical, and financially preferable.

9.2 Comparable Methods

The methodologies described above are those previously used (see details above), and the resulting data will be able to be compared to existing data.

10. SPECIMEN MANAGEMENT

10.1 Laboratories

10.1.1 4-OHT metabolite analysis will be performed at the IIT Research Institute, Chicago, IL in plasma and in tissue.

10.1.2 F VIII, vWB factor, SHBG, lipid profile, CRP, and estradiol will be performed at UT MD Anderson Outpatient Laboratory.

10.1.3 Plasma IGF pathway biomarkers will be performed in Dr. Arun's research laboratory.

10.1.4 Exploratory tissue biomarkers (TDLU involution; collagen structural changes; $SET_{ER/PR}$ index: estrogen related transcription; Ki-67, COX-2, p16, and CD68) will be performed by our pathology collaborator, Dr. Fraser Symmans in the Departments of Pathology and Translational Molecular Pathology at UT MD Anderson Cancer Center.

10.2 Collection and Handling Procedures

Note: data from Month 13-18 visit instead of Month 12 visit will be used if participants were seen under the COVID-19 Contingency Plan. If data were not collected at Month 6 under the COVID-19, such participants will have missing data.

Submission of mammographic data Using TRIAD
TRIAD is the American College of Radiology's (ACR) image exchange application. TRIAD provides sites participating in clinical trials a secure method to transmit DICOM RT and other objects. TRIAD anonymizes and validates the images as they are transferred.

TRIAD Access Requirements
Site staff who will submit images through TRIAD will be provided necessary log in information. To submit images, the site staff must be on the delegation of task log and be assigned the 'TRIAD site user' role.

TRIAD Installations
The TRIAD site user will need to have the TRIAD application installed on his/her workstation to be able to submit images.
TRIAD installation documentation can be found by following this link
https://triadinstall.acr.org/triadclient/
If you have any questions regarding this information, please send an e-mail to the TRIAD Support mailbox at TRIAD-Support@acr.org.

Procedures for Data Submission via TRIAD
Mammograms with their corresponding Diagnostic Imaging Report should be submitted for each participant in this study at the following time points:

1. The original mammogram taken at most 3 months prior to registration.
2. The mammogram taken as near as possible to one year after registration;
3. The mammogram taken as near as possible to two years after registration.

Submission of mammographic data
Digital mammography systems produce images in two forms, commonly referred to as the 'raw' image and the 'processed' images. For analysis of mammographic density **raw images** will be required, both raw images and 'processed' images will be collected.
All centers involved perform Digital Breast Tomography. As the current standard for 3D digital breast tomography is to include 3D images and either a conventional dose 2D mammogram or a synthesized 2D image, both the 3D and 2D images should be submitted. Only the 3D images will be analyzed, but 2D images will also be collected to create a data resource for future studies.
Digital files must be in DICOM format. Ensure that software used to de-identify images does not remove the date of the scan and specifics of the physics/image acquisition such as breast thickness and compression.
These files may be submitted electronically preferably with TRIAD. Alternatively, the images and reports may be submitted via secure portal to UT MD Anderson.

Research Blood collection:
Peripheral blood will be obtained by venipuncture at baseline, at 6 and at 12 months.

For the purpose of tracking samples: a pre-printed label will be attached to identify all sample vials.

Approx. 25 mL of blood will be collected and processed as per the Laboratory Manual. Refer to the Laboratory Manual for storage instructions.

Breast Core Biopsy (see also section 7.6):
The breast core biopsy (image guided) is optional. A 14-gauge needle will be used. Every effort will be made to collect 4 cores of tissue, with the minimum of 2 cores per participant at each time point.

Tissue will be considered adequate for research assays if the H&E stained slide from the block demonstrates at least one TDLU in every tissue core. One H&E slide will be prepared for adequacy and histological assessment. Usually each TDLU contains 200 to 2500 cells. If no TDLUs are seen, deeper sections will be obtained. Subsequent slides will be used for biomarker analysis.

- Samples should be collected and processed in the order below:

Breast Biopsy Sample Priority:	
Sample 1	FF
Sample 2-3	PE
Sample 4	RNALater®

- **Flash Frozen (FF) sample**: Biopsy sample (#1) should be placed directly into pre-labeled cryovial and snap frozen on isopentane, or liquid nitrogen, and then temporary storage on dry ice for transport to freezer at -80°C for storage.
    - If the institution does not have access to isopentane, liquid nitrogen may be used.

- Snap freeze your samples in liquid nitrogen and put them in a cool rack on dry ice for transport to freezer.

- **Paraffin Embedded (PE) samples**: Two core specimens (#2 and #3) will be placed in a pre-labeled container of 10% formalin for a maximum of 24-72 hours prior to embedding and processed using standard institutional procedures.
  - The minimum number of biopsies required is 2.
    - If this is the case, Sample 1 will be flash frozen and Sample 2 will be paraffin embedded.

- **RNALater® sample**: Biopsy sample (#4) should be placed directly into a pre-labeled cryovial containing 5-10 volumes of RNAlater® (we pre-aliquoted at 1.25ml per tube), tube with sample should be placed at 4ºC for at least 24 to 72hrs, then transfer to -80°C for long term storage before it's shipped to MD Anderson on dry ice.

10.3 Shipping Instructions

All samples will be shipped in compliance with the International Air Transport Association (IATA) Dangerous Goods Regulations.

All research specimens will be shipped to the laboratory of the Principal Investigator, Banu Arun, M.D. The laboratory is equipped with -80 degree freezers which serve as repositories for research samples. Ship all specimens to the address below:

Angelica Gutierrez Barrera
6565 MD Anderson Blvd
Z12.4032
Houston, TX 77030
Direct phone: 713-792-1986
Email: angutier@mdanderson.org

Use **Appendix I**: Specimen Inventory Form when shipping specimens. Fill Out One Form Per Patient, Per Time-Point.

Do not ship on Thursday, Friday or the day prior to a public holiday.

10.4 Tissue Banking

Biologic specimens collected during the conduct of this clinical trial that are not used during the course of the study will be considered deliverables under the contract and thus the property of the NCI. At study completion, NCI reserves the option to either retain or relinquish ownership of the unused biologic specimens. If NCI retains ownership of specimens, the Contractor shall collect, verify and transfer the requested biologic specimens from the site to a NCI-specified repository or laboratory at NCI's expense.

11. REPORTING ADVERSE EVENTS

DEFINITION: AE means any untoward medical occurrence associated with the use of a drug in humans, whether or not considered drug related. An AE can therefore be any unfavorable and unintended sign), symptom, or disease temporally associated with participation in a study, whether or not related to that participation. This includes all deaths that occur while a participant is on a study.

Please note that all abnormal clinical laboratory values that are determined to be of clinical significance based on a physician's assessment are to be reported as AEs. Those labs determined to be of no clinical significance or of unknown clinical significance (per the physician's assessment) should not be reported as AEs. Any lab value of unknown clinical significance should continue to be investigated/followed-up further for a final determination, if possible.

A list of AEs that have occurred or might occur can be found in §6.2 Reported Adverse Events and Potential Risks, as well as the Investigator Brochure or package insert.

11.1 Adverse Events

11.1.1 Reportable AEs

All AEs that occur after the informed consent is signed and baseline assessments are completed (including run-in) must be recorded on the AE CRF (paper and/or electronic) whether or not related to study agent.

11.1.2 AE Data Elements:

The following data elements are required for AE reporting.

- AE verbatim term
- NCI Common Terminology Criteria for Adverse Events version 4.0 (CTCAE v4.0) AE term (MedDRA lowest level term)
- CTCAE (MedDRA) System Organ Class (SOC)
- Event onset date and event ended date
- Treatment assignment code (TAC) at time of AE onset
- Severity grade
- Attribution to study agent (relatedness)
- Whether or not the event was reported as a SAE
- Whether or not the participant dropped out due to the event
- Outcome of the event

11.1.3 Severity of AEs

11.1.3.1 Identify the AE using the CTCAE version 4.0. The CTCAE provides descriptive terminology (MedDRA lowest level term) and a grading scale for each AE listed. A copy of the CTCAE can be found at http://ctep.cancer.gov/protocolDevelopment/electronic_applications/ctc.htm

AEs will be assessed according to the grade associated with the CTCAE term. AEs that do not have a corresponding CTCAE term will be assessed according to the general guidelines for grading used in the CTCAE v4.0. as stated below.

CTCAE v4.0 general severity guidelines:

Grade	Severity	Description
1	Mild	Mild; asymptomatic or mild symptoms; clinical or diagnostic observations only; intervention not indicated.
2	Moderate	Moderate; minimal, local or noninvasive intervention indicated; limiting age-appropriate instrumental activities of daily living (ADL)*.
3	Severe	Severe or medically significant but not immediately life-threatening; hospitalization or prolongation of hospitalization indicated; disabling; limiting self-care ADL**.
4	Life-threatening	Life-threatening consequences; urgent intervention indicated.
5	Fatal	Death related to AE.

ADL

*Instrumental ADL refers to preparing meals, shopping for groceries or clothes, using the telephone, managing money, *etc*.

**Self-care ADL refers to bathing, dressing and undressing, feeding self, using the toilet, taking medications, and not bedridden.

11.1.4 Assessment of relationship of AE to treatment

The possibility that the AE is related to study agent will be classified as one of the following: not related, unlikely, possible, probable, definite.

11.1.5 Follow-up of AEs

All AEs, including lab abnormalities that in the opinion of the investigator are clinically significant, will be followed according to good medical practices and documented as such.

11.2 Serious Adverse Events

11.2.1 DEFINITION: Regulations at 21 CFR §312.32 (revised April 1, 2014) defines an SAE as any untoward medical occurrence that at any dose has one or more of the following outcomes:

- Death
- A life-threatening AE
- Inpatient hospitalization or prolongation of existing hospitalization
- A persistent or significant incapacity or substantial disruption of the ability to perform normal life functions
- A congenital anomaly or birth defect

- Important medical events that may not be immediately life-threatening or result in death or hospitalization should also be considered serious when, based upon appropriate medical judgment, they may jeopardize the participant and may require intervention to prevent one of the other outcomes.

11.2.2 Reporting SAEs

The organization that experiences the serious adverse event (SAE) should report the SAE to the following four (4) entities: 1) NCI DCP; 2) DCP's regulatory contractor CCSA; 3) MDACC, the CLO; and 4) BHR Pharma, LLC. Detailed reporting instructions are provided below. In addition, all participating organizations will follow Central IRB (CIRB) and their local IRB requirements for SAE reporting.

11.2.2.1 The Lead Organization and all Participating Organizations will report SAEs on the DCP SAE Report Form found at https://prevention.cancer.gov/clinical-trials/clinical-trials-management/protocol-information-office/pio-instructions-and-tools/2012-consortia.

11.2.2.2 Contact the DCP Medical Monitor by phone within 24 hours of knowledge of the event.

Marjorie Perloff, MD
Division of Cancer Prevention
National Cancer Institute
9609 Medical, Rm 5E544
Rockville, MD 200850
Office Tel (240)276-7097
Cell (240)731-1772
FAX (240)276-7847
Email: perloffm@mail.nih.gov

Include the following information when calling the Medical Monitor:

- Date and time of the SAE
- Date and time of the SAE report
- Name of reporter
- Call back phone number
- Affiliation/Institution conducting the study
- DCP protocol number
- Title of protocol
- Description of the SAE, including attribution to drug

11.2.2.3 Contact the Consortium Lead Organization (CLO) PI, Dr. Powel Brown, or designee by phone, fax or email listed on the protocol face page within 24 hours of knowledge of the event. The same information reported to the DCP Medical Monitor should be provided to the CLO PI or designee via email, phone or fax within 24 hours of knowledge of the event.

11.2.2.4 The Lead Organization and all Participating Organizations will email written SAE reports to DCP's Regulatory Contractor CCS Associates, Inc. (CCSA; phone: 650-691-4400) at safety@ccsainc.com within 48 hours of learning of the event using the fillable SAE Report Form.

11.2.2.5 The CLO PI, Dr. Brown, or designee must be copied on the email sent to DCP's Regulatory Contractor CCS Associates, Inc.

11.2.2.6 BHR Pharma, LLC and Besins Pharmacovigilance (pharmacovigilance@besins-healthcare.com) must be copied on the email sent to DCP's Regulatory Contractor CCS Associates, Inc.

11.2.2.7 The DCP Medical Monitor and CCSA regulatory and safety staff will make an initial assessment of the SAE and communicate with BHR Pharma, LLC who will determine which SAEs require FDA submission as IND safety reports.

11.2.2.8 BHR Pharma, LLC will provide a standard report for the Lead Organization and all Participating Organizations to comply with applicable regulatory requirements related to reporting SAEs to the CIRB/IRB/IEC.

11.2.3 Follow-up of SAE

Site staff should send follow-up reports as requested when additional information is available. Additional information should be entered on the DCP SAE Report Form in the appropriate format. Follow-up information should be sent to DCP as soon as available, with a copy to BHR Pharma, LLC and Besins Pharmacovigilance (pharmacovigilance@besins-healthcare.com). SAEs related to the study agent will be followed until resolved.

12. STUDY MONITORING

12.1 Data Management

This study will report clinical data using the Data Management Initiative (DMI) web-based application managed by the Consortium Biostatistics and Data Management Core. Data Management Initiative (DMI) infrastructure has been developed in the Division of Quantitative Sciences (DQS), MD Anderson Cancer Center. This infrastructure supplies integrated database and software services for web-based data collection, randomized treatment assignment, reporting, query, data download, and data quality management. The DMI will be the database of record for the protocol and subject to NCI and FDA audit. All DMI users will be trained to use the DMI system and will comply with the instructions in the protocol-specific "DMI User Manual" as well as applicable regulatory requirements such as 21 CFR; Part 11. Data management procedures for this protocol will adhere to the Data Management Plan (DMP) on file at the DCP for contract HHSN261201200034I.

12.2 Case Report Forms

Participant data will be collected using protocol-specific CRF developed from the standard set of DCP Chemoprevention CRF Templates and utilizing NCI-approved Common Data Elements (CDEs). The approved CRFs will be used to create the electronic CRF (e-CRF) screens in the DMI application. Site staff will enter data into the e-CRFs in DMI. CRF amendments, if needed, will be submitted to the DCP Protocol Information Office for review and approval prior to deployment in DMI. Approved changes will be programmed into the DMI database by the Consortium Biostatistics and Data Management Core.

12.3 Source Documents

Source documentation will include only those documents containing original forms of data, including clinic charts, shadow files, hospital charts, and physician notes. Data recorded directly on the CRFs designated

as source documents (i.e., no prior written or electronic record of data) will be considered source data. All other data recorded on the CRFs will not be considered source documentation.

12.4 Data and Safety Monitoring Plan

The Data and Safety Monitoring Plan for the MD Anderson Consortium is on file at the DCP. This study will be monitored yearly by the MDACC Data and Safety Monitoring Board (DSMB), the data and safety monitoring board of record for this study. The DSMB reports to the President, or his designee, as the on-campus representative of The University of Texas Board of Regents. It oversees the data and patient safety issues for randomized clinical trials that originate at MD Anderson; that are coordinated or analyzed by MD Anderson and are not being monitored by any other DSMB; or have been designated as requiring DSMB monitoring at the request of the IRB, the CRC, or institution. The primary objectives of the DSMB are to ensure that patients' rights pertaining to participation in a research study are protected, and that patients' interests are prioritized over the interests of the scientific investigation. Responsibilities include:

(a) Review interim analyses of outcome data (prepared by the study statistician or other responsible person at the time points defined in the study) approved by the IRB and additional time points as determined by the DSMB, and to recommend, if necessary, whether the study needs to be changed or terminated based on these analyses;
(b) Determine whether, and to whom, outcome results should be released prior to the reporting of study results;
(c) Review interim toxicity data and efficacy of treatment;
(d) Review major research modifications proposed by the investigator or appropriate study committee prior to implementation (*e.g.*, termination, dropping an arm based on toxicity results from the study or results of other studies, increasing target sample size).

Refer to the Data and Safety Monitoring Plan for the MD Anderson Consortium on file at the DCP for further details.

12.5 Sponsor or FDA Monitoring

The NCI, DCP (or their designee), pharmaceutical collaborator (or their designee), or FDA may monitor/audit various aspects of the study. These monitors will be given access to facilities, databases, supplies and records to review and verify data pertinent to the study.

12.6 Record Retention

Clinical records for all participants, including CRFs, all source documentation (containing evidence to study eligibility, history and physical findings, laboratory data, results of consultations, *etc*.), as well as IRB records and other regulatory documentation will be retained by the Investigator in a secure storage facility in compliance with Health Insurance Portability and Accountability Act (HIPAA), Office of Human Research Protections (OHRP), Food and Drug Administration (FDA) regulations and guidances, and NCI/DCP requirements, unless the standard at the site is more stringent. The records for all studies performed under an IND will be maintained, at a minimum, for two years after the approval of a New Drug Application (NDA). For NCI/DCP, records will be retained for at least three years after the completion of the research. NCI will be notified prior to the planned destruction of any materials. The records should be accessible for inspection and copying by authorized persons of the Food and Drug Administration. If the

study is done outside of the United States, applicable regulatory requirements for the specific country participating in the study also apply.

12.7 Cooperative Research and Development Agreement (CRADA)/Clinical Trials Agreement (CTA)

The agent(s) supplied by DCP, NCI, used in this protocol, is/are provided to the NCI under a Collaborative Agreement (CRADA, CTA) between the Pharmaceutical Company(ies) (hereinafter referred to as Collaborator(s)) and the NCI Division of Cancer Prevention. Therefore, the following obligations/guidelines, in addition to the provisions in the "Intellectual Property Option to Collaborator" contained within the terms of award, apply to the use of Agent(s) in this study:

12.7.1 Agent(s) may not be used for any purpose outside the scope of this protocol, nor can Agent(s) be transferred or licensed to any party not participating in the clinical study. Collaborator(s) data for Agent(s) are confidential and proprietary to Collaborator(s) and shall be maintained as such by the investigators. The protocol documents for studies utilizing investigational agents contain confidential information and should not be shared or distributed without the permission of the NCI. If a participant taking part in the study or participant's family member requests a copy of this protocol, the individual should sign a confidentiality agreement. A suitable model agreement can be downloaded from the DCP website.

12.7.2 For a clinical protocol where there is an Investigational Agent used in combination with (an) other investigational Agent(s), each the subject of different collaborative agreements, the access to and use of data by each Collaborator shall be as follows (data pertaining to such combination use shall hereinafter be referred to as "Multi-party Data").

12.7.3 NCI must provide all Collaborators with prior written notice regarding the existence and nature of any agreements governing their collaboration with NIH, the design of the proposed combination protocol, and the existence of any obligations that would tend to restrict NCI's participation in the proposed combination protocol.

12.7.4 Each Collaborator shall agree to permit use of the Multi-Party Data from the clinical trial by any other Collaborator solely to the extent necessary to allow said other Collaborator to develop, obtain regulatory approval, or commercialize its own investigational agent.

12.7.5 Any Collaborator having the right to use the Multi-Party Data from these trials must agree in writing prior to the commencement of the trials that it will use the Multi-Party Data solely for development, regulatory approval, and commercialization of its own investigational agent.

12.7.6 Clinical Trial Data and Results and Raw Data developed under a collaborative agreement will be made available exclusively to Collaborator(s), the NCI, and the FDA, as appropriate. All data made available will comply with HIPAA regulations.

12.7.7 When a Collaborator wishes to initiate a data request, the request should first be sent to the NCI, who will then notify the appropriate investigators of Collaborator's wish to contact them.

12.7.8 Any manuscripts reporting the results of this clinical trial must be provided to DCP for immediate delivery to Collaborator(s) for advisory review and comment prior to submission for publication. Collaborator(s) will have 30 days (or as specified in the CTA) from the date of receipt for review. Collaborator shall have the right to request that publication be delayed for up to an additional 30 days in

order to ensure that Collaborator's confidential and proprietary data, in addition to Collaborator(s)'s intellectual property rights, are protected. Copies of abstracts must be provided to DCP for forwarding to Collaborator(s) for courtesy review as soon as possible and preferably at least three days prior to submission, but in any case, prior to presentation at the meeting or publication in the proceedings. Press releases and other media presentations must also be forwarded to DCP prior to release. Copies of any manuscript, abstract, and/or press release/ media presentation should be sent to the Protocol Information Office at NCI_DCP_PIO@mail.nih.gov.

The Protocol Information Office will forward manuscripts to the DCP Project Officer for distribution to the Collaborator(s). No publication, manuscript or other form of public disclosure shall contain any of Collaborator's confidential/proprietary information.

13. STATISTICAL CONSIDERATIONS

Note: Mammogram, blood, and tissue samples for participants seen under COVID-19 contingency plan will have a range of Month 13-18. Laboratory tests and research bloods not collected at Month 6 under the COVID-19 contingency plan, will result in missing data.

13.1 Study Design/Description

A total of 198 potentially eligible participants will be consented and screened for eligibility and, with a conservative estimate, 158 (80%) participants are expected to be eligible. These 158 women will be randomized 1:1 into one of two groups: placebo or 4-OHT gel 2 mg per breast. Treatment will last for 52 weeks. Considering an attrition rate of approximately 19%, we expect to have 128 evaluable women who will have both baseline and 52-week measurements of percent MD of the breast, 64 women in each group.

With 64 women in each group, we will have an 80% power to detect a decrease of percent MD of 6% in treatment group against 2% in placebo group, with a common standard deviation of 8%, using a two-sided t-test with a significance level of 0.05. The proposed difference between the active treatment and placebo groups and the standard deviation are based on conservative estimates from the published work by Cuzick et al [8]. The sample size calculation is performed using nQuery + nTerim 3.0.

13.2 Randomization/Stratification

Participants will be assigned a randomization number once the following has been accomplished: eligibility has been verified at the site level, eligibility has been confirmed by the site PI, and eligibility CRF has been entered into the DMI web application. The randomization number will be generated by the database and assigned to the participant.

A total of 158 women will be enrolled and randomized 1:1 into one of two groups. Treatment will last for 52 weeks. Considering an attrition rate of approximately 19%, we expect to have 128 evaluable women who will have both baseline and 52-week measurements of percent MD of the breast, 64 women in each group.

We will stratify the randomization by study site (UT MD Anderson Cancer Center, Dana Farber Cancer Institute, Northwestern University, Moffitt Cancer Center, University of Arizona Cancer Center, University of Minnesota, and University of Wisconsin) and baseline breast density category (heterogeneously dense (C), extremely dense (D)) using random permutated block design with the block size of 4.

13.3 Accrual and Feasibility

We plan to consent and screen 198 potential participants over 25 to 30 months at seven clinical centers. Our goal is to randomize 158 eligible participants. The randomized participants will receive the intervention for 52 weeks. They will be followed closely to monitor the toxicity and drug compliance.

13.4 Primary Objective, Endpoint(s), Analysis Plan

The primary objective of this study is to evaluate the change in percent MD (using Cumulus software) from baseline to the week 52 in women applying 4 mg OHT gel versus placebo based on the intent-to-treat principle. The intent-to-treat population is defined as all participants who are randomized and have MD measurements from both baseline and 52 weeks. The primary analysis will be performed comparing the active group versus placebo group per randomization, regardless of the compliance of each participant.

The percent MD is the MD (percent dense area) that is visually estimated using both the mediolateral oblique (MLO) view and the craniocaudal (CC) view as the proportion of the total breast area that was composed of dense tissue (to the nearest 5%). For the study purpose, the densest breast will be the target lesion for the primary endpoint. Breast biopsy for secondary and exploratory endpoints will be performed in the opposite (non-target) breast.

At the end of the study, all participants who have MD measurements from both baseline and 52 weeks, regardless of the compliance, will be included in the primary analysis. Change in percent MD of the breast from baseline to 52 weeks will be summarized using descriptive statistics, such as mean, standard deviation, median and range for each arm. With visual assessments of MD from both the MLO and CC views for evaluating the primary endpoint, Hotelling's T^2 test will be used to conduct multivariate hypothesis testing which includes both views. Hotelling's T^2 test represents the multivariate generalization to the independent two-sample t-test in the univariate setting. The correlation and variance-covariance matrix will be assessed to estimate the level of dependency between the MLO and the CC views. The mean change in percent MD at 52 weeks incorporating both the MLO and CC views will be compared between the two groups utilizing Hotelling's T^2 test. Q-Q plots, used to visually assess the assumption of normality, will be constructed for the marginal distributions associated with observations emanating from both the MLO and the CC view. Assuming the mean difference in density between the observations between the 4-OHT and the placebo groups is approximately normally distributed, then the primary analysis will be conducted as previously described. Additionally, a 95% confidence ellipse (simultaneous confidence intervals) for the mean vector containing information from both views will be provided. If the change in density is not normally distributed, then we will try to rescale to achieve approximate normality by using the Box-Cox transformation. In our prior experience evaluating change in density in response to oral tamoxifen,[12] change in density was scaled and raised to the 1.75^{th} power to approximate normality. We will have to determine what power to use in the Box-Cox transformation based on the data that we have on hand. Transformations may be performed when necessary to make the measure of MD close to normal distribution before evaluating the change of MD over time between two groups. If the Hotelling's T^2 test is significant, we will further examine whether the significant difference between the 4-OHT gel group and the placebo group is in the MLO view or the CC view or both. Note that the sample size calculation is based on the two-sample t-test. It provides a conservative estimate of the statistical power because the Hotelling's T^2 test is more efficient than the two-sample t-test.

We will evaluate if the change of breast density is different in patients with heterogeneously dense (C) vs extremely dense (D) breast at baseline, by incorporating this covariate and its interaction with treatment over time in the repeated measures analysis.

Due to the COVID-19 closures of clinical research activities, a small number of participants (less than 20) will have their month 12 visits by telephone. These participants will continue the randomized treatments and have mammograms in the month 13-18 visits. The measurements of breast density from the month 13-18 (instead of month 12) visits will be used as the primary endpoint. We will first provide summary statistics for the breast density from the month 13-18 visits, from the month 12 visit, and then graphically explore any impact of potentially longer exposures of the treatment on the primary endpoint of mammographic breast density. If appropriate, we will incorporate the timing of the measurement as a covariate in the analysis to estimate the effect of prolonged exposure of the treatment on mammographic breast density. We will also perform a sensitivity analysis by including month 13-18 measures as primary endpoint, and by excluding month 13-18 measures to evaluate the impact of the different timing of measurements on the primary endpoint analysis. For the sample size calculation, we have factored in a 19% inevaluable rate. Based on the current estimate, even including the follow-up delayed caused by COVID-19, the inevaluable rate is still within 19%. Note that patients who were randomized but never started treatments will be replaced if the inevaluable rate is above 19%.

13.5 Secondary and Exploratory Objectives, Endpoints, Analysis Plans

Note: data from Month 13-18 visit instead of Month 12 will be used if participants were seen under the COVID-19 Contingency Plan.

The secondary objectives are:

1. To estimate and compare the percent change in mammographic breast density (using Volpara) from Baseline to Month 12 in women applying 2mg 4-OHT gel per breast versus placebo.
2. To estimate and compare the percent change in mammographic breast density (using LIBRA) from Baseline to Month 12 in women applying 2mg 4-OHT gel per breast versus placebo.
3. To compare the percentage of women who underwent a change in BIRADS category, comparing pre-and post- treatment measurements, for recipients of active agent versus placebo.
4. To conduct subgroup analyses including BIRAD category, menopausal status, cup size, and total breast volume.
5. To estimate percentage of women with ≥ 10% absolute decrease in quantitative mammographic density percentage between baseline and 12 months, comparing between treated group 2mg per breast 4-OHT gel to placebo based on Cumulus method.
6. To describe symptoms assessed by BCPT Eight Symptom Scale (BESS) questionnaire and laboratory toxicity assessment (Factor VIII (F VIII), Von Willebrand (vWB) factor, Sex Hormone-Binding Globulin (SHBG), lipid profile).
7. To evaluate plasma measurements of 4-OHT and related metabolite levels and factors related to tamoxifen exposure.
8. To evaluate tissue measurements of 4-OHT and related metabolite levels and factors related to tamoxifen exposure.

The exploratory objectives are:

1. To compare the Cumulus vs. Volpara vs. LIBRA breast density measurement methods to estimate percent change in mammographic breast density from baseline to Month 12 in women applying 2mg of 4-OHT gel per breast vs. placebo.
2. To perform correlation of drug concentrations in the plasma and the tissue.
3. To evaluate plasma measurements of Insulin-like growth factor (IGF) pathway members, C-reactive protein (CRP), and estradiol.
4. To compare the 2D natural vs. 2D synthetic breast density measurement using the Cumulus method to estimate percent change in mammographic breast density from baseline to Month 12 in women applying 2mg of 4-OHT gel per breast vs. placebo.
5. To evaluate tissue biomarkers (among women undergoing optional pre- and post-treatment biopsies):
   Terminal duct lobular unit (TDLU) Involution
   Collagen structural changes
   $SET_{ER/PR}$ index: estrogen related transcription
   Ki-67, COX-2, p16, CD68.
6. To examine whether any reductions in mammographic density seen after 1 year of 4-OHT vs. placebo gel application persist at 24 months (or longer under the COVID-19 Contingency Plan), one year after gel application has stopped.

All available data will be summarized and analyzed for the secondary analysis. At the end of the study, participant demographic characteristics; the following measurements at baseline and 52 weeks: 1) MD of the breast, 2) tissue biomarkers (among women undergoing optional pre- and post-treatment biopsies: TDLU Involution, Collagen structural changes, SET index, Ki-67, COX-2, p16, CD68, etc.), 3) plasma measurements of parent drug and related metabolite levels and factors related to 4-OHT exposures, such as IGF pathway members, CRP, estradiol, bisphenol and 4-OHT; and the percentage of women with ≥ 10% absolute decrease in percent MD between baseline and 52 weeks will be summarized using descriptive statistics, such as mean, standard deviation, median and range for continuous variables and frequency/percentage for discrete variables. The difference of continuous variables between baseline and 52 weeks may be compared between two groups using t-test or Wilcoxon rank sum test, whichever is appropriate. Multivariate analogues to the before mentioned univariate tests, such as Hotelling's T^2 test and multivariate multiple regression will be employed when appropriate. Transformation may be performed when necessary to make continuous variables close to normal distribution before evaluating the change of these continuous variables over time between two groups using repeated measures analysis where the intra-participant correlation is taken into consideration, adjusting for other important covariates. In addition, longitudinal data analysis such as the linear mixed effect model will be applied to evaluate the mammogram density change over time. The treatment effect will be evaluated adjusting for other important covariates. The effect of compliance will be evaluated in the per protocol analysis.

The percentage of women with at least 10% decrease in percent change of MD will be estimated and compared between two groups using chi-squared or Fisher's exact test whichever appropriate. We will also explore the correlation between percent change in MD and other markers using Spearman correlation at baseline or by employing repeated measures analysis when both baseline and 52-week data are included and intra-participant correlation is accounted for. We will also monitor and summarize any adverse events observed in women on this study.

The change in percent MD of the breast from the end of the treatment at 1 year to 1 year after the treatment ends will also be summarized using descriptive statistics, such as mean, standard deviation, median and range for each arm. The difference of this change in percent MD will be compared between two treatment groups using t-test or Wilcoxon rank sum test, when appropriate. Multivariate methods such as simultaneous 95% confidence intervals will be used for estimation purposes when appropriate.

We will define three groups of participants by the menopausal status change during the study, pre-menopausal/pre-menopausal, pre-menopausal/post-menopausal and post-menopausal/post-menopausal, and compare the change of MD and other secondary endpoints among these three groups of participants by Kruskal-Wallis test. We may incorporate this grouping covariate in the repeated measures analysis to evaluate any differential effect of menopausal status change on the change of endpoints by treatment over time. Sensitivity analysis on the treatment effect with and without including women who undergo menopausal transition will be performed. Sensitivity analysis on the treatment effect with and without including women on hormone replacement therapy will be performed. The treatment effect will be analyzed by incorporating the longitudinally measured compliance data as a covariate.

If prior imaging is available (mammogram or other modalities), the data will be collected and summarized. Other statistical methods, such as the MANOVA and multivariate multiple regression, may be used when appropriate.

13.6 Reporting and Exclusions

Every reasonable attempt will be made to recover any missing data. If any data for the primary efficacy measure remains missing, then the change of the primary endpoint will not be calculated, and the participant will not be evaluable for the primary endpoint. We will also consider multiple imputations using predictive values based on regression models under the assumption of missing at random to supplement the primary analysis. In addition, any available data will be included in the repeated measure analysis for both primary and secondary endpoints, which allows for all participants with complete or partial data.

13.7 Evaluation of Toxicity

All participants will be evaluable for toxicity from the time of their first dose of 4-OHT gel 4 mg or placebo. The grade, attribution, onset and resolve date of all toxicities will be recorded and summarized for each arm and reported to the DCP monthly and to the DSMB annually as well as at the end of the study.

13.8 Evaluation of Response

All participants included in the study must be assessed for response to intervention, even if there are major protocol deviations or if they are ineligible.

All of the participants who met the eligibility criteria (with the possible exception of those who did not receive study agent) will be included in the main analysis. All conclusions regarding efficacy will be based on all eligible participants.

Subanalyses may be performed on the subsets of participants, excluding those for whom major protocol deviations have been identified (e.g., early death due to other reasons, early discontinuation of intervention, major protocol violations, etc.). However, subanalyses may not serve as the basis for drawing conclusions

concerning efficacy, and the reasons for excluding participants from the analysis should be clearly reported. For all measurements of response, the 95% confidence intervals should also be provided.

13.9 Interim Analysis

There is no interim analysis planned.

13.10 Ancillary Studies

No ancillary studies are planned.

14. ETHICAL AND REGULATORY CONSIDERATIONS

14.1 Form FDA 1572

Prior to initiating this study, the Protocol Lead Investigator at the Lead or Participating Organization(s) will provide a signed Form FDA 1572 stating that the study will be conducted in compliance with regulations for clinical investigations and listing the investigators, at each site that will participate in the protocol. All personnel directly involved in the performance of procedures required by the protocol and the collection of data should be listed on Form FDA 1572.

14.2 Other Required Documents

14.2.1 Current (within two years) CV or biosketch for all study personnel listed on the Form FDA 1572 and Delegation of Tasks form for the Lead Organization and all Participating Organizations.

14.2.2 Current medical licenses (where applicable) for all study personnel listed on Form FDA 1572 and Delegation of Tasks form for the Lead Organization and all Participating Organizations.

14.2.3 Lab certification (*e.g.*, CLIA, CAP) and lab normal ranges for all labs listed on Form FDA 1572 for the Lead Organization and all Participating Organizations.

14.2.4 Documentation of training in “Protection of Human Research Subjects” for all study personnel listed on the FDA Form 1572 and Delegation of Tasks form for the Lead Organization and all Participating Organizations.

14.2.5 Documentation of training in “Good Clinical Practice” for all study personnel listed on the FDA Form 1572 and Delegation of Tasks form for the Lead Organization and all Participating Organizations.

14.2.6 Documentation of Federalwide Assurance (FWA) number for the Lead Organization and all Participating Organizations.

14.2.7 Signed Investigator’s Brochure/Package Insert acknowledgement form.

14.2.8 Delegation of Tasks form for the Lead Organization and all Participating Organizations signed by the Principal Investigator and all study personnel listed on the form for each site.

14.2.9 Signed and dated NCI, DCP Financial Disclosure Form for all study personnel listed on Form FDA 1572 for the Lead Organization and all Participating Organizations.

14.3 Institutional Review Board Approval

Prior to initiating the study and receiving agent, the Investigators at the Lead Organization and the Participating Organization(s) must obtain written approval to conduct the study from the CIRB. Should changes to the study become necessary, protocol amendments will be submitted to the DCP PIO according to DCP Amendment Guidelines. The DCP-approved amended protocol must be approved by the CIRB prior to implementation

14.4 Informed Consent

All potential study participants will be given a copy of the CIRB and IRB-approved Informed Consent to review. The investigator will explain all aspects of the study in lay language and answer all questions regarding the study. If the participant decides to participate in the study, she will be asked to sign and date the Informed Consent document. The study agent(s) will not be released to a participant who has not signed the Informed Consent document. Individuals who refuse to participate or who withdraw from the study will be treated without prejudice.

Participants must be provided the option to allow the use of blood samples, other body fluids, and tissues obtained during testing, operative procedures, or other standard medical practices for further research purposes. If applicable, statement of this option may be included within the informed consent document or may be provided as an addendum to the consent. A Model Consent Form for Use of Tissue for Research is available through a link in the DCP website.

Prior to study initiation, the informed consent document must be reviewed and approved by NCI, DCP, the Consortium Lead Organization, and the IRB at each Organization at which the protocol will be implemented. Any subsequent changes to the informed consent must be approved by NCI, DCP, the Consortium Lead Organization's IRB, and then submitted to each organization's IRB for approval prior to initiation.

14.5 Submission of Regulatory Documents

All regulatory documents are collected by the Consortia Lead Organization and reviewed for completeness and accuracy. Once the Consortia Lead Organization has received complete and accurate documents from a participating organization, the Consortium Lead Organization will forward the regulatory documents to DCP's Regulatory Contractor:

Paper Document/CD-ROM Submissions:
Regulatory Affairs Department
CCS Associates, Inc.
2001 Gateway Place, Suite 350 West
San Jose, CA 95110
Phone: 650-691-4400
Fax: 650-691-4410

E-mail Submissions:
regulatory@ccsainc.com

Regulatory documents that do not require an original signature may be sent electronically to the Consortium Lead Organization for review, which will then be electronically forwarded to DCP's Regulatory Contractor.

14.6 Other

This trial will be conducted in compliance with the protocol, Good Clinical Practice (GCP), and the applicable regulatory requirements.

15. FINANCING, EXPENSES, AND/OR INSURANCE

Participants will not be responsible for non-standard of care costs of this study. Study agent will be provided at no cost to the participant. There will be compensation for additional expenses associated with study visits, such as additional travel expenses, time missed from work or other expenses. The amount of compensation will be $100 associated with the Baseline study visit, Month 6 and Month 12, and $150 at the time of each biopsy for those who agree to the procedure. Under the COVID-19 Contingency Plan, the amount of compensation will be $100 associated with the Baseline study visit, Month 6 telephone call and Month 13-18 visit, and $150 at the time of each biopsy for those who agree to the procedure. If, as a result of participation in this study, an individual experiences injury from known or unknown risks of the research procedures as described in the informed consent, immediate medical care and treatment, including hospitalization, if necessary, will be available. No monetary compensation is available for the costs of medical treatment for an injury, thus, the participant will be responsible for the costs of such medical treatment, either directly or through their medical insurance and/or other forms of medical coverage.

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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.
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

GEL APPLICATION INSTRUCTIONS

1. Flammable: do not apply near fire, flame or heat, or while smoking.

2. If you had a biopsy, start applying the gel at least 24 hours after the biopsy.

3. Apply the gel to your own breasts after bathing, preferably in the morning and at approximately the same time each day. Night-time application is acceptable, but caution should be taken to limit transfer to other individuals.

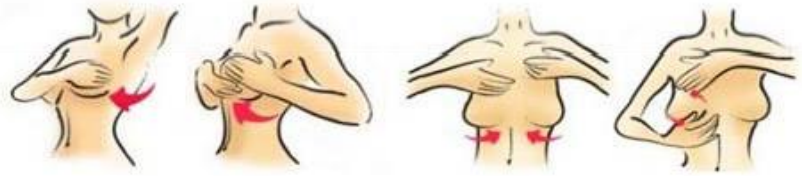
4. To apply, remove the cap from the bottle. When you use a bottle for the first time, you must prime it by pressing the pump fully several times until gel is dispensed (point the spout toward a sink or wastebasket and do not use the first dose, which may be incorrect).

5. Once the bottle is primed, hold it in one hand and place the palm of your other hand under the pump to catch the gel. Be sure to press down completely on the pump and release it completely to dispense one dose of gel.

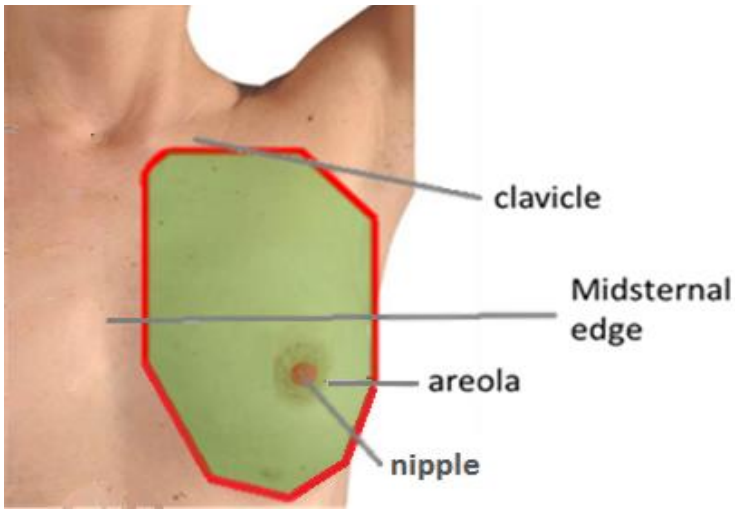
6. Apply **one pump worth of gel to each breast** at one time. Do not apply more or less than this amount to each breast. Be sure to release the pump completely between actuations.

7. If you accidentally pump more than is needed for one breast, please discard this dose and try again to get the correct dose. If you do discard pumped doses, please record this on your study diary.

8. Start around the nipple. Spread outwards until you reach edge of breast bone, below collar bone (clavicle), to the fold under the breast, and to the outer edge of the breast (see picture below).



9. Do not apply to the nipple. Starting at the areola spread the gel evenly over the entire surface of your breast (see green area in the picture below). Do not rub the gel into the skin.



10. The gel should not be applied to any other part of the body.

11. Wash your hands with soap and water immediately after applying the gel.

12. Allow the gel on your breasts to air dry for 2 minutes and then immediately cover with clothing (the gel is colorless and will not stain your clothing). Once dry, the gel is no longer flammable. Do not expose your bare breasts to sunlight at any time.

13. Do not apply any other cream, lotion or moisturizer to your breasts for at least 4 hours following application of the study gel. If cream, lotion, or moisturizer is used between applications, wash the breast skin before applying the next dose.

14. Do not wash your breasts or immerse in water (bath, swim) for at least 4 hours following application of the gel. If this is not possible, delay application of the gel that day until after immersion, and be sure to follow all the above instructions. If you regularly swim in the morning, it is better to apply the gel afterwards, after your shower.

15. After use, replace the cap on the bottle.

16. Avoid exposure of the treated breast skin to natural or artificial sunlight. This includes sunbathing or the use of tanning beds with the breasts exposed. Also, if you have dermatologic conditions causing the breakdown of skin in the area of gel application, you should not use 4-OHT gel.

RECOMMENDATIONS:

1. If you forget to apply a dose, do not double the dose to “catch up”. If your next dose is scheduled within the next 12 hours, it is best just to wait; if it is more than 12 hours until your next dose, apply the dose you missed and resume your normal dosing after that.

2. For the duration of the study, avoid contact between the application area and the skin of other individuals (i.e. your child, your sexual partner, or other persons). If necessary, skin contact is allowable after the breasts have been washed. As noted above, you must wait at least 4 hours following application before washing the application area, otherwise, delay application until after washing and contact.

3. Do not ingest or swallow the gel. For external use only.

4. Please record your use of 4-OHT in your 4-OHT diary.

5. If the pump doesn't come back up correctly or if there's no gel delivered when you press down on the pump, do not use this bottle and notify your doctor immediately.

6. After the end of study treatment, be sure to take back to your doctor **all** the gel bottles you have been given (even if empty or not used). **This is very important for the success of the study**.

STORAGE INSTRUCTIONS:

1. Keep your gel bottles in a cool room temperature, between 59°F and 77°F.

2. Avoid extreme heat or cold during transportation from the clinic to home, and when traveling.

3. Keep the gel bottles out of the reach of children.

TRAVEL:

Your gel is a prescription medication. You may bring the gel in your carry-on bag. Please refer to the Transportation Security Administration web site for further details: https://www.tsa.gov/travel/special-procedures

APPENDIX C

COMPLIANCE DRUG LABELS

(Use this Form at Randomization and Month 6)

INSTITUTION CODE	PARTICIPANT ID	VISIT TYPE	VISIT DATE *(MM/DD/YYYY)*
_____	_____	_____	__ __/__ __/__ __ __ __

Affix Labels Here:

Were Labels Affixed? ☐ Yes ☐ No

Investigator's Signature: _____ Date of Investigator's Signature: _ _ / _ _ / _ _ _ _
(MM/DD/YYYY)

Investigator's Name (Please Print): _____

APPENDIX D

4-OHT Diary

4-OHT Diary [IRB #:]

PID: __ __ __ __ __ __ __ __ __ __ Site: __ __ __ __ __

Amount of Agent Provided: _____ canisters

Canister ___of 5

Total Daily Dose: 1 pump actuation to each breast once a day [4 mg total (2 mg per breast)]

Number of Canisters Returned: _____

Weight of Canisters Returned: _____(grams)

Agent Returned: □ Yes □ No

Visit#: _____

INSTRUCTIONS:

1. Please complete daily. You should apply 1 pump actuation to each breast once a day.
2. If you forget to apply a dose, do not double the dose to "catch up". If your next dose is scheduled within the next 12 hours, it is best just to wait; if it is more than 12 hours until your next dose, apply the dose you missed and resume your normal dosing after that.
3. Each canister provides 40 days of gel application for both breasts. After 40 days of gel application, switch to a new canister and calendar. Do not discard any canisters. Bring all used and unused canisters and calendars to your next appointment.
4. Store the study gel at home at room temperature and avoid extreme heat or cold during transportation from the clinic to home.

4-OHT Diary Continued

Please Bring this Sheet to Your Next Visit

Day	Date	Initials	# of pump actuations to right breast	# of pump actuations to left breast	Did you experience any symptoms, if "Yes" list below
1			_____ Dose or □Missed	_____ Dose or □Missed	
2			_____ Dose or □Missed	_____ Dose or □Missed	
3			_____ Dose or □Missed	_____ Dose or □Missed	
4			_____ Dose or □Missed	_____ Dose or □Missed	
5			_____ Dose or □Missed	_____ Dose or □Missed	
6			_____ Dose or □Missed	_____ Dose or □Missed	
7			_____ Dose or □Missed	_____ Dose or □Missed	
8			_____ Dose or □Missed	_____ Dose or □Missed	
9			_____ Dose or □Missed	_____ Dose or □Missed	
10			_____ Dose or □Missed	_____ Dose or □Missed	
11			_____ Dose or □Missed	_____ Dose or □Missed	
12			_____ Dose or □Missed	_____ Dose or □Missed	
13			_____ Dose or □Missed	_____ Dose or □Missed	
14			_____ Dose or □Missed	_____ Dose or □Missed	
15			_____ Dose or □Missed	_____ Dose or □Missed	
16			_____ Dose or □Missed	_____ Dose or □Missed	
17			_____ Dose or □Missed	_____ Dose or □Missed	
18			_____ Dose or □Missed	_____ Dose or □Missed	
19			_____ Dose or □Missed	_____ Dose or □Missed	
20			_____ Dose or □Missed	_____ Dose or □Missed	

21			_____ **Dose** **or** □**Missed**	_____ **Dose** **or** □**Missed**	
22			_____ **Dose** **or** □**Missed**	_____ **Dose** **or** □**Missed**	
23			_____ **Dose** **or** □**Missed**	_____ **Dose** **or** □**Missed**	
24			_____ **Dose** **or** □**Missed**	_____ **Dose** **or** □**Missed**	
25			_____ **Dose** **or** □**Missed**	_____ **Dose** **or** □**Missed**	
26			_____ **Dose** **or** □**Missed**	_____ **Dose** **or** □**Missed**	
27			_____ **Dose** **or** □**Missed**	_____ **Dose** **or** □**Missed**	
28			_____ **Dose** **or** □**Missed**	_____ **Dose** **or** □**Missed**	
29			_____ **Dose** **or** □**Missed**	_____ **Dose** **or** □**Missed**	
30			_____ **Dose** **or** □**Missed**	_____ **Dose** **or** □**Missed**	
31			_____ **Dose** **or** □**Missed**	_____ **Dose** **or** □**Missed**	
32			_____ **Dose** **or** □**Missed**	_____ **Dose** **or** □**Missed**	
33			_____ **Dose** **or** □**Missed**	_____ **Dose** **or** □**Missed**	
34			_____ **Dose** **or** □**Missed**	_____ **Dose** **or** □**Missed**	
35			_____ **Dose** **or** □**Missed**	_____ **Dose** **or** □**Missed**	
36			_____ **Dose** **or** □**Missed**	_____ **Dose** **or** □**Missed**	
37			_____ **Dose** **or** □**Missed**	_____ **Dose** **or** □**Missed**	
38			_____ **Dose** **or** □**Missed**	_____ **Dose** **or** □**Missed**	
39			_____ **Dose** **or** □**Missed**	_____ **Dose** **or** □**Missed**	
40			_____ **Dose** **or** □**Missed**	_____ **Dose** **or** □**Missed**	

Participant Signature: _____ Date: _____

Reviewer's Signature: _____ Date: _____

Comments:

_____

_____

_____

APPENDIX E

BESS QUESTIONNAIRE

The completed form is to be used as source

BESS QUESTIONNAIRE

INSTITUTION CODE	PARTICIPANT ID	VISIT TYPE	VISIT DATE *(MM/DD/YYYY)*
_____	_____	_____	__ __ / __ __ / __ __ __ __

We are interested in knowing whether you have had any of the following problems during the **PAST TWO WEEKS**. Please mark the number which best describes how much each problem bothered you.

	PROBLEM	**Not at all**	**Slightly**	**Moderately**	**Quite a bit**	**Extremely**
C1	Difficulty concentrating	0	1	2	3	4
C2	Easily distracted	0	1	2	3	4
C3	Forgetfulness	0	1	2	3	4
M1	Joint pain	0	1	2	3	4
M2	Muscle stiffness	0	1	2	3	4
M3	General aches and pains	0	1	2	3	4
V1	Night sweats	0	1	2	3	4
V2	Hot flashes	0	1	2	3	4
V3	Cold sweats	0	1	2	3	4
Ga1	Vomiting	0	1	2	3	4
Ga2	Nausea	0	1	2	3	4
Ga3	Diarrhea	0	1	2	3	4
D1	Vaginal dryness	0	1	2	3	4
D2	Pain with intercourse	0	1	2	3	4
W1	Weight gain	0	1	2	3	4
W2	Unhappy with the appearance of my body	0	1	2	3	4
Gy1	Vaginal discharge	0	1	2	3	4
Gy2	Genital itching/irritation	0	1	2	3	4
Gy3	Vaginal bleeding or spotting	0	1	2	3	4
B1	Difficulty with bladder control (when laughing or crying)	0	1	2	3	4

BESS QUESTIONNAIRE (cont'd)

INSTITUTION CODE	PARTICIPANT ID	VISIT TYPE	VISIT DATE *(MM/DD/YYYY)*
_____	_____	_____	__ __ / __ __ / __ __ __ __

	PROBLEM	Not at all	Slightly	Moderately	Quite a bit	Extremely
B2	Difficulty with bladder control (at other times)	0	1	2	3	4
P1	Headaches	0	1	2	3	4
P2	Blind spots, fuzzy vision	0	1	2	3	4
P3	Constipation	0	1	2	3	4
P4	Cramps	0	1	2	3	4
P5	Breast sensitivity/tenderness	0	1	2	3	4
P6	Ringing in ears	0	1	2	3	4
P7	Chest pains	0	1	2	3	4
P8	Swelling of hands or feet	0	1	2	3	4
P9	Difficulty breathing	0	1	2	3	4
P10	Dry mouth	0	1	2	3	4
P11	Weight loss	0	1	2	3	4
P12	Decreased appetite	0	1	2	3	4
P13	Feeling of suffocation	0	1	2	3	4
P14	Excitability	0	1	2	3	4
P15	Short temper	0	1	2	3	4
P16	Tendency to take naps; stay in bed	0	1	2	3	4
P17	Tendency toward accidents	0	1	2	3	4
P18	Avoidance of social affairs	0	1	2	3	4
P19	Dizziness, faintness	0	1	2	3	4
P20	Numbness, tingling	0	1	2	3	4

BESS QUESTIONNAIRE (cont'd)

INSTITUTION CODE	PARTICIPANT ID	VISIT TYPE	VISIT DATE *(MM/DD/YYYY)*
_____	_____	_____	__ __/__ __/__ __ __ __

P21	Early awakening	0	1	2	3	4
P22	Abdominal pain	0	1	2	3	4
P23	Pain or cramps in the legs or feet	0	1	2	3	4
P24	Back pain or problems	0	1	2	3	4
P25	Low energy	0	1	2	3	4
P26	Blurred vision	0	1	2	3	4
P27	Any other problems?	Please Specify:_____ _____ _____				

_____
Participant Name

_____
Participant Signature

_____
Date

APPENDIX F

Alcohol and Tobacco Use Assessment Questionnaires – Baseline

ALCOHOL ASSESSMENT – BASELINE

INSTITUTION CODE	PARTICIPANT ID	VISIT TYPE	VISIT DATE *(MM/DD/YYYY)*
_____	_____	_____	___ ___ / ___ ___ / ___ ___ ___ ___

Instructions:

For the following questions about drinking alcoholic beverages, a drink means a 12 oz. beer, a 5 oz. glass of wine, or one and a half ounces of liquor.

1. In your entire life, have you had at least 12 drinks of any kind of alcoholic beverage?

   ☐ Yes
   ☐ No **(End)**
   ☐ Refused **(End)**
   ☐ Don't know/Not sure

2. In the past 12 months, on average, how often did you drink any type of alcoholic beverage?

   _____ (Enter the number of days you drank based on the timeframe checked below. Enter 0 if you never drank and skip to Question 6.)
   ☐ Week
   ☐ Month
   ☐ Year
   ☐ Refused
   ☐ Don't know/Not sure

3. In the past 12 months, on those days that you drank alcoholic beverages, on average, how many drinks did you have per day?

   _____ (Enter the average number of drinks per day)

   ☐ Refused
   ☐ Don't know/Not sure

4. In the past 12 months, on how many days did you have 5 or more drinks of any alcoholic beverage?

   _____ (Enter the number of days you had 5 or more drinks, or enter 0 if none.)

   ☐ Refused
   ☐ Don't know/Not sure

5. Was there ever a time or times in your life when you drank 5 or more drinks of any kind of alcoholic beverage almost every day?

   ☐ Yes
   ☐ No
   ☐ Refused

☐ Don't know/Not sure

6. If you do not currently drink alcoholic beverages, but did in the past, how long has it been since you last drank regularly?

☐ Within the past month (0 to 1 month ago)
☐ Between 1 and 3 months (1 to 3 months ago)
☐ Between 3 and 6 months (3 to 6 months ago)
☐ Between 6 and 12 months (6 to 12 months ago)
☐ Between 1 and 5 years (1 to 5 years ago)
☐ Between 5 and 15 years (5 to 15 years ago)
☐ More than 15 years ago
☐ Don't know/Not sure
☐ Never drank regularly

7. At the heaviest point, either now or in the past, on the days when you drank, about how many drinks did you drink a day on the average?

_____ (Enter the number of drinks a day)
☐ Refused
☐ Don't know/Not sure

8. How many years have you been drinking (or did drink) regularly?

_____ years
☐ Refused
☐ Don't know/Not sure

9. At what age did you begin drinking regularly?

_____ years of age
☐ Refused
☐ Don't know/Not sure

10. What type(s) of alcohol do you drink? (Mark ALL that apply)

☐ Wine
☐ Liquor
☐ Beer
☐ Wine cooler

Signature of Individual Completing This Form_____ Date __ __ / __ __ / __ __ __ __
(MM/DD/YYYY)

Name of Individual Completing This Form (please print) _____

TOBACCO ASSESSMENT – BASELINE

INSTITUTION CODE	PARTICIPANT ID	VISIT TYPE	VISIT DATE *(MM/DD/YYYY)*
_____	_____	_____	___ ___ / ___ ___ / ___ ___ ___ ___

Section A. Basic Cigarette Use Information

1. Have you smoked at least 100 cigarettes (5 packs = 100 cigarettes) in your entire life?

☐ Yes
☐ No → **Skip to Section B**
☐ Don't know/Not sure → **Skip to Section B**

2. How old were you when you first smoked a cigarette (even one or two puffs)?

_____ Years old

3. How old were you when you first began smoking cigarettes regularly?

_____ Years old
☐ Check here if you have never smoked cigarettes regularly.

4. How many total years have you smoked (or did you smoke) cigarettes? Do not count any time you may have stayed off cigarettes.

_____ Years (If you smoked less than one year, write "1.")

5. On average when you have smoked, about how many cigarettes do you (or did you) smoke a day? (A pack usually has 20 cigarettes in it).

_____ Number of cigarettes per day

6. Do you NOW smoke cigarettes?

☐ Everyday
☐ Some days
☐ Not at all → **Skip to question 8**

7. How soon after you wake up do you smoke your first cigarette?

☐ Within 30 minutes
☐ After 30 minutes

8. How long has it been since you last smoked a cigarette (even one or two puffs)?

First check which one of the following choices applies to you. Then, if applicable, write a number on the line
for how many days, weeks, months, or years it has been since your last cigarette.

☐ I smoked a cigarette today (at least one puff)
☐ 1-7 days → Number of days since last cigarette _____
☐ Less than 1 month → Number of weeks since last cigarette _____
☐ Less than 1 year → Number of months since last cigarette _____
☐ More than 1 year → Number of years since last cigarette _____
☐ Don't know/Don't remember

Section B. Use of Other Forms of Tobacco

9. Have you ever used other forms of tobacco, not including cigarettes?

☐ Yes
☐ No → **Skip to Section C**

10. How often do you/did you use other forms of tobacco?

☐ Every day → Number of times per day _____
☐ Some days → Number of days _____ per ☐ Week ☐ Month ☐ Year

11. Which of the following products have you ever used regularly?
Check all that apply

☐ Cigarettes
☐ E-cigarettes or other electronic nicotine delivery system
☐ Traditional cigars, cigarillos or filtered cigars
☐ Pipes
☐ Hookah
☐ Clove cigarettes or kreteks
☐ Bidis
☐ Smokeless tobacco, like dip, chew, or snuff
☐ Snus
☐ Paan with tobacco, gutka, zarda, khaini
☐ Other, Please specify:_____

12. If you do not currently use other forms of tobacco, but did in the past, how long has it been since you last used other forms of tobacco regularly?

- ☐ Within the past month (0 to 1 month ago)
- ☐ Between 1 and 3 months (1 to 3 months ago)
- ☐ Between 3 and 6 months (3 to 6 months ago)
- ☐ Between 6 and 12 months (6 to 12 months ago)
- ☐ Between 1 and 5 years (1 to 5 years ago)
- ☐ Between 5 and 15 years (5 to 15 years ago)
- ☐ More than 15 years ago
- ☐ Don't know/Not sure
- ☐ Never used other forms of tobacco regularly

Section C. Second-Hand Smoke Exposure

13. Are you currently living with a smoker?

- ☐ Yes
- ☐ No

14. In the past 30 days, have you lived in a place where other people smoked cigarettes indoors?

- ☐ Yes
- ☐ No

15. In the past 30 days, have you worked in a place where other people smoked cigarettes indoors?

- ☐ Yes
- ☐ No

16. Thinking of all your childhood and adult years, have you ever lived in a place where other people smoked cigarettes indoors?

- ☐ Yes In total, for about how many years?_____ If less than 1, write "1."
- ☐ No

17. Thinking of all the years you have worked, have you ever worked in a place where other people smoked cigarettes indoors?

- ☐ Yes → In total, for about how many years?_____ If less than 1, write "1."
- ☐ No

Signature of Individual Completing This Form_____ Date __ __ / __ __ / __ __ __ __
(MM/DD/YYYY)

Name of Individual Completing This Form (please print) _____

APPENDIX G

Alcohol and Tobacco Use Assessment Questionnaires – Month 12

ALCOHOL ASSESSMENT – MONTH 12

INSTITUTION CODE	PARTICIPANT ID	VISIT TYPE	VISIT DATE *(MM/DD/YYYY)*
_____	_____	_____	___ ___ / ___ ___ / ___ ___ ___ ___

Instructions:

For the following questions about drinking alcoholic beverages, a drink means a 12 oz. beer, a 5 oz. glass of wine, or one and a half ounces of liquor.

1. During the past 30 days, did you drink any alcoholic beverages?

☐ Yes
☐ No **(End)**
☐ Refused **(End)**
☐ Don't know/Not sure

2. During the past 30 days, how many days per week or per month did you drink any alcoholic beverages, on the average?

_____ (Enter number of days you drank based on the timeframe checked below. Enter 0 if you did not drink.)

☐ Week
☐ Month
☐ Refused
☐ Don't know/Not sure

3. On the days when you drank, on average, about how many drinks did you have?

_____ (Enter the average number of drinks you had per day.)

☐ Refused
☐ Don't know/Not sure

4. In the past 30 days, on how many days did you have 5 or more drinks per day?

_____Number of times

☐ None
☐ Do not know/Not sure

Signature of Individual Completing This Form_____ Date __ __ / __ __ / __ __ __ __
(MM/DD/YYYY)

Name of Individual Completing This Form (please print) _____

TOBACCO ASSESSMENT – MONTH 12

INSTITUTION CODE	PARTICIPANT ID	VISIT TYPE	VISIT DATE *(MM/DD/YYYY)*
_____	_____	_____	___ ___ / ___ ___ / ___ ___ ___ ___

1. Do you NOW smoke cigarettes?

☐ Everyday
☐ Some days
☐ Not at all → **Skip to Question 3**
☐ Never Smoked → **Skip to Question 4.**

2. On average, when you smoked, about how many cigarettes do you (or did you) smoke a day? (A pack usually has 20 cigarettes in it).

_____ Number of cigarettes per day

3. How long has it been since you last smoked a cigarette (even one or two puffs)?

First check which one of the following choices applies to you. Then, if applicable, write a number on the line
for how many days, weeks, months, or years it has been since your last cigarette.

☐ I smoked a cigarette today (at least one puff)
☐ 1-7 days → Number of days since last cigarette _____
☐ Less than 1 month → Number of weeks since last cigarette _____
☐ Less than 1 year → Number of months since last cigarette _____
☐ More than 1 year → Number of years since last cigarette _____
☐ Don't know/Don't remember

4. Since your last visit, have you used other forms of tobacco, not including cigarettes?

☐ Yes
☐ No (**End)**

5. How often do you/did you use other forms of tobacco?

☐ Every day → Number of times per day _____
☐ Some days → Number of days _____ per ☐ Week ☐ Month ☐ Year

6. Since your last visit, which of the following products have you used? ***Check all that apply***

- ☐ Cigarettes
- ☐ E-cigarettes or other electronic nicotine delivery system
- ☐ Traditional cigars, cigarillos or filtered cigars
- ☐ Pipes
- ☐ Waterpipe
- ☐ Hookah
- ☐ Clove cigarettes or kreteks
- ☐ Bidis
- ☐ Smokeless tobacco, like dip, chew, or snuff
- ☐ Snus
- ☐ Paan with tobacco, gutka, zarda, khaini
- ☐ Other, Specify_____

7. If you do not currently use other forms of tobacco, but did in the past, how long has it been since you last used other forms of tobacco regularly?

- ☐ Within the past month (0 to 1 month ago)
- ☐ Between 1 and 3 months (1 to 3 months ago)
- ☐ Between 3 and 6 months (3 to 6 months ago)
- ☐ Between 6 and 12 months (6 to 12 months ago)
- ☐ Between 1 and 5 years (1 to 5 years ago)
- ☐ Between 5 and 15 years (5 to 15 years ago)
- ☐ More than 15 years ago
- ☐ Don't know/Not sure
- ☐ Never used other forms of tobacco regularly

The following instructions pertain to questions 8 - 10. During each of the following time frames, please indicate whether you smoked cigarettes every day, some days, or not at all.

8. During study treatment

- ☐ Smoked every day
- ☐ Smoked some days
- ☐ Did not smoke at all
- ☐ Don't know/not sure
- ☐ Not applicable

9. After the end of study treatment

- ☐ Smoked every day
- ☐ Smoked some days
- ☐ Did not smoke at all
- ☐ Don't know/not sure
- ☐ Not applicable (I have not completed the study treatment)

10. Since your last visit to this clinic

- ☐ Smoked every day
- ☐ Smoked some days
- ☐ Did not smoke at all
- ☐ Don't know/not sure

Signature of Individual Completing This Form_____ Date __ __ / __ __ / __ __ __ __
(MM/DD/YYYY)

Name of Individual Completing This Form (please print) _____

APPENDIX H

Resources for tobacco and alcohol quitting

National and local resources to help with alcohol abuse and alcoholism

NIAAA's online guide ***Treatment for Alcohol Problems: Finding and Getting Help*** is written for individuals, and their family and friends, who are looking for options to address alcohol problems. It is intended as a resource to understand what treatment choices are available and what to consider when selecting among them.
https://pubs.niaaa.nih.gov/publications/treatment/treatment.htm

Other resources:

National Institute on Alcohol Abuse and Alcoholism www.niaaa.nih.gov
301–443–3860

National Institute on Drug Abuse www.nida.nih.gov
301–443–1124

National Clearinghouse for Alcohol and Drug Information www.samhsa.gov
1–800–729–6686

Substance Abuse Treatment Facility Locator www.findtreatment.samhsa.gov
1–800–662–HELP

Alcoholics Anonymous (AA) www.aa.org
212–870–3400 or check your local phone directory under "Alcoholism"

Moderation Management www.moderation.org
212–871–0974

Secular Organizations for Sobriety www.sossobriety.org
323–666–4295

SMART Recovery www.smartrecovery.org
440–951–5357

Women for Sobriety www.womenforsobriety.org
215–536–8026

Al-Anon Family Groups www.al-anon.alateen.org
1–888–425–2666 for meetings

Adult Children of Alcoholics www.adultchildren.org
310–534–1815

National and local resources to help with quitting smoking

NCI's Smokefree.gov offers science-driven tools, information, and support that has helped smokers quit. You will find state and national resources, free materials, and quitting advice from NCI.

Smokefree.gov was established by the Tobacco Control Research Branch of NCI, a component of the National Institutes of Health, in collaboration with the Centers for Disease Control and Prevention and other organizations.

Publications available from the Smokefree.gov Web site include the following:

- Clearing the Air: Quit Smoking Today for smokers interested in quitting.
- Clear Horizons for smokers over age 50.
- Forever Free™ for smokers who have recently quit.
- Forever Free for Baby and Me™, in English and Spanish, for pregnant smokers who have recently quit.
- Pathways to Freedom: Winning the Fight Against Tobacco for African American smokers.

NCI's Smoking Quitline at **1–877–44U–QUIT (1–877–448–7848)** offers a wide range of services, including individualized counseling, printed information, referrals to other resources, and recorded messages. Smoking cessation counselors are available to answer smoking-related questions in English or Spanish, Monday through Friday, 8:00 a.m. to 8:00 p.m., Eastern time. Smoking cessation counselors are also available through LiveHelp, an online instant messaging service. LiveHelp is available Monday through Friday, 8:00 a.m. to 11:00 p.m., Eastern time.

Your state has a toll-free telephone quitline. Call **1–800–QUIT–NOW (1–800–784–8669)** to get one-on-one help with quitting, support and coping strategies, and referrals to resources and local cessation programs. The toll-free number routes callers to state-run quitlines, which provide free cessation assistance and resource information to all tobacco users in the United States. This initiative was created by the Department of Health and Human Services. For more information about quitlines, speak to an expert on the Smokefree.gov Web site.

Appendix I.

Specimen Inventory Form.

Dr. Banu Arun's Laboratory
ATTN: Angelica Gutierrez Barrera

6565 MD Anderson Blvd., Z12.3032
Houston, TX 77030
Direct phone: 713-792-1986
Email: angutier@mdanderson.org

MDA2016-07-02: Specimen Inventory Form

(Fill Out One Form Per Patient, Per Time-Point)

Collection Institution: _____

Unique Specimen ID: _____

Study Staff Name: _____

Date Shipped: _____

Specimen Collected:

Indicate the quantity of each sample collected:

☐ NaCit/Blue Top Plasma _____ (1 mL aliquots) _____/_____ (Other aliquots/volume)

☐ EDTA/Purple Top Plasma _____ (1 mL aliquots) _____/_____ (Other aliquots/volume)

☐ Fresh Frozen Biopsy Tissue _____ (# of cryovials)

☐ RNALater: _____ (# of cryovials) Room Temp Time: _____:_____(am / pm)

Refrigeration Time (4°C or on ice): _____:_____(am / pm)

Date/Time frozen (-80°C): ____/____/____ _____:_____(am / pm)

☐ Paraffin embedded Biopsy Blocks: _____ (# of blocks)

Date/Time Embedded: _____/_____/_____ _____:_____(am / pm)

☐ Formalin Fixed for paraffin embedding: _____ (# of specimens)–**MDA only**

Date/Time in Formalin: _____/_____/_____ _____:_____(am / pm)

Notes/Comments:

_____
_____
_____

For the Receipt at the Lab:

STATE OF BLOOD SAMPLES/TISSUE: □ good condition □ poor condition, specify:

_____
_____
_____
_____

DATE RECEIVED: _____ RECEIVED BY: _____

[Return a copy of this form to the Sender]