Low-dose Tamoxifen For Radiation-Induced Breast Cancer Risk Reduction: A Phase IIB Randomized Placebo-Controlled Trial

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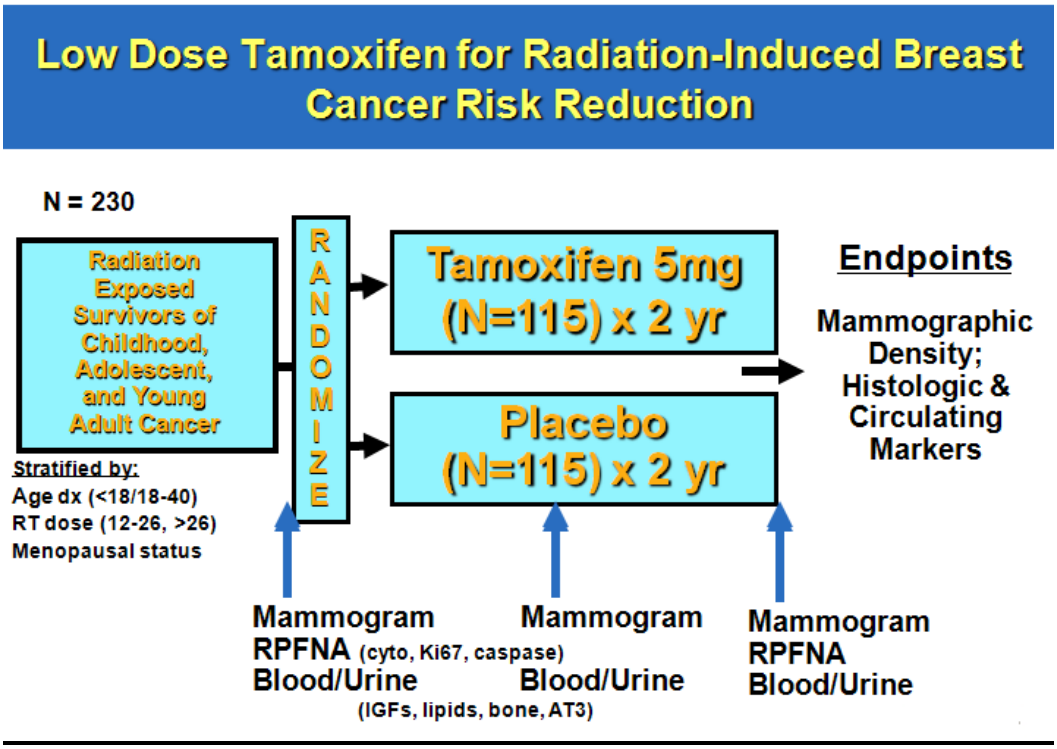
Schema



230 patients will be randomized 1:1 to take a tablet containing 5mg tamoxifen (TAM) versus a placebo of equal size and shape daily for two years. The primary endpoint is mammographic density, which will be measured at baseline and on an annual basis (at Year 01 and 02). Blood and urine collections will also be scheduled annually for measurement of circulating markers of efficacy and safety. Normal breast tissue will be sampled before and after the two-year treatment course for measurement of tissue-based efficacy markers (breast tissue sampling by RPFNA no longer collected from patients).

1.0 OBJECTIVES

1.1 To determine the impact of a two-year course of low-dose tamoxifen administered at 5mg per day on surrogate endpoint biomarkers of breast cancer (BC) risk, including:

- mammographic breast density (MBD), an established radiographic biomarker of BC risk,
- cytomorphology and proliferative index, tissue biomarkers closely linked to BC risk, and
- sex steroid hormones and insulin growth factors, circulating biomarkers of BC risk.

1.2 To establish safety and tolerability of this low-dose tamoxifen regimen, assessing both objective measures (lipid profiles, clotting factors and bone metabolism markers) and patient-reported outcomes.

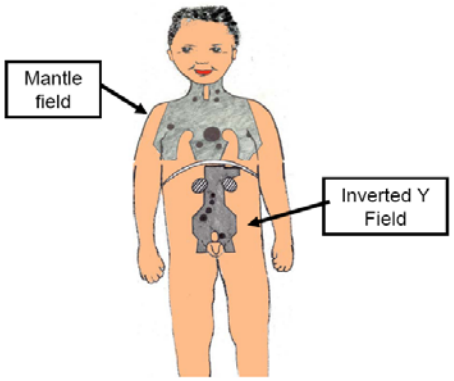
1.3 To examine the modifying effect of demographic, clinical, and molecular characteristics on the risk: benefit ratio from this two-year low dose tamoxifen intervention.

1.4 To explore the relationship between this low-dose tamoxifen regimen and clinical measures of efficacy (new breast cancer and DCIS diagnoses) and toxicity (thromboembolic events, reports of hot flashes and gynecological symptoms, liver function abnormalities, and other cancer diagnoses).

2.0 BACKGROUND AND HYPOTHESES

2.1 **BC risk in radiation exposed childhood, adolescent, and young adult cancer (CAYAC) survivors.** Radiation therapy (RT) is a cornerstone of treatment for several cancers that commonly occur during childhood, adolescence, and young adulthood. These include Hodgkin lymphoma (HL), non-Hodgkin lymphoma (NHL), Ewing sarcoma (ES), rhabdomyosarcoma, and Wilms tumor. Occasionally other lymphomas and sarcomas are treated with RT as well. RT for HL and NHL with bulky mediastinal involvement typically involves the chest. Among sarcomas of the upper extremities treated with RT, the radiation port may include the axilla, from which there may be scatter to the chest wall. While RT for Wilms tumor typically involves the abdomen, occasionally the radiation RT port may cross the diaphragm. RT is also delivered as part of the total body irradiation (TBI) conditional regimen for hematopoietic cell transplant (HCT), commonly used in the treatment of acute leukemia and as salvage therapy for germ cell tumors and other solid cancers that may occur in children, adolescents, and young adults.

Figure 1: Radiation Therapy Fields Used for Hodgkin Lymphoma



Over the past 20 years, the incidence of childhood cancer has increased modestly from 11.5 cases per 100,000 children in 1975 to 14.8 per 100,000 in 2004 [3]. During this same time, however, death rates dramatically declined for most childhood cancers. For instance, 5-year survival rates for all childhood cancers combined increased from 58% in

1975–77 to 80% percent in 1996–2003 [3]. This trend has been attributed to therapeutic advances, including the use of RT.

For example, until the late 1980s, standard treatment of pediatric patients with HL involvement of mediastinal lymph nodes (constituting three-quarters of patients) included RT at a dose of 40 to 45 Gy radiation to the "mantle" field, which encompasses the primary lymph node regions of the neck, supraclavicular, infraclavicular, axillary, and mediastinal areas (Figure 1) [2]. With this treatment, nearly 90% of patients diagnosed with HL, and treated with mantle radiation prior to age twenty survived beyond five years [3]. After the first late effects of HL therapy, such as growth deformities, began to be recognized, the field and dose of RT was reduced and radiation was combined with chemotherapy [4]. The combined modality approach has maintained high cure rates, such that late effects like second malignant neoplasm (SMNs) and cardiovascular disease now account for more deaths than those attributed to progressive HL [5]. In fact, of approximately 120,000 HL survivors in the United States, SMNs are the leading cause of death, with BC the most frequent solid tumor occurring in females treated for HL [6].

Magnitude of risk of BC after radiation exposure: Reported standardized incidence ratios (SIR) or relative risks (RR) for BC in this population range from 11.6 to 136.0, with the highest risk estimates generally from relatively smaller single institution studies [7-9]. From the single institution study with the largest number of women exposed to chest RT prior to 21 years of age (N=307), Wolden *et al* reported a RR of 26.2 [10]. A population-based study by Metayer *et al* reported a RR of 11.6 derived from 2,737 women who were diagnosed with HL prior to 21 years of age identified from nine U.S. NCI SEER registries and selected European countries [11]. Within this cohort, 62 girls and young women diagnosed between ages 10 and 21 subsequently developed BC. Another population-based study, the British Childhood Cancer Survivor Study (British CCSS), which included 16 cases of BC among 383 women who were treated for HL prior to 15 years of age [12], reported a similar SIR of 13.3. In contrast, in an international population-based study, Maule *et al* reported a SIR of 20.9 and an absolute excess risk of 40.6 based on 6 cases of BC that developed in 422 women with a prior diagnosis of HL occurring before age 15 [13]. Bhatia *et al* from the multi-institutional Late Effects Study Group (LESG) reported an excess risk of 55.5 based on 29 cases of BC among 480 women diagnosed with HL prior to age 17 [14]. On the other hand, Kenney and colleagues reported an SIR of 24.7 in women who were treated for a pediatric cancer with chest RT prior to age 21 from the 26-institution U.S. Childhood Cancer Survivor Study (CCSS) [15]. With 95 cases of BC among 6,068 female pediatric cancer survivors, this study had a significantly larger number of women with BC, and thus may provide a more robust estimate. In addition, the risk estimate from the CCSS study included all pediatric cancers prior to BC, with a consequent heterogeneity of radiotherapy doses and fields, while the other risk estimates were based only on HL survivors. Thus, it is clear that the risk of BC is elevated in all radiation-exposed pediatric cancer survivors.

Long latency between radiation exposure and diagnosis of BC: The risk of BC does not begin to increase until approximately 8 years after chest RT, and the median age at BC diagnosis is 32 to 35 years, based on the LESG and CCSS studies [14, 15]. The British CCSS reported that by twenty-five years of follow-up, the cumulative risk of BC was 12.2%.

Risk of BC with attained age: Both the LESG and CCSS studies reported cumulative incidence of BC to be 13.9% and 12.9% by age 40 and 20% and 22% by age 45, respectively. Cumulative incidence rates reported by Travis *et al* ranged from 4.1% to 39.6% by age 30, depending on the age at diagnosis and amount of radiation received

[16]. For perspective, among *BRCA1* or *BRCA2* gene mutation carriers, the estimated cumulative incidence of BC at age 30 ranges from 1-5% and at age 40 ranges from 10-19% [17-20]. Thus, the risk for developing BC among HL survivors appears to exceed that of *BRCA* mutation carriers, especially before age 30 (4-40% in HL survivors compared to 1-5% in *BRCA* carriers). For survivors of other cancers, the risk appears to be elevated approximately 2-fold, similar to the degree of elevated risk for which standard-dose tamoxifen is typically offered. Importantly, in none of the studies of women treated with chest RT was a plateau of BC risk with increasing interval of follow-up observed (Figure 7, page 14).

Risk modification by radiation dose, age, endogenous hormones, and family history: Bhatia *et al* demonstrated preliminary evidence for an increasing risk of BC with a radiation dose of 26 Gy or more [14]. This has been confirmed by the CCSS, where a clear dose-response relationship is evident between the dose of RT to the chest and the risk of subsequent BC down to exposures of 15 Gy (Figure 2) [15]. Using a case-control design, Travis *et al* confirmed that BC risk was related to the dose of RT [21]. Following 23.1-37.1 Gy and 37.2-61.3 Gy radiation delivered to the site where a secondary BC later appeared, the RR was 8.5 and 10.5, respectively. In Figure 3, Travis *et al* clearly demonstrates that BC risk increases with dose of radiation and latency from exposure to radiation. A novel finding in this study was a protective effect of exposure to an alkylating agent (AA) or radiation to the ovaries with respect to the development of BC following chest RT (Figure 3). While still having an increased BC risk compared to population rates, those who received chest RT plus an AA or pelvic RT had a significantly lower BC

Figure 2: Breast Cancer Risk by Dose of Radiation Exposure to the Chest

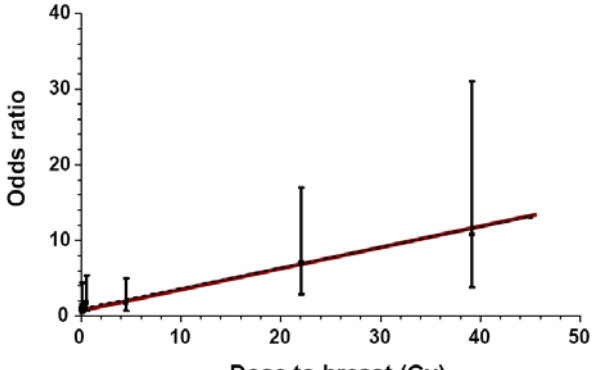


Figure 3: Modification of Breast Cancer Risk by Chest Radiation Dose with or without Alkylating Agent

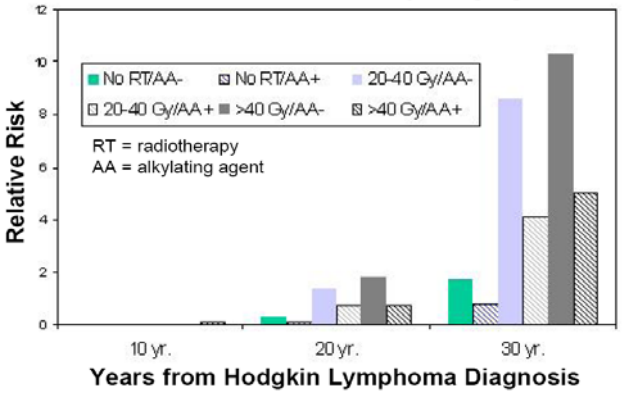


Figure 4: Modification of Breast Cancer Risk by Other Factors

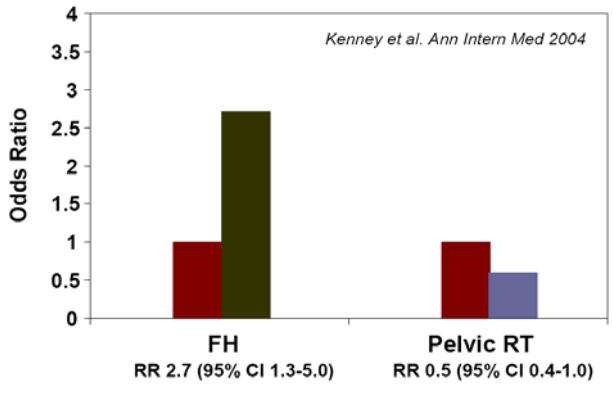
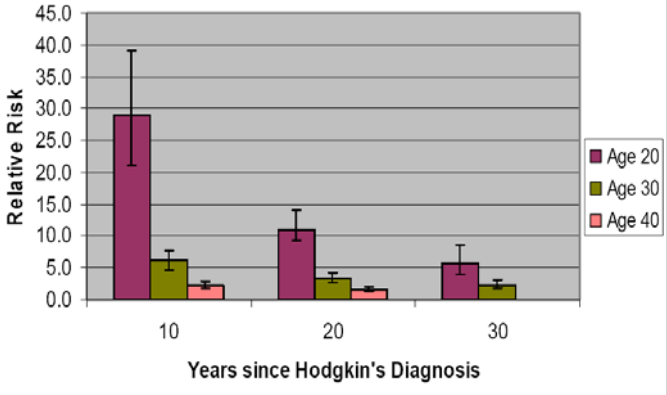


Figure 5: Risk of Breast Cancer By Age at Hodgkin's Diagnosis



risk than women treated with chest RT alone. Hill *et al* reported that parity appeared to contribute to BC risk among women who did not receive ovarian-damaging therapy [22]. The finding of a reduced risk in women with pelvic RT was confirmed in the CCSS (Figure 4) [15]. The findings across these studies highlight that endogenous estrogen may modify BC risk in this population. Although both the CCSS study and the report by Travis *et al* found that a family history (FH) of breast or ovarian cancer was associated with an increased risk of secondary BC among CAYAC survivors exposed to chest RT (Figure 4) [15, 22], multiple regression models suggested that additional risk of BC after chest RT is unlikely to be larger among women with a FH of breast or ovarian cancer. Lastly, age at radiation exposure also is an important risk factor, with chest RT exposures prior to age 30 impacting most significantly on BC risk (Figure 5).

A synergistic effect between radiation and estrogen exposures was, in fact, originally described in a rat mammary carcinoma model in the 1970s [23]. This effect was related to hormonal dose [24], radiation dose [25], and age at radiation exposure [26]. All of these laboratory observations are consistent with the epidemiologic observations among radiation exposed CAYAC survivors described above. Therefore, estrogen appears to play an important role in the etiology of radiation-induced BCs, and an estrogen-blocking intervention is expected to prevent radiation-induced BCs.

2.2 **Current options for BC risk reduction.** In other populations with an elevated risk for developing BC, such as women with a family history of BC, those with reproductive risk factors associated with BC, women with a personal history of premalignant breast disease, or those who are *BRCA* carriers, risk reduction strategies have included surgical and pharmacologic interventions [27].

Surgical interventions: Prophylactic bilateral mastectomy is associated with a 90-95% reduction in risk in women with familial BC [28]. This risk reduction measure has been the intervention of choice in women at high risk for BC who also have high cancer worry; however, not all women feel comfortable with this surgical option [29]. Alternatively, a 50-70% reduction in BC risk has been observed with bilateral oophorectomy, in addition to a 80-90% reduction in ovarian cancer risk [30, 31]. Prophylactic bilateral salpingo-oophorectomy (BSO) is a standard recommendation for women with a germline *BRCA1* or *BRCA2* mutation after they have completed childbearing primarily because of the associated elevated ovarian cancer risk. However, due to increased risks of osteoporosis, dyslipidemia and cardiovascular disease resulting from early menopause, BSO is not a procedure that is recommended for BC risk reduction in other patient populations in which an elevated BC risk is not associated with an elevated ovarian cancer risk (eg. carriers of germline mutations in *PTEN* or *TP53*) [32].

Pharmacologic interventions: Tamoxifen, a synthetic selective estrogen receptor modifier (SERM), was demonstrated to decrease the risk for BC by approximately 50% in women at moderately increased risk for developing BC as defined by the Gail model, a validated statistical tool which estimates the risk of developing BC by age 90 and within 5 years based on the following risk factors: current age, age at menarche, age at first birth, number of first degree relatives with BC, and number of prior breast biopsies [33, 34]. This finding led to the approval of tamoxifen for BC risk reduction by the Food and Drug Administration (FDA) in 1998. Raloxifene, another agent in the SERM class originally developed for the treatment of postmenopausal osteoporosis, was also noted to be associated with dramatic reductions in breast cancer risk [35, 36]. The NSABP P-2 Study of Tamoxifen and Raloxifene (STAR) trial compared the relative efficacy of the two SERMs, tamoxifen and raloxifene, for BC prevention in postmenopausal women at

moderately elevated risk for BC and found that raloxifene was equivalently efficacious but was associated with a better side effect profile [37]. However, raloxifene has only been tested in postmenopausal women, and is only FDA approved for either osteoporosis or BC prevention in women who have reached menopause.

Aromatase inhibitors (AI) are more efficacious and are better tolerated than tamoxifen in the treatment of postmenopausal hormone receptor positive BC. In the adjuvant setting, a reduction in the incidence of contralateral BC was observed for all three currently marketed AIs, anastrozole, letrozole, and exemestane [38-40], suggesting that AIs may also be useful for BC chemoprevention. Clinical trials are now underway to test this hypothesis; however, their efficacy is limited to postmenopausal women, since negative feedback to the hypothalamus and the pituitary further drives, rather than suppresses, ovarian estrogen production in premenopausal women.

Therefore, the only FDA-approved BC chemopreventive option in premenopausal women is tamoxifen. However, tamoxifen administered at a 20 mg daily dose is associated with uterine malignancies, stroke and venous thromboembolism. Although these adverse events occurred in less than 1% of the nearly 13,000 women studied, concerns about these side effects have been a deterrent to the widespread use of tamoxifen at a standard dose of 20 mg for BC chemoprevention. In addition, tamoxifen at this dose results in vasomotor and gynecological symptoms in approximately two-thirds of users, which can interfere with patient compliance with their use [33].

These concerns led some investigators in Italy to evaluate lower doses of tamoxifen in a four-arm double-blind placebo-controlled randomized trial of 210 healthy postmenopausal women, hoping to find a dose that would be better tolerated yet still efficacious for breast cancer risk reduction [41]. Participants were randomized to one of four treatment arms: 1) tamoxifen at 1 mg/day and a weekly placebo, 2) a daily placebo and tamoxifen at 10 mg/week, 3) tamoxifen at 5 mg/day and a weekly placebo, or 4) both daily and weekly placebos for 12 months. Plasma insulin-like growth factor 1 (IGF-I), which is positively correlated with BC risk, was the primary endpoint, evaluated before and after the 12-month treatment. Secondary endpoints included MBD, IGF binding protein-3 (IGFBP-3), fibrinogen, antithrombin-III (AT3), C-telopeptide (CTX), and endometrial proliferation assessed by Pipelle biopsy. MBD is positively correlated with BC risk, while IGFBP-3 is negatively correlated with BC risk. Along with IGF-1, they served as surrogate endpoints of efficacy. Fibrinogen and AT3 are markers of clotting risk, CTX, which is positively correlated with bone resorptive activity, is a marker for osteoporosis risk, and endometrial proliferation is a marker of uterine cancer risk. Collectively, these were safety endpoints in the trial. The investigators reported a significant decrease in IGF-I in all tamoxifen arms relative to placebo ($p = 0.005$), with the greatest change observed in the 5 mg/day treatment arm ($p = 0.019$). Low-dose tamoxifen also lowered MBD and increased IGFBP-3, with optimal effects observed in the 5 mg/day arm. These findings all consistently suggest that 5 mg/day dosing is the most effective dose schedule of low-dose tamoxifen. No significant change was observed with CTX, suggesting that low-dose tamoxifen does not adversely affect bone health. Interestingly, a significant decrease in AT3 ($p = 0.006$), particularly with the 5 mg/day dose schedule, was also seen, suggesting that low-dose tamoxifen may be associated with a lower clotting risk than even placebo. While there was an association between low-dose tamoxifen and endometrial thickness, no association was found with endometrial proliferation measured with Ki67 expression. In fact, Ki67 was lowest in patients receiving the 5 mg/day dose, suggesting that low-dose tamoxifen at that dose would likely not be associated with an increased risk of endometrial carcinoma as is observed with standard dose tamoxifen. Also, menopausal symptoms were not

significantly worsened by low-dose tamoxifen. In summary, low-dose tamoxifen, especially when taken at 5 mg/day is associated with a decreased risk of BC and without the typical adverse effects associated with standard dose tamoxifen.

A follow-up trial randomized 235 premenopausal women with intraepithelial neoplasia (IEN) or 5-year Gail risk >1.3% to tamoxifen 5 mg daily versus fenretinide 200 mg daily in a 2x2 factorial fashion for two years. As with the previous study, a two-year course of 5 mg daily of tamoxifen was associated with a significant decrease in MBD and IGF-1 [42, 43]. Unlike the first study, the second study included long-term follow-up for outcomes, and after a median of 38 months, 24 women developed BC [42]. Trends toward lower annual BC rates were observed in the tamoxifen and fenretinide single agent arms, compared to placebo (2.0, 2.7, and 5.4 per 100, respectively) [42]. Based on the findings in both studies, we believe that a 5mg daily dose of tamoxifen will be a better tolerated, efficacious chemopreventive option for a mixed population of pre- and post-menopausal high-risk women.

A preclinical tamoxifen chemoprevention trial resulted in both decreased incidence and prolonged latency of radiation-induced mammary carcinomas [44]. In this study, female Sprague-Dawley rats were exposed to 300, 500, or 900 cGy total body irradiation (TBI) vs sham and 30 days later half of animals in each radiation treatment group received tamoxifen. Tumor formation after 12 months of follow-up was completely inhibited, and tumor incidence after 24 months follow-up was reduced by 50% in all TBI groups combined. The tamoxifen intervention was most efficacious among animals who received 300 or 500 cGy TBI.

Thus, there is strong preclinical, epidemiologic, and clinical trial evidence supporting our choice tamoxifen to reduce BC risk among young predominantly premenopausal female radiation-exposed HL survivors.

2.3 **Clinical characteristics of radiation-associated BC.** Little information exists regarding clinical characteristics and outcomes of women diagnosed with BC after radiation exposure. In the only peer-reviewed published study evaluating BC outcomes after RT for prior lymphoma – a single institution experience described by Sanna *et al* [45], BCs in 53 women with either a prior diagnosis of HL or NHL treated at the European Institute of Oncology were compared to sporadic BC cases treated at the same institution matched for age, year of diagnosis, stage, and hormone receptor status. The cases were not significantly different in terms of biologic features (eg. Her-2 expression), but there was a trend toward more lobular histology, higher grade, and higher Ki67 proliferation index in the lymphoma-associated breast cancers. Interestingly, Sanna and colleagues reported that in their patient sample, both 5-year disease free survival (54% versus 91% in controls) and overall survival (87% versus 99%) were statistically significantly decreased ($p < 0.0001$ and $p = 0.03$, respectively) for BC diagnosed after lymphoma. Although no significant differences in medical and surgical therapy could be detected overall, anthracycline use was significantly lower among women with node-positive disease ($p = 0.03$). In addition, significant differences in RT among the lymphoma survivors were observed, with over a third receiving intraoperative electron beam radiotherapy, compared to only 10% of controls ($p = 0.0001$). Such treatment differences are likely due to concerns for toxicity due to prior treatment exposures, and probably underscore the differences in outcome, since histologic differences observed were minor. Regardless, the finding of worse clinical outcomes from secondary BC in lymphoma survivors provides a strong rationale to support the importance of finding ways to prevent this

disease in women who have survived HL, who are at enormously high risk for developing BC with limited ability to tolerate full treatment for BC, if it occurs.

Because the control group for the patient cohort described by Sanna and colleagues was matched for hormone receptor status, nothing can be said about ER expression in HL-associated BC compared to sporadic BC. Only three studies have reported hormone receptor expression and are listed in Table 1. In all three studies, ER expression data was available on less than half of BC cases. Nevertheless, the two larger studies, including the CCSS study and one case series from Stanford, report a similar proportion of ER-expressing breast tumors among cases of secondary BC after HL compared to what would be expected with sporadic BC [10, 15]. Furthermore, several animal models have demonstrated no reduction in ER expression among radiation-induced rodent mammary tumors [46, 47].

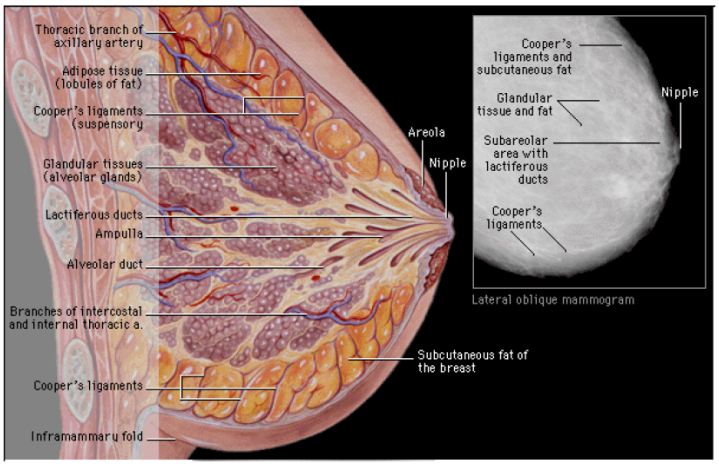
In addition, as reviewed above, it has been observed that CAYAC survivors who had received chest RT plus an alkylating agent or radiation to the ovaries, both of which are associated with premature menopause, had a significantly lower BC risk than CAYAC survivors treated with chest RT without these exposures [15, 16]. Similarly, surgical menopause after BSO has been shown to decrease sporadic and BRCA-associated BC [30, 31]. Thus, it is expected that a hormonal intervention would be appropriate for chemoprevention of radiation-associated BC, just as it is for sporadic and *BRCA*-associated BC [48].

Table 1: Breast Cancer After Hodgkin's Disease – Tumor Characteristics

Patient Population	Female BC cases	Average age at BC dx	Histology	Grade	Stage	ER/PR	Reference
MSKCC, Breast Service records 1969-1991	45	43	IDC 31 (69%), ILC 2 (9%), mixed 1 (2%), colloid 2 (9%), medullary 1 (2%) DCIS 7 (16%), LCIS 1 (2%)	Of n=31: Grade 1=1 (3%), Grade 2=21 (68%), Grade 3=9 (29%).	NR	Of n=18: 8 (44%) positive	[49]
Stanford Hodgkin Disease radiation oncology clinical database 1960-1997	71	42.6	IDC 60 (85%), ILC 2 (3%); DCIS 8 (11%), LCIS 1 (1%)	Of n=50: Grade 1=9 (13%), Grade 2=32 (45%), Grade 3=19 (27%)	Of n=56 invasive: 31 (55%) stage I, 18 (32%) stage II, 5 (9%) stage III, & 2 (4%) stage IV.	Of n=30: 19 (63%) positive	[50]
26-center CCSS 1970-1986	111	35	IDC 77 (86%), ILC 4 (4%), mixed 3 (3%), phylloides 2 (2%), angiosarcoma 1 (1%), fibrosarcoma 1 (1%)	NR	Of n=66 invasive: 30 (45%) Stage I; 27 (41%) Stage II; 4 (6%) Stage III; & 5 (8%) Stage IV	Of n=37: 28 (76%) positive	[15]

2.4 **Biomarkers of BC risk reduction.** Because Phase III prevention trials require very large numbers of participants in order to show significant differences in clinical endpoints, many chemoprevention trials rely on biomarker endpoints [51]. There are several promising markers of BC risk. Mammographic breast density (MBD), proliferative index measured by Ki67 expression, and intra-epithelial neoplasia (IEN, includes lobular carcinoma in situ (LCIS), atypia and hyperplasia without atypia) are all considered biologically plausible surrogate endpoint biomarkers which both have a strong statistical association with BC and have been shown to be modulated by BC prevention intervention strategies [52]. Of these, the marker most easily and reliably measured is the radiographic marker, mammographic breast density (MBD).

Figure 6: Breast Anatomy as Visible on Mammogram



A normal mammographic image shows the diffuse soft tissue density of glandular tissue (relatively white) dispersed within fatty structures (relatively dark) organized by Cooper's ligaments, all surrounded by a thin skin line.

Mammographic breast density. On mammographic images, fibroglandular elements of the breast appear white, or dense, on a dark background of fatty tissue (Figure 6). Multiple epidemiologic studies have demonstrated an association between increased MBD and increased BC risk [53-60]. A meta-analysis reported that high MBD was associated with a nearly 4-fold increased risk in BC [61]. The earliest studies of MBD used a semi-quantitative scale, and reported an increased risk of BC in individuals with the highest MBD. Specifically, Boyd *et al* found that women with dense breast tissue in > 75% of their breast had an odds ratio (OR) of up to 6.0 (95% confidence interval: 2.5-14.1) [62]. However, when three independent radiologists performed the readings, the ORs were 6.0, 3.7, and 2.8 for each of the readers. This suggests that MBD is a strong risk factor for BC, but that the semi-quantitative measurement has a large degree of variation between readers. Such variations led to the development of more quantitative measurements of MBD. Three separate groups have developed techniques to assess MBD using digitized mammograms [63-65]. These techniques allow for quantitative measurements, allowing for greater inter-reader reliability. Large cohort studies nested within the BC Detection and Demonstration Project and the Canadian National Breast Screening Study using these techniques demonstrate a clear positive linear relationship between MBD and BC risk (Table 2) [54, 55].

Table 2: Association between breast cancer risk and percentage of breast area containing mammographic densities

Breast Cancer Detection and Demonstration Project *a screening study of >280,000 followed 1973-1980*					
% Densities	**Cases (n)**	**Controls (n)**	**Unadjusted OR (95% CI)**	**Adjusted OR (95% CI)**	**Cumulative lifetime BC risk**
0	141	309	1.0	1.0	4.1%
1-24	445	632	1.6 (1.3-2.1)	1.6 (1.2-9.0)	7.0%
25-49	490	489	2.5 (2.0-3.2)	2.5 (1.9-3.2)	10.3%
50-74	576	554	2.9 *2.2-3.6)	2.8 (2.1-3.6)	15.6%
75+	194	136	4.5 (3.3-6.3)	4.4 (3.1-6.1)	17.7%
	1,846	2,120			*Byrne, JNCI, 1995*

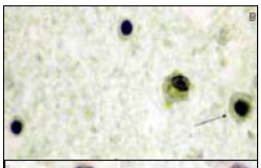
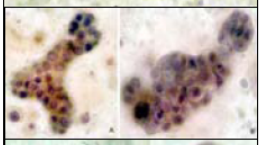
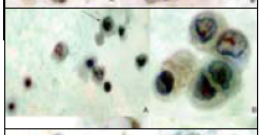
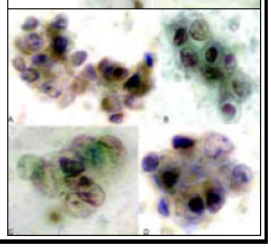
More recently, Ursin *et al* at University of Oslo have successfully adapted their method to work with digital mammography, which is currently more widely used than traditional film mammography, and avoids measurement error that can be introduced by variability in the acquisition and subsequent digitization of mammographic films [65, 66]. With the ability to better quantify MBD using standard digital mammograms, investigators can now use it as a modifiable biomarker of BC risk [67, 68].

Endogenous or exogenous estrogen, which is associated with stimulation of normal and premalignant breast epithelia, is associated with increases in MBD [69], and estrogen withdrawal is associated with a decline in MBD [70]. The degree of modulation of MBD parallels the magnitude of risk modification as well. In the Postmenopausal Estrogen/Progestin Interventions (PEPI) Trial, MBD was increased by combined estrogen (E) and progesterone (P) hormone replacement therapy (HRT), but not by estrogen alone [71]. Similarly, the Women's Health Initiative (WHI) found that the use of E+P HRT was associated with a 25% increased incidence of BC, while HRT with E alone did not have any significant impact on BC incidence [72, 73]. Also of note, the effect of E+P on MBD could be observed as early as after one year of treatment, preceding its effect on BC incidence after five years of use [74] Based on these findings, estrogen blockade would be predicted to suppress MBD. Indeed, several reports have found that the SERMs, tamoxifen and raloxifene, both of which have been associated with decreased BC incidence in large clinical trials [33, 36], are both associated with decreases in MBD after 1-2 years of treatment, preceding effects on clinical endpoints seen after five years of treatment [75-79].

Recently, a MBD reduction of 10% or more 12-18 months after initiating therapy was shown to predict who achieved clinical benefit from standard-dose tamoxifen [80]. While that study used subjective MBD readings in which MBD was scored in 5% increments, the Ursin method, a computer-assisted method that reports MBD on a continuous scale, was shown to be very sensitive to small changes in the PEPI trial [71].

Thus, biomarker observations within clinical trials suggest that two years is a sufficient duration to detect significant differences in MBD as a primary endpoint, that the MBD biomarker endpoint will predict who will have clinical benefit from tamoxifen BC risk reduction, and that the Madena method will sensitively detect MBD changes in response to tamoxifen.

Table 3: Breast aspiration fluid cytology classification and Masood cytomorphology score

Cytologic classification	Characteristics	Masood score	
Benign (Category I)	Duct epithelial cells within normal limits. Foam cells. Apocrine metaplastic cells.	6-10	
Hyperplasia (Category II)	Minimal changes including slight cell and nuclear enlargement. Chromatin remains finely granular and evenly distributed. Small and regular nucleoli sometimes present. Cell distribution predominately in groups and cohesive with >10–50 cells (papillary and apocrine subcategories).	11-14	
Atypical Hyperplasia (Category III)	Moderate to severe abnormalities with distinct nuclear enlargement, increasing nuclear to cytoplasmic ratio, irregular nuclear borders, and nuclear variation. Coarsely granular chromatin. Prominent chromocenters. Cell distribution in groups with some papillary formations. Increased numbers of single atypical cells (apocrine type subcategory).	15-18	
Malignancy (Category IV)	Single cells and groups of cells with unequivocal nuclear features of cancer.	19-24	

Cytomorphology and proliferation. According to well-established models of carcinogenesis, the transition of normal cells to invasive cancer involves the following progressive intermediate morphologic steps: from typical hyperplasia, to atypical hyperplasia, and then carcinoma-in-situ [81]. Each of these premalignant states are associated with increased BC risk, as well as lower category, or non-IEN, benign breast disease [82]. In a study of normal and premalignant breast lesions, the percentage of Ki67-expressing cells was significantly increased in all of the premalignant lesions in comparison to normal lobules, with the following trend observed: the highest expression was seen in DCIS, followed by LCIS and atypical hyperplasia [83]. Among women with typical hyperplasia, patients with benign breast lesions that progressed to BC had a significantly higher proliferative index, measured by Ki67 expression in their lesions, compared to patients who did not [84]. Cytomorphology may also be assessed from fine needle aspiration samples using the 24-point Masood scoring classification (Table 3) [85]. Cytomorphology classified in this manner has also been shown to be significantly associated with Ki67 expression [86]. Control of cellular proliferation is important for cancer prevention since active proliferation has an integral role in carcinogenesis, including the processes of initiation and promotion [87].

Although no data exists of the impact of tamoxifen on Ki67 *in the prevention setting*, a decrease in Ki67 index has been shown to correlate with a clinical response to neoadjuvant therapy with tamoxifen [88]. In fact, early changes in Ki67 index during neoadjuvant therapy with another hormonal agent, letrozole, has been noted to precede tumor response [89, 90]. According to Ki67 reproducibility studies, a > 36% change in Ki67 index after 2-3 weeks of therapy is necessary to predict a statistically significant improvement in response to primary chemo-endocrine therapy at 3 months [91]. In the prevention setting, letrozole has been reported to significantly decrease Ki67 index

determined from fine needle aspirations when women with atypia were treated for 6 months [92]. As far as breast pathology itself is concerned, tamoxifen has been associated with a decrease in DCIS, LCIS, atypical hyperplasia, and other benign breast disease [33, 93].

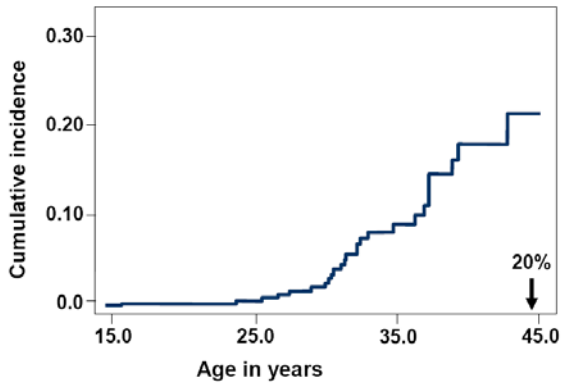
Several methods are available for sampling breast epithelial cells: *random periareolar fine needle aspiration (RPFNA)*, which has been used extensively by Dr. Carol Fabian's group, *core needle biopsy*, used in several of our previous biomarker modulation BC prevention studies, and *ductal lavage* [94, 95]. Each of these techniques is well tolerated by healthy women, and each has unique advantages and disadvantages [96]. Both RPFNA and core biopsy have the highest epithelial yields, sufficient for multiple biomarker assays, but RPFNA is slightly less invasive. In addition, RPFNA allows cytomorphology evaluation using the standardized continuous-scale Masood scoring method described above, which is highly reliable and has been shown to reflect changes in response to hormonal chemopreventive interventions [92].

Circulating biomarkers of BC risk - Dose-response relationships have been noted between tamoxifen and multiple serum biomarkers of BC risk. These include decreased IGF-1 and increased IGFBP-3, as discussed earlier, as well as increased sex hormone-binding globulin (SHBG) levels [97, 98]. While these circulating biomarkers are correlated to BC risk, cytomorphology and proliferative Ki67 index are the only direct measures of breast tissue response to BC risk-reducing interventions. Sampling of normal breast epithelia to assess both biomarkers is feasible through a well tolerated method, RPFNA (please see discussion above). Nevertheless, this procedure is not considered standard of care for radiation exposed female CAYAC survivors, while annual screening mammography is. In addition, while MBD is evaluable on all digital mammograms, some RPFNA samples will not yield sufficient cells for Ki67 assessment. Therefore, we have chosen MBD to be the primary endpoint in the proposed chemoprevention trial.

2.5 Significance

In summary, evidence from several high quality studies indicates that women treated with chest RT by the age of 40 years have a very high risk of developing BC [7-16]. The risk begins to increase within 8 years following RT, and a substantial proportion of these women will be diagnosed with their BC before the age of 40. Indeed, by the age of 40-45 years, about 20% of women treated with moderate to high dose chest RT for childhood HL will be diagnosed with BC, mirroring the magnitude of BC risk seen in women who carry germline mutations in the *BRCA1* or *BRCA2* genes (see Figure 7). Options to reduce BC risk include prophylactic mastectomy or oophorectomy, but not all women are interested in invasive procedures. Non-surgical interventions include a variety of drugs which interfere with estrogen signaling to the breast; however, the only one which is FDA approved for young women who

Figure 7: Breast Cancer after Hodgkin's Lymphoma in Girls Receiving Radiation to the Chest



have not yet reached menopause is tamoxifen. Tamoxifen taken at 20 mg/day for five years is associated with a 50% reduction in BC risk [33]. However, when taken at that dose, tamoxifen is associated with rare but concerning side effects that interfere with patient compliance with the medication. On the other hand, tamoxifen taken at 5 mg/day is associated with a decreased risk of BC without the typical adverse effects associated with standard dose tamoxifen [41]. Findings across several studies suggest that a hormonal intervention would be appropriate for chemoprevention of radiation-associated BC [15, 16, 50]. Because definitive Phase III BC prevention trials require tens of thousands of participants to show significant differences in clinical endpoints, many chemoprevention trials make use of biomarker surrogate endpoints [51]. MBD, Ki67 proliferative index, and evidence of IEN on cytomorphology are all considered biologically plausible surrogate endpoint biomarkers with both a strong statistical association with BC and evidence of modulation by BC prevention intervention strategies. Of these, the marker most easily and reliably measured is MBD, which we have chosen to be our primary endpoint, although we will also measure the other two as secondary endpoints. Tamoxifen has been shown to decrease both MBD and the incidence of IEN in the prevention setting in an epidemiologically defined BC risk population [33]. We now aim to demonstrate a similar effect in female CAYAC survivors at high risk for developing BC due to chest RT exposure at a young age. Considering that SMNs are the leading cause of death in CAYAC survivors, with BC the most frequent secondary solid tumor [6], we are compelled to develop targeted intervention studies for those at highest risk. Thus, we propose the first BC chemoprevention trial for female CAYAC survivors who received chest RT as part of their treatment regimen by the age of 40.

2.6 **Hypotheses**

We hypothesize that low-dose tamoxifen delivered at a dose of 5 mg per day for two years will be an efficacious and a safe option for BC risk reduction in young women at an extremely high risk for developing BC due to exposure to chest RT during childhood or early adulthood. We also hypothesize that there exists a subgroup within the patient population who will derive the most benefit from taking low-dose tamoxifen for BC prevention with the least risk.

3.0 DRUG INFORMATION: TAMOXIFEN

3.1 **Chemistry**

The chemical name for tamoxifen citrate is (Z)2-[4-(1,2-diphenyl-1-butenyl) phenoxy]-N, N-dimethylethanamine 2-hydroxy-1,2,3- propane-tricarboxylate (1:1). The structural and empirical formulas are: (C32H37NO8). Tamoxifen citrate has a molecular weight of 563.62, the pKa is 8.85, and the equilibrium solubility in water at 37°C is 0.5 mg/mL and 0.2 mg/mL in 0.02 N HCl at 37°C. Commercially available tamoxifen tablets also contain the following inactive ingredients: anhydrous lactose, colloidal silicon dioxide, crospovidone, magnesium stearate, microcrystalline cellulose and sodium lauryl sulfate.

3.2 **Mechanism of Action**

Tamoxifen is a nonsteroidal agent that has demonstrated potent antiestrogenic properties in animal test systems. The antiestrogenic effects are thought to be related to its ability to compete with estrogen for binding sites in target tissues such as breast.

3.3 Human Toxicity

Toxicity data from prevention trials in healthy women are reviewed in detail here [33, 41].

Standard dose tamoxifen: The Breast Cancer Prevention Trial (BCPT, NSABP P-1) was a double-blind randomized placebo-controlled trial with a primary objective to determine whether 5 years of tamoxifen therapy at 20 mg per day would reduce the incidence of invasive breast cancer in women at high risk for the disease. Secondary objectives included an evaluation of the incidence of ischemic heart disease; the effects on the incidence of bone fractures; and other events that had been associated with the use of tamoxifen in breast cancer therapeutic trials, including: endometrial cancer, pulmonary embolus, deep vein thrombosis, stroke, and cataract formation and surgery.

In the BCPT, NSABPP-1 trial, 13,388 women of at least 35 years of age, who had a 5-year risk for developing breast cancer of 1.67% or more estimated by the Gail model, were randomized to receive either tamoxifen 20 mg per day or placebo for five years. After a median follow-up of 54.6 months, the incidence of invasive breast cancer was reduced by 49% among women assigned to tamoxifen compared to placebo ($p<0.00001$; relative risk (RR)=0.51, 95% CI: 0.39-0.66). In addition, a significant decrease in the incidence of ductal carcinoma in situ (DCIS) was seen ($p<0.002$; RR = 0.50, 95% CI 0.33-0.77). There was no statistically significant difference in the number of myocardial infarctions, severe angina, or acute ischemic cardiac events between the two groups. With regards to skeletal fractures, several beneficial nonsignificant trends were noted: there was a 45% reduction in fractures of the hip (RR = 0.55; 95% CI 0.25–1.15), a 39% reduction in wrist fractures (RR = 0.61; 95% CI 0.29–1.23), and a 26% reduction in fractures of the spine (RR = 0.74; 95% CI 0.41–1.32).

The risks associated with 5 years of 20 mg per day of tamoxifen in this trial include endometrial cancer, deep venous thrombosis (DVT), pulmonary embolus (PE), stroke, cataract formation and cataract surgery, as shown in the table below, with statistical comparisons between treatment groups immediately following:

	Cases per year out of 1000 women taking NOLVADEX	Cases per year out of 1000 women taking PLACEBO
Breast Cancer	**3.6**	**6.5**
Endometrial Cancer	**2.3**	**0.9**
Blood clot in the lungs	**0.8**	**0.3**
Blood clot in the veins	**1.3**	**0.8**
Stroke	**1.4**	**1.0**
Cataracts	**25.4**	**22.5**
Cataract surgery	**46.6**	**31.4**

Participants who received tamoxifen in this trial had a 2.53 times greater risk of developing an invasive endometrial cancer (95% CI 1.35–4.97) than did women who received placebo. Of note, the increased risk was predominantly in women 50 years of age or older. The RR of women aged 49 years or younger was 1.21 (95% CI 0.41–3.60), whereas it was 4.01 (95% CI 1.70–10.90) in women aged 50 years or older.

Pulmonary emboli ("blood clot in the lungs" in the table above) were rare, fewer than 1 case per 1000, but were observed in almost three times as many women in the

tamoxifen group as in the placebo group (total number of cases per treatment group: 18 versus 6, respectively; RR = 3.01; 95% CI 1.15–9.27). When the incidence of pulmonary embolism was related to the age of participants, it appeared that most of the risk was confined to the older subgroup. Among women aged 49 years or younger, only one event occurred in the placebo group and two events occurred in the tamoxifen group (RR = 2.03; 95% CI 0.11–119.62), in contrast to those aged 50 years or older, in whom 5 events occurred in the placebo group and 16 in the tamoxifen group (RR = 3.19; 95% CI 1.12–11.15).

More women who received tamoxifen developed deep venous thrombosis ("blood clot in the veins" in the table above) than did women who received placebo (35 versus 22 cases, respectively; RR = 1.60; 95% CI 0.91–2.86). Again, the excess risk appeared to be greater among women aged 50 years or older. For women aged 49 years or younger, the number of cases was 8 in the placebo group versus 11 in the tamoxifen group (RR = 1.39; 95% CI 0.51–3.99). In women 50 years of age or older, the number of cases was 14 versus 24, with an RR of 1.71 (95% CI 0.85–3.58).

The rate of cataract development among women who were cataract-free at the time of randomization was 21.7 per 1000 women in the placebo group and 24.8 per 1000 women in the tamoxifen group (the rates in the table above represent total number of cases per treatment group). This represents an RR of 1.14, with confidence intervals that indicate marginal statistical significance (95% CI 1.01–1.29). There was also a difference by treatment group with respect to cataract surgery. In the placebo and tamoxifen groups, the rates of developing cataracts and undergoing cataract surgery were 3.00 and 4.72 per 1000 women, respectively (RR = 1.57; 95% CI 1.16–2.14).

In breast cancer treatment trials, tamoxifen at 20 mg per day has been associated with changes in liver enzyme levels, and on rare occasions, a spectrum of more severe liver abnormalities including fatty liver, cholestasis, hepatitis and hepatic necrosis. In most reported cases the relationship to the drug was uncertain.

Common yet less serious side effects associated with standard dose tamoxifen include hot flashes, vaginal discharge, and irregular menstrual bleeding. On the NSABP P-1 trial, hot flashes occurred in 68% of women on placebo and in 80% of women on tamoxifen. Vaginal discharge occurred in 35% and 55% of women on placebo and tamoxifen, respectively. There was no difference in the incidence of vaginal bleeding between treatment arms.

During postmarketing surveillance, there have been some reports of occasional headaches or skin rashes on tamoxifen at 20 mg/day. T4 elevations were reported for a few postmenopausal patients which may be explained by increases in thyroid-binding globulin, but these laboratory findings were not accompanied by clinical hyperthyroidism.

Low dose tamoxifen: As reviewed in the background section, Decensi *et al* performed a trial of several lower doses of tamoxifen in healthy postmenopausal women. Findings suggested that 5 mg per day dosing is the most effective dose schedule of low-dose tamoxifen compared to placebo. No significant change was observed with C-telopeptides, suggesting that low-dose tamoxifen does not adversely affect bone health. A significant decrease in antithrombin-III ($p = 0.006$) suggested that low-dose tamoxifen may still be associated with a higher clotting risk compared with placebo; but, this may be improved compared with standard-dose tamoxifen. While there was an association between low-dose tamoxifen and endometrial thickness, no association was found with endometrial proliferation measured with Ki67 expression. In fact, Ki67 was lowest in patients receiving the 5 mg/day dose, suggesting that low-dose tamoxifen at that dose

would likely not be associated with an increased risk of endometrial carcinoma as is observed with standard dose tamoxifen. In addition, menopausal symptoms were not significantly worsened by low-dose tamoxifen. A follow-up study of premenopausal women found similar results.

In summary, because the dose that we will be administering will be 5 mg per day rather than 20 mg per day, we expect no significant increase in these adverse events in the treatment group compared to the placebo group. In addition, the median age of the patient cohort from which trial participants will be drawn is 36 years old, while the adverse effects associated with the 20 mg per day of tamoxifen were only significantly elevated above placebo among women who were 50 years old or older at the time of treatment initiation. However, precautions will be taken, nevertheless (see Section 8.2 for guidelines regarding adverse event reporting and for data safety monitoring). In addition, women with childbearing potential will be warned that they should avoid pregnancy while taking study medication and for two months after discontinuation.

3.4 Pharmaceutical Data

Absorption and Distribution: Following a single oral dose of 20 mg tamoxifen (standard-dose tamoxifen), an average peak plasma concentration of 40 ng/mL (range 35 to 45 ng/mL) occurred approximately 5 hours after dosing. The decline in plasma concentrations of tamoxifen is biphasic with a terminal elimination half-life of about 5 to 7 days. The average peak plasma concentration of N-desmethyl-tamoxifen is 15 ng/mL (range 10 to 20 ng/mL). The average steady-state plasma concentrations of tamoxifen and N-desmethyl-tamoxifen after administration of 20 mg tamoxifen once daily for 3 months are 122 ng/mL (range 71-183 ng/mL) and 353 ng/mL (range 152-706 ng/mL), respectively. Chronic administration of 10 mg tamoxifen given twice daily for 3 months to patients results in average steady-state plasma concentrations of 120 ng/mL (range 67-183 ng/mL) for tamoxifen and 336 ng/mL (range 148-654 ng/mL) for N-desmethyl tamoxifen. After initiation of therapy, steady state concentrations for tamoxifen are achieved in about 4 weeks and steady-state concentrations for N-desmethyl tamoxifen are achieved in about 8 weeks, suggesting a half-life of approximately 14 days for this metabolite. In a steady-state, crossover study of 10 mg tamoxifen tablets given twice a day vs. a 20 mg tamoxifen tablet given once daily, the two schedules were found to be bioequivalent.

Metabolism: Tamoxifen is extensively metabolized after oral administration. N-desmethyl tamoxifen is the major metabolite found in patients' plasma. The biological activity of N-desmethyl tamoxifen appears to be similar to that of tamoxifen. 4-Hydroxytamoxifen and a side chain primary alcohol derivative of tamoxifen have been identified as minor metabolites in plasma. Tamoxifen is a substrate of cytochrome P-450 3A, 2C9 and 2D6, and an inhibitor of P-glycoprotein.

Excretion: Studies in women receiving 20 mg of ^{14}C-tamoxifen have shown that approximately 65% of the administered dose was excreted from the body over a period of 2 weeks with fecal excretion as the primary route of elimination. The drug is excreted mainly as polar conjugates, with unchanged drug and unconjugated metabolites accounting for less than 30% of the total fecal radioactivity.

Pregnancy: **Category D.** Tamoxifen may cause fetal harm when administered to a pregnant woman. Women should be advised not to become pregnant while taking tamoxifen or within 2 months of discontinuing tamoxifen and should use barrier or other

nonhormonal contraceptive measures if sexually active. Tamoxifen does not cause infertility, even in the presence of menstrual irregularity.

3.5 Supplier

Tamoxifen is commercially available in 10 mg and 20 mg tablets. For this trial, generic 5mg capsules and matching placebo capsules will be manufactured by Sharp Clinical Services.

Tamoxifen and matching placebo will be manufactured as a capsule for oral administration. Each 5 mg tamoxifen capsule contains 7.6 mg of tamoxifen citrate, which is equivalent to 5 mg of tamoxifen, as well as anhydrous lactose, colloidal silicon dioxide, crospovidone, magnesium stearate, microcrystalline cellulose and sodium lauryl sulfate, as inert excipients. Matching placebo capsules will contain anhydrous lactose.

For this study, blistered cards will contain a month's supply of tamoxifen 5mg or matching placebo capsules.

3.6 Storage and Stability

Tamoxifen 5 mg capsules and matching placebo will be shipped at room temperature. The capsules should be stored at a controlled room temperature of 20-25°C (68-77°F) in the supply kits containing the blister cards. They will be stored and dispensed in the original containers.

3.7 Administration

Trial participants will be instructed to take one capsule per day orally. They will be instructed to swallow the capsule whole, with water or another non-alcoholic liquid, preferably the same time each day. They may take the capsules with or without food. If they forget a dose, participants will be asked to take it when they remember, and then take the next dose as usual, but if they do not remember until their next dose, to not take extra capsules to make up the missed dose.

For premenopausal subjects, they will be instructed to begin their study medication course while menstruating or after negative beta-HCG test as below. For premenopausal subjects who do not have regular menstrual periods or are between periods, a beta-HCG level will be performed to confirm that the patient is not pregnant before starting study medication. Participants will be warned that they should avoid pregnancy while taking study medication and for two months after discontinuation. They will be advised to use barrier or nonhormonal contraceptive measures.

4.0 RECRUITMENT STRATEGY

Via the Consortium for Pediatric Interventional Research (CPIR), a consortium of five institutions, including the *City of Hope National Medical Center (COH), St. Jude Children's Research Hospital (SJCRH), Emory University (EU), University of Michigan (UM),* and *Princess Margaret Hospital in Toronto (PMH),* we have constructed a cohort of HL survivors who were exposed to chest RT during childhood or young adulthood, at least 5 years prior to study participation. This cohort will form the initial basis from which accrual in this trial will be conducted. Other sites and additional CAYAC diagnoses treated with RT involving the chest may be added as needed to complete accrual for the trial.

Recruitment will be conducted both prospectively and retrospectively as described below. Because this is a highly motivated patient population, at study activation we anticipated that it would be feasible to achieve a recruitment yield of 30%, and that the accrual goal of 230 would be achievable across the CPIR participating institutions described above. After the initial two years of accrual, we observed an average recruitment yield of only 14%. Therefore, new sites were recruited, and additional sites have been identified and will be recruited as needed to reach the accrual goal.

4.1 Retrospective recruitment

Participants in any long-term cancer survivorship clinic, database or registry associated with a participating site, who are alive and eligible based on medical record review will be mailed a letter and study brochure describing the study. The letter lets them know that a representative from the study will be calling to tell them more about the study, unless they opt out (i.e. passive consent). Upon contact, the patient will be given another opportunity to opt out. Patients who agree to be prescreened will be interviewed per the Intake Form. Subjects who are screened by this method will form *cohort 1.*

4.2 Prospective recruitment

Alternatively, the study will be introduced to eligible patients at their survivorship and/or high risk screening and/or prevention clinic visit and given a brochure describing the study to take home to read. The study clinical research associate (CRA) or clinical research nurse (CRN) will then follow up with a call the following week to assess interest and confirm eligibility. Subjects who are screened by this method will form *cohort 2.* Patients may also self-identify and call in to a local site via recruitment advertising on the internet or from a flyer placed in the community (such as community centers, libraries and churches). Self-identifying subjects will form *cohort 3.*

4.3 Informed consent process

Eligible and interested individuals will be approached for informed consent. One of the members of the Protocol Management Team (PMT) at each site will introduce the study to potentially eligible patients, either at a survivorship clinic visit or via a mailing as described. The PMT at each site includes the consortium PI, the co-investigator, and the clinical research nurse (CRN), and/or clinical research associate (CRA). All potential risks and benefits of the study, as well as alternatives, will be fully explained. Whenever possible, the informed consent process will be conducted in the patient's preferred language. If that is not possible, a professional translator will be used.

5.0 ELIGIBILITY CRITERIA

5.1 Inclusion Criteria

The following are requirements for entry onto this Phase IIb trial:

- Females, 25 years of age or older at the time of registration
- Exposure to RT delivered to the chest, axilla, and/or supraclavicular areas at a cumulative dose of 12 Gy or more by age 40 years. In addition, patients who received total body irradiation by age 40 may be considered.

- No evidence of active disease from their primary cancer for at least 2 continuous years prior to registration. The indication for RT is not specified but cannot be for primary breast cancer. Common examples of primary cancer diagnoses include, but are not limited to: lymphoma, leukemia, sarcoma, and Wilms tumor occurring in pediatric patients, and lymphoma, leukemia, and sarcoma occurring in young adults. Primary cancer therapy must have been completed at least 6 months prior to registration.
- Well-defined menopausal status falling into one of the following categories:
    - Premenopausal, defined as age at registration 45 years old or younger with regular monthly period for at least 6 consecutive months prior to registration.
    - Postmenopausal, defined as continuous absence of menstruation for 12 months *OR* status-post bilateral oophorectomy *OR* follicle stimulating hormone (FSH) level in the postmenopausal range.

5.2 **Exclusion Criteria**

Subjects will be excluded if any of the following characteristics are present:

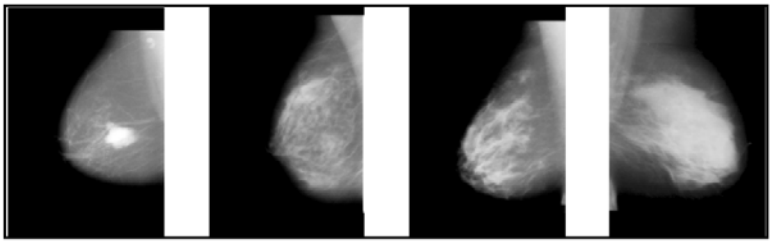
- SMN other than those listed below diagnosed within 2 years of study entry. Patients with the listed indolent or pre-invasive neoplasms may be eligible if diagnosed within 2 years and all treatment was completed at least 6 months prior to registration:
    - non-melanoma cancers of the skin
    - thyroid cancer
    - cervical cancer confined to the cervix or cervical intraepithelial neoplasia (CIN)
    - ductal carcinoma in situ (DCIS) or breast IEN (includes atypical hyperplasia and LCIS)
    - superficial or non-invasive transitional cell carcinoma of the bladder

  For women with a prior history of ductal carcinoma in situ (DCIS) or breast IEN, only one breast could have been involved and all therapy must have been completed at least 6 months prior to registration. In addition women with a prior history of invasive breast cancer may also be eligible, as long as only one breast was involved, they were diagnosed at least 2 years prior to study entry, and therapy was completed at least 6 months prior to study entry.
- Bilateral breast implants or status-post bilateral prophylactic mastectomy.
- Evidence of malignant breast disease on any form of breast imaging. The study only requires annual mammography; however, annual breast MRI is considered standard of care in this patient population (per COG or NCCN follow-up guidelines), and breast ultrasound may be indicated if a palpable lesion is detected on screening clinical breast exam. Abnormal imaging may require additional radiographs and/or breast biopsy. Patients who are found to have benign breast disease with or without atypia may continue on study as long as there is no evidence of malignancy. If there is evidence of malignancy, and only one breast is involved, they may be reapproached 6 months after completion of therapy for consideration of the trial.
- Baseline categorical mammographic density scored as BIRAD 1, or extremely fatty, in both breasts (see Figure 9). If the patient has a prior history of IEN (DCIS, LCIS, or atypical hyperplasia), the contralateral breast must not have a mammographic density score of BIRAD 1. This determination will be made at the local site.

Figure 9: Mammographic breast density (MBD) classification with the 4-category BIRADS density score

The American College of Radiology (ACR) Breast Imaging Reporting and Data System (BIRADS) identifies four major groups for classifying breast density:

1 = predominantly fat;
2 = scattered fibroglandular tissue;
3 = heterogeneously dense; and
4 = extremely dense.



1 2 3 4

D. B. Kopans. *Breast Imaging*. Lippincott-Raven Publishers, 1998

- Current or recent use (within 6 months of registration or baseline mammogram, whichever is first) of any of the following hormonal agents:
    - systemic hormone replacement therapy (includes oral or transdermal formulations). Vagifem and Estring, two formulations of locally applied vaginal estrogen associated with minimal systemic absorption, may be allowed. Other estrogen-containing vaginal creams, while not an exclusion, should be avoided whenever possible. Patients with a history of hormone modifying herbal supplements (see section 11.14) are eligible, but patients will be asked to avoid their use after on study.
    - hormonal forms of contraception (includes oral, transdermal, implanted, and injectable formulations),
    - selective estrogen receptor modifiers (such as tamoxifen and raloxifene),
    - aromatase inhibitors,
    - GnRH analogs,
    - prolactin inhibitors, or
    - androgens or antiandrogens.
- Concurrent use of warfarin and strong inhibitors of CYP2D6 (see Section 11.15 for a list) will not be allowed.
- A personal history or a strong family history of venous thromboembolism, (VTE), including deep venous thrombosis (DVT) or pulmonary embolus (PE). A one-time personal history of catheter-associated DVT may be allowed, as long as there were no subsequent VTE events and a strong family history is not present. Examples of a strong family history include (but are not limited to): one first degree relative with more than one VTE event in the same individual, and two family members in the same lineage with unexplained VTE or two VTE events in the same individual. If a family history is present, but it is not clear whether or not it is clinically significant, consideration of genetic testing to rule out a hereditary clotting syndrome (e.g. FVL, prothrombin G2010A mutations) may be considered. Because of a drug-drug interaction with tamoxifen, concurrent with warfarin use for any reason is not allowed.

For this reason, patients with atrial fibrillation may not participate. However, patients with coronary artery disease or congestive heart failure without atrial fibrillation may be allowed to participate. Patients with a personal history of a cerebrovascular accident (CVA), transient ischemic attack (TIA), or retinal vein thrombosis will not be allowed to participate.

- Current intrauterine pregnancy or plans to become pregnant within two years. In addition, currently nursing mothers will be excluded.
- Renal or hepatic insufficiency, defined as having a serum creatinine, total bilirubin, SGOT, or SGPT greater than 2x the institutional norm.
- Unable to provide consent.

6.0 DOSE SCHEDULE AND RULES FOR TREATMENT DISCONTINUATION

6.1 **Dose Schedule**

Randomization will be stratified according to the rules outlined in Section 12.3. Subjects will be randomized 1:1 to receive low-dose tamoxifen 5 mg or placebo daily for 24 months. Registration will be done via a central database, Interactive Web Response System (IWRS), which will also perform stratified treatment assignment. This system is maintained by Sharp Clinical Services, which is separate from the coordinating center and all participating sites such that all study staff (except for the statistician when preparing reports for the DSMB) will remain blinded.

6.2 **Dose Escalation**

There will be no dose escalation in this Phase IIb study.

6.3 **Criteria for Discontinuation of Study Protocol Therapy**

- Patient requests to withdraw from study
- Treatment interruption longer than 90 consecutive days
- A breast or gynecologic event (see definitions below)
- Pregnancy
- The occurrence of a grade 2 toxicity with *probable* or greater attribution without resolution within 72 hours, or any grade 3 or 4 toxicity with *possible* or greater attribution meeting definitions below
- Death

A *breast event* is defined as a new diagnosis of DCIS or invasive BC. These events are expected to occur as a consequence of the high risk study population. In the case that it should occur, the subject will be taken off study and referred to the appropriate breast surgeons and medical oncologists for proper diagnostic work-up and treatment. For subjects who develop atypical hyperplasia (AH) or lobular carcinoma in-situ (LCIS), or any other form of benign breast disease, their specific histologic diagnosis and the date of diagnosis will be recorded, but they may continue on study.

A *gynecologic event* is defined as a new diagnosis of endometrial carcinoma or uterine sarcoma. Although these events may occur sporadically, they have been associated with standard dose tamoxifen and therefore are listed among criteria for discontinuation of study treatment. In the case that a subject should experience dysfunctional uterine

bleeding of any etiology, the subject will be referred to a gynecologist for proper diagnostic work-up. Should such a work-up lead to a diagnosis of endometrial carcinoma or uterine sarcoma, the subject will be taken off study and referred to a gynecologic oncologist for appropriate treatment. Otherwise, their specific histologic diagnosis and the date of diagnosis will be recorded, but they may continue on study.

Participants must not be planning to become pregnant for two years to be eligible for the study, and they will be warned about the importance of not becoming pregnant while on study at the time of enrollment. They will be advised to use either a barrier method or another nonhormonal form of contraception (eg. hormone-free IUD) for all sexual activity during the course of the study. Nevertheless, occurrence of an unplanned intrauterine pregnancy while on study will be an indication for immediately stopping treatment and the patient must withdraw from the study. If the patient should become pregnant while on study, or within two months after discontinuing therapy, her treatment assignment will be unblinded immediately. If the patient was randomized to tamoxifen, then they must be apprised of the potential risk of spontaneous abortion, birth defects, or a DES-like syndrome.

The Principal Investigator (PI) at each site is responsible for monitoring protocol conduct and reporting to their Institutional Review Board (IRB) any adverse events (AEs) related to either the study medication per their institutional guidelines. AEs must also be reported to the Coordinating Center (UAB) per instructions in Section 8.0. The NCI common toxicity criteria for adverse events Version 4.0 (CTCAE v.4.0, Publish Date 10/19/2009) will be used to assess toxicity.

With the exception of vasomotor and gynecologic symptoms commonly associated with menopause, all grade 2 or greater toxicities of clinical significance with *possible* or greater attribution (please refer to Section 8.3 for definition) to the study drug will be reviewed by the Protocol Management Team (PMT) at the local site. The PMT includes the consortium PI, the consortium co-investigator, and the CRA/CRN.

- Any grade 2 toxicity besides vasomotor and/or gynecologic symptoms commonly associated with menopause with *probable* or greater attribution to the study drug will be brought to the attention of the local PI (within 24 hours of knowledge of the event) for close monitoring. If the symptom resolves or improves within 72 hours, the patient may continue on the study medication. Otherwise, the patient will be unblinded and taken off therapy.
- A patient reporting grade 3-4 toxicity with *possible* or greater attribution to the study drug will be unblinded and taken off protocol therapy.

Serious adverse events (SAE) and all grade 3-4 toxicities with *possible* or greater attribution to the study drug must be submitted to the coordinating center PI and study staff immediately (within 24 hours of knowledge of the event), to allow reporting to the local and central IRBs and the central DSMB. If a Grade 3-4 AE is determined to have *possible* attribution to low-dose tamoxifen, an AE report will be submitted to the Food and Drug Administration (FDA) as described in Section 8.0.

Because venous thromboembolic events have been associated with standard dose tamoxifen, we include CTCAE specific to DVT, PE, and stroke below.

CATEGORY: VASCULAR
Adverse Event: Thrombosis/embolism (vascular access-related)
MedDRA Code: 10062169

Grade	Description
1	Venous thrombosis (e.g., superficial thrombosis)
2	Venous thrombosis (e.g., uncomplicated deep vein thrombosis), medical intervention indicated
3	Thrombosis (e.g., uncomplicated pulmonary embolism [venous], non-embolic cardiac mural [arterial] thrombus), medical intervention indicated
4	Life-threatening (e.g., pulmonary embolism, cerebrovascular event, arterial insufficiency); hemodynamic or neurologic instability; urgent intervention indicated
5	Death

The following are considered clear indications for discontinuation of study protocol therapy (confirmed by clinical diagnosis):

- Deep venous thrombosis (DVT)
- Pulmonary embolism (PE)
- Transient ischemic attack (TIA)
- Cerebral vascular accident (CVA)
- Retinal vein thrombosis
- Cardiac ischemia/infarction

6.4 Study Stopping Rules

6.4.1 Toxicity criteria for study stopping

If two or more cases of the same related and unexpected grade 3-4 toxicity with *probable* or greater attribution are reported in separate patients, accrual will be held until further review by the central DSMB to determine whether or not the study should be stopped based on the unblinded data available at that time. Because low-dose tamoxifen is expected to have lower toxicity than standard-dose tamoxifen, if two separate cases of grade 4 thromboembolism as listed in Section 6.3 occur and are attributed to the tamoxifen intervention in the unblinded DSMB review, with none observed in the placebo group, this will be a clear indication for stopping the study. If cases of grade 4 thromboembolic events are observed in the placebo group, then if occurrences in the tamoxifen group reach a risk ratio of 1.4, the trial will be stopped. Similarly, if 2.5-fold more endometrial cancers are observed in the tamoxifen group compared to the placebo group, the trial will be stopped. Both of these cut-offs are derived from the NSABP P-1 trial [33].

6.4.2 Efficacy criteria for study stopping

We do not expect to see a greater efficacy of low-dose tamoxifen compared to the standard tamoxifen regimen for breast cancer risk reduction in any risk population. The NSABP P-1 trial found an overall magnitude of risk reduction of 49% with the standard tamoxifen regimen of 20mg per day for 5 years (risk ratio 0.51, 95% CI 0.39–0.66), with higher efficacy observed among women with

higher baseline risk. Specifically, the subgroup of women with a prior history of atypical hyperplasia had a reduction in breast cancer risk of 86% (risk ratio 0.14, 95% CI 0.03–0.47) [33]. We believe that a similar risk reduction among radiation exposed CAYAC survivors would be of sufficient clinical significance to warrant early trial stopping and reporting of results. Thus, if a risk ratio of 0.14 or less is observed during DSMB review, the trial will be halted and subjects unblinded.

7.0 TREATMENT PLAN

7.1 **Subject Treatment**

Subjects will be randomized 1:1 to receive low-dose tamoxifen 5 mg or placebo daily for 24 months.

Subjects will be instructed to swallow the capsule whole, with water or another non-alcoholic liquid, preferably the same time each day. They may take the tamoxifen capsules with or without food. If they forget a dose, participants will be asked to take it when they remember, and then take the next dose as usual, but if they do not remember until their next dose, to not take extra capsules to make up the missed dose.

For premenopausal subjects, they will be instructed to begin their study medication course while menstruating or after a negative beta-HCG or serum pregnancy test as below. For premenopausal subjects who do not have regular menstrual periods or are between periods, a beta-HCG level will be performed to confirm that the patient is not pregnant before starting study medication. Participants will be warned that they should avoid pregnancy while taking study medication and for two months after discontinuation. If they are sexually active, they will be advised to use barrier or nonhormonal contraceptive measures while on study.

7.2 **Concomitant and Repeat Therapy**

No concomitant therapy is allowed with the exception of continued medications for chronic illnesses that are not excluded in Section 5.0, and necessary medications for unrelated acute illnesses that may occur during the study (cold, flu, infection, etc.). Any such medications must be recorded. Ingestion of certain natural products should also be monitored while on trial, as outlined in Section 11.14.

7.3 **Criteria for Removal from Treatment**

7.3.1 Treatment will be discontinued if a subject experiences an adverse event which the investigator deems related to the study medication or which will interfere with the ability of the subject to comply with the protocol. See section 6.3 for specific rules.

7.3.2 A subject may always voluntarily withdraw from treatment whenever she wishes.

8.0 TOXICITIES TO BE MONITORED AND DOSAGE MODIFICATIONS

NCI CTCAE v.4.0 will be used to assess toxicity, study and medication stopping rules are outlined in Sections 6.3 and 6.4, respectively. The study will utilize Adverse Event

Collection Form for reporting all AEs and the FDA MedWatch for reporting serious adverse events as defined below.

8.1 Dosage Changes Based on Toxicity

There will be no dose modifications on this trial. See Section 6.3 for discontinuation rules.

8.2 Data Safety and Monitoring

8.2.1 Monitoring and Personnel Responsible for Monitoring

This is a multi-center phase II trial conducted at the University of Alabama at Birmingham and participating institutions, and monitored by the Clinical Trials Monitoring Committee (CTMC) housed at the UAB Comprehensive Cancer Center.

8.2.2 Adverse Events: List and Reporting Requirements

Adverse event (AE) monitoring and reporting is a routine part of every clinical trial. Serious Adverse Events (SAEs) are reported by the site PI within 24 hours to the coordinating center (survivorshiptrial@peds.uab.edu, phone: 205-638-2127). The Coordinating Center is then responsible for reporting SAEs to the UAB IRB and Clinical Trials Monitoring Committee in accordance with study-specific requirements. SAEs occurring at participating sites are reported to the UAB IRB as “non-UAB” events.

A Serious Adverse Event (SAE) is an AE that 1) results in patient hospitalization or prolongation of hospitalization; 2) results in persistent or significant disability or incapacity; 3) results in death; 4) is a cancer or congenital abnormality or 5) results in the development of drug dependence or abuse. An AE must be considered an SAE when the nature or severity of the event is not consistent with the current Investigator’s Brochure. Participating site SAEs must be reported by the site PI to the coordinating center as described above. It is also the responsibility of the site PI to report SAEs to the site IRB and to submit copies of that report to the coordinating center. It is the Coordinating Center’s responsibility to report the SAE to the appropriate regulatory agency and / or industry sponsor. This submission of IND Safety Reports will be cross referenced according to local regulations to Onyx Investigational Compound Number (IND) at the time of submission.

8.2.3 Data and Safety Monitoring Plan

Participants enrolled on this study will be monitored by the UAB Comprehensive Cancer Center’s Clinical Trials Monitoring Committee (CTMC). Adverse reactions observed during treatment will be closely monitored by the Clinical Trials Monitoring Committee (CTMC) on a monthly basis. The CTMC is responsible for data and safety monitoring of the trial and adherence to the DSMP, and is serving as the Central DSMB for this study. The independent Quality Assurance Committee (QAC) is responsible for oversight of the operation of CTMC, including adherence to the DSMP. Reports from the CTMC are reviewed monthly by the QAC.

9.0 STUDY PARAMETERS AND CALENDAR

These procedures are fully outlined in the study calendar and summarized below.

9.1 **Screening visit**

After informed consent is obtained, a screening visit will be scheduled. This visit will include a **history and physical**, **clinical breast exam**, a **complete blood count** (includes platelet count, but no differential necessary)**, PT/PTT, metabolic panel** (includes electrolytes, creatinine, and liver function tests), and a **screening mammogram**.

For premenopausal patients, the screening mammogram should be performed between days 2 and 10 of their menstrual cycle (follicular phase). For premenopausal subjects who are unable to schedule a mammogram during the follicular phase of their menstrual cycle, the days since last menstrual period (LMP) will be recorded in the Breast Image Submission Form, and follow up mammograms will be scheduled on the same day of their cycle in which their screening mammogram was completed +/- 5 days. All mammograms must be performed on a digital mammography machine using large paddles. Images should be taken using on-site mammography equipment (rather than off-site facilities), and serial measurements should be taken from the same mammography unit whenever possible. Each view from each breast must be saved as a single electronic image in raw DICOM (Digital Imaging and Communications in Medicine) format as instructed in Section 11.1.1. A site radiologist will perform a clinical reading of the screening mammogram; including a density score per American College of Radiology (ACR) BIRADS guidelines (Figure 9). If a screening mammogram was performed within 120 days of study registration as part of the patient's routine clinical care, it can be estimated how many days into the menstrual cycle the patient was when it was performed (or the patient is postmenopausal), and it was obtained using a digital mammography unit, a digital copy of the original film will suffice, as long as that digital copy includes whole electronic images in raw DICOM format as outlined in Section 11.1.1. In that case, a copy of the radiology report should be retrieved at the same time as the digital mammogram. If both clinical reading and clinical density assessment were provided on that report, that will suffice. Otherwise, the outside film must be submitted for a reading by a site radiologist who will provide a reading and density assessment according to Figure 9 in Section 5.2. Detailed instructions on electronic submission of study mammograms are in Section 11.1.1.

For subjects whose menopausal status is unclear based on clinical history alone (see definitions in inclusion criteria below), a **FSH** level will be drawn at the time of the screening visit. For subjects who are premenopausal and have not had a menstrual period within 28 days, a blood **beta-HCG** test will also be performed.

9.2 **Other baseline study parameters**

Screen-eligible patients will be scheduled for baseline **RPFNA** (for consenting patients at FNA-participating sites) between days 2 and 10 of their menstrual cycle (follicular phase) and phlebotomy for circulating biomarker assays (all sites) to be performed within 60 days of completion of the screening visit (for patients consented prior to protocol version 3/9/2015 and all previously consented patients as of 10/26/15). A *urine collection* and a *blood draw* after at least an 8 hour fast must also be scheduled within the same time window for **lipid profile**, **anti-thrombin III**, **bone turnover markers**, and **circulating**

biomarkers (including insulin growth factors, sex steroid hormones, CYP2D6 SNPs, and tamoxifen metabolites). Please refer to Sections 11.2 [RPFNA], 11.3 [blood] and 11.4 [urine] for details regarding specimen collection, processing, and transfer.

In addition, if the patient is followed with annual screening **breast MRI** as part of their standard of care (applies to patients who have survived at least 8 years beyond their chest radiation exposure; please refer to the COG cancer survivorship guidelines [www.survivorshipguidelines.org] for those diagnosed with CAYAC prior to age 21 or the NCCN guidelines [www.nccn.org] for those diagnosed after age 21), then the most recent pre-registration MRI must be obtained for submission with the baseline screening mammogram described above. Patient reported symptoms will be assessed via standardized **Symptom Log**.

For sites who prefer to combine screening and baseline enrollment visits, the screening blood collection must be performed after an 8 hour fast and include the additional tests outlined in this section. The tubes drawn for most of the baseline tests below may be held until screen eligibility is confirmed; however, the anti-thrombin III level must be processed right away. The baseline RPFNA (for FNA-participating sites, for patients consented prior to protocol version 3/9/2015 and all previously consented patients as of 10/26/15) can be tentatively scheduled to follow completion of all screening activities, including registration in the Interactive Web Response System (IWRS), as described in Section 13.0, and then cancelled if eligibility is not confirmed by normal breast exam, benign mammogram, breast density reading of 2-3, and lab tests within stated parameters (see Section 5.0), or postponed if more time is needed. Study drug must be available for the participant to take home with them on the day the last baseline activity is performed.

9.3 Treatment initiation

The study CRA or CRN will register subjects as outlined in Section 13.0. The CRA or CRN will perform the randomization using the Sharp Clinical Services IWRS according to specifications in Section 12.3. Upon completion of the randomization, the Sharp Clinical Services IWRS will assign kit numbers to be dispensed. Kit labels include instructions on how to properly store and take the capsules as well as a contact number should any concerns regarding either the study medication or procedures arise.

9.4 Follow-up visits

For patients who consented to the RPFNA procedure (prior to protocol version 3/9/2015 and all previously consented patients as of 10/26/15), the study CRA or CRN will follow up with a call after the first week on study (**Day 8**) to confirm that the baseline RPFNA was tolerated well All patients will be called after the fourth week (**Day 28**) to confirm that the patient is tolerating the medication well. Patient reported symptoms will be assessed via standardized **Symptom Log** administered via phone interview.

The patient will be asked to return their pill kits on **Day 90** for a pill count (reported on the **Adherence Tracking Form**) and refill dispensal, and patient reported symptoms will be assessed via standardized **Symptom Log** administered via phone interview.

A return visit will be scheduled for **Day 180**, during which a clinical breast exam will be performed and reported on a **Clinical Breast Exam Form**. Patient reported symptoms will be assessed via standardized **Symptom Log** form administered via in-person

interview. Patient reported health status changes will be assessed using the **Health Assessment Form**. In addition, health-related quality of life (QOL) will be assessed using the patient self-administered **NSABP Quality of Life Form**. A medication refill will be dispensed at that time, and pill count will be reported on the **Adherence Tracking Form**.

A phone follow-up will be conducted 3 months later (**Day 270**), including pill kit collection for assessment of adherence via pill count pill count (reported on the **Adherence Tracking Form**) and another refill dispensal. Patient reported symptoms will be assessed via standardized **Symptom Log** administered via phone interview.

A return visit will be scheduled for **Day 365**, during which a clinical breast exam (reported on a **Clinical Breast Exam Form**), fasting blood draw and urine collection, and patient reported symptom assessment will be performed via in-person interview documented on the **Symptom Log** form. Patient reported health status changes will be assessed using the **Health Assessment Form**. In addition, the patient will be given the self-administered **NSABP Quality of Life Form** to fill out. All lab results will be reported using a **Lab Values Form**. A repeat mammogram using a digital mammography machine with large paddles recommended to be timed to the follicular phase of the menstrual cycle (Days 2-10) will also be performed. The patient will be encouraged to have their mammogram at the same facility that did the initial study mammogram. If indicated (per COG or NCCN follow-up guidelines), a screening breast MRI should also be performed. If done while on study, it is suggested to time it to the follicular phase of the menstrual cycle (Days 2-10). A medication refill will be dispensed at that time, and pill count will be reported on the **Adherence Tracking Form**.

A phone follow-up will be conducted 3 months later (**Day 455**), including pill kit collection for assessment of adherence, collection of patient reported symptoms via standardized **Symptom Log** administered via phone interview, and refill dispensal, with pill count reported on the **Adherence Tracking Form**.

Another in-person visit will be scheduled for **Day 540** for clinical breast exam (reported on a **Clinical Breast Exam Form**) and assessment of patient reported symptoms via **Symptom Log**. Patient reported health status changes will be assessed using the **Health Assessment Form**. In addition, the patient will be given the self-administered **NSABP Quality of Life Form** to fill out. The pill kit will be collected for assessment of adherence via pill count pill count, reported on the **Adherence Tracking Form**, and another refill will be dispensed.

This will be followed by a phone follow-up 3 months later (**Day 630**) for pill adherence (reported on the **Adherence Tracking Form**), patient reported symptom evaluation via **Symptom Log**, and the last refill dispensal.

The final study visit will be conducted on **Day 730**, during which a final history and physical (reported on the **General History and Physical Form**), fasting blood draw, and urine collection will be performed. For patients who completed a baseline RPFNA, a post-treatment RPFNA will also be performed. Adherence will be reported on the **Adherence Tracking Form**, and patient-reported symptoms will be assessed via **Symptom Log**. Patient reported health status changes will be assessed using the **Health Assessment Form**. All lab results will be reported using a **Lab Values Form**. A repeat mammogram using a digital mammography machine with large paddles and

timed to the follicular phase of the menstrual cycle (Days 2-10) will also be performed. The patient will be encouraged to have their mammogram at the same facility that did the initial study mammogram. If annual screening breast MRI is indicated (per COG or NCCN follow-up guidelines), a repeat breast MRI should also be performed. If done while on study, it is suggested to time it to the follicular phase of the menstrual cycle (Days 2-10). A final telephone follow-up will occur one week later (**Day 738**) to ensure that the post-treatment RPFNA was well tolerated (only for patients consenting to the RPFNA procedure before protocol version 3/9/2015 and all previously consented patients as of 10/26/15).

All forms are due within 28 days of each scheduled visit and should be entered on the study Electronic Data Capture (EDC) website. Please refer to the Treatment Monitoring and Follow-up SOP for detailed instructions.

9.5 Long-term Follow-up

After trial completion, the patient should be referred back to their local long-term survivorship clinic for continued follow-up, with their first return visit scheduled one year after their Day 730 study visit. Their first return visit will represent a new baseline survivorship clinic visit, and all organ function baseline assessments included in the COG survivorship guidelines should be repeated. This includes a comprehensive metabolic panel, which is recommended to be performed at baseline and as needed. Because of the association between standard dose tamoxifen and hepatic disease, special attention should be paid to symptoms and signs of hepatic disease on all subsequent annual follow-up survivorship clinic visits.

To monitor for significant medical events, including second malignancies that may occur after the two-year intervention, sites will submit an **Annual Follow-Up Form** for up to 10 years post-study completion, including participants that complete the two-year intervention and those that withdrew prior to completion. Subjects who are lost to follow-up will be reported to the coordinating center using this form. New cancer and any inpatient or outpatient procedures potentially related to the study will be reported using the **Breast Event Form** and **Other Event Form**.

STUDY CALENDAR*

Treatment plan:	Prior to Treatment		Days on Study Treatment										Off
STUDIES	**Screening**	**Baseline (Day 0)****	**8**	**28**	**90**	**180**	**270**	**365**	**455**	**540**	**630**	**730**	**738**
Primary Cancer Treatment History	X												
General History and Physical	X											X	
Health Assessment						X		X		X		X	
Breast Evaluations													
Clinical Breast Exam	X					X		X		X		X	
Breast Imaging [a]	X							X				X	
Breast Tissue Sampling [b]		X										X	
Blood/Urine Tests													
CBC and Metabolic Panel [c]	X							X				X	
PT/PTT (pre-RPFNA)	X											X	
Beta-Human Chorionic Gonadotropin (β-HCG), *if needed* [d]	X												
FSH level, *if needed* [e]	X												
Blood for circulating biomarkers [f]		X						X				X	
Fasting lipid profile [g]		X						X				X	
Anti-thrombin-III [h]		X						X				X	
Blood and urine to assess bone turnover [i]		X						X				X	
Self-Administered Questionnaires													
Demographics and BC risk Form [J]	X												
Family History Form [J]	X												
NSABP Quality of Life Form [J]		X				X		X		X		X	
Treatment Management													
Study tablet dispensal [K]		X			X	X	X	X	X	X	X		
Study tablet collection[m]						X		X		X		X	
Symptom log [J]		X		X	X	X	X	X	X	X	X	X	
Adverse Event Collection Form [J]						X		X		X		X	
Follow up phone call [L]			X	X	X		X		X		X		X

[a] Annual mammograms will be required as standard of care follow-up for this patient population per COG cancer survivorship or NCCN guidelines. For patients who are at least 8 years beyond their RT exposure, annual screening breast MRIs are recommended. Both forms of breast imaging will be timed to the menstrual cycle and submitted electronically as described in section 11.1.1.

[b] Will be done via RPFNA described in section 11.2, for patients consented prior to protocol version 3/9/2015 and discontinued for all participants 10/26/15.

[c] Platelet count should be included in the CBC panel, differential is not needed. The metabolic panel should include liver function tests.

[d] Only if premenopausal with irregular menstrual cycles, to rule out pregnancy (see eligibility criteria).

[e] Only if status-post hysterectomy without bilateral salpingo-oophorectomy, or does not have regular periods and pregnancy has been ruled out, to determine menopausal status (see eligibility criteria).

[f] Insulin growth factor-1 (IGF-1) and insulin growth factor binding protein-3 (IGFBP-3) will be determined (assay methods described in Section 11.7). Future analyses of sex steroid hormone levels, CYP2D6 SNPs and tamoxifen metabolite levels are also planned.

[g] Lipid profile includes total cholesterol, LDL-cholesterol, HDL-cholesterol, and triglycerides, determined using standard clinical assays.

[h] Anti-thrombin III (AT-III) is a measure of clotting propensity (a lower level suggests higher risk of clotting).

[i] Submit for serum bone-specific alkaline phosphatase (BSAP) and urine N-telopeptide crosslinks (NTX), which measure bone formation and bone resorption, respectively.

[J] Included in the appendix.

[K] The initial Day 0 pill kit will be dispensed at the time of blood and/or tissue sampling. The Day 180, 365, and 540 refills will be dispensed at the time of their follow-up clinical breast exams. Study capsules may be dispensed without waiting for lab clearance.

[L] Follow up calls are to confirm the patient is tolerating the RPFNA and to determine adherence and arrange medication refill dispensal.

[m] Patients will return 2 study kits at the time of their visit

*** The protocol allows for a +/- 60 business day window for all in person visits and +/- 5 business days for scheduled phone visits**.

**** The Day 0 visit (must be done within 60 days of completion of the screen visit if scheduled separately)**

10.0 CRITERIA FOR EVALUATION AND ENDPOINT DEFINITIONS

A subject will be considered evaluable if they complete at least one year on the study, including both baseline and one-year study procedures. The outcome status (in terms of toxicity and biological response) of all eligible subjects will be reported. Subjects who begin treatment will be accounted for in the summaries of patient reported outcomes, regardless if they are considered evaluable or not.

10.1 **Parameters to be Measured Prior to Initiation of Treatment**

There will be two visits prior to the initiation of treatment: a screening visit to identify eligibility, and a baseline visit. With careful planning and coordination, these visits may be combined as described in Sections 9.1 and 9.2. At the screening visit, a history and physical, CBC (with platelets), comprehensive chemistry panel will be performed. A mammogram will also be performed at that time. (If the patient had a digital mammogram using a large-paddle digital mammography machine performed within 120 days, the patient is either postmenopausal or is premenopausal and can estimate the day in the menstrual cycle when the mammogram was performed, and a raw DICOM format image of that mammogram can be obtained, then their prior mammogram may be used as the study mammogram). A FSH and/or HCG level will be measured at that time if it is needed to determine menopausal status (see definition in Section 5.0). For confirmed screen-eligible subjects enrolled at FNA-participating sites, an RPFNA procedure will be performed within 60 days of the screening visit to establish baseline breast tissue markers (for patients consenting prior to protocol version 3/9/2015 and all previously consented patients as of 10/26/15). Blood will be drawn after an 8-hour fast and urine collected within 60 days of the screening visit to measure circulating biomarkers as outlined in the study calendar.

10.2 **Specifications by Methods of Measurements**

The same method and laboratory will be used to characterize each identified and reported biological endpoint at baseline and during follow-up (see Section 11.0).

10.3 **Parameters to be Measured During Treatment**

All evaluations will be measured per the study calendar schedule using the assay methodology outlined in Section 11.0. Analysis of results is fully described in Section 12.0.

The following are biological response endpoints:

1. Mammographic Breast Density
2. Tissue biomarkers of BC risk – Cytomorphology, proliferative (Ki67) index, and apoptotic (caspase) index
3. Insulin Growth Factors – IGF-1 and IGFBP-3

The following endpoints are measures of safety and tolerability:

1. Bone metabolism markers - serum bone specific alkaline phosphatase (BSAP) and urine N-telopeptide crosslinks (NTX)
2. Fasting lipid panel – total cholesterol, HDL-cholesterol, LDL-cholesterol, and triglycerides
3. Clotting propensity – anti-thrombin III (AT-III)
4. Symptom logs

11.0 SPECIAL INSTRUCTIONS

11.1 **Mammographic Breast Density (MBD)**

Mammograms will be collected within 120 days prior to treatment initiation and at one and two years after initiation of treatment as described in the study calendar. After a clinical reading by a local radiologist, who will evaluate density according to the ACR BIRAD scoring system used to determine eligibility, MBD will be determined from digital mammogram images by study radiologists experienced in breast imaging. This density assessment method, fully outlined below, yields a value expressed as a continuous percentage, which is more sensitive to modulation by an intervention than the categorical BIRADS density score. Thus, while BIRAD score will be used for determining eligibility, percent breast density will be subjectively evaluated by study radiologists, and objectively measured using area- and volume-based mammographic density software. The area-based software measurement will be the primary endpoint. MBD will be determined from each breast. If the patient had a history of DCIS or IEN prior to study entry, or if one breast had a baseline BIRAD density score of 1, only the contralateral breast measurement will be used for analysis. Otherwise, the two measurements will be modeled as described in the statistical section.

11.1.1 Digital mammogram handling and transfer:

Mammography images will be performed on digital mammography machines using large paddles. Serial mammograms for each patient will be performed at the same facility using the same machine whenever possible. Sites will save post-processed and pre-processed or raw mammography images whenever both are available. The pre-processed or raw image is also called the “for processing” image, and may require coordination with radiology staff to obtain before being cleared from the PACS system. The post-processed image (also called “for presentation” image) is the file typically displayed by a standard PACS system. Each digital image (up to 8 mammogram files per patient time point) should be de-identified (headers excluded) and submitted on CD-ROM to the address below within 28 days of the screening, Day 365, and Day 730 visits.

If the subject has been followed with screening breast MRI as part of their standard of care screening regimen, the full electronic MRI file from the last breast MRI procedure performed prior to starting study drug and any breast MRI procedures performed during the 2-year protocol treatment period will be saved in de-identified DICOM format. All clinical MRI series obtained during the pre-treatment breast MRI procedure will be submitted at the same time as the screening mammogram. All clinical MRI series obtained during all breast MRI procedures performed on protocol treatment (up to two annual exams per participant) may be submitted at the end of study with the Day 730 mammogram.

The CD-ROM will be labeled with the subject’s name, central participant ID, site, and the date of preparation of the CD-ROM. A **Breast Imaging Submission Form** will be submitted along with the CD-ROM, on which the CRA/CRN will specify the date(s) of procedure(s) and the imaging equipment used. The electronic MRI will only be referred to as needed in the case of a breast event on trial. The MRI may also be stored for future research.

Address for site submission of breast imaging:

Smita Bhatia, MD, MPH
ATTN: LDtam study coordinator
UAB Division of Peds Hem/Onc
1600 7th Ave S, Lowder 500Birmingham, AL 35233
United States
Phone: 205-638-2127
Fax: 205-638-2121
Email: Survivorshiptrial@peds.uab.edu

After the CD-ROM arrives, study staff at the coordinating center will remove any electronically embedded patient identifiers and procedure dates from the header of each image file. Then, film IDs will be assigned to each mammogram image, consisting of the participant ID plus a dummy code in random order with respect to timing. The procedure date for each mammogram will be tracked in a database that documents which dummy code was assigned to the serial images. Anonymized films will sent to the study radiologists, organized in batches at multiple reading time points: when the 10th patient completes the study (initial data check), and subsequently when the 25th, 50th, 100th (halfway point), 150th, and the last patient completes the study.

MBD will be assessed in 12 batches (2 batches at each of the 6 reading time points) that contain all raw (batch A) and processed (batch B) films collected for 10-80 subjects (up to 320 films per reading time point). Views for 10% of subjects in each batch will be included a subsequent batch to obtain a second reading to assess reader reliability.

11.1.2 MBD assessment:

MBD will be assessed by study radiologists using previously published area [65] and volumetric [66] software methods.

11.1.3 MBD quality assurance:

Since all subject identifiers and dates will be removed from the DICOM file prior to transfer, study radiologists will be blinded to treatment arm as well as timing of the mammograms. To minimize random measurement error, all mammograms from each subject will be read in the same session, but they will be in random order. A random 10% of subject's films will be read twice to estimate quality control, as described above. Results will be delivered electronically, according to the batching procedures described above.

11.2 Breast tissue sampling

Breast epithelia will be sampled at baseline and after the two-year treatment period. Normal breast epithelial tissue will be acquired via RPFNA, which is a well-tolerated procedure that has been found to yield adequate tissue for multiple biomarkers [52]. The RPFNA procedure is fully described below, as well as the assays planned for tissue specimens obtained. The tissue biomarkers will be considered as secondary endpoints.

NOTE: The study was amended to discontinue this procedure for new patients consenting to the study (protocol version 3/9/2015) and all previously consented patients as of 10/26/15.

11.2.1 RPFNA procedure

After local anesthesia, a 21-gauge needle attached to a 10-mL syringe will be used to sample normal breast glandular tissue of the upper outer and upper inner quadrants by entering just immediately adjacent to the areola at approximately 3 o'clock and 9 o'clock, varying the position slightly as needed to avoid superficial blood vessels. Tissue is probed deeply to sample the terminal lobular–duct unit, where most cancers are thought to arise, obtaining 8-10 aspirations per breast; half from the upper outer quadrant site and half from the upper inner quadrant site. After the procedure, cold packs are applied to the aspiration sites for approximately 10 minutes, the breasts and chest wall are firmly bound in gauze, and a tight-fitting sports bra was worn over the gauze wrap to minimize breast movement and to decrease the chance of hematoma formation. Performing the RPFNA procedure in this manner typically yields 1000 epithelial cells per cytology slide [94, 95]. Minimum epithelial cell yields to allow evaluation are 10 for cytomorphology and 25 for IHC per slide.

The RPFNA procedure is well tolerated by most women, with any temporary discomfort experienced typically relieved with acetaminophen. In a large study including 480 women, 408 of 439 women invited for a repeat aspiration (93%) agreed to a second procedure, with 225 (51%) doing so as soon as 6 months after the first procedure. Infection requiring oral antibiotics or large hematoma formation requiring surgical evacuation occurred in fewer than 1% of the procedures performed [94]. Women will be asked to stop any medication that they may be taking that might increase their chances of bruising for at least ten days prior to the procedure (aspirin, non-steroidal, anti-inflammatory, or anti-coagulant medications, as well as any herbal supplements). In addition, as long as there is no history of venous thromboembolism, they may be given vitamin K 10mg to take daily for 3 days prior to the procedure to avoid bleeding and hematoma formation. Patients should be offered lorazepam 2 mg for anxiety and be instructed to take it 30 minutes before the procedure if they feel they need it. In that case, they should arrange for transportation and avoid driving that day. Also, the patient should be provided with four extra-strength Tylenol after the procedure in case of discomfort.

11.2.2 RPFNA specimen collection

All cells collected by the RPFNA procedures will be pooled into one tube containing 6 mL of Modified CytoLyt per the Specimen Handling SOP. Briefly, the sample is labeled with participant ID, date of procedure, and sample type (RPFNA) and then capped and gently inverted 2-3 times to mix. The sample is placed on a test tube rocker at low speed (if test tube rocker is not available, then leave tube in wet ice). Within 24 hours, the sample must be shipped with the **RPFNA Cytology Sample Submission Form** via FedEx priority overnight service on a cold pack to the central processing lab at KUMC at the address below.

Address for site submission of RPFNA samples:

Carol J. Fabian, M.D.
c/o Trina Metheny
University of Kansas Medical Center
Breast Cancer Prevention Center Laboratory

3901 Rainbow Blvd
1005 WHE, Mail Stop 3003
Kansas City, KS 66160
Phone: 913-588-3917
Email: tmetheny@kumc.edu

11.2.3 Processing the cytology sample at the central pathology lab:

Upon arrival to the KUMC central processing lab, one third of the CytoLyt suspended cellular sample will be processed for cytomorphology, and one third for evaluation of proliferative index and apoptotic index via Ki67 and caspase immunohistochemistry (IHC) staining, respectively. The remainder of the sample will be designated for tissue banking will be stored at -80C in RNAlater®. The cytomorphology sample will be processed first as outlined below, and that will be used to determine how the IHC slides will subsequently be prepared.

Cytomorphologic preparations are filtered through a 25-mm Millipore filter (5-m pore size) and cytocentrifuged, rather than smeared, to reduce cellular distortion and maximize cell yield per slide. If there are fewer than 10 cells on the cytomorphology slide, this will be noted in the pathology report, and the sample will be considered unevaluable for cytomorphology. The method for cytomorphologic assessment is described in Section 11.5.1.

Slides designated for IHC are sequentially fixed in 10% buffered formalin, methanol, and acetone. If there were fewer than 100 cells on the cytomorphology slide, the aliquots designated for Ki67 and caspase will be combined and used together for determination of proliferative index via IHC. In that case, the sample will be considered unavailable for apoptotic index. Proliferative index (and apoptotic index, when available) will be read as the number of cells with unequivocal nuclear staining per total number of ductal epithelial cells. If there were fewer than 500 cells on the slide, but more than 100, results will be considered evaluable but suboptimal. If there were fewer than 100 cells on the slide, that IHC measure will be considered unevaluable. Both of these IHC measures a described in detail in Sections 11.5.2 and 11.5.3, respectively.

RPFNA specimens processed in this manner typically result in slides with approximately 1000 epithelial cells per slide. Thus, we expect that cytomorphology, Ki67, and caspase, will be evaluable from nearly all breast epithelial specimens obtained by RPFNA.

11.3 Blood collection and processing

Peripheral blood will be sampled at baseline and annually while on treatment. For premenopausal participants, blood draws will be timed to occur during the follicular phase of the menstrual cycle (Days 2-10). All subjects will be asked to fast for at least 8 hours prior to blood draw.

Whole blood will be drawn into the following tubes:

- one 10 mL EDTA-containing lavender-top
- one 5 mL citrate-containing blue-top
- one 10 mL red -top vacutainer tube
- two 10 mL red tiger-top vacutainer SST tubes

All tubes are gently inverted 2-3 times after being drawn. Two of the red tiger-top tubes will require processing at the local site and should be placed on wet ice immediately after blood draw for transport to the specimen processing center. Specimen processing and handling are fully detailed in the Specimen Handling SOP, and briefly discussed below.

11.3.1 Processing and handling the K2EDTA-coated lavender-top tube

This tube should be inverted 8-10 times and then labeled with type of sample (whole blood), participant ID, and date of blood draw. Pre-printed cryogenic labels with the sample type will be provided. PLEASE WRITE IN THE PTID AND THE DATE OF THE SAMPLE WITH A FINE TIP SHARPIE PERMANENT MARKER. It will then be shipped ON DRY ICE (≥10 pounds of dry ice) in the provided shipping container via FedEx priority overnight service (ship Monday thru Wednesday only), accompanied by the Blood and Urine Specimen Submission Form. If there is a delay between blood draw and shipment, the tube must be kept frozen at -80°C. Do NOT store the blood at -20°C for any length of time. This sample will be used for pharmacogenetic studies.

Address for site submission of blood and urine samples. The study coordinator will document the receipt of the samples and transfer to the coordinating center laboratory:

Smita Bhatia, MD, MPH
ATTN: LDtam study coordinator
UAB Division of Peds Hem/Onc
1600 7th Ave S, Lowder 500
Birmingham, AL 35233
Phone: 205-638-2127
Email: Survivorshiptrial@peds.uab.edu

11.3.2 Handling the blue-top tube

The **blue-top tube** should be inverted 2-3 times to gently mix the blood in citrate and immediately transported to the CLIA-approved hematology laboratory at the local site for determination of anti-thrombin III (AT3) per institutional protocol. Results will be entered into a secure study website.

11.3.3 Processing and handling the red-top tubes:

The **red-top tube** will be immediately transported to the CLIA-approved chemistry laboratory at the local site for determination of a fasting lipid profile per institutional protocols. Lipid profiles consist of triglycerides (TG), total cholesterol (TC), high-density lipoprotein (HDL), and low-density lipoprotein (LDL). Results will be entered into a secure study website.

The two red tiger-top tubes should be inverted five times immediately after blood draw to ensure mixing of clot activator with blood (do not shake vigorously). Label the tubes with participant ID and date of blood draw using a permanent marker. Tubes should be allowed to clot at room temperature, upright in a test tube rack for 30 minutes. The sera is then harvested by spinning in a balanced centrifuge at 1200 x g for 10 minutes. Sera is aliquoted into up to 10 study-labeled cryovials with 1 ml per aliquot and placed in a -80°C freezer within 2 hours of collection time. All aliquots will be labeled with the provided cryogenic

labels (pre-printed with the aliquot number and sample type). PLEASE WRITE IN THE PTID AND THE DATE OF THE SAMPLE WITH A FINE TIP SHARPIE PERMANENT MARKER. Frozen serum samples should be shipped on DRY ICE (≥10 pounds of dry ice) via FedEx priority overnight service (ship Monday thru Wednesday only) to the coordinating center using the provided shipping container, FedEx shipping label, and accompanied by the **Blood and Urine Specimen Submission Form**.

11.3.4 Storage of blood samples

The whole blood and serum samples will be transferred to the UAB Institute for Cancer Outcomes and Survivorship laboratory, where they will be bar-coded, entered into a specimen tracking database, and stored at -80°C until assays are performed.

11.4 Urine collection and processing

A spot urine sample will be collected at baseline and annually while on treatment. Two aliquots (3.5mL each) will be frozen at -20°C and then shipped on dry ice as described in the Specimen Handling SOP to the address in Section 11.3.1, accompanied by a **Blood and Urine Specimen Submission Form**. This sample will be used for evaluation of N-telopetides (NTX) as described in Section 11.8.2.

11.5 Tissue-based biomarkers

11.5.1 Cytomorphologic scoring

Cytomorphology will be scored according to the Masood classification from epithelial cells retrieved via RPFNA. This scoring system assigns a score of 1 to 4 points to each of 6 morphologic characteristics: cellular arrangement, cellular pleomorphism, prevalence of myoepithelial cells, anisonucleosis, nucleoli, and chromatin clumping. A score of 6 to 10 is associated with nonproliferative specimens, 11 to 14 with hyperplasia without atypia, 15 to 18 with hyperplasia with atypia, and 19 to 24 with malignancy (Table 2) [99]. For cytology, intraobserver variance has been reported to range from 8-24% [100]. Slides will be hand scored by two readers. In the case of a difference between the two readers, the scores will be averaged. The first reading and second reading will be performed by KUMC-associated cytopathologists. De-identified stained slides will be sent to each reader for blinded review:

11.5.2 Determination of tissue proliferative index

Proliferative Index will be determined using an immunohistochemistry (IHC) assay to evaluate Ki-67 expression from epithelial cells contained in RPFNA samples. A categorical estimate of the number of ductal epithelial cells present on the slides designated for proliferative index evaluation will first be assessed: less than 100, 100 to 500, 500 to 1,000; 1,000 to 5,000; or more than 5,000. Slides containing at least 100 epithelial cells will be processed for Ki-67 staining; if no slides meet this criterion, the subject will be deemed unevaluable for this endpoint at that time point. Antigen retrieval will be performed with 10 mmol/L citrate buffer (pH 6) and then will be stained with Ki67 clone MiB-1 monoclonal antibody (Dako North America, Carpinteria, CA) at a 1:20 dilution. Hyperplastic cell clusters will be preferentially assessed for nuclear staining. The number of cells with unequivocal nuclear staining out of 500 cells assessed will be recorded

as the proliferative index. Slides will be hand scored by two readers as described in Section 11.5.1, and in the case of a difference between the two readers, the scores will be averaged. Agreement between two readers using this method is excellent (Cronbach's α = 0.99; reported in [86]). Within-person reliability is also superb, with a previously reported intraobserver variance of 4% [100].

11.5.3 Determination of tissue apoptotic index

RPFNA cytology samples will be stored for future analysis of tissue apoptotic index. *Apoptotic index* will be determined using an IHC assay to evaluate cleaved caspase-3 expression from epithelial cells contained in RPFNA samples. After categorical estimation of the number of ductal epithelial cells present on the slides designated for apoptotic index, antigen retrieval will be performed as described above and slides stained with cleaved Caspase 3 rabbit polyclonal antibody (Cell Signaling Technologies, Boston, MA). The number of cells positively stained out of 100 will be recorded as the apoptotic index, with the subject deemed unevaluable for this endpoint if less than 100 cells are present on the slide. As above, slides will be scored by two readers as described in Section 11.5.1, and scores will be averaged if discrepant.

11.6 Estrogen Assays

Serum and RPFNA samples collected into saline will be stored at the coordinating center for future analysis of sex steroid hormone levels. At the time of analysis, the samples will be shipped to the Reproductive Endocrinology Research Laboratory of Dr. Frank Z. Stanczyk at the University of Southern California. The methodology used is a radioimmunoassay (RIA) after a unique in-house extraction using column chromatography as outlined below. This technique is highly sensitive for monitoring circulating estrogens in postmenopausal women.

Total Estradiol (E_2) – One-tenth of a ml of serum is incubated with ^{3}H-E_2 and extracted with hexane and ethyl acetate to remove unconjugated steroids. After evaporating under nitrogen, the residue is redissolved in isooctane and applied on a column of Celite impregnated with ethylene glycol. E_2 is eluted in ethyl acetate in isooctane and redissolved in buffer. Then it is incubated with ^{125}I-E_2 and anti-E_2 serum. Antibody-bound and unbound ^{125}I-E_2 are separated by adding a second goat anti-rabbit antibody, centrifuging, and aspirating the supernatant. Total estradiol is then quantitated using a gamma counter.

Bioavailable E_2 – is calculated by determining the *sex hormone binding globulin* (SHBG) level and subtracting that from the total estradiol level. SHBG is measured by a solid-phase, two-site chemiluminescent immunoassay using the Immulite analyzer. Alkaline phosphatase conjugated anti-SHBG polyclonal antibodies are introduced into the reaction tube, and the tube is incubated for 30 minutes at 37°C. SHBG in the sample is bound, forming an antibody sandwich complex. Unbound conjugate is then removed by a centrifuge wash, after which the chemiluminescent substrate is added, and the reaction tube is incubated for another 5 min. The chemiluminescent substrate undergoes hydrolysis in the presence of alkaline phosphatase, yielding an unstable intermediate. Continuous production of this intermediate results in the sustained emission of light. The light is measured by an illuminometer and is proportional to the concentration of SHBG in the sample

Estrone (E_1) – is determined using 1 ml of serum, which is incubated with ^3H-E_1 and treated with the same extraction and column chromatography methods as with total estradiol but using ^{125}I-E_1 and anti-E_1 serum with a second goat anti-rabbit antibody. Estrone is quantitated similarly after centrifugation and separation.

Estrone Sulfate (E_1S) - One-third of a ml of plasma is incubated with ^3H-E_1S and is extracted with hexane and ethyl acetate, then deproteinized with methanol. After evaporating under nitrogen, the residue is reconstituted with sodium acetate buffer and hydrolyzed with arylsulfatase. The hexane:ethyl acetate extraction step is repeated, the extract is redissolved in buffer, and then the E_1 RIA is carried out.

The assay sensitivities are as follows: E_2 = 5 pg/ml = 1.36 pmol/L, E_1 = 5 pg/ml = 1.35 pmol/L, and E_1S = 0.16 ng/ml. Intra-assay and inter-assay coefficients of variation (CV) for all four assays are well below 15%, as tabulated below.

Assay	Intra-assay CV	Inter-assay CV
Total Estradiol	7.0%	9-13%
Sex Hormone Binding Globulin	4.1-7.7%	6-13%
Estrone	7.4%	8-10%
Estrone Sulfate	7.5%	11%

11.7 Insulin Growth Factors (IGFs)

These tests will be performed by ARUP Laboratories at the University of Utah. ARUP is a national full-service reference laboratory that serves more than 50 academic medical centers, as well as many community hospitals, commercial laboratories, military and government facilities, and pharmaceutical firms. One 1mL aliquot will be sent by the coordinating center to ARUP Laboratories for measurement of the following IGFs. A second 1mL aliquot will be held for IGF evaluation in the case extra serum is needed.

11.7.1 IGF-1 will be measured as circulating markers of BC risk. The assay will be conducted using Chemiluminescent Immunoassay. The sensitivity of the assay is 5.1 ng/mL. The expected postmenopausal range is 58-318ng/mL. The expected premenopausal range is 89-397ng/mL.

11.7.2 IGFBP3 will be measured as circulating markers of BC risk. The assay will be conducted using Chemiluminescent Immunoassay. The sensitivity of the assay is 38.2 ng/mL. The expected postmenopausal range is 2514-6014 ng/mL. The expected premenopausal range is 2926-5858ng/mL.

11.8 Bone metabolism marker assays

Blood and urine will be collected at baseline and at the one-year and two-year visits at the local site and sent directly to ARUP Laboratories per instructions in Sections 11.3 and 11.4 (see address in Section 11.7).

11.8.1 *Serum bone-specific alkaline phosphatase (BSAP)* will be measured as a marker of bone formation. It is an isoenzyme of alkaline phosphatase localized on the cell membrane of osteoblasts. The assay employs a solid phase two-site immunoradiometric assay (IRMA). The sensitivity of the assay is 2.0 µg/L. The expected postmenopausal range is 7.0-22.4 µg/L. The expected premenopausal range is 4.5-16.9 µg/L.

11.8.2 *Urine N-telopeptide crosslinks (NTX)* will be measured as a marker of bone resorption. Type I collagen comprises 90% of organic bone. It is a helical protein that is cross-linked at the N-terminal and C-terminal ends. These crosslinks are released during bone remodeling by osteoclasts. They are then found in the urine as a stable end-product of bone metabolism. The procedure is chemiluminescent immunoassay. Final values are reported as bone collagen equivalents (BCE) in nM/L corrected for creatinine clearance in mM/L. The expected range in postmenopausal women is 26-124 nM BCE/mM creatinine. The expected premenopausal range is 17-94 nM BCE/mM creatinine.

11.9 Lipid assays

Blood will be drawn into a red-top tube at baseline before the first dose of treatment and then annually (Day 365 and 730), each time after an 8-hour fast. This blood sample will be sent directly to the CLIA-approved chemistry lab for determination of *triglycerides* (*TG*), *total cholesterol* (*TC*), *high-density lipoprotein* (*HDL*), and *low-density lipoprotein (LDL)*. Results will be faxed back to the coordinating site.

Although lipid profile abnormalities alone will not be considered an indication for stopping study protocol therapy, lipids will be monitored and treated according to NCEP ATP guidelines (http://www.nhlbi.nih.gov/guidelines/cholesterol/atglance.pdf).

11.10 Clotting assays

Blood will be collected into at baseline and at the one-year and two-year visits at the local site and sent directly to the CLIA-approved hematology laboratory at each local site for determination of anti-thrombin III per institutional protocol. Results will be entered into the study EDC website.

11.11 Pharmacokinetic and pharmacogenetic measures

Plasma will be processed from each scheduled blood draw and will be stored for the measurement of tamoxifen levels and levels of the tamoxifen metabolites, N-desmethyl-tamoxifen (also known as endoxifen) and 4-OH-tamoxifen, at the time of trial completion.

Blood will be also processed for DNA to allow for analyses of P450 SNPs including CYP2D6 polymorphisms after trial completion and unblinding. Stored DNA may also be used to explore DNA adduct formation during the course of tamoxifen treatment.

The SNP studies will be performed using commercial assays developed by Roche Laboratories.

11.12 Specimen storage

Unused biological materials will not be discarded, and will be stored for possible future research purposes in the UAB Institute for Cancer Outcomes and Survivorship laboratory. IRB approval will be sought prior to their use for purposes other than those specifically outlined in this protocol. In addition, ancillary studies must be approved by the Consortium for Pediatric Interventional Research (CPIR) and all other participating institutions.

11.13 Questionnaire data

Self-reported and CRA/CRN administered questionnaires will be used to obtain baseline data on demographics, family history, reproductive history, and other BC risk factor exposure, as well as to assess patient reported symptoms during the course of the study. The self-reported questionnaires (**Demographic and Breast Cancer Risk Factors Form, QOL Form, Family History Form and the Symptom Log**) are considered source documents themselves and are not required to be accompanied by a source document, because of the design of the study. Utilizing the study EDC website, these questionnaires will be entered electronically, in compliance with FDA Regulation 21 CFR Part 11.

Patient demographics and baseline BC risk factors:

Standardized questionnaires will be used to obtain the following prior to study entry:

- demographic reproductive history, and BC risk factors information (**Demographics and Breast Cancer Risk Factors Form**),
- health and physical (**H&P Form**)
- family history (**COH DPS Family History Form**),

The salient domains captured by these questionnaires include date of birth, gender, race/ethnicity, socioeconomic status, and both familial and environmental BC risk factors. This questionnaire will be administered at the time of initial registration.

Clinical data regarding prior Cancer diagnosis and treatment:

The following information will be obtained from medical records using a standardized data abstraction form (the **Primary Cancer Treatment History Form**): date of diagnosis, stage of disease (with or without "b" symptoms), histological subtype, data elements regarding chemotherapy including dates, protocols/regimens, cumulative doses of therapeutic agents per square meter; and data regarding radiation therapy including dates, total lifetime dose, field, fractions, dose per fraction. A copy of institutional radiation oncology summary report will be obtained whenever possible.

Patient reported symptoms and adherence to the chemopreventive agent:

The National Surgical Breast and Bowel Project (NSABP) developed a standardized quality of life (QOL) questionnaire to assess patient reported symptoms in response to hormonal interventions, which they used in the NSABP P-1 and P-2 breast cancer prevention trials (**NSABP QOL questionnaire**). The most recent version of this questionnaire, which was used in the NSABP P-2 trial [101], will be used. The salient domains captured by this questionnaire include both physical and mental well-being. In addition, a **Symptom Log** including the HEAL hormone-related symptoms instrument will be used [102]. This questionnaire captures general symptoms along with specific hormone-related symptoms along the following scales: vasomotor, vaginal, incontinence, cognitive/mood, and weight gain/appearance. Both questionnaires will be administered at baseline and during the two-year treatment period according to the study calendar. Pill counts will also be used to determine adherence to the prescribed intervention using the **Adherence Tracking Form**.

Patients who report intolerable hot flashes may be managed with any of the following interventions (please refer to the Treatment and Follow-up SOP for recommended algorithm):

- Vitamin E 800 IU po daily
- Effexor (venlafaxine) XL 37.5-75 mg po daily

- Neurontin (gabapentin) 300mg po TID
- Lyrica (pregabalin) 75mg po BID

Patients who report vaginal atrophic symptoms may be managed with any of the following interventions (please refer to the Treatment and Follow-up SOP for recommended algorithm):

- Replens
- Astroglide
- Vagifem
- Estring

11.14 Natural products known to modulate estrogens

The following herbal supplements have been associated with hormonal activity. The primary endpoint, mammographic density, is positively correlated with estrogen levels. Therefore, subjects will be asked to avoid these items before during the treatment period. Any use will be recorded along with medication changes during the course of the trial.

- Alfalfa
- Bee Pollen
- Black Cohosh
- Blue Cohosh
- Cats Claw
- Chasteberry
- Chysin
- Coumestans
- Cramp Bark
- Dong Quai, or Chinese Angelica
- DHEA
- Evening Primrose
- Feverfew
- Flaxseed
- Ginseng
- Hops
- Isoflavones
- Kava
- Kudzu
- Licorice
- Lignans
- Passionflower
- Propolis
- Red clover or other clover extracts
- Sarsaparilla
- Saw palmetto
- Shepherds purse
- Soy extracts
- St. Johns Wort
- Valeriana
- Wild yam

11.15 Drugs described to modulate CYP2D6

The following drugs have been reported as inhibitors or inducers of CYP2D6, which is the major P450 enzyme that metabolizes tamoxifen. Concomitant use of strong and clinically significant moderate inhibitors will not be allowed while on trial. Other moderate inhibitors should be avoided while on study.

Strong CYP2D6 inhibitors (not allowed on study):
bupropion (Wellbutrin)
fluoxetine (Prozac)
paroxetine (Paxil)
quinidine (Quinidex)

Clinically significant moderate CYP2D6 inhibitor (not allowed on study):
duloxetine (Cymbalta)

Other moderate CYP2D6 inhibitors

(should be avoided while on study):
sertraline (Zoloft)
terbinafine (Lamisil)

Weak CYP2D6 inhibitors (will be tracked on study):
amiodarone (Cordarone)
cimetidine (Tagamet)
thioridazine (Mellaril)

Possible CYP2D6 inhibitors (will be tracked on study):
celecoxib (Celebrex)
chlorpheniramine*
chlorpromazine (Thorazine)
citalopram (Celexa)
clemastine (Tavist)*
clomipramine (Anafranil)
diphenhydramine (Benadryl)*
doxepin (Sinequan)
doxorubicin (Adriamycin)
escitalopram (Lexapro)
halofantrine (Halfan)
haloperidol (Haldol)
hydroxyzine (Vistaril, Atarax)*
levomepromazine (Nozinan)
methadone
metoclopramide (Reglan)
midodrine (ProAmantine)
moclobemide (Aurorix, Manerix)
perphenazine (Trilafon)
ranitidine (Zantac)*
ritonavir (Norvir)
ticlopidine (Ticlid)
tripelennamine (Pyribenzamine)
other histamine H1 receptor antagonists**

Possible CYP2D6 inducers (will be tracked on study):
dexamethasone (Decadron, Hexadrol)
rifampin (Rifadin, Rimactane)

Virtually no effect on CYP2D6:
venlafaxine (Effexor) – *preferred antidepressant while on study*

*available over the counter
**examples: cetirizine (Zyrtec), dimenhydrinate (Dramamine), doxylamine (included in NyQuil), fexofenadine (Allegra), loratidine (Claritin), meclizine (Antivert, Dramamine [less drowsy formulation]), pheniramine (Avil)

11.16 Other drugs to avoid while on trial

- Warfarin or other coumarin-type anticoagulants
- Systemic exogenous hormonal agents, including
    - Other selective estrogen receptor modifiers, such as raloxifene (Evista).
    - Systemic hormone replacement therapy (includes oral or transdermal formulations). Vagifem and Estring, two formulations of locally applied vaginal estrogen associated with minimal systemic absorption, may be allowed.

Other estrogen-containing vaginal creams, while not an exclusion, should be avoided whenever possible. Patients with a history of hormone modifying herbal supplements (see section 11.3) are eligible, but patients will be asked to avoid their use after on study.

- Hormonal forms of contraception (includes oral, transdermal, implanted, and injectable formulations),
- Aromatase inhibitors,
- GnRH analogs,
- Prolactin inhibitors, and
- Androgens or antiandrogens.

12.0 STATISTICAL CONSIDERATIONS

12.1 **Study design**

This is a Phase IIb randomized placebo controlled clinical trial.

12.2 **Accrual plan**

This study is expected to accrue 230 evaluable subjects.

12.3 **Stratified randomization**

Central participant IDs will be generated by the coordinating center and will be issued to sites as they are activated. Participant IDs for all sites will be sent to Sharp Clinical Services, along with the site names and strata as defined below. Sharp Clinical Services designed a system (IWRS) that will manage blocked stratified randomization, with menopausal status (2 strata: pre- and post-menopausal), radiation dose to the chest (2 strata: 1200-2599 cGy, ≥2600 cGy), and age at radiation exposure (2 strata: <18y, 18-40y) as stratification factors (8 strata total) and a block size of 4 to balance the number of participants in each arm. Blinded study drug will be shipped to sites as they are activated by Sharp Clinical Services. Once a subject has completed all screening assessments and is eligible, the site CRA or CRN will randomize the subject to low-dose tamoxifen or placebo using the Sharp Clinical Services IWRS, which will assign a blinded study kit. This will generate an e-mail notification to the site and the coordinating center that randomization has been completed (as described in Section 9.3).

12.4 **Data analytic plan**

Aim 1: *To determine the impact of low-dose tamoxifen on surrogate endpoint biomarkers of BC risk.*

Mammographic breast density (MBD). MBD is the ratio of absolute density to the total breast area. Since participants are randomized, we expect MBD to not differ by treatment status at baseline (t0). However, we will evaluate the success of randomization by examining between group differences in demographic and clinical characteristics (i.e. age, radiation dose, age at radiation exposure), study site, as well as the average MBD of the two breasts (or the available one) at baseline. If imbalance is evident, analysis will take into account the covariates with significant imbalance.

Using an intention-to-treat analysis, the efficacy of low dose tamoxifen in reducing MBD will be compared between patients in the low dose tamoxifen intervention and placebo group by applying the linear mixed effects model for bivariate normally distributed data [103]. All patients with a minimum of baseline (t0) mammographic data will be included in the analysis. MBD data from each breast will be square root transformed (RTMBD) to normality before model fitting. RTMBD for each breast will be modeled by the unstructured mean model using two indicator variables of time. We will assume the effects of time on RTMBD to be the same in both breasts by setting their coefficients equal. Correlation between the breasts will be introduced by assuming a bivariate normal random intercept model with a common fixed effects intercept and breast-specific random effects. Residual errors will be assumed to be bivariate normally distributed with mean zero and non-zero correlation. Unstructured and compound symmetry covariance structures will be considered for residual errors. The fixed effects intercept will be allowed to differ between treatment groups. Interactions of the time indicators with treatment indicator (0 for placebo, 1 for tamoxifen) will be included to allow for treatment

differences at t1 and t2, and their significance tested using a 2-df chi-square test. Low dose tamoxifen will be considered effective if 1) the 2-df test is significant and the expected RTMBD is lower both at t1 and t2 or at t2 compared to that of the placebo group, or 2) the interaction term at t2 is significant and the expected RTMBD there is lower than that of placebo. It should be noted that because common fixed effects for intercept and time coefficients are assumed for both breasts, the expected RTMBD is the same for both breasts within a treatment group. This model enables use of the data from both breasts to estimate the common treatment effect while accounting for the between-breast correlation and the correlation among repeated measurements over time within a woman. The validity of these restrictions, e.g. common intercept and treatment effect between left and right breast, while biologically reasonable, will be examined and tested. This method also enables women with incomplete RTMBD data, i.e. data from only one breast or missing data at specific time points, to be included in the analysis. In addition to the unstructured mean model, we will also fit a linear model in time, with an interaction of time by group indicator to allow for treatment difference. A significant time by group interaction, with a larger negative slope for the low dose tamoxifen group compared to placebo, will indicate the effectiveness of low dose tamoxifen in reducing MBD.

In addition to unadjusted analyses which are appropriate under the randomization principal, adjusted analysis also will be performed. Significantly imbalanced covariates and covariates known to be prognostic for BC, such as radiation dose, age at irradiation, premature menopause, and family history for BC, will be incorporated in the longitudinal models before including and testing the time by treatment group interaction.

To address potential noncompliance, we will also conduct an as-treated analysis, considering patients' actual treatment uptake. Instead of the treatment indicator (PBO or LDTAM), we will include adherence rate, i.e. 0 for PBO and a number between 0 and 1 for LDTAM determined by pill count, in the LME model. Time-specific adherence rate and the overall adherence rate will be considered. If LDTAM is effective, we may expect to see a decrease in MBD with adherence rate.

The estimates from linear mixed effects model are valid in the presence of incomplete response data if missing data occurs at random. However, non-participation and dropouts may occur differentially between treatment groups for various reasons, possibly resulting in non-ignorable missing data. In addition to collecting information on reasons for nonparticipation, we will apply two analytic approaches to examine the effects of missing data on study results [104]: 1) pattern mixture models and 2) selection models. In pattern mixture models, the four possible missing data patterns (arising from 3 time points with no missing data at t0 and considering a response as missing if data from both breasts are missing) will be used as covariates in the above-described bivariate linear mixed effects model. The effects of missing data on the estimate of treatment effect can be assessed. An overall estimate of the treatment effect may be obtained by averaging the estimates over the missing pattern groups. In selection model, we will model the probability of dropout using baseline covariates to estimate the propensity for dropout. The propensity scores will be used as a covariate in the bivariate linear mixed effects model to adjust for the effects of dropout on treatment effects.

Secondary efficacy objectives will be evaluated by applying the methods for longitudinal analysis as described below. Significance of the low dose tamoxifen effects is evaluated in the manner described above based on testing the significance of the interaction of time by group indicator variables. The distribution of continuous variables will be

examined graphically and appropriate transformations made before applying analytical methods based on normal assumption.

Cytomorphology and proliferative index. Samples for cytomorphology and proliferative index will be collected at two time points, at pre-treatment (t0) and at post-treatment (t2). The Masood score for cytomorphology ranges from 6 to 24, which can be classified into 4 groups of worsening morphology: 1) nonproliferative (6-10), 2) hyperplasia without atypia (11-14), 3) hyperplasia with atypia (15-18), 4) malignancy (19-24). A patient is considered to have cellular atypia if their Masood score is 15 or above. We will compare the effects of low dose tamoxifen versus placebo on changes in Masood score between pre- and post-treatment. Masood score will be treated as a continuous variable, transformed to normality as appropriate, and the linear mixed effects model applied using an indicator variable for pre- and post-treatment time points. We will also consider Masood score as an ordinal outcome with 4 categories and as a dichotomous outcome (cellular atypia or not), and apply the generalized linear mixed model (GLMM) for non-normal data [105].

Proliferative index, defined as the number of cells with unequivocal nuclear staining (Ki-67 expression) out of a total number of ductal epithelial cells contained in RPFNA samples, will be analyzed using longitudinal logistic regression with two time points. If cell counts are better approximated by a Poisson distribution, we will apply the longitudinal Poisson regression method.

Insulin growth factors. Insulin growth factor-1 (IGF1) and insulin growth factor binding protein-3 (IGFBP3) will be treated as continuous measures. We will apply the linear mixed effects model for between group comparisons of measures from the three time points. The unstructured mean model and linear in time model will be employed.

Aim 2: *To establish safety and tolerability of low-dose tamoxifen.*

Objective safety measures:

Adverse events. The number of grade 2-4 toxicities observed will be tabulated by treatment arm. Differences by treatment arm will be evaluated using Fisher exact tests.

Biomarkers. Total cholesterol, low and high density lipoprotein, triglycerides, anti-thrombin III enzymatic assay (for clotting propensity), and serum bone-specific alkaline phosphatase (BSAP, for bone formation) and urine N-telopeptides (NTX, for bone resorption) measurements will be treated as continuous variables. Transformed to normality as appropriate, the linear mixed effects model with be applied, using the unstructured mean model using and linear in time model, to assess the effects of low dose tamoxifen on these measurements over time.

Subjective safety and tolerability measures:

Treatment adherence. Compliance will be measured by pill counts performed every 3 months. The number of pills taken out of the total prescribed in a 3-month period will be modeled as a random effects binomial regression model [105]. The binomial rates from 8 time points (month 3 to 24) will be modeled as unstructured mean model with 7 indicator variables as well as polynomial models over time. The random-intercept and the random intercept and slope models will be considered. The significance of the time indicators or parameters by treatment interaction will be evaluated for treatment difference in compliance.

Voluntary withdrawals will be examined at the end of the study by comparing the percent of withdrawals between the treatment groups using a chi-square test or Fisher's exact test.

Patient reported symptoms. The outcomes will be scored as a 5-point Likert-type scale (0 to 4) in response to questions on how much the patients are bothered by certain symptoms. The questionnaire will be administered every six months, for a total of five time points. The responses will be treated as normally distributed, as ordinal or dichotomized variable, and we will apply the linear mixed effects model or GLMM methods to compare changes between treatment groups. Because of additional time points available, in addition to the unstructured mean model, we will also fit piecewise models with join point at 6 months, considering linear and curvilinear trajectories between 6m and 24m time points.

Exploratory Aim 1: *To examine the modifying effects of demographic, clinical, and molecular characteristics on both efficacy and safety markers.*

As the primary aim of this trial is to examine the main effects of low dose tamoxifen treatment on MBD, we will examine the modifying effects of demographic, clinical, and molecular characteristics as exploratory analysis. Even if time by treatment interaction (main treatment effect) is not significant, examining the modifying effects of covariates may reveal subgroups that respond differently to low dose tamoxifen. A three-way interaction of time by treatment by modifying variable will be included in the longitudinal model containing a two-way time by treatment group interaction. Modifying effects will be considered statistically significant if the three-way interaction is significant. The covariates of interest are described below.

A. Patient demographics.

The following variables are known to affect breast cancer risk and MBD.

Attained age – continuous, time-varying variable.

Menopausal status – status at study enrollment (premenopausal or postmenopausal, as defined in Section 5.0) will be considered as constant over the study period. We will also consider trichotomized status (pre-menopausal, typical post-menopausal, and early post-menopausal), constant over the study period. Menopause will be defined as "early" if onset was before age 50.

Prior hormone use – categorical variable (estrogen plus progestin menopausal hormone therapy, estrogen-only menopausal hormone therapy, hormonal contraception and/or fertility drugs, no prior hormonal therapy). We will also consider duration of use, as a continuous and dichotomous variable (5+ years, <5 years); and most recent use (within 1 year, >1 year).

Body mass index (BMI) – continuous, time-varying variable. It will also be grouped into ordinal categories: underweight (<19), normal weight (19-24), overweight (25-29), obese (30-39), morbidly obese (40+).

Personal history of benign breast disease – dichotomous (yes/no), constant over the study period. We will also examine whether the number of prior breast biopsies, and the presence of atypia or LCIS on a prior biopsy further modifies the intervention effect.

Family history of cancer – dichotomous (yes/no), constant over the study period. We will also examine the frequency of the following classifications: 0 = no family members with breast cancer; 1 = one or more first or secondary degree relative(s) with BC all diagnosed at 50+ years of age; 2= one first or second degree relative with BC diagnosed

before age 50 (or two, but on different sides of the family); 3 = two or more first and/or second degree relatives with BC diagnosed before age 50 and/or ovarian cancer on the same side of the family; 4 = carrier of a germline mutation in a highly penetrant BC susceptibility gene (BRCA1, BRCA2, PTEN, p53), or a known mutation in one of these genes in the patient's family and patient has not had genetic testing. If this results in sparse cells, we will consider collapsing them: A = no family history, B = some family history (group 2 above), and C = strong family history (groups 3 and 4 above). We will also examine if the number of first & second degree relatives (constant over the study period) is a significant effect modifier.

B. Clinical primary cancer treatment characteristics.

The following variables are known to influence breast cancer risk among radiation exposed CAYAC survivors.

Chest radiation dose – continuous, time-invariant. We will also dichotomize dose levels as described in Section 12.3.

Age at exposure to chest radiation – continuous, time-invariant. We will also categorize as described in Section 12.3.

Latency from radiation therapy – continuous, time-varying. We will examine if intervention effects vary by time since exposure to therapeutic radiation.

Pelvic radiation – continuous, time-invariant. Depending on the range of doses, we will also consider dichotomizing or categorizing the dose levels.

Alkylator containing chemotherapy regimen – dichotomous (yes/no), time-invariant.

C. Molecular characteristics.

The following variables are known to influence efficacy of tamoxifen in the treatment of BC patients and will be analyzed at a later date.

Endoxifen levels – continuous, time-varying. These data will be available at t1 and t2.

CYP variants - dichotomous (yes/no), constant over the study period.

In addition to testing their significance as effect modifiers, we will also examine their relationship with the outcome variables (MBD, cytomorphology, proliferative index, etc) to validate known relationships. Proc Mixed for normal data and Proc GLMMIX for non-normal data in SAS 9.1 (Cary, North Carolina) will be used for analysis.

Exploratory Aim 2: *To examine the relationship between a low-dose tamoxifen regimen and clinical measures of efficacy and toxicity.*

For measures of clinical efficacy, we will document the occurrence of new breast cancer and DCIS diagnoses.

The assessment of clinical toxicity will include the following outcomes/measures:

1) incidence of uterine and other cancers during the study;

2) gynecological symptoms: any and the frequency of hot flashes, night sweats, vaginal discharge, vaginal bleeding or spotting, missed menstrual periods for pre-menopausal women, genital itching/irritation, and pain with intercourse, to be obtained from symptom log administered 10 times (baseline, days 28, 90, 180, 270, 365, 455, 540, 630, 730) ;

3) incident thromboembolic events (DVT, PE, TIA or stroke, and retinal vein thromboses);

4) incidence of cataract;

5) liver function abnormalities: total bilirubin, SGOT/AST, SGPT/ALT, measured at baseline, year 1, and year 2.

The *incidence* of new breast cancer, DCIS, non-breast malignancies, thromboembolic events, and cataract are expected to be low (≤5 events per arm) during the study period given the sample size, based on statistics quoted in Investigational Brochure. Thus, we will initially compare the frequency of events by treatment group in a 2x2 table using the Fisher exact test, stratified on CYP2D6 status. We will also use poisson regression in cohort analysis to examine treatment differences in these outcomes adjusted for covariates, including CYP2D6.

The presence/absence of gynecological symptoms (for each symptom mentioned above) will be compared between treatment groups using 2x2 tables using the Fisher exact test, stratified on CYP2D6 status. Logistic regression will be used to compare the frequency between treatments adjusted for covariates, including CYP2D6. Longitudinal analysis also will be conducted to compare the trends of the presence/absence of each of the reported gynecological symptoms over the 2 year period using the generalized linear mixed-effects model for binary outcomes.

For each liver function measure, a woman will be considered to have elevated liver function if the measurements at years 1 and/or 2 are above the upper limit of the institutional reference range and higher than her baseline pre-treatment level. These data will be coded such that those who are greater than 2-times the upper limit of the reference range will be considered to be clinically significant (grade 1 if asymptomatic and requiring no intervention; grade 2 if minimal/local/non-invasive intervention required). The frequency of women with elevated liver function measures will be compared between treatment groups using an exact test on 2x2 tables, stratified on CYP2D6. Logistic regression analysis will also be used to compare the frequency of elevated liver function between treatments, adjusted for covariates. Linear mixed-effects model for normally distributed data will also be used to compare the trends in liver function levels between the treatment groups. Procs MIXED and GLIMMIX will be used for longitudinal analysis of normally and non-normally distributed data, respectively. Poisson regression analysis will be conducted using Proc GENMOD, all in SAS 9.1 (Cary, North Carolina).

12.5 **Power and sample size**:

Aim 1: *To determine the impact of low-dose tamoxifen on surrogate endpoint biomarkers of BC risk.*

Mammographic breast density (MBD): The primary efficacy endpoint of the trial is MBD. The efficacy of low dose tamoxifen will be examined by comparing the slopes in the longitudinal linear regression of the MBD on time since treatment initiation between the low dose tamoxifen group and placebo control group. For sample size calculation, we let Y, the response, as the mean of the square root of MBD (RTMBD) from both breasts. Because of randomization, we expect no difference between the expected values of Y at t0 between treatments but expect the linear slopes of the two groups to diverge over time, if the intervention is efficacious. Power/sample size calculation was conducted by assuming a linear change in RTMBD over time and various magnitudes of RTMBD difference expected at t2 between the treatment and control groups.

Longitudinal power calculation requires specification of the expected linear trend (thus the expected values of Y at t0, t1, t2) for the controls, the variance/covariance of the repeated measurements, the expected difference in Y at t2 between the treatment groups, and the correlations among repeated measurements. These estimates were obtained from 41 control women in the Puget Sound NSABP P-1 participating sites, [106] 16 pre-menopausal women (median age=50, range=38-50) and 25 post-menopausal women, (median age=57, range=36-74), with data on MBD expressed as a percent breast density at pre-, 1-yr, and 2-yr post-treatment. Although this entire control sample (median age=50, range=36-74) is older than the available CPIR HL cohort (median age=35, range 25-50), based on changes in MBD observed with hormonal fluctuations during the menstrual cycle [66] and after discontinuation of exogenous hormone in postmenopausal women [70], it can be reasonably assumed that estrogen withdrawal associated with menopause has a greater impact on variation in MBD than age alone. Thus, we assumed that the estimates obtained from these women can be applied to the anticipated CAYAC patient control sample.

The expected RTMBD at the three time points in the pre-menopausal and post-menopausal women in the Puget Sound sample were estimated longitudinally using SAS Proc Mixed with a compound symmetry covariance structure. Square root transformation was used to normalize the distribution. A significant decline in MBD with time was detected. Since the covariance matrices of the pre- and post-menopausal women looked very similar, for simplicity, they were assumed to be equal. To determine the variance/covariances of the MBDs and the expected values at t0, t1, t2 in the anticipated CAYAC sample, 1000 random trials of size n were generated from multivariate normal distributions with parameters estimated from the Puget Sound sample to simulate the CAYAC sample expected to contain 67% pre-menopausal and 33% post-menopausal women. Sixty-seven percent of n were drawn randomly from a multivariate normal distribution with expected MBD of (24.7, 23.57, 22.27) at (t0, t1, t2) as predicted for Puget sound pre-menopausal women, and 33% of n were drawn randomly from a multivariate normal distribution with expected Y of (15.49, 11.99, 13.03) as predicted for post-menopausal women. For each of the 1000 simulated samples, a longitudinal linear model was fitted assuming a compound symmetry structure for the covariance. The average of the 1000 estimates of the linear parameter was used to estimate the expected RTMBD at t0, t1, t2 for the CAYAC control sample.

The expected RTMBDs for CAYAC controls obtained from simulations were used to specify the effect size, defined as the difference between expected values at t2 for the two groups divided by the common sd, in the longitudinal power calculations were conducted using the program RMASS2. [107]. We assumed a Type I error of .05, power of .80, 2-sided test, no attrition over time, linear trend, compound symmetry covariance structure, and a correlation of 0.8 between measurements (estimated from the Puget Sound sample).

The size of the difference in MBD that can be detected between the low dose tamoxifen and placebo groups at year 2 (t2) are shown in the table below. Column 4 indicates the expected MBD before randomization (at t0). The expected MBDs at t2 for the control and treatment groups are shown in columns 5 and 6, respectively. Column 7 and Column 8 are the % reduction in MBD at t2 relative to t0 in the placebo control group and low dose tamoxifen treatment group, respectively. The last column shows the % reduction in PD in the treatment group at t2 relative to controls.

Placebo (PBO)	Low Dose Tamoxifen (LDTAM)	Power	Exp(MBD) at t0	Exp(MBD) at t2		% reduction in MBD at t2 relative to t0		% reduction in MBD at t2 for LDTAM group relative to PBO
			Both treatment groups	PBO	LDTAM	PBO	LDTAM	
50	50	.8	21.2	18.75	13.42	11.6%	36.5%	28.4%
		.9	"	"	12.68	"	40.0%	32.4%
75	75	.8	21.1	18.67	14.27	"	32.5%	23.5%
		.9	"	"	13.70	"	35.2%	26.6%
100	100	.8	21.2	18.72	14.89	"	29.6%	20.5%
		.9	"	"	14.32	"	32.3%	23.5%
150	150	.8	21.1	18.64	15.47	"	26.8%	17.0%
		.9	"	"	15.00	"	29.1%	19.5%
200	200	.8	21.1	18.67	15.91	"	24.8%	14.8%
		.9	"	"	15.50	"	26.7%	17.0%

If we anticipate the MBD in the low dose tamoxifen group to change from 21.1 at t0 to 14.89 at t2 compared to 18.72 at t2 in controls (in other words, a reduction of ~30% with low dose tamoxifen versus 11.6% with placebo over a 2 year time, or about 20% reduction in MBD with low dose tamoxifen relative to placebo, or an effect size of 0.25 at t2), a sample size of 100 per arm will be able to detect this magnitude of change with 80% power. With 10% attrition at each annual follow-up visit, 115 women per arm (or a total of 230) would be required. Thus, the goal accrual for this trial will be 115 women per arm, for a total of 230 women at t0.

Tissue and blood biomarkers: Masood score (cytomorphology) and Ki67 expression (proliferative index) evaluated from breast tissue samples will be obtained at t0 and t2. Insulin growth factors (IGF1 and IGFBP-3) will be measured at 3 time points. With a baseline sample size of 115 women per arm (assuming 10% yearly attrition), assuming Type I error=0.05, 2-sided test, and a within-person correlation of 0.5 between pairs of normally distributed measurements, there is 80% power to detect an effect size of 0.34 (absolute difference between treatment and control means divided by the common standard deviation) at year 2. A similar effect size is detectable for Ki67 for which the problem was formulated as that of detecting the difference at t2 in percent of women with positive expression (at least one cell expressing Ki67) between the treatment groups. Kahn et al [86] reported a median Ki67 expression level of 1.4% in RPFNA samples collected from 147 women at high risk for breast cancer. Thus, we hypothesized that 50% (conservative estimate) to 85%.of our cohort will have Ki67 expression in their healthy breast tissue samples collected via RPFNA at their baseline visit.

Detectable values for the various outcomes are shown in the table below. Column 2 shows the assumed common mean (± SD) at t0 for both treatment groups (obtained from the literature, adjusted for the age structure of our cohort, if possible). Column 3 shows the minimum detectable level at t2 for the tamoxifen group in the hypothesized

direction. The mean levels at t2 in the placebo group are assumed to remain the same as those at t0. Column 4 shows the detectable % change at t2 relative to t0 in the tamoxifen group.

Outcome	Assumed levels at t0 for Placebo and Low dose Tamoxifen groups	Detectable levels at t2 (80% power) for Low dose Tamoxifen group	
	Mean ± SD	Mean	% change (from t0)
Masood score [86]	14 ± 1.8	13.4	4% ↓
Women positive for Ki67 expression [86] (%)	50%	32%	36% ↓
	65%	47%	28% ↓
	85%	70%	18% ↓
IGF1 [108] (ng/ml)	260 ± 82.5	230	12% ↓
IGFBP-3 [108] (ng/ml)	3346 ± 1226	3787	13% ↑

Thus, we expect to be able to detect a 5-36% difference in the BC risk biomarkers proposed as secondary endpoints.

Aim 2: *To establish safety and tolerability of low-dose tamoxifen.*

Adverse events: We assume the rate of grade 2-4 toxicities in the placebo group to be low, at 0.5%. At the end of the study, with 94 women in each arm (accounting for 10% attrition per year), the study will have at least 80% power to detect an adverse event rate of 9.1% in the low dose tamoxifen group based on Fisher's exact test and Type I error=0.05.

Adherence to therapy: We will examine the difference in adherence rates of the treatment groups by comparing the percent of pills taken across 8 time points. With 115 women at baseline per arm, assuming a constant adherence rate of 90% in the placebo group and a between-measurement correlation of 0.6 and 2-sided alternative, there will be 80% power to detect an adherence rate of about 77% in the low dose tamoxifen group.

Voluntary withdrawals: If the proportion of patients in the placebo arm who withdraws voluntarily by the end of the study is 10%, then with 115 women in each arm at baseline, assuming type I error=0.05, the study will have 80% power to detect a significant difference in the voluntary withdrawal rate in the low dose tamoxifen group if the withdrawal rate in the intervention group is about 28%.

Safety biomarkers: We focus here on total cholesterol (TC), anti-thrombin-III (AT-III), bone-specific alkaline phosphatase (BSAP), and urine N-telopeptide crosslinks (NTX). With the proposed sample sizes, the detectable effect size (described earlier) is 0.34. A relatively small % change in mean levels is detectable for TC and AT-III but larger % changes are needed to detect a significant treatment difference at t2 for BSAP and NTX.

Outcome	Assumed levels at t0 for Placebo and Low dose Tamoxifen groups	Detectable levels at t2 (80% power) for Low dose Tamoxifen group	
	Mean ± SD	Mean	Relative % change (from t0)

TC* (mg/dL)	199 ± 45	214.3	8% ↑
AT-III** (%)	100 ± 13.3	95.5	5% ↓
BSAP [109] (µg/L)	7.7 ± 4.5	6.2	20% ↓
NTX[110] (nM BCE/mM)	24.4 ± 16.1	18.9	23% ↓

* Mean obtained from [111], sd obtained from [112]

** per ARUP reference laboratory

Thus, we expect to be able to detect a 5-23% difference in the objective safety biomarkers proposed as secondary endpoints.

Exploratory Aim 1: *To examine the modifying effects of demographic, clinical, and molecular characteristics on MBD.*

These analyses will be considered exploratory. We anticipate relatively low power as interaction effects are typically smaller and have relatively larger standard errors than main effects. Examining interactions is similar to comparing treatment effects among smaller sub-samples, thus power is reduced. An example is comparing the treatment effects of low dose tamoxifen between women who are pre- and post-menopausal at the time of study enrollment. Power estimates for this example were obtained by simulation. First, we estimated the linear mixed effects models of RTMBD for the 16 pre- and 25 post-menopausal control women in the Puget Sound NSABP P-1 data [106]. Based on our power calculation which showed that a 20% reduction in MBD is detectable at year 2 at 80% power with 100 women per arm without attrition, linear mixed effects models for the pre- and post-menopausal groups in the treatment arm were set up that included the treatment by time coefficient $\beta=0.2338$ corresponding to the detectable effect size. We also included another treatment by time parameter Δ to allow differential treatment effect by menopausal status ($+\Delta/2$ in pre-menopausal women and $-\Delta/2$ in post-menopausal women, i.e. an assumption of larger treatment effect in pre- than in post-menopausal women). Random sets of RTMBD at (t0, t1, t2) were generated from the corresponding trivariate normal distributions for the four groups (67 pre- and 33 post-menopausal women in each treatment arm). Menopause-specific covariance matrices (assuming compound symmetry) estimated from the Puget Sound NSABP P-1 data were used. Power for detecting a significant Δ under the alternative hypothesis of 100%, 75%, and 50% of the main treatment effects ($\beta=0.2338$) was estimated from 1000 random samples of the trial data by fitting the linear mixed effects model (linear in time) that included the treatment by time by menopausal status term. The model also included menopause status main effects and menopause by time interactions. Since interactions are expected to be smaller than main effects, $\Delta=0.2338$ is likely an overestimate of the interaction effects. Even with this overestimation, the anticipated power is only 40% (corresponds to treatment-related reduction in MBD of 28% relative to placebo at year 2 in the pre-menopausal group versus 13% reduction in the post-menopausal group). For the 75% and 50% alternative for Δ, power is even lower (24% and 12%, respectively). Thus, power for examining other modifying effects are anticipated to be similarly low or lower.

Exploratory Aim 2: *To examine the relationship between a low-dose tamoxifen regimen and clinical measures of efficacy and toxicity.*

This aim will also be considered exploratory as the study was not powered to detect treatment difference in these adverse outcomes. As the events of interest have very low incidence rates in both treatment groups (see Table 3 of Investigational Brochure), the study will not have any power (<5%) to detect the relative risks seen in NSABP P-1 Trial. The power for detecting treatment differences in the proportion of women anticipated to experience hot flashes and gynecological symptoms is somewhat better, 90% for vaginal discharge (prevalence rates of 35% in placebo and 55% in treatment groups), but only

41% for hot flashes (prevalence rates of 68% in placebo and 80% in treated groups), and 5% for vaginal bleeding (22% versus 23%).

13.0 REGISTRATION GUIDELINES

Patients will be registered using an online secure website developed by Sharp Clinical Services (IWRS). To register a subject, the CRN or CRA must complete the intake form and eligibility worksheet online. This process is fully described in the Screening and Enrollment SOP. After verification of eligibility by the coordinating center, randomization will be performed and treatment assigned using the Sharp Clinical Services IWRS. This procedure is fully described above in Section 12.3.

13.1 Procedures for On-Study and Treatment Deviations

Protocol waivers and/or treatment deviations are generally not allowed. However, should the need arise to amend the protocol in order for the protocol document to more accurately reflect the initial intent of the investigator(s), then the treating physician must contact the PI obtain approval. If approved, the protocol PI must immediately submit an amendment to the protocol. A backup for the originating PI has been designated and is specified in the registration worksheet.

14.0 RECORDS TO BE KEPT AND DATA SUBMISSION SCHEDULE

14.1 Confidentiality of Records

All research records will be stored in locked file cabinets at each site. Computer files with coded subject information will have restricted access passwords available only to the local PMT.

14.2 Subject Consent Form

The original signed **Consent Form** will be placed in the medical record. A copy will be given to the subject and a copy will be filed in the research record.

14.3 Data Collection Forms and Submission Schedule

Specific patient-administered forms to be used and the timing that they will be submitted are outlined in Section 9.0. In addition, the CRA/CRN will be responsible for completing the forms below and entering the information into the study EDC website.

Screening and Baseline Forms:

The CRA/CRN will fill out the **Intake Form** at the time of the prescreen interview, and the **Eligibility Worksheet** at the time of the patient's screening visit for study enrollment. These forms and associated documentation must be fully submitted *within 14 days* of the prescreening interview and screening visit, respectively. Should the patient be registered as a participant, the CRA/CRN will complete the baseline **Primary Cancer Treatment History Form**, the **General History and Physical Form**, and the **Clinical Breast Exam Form**. These should be submitted *within 28 days* of the baseline visit. Please refer to the Screening and Enrollment SOP for submission instructions.

Breast Imaging and Specimen Submission Forms:

The CRA/CRN will complete the **Breast Imaging Submission Form** to accompany submission of the baseline and annual screening mammograms and, if applicable, annual breast MRIs. Scheduling of these imaging tests are outlined in Section 9.1. RPFNA-protein, RPFNA-Cytology, and **Blood and Urine Specimen Submission Forms** will be completed to accompany submission of RPFNA tissue and blood specimens as outlined in Sections 11.2.3 (tissue) and 11.3.1–11.3.3 (blood).

Breast Event Form:

The NSABP developed a data capture form to record breast events, which they used in the NSABP P-1 and P-2 breast cancer prevention trials. This form has been adapted for use in this study. The CRA/CRN will complete the **Breast Event Form** should a subject receive a diagnostic breast biopsy while on study, or during follow-up. Should such a biopsy reveal DCIS or invasive breast cancer, the subject will be withdrawn from the study treatment as outlined in Section 6.3. For other breast events, the subject may continue on the trial.

Other Event Form:

The NSABP developed a data capture form to record other cancer and other potentially protocol-related medical events, which they used in the NSABP P-1 and P-2 breast cancer prevention trials. This form has been adapted for use in this study. The CRA/CRN will fill out the **Other Event Form** should a subject receive a cancer diagnosis other than breast cancer or require inpatient or outpatient procedure that could be potentially related to protocol while on trial or during follow-up. Only for specific events outlined in Section 6.3 will the subject necessitate withdrawal from the study. Otherwise, the subject may continue on the trial.

Treatment Interruptions:

The CRA/CRN will fill out a **Treatment Withholding Form** should a subject report an interruption for any reason while on trial. The patient-reported reason and both hold and restart dates will be recorded. If the subject voluntarily chooses to withdraw from study, then a **Study End Form** will be used instead. However, if the subject chooses to continue on trial, then the **Treatment Withholding Form** will be submitted. If a patient is off study medication for more than 90 consecutive days, then they must be withdrawn from the study. In that case, the CRA/CRN will submit both **Treatment Withholding** and **Study End Forms**.

Study End Form:

The CRA/CRN will fill out the **Study End Form** at the time the subject goes off trial, even if they complete the full treatment course. This form records whether or not the subject completed the trial per protocol, voluntarily withdrew, or was withdrawn for another reason. For voluntary withdrawals, subjects will be asked to clarify if it was due to intolerable side effects of treatment or if it was due to another reason.

Annual Follow-up Form:

To monitor for significant medical events, including second malignancies that may occur after the two-year intervention, the CRA/CRN will submit an **Annual Follow-Up Form** for up to 10 years post-study completion, including for participants who withdrew prior to completing the study. Subjects who are lost to follow-up will be reported to the coordinating center using this form. New cancers and any inpatient or outpatient procedures potentially related to the study will be reported using the **Breast Event Form** and **Other Event Form**.

15.0 MINORITIES AND WOMEN STATEMENT

Female subjects of all racial/ethnic groups are eligible for this study if they meet the eligibility criteria outlined in Section 5.0.

Efforts to accrue an ethnically diverse sample are outlined in Section 4.0. Ethnic distribution will likely make up a representative ratio of participants, based on the diversity of the communities in Los Angeles, Memphis, and Toronto. Specific minority groups for which each center has particular outreach include African Americans for SJCRH and EU, Asian Americans for PMH, and Latino Americans for COH.

To date, there is no information that suggests that differences in drug metabolism or response would be expected in one group compared to another. If differences in outcome appear to be associated with gender or ethnic identity, then a follow-up study will be designed to investigate those differences more fully.

16.0 ETHICAL AND REGULATORY CONSIDERATIONS

All subjects will have signed an informed consent for participation in research activities in accord with all institutional, National Cancer Institute (NCI) and Federal regulations, and will have been given a copy of the Experimental Subject's Bill of Rights.

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