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Phase IIB pre-surgical trial of oral tamoxifen versus transdermal 4-hydroxytamoxifen in women with DCIS of the breast

Consortium Name: Northwestern Cancer Prevention Consortium

Organization Name: Northwestern University
Name of Consortium Principal Investigator: Seema A. Khan, MD
303 E. Superior, Suite 4-111
Chicago, IL 60611
Tel: (312) 503-4236
Fax: (312) 503-2555
E-mail: skhan@nm.org

Organization Name: Northwestern University
Protocol Principal Investigator and Protocol Chair: Seema A. Khan, MD
Professor of Surgery
303 E. Superior, Suite 4-111
Chicago, IL 60611
Tel: (312) 503-4236
Fax: (312) 503-2555
E-mail: skhan@nm.org

Organization: Northwestern University
Investigator: Luis Blanco, MD
Pathologist
201 E. Huron
Galter #7-132A
Chicago, IL 60611
Tel: (312) 926-4436
Fax: (312) 926-7537
E-mail: luis.blanco@northwestern.edu

Organization: Duke University
Investigator: E. Shelley Hwang, MD
Chief, Breast Surgical Oncology
Professor, Dept. of Surgery
465 Seely Mudd Bldg
Durham, NC 27710
Tel: (919) 684-6849
Fax: (919) 684-6044
E-mail: shelley.hwang@duke.edu

Organization: Memorial Sloan Kettering Cancer Center
Investigator: George Plitas, MD
Assistant Professor of Surgery
Evelyn H. Lauder Breast Center
300 East 66th Street
New York, NY 10065
Tel: (646) 888-4587
Fax: (646) 888-4921
E-mail: plitasg@mskcc.org

Organization: Cleveland Clinic
Investigator: Zahraa AlHilli, MD, FRCSI, FACS
Breast Surgery
9500 Euclid Ave./A80
Cleveland, OH 44195
Tel: (216) 445-0664
E-mail: ALHILLZ@ccf.org

Organization: Mayo Clinic
Investigator: Amy Degnim, MD
Professor of Surgery
200 First Street SW
Rochester, MN 55905
Tel: (507) 284-4499
E-mail: degnim.amy@mayo.edu

Organization: St. Elizabeth HealthCare
Investigator: Joseph Michael Guenther, MD
Clinical Director, St. Elizabeth Breast Center
580 South Loop Road, Ste 201
Edgewood, KY 41017
Tel: (859) 344-1600
Fax: (859) 344-0091
E-mail: Joseph.Guenther@stelizabeth.com

Organization: The University of Kansas Cancer Center
Investigator: Kelsey E. Larson, MD FACS
Breast Surgical Oncologist
4000 Cambridge, Mail Stop 2005
Kansas City, KS 66045
Tel: (913) 945-6692
E-mail: klarson6@kumc.edu

Organization: Northwestern University
Statistician: Masha Kocherginsky, PhD
680 N. Lake Shore Dr., Suite 1400
Chicago, IL 60611
Tel: (312) 503-4224
Fax: (312) 908- 9588
Email: mkocherg@northwestern.edu

Organization: **National Cancer Institute**
Scientific Monitor: Brandy Heckman-Stoddard, PhD, MPH
Breast and Gynecologic Cancer Research Group
Division of Cancer Prevention
9609 Medical Center Dr.
Bethesda, MD 20892-9783
Tel: (240) 276-7048
Fax: (240) 276-7828
Email: heckmanbm@mail.nih.gov

Organization: **National Cancer Institute**
Medical Monitor Marjorie Perloff, MD
Chemopreventive Agent Development Research Group
Division of Cancer Prevention
9609 Medical, Rm 5E544
Rockville, MD 20850
Tel: (240) 276-7097
Cell: (240) 731-1772
Fax: (240) 276-7847
Email: perloffm@mail.nih.gov

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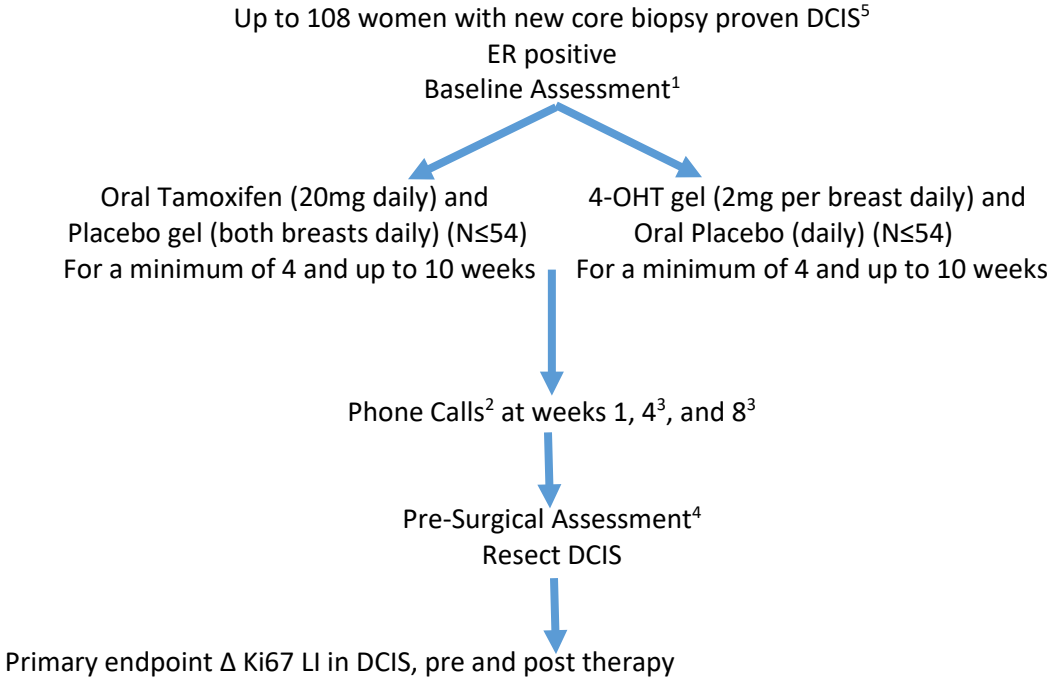
SCHEMA

Phase IIB pre-surgical trial of oral tamoxifen (TAM) versus transdermal 4-hydroxytamoxifen (4-OHT) in women with DCIS of the breast.



Primary endpoint Δ Ki67 LI in DCIS, pre and post therapy

Secondary Endpoints

- Δ in Oncotype DCIS Score
- Breast tissue: 4-OHT & ENX (E & Z isomers), TAM, NDT
- Plasma levels: 4-OHT & ENX (E & Z isomers), TAM, NDT
- Δ IGF-1, IGFBP-3, SHBG, Factors VIII, IX, vWF, total protein S
- Δ Ki67 LI in Terminal Duct Lobular Units, pre and post therapy
- BESS questionnaire, pre and post therapy

Exploratory Endpoints

- Δ in COX2, p16, CD-68 on immunohistochemistry
- Breast tissue: estradiol, progesterone
- Plasma levels: estradiol, progesterone
- Subjects with no residual DCIS

1 Baseline Assessment: Medical history, physical exam, medication review, pregnancy test (for pre-menopausal women of child-bearing potential), review of last menstrual period, clinical lab blood draw, research blood draw, request for core biopsy block or sections and core, and BESS questionnaire.
2 Phone Calls: Medication, compliance, and toxicity review
3 Week 4 and 8 calls as appropriate based on surgery date
4 Pre-Surgical Assessment: Medical history, physical exam, medication review, pregnancy test (for pre-menopausal women of child-bearing potential), review of last menstrual period, clinical lab blood draw, research blood draw, tissue collection, and BESS questionnaire.
5 Up to 108 eligible patients will be enrolled and no more than 100 subjects will start intervention after being randomized.

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1. OBJECTIVES

1.1 Primary Objectives – The primary objective of this study will be to demonstrate that 2mg once daily per breast of 4-OHT topical gel results in a reduction in the immunohistochemical (IHC) Ki-67 labeling index of DCIS lesions that is not inferior to that seen with 20mg daily oral TAM for 4 to 10 weeks, when comparing the base-line diagnostic core biopsy (pre-therapy) sample to the therapeutic surgical excision (post-therapy) sample.

1.2 Secondary Objectives –

1. To compare changes from pre-therapy to post-therapy in the Oncotype DCIS-Score between arms (this is a validated RT-PCR assay for 12 genes).
2. To compare between group post-therapy breast tissue and plasma levels of TAM and its metabolites [N-desmethyl tamoxifen (NDT), (E) and (Z) isomers of 4-hydroxytamoxifen (4-OHT), N- desmethyl-4-hydroxytamoxifen (Endoxifen)].
3. To compare the Ki-67 labeling index in the terminal duct lobular units (TDLUs), evaluated in the same manner as the primary endpoint.
4. To compare changes from pre-therapy to post-therapy in plasma proteins involved in coagulation: Factors VIII and IX, von Willebrand Factor, total protein S between arms.
5. To compare changes from pre-therapy to post-therapy in plasma markers of systemic estrogenic effect (IGF-1, IGFBP-3, and SHBG).
6. To compare changes from pre-therapy to post-therapy in symptoms as captured in the Breast Cancer Prevention Trial (BCPT) Eight Symptom Scale (BESS) questionnaire and skin reactions to 4-OHT gel).

1.3 Exploratory Objectives –

1. To compare changes in DCIS lesions in the expression of protein IHC markers (CD-68, p16, COX-2).
2. To compare changes from pre-therapy to post-therapy in breast tissue and plasma levels of steroid hormones (estradiol and progesterone).
3. To compare the fraction of subjects with no residual DCIS in the surgical specimen.

2. BACKGROUND

2.1 Study Disease: Duct carcinoma in situ of the breast.

Natural history: Annually, about 60,000 American women are diagnosed with DCIS of the breast, the great majority of which is hormone sensitive. Although DCIS is considered to be a precursor lesion with a distinct capacity to progression to invasive disease, the frequency of such progression is a question of debate, and the natural history of this condition is uncertain [1], leading to concerns about over-treatment [2]. Historically, estimates of the fraction of DCIS that may progress to invasive disease without therapy other than excision, have been in the range of 20-40%[3, 4], However, a recent analysis of SEER (Surveillance Epidemiology and End-Results) data revealed that with more extensive local therapy (margin-free excision plus radiation, or mastectomy) 5-year survival exceeds 99% regardless of the therapy; but among women diagnosed under age 50, 1/3 of the deaths are attributed to breast cancer [5]. A second recent SEER analysis focused on the comparison of women who received surgery and those who did not; when DCIS was low grade, the use of surgery did not result in improved survival, with a 10-year survival of 98.8% for the non-surgical group [6]. On the other hand, DCIS clearly has a potential for lethality, even when low grade; Page et al. have examined the Vanderbilt cohort for the natural history low grade DCIS that was not diagnosed and therefore not treated following excision. Of 28 women identified, 11 developed invasive breast cancer, and 5 died of metastatic disease [7]. The risk of mortality is of course related to the subsequent markedly increased risk of invasive cancer which occurs at a rate of

approximately 1% annually, even when relatively small DCIS lesions are excised with wide margins without post-operative radiotherapy, as illustrated by the recent updated 12-year results of Eastern Cooperative Oncology Group (ECOG) 5194 [8]. This risk appears to be a particular concern for young women and those of African descent, but an increased standardized mortality ratio is observed across all ages, ranging from 17 for women aged 30-34, to 1.4 in the 65-69 age group [2].

Role of tamoxifen therapy for women with DCIS
Tamoxifen (TAM) has been proven to reduce risk of both local recurrence and new primary breast cancer in women with DCIS, providing both preventive and therapeutic benefit [9, 10]. Oral TAM also has proven risk reduction value for women at increased risk for breast cancer, numbering over a million women in the United States alone [11, 12]. However, tamoxifen acceptance by DCIS and high-risk patients has been lower than expected, mainly because of toxicity concerns [13-16]. Serious adverse effects of TAM are related to its estrogen agonist activity on the endometrium, leading to an increased risk of endometrial cancer in postmenopausal women; and the activation of coagulation pathways leading to an increased risk of thromboembolism [11]. Hot flashes and vaginal symptoms were 20-30% more common in women aged 35-49 on tamoxifen than in the placebo group of the BCPT [17]. Together, these side effects are significant barriers to wide acceptance of TAM by DCIS patients [13] and high-risk women [18]. Although aromatase inhibitors are now proven to be efficacious for post-menopausal women [19, 20], these too have a burden of skeletal and possibly cardiovascular toxicity. There are no endocrine agents close to Phase 3 testing for pre-menopausal women. A particular challenge for primary and secondary breast cancer prevention efforts, therefore, is to devise an efficacious and non-toxic intervention which is likely to be widely accepted by women with DCIS and those at high risk for developing breast cancer. A potential solution is the development of delivery methods that preserve efficacy through targeted local delivery to the breast, but minimize toxicity because of low systemic exposure, as discussed in Sections 2.2 and 2.3.

2.2 Study Agent: 4-hydroxytamoxifen (4-OHT) transdermal gel formulation

A potential alternative to oral tamoxifen is suggested by studies going back to the 1980s, which showed that 4-OHT, the monohydroxy metabolite of oral tamoxifen, has a far greater affinity for the estrogen receptor α (ER) and is more effective than TAM in suppressing breast cancer cell growth [21]. Furthermore, 4-OHT has been shown to inhibit the growth of normal human breast cells [22], and cancerous cell lines in tissue culture with two-log greater potency than tamoxifen [23-25]. 4-OHT has approximately 100 times the potency of TAM in reducing proliferation of breast cancer cells *in vitro* [21]. Its binding affinity to ER is similar to estradiol (E2) and 25-to-50 times higher than tamoxifen [26]. Its efficacy in modulating ER expression is similar to the other major TAM metabolite, endoxifen [27]. However, 4-OHT has little activity when delivered orally, due to its rapid conjugation and inactivation by the liver. Additionally, plasma concentrations of 4-OHT are only about 2% of the parent drug levels [28]. The recognition by Mauvais-Jarvis et al. in 1986 that transdermal administration of 4-OHT may be possible, led to a study where *trans*-[^{3}H]-4-OHT (9 women) and *trans*-[^{3}H]TAM (3 women) was applied to the skin of the breast and tumor samples were obtained prior to, and 12 hours to 7 days following application [29]. Serial blood and urine samples were also collected. The results showed significantly greater retention of 4-OHT in breast tissue than TAM and appearance of TAM metabolites in the plasma, but little evidence of 4-OHT metabolism in the breast. These findings led the authors to hypothesize that 4-OHT may be a suitable compound for trans-dermal delivery in women with benign breast problems, where local delivery and avoidance of systemic toxicity are the goals. The same may be said for primary and secondary breast cancer prevention in unaffected high risk women and those with pre-invasive malignancy.

Pharmacokinetics and metabolism: 4-OHT is found in all tissues examined after administration of oral TAM and, along with endoxifen, is believed to be responsible for a major part of the anti-cancer efficacy of tamoxifen *in vivo* [30]. The concentrations of 4-OHT in breast tumor tissue are generally higher than

simultaneous concentrations in normal breast tissue, and there is minimal metabolism in the breast, where tissue levels are stable for 2 days and detectable for 7 days [29]. At transdermal doses of 1, 2, and 4 mg daily, plasma levels of 4-OHT were 0.05, 0.13, and 0.2 ng/mL respectively, and were thus approximately 1/30 to 1/12, and 1/6 respectively, of those seen after 20 mg of oral TAM [31]. Metabolism of 4-OHT occurs in the liver. Clinically significant adverse effects of topical 4-OHT have not been reported to-date at the doses that have been studied (0.25 mg/day, 0.5 mg/day, 1 mg/day, 2 mg/day, and 4 mg/day). In addition, the topical application of 4-OHT may circumvent one source of tamoxifen resistance: the failure of women with CYP2D6*4 polymorphisms to achieve sufficient levels of the active metabolites of tamoxifen when the agent is delivered orally [32, 33]. Together, these considerations provide strong rationale for testing 4-OHT in women with non-invasive breast cancer and those at increased risk of breast cancer.

2.3 Rationale

2.3.1 Overall rationale for testing 4-hydroxytamoxifen gel in DCIS patients

For drugs with single-organ cancer prevention value, such as tamoxifen, local delivery to the target organ is an attractive preventive strategy since effective drug concentrations are only required in the breast and systemic exposure constitutes an opportunity for collateral damage. Local therapy can be accomplished either by cannulation of ductal orifices [34], or transdermal delivery [35, 36]. Although data on intraductal delivery have been published, this requires an office procedure by a specialist, the feasibility of repeat duct cannulation is untested, and dissemination across multiple practice settings and varied clinical environments seems dubious. Transdermal delivery on the other hand is challenging to develop in that the stratum corneum must be breached, drugs need to be disseminated though the breast, and retained selectively without significant leakage into the circulation. However, once developed, transdermal therapy will be far more suitable for wide dissemination, and is therefore a more suitable option on a population level. Pilot data, summarized below, support the potential of transdermal therapy as being feasible and effective, but represent short-term therapy, on a scale of weeks. The demonstration of this general concept now requires trials of longer duration that will consolidate the evidence that local transdermal delivery is safe, effective, and acceptable to women who need medical therapy for prevention of malignant events in the breast (healthy women at high risk for breast cancer, or those with carcinoma in situ). Of these two populations, women with DCIS are an ideal population for the next step in development, for both biological and logistical reasons. The modulation of DCIS cell proliferation (Ki67) by endocrine agents has been demonstrated in previous studies [35, 37], and recent lay discussions of DCIS (8 stories in the New York Times, August to September 2015) have refocused attention on the fact that the goal of therapy is the prevention of invasive disease; and that the DCIS lesion in and of itself is not a threat to health or life. Therefore women with newly diagnosed DCIS are now more likely to accept a period of medical therapy prior to surgery, and in fact such a trial has completed accrual (CALGB 40903, NCT01439711). Therefore, a comparison of transdermal and oral therapy for DCIS would be very timely and well received by patients, and by the medical community.

Based on preliminary data discussed below, we hypothesize that once daily topical application of a gel formulation of an active metabolite of tamoxifen (4-OHT) to the breasts will result in a reduction in the Ki-67 labeling index of DCIS lesions that is not inferior to that seen with oral TAM 20 mg daily for 4 to 10 weeks, when comparing the base-line diagnostic core biopsy (pre-therapy sample) to the therapeutic surgical excision (post-therapy) sample.

2.3.2 Clinical data supporting the development of transdermal 4-OHT for DCIS and primary prevention

Pilot studies of 4-OHT gel: this agent has been studied in more than 350 women at daily doses ranging from 0.25 mg to 2 mg/breast (4 mg/day) for periods up to two years. 4-OHT gel was well tolerated in these patients, with no important drug-related adverse events. *The effect is topical since the application of 4-OHT on the abdomen, arms, and shoulders resulted in substantially lower breast tissue levels than those obtained*

by application to the skin of the breasts [29, 38]. Trials in the area of breast cancer, mastalgia, and mammographic density are summarized below.

Pre-surgical study in post-menopausal women with ER positive tumors: A Phase 2 multi-center, randomized, open-label clinical trial of 4-OHT gel was performed in 55 postmenopausal women with newly diagnosed, ER-positive (≥10%), invasive breast cancer by Rouanet et. al. [31]. Participants were randomized to 4-OHT gel (0.5, 1, or 2 mg/day), or 20 mg/day of oral tamoxifen, or observation, followed by tumor resection within 21 days. The primary endpoint was tumor cell proliferation (Ki67 index and proliferative cell nuclear antigen (PCNA)). Pre- and post-therapy blood samples were analyzed for 4-OHT and estradiol concentrations. Breast tumor samples were obtained by core needle biopsy at the baseline and from the excised tumor. Normal breast tissue (500 mg sample) was obtained from the same breast at least 2 cm away from the excised tumor. Efficacy was evaluable in 49 women, and safety in 53. The 1 mg/day, 2 mg/day and the oral tamoxifen groups had significantly lower mean Ki-67 index values after treatment compared to the untreated group (Dunnett's test, $p<0.007$, $p<0.008$ and $p<0.000$, respectively). Mean Ki-67 index after treatment was dose-dependent with values of 6.3%, 4.1%, 3.5%, 3.3%, and 2.5% for the untreated, 0.5 mg/day, 1 mg/day, 2 mg/day, and oral tamoxifen groups, respectively. These results demonstrate a dose response to 4-OHT gel and approximate equality of the 2.0-mg/day 4-OHT gel group and the 20-mg oral tamoxifen group. In tumor tissue, 4-OHT mean concentrations were clearly dose-dependent (0.63 ng/g for 0.5 mg/day, 1.55 ng/g for 1 mg/day, 1.87 ng/g for 2 mg/day of 4-OHT gel and 4.32 ng/g for oral TAM). Normal tissue concentrations were roughly half those of the tumor tissue. The mean plasma concentration on the evening prior to surgery was 1.53 ng/mL for the oral tamoxifen group, compared to 0.059, 0.050, and 0.131 ng/mL for the 0.5-, 1-, and 2-mg/day 4-OHT groups, respectively. *This study demonstrated that short-term administration of topical 4-OHT gel had a dose-dependent effect on cellular proliferation of breasts cancer cells in situ equivalent to that of oral tamoxifen administered at the recommended dose of 20 mg/d.*

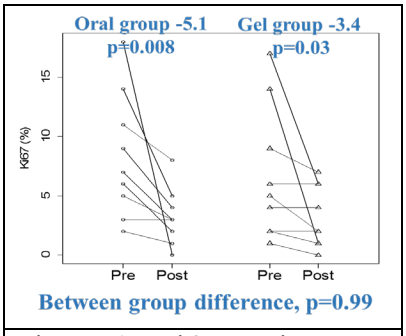

Figure 1. Ki67 LI in DCIS patients, at baseline and following >6 weeks of oral tamoxifen 20 mg daily or 4-OHT gel applied to the breast (NWU 07-9-02, Lee et. al. Clin Cancer Res 2014).

Pre-surgical Phase IIB study in women with DCIS (NWU 07-9-02) In a DCP-funded study, we confirmed equivalent reduction in Ki67 labelling of DCIS lesions by oral tamoxifen 20 mg and 4-OHT 2 mg/breast, in women treated for at least 6 weeks pre-operatively, see Figure 1 [35]. We also measured plasma levels of proteins involved in coagulation, and those reflecting systemic estrogenicity (serum IGF-1 and SHBG); we found the expected increases in the TAM group, but no significant changes in the 4-OHT gel group. Compliance was good and the gel was well-tolerated. The concentrations of (Z) 4-OHT in breast adipose tissue were equivalent in the two groups (5.4±2.8 ng/g in oral TAM group and 5.8 ± 9.3 ng/g in 4-OHT gel group, p=0.88), but plasma (Z) 4-OHT concentrations were significantly lower in the gel group (1.1± 0.7 ng/mL vs. 0.2 ± 0.2 ng/mL, p= 0.0003).

Mastalgia: A Phase 2 Study (01-4OHT-02) was conducted to assess the safety and efficacy of topical 4-OHT gel in treating cyclical mastalgia in pre-menopausal women [39], and tested 2-mg 4-OHT gel (n=40), 4-mg 4-OHT gel(n=39) or placebo(n=35) applied daily; 114 subjects were evaluated at 4-months. Those receiving 4 mg 4-OHT demonstrated statistically significant improvement in pain scores relative to placebo (-19.34 mm for placebo, -25.76 mm for 2-mg 4-OHT, and -32.06 mm for 4-mg 4-OHT). The gel was well tolerated at both doses. Mean plasma concentration of 4-OHT for the 2- and 4-mg treatment groups after 4weeks (one month) of treatment were 17 ± 19 pg/mL, and 60 ± 65 pg/mL, while mean plasma levels following 16 weeks (4 months) of treatment were 20 ± 28 pg/mL and 50 ± 64 pg/mL, respectively [39]. The systemic exposure to 4-OHT following oral TAM administration of 20 mg/day has been reported to be between 2-6 ng/mL [30-32].

Data on Hot Flashes: In a double-blind, placebo-controlled 4-OHT breast density study conducted by Besins, 80 pre-menopausal women were randomized to 2 mg/day of 4-OHT gel or placebo for six months; hot flashes did not change from baseline in either the placebo or the 4-OHT groups. Similarly, in the 4-OHT breast pain study, where 41 women were treated with 2 mg/day and 45 women with 4 mg/day 4-OHT gel for four months, there was only a small indication of increased hot flushes as compared to the placebo arm. At Visit 4 (Week 8 of 16), four patients (9%) in the 4 mg group experienced 1-5 episodes, one patient (2.4%) in the placebo group experienced 11-15 episodes, and no patients in the 2 mg group experienced hot flushes or night sweats. In contrast, in the NSABP P-1 trial, the proportion of women reporting hot flushes as being quite or extremely bothersome was 45.7% in the tamoxifen group (total of post-menopausal and pre-menopausal women) as compared with 28.7% in the placebo group. Among postmenopausal women, the data are insufficient to assess hot flash frequency.

The attitudes of women to drug gel application to the breast: We have conducted a focus group study where 32 healthy women attending the Lynn Sage Breast Center for mammography or benign breast problems participated in four focus groups of about 8 women each [40]. The major findings from this study were that 1) self-perceptions of risk are inaccurate in both directions (underestimation and overestimation of risk were common); 2) women were surprisingly unaware of medication for breast cancer prevention; 3) 90% of women would prefer a gel to a capsule, anticipating fewer side effects with this approach.

Summary of pilot treatment data: This series of pre-surgical studies indicate that the anti-proliferative activity of 4-OHT gel at 1, 2, and 4 mg/day in invasive breast tumors and DCIS is similar to that of oral TAM at 20 mg/day. 4-OHT is a potent antiestrogenic metabolite of oral TAM, an agent with an unparalleled record of therapeutic and preventive breast cancer efficacy. The pharmacokinetic rationale for the use of a topical transdermal formulation of 4-OHT is justified by: 1) the inability to give 4-OHT orally due to poor bioavailability, 2) the significant percutaneous bioavailability of 4-OHT achieved with hydroalcoholic gel application, and 3) the ability to attain high tissue levels of 4-OHT with low systemic levels, which should translate into little (if any) systemic toxicity. Preliminary data are encouraging that the desired local tissue effects in the breast can be achieved with well-tolerated topical delivery.

NWU 07-9-02 was designed to address these hypotheses, with a planned sample size of 112 DCIS patients. Unfortunately this was closed prematurely due to interruption of supply of the study drug (28 women were enrolled and 18 were evaluable for the primary endpoint). The present protocol is intended to extend that study to up to 108 DCIS patients, aiming to obtain further data regarding feasibility and clinical outcomes of efficacy and toxicity.

2.3.3 Biomarkers predicting recurrence: Given the variable natural history of DCIS, there has been much discussion regarding the need for biomarkers that predict for invasive recurrence, and some progress has been made recently in this area.

Our **primary endpoint** of Ki67 response to tamoxifen therapy in ER positive DCIS lesions is based on the known correlation between early reduction in Ki67 and long-term cancer outcomes in studies of neoadjuvant endocrine therapy for invasive breast cancer [41, 42]. As a result of these and other studies [43] Ki67 has become a widely recognized, valid endpoint biomarker of response of primary breast cancer to a variety of agents. In the setting of the present study, we considered two alternative candidate primary endpoints: mammographic density and DCIS size. However, it is not clear that mammographic density will change with 2-3 months of treatment; and robust evaluation of this endpoint would require a larger sample size than is presently possible. The measurement of pre-therapy lesion size is attractive, but uncertain with mammography; MRI is very likely more accurate than mammography for this purpose, but is fraught with sources of variation in terms of MRI equipment and image processing algorithms; and would add considerable expense and logistical difficulty. Building on our previous trial therefore, Ki67

labelling is the most feasible endpoint, and the one we have selected, with the hypothesis that reduction in Ki67 labelling will be similar in following therapy with oral tamoxifen and 4-hydroxytamoxifen.

In addition, we have selected a series of **secondary and exploratory endpoints**, that are designed to complement the primary endpoint of Ki67 response in the DCIS lesion, with the expectation that these additional biomarkers will identify tamoxifen sensitive DCIS lesions.

Secondary endpoints:

1. A 12-gene biomarker panel (the DCIS Score) derived from the 21-gene Oncotype DX recurrence score has been tested in a subset of patients recruited to ECOG 5194 [44]; this shows good predictive value, with clear discrimination of women who develop subsequent ipsilateral breast events (IBE) from those who do not. In particular, the 10-year risk of invasive IBE was 12% and 19% in women with intermediate and high DCIS scores. This panel has been subsequently validated in a population-based cohort from Ontario, Canada [45]. However, in neither of these studies was it possible to examine the predictive value of the DCIS Score, either for benefit of radiotherapy, or for the use of tamoxifen. Both study populations consisted of patients who did not undergo radiation, and tamoxifen was used by only 25% of E5194 patients. Tamoxifen use data was not available on the Ontario cohort. However, since the DCIS Score was derived from a gene panel that was developed for ER positive invasive breast cancer and includes genes involved in proliferation and estrogen response, there is clear relevance of the DCIS Score to the question of whether or not DCIS patients would benefit from tamoxifen. This is a compelling question, since the uncertainties about the natural history of DCIS and the worries about tamoxifen toxicity conspire to distress women about the value of this medication in terms of overall health. We therefore hypothesize that DCIS Score will relate to tamoxifen benefit, as measured by Ki67 response and mammographic density.
2. Breast tissue and plasma levels of TAM and its metabolites [N-desmethyl tamoxifen (NDT), (E) and (Z) isomers of 4-hydroxytamoxifen (4-OHT), N- desmethyl-4-hydroxytamoxifen (Endoxifen)] are a necessary component of this trial since a key question about transdermal delivery pertains to the ability to achieve effective breast tissue drug concentrations. Although prior data suggest that this is in fact true, the number of samples tested so far is small and testing of multiple samples across the breast has not yet been performed. Therefore, it is important in the present study to measure drug concentrations at a minimum of two locations in women undergoing breast conserving surgery, and more extensively in women undergoing mastectomy.
3. Ki67 labeling index in terminal duct lobular units (TDLUs) will be evaluated in the same manner as the primary endpoint (KI67 LI in the DCIS lesions). We expect 10 to 20 percent of participants would have no residual DCIS in the surgical samples and they cannot be included in the main analysis for the primary endpoint since Ki67 in DCIS at both time points is required for that purpose. However, TDLUs are present in most tissue sections. Existing data suggest that breast cancer risk is higher in women whose TDLUs show increased Ki67 LI[47, 48], and is reduced by short-term tamoxifen use, even at lower doses[49, 50]. Therefore, we have now included a secondary endpoint of Ki67 reduction in TDLUs, to be compared in both arms, and we expect that changes in TDLU Ki67 LI will be non-inferior in the gel arm.
4. As secondary endpoints related to toxicity, we have identified three areas for measurement: a) Plasma proteins involved in coagulation: Factors VIII and IX, von Willebrand Factor, total Protein S. We expect that the oral tamoxifen group will show significant changes and the 4-OHT gel group will not. B) Plasma markers of systemic estrogenic effect (IGF-1, IGFBP-3, and SHBG); we expect that these will change in the oral tamoxifen group but not in the 4-OHT gel group. C) Symptoms as captured in the BESS questionnaire and skin reactions to 4-OHT gel. We expect that symptoms will be less severe in the 4-OHT gel group, and skin toxicity will be minor or none.

Exploratory endpoints:

1. Other immunohistochemical (IHC) markers have been investigated in a DCIS cohort in California; Tlsty and colleagues have identified Ki67, COX2, and p16 expression as predictors of invasive recurrence [46]. In a different, presurgical endocrine therapy study, Hwang and colleagues have identified CD-68 (a macrophage marker) as a surrogate for DCIS response to endocrine therapy. We hypothesize that this panel of markers will complement the evaluation of Ki67 response, and aid interpretation of responses that fall at the extremes (excellent or no change in Ki67).
2. Changes in plasma levels of steroid hormones (estradiol and progesterone) will be greater in the oral tamoxifen arm but no change is expected in the gel arm. Breast tissue levels of the hormones in post-therapy samples will be compared between arms. We expect estradiol level will be higher in the oral tamoxifen arm. Systemic estradiol increase due to oral Tamoxifen treatment was evident in premenopausal breast cancer patients[51, 52]. The response to tamoxifen may relate to ambient levels of these steroid hormones; furthermore, data on breast tissue hormone levels are scant and will also inform future studies of endocrine agents. This will be measured at the Hormone Laboratory of Haukeland University Hospital, Norway.
3. When DCIS is diagnosed on core biopsy, there is no residual lesion in the subsequent surgical specimen in about 10% of patients. If this fraction exceeds 20% in our study (across both arms) we will take it as evidence of lesion regression related to tamoxifen/4-OHT therapy. In these subjects, we will compare the fraction of subjects with no residual DCIS between arms.

3. SUMMARY OF STUDY PLAN

3.1 Study design: We propose a randomized, double-blind, placebo-controlled pre-surgical trial of 0.228% 4-hydroxy-tamoxifen (4-OHT) gel vs. oral tamoxifen (TAM) 20 mg daily in up to 108 women with a core needle biopsy diagnosis of ER positive DCIS, regardless of grade, with 1:1 randomization to oral TAM and 4-OHT gel. We will consider the following strata: 1) Recruitment site, of which there will be seven, and 2) Pre- or post-menopausal status, within each site. The 4-OHT group will apply active gel 2 mg daily to each breast and take oral placebo for a minimum of 4 and a maximum of 10 weeks. The TAM group will take 20 mg TAM orally daily and apply gel placebo daily to each breast for 4 to 10 weeks. The primary endpoint is Ki67 labeling index in the DCIS core (pre-therapy sample) compared to the surgical sample (post-therapy sample), with the expectation that the reduction in this parameter will be equivalent in the two arms.

3.2 Number of participants to be enrolled: A maximum of 108 participants with estrogen receptor (ER) positive DCIS of the breast, newly diagnosed on core needle biopsy, will be accrued into two intervention arms: oral tamoxifen 20 mg daily, and 4-hydroxytamoxifen gel, 4 mg daily (2 mg to each breast). Due to early drop-out after randomization, we expect no more than 100 women to start intervention. In addition, we assume that there will be an attrition of 10 women (10%) related to lack of compliance to medication or other logistical reasons, and an attrition of another 10 women (10%) related to lack of sufficient DCIS tissue for analysis of the primary endpoints. Therefore a total of 90 women (45 in gel arm and 45 in oral arm) will be evaluated for study endpoints, and 80 women (40 per arm) will provide data for the primary endpoint. Assuming a screening rate of approximately 60 participants per month and an average accrual rate of 4.5 participants per month, we expect the study to be complete within 24 months. Accrual is competitive and sites should accrue as many participants as possible until total accrual is met.

3.3 The study population: newly diagnosed adult DCIS patients will be enrolled at 7 centers. Eligible participants will be approached following diagnostic core needle biopsy at the time of initial surgical consultation. The centers are:

- Northwestern University/Northwestern Medicine (NU)

- Duke University Medical Center (DUMC)
- Memorial Sloan Kettering Comprehensive Cancer Center (MSKCC)
- Cleveland Clinic Comprehensive Cancer Center (CCCCC)
- Mayo Clinic Rochester (MCR)
- St. Elizabeth Hospital, Kentucky (SEH)
- University of Kansas Cancer Center (KUCC)

3.4 Intervention plan

The intervention phase for consenting participants will begin within 5 business days following randomization and end on the day prior to surgery. At baseline (Screen 1) visit, participants will be given 120 placebo or active capsules (20 mg tamoxifen) and two canisters of placebo or active 4-OHT gel, each containing 80 reliable doses. Participants will take study agent for a minimum of 4 and a maximum of 10 weeks. The intervention phase for consenting participants will begin within 5 business days following randomization and end on the day prior to surgery. The 4-OHT group will apply active gel 2 mg daily to each breast and take oral placebo daily for 4 to 10 weeks. The TAM group will take 20 mg TAM orally daily and apply gel placebo daily to each breast for 4 to 10 weeks. There will be two study visits, the day of surgery and the post-op visit.

If a participant opts to proceed to surgery prior to the planned end of intervention, but has completed 4 weeks of intervention she will be asked to complete all study visits/procedures as specified in section 3.8, and she will be included in the final statistical evaluation.

3.5 Run-in procedures
No run-in period is planned

3.6 Assessment time points are:

3.6.1 Baseline .....(Screen 1 visit)
3.6.2 Day of surgery ..... (Study Visit 1, post-intervention sample collection)
3.6.3 Post-operative visit .....(Study Visit 2, symptom assessment)

3.7 Description of measurements taken to meet study objectives

Screen 1: at entry, for consent, medical history including date of last period and menopause determination, pregnancy test (if needed), breast cup size, body mass index, explanation of gel application, baseline symptom assessment (BESS questionnaire), and blood draw. After randomization, the study coordinator will request the paraffin blocks of the diagnostic core needle biopsy (DCNB). The study consent will include specific permission from the subject, to allow the DCNB block, and a selected surgical block to be released to Northwestern University (NU). These will be mailed to NU per instructions in the lab manual. Institutions that do not allow release of DCNB blocks, will provide multiple unstained sections of the DCNB block as detailed in Section 10.2.3.

Study Visit 1: on the day of surgery for blood draw, BESS questionnaire, medical history, symptoms assessment, and collection of surgical tissue sample (since the blood and surgical samples must be collected on the same day). Breast adipose and parenchymal tissue excised from multiple locations will be snap frozen with liquid nitrogen and stored at -80^0C for measurement of TAM metabolites. A portion of each sample will be formalin-fixed for assessment of tissue composition (see Section 10 and Figure 2A).

Study Visit 2: the post-operative visit, up to 21 days post-surgery, to document resolution of study related

symptoms. Visit 2 will also serve as the trigger for the study coordinator to request the paraffin blocks of the surgical sample, since the final pathology report will have been issued at this point. These will be mailed, along with other study samples, in a single shipment as each participant completes the study, per instructions in the lab manual. Institutions that do not allow release of paraffin blocks, will provide multiple unstained sections of the paraffin block as detailed in Section 10.2.3. A final phone call will be made up to 35 days following Study Visit 2 to premenopausal women whose last menstrual period did not occur between surgery and Study Visit 2.

3.8 *Handling of subjects who elect not to complete protocol-specified therapy.*
Every attempt will be made to retain all subjects for the entire duration of protocol-specified therapy. However, some participants may opt for early surgery. In this case, endpoints will be measured if more than 4 weeks of therapy have been completed (blood, tissue biomarkers and drug concentrations).

3.9 Safety monitoring: since oral tamoxifen is a widely used agent with a well-known safety/toxicity profile, we have been able to define exclusion criteria to avoid women for whom there will be safety concerns (e.g. those with a history of thromboembolism, and current smokers). Given the duration of intervention (maximum 10 weeks) it will not be necessary to use laboratory tests to monitor toxicity of oral tamoxifen other than the comprehensive chemistry panel at the pre- and post-treatment visits. The safety of the 4-OHT gel is also well established, since it is a metabolite of tamoxifen; the only unique toxicity to be expected would be skin irritation, which was observed for one participant in our previous trial, and has been reported to be infrequent in other, larger studies [39]..

3.10 Duration of study: we anticipate that overall recruitment will cover months 1-24. The last subject should complete participation by month 27. Data assembly, cleaning, and biomarker evaluation will occur in months 24-36.

4. PARTICIPANT SELECTION

4.1 Inclusion Criteria

4.1.1 Screen-detected, ER positive DCIS of the breast proven on core needle biopsy, defined as 10% ER positive cells. Microinvasion will be allowed. The size of the DCIS in the core biopsy sample must be at least 4mm for a single core or total at least 5 mm if multiple cores are summed and must be estimated on the deepest step section (if step sections are taken).

4.1.2 Age ≥18 years. DCIS of the breast is almost exclusively an adult condition. Because no dosing or adverse event (AE) data are currently available on the use of tamoxifen in participants <18 years of age, children are excluded from this study.

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

4.1.4 Participants must have acceptable organ and marrow function as defined below:
Baseline lab parameters are not standard of care for initiation of tamoxifen therapy; a minimal panel will therefore be appropriate.

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

4.1.5 The effects of topical 4-OHT gel on the developing human fetus at the recommended therapeutic 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.]

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

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

4.2 Exclusion Criteria

4.2.1 Exogenous sex steroid use within 4 weeks prior to diagnostic core needle biopsy (DCNB). Use of vaginally administered estrogens and hormone coated IUD such as Mirena is permitted and use should continue until surgery.

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

4.2.3 History of other prior breast cancer-specific therapy within the previous 2 years (chemotherapy, anti-HER2 agents, endocrine agents, everolimus, CDK4-6 inhibitors).

4.2.4 Skin lesions on the breast that disrupt the stratum corneum (eg eczema, ulceration).

4.2.5 History of endometrial neoplasia

4.2.6 History of thromboembolic disease (history of varicose veins and superficial phlebitis is allowed)

4.2.7 Current smokers.

4.2.8 Current users of potent inhibitors of tamoxifen metabolism must be willing and able to discontinue use and switch to an alternative medication for the duration of participation, under the advice of their physician. If the physician believes the current medication is medically necessary, the participant will not be eligible. The potent inhibitors of tamoxifen metabolism are: bupropion, cinacalcet, fluoxetine, paroxetine, quinidine.

4.2.9 Prior use of SERMS or AIs including tamoxifen, raloxifene, anastrozole, letrozole, or exemestane for prevention or therapy within 5 years.

4.2.10 Participants may not be receiving any other investigational agents within 30 days of enrollment or during this study.

4.2.11 History of allergic reactions attributed to tamoxifen or compounds of similar chemical or biologic composition.

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

4.2.13 Pregnant women are excluded from this study. Because there is an unknown but potential risk for AEs in nursing infants secondary to treatment of the mother with tamoxifen, breastfeeding should be discontinued by nursing mothers who agree to participate in the study.

4.2.14 Men are excluded from this study since DCIS of the breast is exceedingly rare in men, and there are no data regarding skin penetration of 4-OHT though male chest wall skin (which is thicker and hairier than female chest wall skin).

4.3 Inclusion of Women and Minorities

Women of all races and ethnic groups are eligible for this trial. Men are not included since DCIS of the breast is exceedingly rare in men, and there are no data regarding skin penetration of 4-OHT though male chest wall skin (which is thicker and hairier than female chest wall skin).

4.4 Recruitment and Retention Plan

4.4.1 Recruitment brochures and a DCIS Fact Sheet will be developed and approved by the Central Institutional Review Board (CIRB); these materials will be posted in the breast center waiting rooms at each institution, the mammography waiting rooms, the cancer center and institutional websites, and advertised through Google ads. Physician letters will be sent to the referral base at each institution.

4.4.2 Recruitment to similar studies is well established at the seven participating institutions, and will be standardized as far as possible. In brief, the breast surgery clinic schedule will be screened by the study coordinator 2-3 days ahead of the scheduled consultation visit. The medical records of newly diagnosed DCIS patients will be reviewed, if preliminary eligibility criteria are met, pre-screening will be initiated. The coordinator will contact the radiologist and pathologist reviewing the case to assess quality of diagnostic films, and the treating physician regarding suitability of the patient as a potential participant. The coordinator will then be present at the visit of the patient who appears eligible on pre-screen, and once the surgeon has broached the study to the patient, the coordinator will approach the potential participant to provide more information, including the informed consent document. The treating physician will have to remain involved in this process, advising and encouraging the patient as needed. All efforts will be made to align the study visits with usual care visits so as to reduce the burden of the study on the patient.

4.4.3 The proposed study intervention is 4 to 10 weeks prior to surgery, which is a reasonable time frame for newly diagnosed DCIS patients. All of the study materials will explain to subjects why a little delay is not felt to be a safety concern.

4.4.4 Retention is not expected to be a challenge as coordinators will make periodical phone calls to monitor compliance and address subjects' concerns. The phone app used in a previous topical gel study will be modified for this study, and will aid in retention.

4.4.5 Recruitment and retention experience at each institution will be reviewed at monthly conference calls of the site PIs and coordinators, and through AQUIP reports, to identify problems and successful strategies. If protocol-related barriers are identified, strong consideration will be given to protocol revision to address these (unless this would compromise the scientific integrity of the study).

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

Experimental Arm: (Group 1)
4-hydroxytamoxifen gel applied to both breasts (2mg/breast) daily
Oral placebo taken daily
4 to 10 weeks of treatment between randomization and surgical resection

Control Arm: (Group 2)
Placebo gel applied to both breasts daily
20mg oral tamoxifen taken daily (taken as one 20mg capsule)
4 to 10 weeks of treatment between randomization and surgical resection

5.2 Tamoxifen and 4-OHT gel Administration

Participants will self-administer the oral tamoxifen/placebo capsules and 4-hydroxytamoxifen/placebo gel. Participants will be asked to take oral and topical dosing at the same time, preferably in the morning, for consistency and comparable pharmacokinetics. The subjects will be instructed to take the study capsule with some water around the same time every day. The gel will be applied daily to both breasts, freshly washed, either by hand 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 subject 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 swim. No washing or immersion in water should occur for at least 4 hours following gel application. The timing of administration of capsule and gel should be as close to each other as possible (to aid memory). 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 Screen 1 visit. 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 gel canister will contain 80 reliable doses of product metered to dispense 1mL per actuation, 2mg of 4-OHT gel per actuation (~40 days of therapy). The dose is 2mg (1mL) per breast, 4mg (2mL) total per day. A kit containing two canisters of 4-OHT/placebo gel and one bottle of 120 tamoxifen/placebo capsules will be given/shipped to the patient at the time of randomization.

5.3 Run-in Procedures

No run-in phase is planned.

5.4 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.

5.5 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, dose and route of administration, and indication. Medications taken for surgery should not be included.

5.6 Dose Modification

None planned

5.7 Adherence/Compliance

5.7.1 Participants will be considered compliant if they have taken 80% of the prescribed doses and have missed no more than 3 doses in the final 14 days prior to surgery.

5.7.2 The participants will be asked at registration if they would like to utilize a phone application in place of paper forms. According to subject preference, participants will either utilize phone application or fill out paper forms daily to monitor compliance. For participants utilizing the phone application, the study coordinator will be notified after 2 days of missed entries. For individuals using paper forms, email messages will be sent daily for the first week; in following weeks, messaging will be targeted to women having difficulty remembering their dose.

5.7.3 Capsule counts and weighing of gel dispensers will be performed at the conclusion of therapy. Drug and metabolite levels will be measured in blood and tissue on the last day of administration. Prescribed doses include one capsule per day, or one pump of the gel on each breast per day. If the capsule count consumed by the subject is lower than D*80%, or the amount of gel consumed is less than 2D*80%*[m – sd(m)], where D is the number of days between visits, m and sd(m) are the mean and standard deviation of the weight of one pump of the gel provided by the manufacturer, or if more than three doses of therapy are missed during the final 14 days, the subject will be considered non-compliant. For subjects with only one breast, the lower limit of the amount of gel consumed for compliance is D*80%*[m – sd(m)]. All participants who receive a study agent for any period of time will be evaluable for toxicity. Treatment compliance assessment will be based on the active ingredient component of the treatment regimen, i.e. the pill count for the Oral Tamoxifen group and gel dispenser weight for the 4-OHT gel group.

6. PHARMACEUTICAL INFORMATION

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

Structures of Tamoxifen [as (Z) isomer], 4-OHT [as (Z) isomer], and the Primary Metabolites of 4-OHT

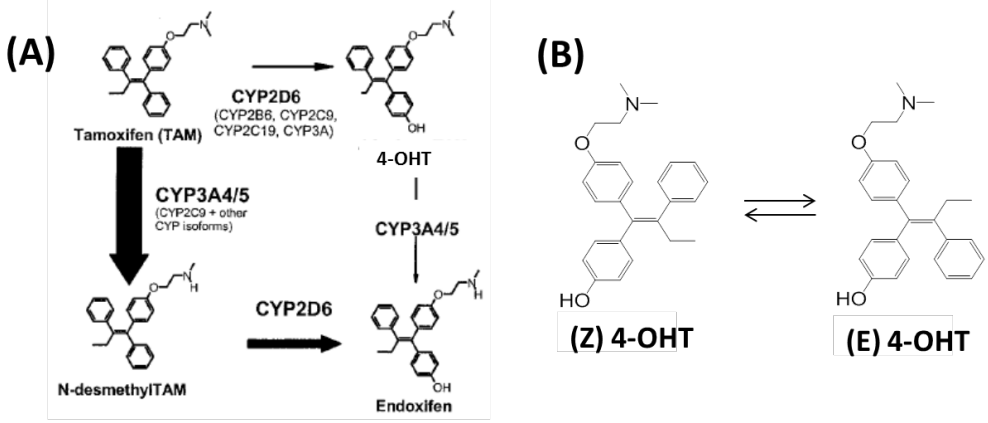

Figure 2. Tamoxifen and its metabolites. (A) chemical structures [as Z isomer] and metabolism [Jin Y, JNCI 2005 p30-39] (B) rapid conversion between two isomers of 4-OHT by light exposure (non-enzymatic) leads to equilibrium.

Table 1: Composition of 4-OHT Gel [0.228% 4-OHT (w/w)] Formulation for present study

Ingredient	Quantity per 100 g gel
4-OHT	0.228 g
95% ethyl alcohol, EP	72 g
Isopropyl myristate, EP	1 g
Hydroxypropylcellulose, EP	1.5 g
Phosphate buffer ¼ (v/v), pH 7, consisting of: Potassium dihydrogen phosphate(KH_2PO_4) Disodium monohydrogen phosphate (Na_2HPO_4,$12H_2O$)	26.372 g 0.023 g 0.092 g

4-OHT is produced by a step-wise synthetic process from the commercially-available raw materials anisole, 2-phenylbutyric acid chloride, 4-bromophenol, and 2-dimethylaminoethyl chloride: HCl. The crude 4-OHT is purified by recrystallization from methanol, resulting in two geometric isomer forms, E and Z. The drug substance is tested for identity, content, isomer ratio and purity prior to formulation into a hydroalcoholic gel product.

The agent to be supplied by the NCI, DCP Repository for this study, is based on the Besins Healthcare gel formulation containing 0.228 g of 4-OHT per 100 g of gel [0.228% on a weight-to-weight (w/w) basis] or 200 mg of 4-OHT in 100 mL of gel [0.2% on a w/v basis]

Chemically, tamoxifen is the trans-isomer of a triphenylethylene derivative. The chemical name is (Z) 2-[4-(1,2-diphenyl-1-butenyl) phenoxy]-N, N-dimethylethanamine 2-hydroxy-1,2,3- propanetricarboxylate (1:1).
Tamoxifen (20 mg) will be incorporated into opaque gelatin capsules and filled with microcystalline cellulose powder.

Placebo capsules will be manufactured by incorporating only microcrystalline cellulose powder into identical opaque capsules.

6.2 Reported Adverse Events and Potential Risks

The toxicokinetics of 4-OHT have been investigated in seven studies in rats and dogs, treated with

repeated doses administered by subcutaneous or dermal routes. In general, plasma concentrations of 4-OHT increased in a dose-dependent manner following subcutaneous and dermal administration in rats and dogs. The intravenous and dermal administrations of 4-OHT were well tolerated by rats.

Repeated dermal doses of up to 1000 μg/kg/day of 4-OHT were administered to female rats. Repeated subcutaneous doses of up to 200 μg/kg/day of 4-OHT were administered to female rats and repeated subcutaneous doses of up to 150 μg/kg/day of 4-OHT were administered to female dogs. Dermal and subcutaneous doses of up to 200 μg/kg/day were generally well tolerated by rats, while subcutaneous doses of up to 150 μg/kg/day were shown to be well tolerated in dogs. There were no clinical signs of toxicity. However, decrease in body weight, reduced uterus and ovary size, and changes in the genital tract were observed.

The reproductive toxicity of 4-OHT was evaluated in 7 studies with rats and 3 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. When 4-OHT was cutaneously applied before mating until day 6 of pregnancy, impaired female fertility and retardation in fetal body weight were observed, but these effects were reversible when females were mated after a 14-day treatment-free period. Topical doses of up to 50 μg/kg/d in rats and 100 μg/kg/d in rabbits during organogenesis resulted in no embryotoxicity. Higher doses during organogenesis reduced body weight gain and increased the incidence of resorptions, and the highest dose of 200 μg/kg/d induced wavy ribs in some fetuses. Subcutaneous injection of 4-OHT at 20 μg/kg/d to F0 female rats following implantation to weaning of the progeny resulted in a reduced number of pregnant F0 females, a reduced number of delivered pups, and pups surviving the early post-partum. However, the physical and behavioral development of the F1 generation and its fertility were not affected at any dose.

The carcinogenic toxicity of 4-OHT was evaluated in one study in female rats. 4-OHT was not carcinogenic in rats treated with up to 1000 μg/kg/day topically for 101 weeks. Notably, during the carcinogenicity study, reduced incidence in mammary tumors was observed in treated rats, leading to lower mortality in treated rats.

4-OHT is the most active metabolite of the well-characterized antiestrogen compound, tamoxifen, which has been used in the treatment of estrogen receptor (ER) positive breast cancer for over 25 years. Tamoxifen was approved in 1998 by the FDA for prevention of breast cancer in high-risk women on the basis of a large, randomized, placebo-controlled study sponsored by the National Cancer Institute, which demonstrated a 50-percent reduction in the risk of ER positive breast cancers in women treated with tamoxifen (Fisher et al. 1998). Although its efficacy is well established in the treatment and prevention of certain breast cancers, tamoxifen increases the risk of several serious adverse events, particularly when used for several years. The most serious adverse events include endometrial cancer, deep-vein thrombosis, pulmonary embolism, stroke, and cataracts. However, thromboembolic events are not expected with transdermal 4-OHT use given the lack of first-pass hepatic metabolism.

Clinically significant adverse effects of topical 4-OHT have not been reported to date at the doses that have been studied (0.25 mg/day, 0.5 mg/day, 1 mg/day, 2 mg/day, and 4 mg/day).

Topical application of 4-OHT avoids first pass metabolism in the liver. Thus, the antiestrogenic activity of 4-OHT after percutaneous administration is expressed locally. Preliminary data from the studies described below indicate that 4-OHT at 1 mg/day and 2 mg/day has similar anti-proliferative activity in breast tissue as oral tamoxifen at 20 mg/day, with much lower plasma levels of 4-OHT. These plasma levels (50 and 130 pg/mL, respectively) are approximately 1/30 – 1/12 of those achieved after oral administration of standard doses (20 mg) of tamoxifen.

6.3 Availability

6.3.1 Oral tamoxifen (20mg) capsules and matching placebo capsules are supplied by NCI, DCP Repository. Capsules will be packaged into bottles containing 120 capsules.

6.3.2 4-OHT and matching placebo gel are supplied by the BHR Pharma and distributed by NCI, DCP Repository (see Section 12.7). Each gel canister contains at least 80 reliable doses and will be metered to dispense 1mL per actuation, 2mg of 4-OHT gel per actuation (~40 days of therapy). Placebo canisters will contain an equal weight of placebo gel.

6.3.3 Agent will be supplied in a kit. Each kit contains two canisters of 4-OHT/ placebo gel and one bottle of 120 tamoxifen/placebo capsules. The kit will be given/shipped to the patient at the time of randomization and collected at Study Visit 1.

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) 360-5369
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). The Investigator is required to maintain adequate records of receipt, dispensing and final disposition of study agent. This responsibility has been delegated to the Research Pharmacist at each site, who will maintain receipt records detailing from whom the agent was received and to whom study agent was shipped, date, quantity and batch or lot number. On the dispensing record, quantities and dates study agent was dispensed to and returned by each participant will be noted.

6.6 Packaging and Labeling

4-OHT and matching placebo gel are packaged by the BHR Pharma and distributed by the NCI, DCP Repository. Oral tamoxifen and matching placebo will be packaged by NCI, DCP Repository. Kits will be assembled by the NCI, DCP Repository.

The tamoxifen and placebo capsules will be packaged as 20 mg, or placebo capsules, in bottles of 120 capsules. Each bottle will have a label printed with the treatment kit number, protocol number, number of capsules, expiration date, and "tamoxifen citrate 20mg or placebo".

Transdermal 4-OHT/ placebo gel is packaged in a container-closure system which consists of a pouch in a canister, a 1 mL pump, and a protective cap that covers the pump. The pouch contains 88g of gel. A single actuation delivers 1mL of gel with a 4-OHT concentration of 2mg/mL. Each canister will have a label printed with the treatment kit number, concentration and weight of gel, expiration date, protocol number and "4-Hydroxytamoxifen 2mg/mL or Placebo Gel " along with the statement "Caution: New Drug- Limited by Federal law (US) to investigational use". Each canister is packaged in a carton with the sample label. Detailed instruction for gel application will be provided.

Agent will be supplied in a kit. Each kit contains two canisters of 4-OHT/ placebo gel and one bottle of 120 tamoxifen/placebo capsules. Each kit will have a label printed with the treatment kit number, protocol number and contents and storage temperature along with the statement "Caution: New Drug- Limited by Federal law (US) to investigational use". Each kit (and its contents) will be labelled so that any oral active kit or any gel active kit can be given to any participant. The portion of the label indicating placebo or active drug will be removed by the pharmacy when the kit is dispensed. The pharmacy will attach pre-printed label overlays with the randomization number of the subject receiving the study drug, and canisters numbers provided by MRI Global. Dispensing pharmacist shall open the kit, affix prescription labels to both capsule bottles and dispensers with written direction according to the prescribing instruction. Fill out blank sections on the bottles and dispensers indicating subject number and subject initials. Weigh all dispensers and record final gross weight on the space provided on the dispenser itself and on the form. Detach peel-off label from each kit and affix it to the form, which is kept in the study binder. Pharmacies that use an eDARF shall print out the logs from their electronic accountability system upon request and keep the logs in the study binder.

The kit will be given/shipped to the patient at the time of randomization and collected at Study Visit 1. If the kit is shipped to participant, the pharmacy or coordinator will package the supplies at controlled room temperature. Please see the NWU2015-06-04 pharmacy manual for shipping procedures and specifications.

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 – 25º C [68° to 77°F]); excursions are permitted to 15° to 30°C [59° to 86°F]) in accordance with labeled storage requirements. Each site's supply of study drugs and placebos will be stored at their respective investigational pharmacies. Subjects will be instructed to store the study drug at home at room temperature, and to avoid extreme heat or cold during transportation from clinic to home. Investigational labeling will include instructions to keep the product out of reach of children.

6.8 Registration/Randomization

1. A study coordinator must upload into the Northwestern Clinical Trials Management System, a signed and complete informed consent along with HIPAA authorization and a completed eligibility form for each participant identified as eligible to be entered into the study.
2. All participants must be registered in the Northwestern University Robert H. Lurie Comprehensive Cancer Center Clinical Trials Management System (CTMS). Participants must not start protocol treatment prior to registration in the Lurie Cancer Center CTMS.
3. After registration in the CTMS, participants will be assigned a participant identification number and a randomization number when applicable.
4. An NCPC Quality Assurance Monitor will submit the following into the REDCap Randomization Information Form: (1) Study Number, (2) Site, (3) Pharmacist Email(s), (4) Participant ID [PID], (5) Participant Initials, and (6) Randomization Date.
5. An Automatic Treatment Assignment Notification email will be sent to the research pharmacist(s) containing: (1) Study Number, (2) Site, (3) Participant ID [PID], (4) Participant Initials, (5) Randomization Date, and (6) Treatment Assignment.
6. The clinical research coordinator(s) will receive a Confirmation of Registration containing the PID via email.
7. The following people will have a copy of the un-blinded randomization log: the study statistician at NU, the Quality Assurance Team at NU, and the Investigational Pharmacists at each site. The following people will have access to the REDCap study project containing randomization information: the study statistician at NU and the Quality Assurance Team at NU. The study statistician will set up randomization blocks.

When possible, the study coordinator will notify an NCPC Quality Assurance Monitor and/or send an email to ncpc@northwestern.edu prior to registering a participant. Prior notification is required for participant randomizations outside the normal business hours of Monday-Friday 9:00am-5:00pm CT.

6.9 Blinding and Unblinding Methods

Study participants will receive a prescription, blinded, from the investigational pharmacy. The blind will be maintained through the effort of the research pharmacist and the pharmacy. Unblinding will only occur when it is deemed medically necessary, and will only take place after consultation with the PI and the NCI, DCP Medical Monitor:

Name:	Marjorie Perloff, MD
Address:	Division of Cancer Prevention National Cancer Institute 9609 Medical Center Drive, 5E544 Rockville, MD 20850
Tel:	(240) 276-7097 (during normal business hours)
Cell:	(240) 731-1772
Fax:	(240) 267-7828
Email:	perloffm@mail.nih.gov

After hours consultation will be provided by Dr. Seema Khan, at telephone 312-472-4720 or cell phone 312-307-3646.

6.10 Agent Destruction/Disposal

Tamoxifen capsules and 4-OHT gel: at the completion of investigation, all unused 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", as specified in The Guidelines and form are available on the DCP website.

7. CLINICAL EVALUATIONS AND PROCEDURES

7.1 Schedule of Events

7.1.1 Expected duration of participation in the study: The interval from registration to the start of the intervention period will be a maximum of two weeks; the interval from Visit 1 (day of surgery) to Visit 2 is 1-21 days; and the (potential) interval from Visit 2 to the final phone call is up to 35 days. With an intervention period of 4 to 10 weeks, the minimum duration of participation is 29 days, and the maximum duration is 20 weeks.

SCHEDULE OF EVENTS

Evaluation/ Procedure	Registration/ Screen 1	Randomization[5]	Study visit 1 (day of surgery)[6]	Study visit 2 (within 21 days after surgery)
Informed Consent	X			
Assess Eligibility	X			
Medical History	X		X[7]	
Physical Exam	X		X[7]	X
Vitals[1]	X		X	X
Breast Cup Size	X			
Blood draw for CBC and chemistry profile	X		X	
Research blood draw	X[2]		X[8]	
Pregnancy test[3]	X		X	
Menstrual period date[4]	X		X	X
BESS questionnaire[10]	X		X[12]	
Concomitant Medications	X		X[12]	
Dispense Study Agent		X		
Collect Study Agent			X[12]	
Review Agent Diary/Record		X	X[12]	
Adverse Events			X[12]	X
Fresh breast sample collection			X	
Record location & depth of lesion in breast			X	
Request paraffin blocks of core biopsy & surgery[11]		X (core biopsy)		X (surgical)
Assemble samples & mail to NU PCF				X
Phone calls timed from day 1 of intervention (up to 4 total)[9]		Weeks 1, 4[13], 8[13]		0-5 weeks after Study Visit 2[14]

1. ECOG performance status, height, weight, blood pressure, pulse, and temperature
2. Research blood at Baseline (Screen 1) includes:
    - Two 2.7mL blue top tubes (Citrate) to obtain plasma for Coagulation panel assays.
    - Two 10 mL lavender top tubes (K_2EDTA) to obtain plasma and buffy coat (WBCs for DNA/RNA isolation). Plasma will be obtained for 1) IGF-1, IGFBP-3, and SHBG assays; 2) steroid hormone assays (estradiol, progesterone). Buffy coat will be collected for future assessment of CYP2D6 polymorphisms in tamoxifen metabolism (refer section 10.2 for details).
3. Pregnancy tests will be performed on all women with child-bearing potential.
4. Record last and estimated next menstrual period at the Screen 1 visit. Confirm and record actual menstrual period date by a phone call to participant 1 week after the estimated date. Record last and estimated next menstrual period date at Visit 1. Confirm and record actual menstrual period date at Visit 2 or the final phone call.
5. Participants must be randomized within 90 days of DCNB and must start drug within 5 days after randomization.
6. Surgery must be scheduled to occur between 4-10 weeks after the patient begins the study agent. However, surgery date may be extended for 10 days beyond 10 weeks if required due to scheduling exigencies driven by patient's personal or health safety needs, surgeon's needs, or operating room schedule.
7. Source document will be anesthesiologist or surgeon pre-op history and physical. May have been performed on day of surgery or within 14 days prior.
8. Research blood on the Day of Surgery (Study Visit 1) includes: plasma for drug measurement (tamoxifen, N-desmethyltamoxifen, Z and E isomers of both 4-OHT and endoxifen) and for Coagulation panel assay (Factor VIII, von Willebrand Factor, Factor IX, and total protein S), and for IGF-1, IGFBP-3, and SHBG assays. WBCs will be used for extraction of DNA/RNA.
    - Two 2.7mL blue top tubes (Citrate) to obtain plasma for Coagulation panel assays.
    - Two 10 mL lavender top tubes (K2EDTA) to obtain plasma and buffy coat (WBCs for DNA/RNA isolation). Plasma will be obtained for 1) measuring tamoxifen and its metabolites [tamoxifen, N-desmethyl tamoxifen, (E) 4-OHT, (Z) 4-OHT, (E) endoxifen, and (Z) endoxifen]; 2) IGF-1, IGFBP-3, and SHBG assays; 3) steroid hormone assays (estradiol, progesterone). Buffy coat will be collected for future assessment of CYP2D6 polymorphisms in tamoxifen metabolism (refer section 10.2 for details).
9. For phone calls at weeks 1, 4^{14}, 8^{14}, a window of ±3 days is allowed.
10. Questionnaires may be administered via interview or self-administered.
11. Institutions that do not allow release of paraffin blocks, will provide sections of the paraffin block as detailed in Section 10.2.3
12. Visit 1 BESS questionnaire, collection of study agent, review of agent diary/record, review of concomitant medication, and review of adverse events may be performed at either Visit 1 (day of surgery) or on the day prior to surgery.
13. Week 4 and 8 calls as appropriate based on surgery date.
14. Premenopausal study participants who did not have a period between surgery and Visit 2 will receive a follow-up phone call to assess LMP. This follow-up call will be made 14-21 days after expected period date, or 28-35 days after study visit 2 if no expected period date is known.

7.2 Baseline Testing/Prestudy Evaluation (Screening Visit 1)

7.2.1 Pre-Study procedures include:

- Informed Consent
- Medical History (from surgical consultation note)
- Record Concomitant Medications and any baseline symptoms
- Physical Exam including vitals Record Breast Cup Size
- Verification by surgeon that radiology review does not reveal clinical or radiologic findings suspicious for invasive disease on the ipsilateral side.
- Clinical Labs – including CBC and Chemistry Panel must be completed within 14 days of randomization. Clinical labs may be run as rush tests with results reported same day or within 24 hours.
- Premenopausal women are those who have had the last menstrual period (LMP) within the last 12 months. Premenopausal women with child-bearing potential are those with at least one ovary, an intact uterus, and LMP less than 12 months previously. Premenopausal women who have undergone bilateral tubal ligation are not considered to be of child-bearing potential.

- For Premenopausal women, record last menstrual period and estimated next menstrual period dates. One week after estimated next menstrual period date, call participant to confirm and record actual menstrual period date.
- Pregnancy Test (only offered for premenopausal women with child-bearing potential).
- Blood collection for research purposes
    - Two 2.7mL blue top tubes (Citrate) to obtain plasma for Coagulation panel.
    - Two 10 mL lavender top tubes (K_2EDTA) to obtain plasma and buffy coat (WBCs for DNA/RNA isolation) for the following purposes:
        a) Plasma for measuring hormones (estradiol, progesterone).
        b) Plasma for measuring IGF-1, IGFBP-3, and SHBG.
        c) Buffy coat will be saved for DNA/RNA isolation.
- BESS questionnaire (Appendix C)
- Drug administration instruction and practice

7.2.2 Subjects will be instructed to keep a study diary to document their use of study medication (Appendix B). They will be offered the use of a phone app instead of a study diary. If they do not possess a smart phone, one will be provided for the duration of the study, and will be returned at the end. If not returned, the cost of the phone ($100) will be deducted from the final study compensation payment.

7.2.3 Patients will be randomized when the baseline clinical lab results have been reported and both eligibility and appropriate stratification have been confirmed. Baseline testing and procedures must have been completed within 14 days of randomization. Once the participant has been randomized (within 90 days of DCNB), the participant's study drugs will be given/shipped to her overnight. Patients must begin study agents within 5 days of randomization. Patients will be given/sent 2 canisters of 4-OHT or placebo gel and 1 bottle of 120 tamoxifen or placebo capsules per bottle. Once the participant has been randomized, study team will begin the process of requesting paraffin blocks of core biopsy or sections of the paraffin block, from institutions that do not allow release of paraffin block.

7.3 Evaluation During Study Intervention

7.3.1 Telephone calls (these will occur regardless of use of phone app).
Phone call for compliance, changes in concomitant medications, and adverse event monitoring (call may be substituted by email if convenient for the subject)

- Week 1, week 4, and week 8 (week 4 and 8 calls as appropriate based on surgery date). A window of ±3 days is allowed.

Please refer to Appendix D for suggested language for participant communications.

7.4 Evaluation at Completion of Study Intervention

<u>Study Visit 1: Day of Surgery (following 4-10 weeks of study agent)</u>

- Physical Exam including vitals. The source document for this may be anesthesia or surgery H&P, completed within 14 days prior to surgery.
- BESS questionnaire*
- Toxicity and AE Assessment*
- Record Concomitant Medications*
- Collection of agent and compliance check* - Capsule count and study gel application
- Clinical Labs – including CBC and Chemistry Panel
- Blood collection for research purposes:

- Two 2.7 mL Blue Top tube (citrate) to obtain plasma for coagulation panel.
- Two 10mL Lavender top tubes (K_2EDTA) to obtain plasma and buffy coat or white blood cells (WBCs) for following purposes:
  a) Plasma for measuring tamoxifen and its metabolites [tamoxifen, *N*-desmethyl tamoxifen, *(E)* 4-OHT, *(Z)* 4-OHT, *(E)* endoxifen, and *(Z)* endoxifen].
  b) Plasma for measuring steroid hormones (estradiol, progesterone).
  c) Plasma for measuring IGF-1, IGFBP-3, and SHBG.
  d) Buffy coat will be saved for DNA/RNA isolation.

- Collection of surgical specimen for drug concentration assay – see section 10 for details.
- Pregnancy Test (only offered for premenopausal women with child-bearing potential).
- Recording of depth of lesion from skin, based on wire localization films in subjects undergoing breast conserving surgery, or on direct measurement during grossing of mastectomy slices in subjects undergoing mastectomy.
- For Premenopausal women, record last menstrual period and estimated next menstrual period dates. Confirm and record actual menstrual period date at Visit 2 or the final phone call.

*BESS Questionnaire, toxicity and AE assessment, review of concomitant medications, and compliance check (collection and review of study agent) may be performed at either Visit 1 (day of surgery) or on the day prior to surgery.

7.5 Post-intervention Follow-up Period

Study Visit 2: Post-surgical visit (within 21 days after surgery)

- Physical exam including vitals.)
- Toxicity and AE assessment
- For premenopausal women, confirm and record actual menstrual period date or estimated next period date. Follow up at the final phone call if no period has occurred since surgery.
- Obtain surgical pathology report to document pathologic features including DCIS size, grade, and presence of invasion.
- Start process of requesting paraffin blocks of surgical specimen or sections of the paraffin block, from institutions that do not allow release of paraffin blocks.

Follow-up phone call (up to 35 days after Study Visit 2)

- Assess date of last menstrual period for premenopausal participants whose last menstrual period did not occur between surgery and Visit 2. This call should take place 14-21 days after the expected period date, or 28-35 days after Visit 2 if no expected period date is known.
- Assemble and mail all study-related samples (blood, fresh tissue, FFPE blocks or sections).

Please refer to Appendix D for suggested language for participant communications.

7.6 Methods for Clinical Procedures

7.6.1. Review of radiologic-pathologic concordance by surgeon and radiologist.
The treating surgeon will confer with the breast radiologist to verify that pathologic findings are concordant with imaging assessment, all clinically indicated imaging has been performed, and no evidence of invasive disease on the ipsilateral side is present. The size measured on imaging studies will also be confirmed at this time.

7.6.2 Assessment of pathologic size

The pathologist signing out the surgical pathology specimen will use the following protocol for size assessment: a) using the slide with maximal disease, report the span of disease in two dimensions; b) report the number of blocks with disease; c) estimate the thickness of tissue per block, multiple the number of blocks by the thickness, report this product as the third dimension. The pathologic size of the DCIS lesion will be recorded as the longest dimension.

8. CRITERIA FOR EVALUATION AND ENDPOINT DEFINITION

8.1 Primary Endpoint

The primary objective of this study will be to demonstrate that 2mg once daily per breast of 4-OHT topical gel results in a reduction in the Ki-67 labeling index of DCIS lesions that is not inferior to that seen with 20mg daily oral TAM for 4 to 10 weeks, when comparing the base-line diagnostic core biopsy (pre-therapy) sample to the therapeutic surgical excision (post-therapy) sample.

Ki67 labelling will be assessed by standard immunohistochemistry as used in our previous study [35] and quantified by computer-assisted image analysis at the Northwestern University Pathology Core Facility. Dr. Luis Blanco (study pathologist at NU) will perform designation of regions of interest for digital scoring. The Ki67 LI in the diagnostic core needle biopsy will be compared to that in the excisional specimen, with the pathology team blinded to the study groups.

8.2 Secondary and Exploratory Endpoints

8.2.1 Oncotype DCIS-Score (validated RT-PCR assay for 12 genes)[53]. This will be performed by Exact Sciences, according to their highly reproducible and well-validated methods.

8.2.2 Breast tissue and plasma levels of TAM and its metabolites [N-desmethyl tamoxifen (NDT), (E) and (Z) isomers of 4-hydroxytamoxifen (4-OHT), N- desmethyl-4-hydroxytamoxifen (Endoxifen)] to be performed at Hormone Laboratory of Haukeland University Hospital, Norway.

8.2.3 Ki67 LI in terminal duct lobular units, applying the same methods as described for measurement of the primary endpoint (KI67 LI in the DCIS lesions).

8.2.4 Plasma proteins involved in coagulation: Factors VIII and IX, von Willebrand Factor, total protein S to be performed at the Northwestern Memorial Hospital Hemostasis Lab and Quest Diagnostics.

8.2.5 Plasma markers of systemic estrogenic effect (IGF-1, IGFBP-3, and SHBG) to be performed at the Northwestern University Comprehensive Metabolic Core Lab.

8.2.6 Patient reported symptoms as captured in the BESS questionnaire, and skin reactions to 4-OHT gel, as in our prior study of 4-OHT gel [35].

8.2.7 IHC markers: CD-68 macrophage marker as a surrogate for response to therapy. p16 and COX-2 [46]. Dr. Luis Blanco (study pathologist) will mark the DCIS legion of H&E slides to be evaluated by biomarker IHC assays. IHC staining and scoring will be performed by the Northwestern University Pathology Core Facility.

8.2.8 Plasma levels of steroid hormones (estradiol and progesterone) in pre-therapy and post-therapy samples, to be measured by Hormone Laboratory of Haukeland University Hospital, Norway.

8.2.9 Breast tissue levels of steroid hormones (estradiol and progesterone) in post-therapy samples , to be measured by Hormone Laboratory of Haukeland University Hospital, Norway.

8.2.10 Fraction of subjects with “no residual DCIS” in surgical sample. This will be assessed by the study pathologist (Dr. Blanco) on the surgical excisional sample.

8.3 Off-Agent Criteria

Participants may stop taking study agent for the following reasons: completed the protocol-prescribed intervention, AE or serious adverse event (SAE), inadequate agent supply, noncompliance, concomitant medications, medical contraindication, or decision by the participant to proceed with surgery for DCIS. In this event, the participant will be asked to continue study medication until the day before surgery, as long as she has taken study medication for 4 weeks or more. Participants will continue to be followed, if possible, for safety reasons. Participants who stop agent within 6 days of surgery will continue their study visits to collect endpoint data according to the schedule of events, unless the participant withdraws consent for further study procedures.

8.4 Off-Study Criteria

8.4.1 Participants may go "off-study" for the following reasons: completed the protocol-prescribed intervention, AE or serious adverse event, lost to follow-up, inadequate agent supply, noncompliance, concomitant medications, medical contraindication/pregnancy, withdraw consent, determination of ineligibility (including screen failure),death, or investigator's discretion. Participants will continue to be followed, if possible, for safety reasons. Participants who stop agent within 6 days of surgery will continue their study visits to collect endpoint data according to the schedule of events, unless the participant withdraws consent for further study procedures.
8.4.2 For the primary analysis (intent to treat, ITT) any participant that received study agent and has tissue available will be included. A per protocol analysis will then be completed which removes noncompliant participants or participants who went off study prior to 4 weeks. For these analyses, participants who complete 4 weeks of study agent will be considered evaluable.
8.4.3 Pregnancy would be considered a medical contraindication to continued use of the study agents. If a pregnancy occurs, the patient will be followed through the resolution of the pregnancy and the outcome of the pregnancy will be recorded. Some pregnancy outcomes are considered to be an SAE (e.g. miscarriage, birth defect) and must be reported following the SAE procedures in section 11.

8.5 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

The primary endpoint is Ki67 labelling by IHC; this is a widely used and validated assay, and no other proliferative assay has reached the same level of methodological or biological validation. It was also the primary endpoint for our prior study (NWU 07-9-02) and will therefore be most appropriate for the present protocol. Dr. Luis Blanco (NU pathologist) will mark the DCIS legion of H&E slides to be evaluated by biomarker IHC assays.

Ki67 IHC staining and scoring will be performed at the Northwestern University Pathology Core Facility.

Secondary endpoints include the Oncotype DCIS Score, which will be performed by Exact Sciences and is also highly standardized and quality-controlled; No other methodology is available for measurement of this parameter.

Other IHC staining and scoring of COX-2, CD68, and p16 will be also performed at the Northwestern University Pathology Core Facility.

Tissue and plasma drug concentrations and hormone levels will be measured at the Hormone Laboratory of Haukeland University Hospital, Norway.

9.2 Comparable Methods

Our assays have been carefully selected with comparability in mind, and all are comparable to prior studies, our own as well as those of other investigators.

10. SPECIMEN MANAGEMENT

10.1 Laboratories

- Pathology Core Facility (PCF) of Northwestern University (specimen receiving, logging, tracking, distribution, sectioning of blocks, H&E staining and IHC marker staining and scoring).
- Exact Sciences (DCIS Score)
- Hormone Laboratory of Haukeland University Hospital, Norway (steroid hormone assays and drug concentration assays on tissue and blood)
- Northwestern University Comprehensive Metabolic Core Lab (IGF-1, IGFBP-3, and SHBG)
- Northwestern Memorial Hospital Hemostasis Lab (coagulation panel– Factor IX and von Willebrand Factor assays)
- Quest Diagnostics (coagulation panel – Factor VIII activity and total Protein S antigen assays)

10.2 Collection and Handling Procedures

10.2.1 Amount of sample

- Blood samples (Screen 1 and Study Visit 1) : Blood samples will be collected with an appropriate type of anti-coagulants, and processed as described in session 7.1 (the schedule of event table footnote #5 and #11). 1) **Two** 2.7mL blue top tubes (citrate) to obtain plasma for coagulation panel assays. Each tube must be a full draw. 1mL each will be aliquoted in a 1.8 mL size cryo-vial. We will make as many aliquots as available. The last aliquot may not be enough for 1 mL, but save it as well and record approximate volume available. 2) **Two** 10 mL lavender top tubes (K_2EDTA) to obtain plasma and buffy coat. We will get approximately 3~ 4 mL plasma from one lavender top tube. 1mL each will be aliquoted in a 1.8 mL size cryo-vial. We will make as many aliquots as available. The last aliquot may not be enough for 1 mL, but save it as well and record approximate volume available. Plasma aliquots will be used for IGF-1 and SHBG assays (Screen 1 and Study Visit 1); for steroid hormone assays (estradiol, progesterone, and dehydroepiandrosterone (DHEA), androstenedione, and testosterone (Screen 1 and Study Visit 1); and for measuring tamoxifen and its metabolites [tamoxifen, N-desmethyl tamoxifen, (E) 4-OHT, (Z) 4-OHT, (E) endoxifen, and (Z) endoxifen] (Only Study Visit 1). Finally, buffy coat will be collected into one 1.8 mL cryo-vial. A total volume of buffy coat will be approximately 1 mL for future polymorphisms assays in tamoxifen metabolism

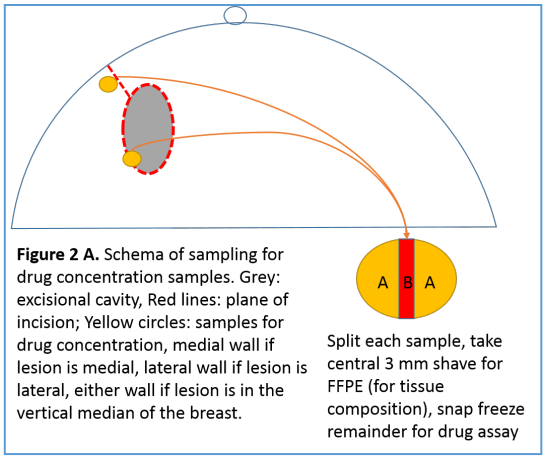

Figure 2 A. Schema of sampling for drug concentration samples. Grey: excisional cavity, Red lines: plane of incision; Yellow circles: samples for drug concentration, medial wall if lesion is medial, lateral wall if lesion is lateral, either wall if lesion is in the vertical median of the breast.

genes (Screen 1 and Study Visit 1). Please refer lab manual for more details on sample handling, storage and shipping.
A minimum of 15 sections will be cut from the core needle biopsy block and the surgical block, 7 for Ki67 and other IHC markers and 8 for DCIS score measurement.

- Drug concentration assays (only for study visit 1): 1) Fresh benign tissue 1x1x1 cm fragments (weight of each fragment is usually 200mg~300mg) from locations defined in **Figure 2**, from surgical cavity, to avoid interfering with margin assessment on the main specimen. The 1x1x1 cm breast tissue sample will be split in half, and a slice of tissue about 3 mm thick, (see Figure 2A, bottom right panel, piece B in red) will be taken from one face, and placed in formalin for at least 6 and at most 72 hours, then embedded in paraffin. The remainder (piece A in yellow) of the fresh breast sample will be flash-frozen for drug assay. 2) An aliquot of plasma from the research blood sample drawn with a 10 mL Lavender top tube (K_2EDTA) at the Day of Surgery (study visit 1) will be to obtain plasma for measurement of circulating drug concentrations. Measurement of drug metabolites (tamoxifen, N-desmethyltamoxifen, E and Z isomers of both 4-OHT and endoxifen) (see the session 7.1, the schedule of event table footnote #9 for details). These fresh frozen tissue samples along with the paraffin block (Sample B in Figure 2), plasma, and buffy coat samples will be sent as a single shipment for each participant (frozen samples on dry ice) to NU from participating sites. FFPE blocks will be shipped in cold condition. At NU-PCF, the Sample B FFPE will be sectioned and stained with H&E to determine the tissue composition of the drug concentration sample. The histology findings from this research sample will not be reported back to clinical teams.

10.2.2 Mastectomy sampling procedures (for subjects who opt for mastectomy)

At five sites (Cleveland Clinic, Mayo Clinic, Duke, St Elizabeth, University of Kansas), the drug concentration samples will be harvested by the grossing pathologist as described above in Section 10.2.1. The location of these two samples will be 1) superficial, one centimeter from the superficial surface of the mastectomy specimen, and 2) deep, one centimeter from the deep aspect of the mastectomy specimen. These samples may be taken from the location that is remote to the known location of the DCIS.

At two sites (NU and MSKCC) the mastectomy specimen will be grossed according to the scheme developed for a previous topical gel study, described above and depicted in Figure 2B.

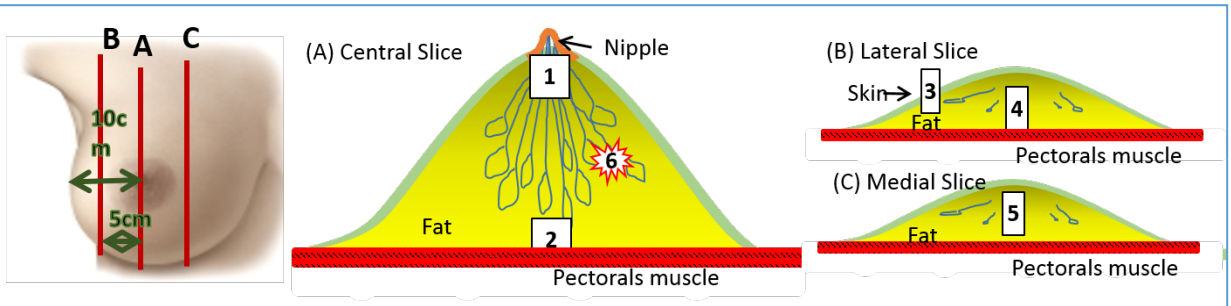

Figure 2B. Mastectomy grossing scheme for measurement of drug concentration at multiple sites
Slice A is through the nipple, slice B is half-way from nipple to lateral edge; slice C is halfway from nipple to medial edge. Samples 1 and 2 are taken from retroroareolar and deepest portion; sample 3 is subcutaneous, lateral; samples 4 and 5 are from the middle of medial and lateral slices. Sample 6 is from tumor, if available; sample 7 is from axillary lymph node, if available.

10.2.3 Paraffin blocks: As each subject is enrolled, the sites will indicate on the pre-enrollment form whether the DCNB was performed at the enrolling institution, or elsewhere. If elsewhere, the name of the

hospital and contact information for the Pathology Department of that hospital will be provided. If DCNB was not done at the enrolling institution, NU-PCF NU PCF will work with the enrolling site as needed to acquire DCNB material from the site where the DCNB was performed. If DCNB was done at the enrolling institution, the study coordinator will place a request for that block to be retrieved as soon as the surgical date is known, to avoid delays caused by block retrieval.

At the end of study participation, for each subject, the paraffin blocks of the DCNB (if done at the enrolling institution) and the surgical samples will be assembled by each site as soon as the final pathology on the surgical sample has been reported. The study consent will include a statement providing permission to the hospitals involved in diagnosis and therapy (which may not be the same) to release the DCNB block and a selected surgical block to NU for study purposes. At NU PCF, the pre- and post-therapy sample blocks will be sectioned at the same time following the completion of participation of each subject. A minimum of 15 sections will be taken for: H&E, Ki67, DCIS-Score and other markers (CD68, COX2, p16), cytokeratin (CK) as epithelial classifier and at least one extra for possible repeats. All sections will be saved, vacuum-sealed and cold, until the conclusion of the study. In this way, pre- and post-therapy sections for each biomarker will be processed in the same batch.

Slide submission for Exact Sciences DCIS-Score: Eight unstained slides of DCBN pre-therapy block will be submitted immediately to GHI (so that the data can be used for radiation therapy decision if needed). Eight unstained slides of post-therapy block will be reserved in the NU PCF and shipped as a single batch after the study is complete and all participant samples have been received at NU.

Institutions that do not allow release of paraffin blocks despite participant consent. These institutions will provide unstained sections of the paraffin block as detailed above.

Institutions that do provide paraffin blocks: these will be returned to the institution where the sample was taken once the protocol-defined sections have been taken, typically within 3 months of submission to NU.

10.2.4 Timing of sample

Specimen is obtained without regard to time of day or fasting, it will be determined by time of study visits. Circulating biomarkers are not significantly affected by time of day.

10.2.5 Labeling of specimen

Labeling will include protocol number and subject ID, 3-letter study site code, date and time of collection, type of sample and sample number as defined in protocol for breast tissue (see above Figure 2B)

10.2.6 Tracking of specimens

Each laboratory will email ncpc@northwestern.edu upon shipment of specimens to another site. Information provided will include PID, visit date, type of specimen, and number of specimens.

10.2.7 Temperature storage requirements

- FFPE samples will be stored and shipped cold
- Fresh tissue will be stored at -80 degrees C and shipped on dry ice
- Blood will be processed and aliquoted to yield serum, plasma, Buffy coat, stored at -80 degrees C and shipped on dry ice.

10.2.8 Storage duration

Samples will be stored until analysis are completed and published, at which point it will be transferred to the DCP repository.

10.3 Shipping Instructions

10.3.1 Shipping of samples to NU

All samples on each participant will be sent in a two simultaneous shipments (one frozen shipping with dry ice, and one cold shipping) to NU following Visit 2. This will consist of i) drug assay samples (A and B in Figure 2A or 1-5 in Figure 2B); ii) pre- and post-intervention blood; iii) pre- and post-therapy paraffin blocks or sections of the paraffin block, from institutions that do not allow release of paraffin blocks. The study consent will specifically include permission for hospitals to release blocks to NU. Thus, as each subject completes participation, her samples will arrive at NU in a single batch, minimizing the chances of missing or misplaced samples.

10.3.2 Shipping of samples from NU

- Unstained paraffin sections from the baseline DCNB sample to Exact Sciences will be sent one at a time (90 shipments).
- Unstained paraffin sections from the post- therapy surgical sample to Exact Sciences will be batch-shipped at the end of the study.
- Plasma samples (lavender top tube) to the Northwestern University Comprehensive Metabolic Core Lab and plasma samples (blue top tube) to the Northwestern Memorial Hospital Hemostasis Lab and Quest Diagnostics will be batch-shipped at the end of the study
- Plasma (lavender top tube) and tissue samples to Hormone Laboratory of Haukeland University Hospital will be batch-shipped at the end of the study.

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

10.4 Tissue Banking

Biologic specimens collected during the conduct of each 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

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

11.1.1.2 AEs not related to surgery that occur from the day of surgery through Study visit 2 (up to 21 days post surgery) must be recorded on the AE CRF (paper and/or electronic) whether or not related to study agent.

11.1.1.3 All SAEs, including all hospitalizations, during the study period will be reported as per DCP SAE reporting procedures, with the following exception:

Hospitalization for planned surgery will not be reported. However, if this hospitalization event lasts longer than the usual period at the institution (as determined by the Site Principal Investigator), it will be reportable as an SAE. Adverse events (AEs) relevant to the prolongation of this hospitalization will be collected and reported on AE CRFs.

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 subject dropped 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.

Grade	Severity	Description
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 patient and may require intervention to prevent one of the other outcomes.

11.2.2 Reporting SAEs to DCP

11.2.2.1 The Lead Organization and all Participating Organizations will report SAEs on the DCP SAE Report Form found at http://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.

Name: Marjorie Perloff, MD
Address: Division of Cancer Prevention
National Cancer Institute
9609 Medical Center Drive, 5E544
Rockville, MD 20850
Tel: (240) 276-7097 (during normal business hours)

Cell: (240) 731-1772
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 The Lead Organization and all Participating Organizations will email written SAE reports to the following recipients within 48 hours of learning of the event using the fillable PDF SAE Report Form

- DCP's Regulatory Contractor, CCS Associates, email (safety@ccsainc.com)
- Lead Organization (Northwestern University), email (nccg@northwestern.edu)
- BHR Pharma, LLC, email (safety@bhr-pharma.com)
- Besins Pharmacovigilance, email (pharmacovigilance@besins-healthcare.com)

11.2.2.4 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.

11.2.2.5 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 the Lead Organization (ncpc@northwestern.edu) and BHR Pharma, LLC (safety@bhr-pharma.com).

12. STUDY MONITORING

12.1 Data Management

Data will be managed by the study statistician, Dr. Kocherginsky, according to standard operating procedures, which meet the guidelines of DCP Requirements for Data Management and which follow the Data Management Plan that Northwestern University has on file with the Division of Cancer Prevention, NCI. The Consortia 2012 Data Management Plan, submitted as part of a contract agreement with the NCI (HHSN261201200035I), was approved.

12.2 Case Report Forms

Participant data will be collected using protocol-specific case report forms (CRFs) developed from the standard set of DCP Chemoprevention CRF Templates and utilizing NCI-approved Common Data

Elements (CDEs). The approved CRFs will be used by Northwestern University to create the electronic CRF (e-CRF) screens in the Robert H. Lurie Comprehensive Cancer Center of Northwestern University (Lurie Cancer Center) Clinical Trials Management System (CTMS). Accrual-site staff will enter data into the e-CRFs for transmission to DCP according to DCP standards and procedures.

12.3 Source Documents

All source documents will be collected and stored in the Clinical Research Office of the site where the participant was accrued. Any data recorded directly on CRFs that constitute no prior written or electronic record of data, will be specifically identified as source data. BESS questionnaires completed in person may be completed directly on the paper CRF and need not be transcribed from separate source documentation.

12.4 Data and Safety Monitoring Plan

A comprehensive Data Safety and Monitoring Plan has been submitted by Northwestern University, approved by the DCP, and is on file there. Any future changes will be forwarded for review.

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 patient participating on 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

13.1 Study Design/Description

This is a Phase IIB randomized, double blind clinical trial testing oral tamoxifen against 4-OHT gel in women with DCIS of the breast. A total of up to 108 women will be accrued from seven institutions, and randomized 1:1 (4-OHT gel: oral tamoxifen). Oral tamoxifen is the standard of care for medical therapy of DCIS, 4-OHT gel is expected to be as effective but less toxic, and therefore a randomized comparison is needed. The blinding will be maintained by the study statistician (Dr. Kocherginsky), who will develop the randomization schema and communicate directly with study pharmacies regarding subject assignment. Unblinding will occur for medical reasons if approved by the DCP study monitor (Dr. Perloff).

13.2 Randomization/Stratification

We will consider the following strata: 1) Recruitment site, of which there will be seven, and 2) Pre- or post-menopausal status, within each site. A maximum of 108 participants with DCIS of the breast, newly diagnosed on core needle biopsy, will be accrued into two intervention arms: oral tamoxifen 20 mg daily, and 4-hydroxytamoxifen gel, 4 mg daily (2 mg to each breast). Due to early drop-out after randomization, we expect no more than 100 women to start intervention.

Of the women who start intervention, we assume an attrition of 20 women (20%) for study drop-out, or absence of residual DCIS in the surgical specimen, so that a total of 80 women (40 in the gel arm and 40 in the oral arm) will be evaluable for the primary study endpoint, and between 80 and 90 will be available for secondary endpoints. Assuming a screening rate of approximately 60 participants per month and an average accrual rate of 4.5 participants per month, we expect the recruitment to be complete within 24 months.

The patients will be enrolled at 7 centers:

- Northwestern University/Northwestern Medicine (NU)
- Duke University Medical Center (DUMC)
- Memorial Sloan Kettering Comprehensive Cancer Center (MSKCC)
- Cleveland Clinic Comprehensive Cancer Center (CCCCC)
- Mayo Clinic Rochester (MCR)
- St Elizabeth Hospital, Kentucky (SEH)
- University of Kansas Cancer Center (KUCC)

Randomization assignments will be provided for each combination of strata (site, meno status) in blocks of four. Excess blocks may be provided as it is not clear whether meno/post meno strata will be balanced.

13.3 Accrual and Feasibility

13.3.1 *Sample Size*: We plan to accrue up to 108 subjects over 24 months. Therefore the monthly rate in the base period is 4.5. The distribution across institutions may be revised as the study progresses. The last participant will go off-treatment in month 27, and complete 30-day follow-up in month 28.

13.3.2 *Gender distribution*: will be entirely female. Although men do develop DCIS, the number is extremely small and it is unlikely that any men with DCIS will be seen at the recruiting sites over an 18 month period. Furthermore, the skin permeation characteristics of 4-OHT gel has never been studied in men, and may be different given different skin thickness in men and women. Finally, the target population for the study intervention is exclusively women; breast cancer prevention therapy for men is not a practical objective at this time.

13.3.3 *Minority populations* will be recruited actively. Minority populations at each site are

- NU, 25.6% of the DCIS population are of non-European ancestry (18% African, 4% Hispanic, 3.5% other).
- Duke has high fractions of African American women with DCIS (24%).
- At Cleveland Clinic, 18% of DCIS patients are AA, 4% other minorities, 78% European American (EA).
- At MSKCC, during 2014, DCIS patients were: 73% EA, 24% minority (12% black, 6% Asian, 6% Hispanic), 4% other or unknown.
- Mayo Rochester has a largely European-American population.
- St Elizabeth's Kentucky also has a largely European-American population.
- At KUCC, approximately 13% of DCIS patients are African American, 1% Hispanic, 1% Asian, and the rest are European-American.

13.4 Primary Objective, Endpoint(s) and Analysis Plan

13.4.1 Hypothesis and Primary Objective:

We hypothesize that the application of 4-OHT gel to the breast at a dose of 2 mg per breast daily will result in a decrease in the Ki-67 labeling index of DCIS lesions that is comparable to the decrease seen with use of oral TAM. Further, that this will occur with 4-OHT concentrations in the breast that are significantly higher than circulating levels. The primary objective of this study is to demonstrate that topical transdermal 4-OHT is not inferior to oral tamoxifen in decreasing Ki-67 labeling index (LI) of DCIS lesions. In the control (oral TAM) group we expect the mean relative decrease in Ki-67 LI in the surgical specimen to be 50% compared to the diagnostic core biopsy samples after therapy. In the study agent group we will accept a relative mean decrease of at least 35% as evidence of non-inferiority.

13.4.2 Proposed Sample Size:

A total of up to 108 women will be accrued and randomized over an 18 month period (1:1 oral:gel), with no more than 100 women to start intervention. Assuming a 20% rate of nonevaluable samples due to insufficient residual DCIS, we expect a total of 80 subjects evaluable for the primary endpoint of Ki67, comparing pre to post-therapy samples. Primary analysis will be conducted in all randomized evaluable patients per intent-to-treat. Additional analyses will be conducted in the per-protocol population of patients who were treatment-compliant as defined in Section 5.7. Adverse events will be evaluated in all patients who received at least one treatment dose.

Sample size calculations: From previous publication (Table 2, Lee et al.) we have an average percent staining for Ki67 at baseline of 7.5% with SD=5.4%. Assuming within-subject correlation between pre- and post-treatment values is ρ=0.5, the pooled SD of the changes will be SD_p=5.4%. A 50% drop (from 7.5%) gives 3.75% while a 35% drop (from 7.5%) gives 4.9% in Ki67 staining. If the drop in the 4-OHT group is non-inferior to the standard of care, we would see it. We use compatible SD from Table 2 in Lee et al, obtaining for the 4-OHT group adequate power to detect a non-inferiority of 35% or bigger drop with a small type 1 error of 10%. Now 2.6% staining. Thus, with a non-inferiority margin M=2.6%, sample size of n=40 evaluable patients per arm will provide 80.5% power with one-sided $\alpha = 0.10$ significance level to detect non-inferiority based on a two-sample equal variance t-test comparing changes in Ki67% staining. Calculations were done using PASS 11.

13.4.3 Statistical analysis plan for primary endpoint:

The primary objective is to estimate and compare changes in %Ki-67 expression from the pre-treatment specimen to the post-treatment specimen between women who received oral Tamoxifen + placebo gel compared with those who receive 4-OHT gel + oral placebo. When using a continuous outcome measured at baseline and after treatment in a randomized trial, it is recommended to use an analysis of covariance (ANCOVA) to model[55, 56]). In addition, the two-sample t-test comparing the change score Y_1 - Y_0 vs.

treatment is prone to incorrect interpretations if the correlation between Y_1 and Y_0 is high, and the ANCOVA model is preferred in this instance.

In this study, we will use the model $Y_1 = \alpha + \beta Y_0 + \gamma G + \epsilon$, where Y_1 and Y_0 are the post- and pre-treatment %Ki-67 expression, and G = 0 or 1 indicates whether the subject belongs to the treatment (4-OHT) or the active control group. This model will be used to test whether the difference between treatment arms (γ) exceeds the pre-specified non-inferiority margin by testing H_0: $\gamma \geq M$ v.s. H_1: $\gamma < M$, where M=2.6% is the pre-specified non-inferiority margin. In order to perform such test, we will modify the outcome as $Y_1^* = Y_1 - M$ when G = 1, or $Y_1^* = Y_1$ otherwise. Thus, the model becomes $Y_1^* = \alpha + \beta Y_0 + \gamma^* G$, and $\gamma^* = \gamma - M$. This corresponds to the hypothesis tests H_0: $\gamma^* \geq 0$ v.s. H_1: $\gamma^* < 0$, which can be obtained from the standard regression model output in a software package. The model will also adjust for the stratification variable menopausal status. The above-described ANCOVA model is different from the non-inferiority test used for the power analysis; however, the power for the ANCOVA models is likely to be greater than power for a t-test comparing change scores [57]. %Ki-67 expression may be may be transformed (e.g. using log-transformation) to satisfy the normality assumption.

13.5 Secondary and Exploratory Objectives and Analysis Plans

13.5.1 Secondary endpoints (efficacy)

1. Oncotype DCIS-Score (validated RT-PCR assay for 12 genes). We expect that changes in this Score will be non-inferior in the gel arm.
2. Breast tissue and plasma levels of TAM and its metabolites [N-desmethyl tamoxifen (NDT), (E) and (Z) isomers of 4-hydroxytamoxifen (4-OHT), N- desmethyl-4-hydroxytamoxifen (Endoxifen)]. We expect that plasma levels will be different between arms, with higher levels in the oral arm but tissue levels will be similar between arms.
3. Ki67 LI in terminal duct lobular units, evaluated in the same manner as the primary endpoint (KI67 LI in the DCIS lesions). We expect that reduction in TDLU Ki67 LI will be non-inferior in the gel arm.

13.5.2 Secondary endpoints (patient-reported symptoms and adverse effects)

4. Plasma proteins involved in coagulation: Factors VIII and IX, von Willebrand Factor, total protein S. We expect that these levels will be different between arms, higher Factor VIII activity and Factor IX, von Willebrand Factor levels but lower total Protein S levels in the oral arm
5. Plasma markers of systemic estrogenic effect (IGF-1, IGFBP-3, and SHBG). We expect that these levels will be different between arms, with significant decreases in IGFI and increases in IGFBP-3[58] and SHBG in the oral arm but not in the gel arm.
6. Patient reported symptoms (BESS Questionnaire): This is a validated instrument that captures patient reported symptoms in a standard format. These data will be compared across arms as reported previously [59]. We expect that these levels will be different between arms, favoring the gel arm.

13.5.3 Exploratory endpoints

7. IHC markers: CD-68 macrophage marker as a surrogate for response to therapy. p16 and COX-2 We expect that changes in these biomarkers will be non-inferior in the gel arm.
8. Plasma levels of steroid hormones (estradiol, progesterone)]. We expect that changes in these biomarkers will be greater in the oral arm.
9. Breast tissue of steroid hormones (estradiol, progesterone) between arms. We expect that changes in these biomarkers will be greater in the oral arm.
10. Fraction of subjects with no residual DCIS in the surgical specimen. We expect that the fraction of subjects with no resual DCIS will be similar in both arms.

13.5.4 Analysis Plan for Secondary Endpoints: Secondary outcomes will be summarized by

treatment arm using descriptive statistics, including mean and confidence interval, or median and interquartile range for continuous outcomes, and proportions and confidence intervals for categorical variables. Outcomes will be compared between arms, with two main types of analyses being conducted: noninferiority testing and testing for differences between arms, depending on the specific hypotheses for each outcome. Noninferiority analyses. Similar to the primary outcome, we expect changes in secondary efficacy outcomes (Oncotype DCIS-Score) to be similar (noninferior) in the 4-OHT gel arm vs. oral tamoxifen arm. Because preliminary data necessary for setting noninferiority margins are not available for all endpoints, we will use a strategy similar to how the non-inferiority margin was set for the primary endpoint, i.e. we will set the margin M to correspond to a 35% change from the average baseline value observed among all patients (i.e. pooling across both study arms). These biomarkers will be compared between arms using ANCOVA models similar to those described for the primary endpoint, with post-treatment measurement as the outcome variable, and with treatment arm and baseline value as predictors, and testing H_0: $\gamma \geq M$ vs. H_1: $\gamma < M$ as described above. Treatment differences. Continuous endpoints for which treatment differences are expected post-treatment (breast and plasma levels of TAM and its metabolites; plasma proteins involved in coagulation; plasma markers of systemic estrogenic effect; and BESS questionnaire) will be compared between arms using a two-sample t-test. Normality assumption will be checked, and data may be transformed (e.g. logarithmically) to satisfy the normality assumption. If no suitable transformation can be found, groups will be compared using the Wilcoxon ranksum test. Additional exploratory analyses will include linear regression models and will adjust for additional covariates. Outcomes for which changes are expected to be different between arms will be analyzed using ANCOVA models as described for the primary endpoint, but testing whether the treatment effect is 0 rather than the non-inferiority margin M, i.e. H_0: $\gamma = 0$ vs. H_1: $\gamma \neq 0$. These tests will be two-sided. Tests of these pre-specified hypotheses will not be adjusted for multiple comparisons, and will be interpreted accordingly.

Adverse Events. AEs will be assessed using CTCAE. Worst grade toxicity for each AE type will be summarized by arm using descriptive statistics. For each AE type, proportions of patients experiencing toxicity of any grade will be compared between arms using Fisher's exact test.

Treatment Compliance. Compliance will be defined as described in Section 5.7. Each patient will be determined as compliant or non-compliant for each treatment component (capsules compliance and gel compliance). Treatment compliance rates based on active ingredient component will be summarized using proportions, and compliance rates will be compared between arms using Fisher's exact test. In addition, in order to determine whether compliance is different for capsule vs. gel administration, within-subject differences in treatment component compliance will be analyzed using the McNemar test among all patients, regardless of treatment arm.

Subgroup analysis. Exploratory analyses will be conducted by menopausal status (pre or peri-menopausal vs. post-menopausal). Descriptive statistics will be used to summarize study endpoints by group. In addition, linear regression models as described above will be used with the addition of menopausal status and possibly its interaction with treatment as predictors. Model-based linear contrasts will be constructed to test relevant treatment noninferiority or superiority hypotheses within subgroups. Similar analyses will be conducted in the subgroup of women with invasion in the post-treatment surgical sample.

13.5.5 Analysis Plan for Exploratory Analyses. Exploratory endpoints will be analyzed similarly to the primary and secondary endpoint analysis, driven by the hypotheses regarding treatment differences or between arms. In an additional exploratory analysis we will examine correlation between mRNA Ki67 expression and Ki67 IHC staining in a subset of study participants with both assays. Graphical tools will be used and data will be summarized using a correlation coefficient. Linear regression models with ki67 IHC assay as the outcome and mRNA ki67 assay as the predictor may be used to further examine the relationship, e.g. if the association appears nonlinear. Linear mixed effects models may also be used if paired assays are performed at both pre- and post-treatment, and will include subject as the random effect to account for the within-subject correlation between the two time points. This analysis will guide planning of future trials and is not intended as a formal endpoint for the present trial.

Pathologic response: Another endpoint of interest is the evaluation of pathologic response, complete (no residual DCIS in the surgical sample). If there is no residual DCIS in the surgical sample, this could mean that the DCIS was small enough at diagnosis that it was ablated by the core needle biopsy; or that the residual DCIS responded completely to therapy. With our present design it is not possible to distinguish between these two possibilities, but we will explore the possibility of pathologic complete response (pCR) in the following ways: the rate of "no residual DCIS" in clinical series and in our institution is about 10%. If we observe that the rate of no residual DCIS is doubled to 20%, we will take this as preliminary evidence of pCR.

13.6 Reporting and Exclusions

Reporting and Exclusions: We expect that 80 subjects will have complete data on the primary endpoint, while about 28 subjects out of 108 (roughly 25%) will be excluded because of not starting study medication, study drop-out, or insufficient DCIS in the surgical specimen. Main analysis will be based on the complete data. Additional descriptive analysis may be performed to see if drop-outs show a systematic property. Thus, we expect the 20% of data missing values will be studied to determine possible patterns and dependence with other relevant variables.

13.7 Evaluation of Toxicity

All participants will be evaluable for toxicity from the time of their first dose.

13.8 Evaluation of Response

13.8.1 *Intent to treat analysis* All samples from all subjects who receive drug will be evaluated and included in the primary analysis, which will be based on intent to treat principle. Per protocol analyses will then be performed as described in Section 8.4.2.

13.8.2 *Missing data* The general approach will be to perform a complete case analysis when the missingness rate is low, and when a missing completely at random (MCAR) or missing at random (MAR) assumption can be reasonably made. To determine whether such assumptions are reasonable, reasons for data missingness will be documented, and clinical and demographic characteristics will be compared between patients with and without missing data for each particular endpoint. If the missing data rate is relatively high but the MAR assumption is reasonable, complete case analyses will be done and carefully interpreted given high missing data rates. To assess the robustness of findings, sensitivity analyses will be performed, including covariate-adjusted regression models, and "best-worst" analyses where all missing values are replaced by either best or worst values for categorical outcomes, and by highest or lowest observed values for continuous outcomes. Multiple Imputation. If data is missing for > 10% of the entries, multiple imputation may be performed via a semiparametric approach based on association between continuous biomarker variables, between binary variables, and between continuous with binary and categorical data [see refs 52-55]. In these approaches, missing data are filled in by plausible values obtained from joint modeling. Observed data are first transformed to normally distributed values so as to satisfy the normality assumptions of the joint modeling approach. Imputed data are then transformed back onto the original scale of the observed data. Imputations are run until convergence criteria are met depending on pairwise correlations between the variables imputed. Performance of the multiple imputation will be assessed via standardized biases (< 50% being acceptable), coverage rates (> 90% being acceptable), small root mean square errors, and average widths of 95% confidence intervals for the estimates from imputed data being comparable to the widths of 95% confidence intervals for the original data pertaining to the pairwise correlations. The goal for the imputed data is to satisfy the assumptions from our power analysis and ensure the convergence of parameter estimation.

13.9 Interim Analysis

None planned.

13.10 Ancillary Studies

None 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. CVs or biosketches do not need to be updated for participating study staff after drug shipment authorization (DSA). “

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 Good Clinical Practice training 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 Federalwide Assurance (FWA) number for the Lead Organization and all Participating Organizations.

14.2.6 Signed Investigator’s Brochure/Package Insert acknowledgement form

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

14.2.8 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 Central IRB. 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 Central IRB prior to implementation

14.4 Informed Consent

All potential study participants will be given a copy of the Central IRB-approved Informed Consent to review. The study coordinator 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, he/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. Subjects 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 Central IRB, 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 Central 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

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

All research related costs associated with participating in this study will be paid for, and will not be the responsibility of the participant. However, it is possible that injury may result from participating in this study. Any expenses incurred as a result of research related injury will be the responsibility of the study participant and/or their insurance carrier.

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CONSENT FORM
Protocol Version Date: v5.16 09/03/2021

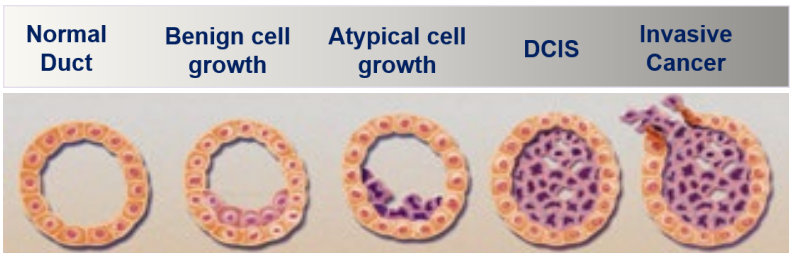
Study Title for Study Participants: Testing an active form of tamoxifen (4-hydroxytamoxifen) delivered through the breast skin to control ductal carcinoma in situ (DCIS) of the breast.

Official Study Title for Internet Search on http://www.ClinicalTrials.gov: NWU2015-06-04 Phase IIB pre-surgical trial of oral tamoxifen versus transdermal 4-hydroxytamoxifen in women with DCIS of the breast.

Introduction

This is a clinical trial, a type of research study. Your study doctor will explain the clinical trial to you. Clinical trials include only people who choose to take part in the research. Please take your time to make your decision about volunteering. You may discuss your decision with your friends and family. You can also discuss this study with your health care team. If you have any questions, you can ask your study doctor for more of an explanation. You should only agree to participate in this study when you are comfortable enough with the information so that you can make an informed decision about joining.

What is the usual approach to my DCIS?



DCIS of the breast is a condition where tumor cells have started to grow inside the breast ducts but have not broken through the duct wall or spread to other organs. The usual course of DCIS varies; sometimes it may grow through the duct wall if left alone long enough, and other times it does not. **The usual treatment for DCIS includes surgical removal of the diseased area of the breast (lumpectomy or mastectomy). Lumpectomy is usually followed by radiation to the breast, and if the DCIS cells have an estrogen-binding protein (estrogen receptor or ER), tamoxifen may be prescribed to help prevent recurrence (return) of the DCIS in the breast. Tamoxifen also reduces the risk of new breast cancers arising in the future in both breasts.** These benefits of tamoxifen are well-proven and have been established in studies with more than 10 years of follow-up.

You are being asked to take part in this study because you have been diagnosed with ER positive DCIS of the breast and are at increased risk for future invasive breast cancer, which can be decreased through the use of tamoxifen after surgical removal of DCIS, and possibly radiation. Recent studies have questioned whether treatment of DCIS with surgery, radiation, and tamoxifen may represent too much treatment and cause unnecessary harm.

The treatment being tested in this study will take place prior to the scheduled surgery for your condition. We are testing this therapy for DCIS, with the expectation that it will decrease the growth rate of the DCIS cells (tamoxifen has been proven to do this in several studies), and therefore shrink the area of DCIS.

What are my other choices if I do not take part in this study?

If you decide not to take part in this study, you have other choices. For example:

- you may choose to have the usual approach described above,
- you may choose to take part in a different study, if one is available,
- or you may choose to do nothing.

Why is this study being done?

For women who have ER positive DCIS, an important part of the treatment after surgery is the anti-estrogen medication, tamoxifen. Tamoxifen is beneficial (see usual treatment paragraph above); but it is an oral capsule (taken by mouth) and therefore circulates through the whole body, and may cause harmful effects to other organs. The goal of our research is to find a safer way to deliver the benefits of tamoxifen. When tamoxifen is taken by mouth, it is broken down by your liver into many active forms, one of which is 4-hydroxytamoxifen or 4-OHT. Tamoxifen is approved by the Food and Drug Administration (FDA) for DCIS while 4-OHT is not and is therefore considered investigational. However, 4-OHT is a break-down product of tamoxifen, and has been developed as an alcohol gel that can be applied to the breast skin that quickly dries. Early results from two previous studies show that 4-OHT applied to the skin gets into the breast, and blocks tumor cell growth in invasive breast cancer, the next stage after DCIS, as effectively as oral tamoxifen. Although the gel was used for 3-6 weeks in these studies, the results were very encouraging. The goal of the study we are asking you to consider is to show that 4-OHT gel works as well as tamoxifen when taken for 4 to 10 weeks, to reduce tumor cell growth, and also reduces the size of DCIS.

The purpose of this study is to compare the safety and effects of tamoxifen taken by mouth to the effects of 4-OHT gel applied directly to the skin of the breast, in women with ER positive DCIS of the breast. We expect that the tamoxifen capsule and the 4-OHT gel will work equally well to reduce the growth of DCIS cells, but side effects will be substantially less with the 4-OHT gel.

Unlike the capsule, the gel is concentrated in the breasts and therefore very little will be circulating throughout the blood stream and the body. Also, much of the benefit of oral tamoxifen is related to its break-down products, which include 4-OHT. Some women lack the proteins that are responsible for the break-down of tamoxifen. It is possible that the use of the 4-OHT gel will be more effective than the tamoxifen capsule in women who lack these enzymes.

In this study, you will get either a tamoxifen capsule and a placebo gel, or a 4-OHT gel and placebo capsule. The placebo looks like the study drug but contains no medication. There will be about 108 women taking part in this study.

What are the study groups?

This is a randomized study and has two study groups.

Group 1 will receive:

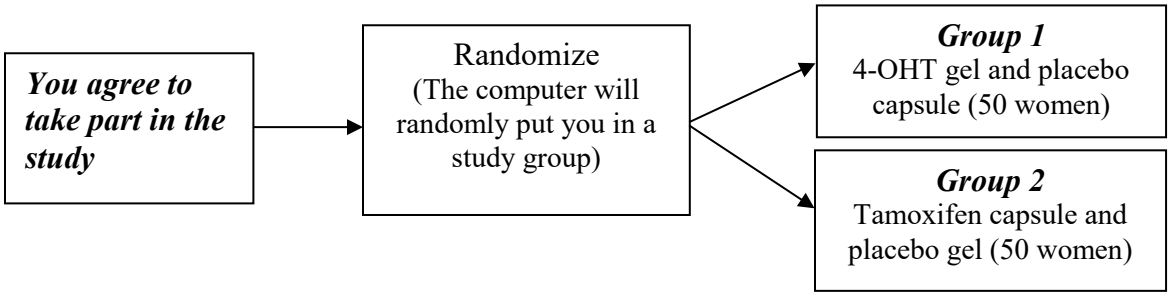
- The 4-OHT gel and will apply the amount in one push of the pump (2 mg) to each breast once every day (preferably in the morning), and
- a placebo capsule that will be taken by mouth once every day at the same time the gel is applied.

Group 2 will receive:

- Placebo gel and will apply the amount in one push of the pump to each breast once every day

(preferably in the morning), and
- a tamoxifen capsule (20 mg) that will be taken by mouth once every day at the same time the gel is applied.

A placebo looks like the study drug but contains no medication. A computer will randomly put you in a study group—like a coin toss—to decide what group you get placed into. This is done because no one knows if one study group is better, the same, or worse than the other group. Your chances of receiving the investigational 4-OHT gel are one out of two; your chances of receiving the FDA approved tamoxifen capsule are also one out of two. Once you are put in a group, you cannot switch to the other group. Neither you nor your doctor will know if you are receiving the study drug or placebo. Your doctor cannot choose which group you will be in.



How long will I be in this study?

If you choose to participate, you will take either the tamoxifen capsule or apply transdermal 4-OHT gel to your breasts every day for 4-10 weeks prior to your surgery. Even if you do not finish the study, your doctor will continue to watch you for side effects and follow your condition up to 56 days after surgery.

What extra tests and procedures will I have if I take part in this study?

Most of the exams, tests, and procedures you will have are part of the usual approach for your condition. However, there are some extra tests (blood draws and surveys) that you will need to have if you take part in this study.

Before you begin the study: You will need to have the following extra tests
- Questions about your medical history
- List of medicines you are currently taking
- Complete a quality of life survey
- Have blood drawn (about 4 tablespoons or 60 mL) to test the hormones in your blood and organ function (how your heart, liver, kidneys, and other parts of your body are working).
- Pregnancy test if you are able to become pregnant.

It is possible that you may not be eligible to participate in remainder of study based on screening procedures.

It is important for the study team to know that you are taking your study drug as directed; we will ask you to keep a study diary to record that you have taken your daily dose. This can be a paper document, or you can opt to use a simple phone app. We will show you how to use the phone app, and if you do not have a smart phone, we can provide you with one for the duration of the study.

In addition, you will receive phone calls to ask how you are doing, and to remind you of events in the study calendar. These will occur at weeks 1, 4, 8 (week 4 and 8 calls as appropriate based on surgery date) and may occur after you have gone off the medication, up to 8 weeks after your surgery.

On the day of surgery:

- Have blood drawn (about 4 tablespoons or 60 mL) to test the hormones in your blood and organ function (how your heart, liver, kidney, and other parts of your body are working), as well as a measurement of the study drug in the blood.
- Quality of life survey.
- List of current medications.
- At this visit you will also return the study drug.
- Pregnancy test
- Breast tissue samples obtained during surgery for measurement of drug concentrations in the breast.

  After the removal of your DCIS is complete, two chick-pea sized pieces of normal breast tissue will be removed during surgery, one close to the skin, and one from the deeper breast. These very small samples will not affect the size or shape of your breast any more than the tissue that needs to be removed for your treatment.

The blood and tissue samples are required in order for you to take part in this study because the research on the sample is an important part of the study. The tissue sample will be used to measure the concentration of tamoxifen and its breakdown products in the breast; the blood sample will be used to measure the concentration of tamoxifen and its breakdown products in the blood.

Breast tissue removed during your core needle biopsy and surgical procedure will be collected by the study team at Northwestern University. This is necessary for us to test the effects of the tamoxifen capsule and the 4-OHT gel on your DCIS. The pathologist routinely embeds your tissue in a wax (paraffin) block for preservation and for examination under the microscope. We would like your permission to obtain these paraffin blocks from the facility where you had you core needle biopsy, and your surgical procedure. Some institutions are reluctant to release the paraffin blocks in case they are needed for future study, but with your permission, we can request these blocks to cut thin shavings (sections) for this study. We will then return the unused portion of the blocks to the institutions they came from, and they will remain there for future tests as needed. If an institution does not allow release of the paraffin blocks, we will request thin shavings (sections) for this study.

Follow up:

The follow up visit for the study will be at your regularly scheduled post-op visit in the clinic to review your progress and you will complete a quality of life survey. The last contact may be within around 5 weeks after your post-surgery visit visit. The date of your last menstrual period (if you are having periods) will be collected during this call.

STUDY VISIT CALENDAR
(Your Screening Visit may occur during one of your treatment planning visits. Visit 2 and 3 will coincide with treatment visits)

What will happen	Visit 1	Phone calls	Visit 2 (day of surgery)	Visit 3 (post-surgery visit)	Follow-up phone call
Agree to participate	X				
Check Eligibility	X				
Medical History	X		X		
Physical Exam, Height and Weight	X		X	X	
Blood sample	X		X		
Pregnancy test	X		X		
Menstrual period date	X		X	X	
BESS questionnaire	X		X		
Check your medications	X		X		
Get study medication same day or a few days later.	X				
Return study medication			X		
Go over your study diary			X		
Go over any side effects			X	X	
Phone calls timed from day you start study treatment (up to 4 total)		Weeks 1, 4, 8 (week 4 and 8 calls based on surgery date)			Within around 5 weeks after your post-surgery visit (for premenopausal women)

What possible risks can I expect from taking part in this study?

There is a low risk that your diagnosis of DCIS may be incorrect and your tumor cells may have broken through the wall of your duct. However, researchers will carefully review your biopsy to lower the chances that you will be invited to participate in this study if that has occurred.

If you choose to take part in this study, there is a risk that you may:

- Lose time at work or home related to the study visits, but we will try as much as possible to schedule these at the same time as your needed medical care.
- Be asked sensitive or private questions which you normally do not discuss (for example in the quality of life survey).
- Experience inconvenience because you have to avoid exposing breast skin to natural or artificial sunlight for the duration of the study.
- Have potential harm resulting from breach of confidentiality.
- You should discuss risks and benefits of participation with your treating physician.

There is also a risk that you could have side effects.

Here are important points about side effects:

- The study doctors do not know who will or will not have side effects.
- Tell the study doctor if you notice or feel anything different so they can see if you are having a side effect.
- The study doctor may be able to treat some side effects.

The tables below show the most common side effects that we know about tamoxifen, some of which may be serious. We expect that the same side effects may result from 4-OHT, but we hope they will be less

common and/or less severe. The studies that have been completed so far suggest that the number of quality of life side effects experienced by women taking 4-OHT gel is about half those experienced by women taking tamoxifen.

Possible Side Effects of tamoxifen

COMMON In 100 people receiving tamoxifen, more than 20 may have
• Hot flashes • Night sweats • Vaginal discharge • Alterations in the menstrual cycle if you are premenopausal

OCCASIONAL, some may be SERIOUS In 100 people receiving tamoxifen, from 4 to 20 may have:
• Cough • Swelling of the hands and feet • Joint pain • Bloody vaginal discharge (you should tell your study doctor even if only a small amount of bleeding occurs because it could be a sign of a serious problem.) • Menstrual changes • Ovarian cysts related to ovulation if you are premenopausal • Depression • Abdominal cramps • Growths in the uterus (can include non-cancerous tumors) which may cause irregular or heavy periods

RARE, SOME MAY BE SERIOUS. In 100 people receiving tamoxifen, 3 or fewer may have:
• Cataract or worsening cataract of the eye (blurring of your vision that slowly gets worse) may happen in fewer than 3 out of every 100 women • Blood clots in the legs (pain, tenderness, or swelling in one or both of your legs) may happen in 1 out of every 100 women. • Blood clots may travel to the lungs (sudden chest pain, shortness of breath, coughing up blood) may happen in 1 out of every 200 women. These are usually successfully treated. • Stroke (sudden onset of: weakness, tingling, or numbness in your face, arm or leg, confusion, trouble speaking or understanding, trouble seeing in one or both eyes, trouble walking, dizziness, loss of balance or coordination, severe headache) may happen in *fewer than* 1 out of every 500 women. • Cancer of the uterus or lining of the uterus may occur in *fewer than* 1 out of every 500 women. • Allergic reaction which may cause itching, rash, low blood pressure, wheezing, shortness of breast, swelling of the face or throat may happen in fewer than 1 out of every 500 women.

- In previous studies, up to 6% of (in 100 people receiving 4-OHT, 6 or fewer) women have experienced skin irritation where the gel has been applied. 4-OHT gel contains 60% alcohol that is flammable so should not be applied near fire, flame, or while smoking. Once dry, the gel is no longer flammable.

Reproductive risks: You should not get pregnant or breastfeed a baby while in this study and for two months after you stop taking tamoxifen. The study medication could be damaging to an unborn baby from conception until the baby is born. The study medication can stop hormonal birth control methods from working. While taking study medication, you and your male partner should use birth control methods that don't use hormones, such as condoms, diaphragms with spermicide, or copper IUDs. Check with the study doctor about what types of birth control, or pregnancy prevention, to use while in this study. If you get pregnant, stop taking study medication right away and call your doctor, and you will be followed to collect data on your pregnancy outcome.

Risks associated with research blood draw: There may be pain from the needle stick, and you may notice a bruise around the vein where your blood is collected. Rarely, some people will feel dizzy or may even faint when blood samples are taken.

What possible benefits can I expect from taking part in this study?

- This study may or may not help you because we do not know how the study drugs will compare to the usual approach for your condition.
- As part of the study, your DCIS tissue will be tested to measure the DCIS-Score by a company in California (Exact Sciences). The DCIS-Score result will be provided to you after surgery and can help you decide (with your radiation doctor) whether breast radiation will be beneficial for you. Some women with a low DCIS-Score may be able to avoid radiation.
- This study will help us learn whether 4-OHT gel is a safer and equally effective alternative to tamoxifen capsules, and this knowledge will help women in the future.

Can I stop taking part in this study?

Yes. You can decide to stop at any time. If you decide to stop for any reason, it is important to let the study doctor know as soon as possible so you can stop safely. If you stop, you can decide whether or not to let the study doctor continue to provide your medical information to the organization running the study.

The study doctor will tell you about any new information or changes in the study that could affect your health or your willingness to continue in the study.

The study doctor may take you out of the study:

- If your health changes.
- If the study is no longer in your best interest.
- If new information becomes available.
- If the study drug is no longer available.
- If you do not follow the study rules.
- If the study is stopped early for any reason by the sponsor, IRB or FDA.

What are my rights in this study?

Taking part in this study is your choice. No matter what decision you make, and even if your decision changes, there will be no penalty to you. You will not lose medical care or any legal rights.

For questions about your rights while in this study, call the National Cancer Institute Central Instiutional Review Board at 888-657-3711.

What are the costs of taking part in this study?

The tamoxifen capsule and 4-OHT gel will be supplied at no charge while you take part in this study. The cost of study-specific blood tests will be paid for by the study, even if you are later determined as ineligible. There might be extra costs that are incurred from the text message reminders from the study, if your phone plan does not cover text messaging.

Some costs associated with your care are standard of care, and will be billed to you or your insurance company. You will have to pay for any costs (including deductibles and co-payments) not covered by your health insurer.

Before you decide to be in the study, you should check with your health plan or insurance company to find out exactly what they will pay for.

The study requires up to three study visits: initial screening visit, the day of your surgery visit, and the post-operative visit. You will be compensated for travel costs and time for these visits to the amount of $100 for each visit that you complete. The maximum compensation that you receive will be $300. If you are provided with a smart phone for use during the study, you are expected to return this at the end of you participation. If you do not return the smart phone, $100 will be deducted from your total compensation at the end of the study.

What happens if I am injured or hurt because I took part in this study?

If you feel you have been injured or hurt as a result of taking part in the study, it is important that you tell the study doctor immediately. You will get medical treatment if you are injured or hurt as a result of taking part in this study.

The study sponsors will not offer to pay for medical treatment for injury. Your insurance company may not be willing to pay for study-related injury. If you have no insurance coverage, you would be responsible for any costs. Even though you are in a study, you keep all of your legal rights to receive payment for injury caused by medical errors.

Who will see my medical information?

Your privacy is very important to us and we will make every effort to protect it. Your information may be given out if required by law. For example, certain states require doctors to report to health boards if they find a disease like tuberculosis. However, we will do our best to make sure that any information that is released will not be able to identify you. Some of your health information, and/or information about your specimen, from this study will be kept in a central database for research.

There are organizations that may inspect your records. These organizations are required to make sure your information is kept private. Some of these organizations are:

- The Institutional Review Board, CIRB, is a group of people who review the research with the goal of protecting the people who take part in the study.
- The Food and Drug Administration and the National Cancer Institute in the US, and similar organizations if other countries are involved in the study.
- The National Cancer Institute will obtain information for this clinical trial under data collection authority Title 42 U.S.C. 285.
- Northwestern University will receive tissue samples from your core needle biopsy tissue and surgical tissue at your final study visit.
- BHR Pharma, LLC and Besins Pharmacovigilance will receive information about side effects you may have.

Where can I get more information?

You may visit the NCI website at http://cancer.gov/ for more information about studies or general information about cancer. You may also call the NCI Cancer Information Service to get the same information at: 1-800-4-CANCER (1-800-422-6237).

A description of this clinical trial will be available on http://www.ClinicalTrials.gov, as required by US law. This website will not include information that can identify you. At most, the website will include a summary of the results. You can search this website at any time.

Who can answer my questions about this study?

You can talk to the study doctor about any questions or concerns you have about this study or to report side effects or injuries. Contact the study doctor _____ *(insert name of study doctor[s])* at _____ *(insert telephone number)*.

This section is about optional studies you can choose to take part in.

This part of the consent form is about optional studies that you can choose to take part in. You will not get health benefits from any of these studies. The researchers leading this optional study hope the results will help other people with cancer in the future.

The results will not be added to your medical records, and you or your study doctor may not know the results. You will not be billed for these optional studies.

You can still take part in the main study even if you say 'no' to any or all of these studies. If you sign up for but cannot complete any of the studies for any reason, you can still take part in the main study.

Circle your choice of "yes" or "no" for each of the following studies.

Optional Sample Collections for Laboratory Studies and/or Biobanking for Possible Future Studies

Researchers are trying to learn more about cancer, diabetes, and other health problems. Much of this research is done using samples from your biopsies, blood, urine, or other fluids. Through these studies, researchers hope to find new ways to prevent, detect, treat, or cure health problems.

Some of these studies may be about genes. Genes carry information about features that are found in you and in people who are related to you. Researchers are interested in the way that genes affect how your body responds to treatment.

If you choose to take part, there may be leftover samples of blood, and tissue from your breast that were collected as part of the study. The researchers ask your permission to store and use your samples and health information for medical research. The research that may be done is unknown at this time. Storing samples for future studies is called "biobanking". The Biobank is being run by the Division of Cancer Prevention and supported by the National Cancer Institute.

WHAT IS INVOLVED?

If you agree to take part, here is what will happen next:

1) A sample from the tissue that was collected at the time of your surgery will be sent to the Biobank.

a) Your sample and some related information may be stored in the Biobank, along with samples and information from other people who take part. The samples will be stored at (*insert name of institution storing samples during study)* until the end of the study, when they may be transferred to the National Institutes of Health.

2) *Qualified researchers can submit a request to use the materials stored in the Biobank. A research committee will review each request.* There will also be an ethics review to ensure that the request is necessary and proper. Researchers will not be given your name or any other information that could directly identify you.

3) Neither you nor your study doctor will be notified if/when research is conducted using your samples.

4) Some of your genetic and health information may be placed in central databases that may be public, along with information from many other people. Information that could directly identify you will not be included.

WHAT ARE THE POSSIBLE RISKS?

1) There is a risk that someone could get access to the personal information in your medical records or other information we have stored about you.

2) There is a risk that someone could trace the information in a central database back to you. Even without your name or other identifiers, your genetic information is unique to you. The researchers believe the chance that someone will identify you is very small, but the risk may change in the future as people come up with new ways of tracing information.

There are laws against the misuse of genetic information, but they may not give full protection. New health information about inherited traits that might affect you or your blood relatives could be found during a study. The researchers believe the chance these things will happen is very small, but cannot promise that they will not occur.

A new Federal law, called the Genetic Information Nondiscrimination Act (GINA), generally makes it illegal for health insurance companies, group health plans, and most employers to discriminate against you based on your genetic information. This law generally will protect you in the following ways:

- Health insurance companies and group health plans may not request your genetic information that we get from this research.

- Health insurance companies and group health plans may not use your genetic information when making decisions regarding your eligibility or premiums.

Employers with 15 or more employees may not use your genetic information that we get from this research when making a decision to hire, promote, or fire you or when setting the terms of your employment. All health insurance companies and group health plans must follow this law by May 21, 2010. All employers with 15 or more employees must follow this law as of November 21, 2009.

Be aware that this new Federal law does not protect you against genetic discrimination by companies that sell life insurance, disability insurance, or long-term care insurance.

3) There are laws against the misuse of genetic information, but they may not give full protection. New health information about inherited traits that might affect you or your blood relatives could be found during a study. The researchers believe the chance these things will happen is very small, but cannot promise that they will not occur.

HOW WILL INFORMATION ABOUT ME BE KEPT PRIVATE?

Your privacy is very important to the researchers and they will make every effort to protect it. Here are just a few of the steps they will take:

1) When your sample(s) is sent to the researchers, no information identifying you (such as your name or social security number) will be sent. Samples will be identified by a unique study code only.
2) The list that links the unique code to your name will be kept separate from your sample and health information. Any Biobank and *(insert name of clinical trials organization)* staff with access to the list must sign an agreement to keep your identity confidential.
3) Researchers to whom *(insert name of clinical trials organization)* sends your sample and information will not know who you are. They must also sign an agreement that they will not try to find out who you are.
4) Information that identifies you will not be given to anyone, unless required by law.
5) If research results are published, your name and other personal information will not be used.

WHAT ARE THE POSSIBLE BENEFITS?

You will not benefit from taking part. The researchers, using the samples from you and others, might make discoveries that could help people in the future.

ARE THERE ANY COSTS OR PAYMENTS?

There are no costs to you or your insurance. You will not be paid for taking part; however, you may receive some funds to defray some of the cost of participating (*e.g.*, parking, child care). If any of the research leads to new tests, drugs, or other commercial products, you will not share in any profits.

WHAT IF I CHANGE MY MIND?

If you decide you no longer want your samples to be used, you can call the study doctor, _____, *(insert name of study doctor for main trial)* at _____ *(insert telephone number of study doctor for main trial)* who will let the researchers know. Then, any sample that remains in the bank will no longer be used. Samples or related information that have already been given to or used by researchers will not be returned.

WHAT IF I HAVE MORE QUESTIONS?

If you have questions about the use of your samples for research, contact the study doctor, _____, *(insert name of study doctor for main trial)*, at _____ *(insert telephone number of study doctor for main trial)*.

Please circle your answer to show whether or not you would like to take part in each option (*include only applicable questions)*:

SAMPLES FOR FUTURE RESEARCH STUDIES:

My samples and related information may be kept in a Biobank for use in future health research.

YES NO

PERMISSION FOR RE-CONTACT:

I agree that my study doctor, or their representative, may contact me or my physician to see if I wish to participate in other research in the future.

YES NO

I agree that my study doctor, or someone on the study team, may contact me or my doctor to see if I wish to learn about results from the study.

YES NO

I agree that my study doctor, or their representative, may contact me or my doctor to see if I wish to learn about the results from this study.

YES NO

This is the end of the section about optional studies.

My Signature Agreeing to Take Part in the Main Study

I have read this consent form or had it read to me. I have discussed it with the study doctor and my questions have been answered. I will be given a signed copy of this form. I agree to take part in the main study. This includes permission for my core needle biopsy and surgical blocks to be used for this research.

Participant's signature _____

Date of signature_____

Signature of person(s) conducting the informed consent discussion

_____

Date of signature_____

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
Diary and instructions for gel use

Study Diary Part I

Protocol Number
Subject ID Name
Site Start date End Date

Dear research participant,
Thank you for participation in this study. It is important that you fill in the diary every day and bring it with you to your visit just before your surgery. If you do not have the diary with you, continue to record information on note paper and copy it onto the diary afterwards. If you lose the diary, please contact the study staff immediately to get another diary.

Please remember to apply the gel every morning. Each canister should be used for only 40 days (even if some gel remains). Your cooperation in this study is greatly appreciated. Bring all canisters back with you at your next visit, whether gel remains in the canister or not.

Canister #1	Date of first use	Date of last use
Canister #2	Date of first use	Date of last use

Study Coordinator's Signature/Date

Study Diary Part II

Protocol Number
Subject ID Name
Site Start date End Date

Day	Monday	Tuesday	Wednesday	Thursday	Friday	Saturday	Sunday
Date							
Time							
How many hot flashes in the past 24 hours above your normal experience?	0 1-5 6-10 11-15	0 1-5 6-10 11-15	0 1-5 6-10 11-15	0 1-5 6-10 11-15	0 1-5 6-10 11-15	0 1-5 6-10 11-15	0 1-5 6-10 11-15
Night sweats in the past 24 hours above your normal experience?	Yes No	Yes No	Yes No	Yes No	Yes No	Yes No	Yes No
Did you apply your gel to both breasts, a total of 2 complete pumps (1 complete pump per breast?	Yes No	Yes No	Yes No	Yes No	Yes No	Yes No	Yes No
Did you discard any pumped doses of gel?	Yes No	Yes No	Yes No	Yes No	Yes No	Yes No	Yes No
If yes, how many pumped doses did you discard?							
Did you take your capsule?	Yes No	Yes No	Yes No	Yes No	Yes No	Yes No	Yes No
Are you having a period?	Yes No	Yes No	Yes No	Yes No	Yes No	Yes No	Yes No

Study Coordinator's Signature/Date

To be repeated, for each week, until end of intervention

GEL APPLICATION INSTRUCTIONS:

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

2. Apply the gel to your own breasts after bathing, preferably in the morning and at approximately the same time each day.

3. To apply, remove the cap from the canister. When you use a canister 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).

4. Once the canister 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.

5. Apply **one dose of gel to each breast** (dosage is indicated on the canister label). Do not apply more or less than one dose to each breast. Be sure to release the pump completely between both actuations.

6. If you accidentally pump twice for one breast, please discard this dose and try again to get one pump. If you do discard pumped doses, please record this on your study diary Part II

7. Spread the gel evenly over the entire surface of your breast, without rubbing.

8. Wash your hands immediately after applying the gel.

9. 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.

10. Do not apply any other cream, lotion or moisturizer to your breasts at any time during the study.

11. 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.

12. After use, replace the cap on the canister.

13. Switch to a new canister of gel every 40 days.

RECOMMENDATIONS:

1. Be sure to apply **after bathing or showering, each day** during the study and preferably in the morning.

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. 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.

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

5. Please note on each canister label, the first and last day of use. Stop using a canister after 40 days, even if the canister is not empty.

6. 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 canister and notify your doctor immediately.

7. After the end of study treatment, be sure to take back to your doctor **all** the gel canisters 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 canisters at room temperature.

2. Keep the gel canisters out of the reach of children.

Instructions for capsules

1. Store at room temperature
2. Keep bottles out of reach of children
3. If you can, take your capsule at the same time that you apply the gel. If you forget to take it at the same time, take it any time that you remember.
4. If you prefer to take the capsule at another time (for example, when you take your other medications) that is OK, as long as you remember to take it daily.
5. It does not matter whether you take it with food or not; there are no specific foods to avoid while you are on the study medicine.
6. If you start any new medications while you are on study, please call your study coordinator. She will check with the study doctor and if needed, with your personal physician so that possible interactions can be managed safely.
7. If you develop hot flashes, here are some suggestions for managing them:
    a. Dress in layers, wear cool clothes at night and keep your bedroom cool.
    b. Avoid hot flash triggers: Alcohol and caffeine; Hot food (either in terms of temperature, spiciness, or both); a warm room or bedroom; hot tubs, hot showers, and saunas.
    c. Try to dress in cotton or linen and avoid wool and synthetic material. Avoid turtle necks.
    d. Keep iced water nearby
    e. A healthy, simple diet without heavy sauces or spices may help
    f. Physical activity may also help… take the stairs, walk the long way to wherever you are going, develop an exercise routine, etc.

APPENDIX C
BESS questionnaire

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

	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
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

	PROBLEM	Not at all	Slightly	Moderately	Quite a bit	Extremely
P25	Low energy	0	1	2	3	4
P26	Blurred vision	0	1	2	3	4
P27	Any other problems?	Please Specify:				

Study Coordinator's Signature: _____ Date: _____

APPENDIX D

Please use the following suggested language for participant communications.

For Phone Calls or email reminders at Week 1, 4, and 8 (week 4 and 8 calls as appropriate based on surgery date) to assess compliance, toxicity, and changes in medication please use the suggested language:

"Hello my name is **[insert name]**, Research Coordinator at [site name/department]. I wanted to check in with you briefly about the NWU2015-06-04 TopTam2 study. Is this a good time to talk?"

If no: "When would be a better time for me to call you?"

If yes: "As part of your study follow up, I am calling you to briefly assess your study compliance and symptoms. Since we last spoke on <<date>>, have you missed your study medication on any days?"

If yes: "How many days did you miss your study medication? On what dates did you miss your study medication?"

"Since we last spoke, have there been any changes to your current medications? For example, a change in dose, stopping a medication, or starting a new medication."
If yes: "Please tell me what has changed."

"Since we last spoke on <<date>>, have you experienced any symptoms?

"Please remember to take your study agent, apply your study gel to both breasts, and fill out the study diary or phone application. Thank you for your time."

For the Last Phone for premenopausal participants (up to 35 days post-surgery), please use the following suggested language:

"Hello my name is [insert name] from [site name/department], regarding NWU2015-06-04 TopTam2 research study. Is this a good time to talk?"

If no: "When would be a better time for me to call you?"

If yes: "Have you had a period since we last met? If so, what was the start date?"

"Thank you for your time and for participating in this study."