Elm & Carlton Streets
Buffalo, New York 14263

Institutional Review Board

Approval Notice

This institution has an approved assurance of compliance on file with HHS which covers this activity FWA 00006731 Federal Wide Assurance identification number

December 29, 2016

Dr. Gokul Das

Gokul.Das@RoswellPark.org

Dear Dr. Gokul Das:

On 12/23/2016, the IRB reviewed the following submission:

Type of Review:	Modification/Update
Title of Study:	Pilot Study to Analyze a Novel Mechanism Underlying Response to Tamoxifen Therapy in Breast Cancer Patients
Investigator:	Gokul Das
IRB ID:	MOD00000518
Funding:	Name: ROSWELL PARK CANCER INSTITUTE; Name: NATIONAL INSTITUTES OF HEALTH
Grant ID:	None
IND, IDE, or HDE:	IND #000000, HOLDER Other
Documents Reviewed:	• I 110907 PROT AMD 23 CLN 120516, Category: IRB Protocol;

The IRB approved the study from 12/23/2016 to 12/11/2017 inclusive. Before 12/11/2017 or within 30 days of study closure, whichever is earlier, you are to submit a continuing review with required explanations. You can submit a continuing review by navigating to the active study and clicking Create Modification / CR.
If continuing review approval is not granted on or before 12/11/2017, approval of this study expires after that date.
. **Please be advised that only the IRB approved and stamped consent form can be used to enroll subjects.**

The principal investigator is responsible for ensuring that the research complies with all applicable regulations. Any modifications in the research project are subject to approval by the Board prior to initiation by the investigator. The Board reserves the right to stop the research for violations of regulatory or IRB requirements.

Unanticipated Problems which occur during the course of the research study must be reported to the CRS office to be reported to the IRB in accordance with the RPCI unanticipated problem reporting policy.

The protocol and consent forms, along with a brief progress report must be resubmitted to the IRB at least one month prior to the expiration date noted above for continuing review as required by the federal regulations. Please consult the CRS policies and SRC/IRB calendar for the submission date for the IRB meeting date prior to your review date.

Please be advised that your research study may be audited periodically by the IRB for compliance.

This activity has been reviewed and approved by an IRB in accordance with the requirements of 45 CFR 46, including its relevant Subparts. This protocol fulfills, when applicable, requirements for certifying FDA status for each investigational new drug or device.

The study documents have been submitted to Clinical Research Services (CRS) Compliance Office for processing prior to release and protocol implementation. Please contact CRS Compliance for information regarding the protocol implementation release date.

In conducting this protocol you are required to follow the requirements listed in the INVESTIGATOR MANUAL (HRP-103).

Sincerely,
Donald Handley MSc, MBA
Camille P Wicher, PhD, Esq., RN, MSN

Clinical Protocol (IRB approved- Protocol Identification: RPCI # I 110907)

Pilot study to analyze a novel mechanism underlying response to tamoxifen therapy in breast cancer patients

Principal Investigator

Gokul Das. PhD

Amendment 11 05-09-11
Amendment 12 01-23-12
Amendment 13 03-16-12
Amendment 14 06-06-12
Amendment 15 02-07-13
Amendment 16 05-03-13
Amendment 17 06-27-13
Amendment 18 11-07-13
Amendment 19 12-06-13
Amendment 20 12-09-14
Amendment 21 02-24-15
Amendment 22 10-28-15
Amendment 23 12-05-16

Network Investigator Signature Page

I 110907 Pilot study to analyze a novel mechanism underlying response to Tamoxifen therapy in breast cancer patients

PROTOCOL APPROVAL AND INVESTIGATOR AGREEMENT
I have read and familiarized myself with this protocol and I agree to conduct the study as described according to GCP and ICH guidelines.

Principal Investigator

_____ _____
Signed Date

_____
Printed

Address: _____

_____

_____

_____

Phone: _____

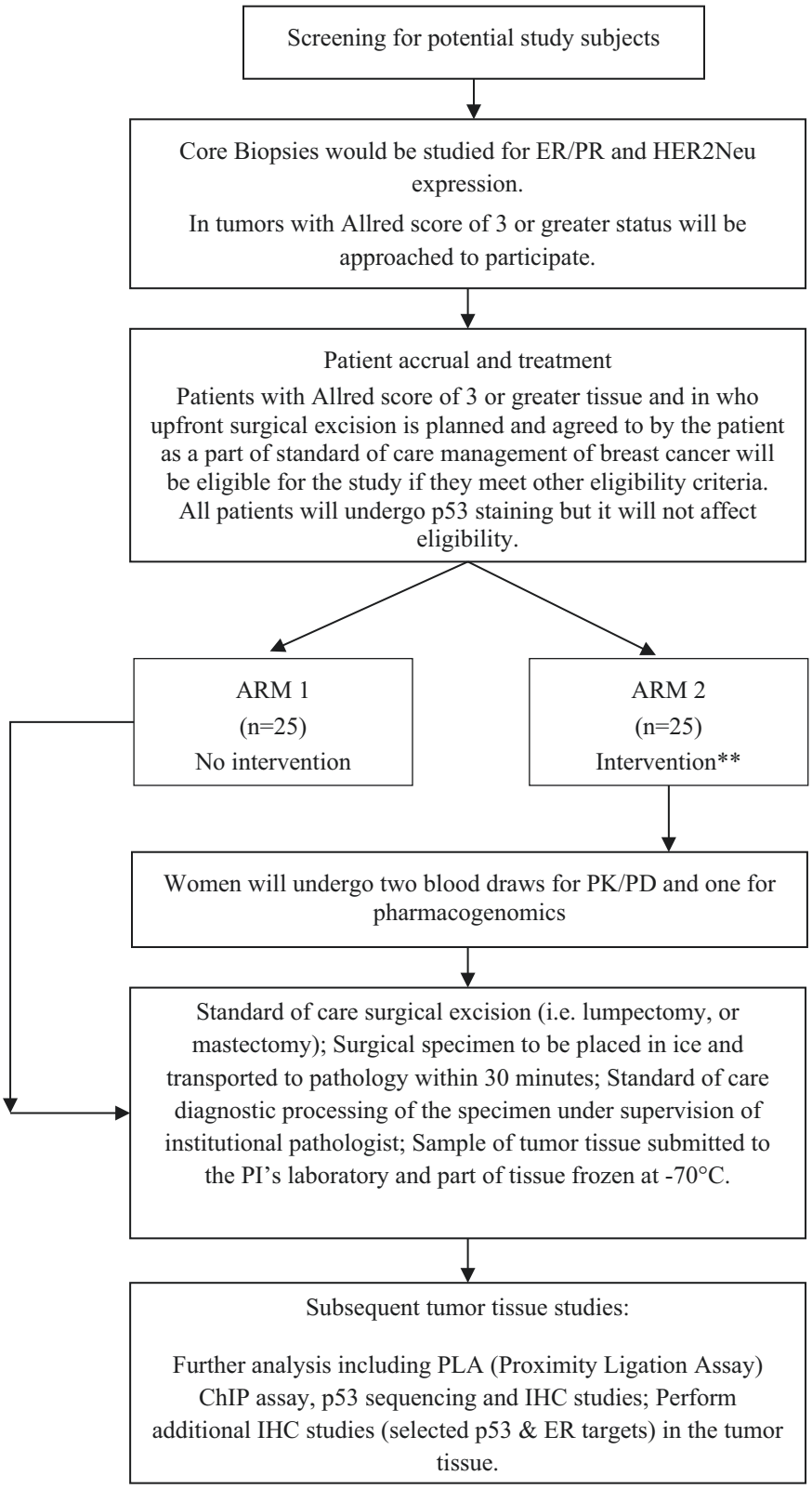
Fax: _____

E-mail: _____

NOTE: Please see Appendix 2 for Network-specific instructions that will apply to your site.

TABLE OF CONTENTS

I. SCHEMA



**Tamoxifen 20mg PO daily for 28 days and 5000 units subcutaneous heparin on day 29

II. STUDY OBJECTIVES

(1) Investigate the status of ERα-p53 interaction in ERα-positive, p53-wild type breast tumors in untreated patients and examine how tamoxifen therapy modifies this interaction in relation to existing estradiol and estrone levels.

(2) Confirm the wild type status of p53 and analyze the functional status of p53 pathway by monitoring expression of selected p53-target genes in tumors in patients who have or have not been treated with tamoxifen.

Approximately 60-80% of breast cancers are estrogen receptor alpha (ERα)-positive and estrogen-dependent. The anti-estrogenic drug tamoxifen is an approved treatment for women with both advanced and early stage ERα-positive breast cancer and for prevention of breast cancer in l women. However, a large number of patients with ERα positive breast tumors either do not respond to tamoxifen therapy or develop resistance to it. The mechanisms of such resistance remain largely unclear. ERα promotes proliferation of breast cancer cells, whereas p53 suppresses tumorigenesis by impeding proliferation and/ or promoting apoptosis of cells with genomic damage. Compared to many other cancers, breast cancers do not have high frequency of p53 mutations. In fact, most ERα (+) breast tumors have wild type p53. **Why wild type tumor suppressor protein p53 in such tumors remain functionally inactive has been unclear.** We have recently reported that ERα binds to p53 and represses its function in human breast cancer cells. Our preliminary data show that estrogen enhances the ERα-p53 interaction, whereas tamoxifen and fulvestrant disrupt it. Our findings provide a likely mechanism for the functional repression of wild type p53 in ERα-positive breast cancer. It appears that abrogation of p53 pathway is a major event both in the ERα-positive and ERα-negative tumors; in the former, wild type p53 is functionally repressed by ERα and in the latter case, p53 is inactivated by mutations. Our cellular and molecular data that antiestrogens such as tamoxifen can disrupt ERα-p53 interaction resulting in alleviation of repression of p53 function caused by ERα strongly suggests that disruption of ERα-p53 interaction may explain the clinical observation that breast cancer patients with ERα-positive tumors carrying wild type p53 respond better to tamoxifen therapy as compared to ERα-positive tumors carrying mutant p53. Our observation in retrospective clinical studies is consistent with results from other studies with independent patient cohorts (1, 2).

Based on these observations, we hypothesize that tamoxifen, by relieving functional suppression of wild type p53 by ERα, could enable p53 to regain its tumor suppressor activity. Tamoxifen may prevent ERα's ability to repress p53 function resulting in the activation of tumor suppressor pathways to prevent disease progression. This advantage of tamoxifen therapy becomes irrelevant in tumors containing mutant inactive p53, thereby contributing to tamoxifen resistance. If so, mutant p53 that is inactive with or without ER alpha bound to it could contribute to resistance to tamoxifen therapy. The Specific aims to test this hypothesis are: (1) investigate the status of ERα-p53 interaction in ERα-positive, p53-wild type breast tumors in untreated patients and examine how tamoxifen therapy modifies this interaction. (2) Confirm the wild type status of p53 and analyze the functional status of p53 pathway by monitoring expression of selected p53-taget genes in tumors in patients who have or have not been treated with tamoxifen. The proposed prospective randomized clinical trial is essential to address ERα-p53 interaction in tumors. Non-formalin fixed (fresh) tumor tissue is required to accurately examine ERα-p53 interaction. Our strategy is to analyze protein-protein interaction on endogenous p53-target gene promoter in the chromatin environment of the whole genome in vivo in cells of the fresh tumor tissue from patients who have and have not undergone treatment with tamoxifen. This novel strategy will allow us to dissect tamoxifen effect on ERα-p53 interaction in vivo in patient tumors.

Potential outcomes and benefits of the research to the patients: Testing the unconventional idea that tamoxifen, by disrupting ER alpha-p53 interaction, will enable p53 to regain its tumor suppressor function, brings in a novel dimension to the existing views on resistance to tamoxifen therapy. This study, if it reveals that ER alpha is bound to p53 in untreated patients and that the complex is disrupted in patients treated with tamoxifen resulting in the restoration of tumor suppressor function of p53, will immediately pave the way for undertaking a large randomized clinical trial to validate if ERα-p53 interaction can be used to devise new method to screen patients for tamoxifen therapy that would improve identification of patients who would be benefited from tamoxifen therapy, thereby avoiding unnecessary exposure to this drug with several side effects. Further, results from the proposed work will spawn research on mechanisms and consequences of ERα-p53 interaction that could lead to the development of better diagnostic and intervention strategies.

III. BACKGROUND

Breast cancer is the most commonly diagnosed cancer among women in the United States. Approximately 70% of breast carcinomas are ER-positive and estrogen-dependent. The anti-estrogenic drug tamoxifen is an approved standard of care therapy for women with both advanced and early stage ER-positive breast cancer. In women with early stage breast cancer, 5 years of adjuvant tamoxifen therapy reduces the annual breast cancer death rate by 31% irrespective of the use of chemotherapy, age and other tumor characteristics (3). When compared with placebo 5 years of tamoxifen leads to 28% reduction in death at 5 years (4) and reduces the risk of contralateral breast cancer by 49% (5). Tamoxifen is considered as a competitive inhibitor of estrogen binding to ER thereby affecting ER's properties as a transcriptional regulator. The levels of ER expression along with the levels of progesterone receptor (PR), a transcriptional target of ER, are used as the best predictors of tamoxifen response in patients. However, a major clinical problem is that a large fraction of patients with ERα-positive breast tumors either do not respond to tamoxifen therapy or develop resistance to it. Although several plausible reasons for such resistance have been suggested (6) the mechanisms of resistance to tamoxifen therapy remain largely unclear.

Genetic heterogeneity in tamoxifen metabolism

After oral administration, TAM is oxidized by the cytochrome P450 enzymes, CYP3A4/5 and 2D6 to active metabolites (7, 8). These metabolites, together with the parent tamoxifen, function as competitive inhibitors of estrogen, binding to ER and thereby affecting ER's properties as a transcriptional regulator. The most active metabolites of tamoxifen are 4-hydroxytamoxifen and endoxifen (9, 10). They have a higher affinity for ER and are more potent than tamoxifen in suppressing estrogen-dependent cell proliferation (11-13). Genetic and environmental factors can affect the concentrations of active TAM metabolites.

CYP2D6 genetic polymorphisms:

The CYP2D6 gene encodes the rate-limiting enzyme that catalyzes the conversion of tamoxifen into its metabolites. A highly polymorphic gene, it has at least 63 different major alleles known to date (14). These variant alleles have been associated with increased activity (*CYP2D6*2xn*) or with little (*CYP2D6*10,*17,*41*) or no activity (*CYP2D6*4,*5*). Phenotypes arising from variations of these genotypes have been described as either poor (PM), intermediate (IM), extensive (EM), or ultrarapid (UM) metabolizers. Approximately 10% of the Caucasian population carries variant alleles that code for an impaired enzyme with the most common variant being *CYP2D6*4* having a significantly altered function (15). At an allele frequency of 15–21% in the Caucasians population, this variant ccauses a splicing defect that results in a non-functional protein. Individuals described as UM metabolizers carry gene duplications and multi-duplications of functional alleles, which lead to up to 30% higher CYP2D6 expression and enzyme activity, commonly observed in Ethiopians (16) but occurring at relatively low frequency in Caucasians and Asians (15)**.**

CYP3A4/5 genetic polymorphisms:

CYP3A4 is genetically polymorphic and is thought to explain about 60-90% of observed variation in drug metabolizing capacity of patients and over 30 SNPs have been published (17, 18). CYP3A4*1B, with allele frequency of 2-9% in Caucasians has been associated with worse presentation of prostate cancer (19) **,** however reports on its function are mixed. One report suggests an increase in expression as measured by higher promoter activity (20); another associated CYP3A4*1B with a significantly decreased CYP3A activity (21). Another study reported no significant change (22). On the other hand CYP3A5 which is also polymorphic and has a very common defective variant CYP3A5 *3, that has an allele frequency of about 90% in Caucasians (23). This variant CYP3A5 *3 creates an alternative splice site that results in a frame shift and truncation of the protein hence no activity (24, 25).

CYP2D6, CYP3A4/5 polymorphisms and tamoxifen treatment:

Genetic variability in CYP2D6 has been reported to affect patients treated with tamoxifen. Goetz et al (26) reported that CYP2D6*4 was an independent predictor of a higher risk of relapse and a lower incidence of hot flashes in postmenopausal women. Further studies revealed that patients receiving selective serotonin reuptake inhibitors (SSRIs) that inhibit CYP2D6 activity, for their hot flashes were at an increased risk of breast cancer relapse (26). SSRI antidepressants are commonly prescribed to treat hot flashes in women who take tamoxifen and some such as paroxetine and fluoxetine, are known to inhibit cytochrome CYP2D6 (27). Other studies have associated breast cancer patients carrying the CYP2D6*4 genotype or other genotypes (*5, *10, *41) of reduced or absent enzyme activity with less likelihood of benefiting from tamoxifen chemoprevention (28), or with significantly more recurrences, shorter

relapse-free time interval and worse event-free survival during tamoxifen treatment (29, 30). Contrary to these studies, Nowell et al (31), reported of no association for the CYP2D6*4 genotype with response to tamoxifen or breast cancer prognosis. In addition, other studies (29, 30), also reported a decrease in recurrence rate and a better disease free survival in breast cancer for women with the CYP2D6*4 genotype. These results suggest that more studies including prospective studies with larger cohorts of patients and with consistent criteria for variables such as ER status, tamoxifen doses, length of treatments and chemotherapy regimens are needed (14, 32, 33). CYP2D6 genotype with low or absent enzyme activity has also been associated with significantly low endoxifen concentrations in plasma of tamoxifen-treated patients (34). Moreover Borges et al, (11) showed that patients with CYP2D6*4 genotype had significantly lower endoxifen concentrations (21.9 nM) than those with one (64.2 nM) or two (88.6 nM) CYP2D6 functional alleles. Studies involving CYP3A5*3 and tamoxifen have also been reported with each study investigating different end points. Jin et al (34), did not observe any significant association of CYP3A5*3 with plasma concentration of tamoxifen 4-hydroxytamoxifen, NDM or endoxifen in patients undergoing adjuvant breast cancer treatment. A similar observation was made by Tucker et al (35) in breast cancer patients who had been taking tamoxifen for at least 30 days (20 mg/day) and were not on chemotherapy or radiation at the time of study. In addition no significant association was observed between CYP3A5*3 hot flashes- the major and tamoxifen side effect. In a retrospective analysis of a prospective adjuvant tamoxifen phase III trial, Goetz et al (26), did not observe any difference in relapse time, disease-free survival or overall survival. On the contrary, Wegman et al (29), found a significant association of CYP3A5*3 with improved recurrence-free survival (RFS) of patients treated with tamoxifen for five years although those patients treated for 2 years tended to have an increased risk of recurrence. All of these observations suggest that CYP3A4/5 genetic variations may contribute to clinical outcomes in patients being treated with tamoxifen. Studies to link the metabolic status (plasma metabolite levels) of endoxifen and hydroxytamoxifen with genotype and treatment outcomes are needed to further increase our understanding of the pharmacogenetics of tamoxifen metabolism. Moreover there are no studies on I breast cancer women linking genotype status, treatment outcome and metabolic status. In this study, we propose to genotype 28-30 I women with known ER+ status undergoing tamoxifen treatment for the low or absent CYP2D6 alleles (*3, *4, *5, *6, *7, *8, *10, *14 and *41), the functional allele(*2), CYP3A4*1B and CYP3A5*3. Similar genotyping assays will be performed on a control cohort of patients not receiving tamoxifen. The genotype information will be correlated with measured tamoxifen metabolite serum levels and with treatment outcome.

ERα and p53

ERα mediates effects of estrogen in promoting proliferation of breast cancer cells (6, 36, 37) whereas tumor suppressor protein p53 impedes proliferation and/ or promotes death (apoptosis) of cells with genomic damage. 17β-estradiol (E2), ligand of ERα, is known to act as a mitogen for many breast cancer cell lines (38). Thus, while ERα promotes tumorigenesis, p53 suppresses tumorigenesis by preventing accumulation of cells with genetic damage. The opposing functions of p53 and ERα, while stringently controlled in normal cells, are likely disrupted in cancer cells. Various observations have alluded to the potential for a cross-talk between p53 and ERα signaling pathways (39-44). Recently, we have reported that estrogen receptor-α (ERα) binds to p53 and represses its function in human breast cancer cells (45), thus providing a direct link between these two proteins mediating opposing cellular pathways. Remarkably, ionizing radiation that causes genomic damage disrupts the interaction between ERα and p53. Antiestrogenic drugs tamoxifen and ICI 182,780 (fulvestrant) counteracted the repressive effect of ERα on p53, whereas 17β-estradiol (E2) increases the interaction.

Consistent with these cellular and molecular observations, our retrospective study (in collaboration with researchers at the Dr. Margarete Fischer-Bosch-Institute in Germany) on patients showed that presence of wild type p53 in estrogen receptor-positive breast tumors is associated with better response to tamoxifen therapy suggesting that ERα may be functionally inactivating wild type p53 in tumors and tamoxifen is alleviating that inactivation (46). We analyzed the p53 status in 35 randomly selected ERα positive, tamoxifen-treated breast cancer cases and 36 ERα negative cases with a clinical follow-up of up to 176 months. Importantly, none of the patients responding to tamoxifen treatment (50%) had detectable mutant p53 protein (in other words, they retain wild type p53) in their tumors, whereas all patients treated with tamoxifen and expressing mutant p53 (19%) in their ERα positive tumors developed recurrences. The overall survival (OS) of patients with ERα-positive tumors under tamoxifen treatment was significantly influenced by p53 status in that mutant p53 was associated with a poor response. Multivariate analysis by Cox regression model revealed p53 as a stable predictor of OS for ER-positive patients ($p = 0.012$; Relative Risk 5.4; 95% CI 1.45-20.76). In contrast, mutant p53 was not a predictor for OS of patients with ERα-negative tumors, for whom tamoxifen is not a standard treatment. Our observations as well as those from independent clinically diverse patient cohorts (1, 47) are consistent with our hypothesis that tamoxifen could alleviate inhibition of wild type p53 function by ERα enabling patients with such tumors to be tamoxifen-responsive. Thus, mutational inactivation of p53 is

likely to be one of the critical factors that contribute to resistance to tamoxifen therapy and tumor recurrence depending on particular disease sub group contexts.

Importantly, consistent with our observations in breast cancer cell culture model systems, ***we could detect ERα-p53 interaction in human MCF-7 xenograft tumors grown in mice*** (48). ***More recently, we have succeeded in optimizing the ChIP assay to analyze ER-p53 interaction in patient breast tumors. ChIP assay was performed in ER (+), p53 wild type breast tumor in patients who were not treated with tamoxifen (obtained from RPCI Pathology Resource Network) with antibodies against p53 (mouse monoclonal) and ER (rabbit polyclonal).*** Corresponding IgGs were used as negative controls. PCR was performed with primers specific for human p21 and survivin gene promoter regions containing p53-bing sites. Primers against a non-specific (NS) site were also used as negative control. As we had reported in the case of MCF-7 cells and xenografts, both p53 and ER antibodies pulled down endogenous p21 and survivin gene promoter fragments containing p53 sites, but not the NS site that does not contain any p53 site. ***Having demonstrated as a proof-of-principle that ER-p53 binding in breast tumors can be assayed by the ChIP assay, we are in a strong position to investigate whether ER-p53 interaction occurs in breast tumors in untreated patients and if the interaction is disrupted in patients treated with tamoxifen. Effectiveness of the disruption of the ER-p53 interaction by tamoxifen and its metabolites may be further correlated to 17β-estradiol and estrone (E2), which increases the ER-p53 interaction.***

Several clinical studies, especially those where DNA sequencing was used to detect p53 mutations, have found wild type p53 status to have a positive impact on therapeutic response and prognosis of breast cancer. Conversely, mutant p53 predicted resistance to endocrine therapy (1, 2, 47, 49-51). However, because of a lack of information on the mechanistic basis for relationship between therapeutic response to anti estrogen therapy and p53 status, p53 has not been incorporated into routine treatment decisions in breast cancer thus far. **A large percentage of ERα-positive breast tumors contain p53 with normal structure (wild type p53), albeit functionally debilitated. Why wild type p53 in such tumors has been unable to elicit tumor suppressor function has remained unknown. Further, it is intriguing that most of the ERα-negative breast tumors contain mutant p53** (47, 52, 53).

Study design:

This is a multiple institute study coordinated by Roswell Park Cancer Institute Network Office. See Appendix 2 for Network specific information.

Women will present to the University of Chicago Medical Center (UCMC) Breast Center and Roswell Park Cancer Institute (RPCI) breast center with either an abnormal mammogram, new discrete breast mass, or biopsy proven cancer. Those women with abnormal mammograms (BRIRADS 4 and 5) or suspicious masses will undergo a diagnostic core biopsy after being evaluated in the breast center, the intake point of all new breast cases through the surgical clinic. Once an invasive carcinoma is confirmed on core biopsy by our staff pathologist, the majority of women will undergo definitive surgery to remove the tumor and determine staging. Typically, at University of Chicago and RPCI the interval between biopsy and definitive surgery is between two-four weeks. To examine the interaction between ERα and P53 with and without tamoxifen unfixed tumor tissue (fresh or frozen) is needed. A pilot randomized trial is proposed in ERα positive women to study this interaction. ERα positive disease will be identified at their initial visit, or upon return to the clinic to discuss core biopsy results. A p53 staining will be performed on the diagnostic core biopsy if an ERα positive malignancy is identified (Allred score of 3 or higher). The results of the p53 status will not be used for eligibility. ER/PR/HER-2 analysis of the diagnostic core biopsy tissue is done routinely as a standard of care. Women with tumors which are ERα positive will be enrolled if they meet eligibility criteria. Women will either be randomized to standard of care surgical therapy or a four week intervention prior to surgery; Based on our preclinical studies four weeks of tamoxifen exposure is adequate to study the interaction between ERα, p53 and tamoxifen in the tumor tissue. During the intervention, blood will be drawn from the participants to measure levels of tamoxifen metabolites in the blood and test for polymorphisms that may decrease levels of active metabolites. Estradiol and estrone levels in the blood and tumor tissues of all patients with or without intervention will be measured.

Eligibility Criteria:

Inclusion Criteria:

(a) The patient must consent to be in the study and must have signed an approved consent form conforming to institutional guidelines

(b) The patient must be 18 years or older.

(c) Core biopsy should definitively demonstrate invasive carcinoma.

(d) Invasive carcinoma should be ERα receptor positive

(e) The tumor should be approximately at least 1 cm, to account for variability in imaging and imaging occult disease (physical exam, mammography, ultrasound). We recognize that from time to time because of this variation, there might

not be enough tissue available for analysis after surgical excision but this will allow the greatest opportunity to capture as many eligible patients as possible.
(f) Patients in whom surgical excision of the tumor is part of standard of care management
(g) ECOG score of 0 or 1
(h) Negative serum or urine β-hCG pregnancy test at screening for patients of child-bearing potential (this is routinely done if the patient is premenopausal and having surgery)
(i) Consent to participate in DBBR (RPCI only)

Exclusion Criteria:
(a) Male patients are not eligible for this study
(b) Female patients with inoperable tumors or women with stage 4 disease diagnosed on CT, PET, PET/CT or bone scan.
(c) Patients with diagnosis by FNA cytology only
(d) Pregnant or lactating women
(e) Prior therapy for breast cancer, including irradiation, chemo- immuno- and/or hormonal therapy
(f) Patients receiving any hormonal therapy, e.g. ovarian hormonal replacement therapy, infertility medications etc., are not eligible
(g) Nonmalignant systemic disease (cardiovascular, renal, hepatic, etc.) that would preclude the patient from being subjected to surgical excision
(h) Psychiatric or addictive disorders that would preclude obtaining informed consent
(i) Patients known or suspected to have hypercoagulable syndrome or with history of venous or arterial thrombosis, stroke, TIA, or pulmonary embolism
(j) Women with non-invasive disease or microinvasion are not eligible.
(k) Women undergoing neoadjuvant chemotherapy are not eligible
(l) women currently on tamoxifen and raloxifene for prevention are not eligible
(m) Patients shall not receive any herbal/alternative therapies such as flaxseed or soy products or black cohosh.
(n) Patients with a known mutation in p53 (Li Fraumeni Syndrome)

Unevaluable patients are defined as patients who do not have enough tissue for study or who come off early on tamoxifen, and will be replaced on study.

IV. TREATMENT PLAN AND RESEARCH METHODS:

Patients eligible for this study must have histological diagnosis of invasive breast cancer. The tumor must be ERα positive as defined by having an Allred score 3 or greater for ERα on a core biopsy by immunohistochemistry (IHC). IHC for p53 will be done on the core biopsy specimen, but will not be used as an eligibility criterion. The paraffin block (or 15-5 microns tissue thickness on charged slides) used for ER determination will be recut for p53 immunohistochemical staining using a commercially available antibody detection system (Dako Corporation, Carpinteria, CA) according to the manufacturer's protocol. The results of the p53 status by IHC will not be used for eligibility. The p53 status will be confirmed in resected tumor tissue by high resolution sequence analysis strategy (see under required studies). Inclusion and exclusion criteria for this study are shown above. Only those disease response assessments required for standard of care management will be undertaken. There will be no intervention for patients who are randomized to arm 1. Patients randomized to arm 2 will receive tamoxifen 20 mg by mouth daily for 28 days immediately preceding standard surgical therapy and 5000 units of subcutaneous heparin on day 29 in the holding area on day of surgery.

Women who agree to participate in this study will undergo a complete history and physical examination as part of their clinical care. The history and physical will include questions about prior history of thrombosis and stroke and complete medication history. Gynecologic history will be obtained as well including LMP, history of dysfunctional bleeding, recent pap smears, and smoking history. History and physical will be done standard of care and the study can use history and physical from 4 weeks prior to consent date for baseline. The patients will undergo standard of care blood work for staging of their breast cancer as per national clinical guidelines.

A Symptom Diary and a pill diary (See appendix 3 &4) will be supplied to the patient by the study coordinator to be maintained by the patient throughout participation in the study if randomized to study drug. Symptom Diary will be used as patient tool only. These will be reviewed by the study coordinator with the patient at the 2 week visit and again on the day of surgery.

An initial blood draw for PK/PD studies will be done prior to starting tamoxifen. There will be no PK/PD samples for patients randomized to the observation arm. Pre-dose, 2 green top tubes are to be obtained followed by 1 green top tube of blood for all other samples are to be obtained for PK/PD studies for each time point noted in the Study Schedule (Appendix 1).Once patients are enrolled, they will be seen by the study nurse one time prior to their surgical date at which time they will undergo adverse events assessment. During this appointment, patients will undergo standard of care pre-operative blood work. One additional tube of blood will be obtained from the patient at this time to measure the levels of tamoxifen and tamoxifen metabolites in the blood.

Patients at RPCI must be enrolled in the RPCI DBBR protocol (I 03103) to participate in this study. The DBBR will obtain blood for banking as per standard DBBR protocol. DBBR samples will be drawn prior to surgery. At the completion of the study, the DBBR will provide DNA for polymorphism studies. At the University of Chicago, one EDTA (purple or pink) tube of blood will also be collected for polymorphism studies from all patients participating in the study. At the conclusion of the study the research nurse will do toxicity assessments post tamoxifen, on the day of surgery. An additional tube of blood will be needed for PK/PD studies.

All blood samples collected in this study will be processed and stored at -70 until being shipped via dry ice (overnight delivery) for analysis. Plasma will be separated from the total blood within 30 minutes following the extraction. Shipments should be to the attention of Roswell Park Cancer Institute, Bioanalytics, Metabolomics and Pharmacokinetics (BMPK) Shared Resource, Center for Genetics and Pharmacology, Room L1-140, Elm & Carlton Streets, Buffalo, New York 14263, e-mail address: PKPDCore@RoswellPark.org. For additional information regarding the handling of pharmacokinetic samples please contact RPCI's BMPK Shared Resource at 716-845-3331. Batch shipment every 2-3 patients is preferred. Shipment should only occur Monday-Thursday's.

Standard surgical therapy will be offered to the patient based on the size, location of their tumor or presence of multiple tumors. After the completion of the surgical resection, two core biopsies of the surrounding normal breast tissue will be taken. One will be used to evaluate for tamoxifen and tamoxifen metabolite levels as well as estradiol and estrone levels in the breast tissue. The second will be stored for future pharmacogenomic studies.

Sequential compression devices (SCD's) will be used as part of standard of care for all women undergoing general anesthesia. Even though there is no data to suggest that a short intervention with tamoxifen will increase the risk of thrombosis, only women who get the tamoxifen arm will receive 5000u of subcutaneous heparin in the pre-operative holding area in addition to the use of SCD's for added prophylaxis.

Patients will undergo adjuvant therapy according to the findings of their surgical pathology. If the patient undergoes Oncotype testing as part of their standard of care, we will collect that information as part of our clinical data for analysis.

ENROLLMENT: Patients would be enrolled in this study at both the University of Chicago and the multidisciplinary breast clinic at RPCI. We plan to accrue 50 patients to this study over 9-year study duration. Anticipated rate of accrual would be 2-3 patients a month.

Tamoxifen is commercially available as a 20 mg tablet. The appropriate grant supporting the study will pay for the tamoxifen and will be provided free-of-charge to patients enrolled in this study.

V. REQUIRED STUDIES

Collaborating institutional staff pathologist will supervise all pathology examinations.

Estrogen Receptor, Progesterone Receptor And Her2 Expression Status:

Biopsy specimens: Estrogen and progesterone receptors are critical for inclusion in this protocol and should either be available as a part of routine analysis or will be done as a part of screening for this study in patients otherwise eligible for the study. Enrollment in the study will be predicated on the presence of ER based on an Allred score of 3 or above. The results of the p53 status will not be used for eligibility and will be determined at the end of study. HER2 expression status is not required prior to accrual.

Genotyping:

Genotyping will be performed under the guidance of Dr. Araba Adjei who has experience in genotyping genes involved in tamoxifen metabolism. We will genotype nine CYP2D6 SNPs (*2, *3, *4, *6, *7, *8, *10, *14 and *41) in

addition to the detection of the CYP2D6*5 gene deletion. The two most common CYP3A4/5 SNPs, CYP3A4*1B and CYP3A5*3 will also be genotyped. All genotyping are PCR-based and will be performed, using established methods at Roswell Park Cancer Institute (RPCI). Briefly, the primary genotyping platform for SNP Analysis will use the Sequenom MassARRAY platform. This system utilizes mass spectrometry (MS) in the detection and analysis of primer-extended PCR products (54). Five ng DNA is required for PCR reaction. Multiplexed PCR and then an extension reaction will be performed in a single well. The final PCR product is spotted to the appropriate location on a 384-pad SpectroCHIP (Sequenom) using a ChipSpotter LT nanodispenser (Samsung). Analysis by mass spectrometry and data acquisition using the MassARRAY Analyzer Compact MALDI-TOF MS (Sequenom) completes the assay yielding genotype information. Currently, multiplexing permits determination of up to 36 SNPs in a single well of a 384 well plate. For assays that fail the high throughput platform, the alternative method will be Pyrosequencing using published methods and primers (55) or restriction fragment length polymorphism (RFLP) techniques (34, 56, 57). Positive and negative controls will be utilized for all genotyping assays using the Coriell Cell Repository DNA samples. Similar genotyping assays will be performed on a control cohort of patients not receiving tamoxifen. The genotype information will be correlated with measured tamoxifen metabolite serum levels and with treatment outcomes at the end of the study.

PK/PD studies:

PK/PD analysis will be under the supervision of the Director of the Bioanalytics, Metabolomics and Pharmacokinetics (BMPK) Shared Resource facility at RPCI. Two tubes of blood, 10 ml each, will be drawn 0-30 days prior to the first dose of tamoxifen for PK sampling, estradiol and estrone analysis (tube 1), and genotyping (tube 2), and an additional PK sample of one tube at week 2 or 3 after receiving tamoxifen. An additional PK sample will be collected for all patients on the day of surgery. Also, a core tissue biopsy will be collected at the time of surgery to assess the concentration of tamoxifen and its active metabolites, as well as estradiol, in the surrounding breast tissue. Blood drawn prior to tamoxifen administration will be used to calculate the genotype of CYP2D6 and 3A4/5 and also to ensure tamoxifen levels are zero, and to measure the estradiol concentration. The other 2 samples (2 samples in duplicate) will be used to measure plasma and tissue concentrations of both TAM and its metabolites in comparison to estradiol and estrone concentrations by assessing (a) the area under the curve (AUC; $AUC_{tamoxifen}$, $AUC_{4\text{-}OH\text{-}tamoxifen}$, $AUC_{N\text{-}desmethyl\text{-}tamoxifen}$, and $AUC_{endoxifen}$ and the corresponding metabolic ratios), (b) clearance (CL) of TAM (($CL_{tamoxifen}$), and (c) maximal concentrations (Cmax) of TAM and its metabolites. With the 2 time points, a population PK model will be built and then used to estimate individual AUCs or CL. With the 2 time points, a population PK model will be built and then used to estimate individual AUCs or CL. These measurements, as well as the observed C_{max} , will then be tested for association with genotype and baseline estradiol concentrations. Demographic and clinical data (ethnicity, current age, age at diagnosis, menopausal status) will be collected for these patients. Samples of blood (10 ml) will be collected in heparinized tubes centrifuged at 4° C and plasma is stored frozen at -70 °C or lower until analysis by LC/MS/MS. Derivation of PK parameters and modeling is described below.

PK/PD Modeling:

A PK structural model will be developed for tamoxifen, and potentially including its various metabolites. The physiologic pharmacokinetic models explored will be described by the estimation of mean structural model parameters (e.g., plasma volumes of distribution and clearances), the magnitude of inter-individual variability (IIV) in these parameters, and the magnitude of residual variability (RV). It is anticipated that the appropriate PK model will initially include either a one- or two-compartment model with first-order absorption and (linear) elimination. The PK model could expand to include mechanistic attributes, such as a receptor-mediated clearance mechanisms (58, 59) since tamoxifen binds to the estrogen receptor and may in part, get internalized as a complex, undergoing receptor-mediated clearance as one of the primary clearance mechanisms. In addition, if tamoxifen or active metabolite levels are detected in the core tissue biopsy following 4 weeks of treatment, this data will also be incorporated into the model. Patient demographics, along with CYP3A4/5 and CYP2D6 metabolic status, effect of oral contraceptives, and other laboratory measurements will be evaluated as potential patient covariates that contribute to the inter-individual variability in pharmacokinetics. The final model will be used to conduct simulations to

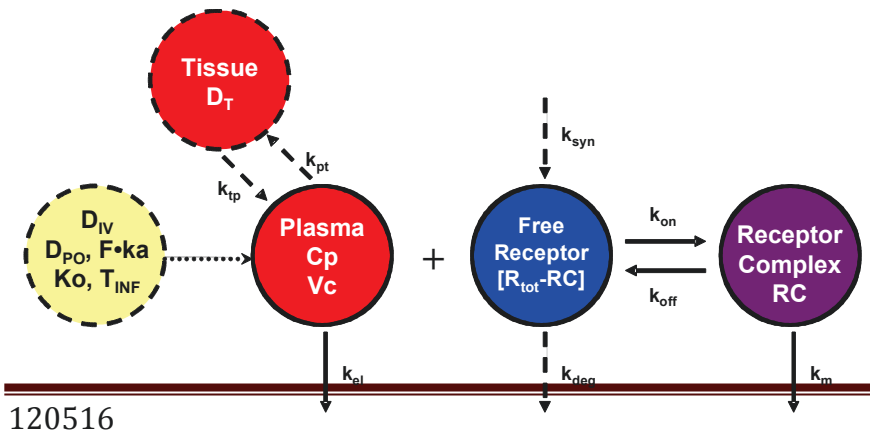


Fig. DM1. General model of target-mediated drug disposition.[2]

predict pharmacokinetic outcomes of various exposure regimens. (58) (59).

Lumpectomy or Mastectomy Specimens:

Surgical specimen upon resection will be transported in ice from the operating room to mammography if the specimen is a needle localization and then to pathology on ice. Mastectomy specimens and lumpectomy specimens will be transported to pathology via a research assistant, the tube system or via HCA to pathology The specimen should be received by pathology within 30 minutes. Specimens will be processed by institutional pathologist as per standards of care which include immediate gross examination. A sample of grossly identified tumor tissue will be frozen to -70^0C. The benign core biopsy samples will also be snap frozen. The frozen tissue from the University of Chicago will be sent via fed-ex (overnight) on dry ice the same day to laboratory of Dr. Gokul Das at Roswell Park Cancer Institute. Tissue shipments should be to the attention of Dr. Gokul Das at Roswell Park Cancer Institute. Shipping address: Dr. Gokul Das, CGP bldg., Room 4-304, Roswell Park Cancer Institute, Elm & Carlton Streets, Buffalo, NY 14263. Please also e-mail Gokul.Das@roswellpak.org. If the surgical sample is harvested on Friday, it will be stored at -70 over the weekend and shipped on Monday morning. .At RPCI, the frozen tissue will be submitted to laboratory of Dr. Das (PI). Further IHC studies of p53 and ER targets will be conducted in the core biopsy samples and surgically excised tumor tissue of the study subjects using a tissue microarray.

Expression analysis of selected ER and p53-target genes:

Expression of selected p53 and ER-target genes will be analyzed. . All IHC staining will be performed utilizing the AutoStainer™ series of automated stainers (Dako-Cytomation, California) retrospectively in the pre-surgical tumor biopsies and in the resected tumors from the breast cancer surgery. The percentage of tumors that are positive for ERα will be recorded, and a tumor will be considered positive if the Allred score is greater than 3. Staining for p21, Bax, puma and Survivin will be evaluated qualitatively and scored on a 0 – 3+ intensity scale (0 = negative staining, 1+ = weak staining, 2+ = moderately intense staining, and 3 + = strong staining). Antibodies against ERα, p53, and Survivin have already been optimized.

Analysis of ERα-p53 interaction in patient tumor tissues:

ChIP and quantitative ChIP (ChIP followed by quantitative RT PCR) assays and proximity ligation assay (PLA) are regularly used in our laboratory to analyze ERα-p53 interaction in cultured breast cancer cells. We have adapted a method others have previously reported for ChIP assay in tissues (60) to analyze tumor tissue in human breast cancer xenograft in mice and was successful in analyzing ERα-p53 interaction. We will use this protocol to analyze ERα-p53 interaction in human breast cancer tissues.

At RPCI, the frozen tissue from surgically excised tumor will be submitted to the laboratory of Dr. Gokul Das. Further IHC studies of p53 and ER targets will be conducted in the core biopsy samples (unstained slides will be requested for biopsies performed outside RPCI) and surgically excised tumor tissue of the study subjects. Tissue microarray (TMA) will be generated from surgically excised tissue and IHC and PLA will be performed on these TMAs.

We have been successful with ChIP assays in both fresh and recently frozen mouse tissues. If a patient consents to fresh tissue collection and the tissue procurement resource contains insufficient sample to release for testing, this testing will not be required. The frozen tissue from the University of Chicago will be sent via FedEx on dry ice the same day to the laboratory of Dr. Das at Roswell Park Cancer Institute Elm/Carlton Streets, Buffalo, NY 14263. If the surgical sample is harvested on Friday, it will be stored at -70 over the weekend and shipped on Monday morning. In addition, PLA for protein-protein interaction will be performed in Dr. Das' laboratory on unstained slides of tumor specimens provided by Dr. Carl Morrison (Department of Pathology, RPCI). Similarly, PLA will be performed on unstained slides of tumor specimens sent (by FedEx, overnight) from University of Chicago to the laboratory of Dr. Das at RPCI.

Determination of p53 sequence in tumor samples:

All evaluations will be done blindly in the absence of any identifying information from IHC as to the intensity of p53 staining. Various studies have indicated that IHC by itself may not be a reliable method to determine p53 status in tumors. As explained above, IHC determination is based on the assumption that mutant p53 is more stable leading to higher signal in IHC. However, that is not always the case. For example, 20% of tumors with a mutant p53 did not show nuclear accumulation of the protein (2). Sequence determination of p53 gene is more precise in determining wild

type vs. mutant status of p53. Importantly, in studies where DNA sequencing was used to detect p53 mutations, mutant p53 was significantly predictive of resistance to endocrine therapy (1, 2, 47). For reliable status determination, we will analyze sequence of p53 in tumors. All evaluations will be done blindly in the absence of any identifying information from IHC as to the intensity of p53 staining. As a means complimentary to IHC to ascertain the wild type versus mutant status of p53, we will determine the sequence of p53 by next generation sequencing technology with Illumina's MiSeq™sequencer housed in the Genomics core facility at RPCI. MiSeq™ sequencer is capable of accurate sequence variant detection and has fast turnaround. All steps starting from DNA isolation to data analysis will be performed according to the instructions provided by the manufacturer (Illumina, Inc.). The sequencing will be performed on DNA isolated from tumor tissue (frozen after surgical resection) of patients enrolled to the study at RPCI and University of Chicago. ***This method will provide accurate sequence information even from a heterogeneous tumor sample containing mixture of normal and tumor cells.***

Analysis of p53-target gene transcription:

First, we will analyze if ERα-p53 interaction in tumors affects p53's function as a transcriptional regulator. Toward this goal, we will analyze a selected group of p53-target genes (based on their reported importance in regulating cell cycle and apoptosis (63,64) that are either activated or repressed by p53 in the resected tumor tissue. . ***We have already demonstrated that Survivin (whose transcription is known to be repressed by p53) expression is upregulated as a consequence of ER binding to p53, and treatment with TAM leads to functional activation of p53, thereby restoring p53's ability to repress Survivin expression (65)***. The mRNA levels of these genes will be analyzed by qRT-PCR, a technique routinely used in the PI's laboratory. Subsequently, we will analyze the expression of these genes at the protein level. Immunohistochemistry (IHC) studies of these proteins will be conducted in the *core biopsy samples (unstained slides will be requested for biopsies performed outside RPCI) and surgically excised tumor tissue* of the study subjects using a tissue microarray (TMA) generated by the Department of Pathology under the supervision of Dr. Carl Morrison. For patients recruited at the University of Chicago, TMA will be generated at the University of Chicago. IHC will be performed under the direction of the pathologist, with all pathology personnel blinded to treatment assignment. The IHC endpoints will be rounded to the nearest 10% by the pathologist who will be blinded to the treatment group assignment. The ER and p53 status will be determined as described in the earlier section (section 4.1.1). Staining for p21, Bax, puma and Survivin will be evaluated qualitatively and scored on a 0 – 3+ intensity scale (0 = negative staining, 1+ = weak staining, 2+ = moderately intense staining, and 3 + = strong staining. It is to be noted that p53-target gene protein expression will be analyzed by IHC both in the core biopsy samples and surgically excised tumor tissue enabling within-patient comparison of expression profile of these proteins *before* and *after* TAM therapy. Ideally, a similar within-patient approach is desirable for other assays (ChIP and qRT-PCR,) as well. However, it is practically impossible given the limited tissue availability from the pre-surgical biopsy.

VI. STATISTICAL CONSIDERATIONS

The bio-statistician at RPCI will perform the statistical analyses and aid in interpretation of results. Exploratory graphical analysis and data confirmation will precede formal inferential analysis. As this is an investigational study, no correction for multiple testing will be made; p-values less than α=0.05 will be deemed statistically significant. While formal power computations are provided, it is expected a larger Phase III trial will be planned to confirm and elaborate promising findings. The demographic and baseline data will be summarized by treatment group. Descriptive statistics such as frequencies and relative frequencies will be computed for all categorical variables. Numeric variables will be summarized using simple descriptive statistics such as mean, standard deviation, quartiles, etc. Ninety-five percent confidence intervals will be computed when appropriate. A variety of graphical techniques will be used to display data.

The primary analyses in Aim 1 are comparisons of ERα-p53 interaction (positive or negative) between independent treatment and control groups. The proportions of patients with positive ChIP assay/PLA will be compared with Fisher's Exact Test, which has 80% power to detect a difference of about 0.41 between the two group; e.g., a difference of 70% in the control group vs. 30% in the TAM group. In Aim 2, mean mRNA levels, as measured by qRT-PCR, will be compared across treatment and control groups. The difference in log fold intensity between treatment and control will be estimated with an independent samples t-confidence interval. The corresponding hypothesis test has

80% power to detect an effect size of 0.91. That is, if the true difference in means is about 0.91 standard deviations, there is an 80% probability our study will correctly reject the null hypothesis of no effect.

Protein expression measured by IHC gives an ordinal variable (0, 1+, 2+, 3+). For each protein, the four-week intensity change ("post – pre" will be between -3 and 3) will be compared between treatment and control groups with the Wilcoxon-Mann-Whitney test. In the control group we expect little change because no treatment has been given between samples. For power calculations we assume 80% of patients in control will have no change and 10% will increase and decrease. As a hypothesis, we estimate half of patients in the treatment group will have no change and the rest will increase one (e.g. from 1+ to 2+) or two categories (e.g. from 1+ to 3+). From simulation we estimate the power to correctly reject the null hypothesis when this alternative is true is about 90%.

It is possible the response variables in Aims 1 and 2 are correlated with clinical factors such as patient age, smoking status, and position in menstrual cycle. Additional exploratory comparisons of response between treatment and control groups will be made after adjustment for clinical factors with linear or logistic regression as appropriate. These secondary analyses may have greater ability to detect a treatment difference by controlling variability unrelated to treatment. PK/PD data modeling was described previously. Pharmacogenomic data will be explored with the general linear model $Y = a + bG + e$, where genotype G is quantified 0, 1, 2 for aa, Aa, AA, respectively; for quantitative response Y, normal linear regression is used; for binary response, logistic regression is used.

Randomization:

Patients will be allocated to treatment or control by block randomization (block size=4). The randomization assignment list will be maintained at RPCI. The nurse/coordinator from University of Chicago will notify the RPCI Network office for a randomization assignment by sending the completed patient registration form to CRSNetworkCoordinators@roswellpark.org. The Network Monitor or designee will randomize the patient and send the randomization assignment to the nurse/coordinator at University of Chicago.

Management of Patients After Completion of Tamoxifen Treatment:

Patient would undergo standard of care surgical excision at completion of four week course of tamoxifen. We do not anticipate the four week therapy with tamoxifen to modify the surgical planning including type of breast surgery, sentinel node biopsy or to affect the clinical outcome. After completion of surgical excision they would be recommended routine adjuvant therapy including adjuvant chemotherapy, radiation therapy and at least 5 years of endocrine therapy as dictated by the patient's final stage.

This protocol does not prohibit patients enrolling in any other future investigational studies after completion of surgical excisions.

Dose Modifications And Delays

There will be no dose modification of tamoxifen therapy. The toxicity profile of four weeks of tamoxifen is unknown. In the event of any Grade 2 or higher toxicity during the study attributed to tamoxifen, it will be discontinued and patient will be permanently taken off the study.

VII. NONPROTOCOL THERAPY

Patients should not receive any cancer therapy other than that specified in the protocol or while on Tamoxifen.

Sex Hormonal Therapy
Patients should not receive any sex hormonal therapy such as birth-control pills, hormone replacement therapy or infertility medications while on this protocol.

Radiation Therapy
Patients may not receive any radiation therapy until the completion of the protocol therapy.

Anti-Hormonal therapy
Patients may not receive any hormonal therapy including Aromatase inhibitors or Raloxifene while on the protocol therapy. Patients should be off of these medications for at least 30 days prior to enrollment. They may receive the appropriate adjuvant therapy after surgery.

Alternative therapy
Patient shall not receive any herbal/alternative therapies such as flaxseed or soy products or other medications such as black cohosh.

Non-cancer therapy
Patient may continue all the therapies intended for other than cancer. Patients shall discuss the safety of receiving the concurrent non-cancer medications with the study investigator. If patients are on SSRI's they are not eligible.

VIII. PHARMACEUTICAL INFORMATION

Tamoxifen [NSC#180973]

Tamoxifen is supplied in tablet form, each containing 20 mg.

Clinical Safety and Adverse Effects

Adverse effects include hot flashes, nausea (vomiting is rare), vaginal bleeding, discharge or dryness, menstrual irregularities, and skin rash. Other rarely seen adverse effects are hypercalcemia, peripheral edema, leucopenia and transient thrombocytopenia, loss of appetite, distaste for food, pruritus vulvae, depression, dizziness, headache, leg cramps, lightheadedness, confusion, and fatigue. There is a small risk of ovarian cysts occurring. Hair thinning and/or hair loss has also been reported in women taking tamoxifen. Liver cancer and other liver toxicities such as fatty liver, cholestasis, hepatitis and hepatic necrosis have been reported in women taking tamoxifen, though such toxicities rarely can be severe or life-threatening. A few of these serious cases have resulted in death, but whether tamoxifen was the cause of these problems remains uncertain. An increased incidence of cataracts has been noted in rats, although such an increase has not been reported in humans. However, other ophthalmic toxicities, such as corneal scarring or retinal changes, have been reported in a few patients. Tumor flare can appear in patients being treated for metastatic disease. Tamoxifen should not be taken during pregnancy due to potential hazard to the fetus. This includes miscarriage, birth defects, or long-term effects on sexual development (which could be similar to the long-term effects caused by diethylstilbestrol [DES]). Women whose mothers took DES during pregnancy have an increased risk of developing cancer of the vagina or cervix, and may have trouble bearing children. The relevance of findings from animal studies to women who may accidentally take tamoxifen during pregnancy is unknown, but it is essential that effective contraceptive methods be used while taking tamoxifen therapy, and for two months after completing or discontinuing therapy. Tamoxifen may cause changes in the lining of the uterus which could potentially lead to uterine cancer. An early sign of abnormal changes in the uterus may be abnormal vaginal bleeding or pelvic discomfort. An increased risk of uterine cancer has been reported with the use of tamoxifen; however, the level of risk is still uncertain. After an average of 8 years of follow-up, the annual risk observed in a large-scale trial of breast cancer patients taking 20 mg of tamoxifen daily is about 2 per 1,000 women (approximately three times greater than that of a similar group of women in the general population.) Uterine cancer is potentially life-threatening, and some breast cancer patients who developed uterine cancer while taking tamoxifen have subsequently died from that disease. Most of the uterine cancers that have occurred have been diagnosed at an early stage when treatment is highly effective. Tamoxifen may cause changes in the lining of the uterus, such as polyps and hyperplasia, and endometriosis. Data from the NSABP B-14 study show no increase in other (non-uterine) cancers among patients receiving tamoxifen. However, other data suggest a possible increase in second cancers of the gastrointestinal tract among women receiving the drug. Whether an increased risk for other (non-uterine) cancers is associated with tamoxifen is still uncertain and will continue to be evaluated. Women on tamoxifen have an increased risk for developing phlebitis and blood clots. Some studies, but not all, have shown that tamoxifen causes about 1% increase in the incidence of thrombotic events. These include superficial phlebitis, deep vein thrombosis, and pulmonary embolism. Rarely, death has occurred from such events in these studies.

However most of the toxicities reported with Tamoxifen is with long term therapy e.g. 5 years of adjuvant therapy. There are no known or reported toxicity with a short course of tamoxifen such as 14 days.

IX. REPORTING OF ADVERSE DRUG REACTIONS/ DATA AND SAFETY MONITORING PLAN

Investigator Requirements and Responsibilities

The principal investigator (PI) of this study at each site is ultimately responsible for every aspect of the design, conduct, and final analysis of this protocol. Each site PI is responsible to ensure continuous, close monitoring of subjects enrolled on this clinical trial.

Adverse Event Reporting (AER)

Investigators are required by Federal Regulations to report serious adverse events. Investigators are required to notify the Clinical Research Services (CRS), CRS will notify RPCI Institutional Review Board if a patient has a reportable serious adverse event. This study will utilize the **Common Toxicity Criteria version**- **3.0** to determine the severity of the adverse event.

Reporting requirements and procedures depend upon:
(1) whether procedure is suspected of causing the adverse event,
(2) whether the possibility of such an adverse event was reported in the protocol, consent form, or manufacturer's literature (expected or unexpected adverse event),
(3) the severity or grade of the adverse event,
(4) the phase of the study and attribution (the determination of whether an adverse event is related to a medical treatment or procedure). All events in a "reportable" category must be reported.

All serious related and unexpected events must be forwarded to the Roswell Park Cancer Institute Institutional Review Board.

Serious Adverse Events (SAE)

A serious adverse event (SAE) is any experience that suggests a significant hazard, contraindication, side effects or precaution. This includes any experience that:

- Results in death.
- Is a life-threatening adverse drug experience
- Requires inpatient hospitalization or prolongation of existing hospitalization.
- Results in persistent or significant disability/incapacity.
- Is a congenital anomaly/birth defect.
- Requires medical or surgical intervention to prevent one of the outcomes listed above.

Reporting Serious Adverse Events

All SAEs occurring during the course of the study or within 30 days of the last administration of study procedure must be reported to each local IRB per institute policy. At RPCI Clinical Research Services (CRS) must be informed within 24 hours of the knowledge of occurrence (this refers to an AE that meets one or more of the aforementioned serious criteria), Network sites please refer to **appendix 2** as well as following your local IRB policy. The principal investigator or designee will complete and submit an FDA Form 3500A Med Watch for any event that meets the above criteria. Forms will be submitted to the CRS Compliance Office via e-mail to CRSCompliance@Roswellpark.org. Follow-up SAE information must be submitted when information is obtained within 24 hrs.

A final assessment of adverse events will be done thirty days after treatment completion (can be plus or minus 2 days).The well-known side effects of tamoxifen increase with increasing duration of use and increasing age. However, women will be specifically asked about any new onset of shortness of breath, unilateral leg swelling, change in vision, abnormal vaginal bleeding or vasomotor symptoms

Continuing Reviews

Required reporting requirements of adverse events annually at Continuing Reviews will be reported per IRB guidelines. At time of continuing review reporting the principal investigator will be required to report to the IRB the number of patients entered on the trial, the number of patients treated, a summary of all adverse events reported to date using CTC 3.0 grading, a specific list of serious adverse events, and significant literature reporting developments that may affect the safety of participants or the ethics of the study.

The IRB will review annual Data Safety Monitoring Board reports and make recommendations on whether the study should continue unchanged, require modifications/amendment, or be closed based on unacceptable risk to participants.

Symptom diaries

Symptom diaries will not be used to track adverse events.

X. REFERENCES FOR CLINICAL PROTOCOL

1. Bergh J, Norberg T, Sjogren S, Lindgren A, Holmberg L. Complete sequencing of the p53 gene provides prognostic information in breast cancer patients, particularly in relation to adjuvant systemic therapy and radiotherapy. Nat Med 1995;1:1029-34.

2. Berns EM, Foekens JA, Vossen R, et al. Complete sequencing of TP53 predicts poor response to systemic therapy of advanced breast cancer. Cancer Res 2000;60:2155-62.

3. Effects of chemotherapy and hormonal therapy for early breast cancer on recurrence and 15-year survival: an overview of the randomised trials. Lancet 2005;365:1687-717.

4. Tamoxifen for early breast cancer: an overview of the randomised trials. Early Breast Cancer Trialists' Collaborative Group. Lancet 1998;351:1451-67.

5. Fisher B, Costantino JP, Wickerham DL, et al. Tamoxifen for prevention of breast cancer: report of the National Surgical Adjuvant Breast and Bowel Project P-1 Study. J Natl Cancer Inst 1998;90:1371-88.

6. Clarke RB, Anderson E, Howell A. Steroid receptors in human breast cancer. Trends Endocrinol Metab 2004;15:316-23.

7. Crewe HK, Ellis SW, Lennard MS, Tucker GT. Variable contribution of cytochromes P450 2D6, 2C9 and 3A4 to the 4-hydroxylation of tamoxifen by human liver microsomes. Biochem Pharmacol 1997;53:171-178.

8. Desta Z, Ward BA, Soukhova NV, Flockhart DA. Comprehensive evaluation of tamoxifen sequential biotransformation by the human cytochrome P450 system in vitro: prominent roles for CYP3A and CYP2D6. J Pharmacol Exp Ther 2004;310:1062-75.

9. Jordan VC. Metabolites of tamoxifen in animals and man: identification, pharmacology, and significance. Breast Cancer Res Treat 1982;2:123-38.

10. Jordan VC, Collins MM, Rowsby L, Prestwich G. A monohydroxylated metabolite of tamoxifen with potent antiestrogenic activity. J Endocrinol 1977;75:305-16.

11. Borges S, Desta Z, Li L, et al. Quantitative effect of CYP2D6 genotype and inhibitors on tamoxifen metabolism: implication for optimization of breast cancer treatment. Clin Pharmacol Ther 2006;80:61-74.

12. Borgna JL, Rochefort H. Hydroxylated metabolites of tamoxifen are formed in vivo and bound to estrogen receptor in target tissues. J Biol Chem 1981;256:859-68.

13. Robertson DW, Katzenellenbogen JA, Long DJ, Rorke EA, Katzenellenbogen BS. Tamoxifen antiestrogens. A comparison of the activity, pharmacokinetics, and metabolic activation of the cis and trans isomers of tamoxifen. J Steroid Biochem 1982;16:1-13.

14. Goetz MP, Kamal A, Ames MM. Tamoxifen pharmacogenomics: the role of CYP2D6 as a predictor of drug response. Clin Pharmacol Ther 2008;83:160-6.

15. Ingelman-Sundberg M, Rodriguez-Antona C. Pharmacogenetics of drug-metabolizing enzymes: implications for a safer and more effective drug therapy. Philos Trans R Soc Lond B Biol Sci 2005;360:1563-70.

16. Aklillu E, Persson I, Bertilsson L, Johansson I, Rodrigues F, Ingelman-Sundberg M. Frequent distribution of ultrarapid metabolizers of debrisoquine in an ethiopian population carrying duplicated and multiduplicated functional CYP2D6 alleles. J Pharmacol Exp Ther 1996;278:441-6.

17. Hesselink DA, van Schaik RH, van der Heiden IP, et al. Genetic polymorphisms of the CYP3A4, CYP3A5, and MDR-1 genes and pharmacokinetics of the calcineurin inhibitors cyclosporine and tacrolimus. Clin Pharmacol Ther 2003;74:245-54.

18. van Schaik RH. Cancer treatment and pharmacogenetics of cytochrome P450 enzymes. Invest New Drugs 2005;23:513-22.

19. Rebbeck TR, Jaffe JM, Walker AH, Wein AJ, Malkowicz SB. Modification of clinical presentation of prostate tumors by a novel genetic variant in CYP3A4. J Natl Cancer Inst 1998;90:1225-9.

20. Amirimani B, Walker AH, Weber BL, Rebbeck TR. RESPONSE: re: modification of clinical presentation of prostate tumors by a novel genetic variant in CYP3A4. J Natl Cancer Inst 1999;91:1588-90.

21. Rodriguez-Antona C, Sayi JG, Gustafsson LL, Bertilsson L, Ingelman-Sundberg M. Phenotype-genotype variability in the human CYP3A locus as assessed by the probe drug quinine and analyses of variant CYP3A4 alleles. Biochem Biophys Res Commun 2005;338:299-305.

22. Ando Y, Tateishi T, Sekido Y, et al. Re: Modification of clinical presentation of prostate tumors by a novel genetic variant in CYP3A4. J Natl Cancer Inst 1999;91:1587-90.

23. Ingelman-Sundberg M. Genetic polymorphisms of cytochrome P450 2D6 (CYP2D6): clinical consequences, evolutionary aspects and functional diversity. Pharmacogenomics J 2005;5:6-13.

24. Hustert E, Haberl M, Burk O, et al. The genetic determinants of the CYP3A5 polymorphism. Pharmacogenetics 2001;11:773-9.

25. Kuehl P, Zhang J, Lin Y, et al. Sequence diversity in CYP3A promoters and characterization of the genetic basis of polymorphic CYP3A5 expression. Nat Genet 2001;27:383-91.

26. Goetz MP, Rae JM, Suman VJ, et al. Pharmacogenetics of tamoxifen biotransformation is associated with clinical outcomes of efficacy and hot flashes. J Clin Oncol 2005;23:9312-8.

27. Otton SV, Ball SE, Cheung SW, Inaba T, Rudolph RL, Sellers EM. Venlafaxine oxidation in vitro is catalysed by CYP2D6. Br J Clin Pharmacol 1996;41:149-56.

28. Bonanni B, Macis D, Maisonneuve P, et al. Polymorphism in the CYP2D6 tamoxifen-metabolizing gene influences clinical effect but not hot flashes: data from the Italian Tamoxifen Trial. J Clin Oncol 2006;24:3708-9; author reply 9.

29. Wegman P, Elingarami S, Carstensen J, Stal O, Nordenskjold B, Wingren S. Genetic variants of CYP3A5, CYP2D6, SULT1A1, UGT2B15 and tamoxifen response in postmenopausal patients with breast cancer. Breast Cancer Res 2007;9:R7.

30. Wegman PP, Wingren S. CYP2D6 variants and the prediction of tamoxifen response in randomized patients: author response. Breast Cancer Res 2005;7:E7.

31. Nowell SA, Ahn J, Rae JM, et al. Association of genetic variation in tamoxifen-metabolizing enzymes with overall survival and recurrence of disease in breast cancer patients. Breast Cancer Res Treat 2005;91:249-58.

32. Desta Z, Flockhart DA. Germline pharmacogenetics of tamoxifen response: have we learned enough? J Clin Oncol 2007;25:5147-9.

33. Sinha G. Gene testing to tailor breast cancer therapy has arrived--is it ready for the clinic? J Natl Cancer Inst 2008;100:1050-1.

34. Jin Y, Desta Z, Stearns V, et al. CYP2D6 genotype, antidepressant use, and tamoxifen metabolism during adjuvant breast cancer treatment. J Natl Cancer Inst 2005;97:30-9.

35. Tucker AN, Tkaczuk KA, Lewis LM, Tomic D, Lim CK, Flaws JA. Polymorphisms in cytochrome P4503A5 (CYP3A5) may be associated with race and tumor characteristics, but not metabolism and side effects of tamoxifen in breast cancer patients. Cancer Lett 2005;217:61-72.

36. Fuqua SA, Cui Y, Lee AV, Osborne CK, Horwitz KB. Insights into the role of progesterone receptors in breast cancer. J Clin Oncol 2005;23:931-2; author reply 2-3.

37. Osborne CK, Schiff R. Estrogen-receptor biology: continuing progress and therapeutic implications. J Clin Oncol 2005;23:1616-22.

38. Prall OW, Rogan EM, Sutherland RL. Estrogen regulation of cell cycle progression in breast cancer cells. J Steroid BiochemMol Biol 1998;65:169-74.

39. Sivaraman L, Conneely OM, Medina D, O'Malley BW. p53 is a potential mediator of pregnancy and hormone-induced resistance to mammary carcinogenesis. Proc Natl Acad Sci U S A 2001;98:12379-84.

40. Martin AM, Weber BL. Genetic and hormonal risk factors in breast cancer. J Natl Cancer Inst 2000;92:1126-35.

41. Liu G, Schwartz JA, Brooks SC. Estrogen receptor protects p53 from deactivation by human double minute- 2. Cancer Res 2000;60:1810-4.

42. Saji S, Okumura N, Eguchi H, et al. MDM2 enhances the function of estrogen receptor alpha in human breast cancer cells. Biochem Biophys Res Commun 2001;281:259-65.

43. Medina D, Kittrell FS. p53 function is required for hormone-mediated protection of mouse mammary tumorigenesis. Cancer Res 2003;63:6140-3.

44. Minter LM, Dickinson ES, Naber SP, Jerry DJ. Epithelial cell cycling predicts p53 responsiveness to gamma-irradiation during post-natal mammary gland development. Development 2002;129:2997-3008.

45. Liu W, Konduri SD, Bansal S, et al. Estrogen receptor-alpha binds p53 tumor suppressor protein directly and represses its function. J Biol Chem 2006;281:9837-40.

46. Konduri SD, Liu W, Srivastava P, et al. ERα Recruits Transcriptional Co-repressors to Inhibit p53 Function: Implications for a Novel Mechanism Determining Response to Tamoxifen Therapy of Breast Cancer. Manuscript Under Preparation 2008.

47. Miller LD, Smeds J, George J, et al. An expression signature for p53 status in human breast cancer predicts mutation status, transcriptional effects, and patient survival. Proc Natl Acad Sci U S A 2005;102:13550-5.

48. Liu W, Ip MM, Podgorsak MB, Das GM. Disruption of estrogen receptor alpha-p53 interaction in breast tumors: a novel mechanism underlying the anti-tumor effect of radiation therapy. Breast Cancer Res Treat 2008.

49. van't Veer LJ, Paik S, Hayes DF. Gene expression profiling of breast cancer: a new tumor marker. J Clin Oncol 2005;23:1631-5.

50. Kai K, Nishimura R, Arima N, Miyayama H, Iwase H. p53 expression status is a significant molecular marker in predicting the time to endocrine therapy failure in recurrent breast cancer: a cohort study. Int J Clin Oncol 2006;11:426-33.

51. Yamashita H, Toyama T, Nishio M, et al. p53 protein accumulation predicts resistance to endocrine therapy and decreased post-relapse survival in metastatic breast cancer. Breast Cancer Res 2006;8:R48.

52. Cattoretti G, Rilke F, Andreola S, D'Amato L, Delia D. P53 expression in breast cancer. Int J Cancer 1988;41:178-83.

53. Olivier M, Langerod A, Carrieri P, et al. The clinical value of somatic TP53 gene mutations in 1,794 patients with breast cancer. Clin Cancer Res 2006;12:1157-67.

54. Tang Y, Hu LA, Miller WE, et al. Identification of the endophilins (SH3p4/p8/p13) as novel binding partners for the beta1-adrenergic receptor. Proc Natl Acad Sci U S A 1999;96:12559-64.

55. Eriksson S, Berg LM, Wadelius M, Alderborn A. Cytochrome p450 genotyping by multiplexed real-time dna sequencing with pyrosequencing technology. Assay Drug Dev Technol 2002;1:49-59.

56. Gaedigk A, Simon SD, Pearce RE, Bradford LD, Kennedy MJ, Leeder JS. The CYP2D6 activity score: translating genotype information into a qualitative measure of phenotype. Clin Pharmacol Ther 2008;83:234-42.

57. Steen VM, Andreassen OA, Daly AK, et al. Detection of the poor metabolizer-associated CYP2D6(D) gene deletion allele by long-PCR technology. Pharmacogenetics 1995;5:215-23.

58. Mager DE. Target-mediated drug disposition and dynamics. Biochem Pharmacol 2006;72:1-10.

59. Mager DE, Jusko WJ. General pharmacokinetic model for drugs exhibiting target-mediated drug disposition. J Pharmacokinet Pharmacodyn 2001;28:507-32.

60. Wells J, Graveel CR, Bartley SM, Madore SJ, Farnham PJ. The identification of E2F1-specific target genes. Proc Natl Acad Sci U S A 2002;99:3890-5.

61. Wu L, Williams PM, Koch WH. Clinical applications of microarray-based diagnostic tests. Biotechniques 2005;39:577-82.

62. L'Esperance S, Popa I, Bachvarova M, et al. Gene expression profiling of paired ovarian tumors obtained prior to and following adjuvant chemotherapy: molecular signatures of chemoresistant tumors. Int J Oncol 2006;29:5-24.

63. Vousden KH, Lu X. Live or let die: the cell's response to p53. Nat Rev Cancer 2002;2:594-604.

64. Riley T, Sontag E, Chen P, Levine A. Transcriptional control of human p53-regulated genes. Nat Rev Mol Cell Biol 2008;9:402-12.

65. Sayeed A, Konduri SD, Liu W, Bansal S, Li F, Das GM. Estrogen Receptor {alpha} Inhibits p53-Mediated Transcriptional Repression: Implications for the Regulation of Apoptosis. Cancer Res 2007;67:7746-55.

66. Konduri SD, Medisetty R, Liu W, Kaipparettu BA, Srivastava P, Brauch H, et al. Mechanisms of estrogen receptor antagonism toward p53 and its implications in breast cancer therapeutic response and stem cell regulation. Proc Nati Acad Sci USA 2010; 107: 15081 1-6.

67. Liu W, Konduri SD, Bansal S, Nayak BK, Rajasekaran SA, Karuppayil SM, et al. Estrogen receptor-alpha binds p53 tumor suppressor protein directly and represses its function. J Biol Chem 2006; 281:9837-40.

68. Sayeed A, Konduri SD, Liu W, Bansal S, Li F, Das GM. Estrogen receptor alpha inhibits p53-mediated transcriptional repression: implications for the regulation of apoptosis. Cancer Research 2007; 67:7746-55.

69. van Landeghem AA, Poortman J, Nabuurs M, Thijssen JH, Endogenous concentration with subcelluar distribution of estrogens in normal and malignant human breast tissue. Cancer Res 1985; 45:2900-6.

70. Miller WR, Dixon JM. Antiaromatase agents: preclinical data and neoadjuvant therapy, Clin Breast Cancer 2000; 1 Suppl 1:S9-14.

71. Masamura S, Santner SJ, Gimotty P, George J, Santen RJ. Mechanism for maintenance of high breast tumor estradiol concentrations in the absence of ovarian function: role of very high affinity tissue uptake. Breast Cancer Res Treat 1997; 42:215-26

72. Lonning PE, Helle H, Duong NK, Ekse D, Aas T, Geisler J. Tissue estradiol is selectively elevated in receptor positive breast cancers while tumor estrone is reduced independent of receptor status. J. Steroid Biochem Mol Biol 2009; 117:31-41.

Appendix 1 Study Calendar

Required studies	Baseline	After Randomization	Week 2 or 3	Day 29 (surgery day)	30 days post-surgery
HCG (serum or urine) Premenopausal women only	X				
H&P [1]	X				
Performance Status	X				
Blood for PK/PD studies (tamoxifen arm only)		X (2 green top)	X (1 green top)	X (1 green top)	
Adverse events assessment (tamoxifen arm only)		X	X	X	X
RPCI will be using DBBR Standard Banking Collection for DNA (Both observation arm and tamoxifen arm) University of Chicago will be collecting one EDTA (purple or pink) tube of blood for polymorphism studies	X(2)				
Core Bx for TAM and TAM metabolites levels, and future pharmacogenomic studies				X	
IHC for p53				X (3)	
ER/PR/HER-2 analysis	X			X	

1. **History and physical will include questions about prior history of thrombosis, stroke, and complete medication history. Gynecologic history will be obtained including LMP, history of dysfunctional bleeding, recent pap smears, and smoking history. Can use history and physical within 4 weeks prior to signing consent.**
2. **Patients need to consent to DBBR. DBBR sample must be collected prior to surgery. DBBR sampling will not be released until the end of study.**
3. **IHC for p53 will not be obtained until day of surgery**

APPENDIX 2: INSTRUCTIONS FOR NETWORK SITES

1. CONTACT INFORMATION

All questions related to the protocol or study implementation should be directed to:
Roswell Park Cancer Institute
CRS Network Office
ASB K 104
Buffalo, New York 14263

Telephone:
716-845-8084 or 716-845-1203 - M-F; 7:00 AM to 4:30 PM
716-845-2300 - After hours, weekends and holidays: request the RPCI Principal Investigator
Fax: 716-845-8743

2. INFORMED CONSENT

- Informed Consent must be obtained by the **Investigator** from any patients wishing to participate, **prior to any procedures or change in their treatment**
- An informed consent template is provided by RPCI and can be amended to reflect institutional requirements
- All consent changes **must** be reviewed by Roswell Park Cancer Institute Network Office prior to submission to the site IRB.
- The informed consent must be IRB approved
- Always check that you are using the correct date and version of the IRB approved consent.

3. PATIENT REGISTRATION AND RANDOMIZATION ASSIGNMENT

Phase II protocol registration instructions:

The **Subject Enrollment Log** must be faxed to the CRS Network Office within 24 hours of the date the patient is consented. Once the Principal Investigator has determined that eligibility has been met, complete the **Patient Registration Form and fax it** to the RPCI Network Office at (716) 845-8743.
Note: The patient completes the **Gender, Race, and Ethnicity form** and this is placed in the study binder.

Roswell Park Cancer Institute does not grant exceptions to eligibility criteria.

Randomization Assignment:
Patients will be allocated to treatment or control by block randomization (block size=4). The randomization assignment list will be maintained at RPCI. The nurse/coordinator from University of Chicago will notify the RPCI Network office for a randomization assignment by sending the completed patient registration form to CRSNetworkCoordinators@roswellpark.org. The Network Monitor or designee will randomize the patient and send the randomization assignment to the nurse/coordinator at University of Chicago.

4. STUDY DEVIATIONS

- If a deviation has occurred to eliminate hazard, this should be reported to the RPCI Network, site IRB and any other regulatory authority involved in the trial.

- ANY study deviation will be recorded on the Study Deviation Log
- Patients who are inadvertently enrolled, with significant deviation(s) from the study-specified criteria, will be removed from the study
- Notify RPCI of any early patient withdrawal and appropriately document the discontinuation and the reason why.

5. STUDY DOCUMENTATION

- Study documents must be filled out completely and correctly. Ditto marks are not allowed.
- If an entry has been documented in error put a single line through the entry and initial and date the change. The auditor must be able to read what has been deleted.
  - Do NOT use white-out, magic marker, scratch-outs
  - Do NOT erase entries
- Use only black ink for documentation on the accountability form and any other study forms.

6. DRUG ACCOUNTABILITY

Drug accountability will be strictly maintained, recording quantities of study drug received, dispensed to patients and wasted, lot number, date dispensed, patient ID number and initials, quantity returned, balance remaining, manufacturer, expiration date, and the initials of the person dispensing the medication.

- Responsibility rests solely with the Principal Investigator but can be delegated as appropriate (e.g. to pharmacy personnel)
- Records must be maintained regarding receipt, dispensing, return, waste and disposition of all investigational agents
- Study drug supply should only be used in accordance with the IRB approved study
- Drug accountability forms are protocol and agent specific, they are study source documents and will be used to verify compliance with the study
- Any discrepancies shall be documented and explained
- An inventory count should be performed with each transaction
- Drug accountability forms shall be stored with study related documents
- Each medication provided for this study and each dosage form and strength must have its own Drug accountability.
- Do **NOT** “transfer”, “borrow” or “replace” supplies between studies
- Dispensing the wrong study supply is considered a **medication error**
- **Never** replace investigational agents with commercial product

7. SERIOUS ADVERSE EVENT REPORTING:

The site Investigator or designated research personnel will report all serious adverse events, whether related or unrelated to the study drug(s) to the **IRB in accordance with their local institutional guidelines.** The site will notify the CRS Network Office within one business day of being made aware of the SAE. A preliminary written report must follow within 24 hours (1 business day) of the oral notification using the following forms:

- SAE report form
- MEDWATCH 3500

A complete follow-up report must be filed within 10 working days.

8. UNANTICIPATED PROBLEM REPORTING:

An Unanticipated Problem (UAP) is any incident, experience, or outcome that meets **all** of the following criteria:

1. Unexpected (in terms of nature, severity, or frequency) given:
   (a) The research procedures that are described in the study- related documents, including study deviations, as well as issues related to compromise of patient privacy or confidentiality of data;
   (b) The characteristics of the subject population being studied;
2. Related or possibly related to participation in the research (possibly related means there is a reasonable possibility that the incident, experience, or outcome may have been caused by the procedures involved in the research);
3. Suggests that the research places subjects or others at a greater risk of harm (including physical, psychological, economic, or social harm) than was previously known or recognized;

For all adverse events occurring that are unanticipated and related or possibly related to the research drug, biologic or intervention the participating physician or delegated research staff from each site will notify **their local IRB in accordance with their local institutional guidelines**. The site must also notify the CRS Network Office within 24 hours of being made aware of the Unanticipated Problem by completing the **RPCI Unanticipated Problem Report Form** and faxing it to the CRS Network office.

I 110907 Pilot Study to Analyze a Novel Mechanism Underlying Response to Tamoxifen Therapy in Breast Cancer Patients

Symptom Diary -Appendix 3

Please record your symptoms in the diary below for each week and bring this with you to your next visit

Use the following scale for recording your symptoms each day:
0 = not at all; 1 = mild (slightly); 2 = moderate (limits activity outside home); 3 = quite a bit (not able to do daily activities); 4 = extremely(severe-unable to get out of bed or hospitalized)

Patient initials _____ Patient # _____ From: ___/___/___ to ___/___/___

Symptoms	Day 1	Day 2	Day 3	Day 4	Day 5	Day 6	Day 7
Date	/ /	/ /	/ /	/ /	/ /	/ /	/ /
Pain (location)							
Pressure or tightness in head/body							
Muscle aches							
Muscle weakness							
Joint pain							
Parts of body feel tingly or numb							
Visual changes							
Ringing in ears							
Headaches							
Feeling dizzy or faint							
Heart beating quickly (palpitations)							
Cough							
Difficulty breathing/shortness of breath							
Difficulty swallowing							
Dry mouth							
Weight gain							
Weight loss							
Diarrhea							
Constipation							
Nausea							
Vomiting							
Heartburn							
Decreased or lack of appetite							
Feeling bloated							
Difficulty with bladder control (when laughing or crying)							
Difficulty with bladder control (at other times)							
Itching skin							
Swelling of hands or feet							
Skin rash or other skin changes							

I 110907 Pilot Study to Analyze a Novel Mechanism Underlying Response to Tamoxifen Therapy in Breast Cancer Patients

Symptom Diary -Appendix 3

Please record your symptoms in the diary below for each week and bring this with you to your next visit

Use the following scale for recording your symptoms each day:
0 = not at all; 1 = mild (slightly); 2 = moderate (limits activity outside home); 3 = quite a bit (not able to do daily activities); 4 = extremely(severe-unable to get out of bed or hospitalized)

Patient initials _____ Patient # _____ From: ___/___/___ to ___/___/___

Symptoms Continued	Day 1	Day 2	Day 3	Day 4	Day 5	Day 6	Day 7
Date	/ /	/ /	/ /	/ /	/ /	/ /	/ /
Chills							
Fever							
Hot flashes							
Irritability							
Night sweats							
Vaginal dryness							
Painful sexual intercourse							
Vaginal discharge/unusual bleeding							
Change in menstrual cycle (irregular)							
Change in menstrual cycle (absent)							
Cramps							
Loss of sexual interest							
Hair loss							
Difficulty concentrating							
Forgetfulness							
Blood clots							
Hospitalizations							
Other:							
Other:							

Symptom Diaries used for patient tool only.

Patient Signature: _____ Date: _____

Protocol #: _____ Patient Name: _____
Drug Name: _____
Cycle: _____ Med. Record #: _____

Study Medication Calendar - Appendix 4

Please complete this calendar on a daily basis. Fill in the date for each day in the 1st row, write the drug dose that you take each day in the 2nd row, and write the total number of pills you take each day in the 3rd row.

On days you do not take any study drugs; please write "0" in drug dose box. If your dose changes record the new dose level.
Start Date: _____

Cycle Day	Day 1	Day 2	Day 3	Day 4	Day 5	Day 6	Day 7
Date							
Dose							
Number of pills taken							

Cycle Day	Day 8	Day 9	Day 10	Day 11	Day 12	Day 13	Day 14
Date							
Dose							
Number of pills taken							

Cycle Day	Day 15	Day 16	Day 17	Day 18	Day 19	Day 20	Day 21
Date							
Dose							
Number of pills taken							

Cycle Day	Day 22	Day 23	Day 24	Day 25	Day 26	Day 27	Day 28
Date							
Dose							
Number of pills taken							

Cycle Day	Day 29	Day 30	Day 31	Day	Day	Day	Day
Date							
Dose							
Number of pills taken							

Please remember to bring this calendar and your pill bottle (including any unused pills) with you to your next study appointment.

Coordinator Use Only
Date of return: _____
# of pills
(dispensed: _____ - returned: _____)
_____ x 100 = % adherence: _____
# of pills scheduled _____

Patient signature: _____ Date: _____

Investigator signature: _____ Date: _____