Update #05

ALLIANCE FOR CLINICAL TRIALS IN ONCOLOGY

PROTOCOL UPDATE TO ALLIANCE A011203

A RANDOMIZED PHASE II TRIAL OF TAMOXIFEN VERSUS Z-ENDOXIFEN HCL IN POSTMENOPAUSAL WOMEN WITH METASTATIC ESTROGEN RECEPTOR POSITIVE, HER2 NEGATIVE BREAST CANCER

[X]	**Update:**	[ ]	**Status Change:**
[ ]	Eligibility changes	[ ]	Activation
[ ]	Therapy / Dose Modifications / Study Calendar changes	[ ]	Closure
[X]	Informed Consent changes	[ ]	Suspension / temporary closure
[ ]	Scientific / Statistical Considerations changes	[ ]	Reactivation
[ ]	Data Submission / Forms changes		
[X]	Editorial / Administrative changes		
[ ]	Other:		

Expedited review is allowed. IRB approval (or disapproval) is required within 90 days. Reconsent is not required, please follow the policy of your IRB of record regarding notifying patients of new information contained in this update.

The changes included in this update to A011203 have been made in response to the NCI Action Letter from Dr. Howard Streicher (streicherh@ctep.nci.nih.gov), dated May 1, 2018. This Action Letter is posted on the A011203 study page on the Alliance web site. A revised CAEPR for Z-Endoxifen HCl with new risk information has been added to the protocol. Therefore, the model consent form has been revised to incorporate this new risk information, consistent with the NCI Model Consent Template instructions. There are no changes to the risk/benefit ratio.

UPDATES TO PROTOCOL

Page 2 (Document History):
The study document history table has been removed. This table now appears as a separate document on the protocol landing page.

Section 3.2.13 (None of the following co-morbid conditions):
A note stating "Serious adverse events will be reported on CTEP-AERS using CTCAE v5.0" has been added to this section.

Section 8.2.1 (Tamoxifen therapy):
A note stating "Serious adverse events will be reported on CTEP-AERS using CTCAE v5.0" has been added at the end of this section.

Section 8.2.2 (Z-Endoxifen HCl):
Note: Serious adverse events will be reported on CTEP-AERS using CTCAE v5.0.

Section 9.2 (Expedited Adverse event reporting):
The third sentence has been revised to state that CTCAE Version 5.0 will be utilized for expedited AE reporting as of April 1, 2018.

Section 9.3 (Z-Endoxifen HCl CAEPR):
The Z-Endoxifen HCL CAEPR has been replaced with the revised CAEPR Version 2.0, September 25, 2017.

- This CAEPR version includes frequency data. The previous version did not have the categories for Likely, Less Likely or Rare but Serious.
- The section below utilizes CTCAE 5.0 language unless otherwise noted.

- **Added New Risk:**
  - Less Likely: Hypertension
  - Also Reported on Z-Endoxifen HCl Trials But With Insufficient Evidence for Attribution: Abdominal distension; Alkaline phosphatase increased; Blood bilirubin increased; Cognitive disturbance; Colonic perforation; Cough; Creatinine increased; Dehydration; Dyspnea; Ear pain; Edema face; Flashing lights; Hyperkalemia; Hypernatremia; Hypokalemia; Hyponatremia; Sinus bradycardia; Sore throat; Stroke; Sudden death NOS; Vaginal dryness
- **Increase in Risk Attribution:**
  - Changed to Less Likely from Also Reported on Z-Endoxifen HCl Trials But With Insufficient Evidence for Attribution: Arthralgia; Constipation; Dizziness; Hypocalcemia; Hypophosphatemia; Rash acneiform; Vomiting
- **Decrease in Risk Attribution:**
  - Changed to Also Reported on Z-Endoxifen HCl Trials But With Insufficient Evidence for Attribution from Possible: Hyperhidrosis; Hypothyroidism; INR increased
- **Provided Further Clarification:**
  - Gastrointestinal disorders - Other (burping) *(CTCAE 4.0 language)* is now reported as Belching.
  - Irritability under the GENERAL DISORDERS AND ADMINISTRATION SITE CONDITIONS SOC (*CTCAE 4.0 language*) is now reported under the PSYCHIATRIC DISORDERS SOC

Section 13.1.6.1 (Adverse Events):
A note stating "Serious adverse events will be reported on CTEP-AERS using CTCAE v5.0" has been added at the end of this section.

UPDATES TO MODEL CONSENT:
In the Section entitled "What possible risks can I expect from taking part in this study?" the following changes have been made under "Possible Side Effects of Z-Endoxifen HCl.

- Added New Risk:
  - Occasional: High blood pressure, which may cause blurred vision, dizziness, headache

- Increase in Risk Attribution:
    - Changed to Occasional from Also Reported on Z-Endoxifen HCl Trials But With Insufficient Evidence for Attribution (i.e., added to the Risk Profile): Constipation; Vomiting; Dizziness; Acne
- Decrease in Risk Attribution:
    - Changed to Also Reported on Z-Endoxifen HCl Trials But With Insufficient Evidence for Attribution from Possible (i.e., removed from the Risk Profile): Increased sweating
    - Changed to Rare from Occasional: Kidney damage which may require dialysis
- Provided Further Clarification:
    - Pain in muscles (under Occasional) is now reported as Pain (under Occasional).

PLEASE NOTE: The potential risks listed in the CAEPR whose relationship to Z-Endoxifen HCl is still undetermined are not required by CTEP to be described in the ICD; however, they may be communicated to patients according to local IRB requirements.

Replacement protocol and model consent documents have been issued.

This study remains closed to accrual.

ATTACH TO THE FRONT OF EVERY COPY OF THIS PROTOCOL

ALLIANCE FOR CLINICAL TRIALS IN ONCOLOGY

ALLIANCE A011203

A RANDOMIZED PHASE II TRIAL OF TAMOXIFEN VERSUS Z-ENDOXIFEN HCL IN POSTMENOPAUSAL WOMEN WITH METASTATIC ESTROGEN RECEPTOR POSITIVE, HER2 NEGATIVE BREAST CANCER

Investigational Agent: Z-Endoxifen HCl (NSC #750393) supplied by CTEP, DCTD, NCI. Commercial Agent: Tamoxifen: (NSC #180973)

Clinical Trials.gov Identifier: NCT02311933

Study Chair
Matthew P. Goetz, MD
Mayo Clinic
200 First Street, SW
Rochester, MN 55905
Tel: 507-284-2511 Fax: 507-284-1803
goetz.matthew@mayo.edu

Community Oncology Co-chair
John T. Reynolds, MD
Sanford Health
222 North 7th Street
Bismarck, ND 58501
Tel: 701-323-5741
john.reynolds@sanfordhealth.org

Pharmacogenomics Co-chair
Joel Reid, PhD
Mayo Clinic
Tel: 507-284-0822
reid@mayo.edu

Solid Tumor Correlative Science Co-chairs
W. Fraser Symmans, MD
The University of Texas MD Anderson Cancer Center
Tel: 713-792-7962
fsymmans@mdanderson.org

John R. Hawse, PhD
Mayo Clinic
Tel: 507-284-4268
hawse.john@mayo.edu

Minetta Liu, MD
Mayo Clinic
Tel: 507-293-9160
Liu.minetta@mayo.edu

Disease Committee Chair
Lisa A. Carey, MD
Tel: 919-966-4431
lisa_carey@med.unc.edu

Ann H. Partridge, MD
Tel: 617-632-3800
ahpartridge@partners.org

Pharmacogenomics and Population Pharmacology Committee Chair
Mark J. Ratain, MD
Tel: 773-702-4400
mratain@medicine.bsd.uchicago.edu

Primary Statistician
Vera J. Suman, PhD
Tel: 507-284-8803
suman@mayo.edu

Secondary Statistician
Travis J. Dockter, MS
507-266-9803
doctor.travis@mayo.edu

Biostatistician
Ann L. Oberg, PhD
Tel: 507-538-1556
oberg.ann@mayo.edu

Protocol Coordinator
Heather P. Becker
Tel: 773-834-2546
hpbecker@uchicago.edu

Data Manager
Amy Oeltjen
Tel: 507-538-0570
oeltjen.amy@mayo.edu

Participating Groups: **Alliance**/Alliance for Clinical Trials in Oncology
ECOG-ACRIN/ECOG-ACRIN Medical Research Foundation, Inc.
NRG/NRG Oncology Foundation, Inc.
SWOG/SWOG

Study Resources:

Expedited Adverse Event Reporting: **http://eapps-ctep.nci.nih.gov/ctepaers/**	Medidata Rave® iMedidata portal **https://login.imedidata.com**
OPEN (Oncology Patient Enrollment Network) https://open.ctsu.org	**Biospecimen Management System** http://bioms.allianceforclinicaltrialsinoncology.org

Protocol Contacts:

A011203 Nursing Contact
Cesar Figueras, BSN, RN
Institution: St. Joseph Mercy Hospital
Tel: 734-712-5628
E-mail: *Cesar.Figueras@stjoeshealth.org*

A011203 Pharmacy Contact
Zoe Ngo, PharmD
Institution: UCSF Helen Diller Family Comprehensive Cancer Center
Tel: 415-353-7052
Email: *Zoe.Ngo@ucsfmedctr.edu*

Alliance Biorepository at Ohio State University
The Ohio State University
Innovation Centre
2001 Polaris Parkway
Columbus, OH 43240
Tel: 614-293-7073 Fax: 614-293-7967
Email: *path.calgb@osumc.edu*

Alliance Biorepository at Mayo Clinic (BAP)
BAP Freezer
Mayo Clinic
St SL-16
150 Third Street SW
Rochester MN, 55902
Biospecimen Resource Manager 507-538-0602

Protocol-related questions may be directed as follows:

Questions	Contact (via email)
Questions regarding patient eligibility, treatment, and dose modification:	Study Chair, Nursing Contact, Protocol Coordinator, and (where applicable) Data Manager
Questions related to data submission, RAVE or patient follow-up:	Data Manager
Questions regarding the protocol document:	Protocol Coordinator
Questions related to IRB issues and model consent revisions:	Alliance Regulatory Inbox regulatory@allianceNCTN.org
Questions regarding AdEERS reporting:	**Regulatory Affairs Manager** regulatory@allianceNCTN.org 773-702-9814
Questions regarding specimens/specimen submissions:	**Appropriate Alliance Biorepository**

CANCER TRIALS SUPPORT UNIT (CTSU) ADDRESS AND CONTACT INFORMATION

For regulatory requirements:	**For patient enrollments:**	**For study data submission:**
Regulatory documentation can be submitted to the CTSU via: **ONLINE:** Regulatory Submission Portal (Sign in at www.ctsu.org, and select the Regulatory Submission sub-tab under the Regulatory tab.) **EMAIL:** CTSURegulatory@ctsu.coccg.org (regulatory documentation only) **FAX:** 215-569-0206 **MAIL:** CTSU Regulatory Office 1818 Market Street, Suite 1100 Philadelphia, PA 19103 For regulatory questions call the CTSU Regulatory Help Desk at 1-866-651-CTSU	Please refer to the patient enrollment section of the protocol for instructions on using the Oncology Patient Enrollment Network (OPEN) which can be accessed at https://www.ctsu.org/OPENSYSTEM/ or https://OPEN.ctsu.org. Contact the CTSU Help Desk with any OPEN-related questions at ctsucontact@westat.com.	Data collection for this study will be done exclusively through Medidata Rave. Please see the data submission section of the protocol for further instructions.
The most current version of the **study protocol and all related forms and documents** must be downloaded from the protocol-specific Web page of the CTSU Member Web site located at https://www.ctsu.org. Access to the CTSU members' website is managed through the Cancer Therapy and Evaluation Program - Identity and Access Management (CTEP-IAM) registration system and requires user log on with CTEP-IAM username and password. Permission to view and download this protocol and its supporting documents is restricted and is based on person and site roster assignments housed in the CTSU RSS.		
For clinical questions (i.e. eligibility or treatment-related questions) see the Protocol Contacts, Page 2.		
For non-clinical questions (i.e. unrelated to patient eligibility, treatment, or data submission) contact the CTSU Help Desk by phone or e-mail: CTSU General Information Line – 1-888-823-5923, or ctsucontact@westat.com. All calls and correspondence will be triaged to the appropriate CTSU representative.		
The CTSU Web site is located at https://www.ctsu.org.		

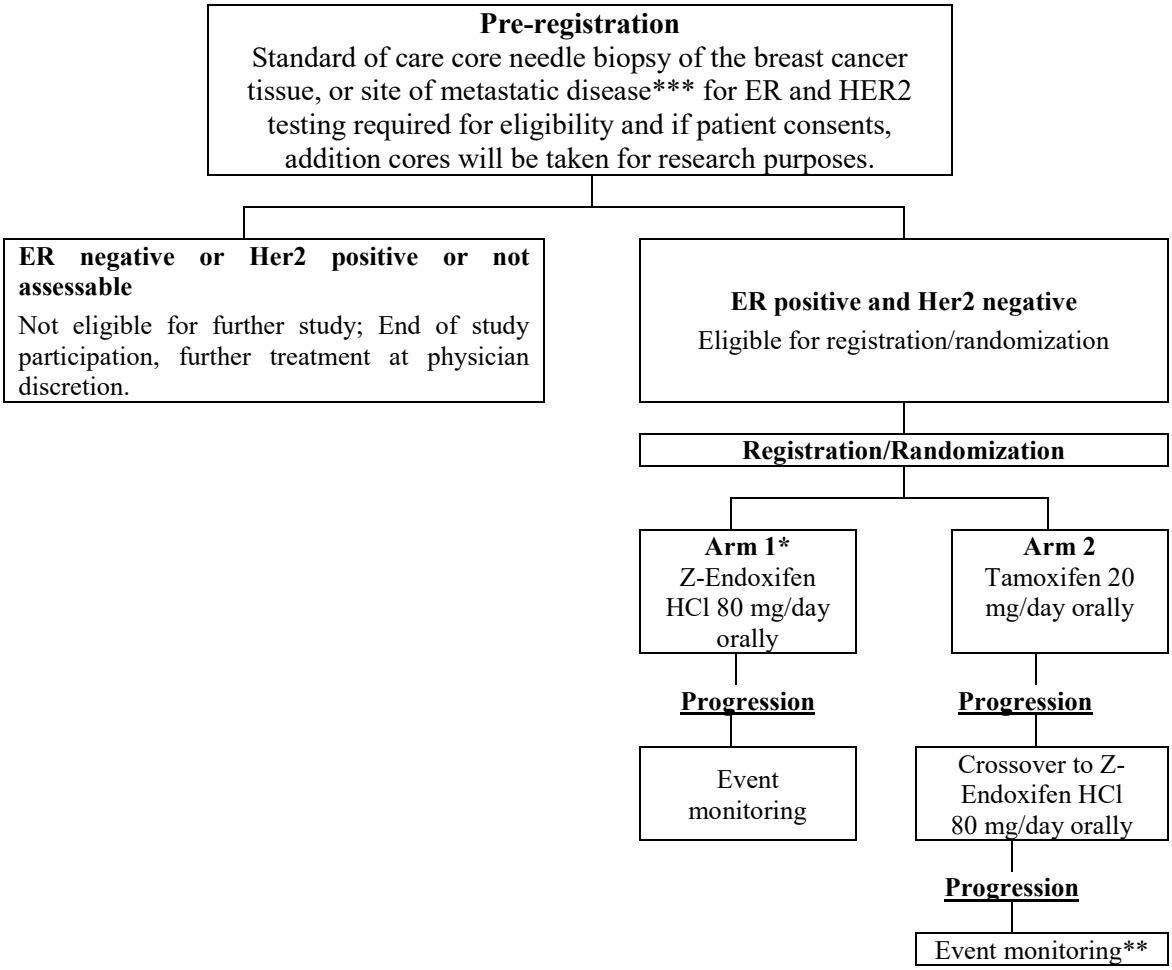
A RANDOMIZED PHASE II TRIAL OF TAMOXIFEN VERSUS Z-ENDOXIFEN HCL IN POSTMENOPAUSAL WOMEN WITH METASTATIC ESTROGEN RECEPTOR POSITIVE, HER2 NEGATIVE BREAST CANCER

Pre-Registration Eligibility Criteria (see Section 3.0)	**Required Initial Laboratory Values**	
Women who agree to undergo a standard of care core biopsy of recurrent or metastatic breast cancer, to confirm ER + and HER2 negative breast cancer (see Sections 3.3.5 and 3.3.6,) Patient previously treated with an AI either in adjuvant or metastatic setting and have clinical resistance as defined in Section 3.2.2. (see Section 3.2.3). Patients with a history of measurable disease as defined by RECIST criteria or bone only disease are eligible. Note: Those patients with non-measureable disease and bone metastases are eligible. No history of tumors involving spinal cord or heart. No current evidence of visceral crisis, lymphangitic spread. No known brain metastasis. Women must be age ≥ 18 years. Women must be postmenopausal (see Section 3.2.8). No more than 2 prior chemo regimens in the metastatic setting. Prior AI treatment in either adjuvant or metastatic setting is required (see Section 3.2.8). Prior endocrine regimens in metastatic setting allowed (see Section 3.2.9) Prior tamoxifen therapy is allowed in the adjuvant setting (see Section 3.2.9). No prior tamoxifen in the metastatic setting. No prior treatment with endoxifen. Patients must have fully recovered from acute, reversible effects of prior therapy (see Section 3.2.9) Not receiving any medications or substances that are strong inhibitors of CYP2D6 (see Appendix II). Not receiving any other investigational agents. No uncontrolled intercurrent illness (see Section 3.2.12) None of the co-morbid conditions described in Section 3.2.13. No other active secondary malignancy (see Section 3.2.14) ECOG performance Status 0-2. Able to swallow oral formulation of the study agent.	Hemoglobin Platelet Count Creatinine Total Bilirubin AST	≥ 9 g/dL ≥ 75,000/mm^3 ≤ 1.5 x upper limits of normal ULN ≤ 1.5 x upper limits of normal (ULN) ≤ 2.5 x upper limits of normal (ULN). For pts with liver mets ≤ 5 x ULN

Schema

Treatment is to continue until disease progression or unacceptable adverse event. Patients will be followed until death, or a maximum of five years after randomization, whichever comes first.

1 Cycle = 21 days



* Note that the cycle length is 3 weeks in order to improve our ability to compare the median progression-free survival times in each treatment arm.

** During the Event Monitoring Phase of the study, all participating NCTN institutions are being asked to provide notification as to whether the participant has progressed, developed a new primary, and/or died. Participants are not be required to return to their registering site to undergo any study related medical examinations or specimen collections, however NCTN institutions are responsible for the collection of follow-up data. All treatment decisions are in the hands of the participant and their medical team (see Section 12.0).

*** See Section 6.2.2.

Table of Contents

1.0 BACKGROUND

1.1 Overview

Over 200,000 women and 2,000 men are diagnosed with breast cancer in the US each year[1]. About 70% of breast cancer expresses estrogen receptors (ER) or progesterone receptors (PR). In this group of patients, endocrine therapy represents the most important treatment modality. Specifically, when administered after surgery, adjuvant hormonal therapy (aromatase inhibitor (AI) or sequential tamoxifen followed by AI) results in a greater than 50% reduction in recurrence and prolongs overall survival compared to no treatment.

Despite the advantage of aromatase inhibitors over tamoxifen, resistance to hormonal therapy remains a public health problem. In the ATAC trial, after a median follow-up of 100 months, disease free survival favored anastrozole, with nearly 26% of patients randomized to anastrozole experiencing a disease event versus 30% on tamoxifen and difference in overall survival [2]. Similar findings were observed with the BIG 1-98 clinical trial [3]. Therefore, new strategies are needed for the treatment of AI resistant breast cancer

Tamoxifen, the selective estrogen receptor modulator (SERM) has been studied and utilized in breast cancer for the last forty years. Tamoxifen undergoes extensive hepatic metabolism to metabolites (4-hydroxy-tamoxifen and 4-hydroxy-N-desmethyl-tamoxifen [endoxifen]) known to be pharmacologically more active (30-100 fold) than tamoxifen in terms of ER binding affinity and ability to suppress estradiol (E2)-stimulated cell proliferation [4]. While the concentrations of 4HT are consistently low < 7 nM), endoxifen concentrations are highly variable (range undetectable to 80 nM) [5]. Cytochrome P450 2D6 (CYP2D6) is the primary hepatic enzyme responsible for the oxidation of N-desmethyl-tamoxifen to endoxifen. While CYP2D6 genetic variation is consistently associated with steady state endoxifen concentrations, secondary analyses of prospective adjuvant tamoxifen studies administering 5 years of tamoxifen (NCCTG 89-30-52 [6], ATAC [7], BIG1-98 [8], and ABCSG 8 [9]) have reached discrepant conclusions on the importance of CYP2D6 genetic variation and its association with disease free survival.

The largest adjuvant study evaluating the association between the steady state concentrations (Css) of tamoxifen and its metabolites and disease recurrence in the adjuvant setting demonstrated that endoxifen Css (but not 4HT or tamoxifen) were associated with the risk of recurrence [10].

In the largest study evaluating the association between CYP2D6 genetic variation and progression free survival in tamoxifen treated women in the metastatic setting, Karle et al demonstrated that patients without any fully functional allele (IM/IM, IM/PM, PM/PM) had a significant shorter progression-free survival (PFS) and overall survival (OS) compared to patients with at least one functional allele (EM/EM, EM/IM, EM/PM) (PFS: p = 0.017; HR = 2.19; 95 % CI 1.15-4.18; OS: p = 0.028; HR = 2.79; 95 % CI 1.12-6.99). The CB rate was 73 % for EM-group and 38.5 % for IM + PM-group (p = 0.019) [11].

We hypothesized that the direct administration of endoxifen would result in acceptable safety, with higher endoxifen concentrations than achievable in tamoxifen treated patients, with antitumor activity in patients with ER+ breast cancer refractory to standard therapies. The latter hypothesis is based on the following:

1) Preclinical *in vitro* and *in vivo* models demonstrating the superior antitumor activity of Z-Endoxifen HCl over tamoxifen, in both AI sensitive and AI resistant models
2) The demonstration of superiority over exemestane + everolimus in a letrozole resistant MCF7 in vivo model

3) The demonstration of a novel mechanism of action of endoxifen as it relates to inhibition of Protein Kinase C β1 (PKCβ1).

4) Preclinical data demonstrating that at clinically achievable concentrations (100 nM – 1000 nM), endoxifen may exhibit antitumor activity in tumors with known activating mutations in ERα, known to confer resistance to tamoxifen and AI's [12].

Z-Endoxifen HCl (NSC #750393) was synthesized and following the completion of toxicology studies and demonstration of acceptable pharmacokinetics in rodent models, an IND was filed and first in humans studies (8821, Mayo) and (8826, NCI) were initiated in 2012. Early data from these trials has demonstrated substantial exposure (300-3000 nM) to endoxifen over the range of 20-160 mg/day, acceptable safety, and repeated evidence for antitumor activity in patients refractory to tamoxifen, AI's, fulvestrant, and everolimus. Based on these data, we propose a randomized phase II of oral endoxifen with oral tamoxifen in patients with metastatic or locally advanced breast cancer refractory to aromatase inhibitors.

1.2 FDA approved endocrine treatments for the adjuvant treatment of breast cancer

1.2.1 Tamoxifen

The SERM, tamoxifen, has been used in the treatment of breast cancer for the last 40 years. It was first FDA approved for metastatic breast cancer in 1977. It received FDA approved for use in post-menopausal women with node positive disease in 1986, pre- and post- menopausal women in the adjuvant setting in 1990, in prevention of breast cancer in 1998 and for resected ductal carcinoma in situ in 2000.

Multiple trials have evaluated tamoxifen as adjuvant therapy in breast cancer. These trials are well summarized in a meta-analysis from the Early Breast Cancer Trialists Cancer Group [13].

Analysis of clinical trials where tamoxifen was used for a median of five years showed reduction of cancer recurrence risk by 50% in ER + patients during the first five years and by 33% during year 5-9 (average risk reduction was 39%, HR 0.61, $P<0.00001$). Breast cancer mortality was also reduced by 30% at year 15 with an absolute difference of 9%. During year 5-9, recurrence rates were reduced by 33% despite the fact that tamoxifen use at that time was similar between the two arms.

Studies evaluating 10 versus 5 years of tamoxifen have demonstrated further reductions in both recurrence and breast cancer mortality [14].

1.2.2 Third generation aromatase inhibitors

The third-generation aromatase inhibitors anastrozole, exemestane and letrozole have become firmly established as important agents for use in adjuvant endocrine therapy and result in superior DFS compared to tamoxifen for the postmenopausal adjuvant treatment of ER+ breast cancer [2, 3]. An American Society of Clinical Oncology (ASCO) Practice Guideline has recommended the use of aromatase inhibitors at some point during adjuvant endocrine therapy either as initial therapy or following some period of tamoxifen therapy [15].

1.3 The endocrine treatment of ER+, metastatic breast cancer

1.3.1 Aromatase inhibitors

In the metastatic setting, aromatase inhibitors are the standard of care for the first line therapy of metastatic breast cancer. A series of randomized phase III trials, have demonstrated the superiority of AIs (anastrozole, letrozole and exemestane) over TAM as first line treatment of metastatic, ER+ breast cancer and a literature based meta-analysis confirmed the superiority as it relates to response rate and clinical benefit rate [16]. However, nearly all patients receiving an AI for metastatic ER + breast cancer will develop progressive disease. Thus, there is a need for new therapeutic and palliative agents for metastatic ER + breast cancer refractory to AIs.

1.3.2 Fulvestrant (Faslodex)

Fulvestrant is an ER antagonist that down-regulates ER and exhibits anti-tumor activity in tumors resistant to TAM. A multicenter, double-blind, randomized trial comparing TAM with fulvestrant in previously untreated metastatic/locally advanced breast cancer demonstrated that TAM tended to be superior to fulvestrant in terms of time to progression (median TTP, 6.8 months and 8.3 months, respectively; P =.088) [17]. Because of inadequate pharmacokinetic exposure with the low dose regimen, multiple studies have evaluated higher doses of fulvestrant. In the phase III EFECT trial [18], the fulvestrant loading dose regimen was compared with the non-steroidal AI, exemestane. Median TTP was 3.7 months in both groups (hazard ratio = 0.963; 95% CI, 0.819 to 1.133; p = 0.6531), with no difference in overall response or clinical benefit rate. However, DiLeo et al compared high dose fulvestrant (500 mg day 1 and 14 of cycle 1 and 500 mg day 1 of subsequent cycles) to fulvestrant (250 mg once monthly) for patients with progressive metastatic ER positive breast cancer [19]. In this study, PFS was significantly longer for fulvestrant 500 mg (n = 362) than 250 mg (n = 374) (hazard ratio [HR] = 0.80; 95% CI, 0.68 to 0.94; P = .006). Based on this data, FDA approved the use fulvestrant 500 mg as the standard dose in postmenopausal women with metastatic HR+ breast cancer progressing following anti-estrogen therapy.

1.3.3 Combination fulvestrant and aromatase inhibitors

Three clinical have tested whether the AI fulvestrant combination approach is superior to AI alone in postmenopausal women with metastatic breast cancer (FACT, SOFEA and SWOG 0226). The FACT trial postmenopausal women with ER+, metastatic breast cancer to receive either anastrozole 1 mg PO daily, or anastrozole 1 mg PO daily in combination with fulvestrant (loading dose of 500 mg on Day 1, 250 mg on Day 14 and 250 mg on Day 28, during the first cycle, followed by 250 mg/month). This study demonstrated no difference in TTP (10.8 months on combination therapy versus 10.2 months on anastrozole alone), HR 0.99 (95% CI 0.81 – 1.20), p=0.91), clinical benefit rate or OS [20].

Similarly, the SOFEA trial (Study of Faslodex with or without concomitant Arimidex vs Exemestane following progression on non-steroidal Aromatase inhibitors) randomized postmenopausal women with hormone-receptor-positive breast cancer who had either relapsed or progressed with locally advanced or metastatic disease on letrozole or anastrozole. In this study, participants were randomly assigned (1:1:1) to receive fulvestrant (500 mg intramuscular injection on day 1, followed by 250 mg doses on days 15 and 29, and then every 28 days) plus daily oral anastrozole (1 mg); fulvestrant plus anastrozole-matched placebo; or daily oral exemestane (25 mg). Median PFS was 4·4 months (95% CI 3·4—5·4) in patients assigned to fulvestrant plus anastrozole, 4·8

months (3·6—5·5) in those assigned to fulvestrant plus placebo, and 3·4 months (3·0—4·6) in those assigned to exemestane [21].

In contrast, the SWOG S0226 trial randomized postmenopausal women with metastatic breast cancer to either fulvestrant alone or fulvestrant + anastrozole, using a similar eligibility as the FACT trial, but with a greater percentage of treatment naïve patients. In this study, the median PFS was 15.0 months (95% CI 13.2 -18.4 months) in the combination arm, compared to the 13.5 months (95% CI 12.1 – 15.1 months) in the anastrozole arm, p = 0.007, HR 0.8 (95% CI 0.68 - 0.94), favoring the combination therapy. Additionally, a significant improvement in OS (combination therapy: 47.7 months (95% CI 43.4 -55.7) versus the anastrozole arm: 41.3 months (95% CI 37.2 -45.0), p = 0.049, HR 0.81 (95% CI 0.65 -1.00) was observed [22].

One potential difference accounting for the discrepant results of these trials has been postulated to be related to a greater number of endocrine naïve cases in the S0226 trial than in the FACT or SOFEA trial.

1.3.4 Tamoxifen

The activity of TAM in the metastatic setting following AI failure has been evaluated in several studies. Osborne et al conducted a randomized phase II trial evaluating the activity of tamoxifen +/- gefitinib in either hormonally naïve (stratum I) or patients who relapsed on AI (stratum II). In stratum II, for patients randomized to TAM alone, the objective response rate was 0% and progression free survival 7.0 month [23]. In the TAMRAD study, patients who had failed a non-steroidal AI were randomized to either tamoxifen alone or tamoxifen + everolimus. Notably, in this trial, time to progression was 4.5 months in patients randomized to the tamoxifen arm with a 6 month complete benefit rate (complete, partial or stable disease) of 42%.

1.3.5 PI3K/mTOR pathway in endocrine resistant breast cancer

Activation of the phosphatidylinositol 3-kinase (PI3K)/AKT/mammalian target of rapamycin (mTOR) pathway has been extensively linked to the development of endocrine resistance in ER positive breast cancer. Specifically, cross-talk between ER signaling pathways and the PI3K/AKT/mTOR pathway is associated with the development of endocrine resistance when ER positive cells undergo long term estrogen deprivation (LTED) mimicking AI resistance [24]. These observations have led to the development of clinical studies which have demonstrated that in patients with acquired endocrine resistance, the addition of an mTOR inhibitor to hormonal therapy prolonged PFS [25, 26].

Bachelot et al randomized postmenopausal women with hormone receptor-positive, human epidermal growth factor receptor 2-negative, AI-resistant metastatic breast cancer to either tamoxifen 20 mg/d plus everolimus 10 mg/d (n = 54) or tamoxifen 20 mg/d alone (n = 57) (randomized phase II). Randomization was stratified by primary and secondary hormone resistance. Primary end point was clinical benefit rate (CBR), defined as the percentage of all patients with a complete or partial response or stable disease at 6 months. The 6-month CBR was 61% (95% CI, 47 to 74) with tamoxifen plus everolimus and 42% (95% CI, 29 to 56) with tamoxifen alone. Time to progression (TTP) increased from 4.5 months with tamoxifen alone to 8.6 months with tamoxifen plus everolimus, corresponding to a 46% reduction in risk of progression with the combination (hazard ratio [HR], 0.54; 95% CI, 0.36 to 0.81). Risk of death was reduced by 55% with tamoxifen plus everolimus versus tamoxifen alone (HR, 0.45; 95% CI, 0.24 to 0.81).

The main toxicities associated with tamoxifen plus everolimus were fatigue (72% v 53% with tamoxifen alone), stomatitis (56% v 7%), rash (44% v 7%), anorexia (43% v 18%), and diarrhea (39% v 11%).

The BOLERO-2 study was a phase 3, randomized trial which compared everolimus and exemestane versus exemestane and placebo (randomly assigned in a 2:1 ratio) in 724 patients with hormone-receptor–positive advanced breast cancer who had recurrence or progression while receiving previous therapy with a nonsteroidal aromatase inhibitor. Progression free survival time was significantly increased with the addition of everolimus to exemestane. The median progression-free survival was 6.9 months with everolimus plus exemestane and 2.8 months with placebo plus exemestane, according to assessments by local investigators (hazard ratio for progression or death, 0.43; 95% confidence interval [CI], 0.35 to 0.54; P<0.001) [26]. Importantly, while there was no difference in quality of life during treatment in the two arms of the study, like the TAMRAD study, there was evidence for an increase in toxicity, including Grade 3/4 stomatitis, anemia, hyperglycemia, fatigue and pneumonitis.

In the only study evaluating mTOR inhibitors in patients with enodocrine sensitive metastatic breast cancer, the addition of temsirolimus to letrozole did not improve PFS [27].

1.3.6 Summary of available treatments for ER+ breast cancer that is AI refractory

In summary, for women with ER+ positive breast cancer, nonsteroidal AI's are the standard of care. However, in patients with disease refractory to non-steroidal AI's, the FDA approved treatments most commonly utilized include fulvestrant, exemestane, or exemestane + everoliumus. The time to progression for single agent tamoxifen in patients with AI refractory breast cancer is similar to exemestane or fulvestrant (range 4-7 months). Everolimus containing regimens result in the most impressive PFS rates (8-10 months); however, the toxicity of everolimus containing regimens (fatigue, stomatitis, pneumonitis, diarrhea, anorexia) are substantial for some patients, leading to treatment discontinuation in 9% of patients enrolled onto BOLERO-2 [28]. In all cases, these drugs simply prolong PFS and resistance develops in all cases. New strategies are therefore needed.

1.4 Preclinical Endoxifen antitumor activity

1.4.1 Activity in hormonally sensitive models

As summarized in Section 1.1, the pharmacology of tamoxifen is complex, and there are conflicting retrospective clinical studies regarding the importance of endoxifen Css in tamoxifen treated patients. Therefore, we sought to evaluate the antitumor activity of endoxifen in multiple different ER+ hormonally sensitive and resistant models to further elucidate and compare the activity and mechanism of action of endoxifen compared with tamoxifen.

First, we assessed the growth inhibition properties of TAM and endoxifen in BT474 cells (ER+/HER2+) and MCF-7 (parental and HER2-expressing). Compared to TAM, endoxifen potently inhibited the growth of estrogen- stimulated BT474 cells and MCF-7/HER2 cells however TAM activated tumor growth.

Multiple laboratories have demonstrated bi-directional cross-talk between the ER and EGFR/HER2 pathways in breast cancer [29-31]. MAPK, which has been activated through EGFR or HER2 signaling, can phosphorylate the ER leading to co-activator recruitment and increased ER transcriptional activity. Concomitantly, ER can induce expression of specific EGFR receptors and ligands, including amphiregulin, leading to activation of this signaling pathway [32]. Increased activity of the MAPK pathway has been demonstrated in long-term estrogen-deprived and estrogen non-responsive human breast cancer cell lines [33] as well as in ER positive cells that express HER2 [29]; and activation of this signaling pathway contributes to anti-estrogen resistance in MCF-7 breast cancer cells [34]. While the role of TAM in regard to the stimulation of the EGFR/HER2/MAPK pathway is well documented, there are no data in regard to endoxifen's effect on this pathway.

In MCF-7 cells (wild type and HER2+) as well as BT474 cells (ER+/HER2+), we examined the effect of endoxifen on EGFR/HER2/MAPK signaling. In MCF-7 wild type and HER2+, TAM (regardless of concentration) and 4-OH TAM [at concentrations (10 nM) observed in TAM treated humans], [35] activates p42/p44 MAPK. In contrast, endoxifen does not activate MAPK, with significantly lower levels of pMAPK.

We then compared TAM with endoxifen using an in vivo model previously used by Dr. Angela Brodie's laboratory to demonstrate the superiority of letrozole over TAM (MCF-7/AC-1) [36]. Tumors were stimulated with androstenedione, and once tumor size reached 300 mm3, mice were randomly assigned to receive once daily subcutaneous injections of vehicle, tamoxifen or letrozole (10 ug/day), or once daily oral gavage of low dose endoxifen (25 mg/kg) or high dose endoxifen (75 mg/kg) for the period indicated (Figure 1). At 4 weeks, both doses of endoxifen resulted in greater anti-tumor activity (in terms of percent change in tumor volume) than either control or TAM (all p values <0.008).

After 4 weeks, treatment on the TAM arm was discontinued; however, the letrozole and endoxifen arms continued. Notably, both doses of endoxifen were substantially superior to letrozole (out to 46 weeks). In the letrozole arm, resistance was observed at 24 weeks, characterized by increase in tumor volume by at least 300% from the starting volumes. At that point, mice were randomly assigned either tamoxifen or endoxifen. The median tumor volume (after 4 weeks of treatment and expressed as a % of its size prior to randomization) was significantly different comparing mice randomized to endoxifen (73.3%; range: 69.3 to 80.75%) versus tamoxifen (148.39%; range: 114.07 to 165.99%) (Wilcoxon rank sum test $p=0.016$).

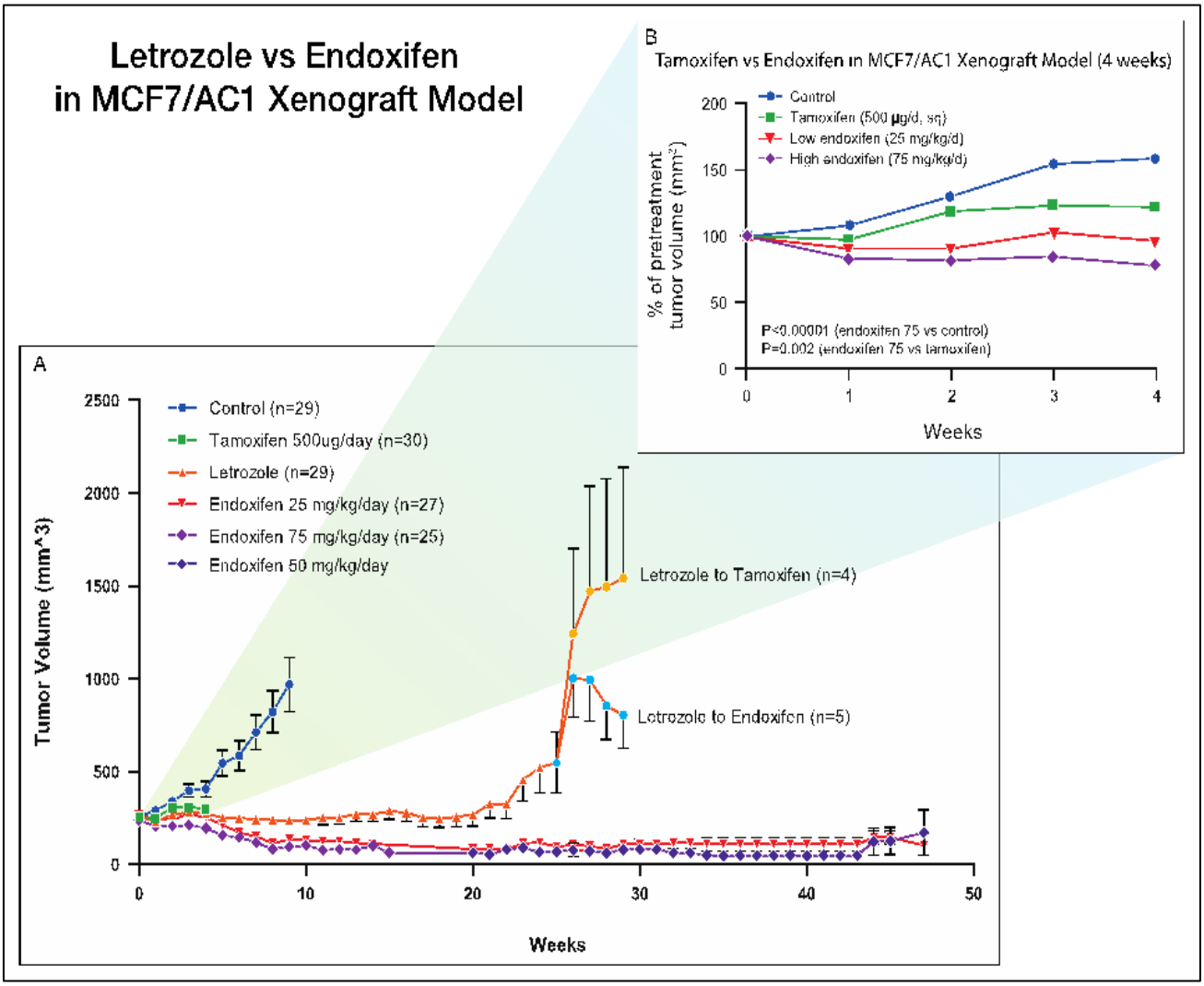


Fig 1. Athymic nude mice bearing established subcutaneous MCF7/AC1 xenografts were treated once daily with subcutaneous injections of vehicle, tamoxifen or letrozole (10 ug/day), or once daily oral gavage of low dose endoxifen (25 mg/kg) or high dose endoxifen (75 mg/kg) for the period indicated. B) Initial 4 week data comparing tamoxifen with either low or high dose endoxifen.

1.4.2 Antitumor activity of Endoxifen compared with Exemestane and Exemestane + Everolimus in a Letrozole resistant breast cancer model

Because a current standard of care is to utilize exemestane plus everolimus in AI resistant breast cancer, we sought to compare the activity of endoxifen with control (letrozole), exemestane alone, and exemestane + everolimus in an aromatase expressing MCF7 letrozole resistant breast cancer. MCF7/AC1 tumors were established were grown in setting of letrozole and androstenedione, and once tumor size reached 200 mm3, all mice continued androstenedione and were randomly assigned to 1) continue letrozole, or 2) exemestane, 3) exemestane + everolimus, or 4) endoxifen (50 mg/kg) (Figure 2).

After 23 weeks, endoxifen and exemestane + everolimus were superior to exemestane alone (endoxifen vs. exemestane p = 0.003; exemestane + everolimus vs. exemestane alone p = 0.13). Additionally, endoxifen tended to be superior to exemestane+everoliumus (p = 0.15).

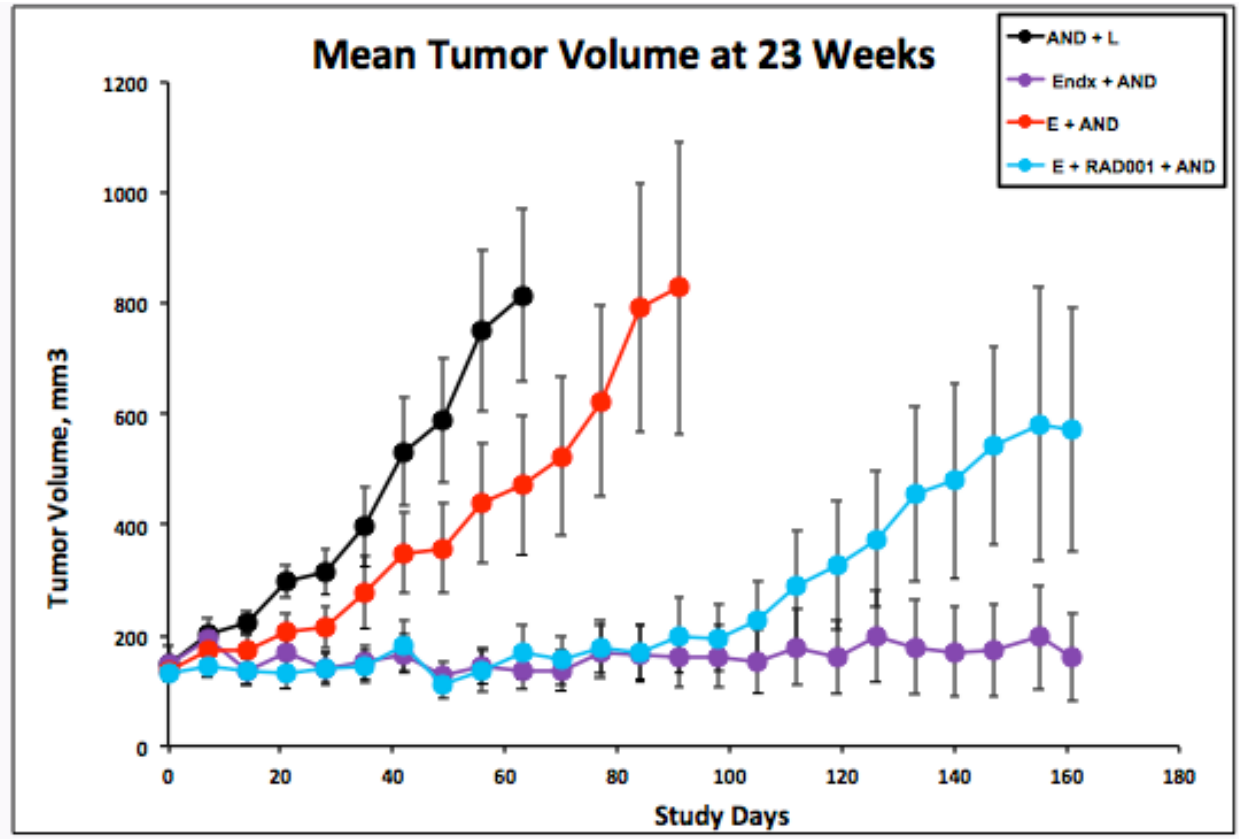


Figure 2. MCF7/AC1 Letrozole (L) -resistant xenografts were established in ovariectomized athymic nude mice by daily sc. injections of Androstenedione (AND) (100 ug/day) and L (10ug/day). Once tumors reached an average volume of ~150 mm^3 , all mice (n=12/group) continued to receive AND and were then randomized to one of the following: L (10 ug sc), Exemestane (E) (250 ug sc), E (250 ug sc) + RAD001 (2.5 mg/kg oral), or Endoxifen (50 mg/kg oral). Volumes of the xenografts were measured weekly with calipers. Mean tumor volumes and their standard error of the mean are shown for the 23 week study.

In summary, we have generated preclinical evidence to suggest that endoxifen is 1) superior to tamoxifen in both an letrozole sensitive and letrozole resistant model; 2) is significantly superior to exemestane in letrozole resistant MCF7 breast cancer 3) a trend towards greater antitumor activity as compared to exemestane + everolimus in letrozole refractory breast cancer.

1.4.3 Endoxifen inhibits PKCB1

In order to further understand how endoxifen's mechanisms of action may differ from TAM, conducted shape screening, pharmacophore modeling and ligand-protein docking (Anne Bode) comparing tamoxifen with endoxifen in order to identify oncogenic kinases Z-Endoxifen HCl may differentially regulate. We demonstrated that protein kinase C (PKC) resulted in a docking energy for Endoxifen as E(End-PROT) < ~-8 kcal/mol, but showed a remarkable increase for TAM as their energy difference ΔE(End/TAM-PROT) > ~2 kcal/mol. Base on this, we performed kinase enzyme assays (Reaction Biology), which demonstrated that endoxifen inhibited PKCβ1 (IC50 350 nM) while the IC50 for TAM was substantially higher (≥ 5 micromolar) (Goetz et al. data unpublished).

These data suggest that this unique non-genomic effect of endoxifen on PKCβ1 may impart and advantage for endoxifen over tamoxifen, especially in hormonally resistant breast cancer that exhibits activated growth factor signaling.

While the overall contribution of PKC to the activity of endoxifen is unknown, it should be noted that the endoxifen plasma concentrations achieved in the *in vivo* mouse studies, as well as in breast cancer patients on the Phase I trial, exceed the in vitro IC50 of endoxifen for this kinase.

1.4.4 The potential activity of endoxifen in endocrine resistant breast cancer harboring ER mutations

While protein levels of ER continue to be the most important factor determining response to endocrine therapy, an emerging area of research has demonstrated that genetic alterations in ESR1, either through mutations in the ligand binding domain, fusions, or the presence of ESR1 amplification, are associated with endocrine resistance. Additionally, a key oncogenic co-activator, SRC3 (AIB1), known to be associated with tamoxifen and AI resistance is more likely to avidly bind ER in these settings, leading to potential resistance and/or greater agnostic effects of SERMS. Therefore, new drug strategies are needed in patients with ESR1 alterations whose tumors continue to express the ER, but are resistant to standard hormonal therapies.

With regard to ESR1 mutations, recent data suggest that these alterations are rare in newly diagnosed breast cancer, but reported in up to 20% of recurrent breast cancers [12, 37]. These mutations lead to a conformational change in the LBD which mimics the conformation of activated ligand-bound receptor and constitutive, ligand-independent transcriptional activity resulting in and resistance to hormonal therapy. Preclinical studies suggest that cell lines that harbor some of these mutations, while insensitive to estrogen deprivation, retain sensitivity to higher doses of fulvestrant or SERMs (endoxifen and 4HT). However, the concentrations of SERMS used in the study by Robinson et al [12] (1 micromolar) are neither observed nor achievable in tamoxifen treated humans [5]. However, we have demonstrated that endoxifen concentrations known to be able to inhibit the growth of *ESR1* mutants in vivo (1 micromolar) were achievable in the endoxifen phase I study (range 300 nM-3000 nM). As a proof of principle, in the phase I endoxifen study, a patient with evidence for the *ESR1 D538G* activating mutation (obtained from liver biopsy) had evidence for a 50% reduction in target lesions in the liver, and was on study for 6 months. Notably, this patient had prior progression on an AI, chemotherapy, and Faslodex.

Additional data suggest that amplification of ESR1, observed more commonly in de novo resistant tamoxifen treated breast cancers and additionally observed in AI resistant breast cancers, may be an important marker of disease resistance. Finally, ESR1 fusions, as described by Li et al, appears additionally to confer resistance to hormonal therapy but are rare [38].

In our MCF7 AC1 letrozole resistant model, we have identified ESR1 amplification in the letrozole resistant MCF7/AC1 cells (but not parental). These findings are consistent with other groups that have demonstrated ESR1 amplification in MCF7 LTED cells [38]. Using the MCF7 letrozole resistant line, we identified that at 1 hour, TAM, 4-hydroxy TAM, and estrogen phosphorylated SRC3, with additional downstream activation of IGF1R, PDK1, and AKT, as well as ERK and AP1 (c-Jun, c-Fos). Conversely, Z-Endx reduced SRC3 phosphorylation as well as IGF1R, PDK1, AKT, c-Jun and c-Fos (data not shown).

In summary, these preclinical data suggest that Endx may be superior to tamoxifen, especially in tumors that contain ER alterations including *ESR1* amplification, as well as activating mutations associated with resistance to tamoxifen and AI's.

1.5 Summary of endoxifen toxicology studies (Endoxifen Investigator's Brochure)

Doses of ≤ 200 mg/kg/day were well-tolerated when given orally for four days to female Sprague-Dawley rats. Microscopic lesions in the liver (hepatocellular cytoplasmic vacuolization and glycogen depletion) were noted in almost all dose groups (2-200 mg/kg/day), and did not appear to be dose-related. Z-Endoxifen HCl given to rats via gavage once daily for 28 days resulted in lethality at doses ≥80 mg/kg/day (≥480 mg/m^2/day). The MTD was 20 mg/kg/day. Clinical signs of toxicity (abdominal distention, dyspnea, hunched posture, and discharge from the nose/mouth) occurred mainly in rats given ≥ 80 mg/kg/day. Changes in clinical pathology parameters were noted in ALT, AST, ALP, calcium, albumin, and total protein; decreased uterine weight also occurred. Target organs were the heart, kidney, liver, lung, GI tract, ovary, skeletal muscle, small and large intestine, adrenal gland, prostate, and lymphoid organs. Female reproductive tract (ovarian) toxicity was dose-limiting in rats. Skeletal muscle necrosis was also noted at the MTD, but was not dose-limiting. Liver, lung, heart, kidney, GI, skeletal muscle, and reproductive tract toxicity were dose-limiting at doses exceeding the MTD. With the exception of ovarian cysts and histiocytic infiltration of the lung, Z-Endoxifen HCl toxicity appeared to be reversible after a 28-day recovery period. A NOAEL was not established.

Z-Endoxifen HCl exhibited dose-limiting emesis in female dogs given daily oral doses of 30 mg/kg/day for four consecutive days for 28 days. Administration of Z-Endoxifen HCl orally to beagle dogs resulted in reproductive tract toxicity in males (atrophy of the prostate and testes, depletion of spermatozoa in the epididymides) and females (vaginal epithelium hyperplasia), and thymic atrophy in both males and females. Reproductive tract lesions were not reversible after a 28-day recovery period. A NOAEL was not established. The MTD of Z-Endoxifen HCl in rats and dogs dosed orally once daily for 28 days was 20 mg/kg/day and > 30 mg/kg/day, respectively. With respect to body surface area, the MTD in dogs (> 600 mg/m^2/day) was at least 5-fold greater than the rat MTD (120 mg/m^2/day). Peak plasma levels and the AUC were also higher in dogs than in rats (10-fold and 5-fold higher, respectively). The relative lack of toxicity at greater plasma levels in the dog compared to the rat may be due to a species difference in the metabolism of Z-Endoxifen HCl.

1.6 Status of NCI IND and Mayo Phase I protocol

The progress regarding the NCI program to develop Z-Endoxifen HCl is listed here. The Z-Endoxifen HCl (NSC #750393) IND was filed by NCI on November 9, 2010 and activated on December 9, 2010. The first patient enrolled onto Mayo's phase I protocol of endoxifen hydrochloride (NCI protocol 8821, MC093C) beginning in March 2011. MC093C is a phase I study evaluating escalating doses of Z-Endoxifen HCl in AI refractory patients. Women with metastatic breast cancer were required to progress on an AI and to have received at least one line of chemotherapy in the metastatic setting. Patients were enrolled to an accelerated titration schedule (2 pts/dose level) until moderate toxicity or DLT, and then to a 3+3 design. Z-Endoxifen HCl was administered orally once daily (28 day cycle). Dilated eye exams were performed at baseline, end of cycle 2, and after 6 cycles to evaluate for retinal toxicity. PK was performed on days 1-2, 3, 7, 14, and 28, and prior to subsequent cycles.

Summary of MC093C (NCT01327781) As of 8/28/2013, a total of 25 patients had been treated at 7 dose levels, including 3 patients at 20 mg (2 evaluable for toxicity), 2 at 40 mg, 8 at 60 mg (6 evaluable for toxicity), 3 at 80 mg, 3 at 100 mg, 3 at 120 mg and 3 at 160 mg.

Thus far, Cycle 1 dose limiting toxicity (pulmonary embolism) was observed in 1/6 patients at the 60 mg dose level, with no additional DLT at the higher dose levels. Dilated ophthalmologic examinations were performed at baseline and following completion of cycle 2 and at the completion of 6 cycles (for patients continuing on study). Thus far, no eye toxicity has been observed.

A similar phase I study is ongoing at NCI (NCT01273168) in patients with hormonally positive cancer.

According to the Z-Endoxifen HCl IB dated June, 2013, a summary of the adverse events in both studies are as follows: A total of 32 patients have been treated on the two DCTD-sponsored trials. Adverse events associated with the administration of Z-Endoxifen HCl in the trials have been mostly grades 1-2 hot flashes (50%), anemia (25%), fatigue (25%), lymphocyte count decreased (22%), nausea (19%), platelet count decreased (19%), hypertriglyceridemia (16%), irritability (16%), anorexia (13%), AST increased (13%), edema limbs (13%), and WBCs decreased (13%). Thromboembolic event (grades 3-4) and hypertriglyceridemia (grade 4) have also been reported.

An analysis of pharmacokinetic data from MC093C is listed on Table 1. This demonstrates the following:

a) Z-Endoxifen HCl Cmax and AUC increased in a dose-dependent manner.

b) Steady-state trough concentrations are reached by Day 7.

c) There is a 3-fold accumulation of drug over the 28-day cycle. The half-life is 45 hours based on accumulation.

d) A 20 and 100 mg/day dose yields Css of 0.39 and 2.48 uM, respectively.

Table 1

Dose (mg)	20		40		60		80		100		120		160	
Day	1	28	1	28	1	28	1	28	1	28	1	28	1	28
N	6	5	7	5	11	9	3	3	3	3	3	3	3	2
T_{max} (h)	4.0	1.8	3.4	2.6	5.2	5.4	4.0	3.0	19.5	2.2	10.0	2.7	3.7	6.0
C_{max} (ng/ml)	66.7	258	143	346	286	547	228	849	460	1280	420	1260	635	1950
C_{24h} (ng/ml)	34.1	146	67.3	248	119	379	138	602	194	926	357	813	333	1360
AUC_{0-24h} (mg/L*h)	1.09	4.22	2.00	6.42	3.74	9.43	3.73	15.4	5.97	26.9	7.04	20.2	9.81	38.0
Accumulation (AUC)		3.7		3.0		3.5		4.1		4.4		3.4		3.8
Half-life (h)		53.1		41.4		55.0		59.9		65.1		47.3		54.8

1.7 Antitumor Activity

Significant anti-tumor activity has been observed at multiple different dose levels.

At the 160 mg/day dose level, a patient who progressed on tamoxifen, anastrozole, faslodex, and exemestane + everolimus had a partial response (including greater than 50% reduction in hepatic metastatic disease). This progressed after 10 months of treatment.

At the 100 mg/day dose, a patient who had progressed on multiple different AI's and faslodex had a confirmed PR lasting 225 days

At the 80 mg/day, a patient who had progressed on both letrozole and fulvestrant had a non-confirmed PR with a PFS 169 days. A second patient treated at 80 mg/day had stable disease lasting > 270 days.

At 60 mg/day, a patient had stable disease lasting for >270 days.

At the 40 mg/day, a patient who had progressed on both letrozole and fulvestrant has a confirmed partial response and has now been progression free for > 480 days.

At the 40 mg/day dose level, a patient who had progressed on exemestane, tamoxifen, fulvestrant, and exemestane + everolimus who has exhibited stable disease for > 280 days.

In summary, Z-endoxifen HCl exhibits superior anti-tumor activity both *in vitro* and *in vivo* compared to TAM and exhibits substantial anti-tumor activity in an *in vivo* tumor bearing model resistant to letrozole. In this same model, we have demonstrated that endoxifen regulates substantially different set of genes than TAM, and have identified that endoxifen has differential effects on important oncogenic biomarkers already implicated in both tamoxifen and AI resistance including our preclinical modeling demonstrates that endoxifen exhibits differential effects on these biomarkers compared to tamoxifen, resulting in effects on downstream signaling (pAKT) (both in vitro and in vivo), With regard to bone effects, we have demonstrated substantial differences in bone remodeling between tamoxifen and Z-Endoxifen HCl.

Based on pharmacokinetics of endoxifen observed in the phase I study, and the preclinical data regarding the IC50 for endoxifen's inhibiton of PKCβ1 (350 nM), we will proceed with the 80 mg/day dose, which achieves Css concentrations of > 1uM, is associated with maximal inhibition of the ER, and associated with robust antitumor activity in the phase I study.

1.8 Rationale for the Required Pre-registration Core Biopsy

As part of A011203, a biopsy of metastatic disease is required prior to enrollment. This biopsy is a standard of care procedure to ascertain ER, PR, and HER2 status. The decision to make this biopsy a requirement prior to registration was based on several notable recent reports that demonstrate that: 1) ER and HER2 status changes over time (not just from adjuvant to metastatic, but over time in the metastatic setting); 2) change in ER and/or HER2 status affects prognosis and 3) patients proven to have either ER negative or HER2 positive breast cancer should not be treated with an endocrine agent, since alternative FDA approved regimens exist for these patients. For further explanation, please review the report by Lindstorm LS et al. in 2012 (http://www.ncbi.nlm.nih.gov/pubmed/?term=22711854).

1.9 Registration Quality of Life (QOL) Measurements

QOL measurements of fatigue and overall perception of QOL are routinely included in Alliance studies and will be assessed upon registration in this study. Evidence has arisen indicating that baseline single-item assessments of fatigue and overall QOL are strong prognostic indicators for survival in cancer patients, independent of performance status. This evidence was derived from two separate meta-analyses recently presented at ASCO, the first involving 23 NCCTG and Mayo Clinic Cancer Center oncology clinical trials, the second involving 43 clinical trials. Routine inclusion of these measures should be considered similar to that of including performance status, either as stratification or prognostic covariates [39] [40]. It will take approximately a minute to complete this measure.

2.0 OBJECTIVES

2.1 Primary Objective

2.1.1 To assess whether progression-free survival with Z-Endoxifen HCl relative to that with tamoxifen is prolonged in postmenopausal women with local advanced or metastatic ER positive/Her2 negative breast cancer.

2.2 Secondary Objectives

2.2.1 To assess the safety profile of each of these agents in this patient population.

2.2.2 To assess whether the tumor response rate (as determined using the RECIST criteria) among those randomized to Z-endoxifen HCl differs from that among those randomized to tamoxifen.

2.2.3 To estimate the median progression-free survival time for those who receive Z-Endoxifen HCl after disease progression with tamoxifen.

2.3 Correlative Science Objectives

2.3.1 To examine whether ERα alterations (defined as either ER activating mutations or ER amplification) are associated with longer PFS or higher response rates in the Z-Endoxifen HCl arm compared to the tamoxifen arm.

2.3.2 To determine changes in lipid profiles comparing tamoxifen and Z-endoxifen HCl.

2.3.3 For each treatment, to evaluate changes in markers of bone formation and absorption after 12 weeks (4 cycles) of treatment.

2.3.4 For all patients and by treatment arm, to determine whether progression-free survival differs with respect to the sensitive to endocrine therapy (SET) index.

2.3.5 To examine whether DNA alterations as measured by Foundation medicine in all coding exons of 287 cancer-related genes as well as 78 polymorphisms in 34 ADME-related genes are associated with longer PFS or higher response rates in the Z-Endoxifen HCl arm compared to the tamoxifen arm.

2.3.6 To assess whether the molecular characteristics identified in the tumor biopsies are detectable in the CTCs and/or cfDNA.

2.3.7 For each treatment arm: to examine changes in ER expression on CTCs, changes in ESR mutations or amplification is CTCs or CfDNA and explore the impact of these changes on PFS and response rates.

2.4 Pharmacokinetics and Pharmacogenomics Objectives

2.4.1 To further characterize pharmacokinetics, pharmacogenetics and metabolism of Z-Endoxifen HCl and tamoxifen.

2.4.2 To determine the impact of the concentrations of tamoxifen and its metabolites on PFS in the tamoxifen arm.

2.4.3 To determine the impact of the concentrations of Z-Endoxifen HCl and its metabolites on PFS in the endoxifen arm.

2.4.4 To determine the impact of genetic variation in the enzymes responsible for tamoxifen and Z-Endoxifen HCl metabolism.

3.0 PATIENT SELECTION

For questions regarding eligibility criteria, see the Contact Information page. Please note that the Study Chair cannot grant waivers to eligibility requirements.

3.1 On-study Guidelines

This clinical trial can fulfill its objectives only if patients appropriate for this trial are enrolled. All relevant medical and other considerations should be taken into account when deciding whether this protocol is appropriate for a particular patient. Physicians should consider the risks and benefits of any therapy, and therefore only enroll patients for whom this treatment is appropriate.

Physicians should consider whether any of the following may render the patient inappropriate for this protocol:

- Psychiatric illness which would prevent the patient from giving informed consent.
- Medical condition such as uncontrolled infection (including HIV), uncontrolled diabetes mellitus or cardiac disease which, in the opinion of the treating physician, would make this protocol unreasonably hazardous for the patient.
- Patients with a "currently active" second malignancy other than non-melanoma skin cancers. Patients are not considered to have a "currently active" malignancy if they have completed therapy and are free of disease for ≥ 3 years.

3.2 Pre-registration Eligibility Criteria

___ **3.2.1** Women who agree to undergo a standard of care core biopsy of recurrent or metastatic breast cancer (see Section 6.2.2 for details of biopsy) to confirm the ER+ (≥10% nuclear staining) and HER2 negative status (see Sections 3.3.5 and 3.3.6).

3.2.2 Patient must have been previously treated with an aromatase inhibitor (either letrozole, anastrozole or exemestane) either in the adjuvant or metastatic setting, and have one of the following types of primary or secondary endocrine resistant disease.

Primary Clinical Resistance is defined as one of the following:

___ • Recurrence within the first 2 years of adjuvant endocrine therapy while on aromatase inhibitor therapy

___ • Progression within first 6 months of initiating first-line endocrine therapy (either aromatase inhibitor or fulvestrant containing regimen) for the treatment of metastatic breast cancer.

Secondary Clinical Resistance is defined as one of the following:

___ • Recurrence after year 2 while receiving adjuvant aromatase inhibitor therapy, or within 12 months of completing adjuvant aromatase inhibitor therapy.

• Progression occurring 6 or more months after initiating the first endocrine therapy for metastatic disease (either fulvestrant or aromatase inhibitor containing regimen).

___ **3.2.3** Patients with a history of measurable disease as defined by RECIST criteria (See Section 11.0) or bone only disease are eligible. Note: Those patients with non-measurable disease and bone metastases are eligible.

___ **3.2.4** No history of tumors involving spinal cord or heart.

___ **3.2.5** No current evidence of visceral crisis or lymphangitic spread.

___ **3.2.6** No known brain metastases.

___ **3.2.7** Women age ≥ 18 years.

___ **3.2.8 Women must be postmenopausal**

Postmenopausal status is verified by:

___ • Prior bilateral surgical oophorectomy, or

___ • Age ≥ 60 years, or

___ • Age < 60 with no menses for > 1 year with FSH and estradiol levels within post menopausal range, according to institutional standard.

___ **3.2.9 Prior treatment**

- No more than two prior chemotherapy regimens in the metastatic setting.
- Prior treatment with an aromatase inhibitor (either anastrozole, letrozole or exemestane), either in the adjuvant or metastatic setting is required.
- Unlimited prior endocrine regimens in the metastatic setting, which may have included everolimus or a CDK4/6 inhibitor (such as a palbociclib, abemaciclib or ribociclib) containing regimens.
- Prior tamoxifen treatment is allowed in the adjuvant setting, but patients must not have experienced relapse within 1 year of stopping tamoxifen.
- No prior treatment with tamoxifen in the metastatic setting.
- No prior treatment with endoxifen.
- Patients who have not fully recovered from acute, reversible effects of prior therapy regardless of interval since last treatment are not eligible to participate in this study. **EXCEPTION:** Neuropathies – if grade 2 neuropathies have been stable for at least 3 months since completion of prior treatment patient is eligible.

___ **3.2.10** Not receiving any medications or substances that are strong inhibitors of CYP2D6 (see Appendix II).

___ **3.2.11** Not receiving any other investigational agents.

___ **3.2.12 No uncontrolled intercurrent illness including, but not limited to:**

- Ongoing or active infection
- Symptomatic congestive heart failure
- Unstable angina pectoris
- Uncontrolled symptomatic cardiac arrhythmia
- Uncontrolled hypertension (defined as blood pressure > 160/90)

___ **3.2.13 None of the following co-morbid conditions:**

- Cataracts of grade 2 or greater as per CTCAE Version 4.0
- Retinopathy of grade 2 or greater as per CTCAE Version 4.0

Note: Patients that have cataracts that do not require surgery are eligible.

Note: Serious adverse events will be reported on CTEP-AERS using CTCAE v5.0.

- DVT/PE within the past 6 months

Note: Patients that are on anticoagulant therapy for maintenance are eligible as long as the DVT and /or PE occurred > 6 months prior to enrollment, and there is no evidence for active thrombosis (either DVT or PE).

___ **3.2.14** No other active second malignancy other than non-melanoma skin cancers within 3 years of pre-registration. A second malignancy is not considered active if all treatment for that malignancy is completed and the patient has been disease-free for at least 3 years prior to pre-registration.

___ **3.2.15 ECOG Performance Status: 0-2**

___ **3.2.16** Able to swallow oral formulation of the study agent.

___ **3.2.17 Required Initial Laboratory Values:**

Hemoglobin	$\geq$ 9 g/dL
Platelet Count	$\geq$ 75,000/mm^3
Creatinine	$\leq$ 1.5 x upper limits of normal ULN
Total Bilirubin	$\leq$ 1.5 x upper limits of normal (ULN)
AST	$\leq$ 2.5 x upper limits of normal (ULN)
	For patients with liver metastasis: $\leq$ 5 x upper limits of normal (ULN)

3.3 Registration Eligibility Criteria

Registration must be completed within 28 days of Pre-registration

___ **3.3.1** Patients with either measurable disease as defined by RECIST criteria (See Section 11.0) or bone only disease are eligible. Note: Those patients with both non-measurable disease and bone metastases are eligible.

- Non-measurable bone only disease: Non-measurable bone only disease may include any of the following: blastic bone lesions, lytic bone lesions without a measurable soft-tissue component, or mixed lytic-blastic bone lesions without a measurable soft-tissue component.
- Lytic bone lesions, with an identifiable soft tissue component, evaluated by CT or MRI, can be considered as measurable lesions if the soft tissue component otherwise meets the definition of measurability previously described.

___ **3.3.2** No tumors involving spinal cord or heart.

___ **3.3.3** No visceral crisis, lymphangitic spread or known brain metastases: Visceral crisis is not the mere presence of visceral metastases, but implies severe organ dysfunction as assessed by symptoms and signs, laboratory studies, and rapid progression of disease.

___ **3.3.4** Histologic confirmation, from the A011203 Pre-registration biopsy, by institutional/local pathologist of either locally advanced or metastatic breast cancer that is estrogen receptor positive and HER2 negative.

Those patients with bone only disease with either no tumor or insufficient tumor for ER/PR and HER2 staining after the bone biopsy are still eligible to participate in this study.

___ **3.3.5** Estrogen receptor positive disease is defined as > 10% nuclear staining.

3.3.6 HER2 Negative Disease as per 2013 ASCO/CAP guidelines, one of the following must apply:

___ 1) 0 or 1+ by IHC and not amplified by ISH

___ 2) 0 or 1+ by IHC and ISH not done

___ 3) 2+ by IHC and not amplified by ISH or

___ 4) IHC not done and not amplified by ISH

___ **3.3.7 None of the following therapies are allowed prior to registration*:**

- Chemotherapy ≤ 2 weeks
- Immunotherapy ≤ 2 weeks
- Biologic therapy ≤ 2 weeks
- Hormonal therapy ≤ 2weeks
- Monoclonal antibodies ≤ 2 weeks
- Radiation therapy ≤ 2 weeks
- Anti-Her-2 or other "targeted" (e.g. mTOR) therapy ≤ 2 weeks

*Note: Any toxicities derived from these therapies must be ≤ grade 2 prior to starting study therapy.

4.0 PATIENT PRE-REGISTRATION, REGISTRATION/RANDOMIZATION AND STRATIFICATION

4.1 CTEP Investigator Registration Procedures

Food and Drug Administration (FDA) regulations and National Cancer Institute (NCI) policy require all investigators participating in any NCI-sponsored clinical trial to register and to renew their registration annually.

Registration requires the submission of:

- a completed Statement of Investigator Form (FDA Form 1572) with an original signature
- a current Curriculum Vitae (CV)
- a completed and signed Supplemental Investigator Data Form (IDF)
- a completed Financial Disclosure Form (FDF) with an original signature

Fillable PDF forms and additional information can be found on the CTEP website at <http://ctep.cancer.gov/investigatorResources/investigator_registration.htm>. For questions, please contact the CTEP Investigator Registration Help Desk by email at <pmbregpend@ctep.nci.nih.gov>.

4.2 CTEP Associate Registration Procedures / CTEP-IAM Account

The Cancer Therapy Evaluation Program (CTEP) Identity and Access Management (IAM) application is a web-based application intended for use by both Investigators (i.e., all physicians involved in the conduct of NCI-sponsored clinical trials) and Associates (i.e., all staff involved in the conduct of NCI-sponsored clinical trials).

Associates will use the CTEP-IAM application to register (both initial registration and annual re-registration) with CTEP and to obtain a user account.

Investigators will use the CTEP-IAM application to obtain a user account only. (See CTEP Investigator Registration Procedures above for information on registering with CTEP as an Investigator, which must be completed before a CTEP-IAM account can be requested.)

An active CTEP-IAM user account will be needed to access all CTEP and CTSU (Cancer Trials Support Unit) websites and applications, including the CTSU members' website.

Additional information can be found on the CTEP website at <http://ctep.cancer.gov/branches/pmb/associate_registration.htm>. For questions, please contact the ***CTEP Associate Registration Help Desk*** by email at <ctepreghelp@ctep.nci.nih.gov>.

4.3 CTSU Site Registration Procedures

This study is supported by the NCI Cancer Trials Support Unit (CTSU).

IRB Approval:

Each investigator or group of investigators at a clinical site must obtain IRB approval for this protocol and submit IRB approval and supporting documentation to the CTSU Regulatory Office before they can be approved to enroll patients. Assignment of site registration status in the CTSU Regulatory Support System (RSS) uses extensive data to make a determination of whether a site has fulfilled all regulatory criteria including but not limited to: an active Federal Wide Assurance (FWA) number, an active roster affiliation with the Lead Network or a participating organization, a valid IRB approval, and compliance with all protocol specific requirements.

Sites participating on the NCI CIRB initiative that are approved by the CIRB for this study are not required to submit IRB approval documentation to the CTSU Regulatory Office. For sites using the CIRB, IRB approval information is received from the CIRB and applied to the RSS in an automated process. Signatory Institutions must submit a Study Specific Worksheet for Local Context (SSW) to the CIRB via IRBManager to indicate their intent to open the study locally. The CIRB's approval of the SSW is then communicated to the CTSU Regulatory Office. In order for the SSW approval to be processed, the Signatory Institution must inform the CTSU which CIRB-approved institutions aligned with the Signatory Institution are participating in the study.

4.3.1 Downloading Site Registration Documents

Site registration forms may be downloaded from the A011203 protocol page located on the CTSU members' website. Permission to view and download this protocol and its supporting documents is restricted and is based on person and site roster assignment housed in the CTSU RSS.

- Go to https://www.ctsu.org and log in to the members' area using your CTEP-IAM username and password
- Click on the Protocols tab in the upper left of your screen
- Either enter the protocol # in the search field at the top of the protocol tree, or
- Click on the By Lead Organization folder to expand
- Click on the NCTN - Alliance link to expand, then select trial protocol #A021202
- Click on the Site Registration Documents link
- Click on LPO Documents, select the Site Registration documents link, and download and complete the forms provided

4.3.2 Requirements for A011203 Site Registration

- CTSU Transmittal Sheet (optional)
- IRB approval (For sites not participating via the NCI CIRB; local IRB documentation, an IRB-signed CTSU IRB Certification Form, Protocol of Human Subjects Assurance Identification/IRB Certification/Declaration of Exemption Form, or combination is accepted)

4.3.3 Submitting Regulatory Documents

Submit required forms and documents to the CTSU Regulatory Office, where they will be entered and tracked in the CTSU RSS.

ONLINE: www.ctsu.org (members' section) Regulatory Submission Portal

EMAIL: CTSURegulatory@ctsu.coccg.org (for regulatory document submission only)

FAX: 215-569-0206

MAIL: CTSU Regulatory Office
1818 Market Street, Suite 1100
Philadelphia, PA 19103

4.3.4 Checking Your Site's Registration Status

You can verify your site registration status on the members' section of the CTSU website.

- Go to https://www.ctsu.org and log in to the members' area using your CTEP-IAM username and password
- Click on the Regulatory tab at the top of your screen
- Click on the Site Registration tab
- Enter your 5-character CTEP Institution Code and click on Go

Note: The status given only reflects compliance with IRB documentation and institutional compliance with protocol-specific requirements as outlined by the Lead Network. It does not reflect compliance with protocol requirements for individuals participating on the protocol or the enrolling investigator's status with the NCI or their affiliated networks.

4.4 Pre-registration Requirements

- Patient pre-registration can occur only after screening evaluation is complete, pre-registration eligibility criteria have been met, and the study site is listed as site is listed as "approved" in the CTSU RSS.
- **Informed consent:** the patient must be aware of the neoplastic nature of his/her disease and willingly consent after being informed of the procedure to be followed, the experimental nature of the therapy, alternatives, potential benefits, side-effects, risks, and discomforts. Current human protection committee approval of this protocol and a consent form is required prior to patient consent and pre-registration.

4.5 Patient Pre-Registration Procedures

Patient enrollment will be facilitated using the Oncology Patient Enrollment Network (OPEN). OPEN is a web-based registration system available on a 24/7 basis. To access OPEN, the site user must have an active CTEP-IAM account (check at < https://eapps-ctep.nci.nih.gov/iam/index.jsp>) and a 'Registrar' role on either the LPO or participating organization roster.

All site staff will use OPEN to enroll patients to this study. It is integrated with the CTSU Enterprise System for regulatory and roster data and, upon enrollment, initializes the patient in the Rave database. OPEN can be accessed at http://open.ctsu.org or from the OPEN tab on the CTSU members' side of the website at https://www.ctsu.org. A user manual is available for OPEN users on the CTSU site.

Following the Pre-registration, and prior to registration/randomization (days -28 to -1 see Section 5.1 and 5.2), a standard of care biopsy of metastatic breast tissue must be taken for local pathologic review. Of note, if the patient has had a recent biopsy (within 28 days prior to pre-registration) of metastatic breast tissue after the most recent progression, this sample can substituted for local pathological review for determination of ER, PR and HER2 status.

For patients who have consented to the correlative tissue studies, additional tissue cores will be obtained at the time of the pre-registration biopsy (see Sections 6.2.2 and 6.2.3).

If the local pathology assessment finds that the patient **does not** have ER positive, HER2 negative disease, the CRA will enter the Alliance ID number obtained at pre-registration; upload a copy of the local pathology report, de-identified (but including the patient's Alliance ID number) into RAVE for documentation of estrogen receptor and HER2 status; and complete the end of pre-registration phase case report form. The patient will not proceed to registration or randomization. No further follow-up is required. Research tissue cores need not be submitted. Future treatment is at the discretion of the patient's medical team.

4.6 Patient Registration/Randomization procedure

If the local pathology assessment finds that the patient **does** have ER positive, HER2 negative disease, CRA will enter the Alliance ID number obtained at pre-registration and the stratification factors into the OPEN registration system and obtain a treatment assignment. The CRA will also enter the Alliance ID number obtained at pre-registration; upload a copy of the local pathology report, de-identified (but including the patient's Alliance ID number), into RAVE for documentation of estrogen receptor and HER2 status; complete the end of pre-registration phase case report forms as well as the baseline patient status case report forms and submit the research tissue cores.

4.7 Registration to Correlative and Companion Studies

4.7.1 Registration to Substudies described in Section 14.0

There are four substudies within Alliance A011203. These correlative science studies **must be offered to all patients** enrolled on Alliance A011203 (although patients may opt to not participate). These substudies do not require separate IRB approval. The substudies included within Alliance A011203 are:

- Evaluation of *ESR1* alterations and markers of endocrine resistance in patients treated with either tamoxifen or Z-Endoxifen HCl Alliance A011203-ST1, (Section 14.1)
- Circulating tumor cells and circulating cell free DNA, A011203-ST2 (Section 14.2)
- Biochemical markers of bone turnover, Alliance A011203-ST3 (Section 14.3)
- Z-Endoxifen HCl and tamoxifen pharmacokinetics and pharmacogenetics, Alliance A011203-PP1 (Section 14.4)

If a patient answers "yes" to "Question #1" in the model consent, they have consented to participate in the substudy described in Section 14.1, the patient should be registered to Alliance A011203-ST1. If a patient answers "yes" to "Question #2" in the model consent, they have consented to participate in the substudy described in Sections 14.2, 14.3 and 14.4, and the patient should be registered to Alliance A011203-ST2, A011203 ST3 and A011203-PP1. Patients should be registered to the correlative studies at the same time they are registered to the treatment trial (A011203).

4.8 Stratification Factors and Treatment Assignments

Endocrine resistant disease

1) Primary endocrine resistant disease*
2) Secondary endocrine resistant disease**

Prior CDK 4/6 inhibitor and/or everolimus

1) Prior CDK 4/6 inhibitor or everolimus or both
2) No prior CDK 4/6 inhibitor and no prior everolimus

Disease type

1) Measureable by RECIST criteria
2) Bone only disease

***Primary endocrine resistant disease is defined as one of the following:**

- Recurrence within the first 2 years of adjuvant endocrine therapy while on aromatase inhibitor therapy
- Progression within the first 6 months of initiating first-line endocrine therapy (either aromatase inhibitor or fulvestrant containing regimen) for the treatment of metastatic breast cancer.

****Secondary Clinical Resistance is defined as one of the following:**

- Recurrence after year 2 while receiving adjuvant aromatase inhibitor therapy, or within 12 months of completing adjuvant aromatase inhibitor therapy.
- Progression occurring 6 or more months after initiating the first endocrine therapy for metastatic disease (either fulvestrant or aromatase inhibitor containing regimens).

4.9 Re-registration at the Time of Progression

Patients who meet the RECIST criteria for disease progression during tamoxifen treatment may switch to Z-Endoxifen HCl until disease progression is documented. Please note that patients who opt to cross over must be re-registered to the study within 14 days of progression documentation.

Re-registration procedures:

OPEN may be accessed at https://open.ctsu.org, from the OPEN tab on the CTSU website at https://www.ctsu.org.

To enroll a patient within OPEN, institution staff must have:

1. A valid and active CTEP-IAM account. This is the same user ID and password used for CTSU's website (for more information see https://www.ctsu.org/public/CTEP-IAM_Factsheet.pdf).

2. Enrollment of patients on Alliance A011203 coordinated protocols requires a "Registrar" role in the ALLIANCE roster. Assignment of the "Registrar" role is managed through the Alliance Central Office.

The OPEN system will provide the registering site with a printable confirmation of re-registration. Please print the confirmation for your records. Further instructional information is provided on the CTSU members' website OPEN tab, or within the OPEN URL. For any additional questions, contact the CTSU Help Desk at 1-888-823-5923, or ctsucontact@westat.com.

5.0 STUDY CALENDAR

Pre-Study Testing Intervals for all patients

- To be completed ≤ 14 DAYS before Pre-registration: All laboratory studies, history and physical.
- To be completed ≤ 28 DAYS before registration: Any imaging utilized for tumor measurement per protocol.

5.1 Study Arm 1 Z-Endoxifen HCl

	Prior to pre-registration	Pre-registration Period (any time during days -28 to -1)	Days 1 of each treatment cycle*	At PD or discontinuation of protocol therapy**
Tests & Observations				
History and physical, weight, PS	X		X	X
Height	X			
Pulse, Blood Pressure	X		X	X
Adverse Event Assessment			X	X
Patient Medication Log			X(1)	X(1)
Registration Fatigue/Uniscale Assessment		A		
Standard of care biopsy of either locally recurrent or metastatic breast cancer is required for determination of ER, PR and HER2		B		
Laboratory Studies				
AST, creatinine, Total Bilirubin	X	X	X	X
Complete Blood Count, Differential, Platelets	X	X	X	X
Albumin, Alk Phos, ALT, calcium, glucose, phosphorous, sodium, potassium,		X	X	X
Fasting lipid profile (total Cholesterol, LDL, HDL, triglycerides)		C	C	
Imaging Tumor Measurements/Evaluation of Indicator Lesions				
CT/MRI		D, E, F	D,	D, E
Bone Scan/PET-CT scan		D, E, F	E	E
Correlative Studies: For Patients who consent to participate				
Tissue and Blood samples	See Sections 6.0 and 14.0.			

* Cycle length is 21 days. Labs completed prior to registration may be used for day 1 of cycle one tests if obtained ≤ 21 days prior to treatment. For subsequent cycles, labs, scans, tests and observations may be obtained no more than +/- 3 days prior to day 1 of treatment.

** After treatment discontinuation, patients will be followed yearly for a maximum of 5 years from study registration/randomization (see Section 12.3).

1. Medication Log must begin on the day the patient begins study medication and must be completed per protocol until treatment discontinuation (see Section 7.4).

A. To be completed after Pre-registration and prior to treatment, and will take approximately a minute to complete (see Section 1.8 and Appendix I).

B. A standard of care biopsy of locally recurrent or metastatic breast cancer during the pre-registration period is required to confirm ER, PR and HER2 status by an institutional/local pathologist and for submission of a research block for optional correlative studies. **However,** if a prior biopsy was performed on a locally recurrent/metastatic site within 90 days of pre-registration, and no anti-cancer therapy was administered during that time period, that specimen can be used for confirmation of ER/PR/HER2 and for submission of tissue for optional correlative studies.

C. Lipid profile is required at Pre-registration (baseline), cycles 2, 4 and 8 and thereafter at the discretion of the treating physician.

D. Acceptable imaging modalities for measurable disease include: a CT, spiral CT, MRI, and chest x-ray. A FDG-PET scanning is allowed to complement CT scanning in assessment of progressive disease.

E. Tumor measurements are required during the pre-registration period (days -28 to -1), at completion of cycles 2, 4, and 6 (approximately, every 6 weeks) then every 4 cycles (approximately every 3 months) thereafter until disease progression, as determined in Section 11.0. The same imaging method utilized at baseline must be used at all disease assessments and must include all target and non-target lesions recorded at baseline.

   For patients without bone metastases, bone scans after the baseline bone scan are at the discretion of the treating physician. See Section 11.0 for acceptable modalities for tumor imaging.

   For patients with non-measurable bone only disease, follow-up imaging either with PET/CT or bone scan should be performed at the time of tumor assessment. The same imaging method utilized at baseline must be used at all tumor assessments.

F. Need not be repeated if performed before pre-registration period, but not more than 28 days prior to registration.

5.2 Study Arm 2 Tamoxifen

	Prior to pre-registration	Pre-registration Period (any time during days -28 to -1)	Tamoxifen treatment phase		Z-Endoxifen HCl Crossover phase	
			Days 1 of each treatment cycle	**At PD or or discontin-uation of tamoxifen ****	**Day 1 of each treatment cycle**	**At PD, or discontinuation of Z-Endoxifen HCl ****
Tests & Observations						
History and physical, weight, PS	X		X	X	X	X
Height	X					
Pulse, Blood Pressure	X		X	X	X	X
Adverse Event Assessment			X	X	X	X
Patient Medication Log			X(1)	X(1)	X(1)	X(1)
Registration Fatigue/Uniscale Assessment		A				
Standard of care biopsy of either locally recurrent or metastatic breast cancer for determination of ER, PR and HER2 in metastatic disease specimen		B				
Laboratory Studies						
AST, creatinine, Total Bilirubin	X	X	X	X	X(2)	X, G
Complete Blood Count, Differential, Platelets	X	X	X	X	X(2)	X, G
Albumin, Alk Phos, ALT, calcium, glucose, phosphorous, sodium, potassium		X	X	X	X(2)	X, G
Fasting lipid profile (total Cholesterol, LDL, HDL, triglycerides)		C	C		C	
Imaging Tumor Measurements/Evaluation of Indicator Lesions						
CT/MRI		D, E, F	D, E		D, E(3)	D, E, G
Bone Scan/PET-CT scan		D, E, F	E		E(3)	E, G
Correlative Studies: For Patients who consent to participate						
Tissue and Blood samples	See Sections 6.0 and 14.0.					

* Cycle length is 21 days. Labs completed prior to registration may be used for day 1 of cycle one tests if obtained ≤ 21 days prior to treatment. For subsequent cycles, labs, scans, tests and observations may be obtained no more than +/- 3 days prior to day 1 of treatment.

** Patients who progress on tamoxifen may crossover to Z-Endoxifen HCl treatment. Patients will be followed for disease outcome during Z-Endoxifen HCl treatment phase where the baseline tumor measurement is considered to be the tumor size at disease progression on tamoxifen. Following the discontinuation of Z-Endoxifen HCl, patients will be followed yearly for a maximum of 5 years from randomization. Future treatment is at the discretion of patient's physician.

Patients who choose not to crossover to Z-Endoxifen HCl will be followed yearly for a maximum of 5 years from randomization. Future treatment is at the discretion of patient's physician (see Section 12.3).

1. Medication Log must begin on the day the patient begins study medication and must be completed per protocol (see Section 7.4).
2. Labs completed prior to crossover may be used for day 1 of cycle one tests if obtained ≤ 21 days prior to start of treatment.
3. Scans are not required day 1 of cycle 1 of Z-Endoxifen HCl. The scans taken at the time of progression on tamoxifen will serve as the baseline scan for the cross-over phase of the study.

A. To be completed after Pre-registration and prior to treatment, and will take approximately a minute to complete (see Section 1.8 and Appendix I).

B. A standard of care biopsy of locally recurrent or metastatic breast cancer site during the pre-registration period is required to confirm ER, PR and HER2 status by an institutional/local pathologist and for submission of a research block for optional correlative studies. However, if a prior biopsy was performed on a locally recurrent/metastatic site within 90 days of pre-registration, and no anti-cancer therapy was administered during that time period, that specimen can be used for confirmation of ER/PR/HER2 and for submission of tissue for optional correlative studies.

C. Lipid profile is required at Pre-registration (baseline), cycles 2, 4 and 8 and thereafter at the discretion of the treating physician.

D. Acceptable imaging modalities for measurable disease include: a CT, spiral CT, MRI, and chest x-ray. A FDG-PET scanning is allowed to complement CT scanning in assessment of progressive disease.

E. Tumor measurements are required during the pre-registration period (days -28 to -1), at completion of cycles 2, 4, and 6 (approximately, every 6 weeks) then every 4 cycles (approximately every 3 months) thereafter until disease progression, as determined in Section 11.0. The same imaging method utilized at baseline must be used at all disease assessments and must include all target and non-target lesions recorded at baseline.

For patients without bone metastases, bone scans after the baseline bone scan are at the discretion of the treating physician. See Section 11.0 for acceptable modalities for tumor imaging.

For patients with non-measurable bone only disease, follow-up imaging either with PET/CT or bone scan should be performed at the time of tumor assessment. The same imaging method utilized at baseline must be used at all tumor assessments.

F. Need not be repeated if performed before pre-registration period, but not more than 28 days prior to registration.

G. Baseline imaging tumor measurements and laboratory studies for patients crossing over to the Z-Endoxifen HCl phase, are those taken at the time of disease progression on the Tamoxifen treatment arm. Z-Endoxifen HCl treatment should start no more than 28 days after documentation of disease progression.

6.0 DATA AND SPECIMEN SUBMISSION

6.1 Data collection and submission

Data collection for this study will be done exclusively through the Medidata Rave clinical data management system. Access to the trial in Rave is granted through the iMedidata application to all persons with the appropriate roles assigned in Regulatory Support System (RSS). To access Rave via iMedidata, the site user must have an active CTEP-IAM account (check at https://eapps-ctep.nci.nih.gov/iam/index.jsp) and the appropriate Rave role (Rave CRA, Read-Only, Site Investigator) on either the LPO or participating organization roster at the enrolling site.

Upon initial site registration approval for the study in RSS, all persons with Rave roles assigned on the appropriate roster will be sent a study invitation e-mail from iMedidata. To accept the invitation, site users must log into the Select Login (https://login.imedidata.com/selectlogin) using their CTEP-IAM user name and password, and click on the "accept" link in the upper right-corner of the iMedidata page. Please note, site users will not be able to access the study in Rave until all required Medidata and study specific trainings are completed. Trainings will be in the form of electronic learnings (eLearnings), and can be accessed by clicking on the link in the upper right pane of the iMedidata screen.

Users who have not previously activated their iMedidata/Rave account at the time of initial site registration approval for the study in RSS will also receive a separate invitation from iMedidata to activate their account. Account activation instructions are located on the CTSU website, Rave tab under the Rave resource materials (Medidata Account Activation and Study Invitation Acceptance). Additional information on iMedidata/Rave is available on the CTSU members' website under the Rave tab at www.ctsu.org/RAVE/ or by contacting the CTSU Help Desk at 1-888-823-5923 or by e-mail at ctsucontact@westat.com.

A Schedule of Forms is available on the Alliance study webpage, within the Case Report Forms section. The Schedule of Forms is also available on the CTSU site within the study-specific Education and Promotion folder, and is named Time & Events.

6.2 Specimen collection and submission Table 1

	Post pre-registration and ≤ 14 prior to registration	Cycle 1, Day 1 prior to start of treatment (Baseline)	At the end of Cycle 2 (prior to start of Cycle 3)	At the end of Cycle 8 (prior to start of Cycle 9)	At time of disease progression	Submit to:
		Standard of care from local, regional or distant site: core biopsies of breast cancer tissue or lymph nodes, or punch biopsy of skin/chest wall, liver , lung or bone for ER, PR and HER2 status **Mandatory for all patients at Pre-registered to A011203:**				
Core biopsies of breast cancer tissue (See Section 6.2.2)	Formalin fixed paraffin embedded block **A**					Local/ institutional pathology laboratory. upload report into RAVE with 1st data submission.
		A011203-ST1				
Core biopsies of breast cancer tissue (See Section 6.2.3	Formalin fixed paraffin embedded block **X(1)**					OSU
		A011203-ST2				
CTC (See Section 6.2.4 **Kit 1**		Two 10 mL whole blood samples in K2-EDTA tubes (provided in ST-2 kit)	Two 10 mL whole blood samples in K2-EDTA tubes (provided in CTC kit)	Two 10 mL whole blood samples in K2-EDTA tubes (provided in ST-2 kit)	Two 10 mL whole blood samples in K2-EDTA tubes (provided in ST-2 kit)	BAP freezer Mayo Clinic
cfDNA (See Section 6.2.4) **Kit 1**		Two 10 mL whole blood samples in Streck Cell Free DNA BCT tubes (provided in ST-2kit)	Two 10 mL whole blood samples in Streck Cell Free DNA BCT tubes (provided in ST-2 kit)	Two 10 mL whole blood samples in Streck Cell Free DNA BCT tubes (provided in ST-2 kit)	Two 10 mL whole blood samples in Streck Cell Free DNA BCT tubes (provided in ST2 kit)	BAP freezer Mayo Clinic
		A011203-ST3				
Bone turnover (FASTING) (See Section 6.2.5)		One 10 mL whole blood sample in Non-additive red top tube	One 10 mL whole blood sample in Non-additive red top tube	One 10 mL whole blood sample in Non-additive red top tube	One 10 mL whole blood sample in Non-additive red top tube	OSU
		A011203-PP1				
PG (See Section 6.2.6)		One 10 mL whole blood sample in EDTA (purple top*)				OSU

A Core biopsy (1-2 cores) to be used for local clinical assessment of ER, PR and HER2 status, as described in Section 6.2.2.

1) Research Cores (2-3 cores) to be used for biomarker analyses described in Section 6.2.2. These cores will be collected at the same time as the clinical cores. For those patients who consented to the correlative studies, research biospecimens are not to be submitted until the patient eligibility has been confirmed and they have been registered and randomized.

* The pharmacogenetic sample may be drawn at any after Pre-registration.

Table 2

	Cycle 1, Day 1 prior to start of treatment (Baseline)	**Cycle 1, Day 1: 2-4 hours post drug administration**	**Cycle 1, Day 1: 4-6 hours post drug administration***	**Cycle 1, Day 2 Prior to drug administration**	**Submit to:**
PK (see Section 6.2.7) **Kit 2**	One 6 mL whole blood sample in EDTA (purple top) tube	One 6 mL whole blood sample in EDTA (purple top) tube	One 6 mL whole blood sample in EDTA (purple top) tube	One 6 mL whole blood sample in EDTA (purple top) tube	BAP Freezer at Mayo Clinic

PK (see Section 6.2.7 **Kit 2**	**End of Cycle 2, (prior to the start of Cycle 3)**	**End of Cycle 8, (prior to the start of Cycle 9)**	**At the time of disease progression**	**Submit to:**
	One 6 mL whole blood sample in EDTA (purple top) tube	One 6 mL whole blood sample in EDTA (purple top) tube	One 6 mL whole blood sample in EDTA (purple top) tube	BAP Freezer at Mayo Clinic

* Do not draw samples within two hours of each other.

** 1) Patient must record the time of day that the dose was taken on the previous day and bring their A011203 Study Medication Log to this visit. 2) Patient should be reminded not to take the dose of study treatment on this day until after the blood sample has been drawn.

For those patients with crossover from tamoxifen to Z-Endoxifen HCl, the following samples will be collected as follows for patients who have consented:

	End of Cycle 2, (prior to the start of Cycle 3)	**End of Cycle 8, (prior to the start of Cycle 9)**	**At the time of disease progression**	**Submit to:**
		A011203-ST2		
CTC (See Section 6.2.4 **Kit 1**	Two 10 mL whole blood samples in K3-EDTA tubes (provided in CTC kit)	Two 10 mL whole blood samples in K2-EDTA tubes (provided in CTC kit)	Two 10 mL whole blood samples in K2-EDTA tubes (provided in CTC kit)	BAP Freezer Mayo Clinic
cfDNA (See Section 6.2.4) **Kit 1**	Two 10 mL whole blood samples in Streck Cell Free DNA BCT tubes	Two 10 mL whole blood samples in Streck Cell Free DNA BCT tubes	Two 10 mL whole blood samples in Streck Cell Free DNA BCT tubes	BAP freezer Mayo Clinic
		A011203-ST3		
Bone turnover (See Section 6.2.5	One 10 mL whole blood sample in Non-additive red top tube	One 10 mL whole blood sample in Non-additive red top tube	One 10 mL whole blood sample in Non-additive red top tube	OSU
		A011203-PP1		
PK (See Section 6.2.7) **Kit 2**	One 6 mL whole blood sample in EDTA (purple top) tube	One 6 mL whole blood sample in EDTA (purple top) tube	One 6 mL whole blood sample in EDTA (purple top) tube	BAP freezer Mayo Clinic

Blood Specimen Collection Kits

Specialized blood specimen collection kits will be provided by the Alliance Biorepository at Mayo Clinic (BAP), for **A011203 ST-2 CTC and cfDNA specimens (Kit 1)** and **A011203 PP1 PK specimens only (Kit 2)**. Kits should be ordered using the Biospecimen Accessioning Processing Fax Supply Order Form, which is available on the A011203 study page of the Alliance and CTSU web sites. Kit 1 and Kit 2 should be ordered at the time of Pre-registration, for patients who have consented to the correlative blood studies. The kits will contain the tubes for blood specimen collection for a single visit, a container for shipping samples to the Alliance Biorepository at Mayo Clinic (BAP) with an affixed return shipping label. For questions regarding kit ordering, contact the Biospecimen Resource Manager at 507-538-0602.

6.2.1 Specimen Submission using the Alliance Biospecimen Management System

USE OF THE ALLIANCE BIOSPECIMEN MANAGEMENT SYSTEM (BioMS) IS MANDATORY AND ALL SPECIMENS MUST BE LOGGED AND SHIPPED VIA THIS SYSTEM.

BioMS is a web-based system for logging and tracking all biospecimens collected on Alliance trials. Authorized individuals may access BioMS at the following URL: http://bioms.allianceforclinicaltrialsinoncology.org using most standard web browsers (Safari, Firefox, Internet Explorer). For information on using the BioMS system, please refer to the 'Help' links on the BioMS web page to access the on-line user manual, FAQs, and training videos. To report technical problems, such as login issues or application errors, please contact: 1-855-55-BIOMS or Bioms@alliancenctn.org. For assistance in using the application or questions or problems related to specific specimen logging, please contact: 1-855-55-BIOMS or Bioms@alliancenctn.org.

After logging collected specimens in BioMS, the system will create a shipping manifest. This shipping manifest must be printed and placed in the shipment container with the specimens.

All submitted specimens must be labeled with the protocol number (A011203), Alliance patient ID number, date and type of specimen collected (e.g., serum, whole blood).

A copy of the shipping manifest produced by BioMS must be printed and placed in the shipment with the specimens.

Instructions for the collection of samples are included below. Please be sure to use a method of shipping that is secure and traceable. Extreme heat precautions should be taken when necessary.

6.2.2 Pre-registration period standard of care core biopsy of breast cancer tissue derived from a local, regional or distant site for mandatory confirmation of ER, PR and HER2 status and for optional translational sub-study A011203-ST1

Core needle biopsies will be collected using a core needle that is 18 gauge or wider that is designed for standard clinical testing. The initial 1-2 cores should be collected for clinical diagnostic purposes, including standard testing of ER, PR and HER2 from all patients. The preferred ER antibody is SP-1 and the preferred PR antibody is SP-2. During the same procedure (for those patients who have consented to A011203-ST1), an additional 2-3 research cores will be collected, immediately formalin-fixed for at least 6 hours and then processed into one paraffin block that is separate from the clinical tissue block. Close proximity to the passes will be attempted. Image guided biopsies are to be

performed per usual standard clinical care. The amount of tissue collected will follow the guidelines listed below.

Patients who undergo a research biopsy procedure, for the purpose of this protocol, and in whom inadequate tissue is obtained for optional correlative studies, are still eligible for the treatment study and are not required to undergo a repeat biopsy for the correlative tissue studies.

Breast: Total goal of 3-5 core biopsy specimens will be obtained using standard institutional guidelines for a diagnostic core biopsy of a breast mass

Skin/chest wall: A goal of 3-5 punch biopsies

Lymph node: A goal of 3-5 core biopsy specimens

Liver: A goal of 3-5 core biopsy specimens will be obtained using standard institutional guidelines

Lung: Because of the risk of pneumothorax associated with core needle biopsies of lung, if a patient has another accessible site of disease (i.e. skin, lymph node, liver), the non-lung site should be biopsied preferentially. If no other sites are available, a standard of care biopsy to confirm ER/PR, and HER2 should be done, and collection of additional 1-2 research cores will be left to the clinical judgment of the physician performing the procedure.

Bone: Because the yield of malignant tissue from bone biopsies tends to be low, if a patient has another accessible site of disease (i.e. skin, lymph node, liver), that site should be biopsied preferentially. If bone is the only biopsy-accessible site, then a goal of 3-5 core biopsy specimens will be obtained institutional guidelines. Note: Patients in which a bone biopsy was performed and no tumor or inadequate tissue was obtained for ER/PR/HER2 are not required to undergo a repeat biopsy and these patients are eligible to participate in this study.

If a patient has more than one site of disease, only one site needs to be biopsied. **For patients who have NOT consented to sub-study A011203-ST1,** tissue will be fixed in formalin and the local site will perform an H&E, ER, PR and HER2 analysis using standard procedures (IHC/ISH) to confirm the presence or ER+, HER2 negative breast cancer.

For patients who have consented to sub-study A011203-ST1, 2-3 research cores will be collected (at the same time as the clinical cores) and all cores will be fixed in formalin, paraffin embedded and labeled as a research block. This research block will be sent to the Alliance Biorepository at Ohio State for evaluation of 1) ESR1 alterations 2) markers of endocrine resistance and additional IHC markers (PKCB1 and SRC3)(A011203-ST1) (see Section 6.2.3) . Note, tissue samples will not be sent to the Alliance Biorepository at Ohio State for those patients that have not consented to the translational tissue component A011203-ST1. For those patients who consented to the correlative studies, research biospecimens are not to be submitted until the patient eligibility has been confirmed and they have been registered and randomized.

6.2.3 Breast tissue collection and processing for Evaluation of *ESR1* alterations and markers of endocrine resistance in patients treated with either tamoxifen or Z-Endoxifen HCl for patients who have consented (Alliance A011203-ST1)

The FFPE research block, as described in Section 6.2.2, will be sent to the Alliance Biorepository at Ohio State University. At the completion of the study, the tumor block(s) will be sectioned and used for nucleic acid extraction for the following

translational objectives: DNA (Foundation medicine) and RNA (SET index). Additional sections will be cut for IHC analyses of SRC3 and PKCβ1, to be performed at the Mayo Clinic.

Formalin fixed paraffin embedded specimens for A011203 ST1 should be sent at ambient temperature to:

Alliance Biorepository at Ohio State University
The Ohio State University
Innovation Centre
2001 Polaris Parkway
Columbus, OH 43240
Tel: 614-293-7073 Fax: 614-293-7967

Shipment on Monday through Thursday by overnight service to assure receipt is encouraged.

6.2.4 Circulating tumor cells (CTC) and circulating cell free DNA (cfDNA) for patients who have consented A011203-ST2

For patients who consent to participate in A011203-ST2, the following specimens will be collected.

Circulating Tumor Cell Capture and Analysis (CTC)

The specimens will be collected at the following time points:

1) Cycle 1, Day 1 prior to the start of treatment
2) End of Cycle 2, (prior to the start of Cycle 3)
3) End of Cycle 8, (prior to the start of Cycle 9)
4) At the time of disease progression

For those patients with crossover from tamoxifen to Z-Endoxifen HCl, CTC samples will be collected as follows:

1) End of Cycle 2, (prior to the start of Cycle 3)
2) End of Cycle 8, (prior to the start of Cycle 9)
3) At the time of disease progression

Two 10 mL blood samples will be collected in K2-EDTA tubes CTC specific kits will be used for this study. **Once a patient has been Pre-registered one ST-2 kit should be ordered for per patients, using the A011203 Biospecimen Accessioning Processing Fax Order Form, which may be downloaded from the A011203 study page of the Alliance and the CTSU web sites.** Instructions for processing these blood samples are provided in the blood specimen collection kits and **must be followed** in order for the samples to be viable.

Samples should be kept at ambient temperature after collection and during shipping. Ship CTC sample and cfDNA samples together to:

BAP Freezer
Mayo Clinic
St SL-16
150 Third Street SW
Rochester MN, 55902
507-538-0602

Samples for the CTC studies should be collected and shipped Monday – Thursday. However, if the subject can only be seen on Fridays, please contact the Biospecimen Resource Manager Roxanne Neumann for additional instructions 507-528-0602, Email: Neumann.roxann@mayo.edu.

Circulating Cell Free DNA Isolation and Analysis (cfDNA)

The specimens will be collected at the following time points:

1) Cycle 1, Day 1 prior to the start of treatment
2) End of Cycle 2, (prior to the start of Cycle 3)
3) End of Cycle 8, (prior to the start of Cycle 9
4) At the time of disease progression

For those patients with crossover from tamoxifen to Z-endoxifen HCl, cfDNA samples will be collected as follows:

1) End of Cycle 2, (prior to the start of Cycle 3)
2) End of Cycle 8, (prior to the start of Cycle 9)
3) At the time of disease progression

Two 10 mL blood samples will be collected in Streck Cell Free DNA BCT tubes. These samples will be processed within 7 days of collection for cfDNA extraction and subsequent analyses. All of these samples must be labeled for immediate processing. The Streck Cell Free DNA BCT tubes will be provided for this study TBD…

The Streck tubes for cfDNA should be kept at 6°C (42.8°F) to an ambient temperature of 37°C (98°F) during shipping. Ship cfDNA sample and CTC samples together to:

BAP Freezer
Mayo Clinic
St SL-16
150 Third Street SW
Rochester, MN 55902
507-538-0602

Samples for the cfDNA studies should be collected and shipped Monday – Thursday. However, if the study participant can only be seen on Fridays, please contact the Biospecimen Resource Manager Roxann Neumann for additional instructions Tel: 507-538-0602 Email: Neumann.roxann@mayo.edu.

6.2.5 Biochemical markers of bone turnover for patients who have consented (Alliance A011203-ST3)

The **FASTING** specimens will be collected at the following time points:

1) Cycle 1, Day 1 prior to the start of treatment
2) End of Cycle 2, (prior to the start of Cycle 3)
3) End of Cycle 8, (prior to the start of Cycle 9)
4) At the time of disease progression

For those patients with crossover from tamoxifen to Z-Endoxifen HCl, bone turnover samples will be collected as follows:

1) End of Cycle 2, (prior to the start of Cycle 3)
2) End of Cycle 8, (prior to the start of Cycle 9)
3) At the time of disease progression

For each time point, 10mL of whole blood will be collected in a Non-additive red top tube. Gently invert approximately 5 times to mix clot activator with blood. Let blood clot for 30 minutes. Observe a dense clot. Centrifuge at approximately 1300g (or in accordance with centrifuge manufacturer's instructions) for 10 minutes at room temperature. After centrifugation, place approximately 1.0 mL aliquots of serum into 2.0mL cryovials. Immediately label and freeze cryovials at –70°C or colder. If –70°C or colder freezer is not available, temporary storage on dry ice or at –20°C prior to shipment is acceptable for up to approximately 48 hours. Samples should be placed in a biohazard bag and shipped on dry ice by overnight express courier to:

Alliance Biorepository at Ohio State University
The Ohio State University
Innovation Centre
2001 Polaris Parkway
Columbus, OH 43240

Tel: 614-293-7073 Fax: 614-293-7967

Shipment on Monday through Thursday by overnight service to assure receipt is encouraged.

6.2.6 Blood submission for pharmacogenetics (A011203-PP)

All patients treated with Z-Endoxifen HCl or tamoxifen, who have consented, to A010203-PP will provide a single blood sample for **pharmacogenetic** analysis as follows:

Day 1, Cycle 1 prior to treatment **or at any time after Pre-registration**, collect a 10-mL sample of blood in an EDTA (Purple top) tube. The tube should be inverted approximately 8-10 times to mix the EDTA. Refrigerate sample until shipping. The sample should be placed in a biohazard bag and shipped the same day on a cold pack by overnight to:

Alliance Biorepository at Ohio State University
The Ohio State University
Innovation Centre
2001 Polaris Parkway
Columbus, OH 43240

Tel: 614-293-7073 Fax: 614-293-7967

Shipment on Monday through Thursday by overnight service to assure receipt is encouraged.

6.2.7 Blood submission for pharmacokinetics for patients who have consented (A011203-PP1)

For all patients who have consented to A011203-PP, blood samples for **pharmacokinetics** will be collected in all patients treated with Z-Endoxifen HCl or tamoxifen, according to the following schedule:

- Cycle 1 Day 1: Prior to drug administration
- Cycle 1 Day 1: 2-4 hours after drug administration
- Cycle 1 Day 1: 4-6 hours after drug administration **Note:** Do not draw samples within 2 hours of each other.)
- Cycle 1 Day 2: Prior to drug administration
- End of Cycle 2, (prior to the start of Cycle 3) prior to drug administration
- End of Cycle 8, (prior to the start of Cycle 9) prior to drug administration
- At the time of disease progression

For those patients with crossover from tamoxifen to Z-Endoxifen HCl, pharmacokinetic samples will be collected as follows:

- End of Cycle 2, (prior to the start of Cycle 3) prior to drug administration
- End of Cycle 8, (prior to the start of Cycle 9) prior to drug administration
- At the time of disease progression

Notes: 1) The patient must record the time of day that the dose was taken on the previous day. 2) The patient should be reminded not to take the dose on this day until after the blood sample is drawn.)

NOTE: Samples are light sensitive. Use amber tubes or cover tubes with aluminum foil to prevent exposure to light.

For each time point, 6mL blood sample will be collected in 6mL EDTA (purple top) tube, wrapped in aluminum foil and immediately cooled in an ice water bath. Blood must be processed for plasma isolation within 20 minutes of collection.

Blood will be subjected to centrifugation (2,000 rpm for 10 minutes) in a refrigerated centrifuge kept at 4°C. Following centrifugation, the plasma will be transferred to a polypropylene tube, capped, wrapped in aluminum foil and immediately frozen at -70°C.

Tube labels must contain the following data:

- Alliance protocol number (A011203), Alliance patient ID number, patient's initials and date and type of specimen collected (plasma).
- Date, cycle number and time of collection (i.e. pretreatment, 1, 2, and 4 hours after the oral dose)

These same data should be recorded in nurse's notes, on the Alliance A011203 Phamacokinetics Study Form that will be uploaded into RAVE. A copy of the form should be sent with the samples.

Send frozen plasma samples on dry ice via overnight courier to the Alliance Pathology Coordinating Office.

Send frozen plasma samples on dry ice, Monday-Wednesday via overnight courier to:

BAP Freezer
Mayo Clinic
St SL-16
150 Third Street SW
Rochester MN, 55902
507-538-0602

7.0 TREATMENT PLAN/INTERVENTION

Patients will be randomized with equal probability to receive daily treatment with Z-Endoxifen HCl or tamoxifen. Treatment on Arms I and II will continue until disease progression or unacceptable adverse event.

Protocol treatment is to begin ≤ 14 days of registration/randomization in order to allow for Z-Endoxifen HCl to arrive for patients assigned to Arm I.

7.1 Arm I Z-Endoxifen HCl

Agent	Dose	Route	Day	Retreatment Cycle
Z-Endoxifen HCl	80 mg	Oral (2 x 40mg capsule)	Days 1-21	Every 21 days (+/- 3 days)

1. Patients will take Z-Endoxifen HCl on an empty stomach (1 hour before or 2 hours after meals).
2. Patients should be instructed that the Z-Endoxifen HCl should be refrigerated. The Z-Endoxifen HCl should be dispensed with a cooler pack that the patient can use to transport this study medication.

7.2 Arm II Tamoxifen

Agent	Dose	Route	Day	Retreatment Cycle
Tamoxifen	20 mg	Oral (1 x 20 mg tablet)	Days 1-21	Every 21 days (+/- 3 days)

1. Patients will take tamoxifen without regard to meals.

7.3 Crossover to Z-Endoxifen HCl for Arm II Patients at Disease Progression

Please note that patients who opt to cross over should be re-registered to the study within 14 days of progression documentation.

Patients with measureable disease who meet the RECIST criteria for disease progression and patients with bone only disease who progress during tamoxifen treatment may switch to Z-Endoxifen HCl.

Those patients with both non-measureable disease and bone metastases are also eligible to cross over to Z-Endoxifen HCl.

Palliative radiation, if administered, should be completed prior to re-registration. Note that imaging studies obtained at the time of disease progression on Tamoxifen treatment Arm should be used to obtain the baseline tumor measurements for the Z-Endoxifen HCl crossover phase. These images must have been taken no more than 28 days prior to re-registration. Also, lesions that have been irradiated must not be used to evaluate tumor response to Z-Endoxifen HCl.

Z-Endoxifen HCl treatment should start no later than 28 days after documentation of progression.

Agent	Dose	Route	Day	Retreatment Cycle
Z-Endoxifen HCl	80 mg	Oral (2 x 40mg capsule)	Days 1-21	Every 21 days (+/- 3 days)

7.4 Adherence

Patients will be taking oral medication at home, without direct supervision. Drug compliance will be monitored until the discontinuation of Z-Endoxifen HCl or tamoxifen using A011203 Study Medication Logs found in Appendix III. Patients should bring the Medication Log to each clinic visit. A study team member will review the Medication Log, discuss any missed dose or discrepancy and document it. The Medication Log should be returned to the treating institution and compliance must be documented in the medical record by any member of the care team.

8.0 DOSE AND TREATMENT MODIFICATIONS

8.1 Ancillary therapy, concomitant medications, and supportive care

8.1.1 Colony stimulating factors

Routine use of colony-stimulating factors (G-CSF or GM-CSF) are not allowed.

8.1.2 Concurrent therapy

Use of other concurrent chemotherapy or anti HER2 therapy, immunotherapy, radiotherapy, or any ancillary therapy considered investigational (utilized for a non-FDA-approved indication and in the context of a research investigation) is not allowed.

Patient who are receiving tamoxifen (Arm II), should not be treated with strong CYP2D6 inhibitors while receiving study treatment (see Section 10.1.5 and Appendix II).

8.1.3 Expected prominent symptoms

Vasomotor symptoms such as hot flashes, sweating, as well as other estrogen withdrawal symptoms including insomnia, depression, and weight gain may be prominent in patients receiving Z-Endoxifen HCl and/or tamoxifen. These symptoms should not be treated with dose reduction, rather, intervention with either an SSRI or SNRI that is not known to be a potent CYP2D6 inhibitor (see Appendix II for a list of acceptable drugs). CYP2D6 catalyzes the formation of endoxifen from tamoxifen. Among SSRIs studied, venlafaxine had the least effect on endoxifen levels. Paroxetine was the most potent inhibitor of CYP2D6, resulting in the lowest endoxifen concentrations.

If the patient continues to have symptoms that are bothersome, a 2nd line intervention such as gabapentin should be initiated. A dose reduction should be considered if these symptoms persist despite 2 lines of intervention.

8.1.4 Full supportive care

Patients should receive full supportive care while on this study. This includes blood product support, antibiotic treatment and treatment of other newly diagnosed or concurrent medical conditions and concomitant medications such as anti-diarrheals, analgesics and anti-emetics. All blood products and concomitant medications received from Cycle 1 Day 1 of study drug until 30 days after the final dose are to be recorded in the medical record.

8.1.5 Not to be considered for any other studies involving pharmacologic agents

Patients participating in this trial are not to be considered for enrollment in any other study involving a pharmacologic agent–(drugs, biologics, immunotherapy approaches, gene therapy) whether for symptom control or therapeutic intent.

8.1.6 Non-protocol hormones or other chemotherapeutic agents may not be administered. Patients who initiate such treatment will be removed from protocol therapy due to either concurrent medical condition or desire for alternative therapy, depending upon reasons these non-protocol medications were given.

8.1.7 Antiemetics may be used at the discretion of the attending physician.

8.1.8 Diarrhea: Diarrhea is not anticipated as a side effect from either drug. However, this could be managed conservatively with loperamide. The recommended dose of loperamide is 4 mg at first onset, followed by 2 mg every 2-4 hours until diarrhea free (maximum 16 mg/day).

In the event of grade 3 or 4 diarrhea, the following supportive measures are allowed: hydration, octreotide, and antidiarrheals.

If diarrhea is severe (requiring intravenous rehydration) and/or associated with fever or severe neutropenia (grade 3 or 4), broad-spectrum antibiotics must be prescribed. Patients with severe diarrhea or any diarrhea associated with severe nausea or vomiting **should be hospitalized** for intravenous hydration and correction of electrolyte imbalances.

8.1.9 Palliative radiation therapy may not be administered during protocol treatment. Patients who require radiation therapy during protocol treatment will be removed from protocol therapy due to disease progression.

8.2 Dose Modifications

CTEP-AERS reporting may be required for some adverse events (see Section 9.0).

8.2.1 Tamoxifen therapy

There will be no dose reductions for Tamoxifen therapy. Tamoxifen therapy may be permanently discontinued at the discretion of the treating physician for other tamoxifen related toxicity.

Tamoxifen will be held for grade 3 or 4 hepatic function impairment. Resume tamoxifen therapy at the previous dose when hepatic toxicity resolves to $\leq$ grade 1 or baseline.

If tamoxifen therapy is held for > 3 weeks (1 cycle), permanently discontinue protocol therapy.

Note: Vasomotor symptoms defined as flushing, hot flashes and sweating will be graded using the CTCAE Version 4.0. Please see Section 8.1.3 regarding instructions for treatment of these symptoms.

Note: Serious adverse events will be reported on CTEP-AERS using CTCAE v5.0.

8.2.2 Z-Endoxifen HCl

If a grade 3 or 4 lymphopenia, thrombocytopenia, or hypertryglyceridemia, hold Z-Endoxifen HCl therapy until toxicity resolves to < grade 2. Reduce dose to 40mg daily. If the toxicities do not resolve to ≤ grade 1 or baseline severity within 21 days (1 cycle), treatment is to be discontinued and the patient will go to event monitoring. If toxicity recurs, treatment is to be discontinued.

If a grade 3 or 4 non-hematologic event that is attributed to Z-Endoxifen HCl occurs, hold therapy until toxicity resolves to ≤ grade 1 or back to baseline (if baseline is grade 2) and reduce dose to 40mg daily. If the toxicities do not resolve to ≤ grade 1 or baseline severity within 21 days (1 cycle), treatment is to be discontinued and the patient will go to event monitoring.

The prompt reporting of adverse events is the responsibility of each investigator engaged in clinical research, as required by Federal Regulations. Adverse events must be described and graded using the terminology and grading categories defined in the NCI's Common Terminology Criteria for Adverse Events (CTCAE), Version 4.0. The CTCAE is available at:

http://ctep.cancer.gov/protocolDevelopment/electronic_applications/ctc.htm. Attribution to protocol treatment for each adverse event must be determined by the investigator and reported on the required forms, using the codes provided.

Note: Serious adverse events will be reported on CTEP-AERS using CTCAE v5.0.

9.0 ADVERSE EVENTS

9.1 Routine Adverse Event Reporting

Adverse event data collection and reporting, which are required as part of every clinical trial are done to ensure the safety of patients enrolled in the studies as well as those who will enroll in future studies using similar agents. Adverse events are reported in a routine manner at scheduled times according to the study calendars in Section 5.0. For this trial, Adverse Events: Baseline, Adverse Events: Solicited and Adverse Events: Other forms are used for routine AE reporting in Rave.

Category (CTCAE SOC)	Adverse Event/ Symptoms	Baseline	Each evaluation	Grading scale (if not CTCAE)
Vascular disorders	Hot flashes	X	X	
	Thromboembolic events			
Metabolism and Nutrition disorders	Hypertriglyceridemia	X	X	
Investigations	Aspartate aminotransferase (AST) increased	X	X	

1. Patients who report visual symptoms in between their baseline and follow-up visits should be evaluated by ophthalmology.

9.2 Expedited Adverse event reporting (CTEP-AERS)

Investigators are required by Federal Regulations to report serious adverse events as defined in the table below. Alliance investigators are required to notify the Investigational Drug Branch (IDB), the Alliance Central Office, the Study Chair, and their Institutional Review Board if a patient has a reportable serious adverse event. The descriptions and grading scales found in the NCI Common Terminology Criteria for Adverse Events (CTCAE) Version 5.0 will be utilized for AE reporting as of April 1, 2018. The CTCAE is identified and located on the CTEP website at: http://ctep.cancer.gov/protocolDevelopment/electronic_applications/ctc.htm. All appropriate treatment areas should have access to a copy of the CTCAE. Expedited AE reporting for this study must only use CTEP-AERS (CTEP Adverse Event Reporting System), accessed via the CTEP home page, http://ctep.cancer.gov. In the rare occurrence when Internet connectivity is lost, a 24-hour notification is to be made to CTEP by telephone at 301-897-7497. Once Internet connectivity is restored, the 24-hour notification phoned in must be entered electronically into CTEP-AERS by the original submitter at the site.

Note: All deaths on study require both routine and expedited reporting regardless of causality. Attribution to treatment or other cause should be provided.

9.2.1 Late Phase 2 and Phase 3 Studies: Expedited Reporting Requirements for Adverse Events that Occur on Studies under an IND/IDE ≤ 30 Days of the Last Administration of the Investigational Agent/Intervention [1, 2]

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

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

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

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

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

<table>
<tr><th>Hospitalization</th><th>Grade 1 Timeframes</th><th>Grade 2 Timeframes</th><th>Grade 3 Timeframes</th><th>Grade 4 & 5 Timeframes</th></tr>
<tr><td>Resulting in Hospitalization ≥ 24 hrs</td><td colspan="3">10 Calendar Days</td><td rowspan="2">24-Hour; 5 Calendar Days</td></tr>
<tr><td>Not resulting in Hospitalization ≥ 24 hrs</td><td colspan="2">Not required</td><td>10 Calendar Days</td></tr>
</table>

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

Expedited AE reporting timelines are defined as:

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

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

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

- All Grade 4, and Grade 5 AEs

Expedited 10 calendar day reports for:

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

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

- Expedited AE reporting timelines defined:

➢ "24 hours; 5 calendar days" – The investigator must initially report the AE via AdEERS ≤ 24 hours of learning of the event followed by a complete CTEP-AERS report ≤ 5 calendar days of the initial 24-hour report.

➢ "10 calendar days" - A complete CTEP-AERS report on the AE must be submitted ≤ 10 calendar days of the investigator learning of the event.

• Use the NCI protocol number and the protocol-specific patient ID provided during trial registration on all reports.

Additional Instructions or Exclusion to CTEP-AERS Expedited Reporting Requirements for Phase 2 and 3 Trials Utilizing an Agent Under a CTEP IND or non-CTEP IND:

• All adverse events reported via AdEERS (i.e., serious adverse events) should also be forwarded to your local IRB.

• Alliance A011203 uses a drug under a CTEP IND. The reporting requirements for investigational agents under a CTEP IND should be followed for all agents (any arm) in this trial.

• Reporting of cases of secondary AML/MDS is to be done using the NCI/CTEP Secondary AML/MDS Report Form. New primary malignancies should be reported using the study form Notice of New Primary.

Secondary Malignancy:

A *secondary malignancy* is a cancer caused by treatment for a previous malignancy (*e.g.*, treatment with investigational agent/intervention, radiation or chemotherapy). A secondary malignancy is not considered a metastasis of the initial neoplasm.

CTEP requires all secondary malignancies that occur following treatment with an agent under an NCI IND/IDE be reported via AdEERS. Three options are available to describe the event:

- Leukemia secondary to oncology chemotherapy (*e.g.*, acute myelocytic leukemia [AML])
- Myelodysplastic syndrome (MDS)
- Treatment-related secondary malignancy

Any malignancy possibly related to cancer treatment (including AML/MDS) should also be reported via the routine reporting mechanisms outlined in each protocol.

• Treatment expected adverse events include those listed in Section 10.0 and in the package insert.

• CTEP-AERS reports should be submitted electronically.

• When submitting CTEP-AERS reports for "Pregnancy", "Pregnancy loss", or "Neonatal loss", the Pregnancy Information Form should be completed and submitted, along with any additional medical information (form is available on the CTEP web site at http://ctep.cancer.gov/). The potential risk of exposure of the fetus to the investigational agent(s) or chemotherapy agent(s) should be documented in the "Description of Event" section of the AdEERS report.

9.3 Z-Endoxifen HCl CAEPR

Comprehensive Adverse Events and Potential Risks list (CAEPR) for Z-Endoxifen HCl (NSC 750393)

The Comprehensive Adverse Events and Potential Risks list (CAEPR) provides a single list of reported and/or potential adverse events (AE) associated with an agent using a uniform presentation of events by body system. In addition to the comprehensive list, a subset, the Specific Protocol Exceptions to Expedited Reporting (SPEER), appears in a separate column and is identified with bold and italicized text. This subset of AEs (SPEER) is a list of events that are protocol specific exceptions to expedited reporting to NCI (except as noted below). Refer to the 'CTEP, NCI Guidelines: Adverse Event Reporting Requirements' http://ctep.cancer.gov/protocolDevelopment/electronic_applications/docs/aeguidelines.pdf for further clarification. *Frequency is provided based on 125 patients.* Below is the CAEPR for Z-Endoxifen HCl.

NOTE: Report AEs on the SPEER **ONLY IF** they exceed the grade noted in parentheses next to the AE in the SPEER. If this CAEPR is part of a combination protocol using multiple investigational agents and has an AE listed on different SPEERs, use the lower of the grades to determine if expedited reporting is required.

Version 2.0, September 25, 2017[1]

Adverse Events with Possible Relationship to Z-Endoxifen HCl (CTCAE 5.0 Term) [n= 125]			Specific Protocol Exceptions to Expedited Reporting (SPEER)
Likely (>20%)	**Less Likely (<=20%)**	**Rare but Serious (<3%)**	
BLOOD AND LYMPHATIC SYSTEM DISORDERS			
Anemia			***Anemia (Gr 2)***
GASTROINTESTINAL DISORDERS			
	Constipation		
	Diarrhea		
	Dry mouth		
	Dyspepsia		
	Gastroesophageal reflux disease		
	Nausea		***Nausea (Gr 2)***
	Vomiting		
GENERAL DISORDERS AND ADMINISTRATION SITE CONDITIONS			
	Edema limbs		***Edema limbs (Gr 2)***
Fatigue			***Fatigue (Gr 2)***
INVESTIGATIONS			
	Alanine aminotransferase increased		***Alanine aminotransferase increased (Gr 2)***
	Aspartate aminotransferase increased		***Aspartate aminotransferase increased (Gr 2)***
	Cholesterol high		
Lymphocyte count decreased			***Lymphocyte count decreased (Gr 2)***
	Neutrophil count decreased		
	Platelet count decreased		***Platelet count decreased (Gr 2)***
	White blood cell decreased		***White blood cell decreased (Gr 2)***
METABOLISM AND NUTRITION DISORDERS			
	Anorexia		***Anorexia (Gr 2)***
	Hyperglycemia		

Adverse Events with Possible Relationship to Z-Endoxifen HCl (CTCAE 5.0 Term) [n= 125]			Specific Protocol Exceptions to Expedited Reporting (SPEER)
Likely (>20%)	**Less Likely (<=20%)**	**Rare but Serious (<3%)**	
	Hypertriglyceridemia		***Hypertriglyceridemia (Gr 2)***
	Hypoalbuminemia		
	Hypocalcemia		
	Hypophosphatemia		
		Tumor lysis syndrome	
MUSCULOSKELETAL AND CONNECTIVE TISSUE DISORDERS			
	Arthralgia		
	Myalgia		
NERVOUS SYSTEM DISORDERS			
	Dizziness		
	Dysgeusia		
	Headache		
	Hypersomnia		
	Peripheral sensory neuropathy		
PSYCHIATRIC DISORDERS			
	Agitation		
	Anxiety		
	Insomnia		***Insomnia (Gr 2)***
	Irritability		***Irritability (Gr 2)***
SKIN AND SUBCUTANEOUS TISSUE DISORDERS			
	Alopecia		
	Rash acneiform		
	Rash maculo-papular		
VASCULAR DISORDERS			
Hot flashes			***Hot flashes (Gr 2)***
	Hypertension		
	Thromboembolic event		

[1]This table will be updated as the toxicity profile of the agent is revised. Updates will be distributed to all Principal Investigators at the time of revision. The current version can be obtained by contacting PIO@CTEP.NCI.NIG.GOV. Your name, the name of the investigator, the protocol and the agent should be included in the e-mail.

Adverse events reported on Z-Endoxifen HCl trials but for which there is insufficient evidence to suggest that there was a reasonable possibility that Z-Endoxifen HCl caused the adverse event:

CARDIAC DISORDERS - Sinus bradycardia
EAR AND LABYRINTH DISORDERS - Ear pain; Vestibular disorder
ENDOCRINE DISORDERS - Hyperthyroidism; Hypothyroidism
EYE DISORDERS - Blurred vision; Dry eye; Flashing lights; Floaters
GASTROINTESTINAL DISORDERS - Abdominal distension; Abdominal pain; Belching; Colonic perforation; Flatulence; Lip pain; Mucositis oral; Oral dysesthesia
GENERAL DISORDERS AND ADMINISTRATION SITE CONDITIONS - Chills; Edema face; Fever; Sudden death NOS
INFECTIONS AND INFESTATIONS - Infections and infestations - Other (infected hair follicle)
INVESTIGATIONS - Alkaline phosphatase increased; Blood bilirubin increased; Creatinine increased; INR increased; Investigations - Other (low HDL); Weight loss

METABOLISM AND NUTRITION DISORDERS - Dehydration; Hypercalcemia; Hyperkalemia; Hypermagnesemia; Hypernatremia; Hypokalemia; Hypomagnesemia; Hyponatremia
MUSCULOSKELETAL AND CONNECTIVE TISSUE DISORDERS - Bone pain; Buttock pain; Generalized muscle weakness
NERVOUS SYSTEM DISORDERS - Cognitive disturbance; Paresthesia; Seizure; Stroke
PSYCHIATRIC DISORDERS - Depression; Libido decreased
REPRODUCTIVE SYSTEM AND BREAST DISORDERS - Breast pain; Vaginal discharge; Vaginal dryness; Vaginal hemorrhage
RESPIRATORY, THORACIC AND MEDIASTINAL DISORDERS - Cough; Dyspnea; Sore throat
SKIN AND SUBCUTANEOUS TISSUE DISORDERS - Dry skin; Hyperhidrosis; Pruritus
VASCULAR DISORDERS - Peripheral ischemia

Note: Z-Endoxifen HCl in combination with other agents could cause an exacerbation of any adverse event currently known to be caused by the other agent, or the combination may result in events never previously associated with either agent.

10.0 DRUG INFORMATION

10.1 Z-Endoxifen HCl (NSC 750393)

10.1.1 Procurement

Z-Endoxifen is an investigational agent in this study and open label supplies will be provided for treatment Arm I and for patients on Arm II who crossover to Z-Endoxifen HCl therapy after disease progression, by the Pharmaceutical Management Branch (PMB), Cancer Therapy Evaluation Program (CTEP), Division of Cancer treatment and Diagnosis (DCTD), National Cancer Institute (NCI). Using the PMB Online Ordering Process (OAOP) application (https://eapps-ctep.nci.nih.gov/OAOP/pages/login.jspx). Access to OAOP requires the establishment of a CTEP Identity and Access Management (IAM) account (https://eapps-ctep.nci.nih.gov/iam/) and the maintenance of an "active" account status and a "current" password." See Section 10.1.8 for further instructions.

Procurement of Z-Endoxifen HCl for Crossover patients

For patients randomized to tamoxifen and who progressed on treatment, open label supplies of Z-Endoxifen HCl may be ordered using the PMB OAOP as described above.

10.1.2 Formulation

Z-Endoxifen HCl as 40 mg (Swedish orange-colored size 2) capsules.

The capsules contain the inactive ingredients microcrystalline cellulose, ascorbic acid, sodium croscarmellose, colloidal silicon dioxide, and magnesium stearate. The capsules are packaged in high-density polyethylene (HDPE) bottles containing 30 capsules each.

10.1.3 Storage and stability

Capsules should be stored in the refrigerator at 2-8ºC. Shelf-life surveillance of the intact bottles is on-going. Capsules are not to be re-packaged for dispensing purposes. Capsules are to be dispensed to study participants in the original manufacturer's container.

Z-Endoxifen HCl should be dispensed with a cooler pack that the patient can use to transport this study medication.

10.1.4 Administration

Capsules should be taken by mouth at approximately the same time each day on an empty stomach, either 1 hour before or 2 hours after meals, in accordance with Section 7.1 of the protocol.

10.1.5 Potential drug interactions

No significant drug interactions with Z-Endoxifen HCl are known at this time. Because the potential of Z-Endoxifen HCl to inhibit and/or induce any CYP450 enzyme is not known, every effort should be made to switch patients taking drugs that are known to be potent inducers/inhibitors of CYP450 enzymes to other medications prior to starting therapy. A list of drugs that are potent CYP450 inhibitors/inducers are included in Appendix II.

10.1.6 Pharmacokinetic information

a) Absorption

In rats, absorption occurs within 15 minutes following oral administration. Mean peak plasma concentrations were observed at 2 hours post dosing. Oral absorption was linear with bioavailability greater than 67% over the range 20 mg/kg to 140 mg/kg in rats. Absorption in dogs was also linear with a bioavailability greater than 50% over the range 15 mg/kg to 100 mg/kg.

b) Distribution

There is no information available at this time.

c) Metabolism

Z-Endoxifen HCl, along with 4-hydroxy-TAM, is the most metabolically active form of the selective estrogen receptor modulator, tamoxifen. Z-Endoxifen HCl concentrations are 20-fold higher than 4HT levels, and display considerable variability between patients, depending on the activity of CYP2D6. Other phase II metabolizing enzymes such as glucuronide (UGT) and sulfate (SULT), may affect endoxifen exposure, since preclinical studies have implicated these enzymes in the conjugation of Z-Endoxifen HCl. Z-Endoxifen HCl is the key metabolite necessary to fully inhibit ERα transcription and proliferation.

d) Excretion

In mice, peak plasma concentrations were maintained for 8 to 24 hours with plasma concentrations of 0.1μM to 2μM respectively. Terminal elimination half-life was 6.5 h.

Using a different formulation of endoxifen (endoxifen sulfate), Jina Pharmaceuticals has presented data regarding single dose endoxifen pharmacokinetics studies in humans. Ahmad et al (2010) reported results of an open label, single oral dose, randomized, dose escalating parallel study in 40 healthy male and female fasting subjects. Eight subjects received a single dose of 0.5, 1, 2, or 4 mg endoxifen tablets. Tamoxifen (Nolvadex) 20 mg tablets were administered to eight subjects as reference. Blood samples were collected at predose and up to 528 hours post dose for PK. Single oral dose of endoxifen at 0.5, 1, 2, or 4 mg was safe and well tolerated. Adverse events were not found and no clinically significant changes in laboratory or other safety variables were noted. Endoxifen displayed near dose-proportional pharmacokinetics, and small deviations from dose. The drug at all doses was rapidly absorbed with Tmax 6±2.9 h and half-life (t1/2) of 54.9±8.6. Increase in Cmax and AUC appeared proportional to dose when consecutive dose increments were compared. Endoxifen administered at 0.5, 1, 2, or 4 mg resulted in 1.36, 3.8, 5.5, and 14.5 ng/mL Cmax and 99.1, 231, 330, 763 ng.h/mL AUC0-inf, respectively. When compared with endoxifen levels in 20 mg tamoxifen administered subjects, 0.5, 1, 2, and 4 mg endoxifen showed 228%, 804%, and 1,216%, 3,385% higher Cmax, respectively, while Tmax was significantly lower in endoxifen treated subjects (Ahmand et al., 2010).

10.1.7 Adverse events

The most common adverse effects of Z-Endoxifen HCl have been mostly grades 1-2 hot flashes (50%), anemia (25%), fatigue (25%), lymphocyte count decreased (22%), nausea (19%), platelet count decreased (19%), hypertriglyceridemia (16%), irritability (16%), anorexia (13%), AST increased (13%), edema limbs (13%), and WBCs decreased (13%).

Thromboembolic event (grades 3-4) and hypertriglyceridemia (grade 4) have also been reported.

10.1.8 Agent ordering and agent accountability

NCI-supplied agents may be requested by the Principal Investigator (or their authorized designee) at each participating institution. Pharmaceutical Management Branch (PMB) policy requires that agent be shipped directly to the institution where the patient is to be treated. PMB does not permit the transfer of agents between institutions (unless prior approval from PMB is obtained). The CTEP-assigned protocol number must be used for ordering all CTEP-supplied investigational agents. The responsible investigator at each participating institution must be registered with CTEP, DCTD through an annual submission of FDA Form 1572 (Statement of Investigator), Curriculum Vitae, Supplemental Investigator Data Form (IDF), and Financial Disclosure Form (FDF). If there are several participating investigators at one institution, CTEP-supplied investigational agents for the study should be ordered under the name of one lead investigator at that institution.

Active CTEP-registered investigators and investigator-designated shipping designees and ordering designees can submit agent requests through the PMB Online Agent Order Processing (OAOP) application (https://eapps-ctep.nci.nih.gov/OAOP/pages/login.jspx). Access to OAOP requires the establishment of a CTEP Identity and Access Management (IAM) account (https://eapps-ctep.nci.nih.gov/iam/) and the maintenance of an "active" account status and a "current" password. For questions about drug orders, transfers, returns, or accountability, call (240) 276-6575 Monday through Friday between 8:30 am and 4:30 pm (ET) or email PMBAfterHours@mail.nih.gov anytime.

Agent Inventory Records – The investigator, or a responsible party designated by the investigator, must maintain a careful record of the inventory and disposition of all agents received from DCTD using the NCI Drug Accountability Record Form for Oral Agents (DARF).

10.2 Tamoxifen citrate (NSC 180973)

Please refer to the FDA-approved package insert for tamoxifen citrate for product information, extensive preparation instructions, and a comprehensive list of adverse events.

10.2.1 Procurement

Tamoxifen is commercially available and will not be supplied by the Pharmaceutical Management Branch (PMB) for this study.

10.2.2 Formulation

Tamoxifen is available in 10 mg and 20 mg tablets for oral administration.

10.2.3 Storage and Stability

Tamoxifen tablets should be stored at room temperature. The product label will contain the expiration date.

10.2.4 Administration

Tamoxifen will be administered orally, at a dose of 20 mg daily without regard to meals.

10.2.5 Potential Drug Interactions

Tamoxifen is extensively metabolized by CYP isoforms. In particular CYP2D6 catalyzes the formation of endoxifen and 4-hydroxytamoxifen, both of which are significantly more potent than tamoxifen. Inhibition of CYP2D6 activity, as a result of variant polymorphisms or concomitant administration of CYP2D6 inhibitors, is associated with decreased endoxifen levels. SSRIs are increasingly used to manage hot flashes, including hot flashes secondary to tamoxifen. Improvement in symptoms may result from decreased generation of endoxifen, and the potential exists for decreased effectiveness of tamoxifen. Among SSRIs studied, venlafaxine had the least effect on endoxifen levels. Paroxetine was the most potent inhibitor of CYP2D6, resulting in the lowest endoxifen concentrations.

10.2.6 Pharmacokinetic information

Following a single oral dose of 20 mg 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). 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. 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. 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 NOLVADEX (tamoxifen citrate) tablets given twice a day vs. a 20 mg NOLVADEX (tamoxifen citrate) tablet given once daily, the 20 mg NOLVADEX (tamoxifen citrate) tablet was bioequivalent to the 10 mg NOLVADEX (tamoxifen citrate) tablets.

10.2.7 Adverse events

The most common adverse events reported in recent trials with tamoxifen include hot flashes, nausea and vaginal discharge. Tamoxifen is also associated venous thrombosis and pulmonary embolism. As a result of tamoxifen's estrogenic effect on the endometrium, endometrial hyperplasia and endometrial cancer have been observed. In contrast, the estrogenic effect of tamoxifen is protective in bone (less osteoporosis and fewer fractures compared to aromatase inhibitors), and tamoxifen also reduces cholesterol.

11.0 MEASUREMENT OF EFFECT

Response and progression will be evaluated in this study using the international criteria proposed by the revised Response Evaluation Criteria in Solid Tumors (RECIST) guidelines (version 1.1) Changes in the largest diameter of the tumor lesions and the short axis measurements in the case of lymph nodes are used in the RECIST guideline.

11.1 Schedule of Evaluations

For the purposes of this study, patients should be re-evaluated every 6 weeks (every 2 cycles) for the first 8 cycles of treatment and then every 12 weeks (every 4 cycles) until progression.

For patients randomized to Arm 2 who crossover to Z-Endoxifen HCl, disease evaluations should occur every 6 weeks after starting Z-Endoxifen HCl for the first 8 cycles of treatment and then every 12 weeks until progression

11.2 Definitions of Measurable and Non-Measurable Disease

11.2.1 Measurable Disease

A non-nodal lesion is considered measurable if its longest diameter can be accurately measured as 2.0 cm with chest x-ray, or as ≥1.0 cm with CT scan, CT component of a PET/CT, or MRI.

A malignant lymph node is considered measurable if its short axis is ≥1.5 cm when assessed by CT scan (CT scan slice thickness recommended to be no greater than 5 mm).

Tumor lesions in a previously irradiated area are not considered measurable disease.

Lytic bone lesions, with an *identifiable soft tissue component*, evaluated by X-ray, CT (with bone windows) or MRI, *can be considered as measurable lesions* if the soft tissue component otherwise meets the definition of measurability described above

11.2.2 Non-Measurable Disease

All other lesions (or sites of disease) are considered non-measurable disease, including pathological nodes (those with a short axis ≥ 1.0 to < 1.5 cm). Bone lesions, leptomeningeal disease, ascites, pleural/pericardial effusions, lymphangitis cutis/pulmonis, inflammatory breast disease, and abdominal masses (not followed by CT or MRI), are considered as non-measurable as well.

Note: 'Cystic lesions' thought to represent cystic metastases can be considered as measurable lesions, if they meet the definition of measurability described above. However, if non-cystic lesions are present in the same patient, these are preferred for selection as target lesions. In addition, lymph nodes that have a short axis < 1.0 cm are considered non-pathological (i.e., normal) and should not be recorded or followed.

For patients enrolled onto A011023 who are determined to have non-measurable bone only disease, follow-up imaging (either with PET/CT or bone scan) should be performed at the time of tumor assessment. The method of assessment used at baseline must be used consistently.

11.3 Guidelines for Evaluation of Measurable Disease

11.3.1 Measurement Methods:

- All measurements should be recorded in metric notation (i.e., decimal fractions of centimeters) using a ruler or calipers.
- The same method of assessment and the same technique must be used to characterize each identified and reported lesion at baseline and during follow-up. For patients having only lesions measuring at least 1 cm to less than 2 cm must use CT imaging for both pre- and post-treatment tumor assessments.

11.3.2 Acceptable Modalities for Measurable Disease:

- **Conventional CT and MRI:** This guideline has defined measurability of lesions on CT scan based on the assumption that CT slice thickness is 5 mm or less. If CT scans have slice thickness greater than 5 mm, the minimum size for a measurable lesion should be twice the slice thickness.

As with CT, if an MRI is performed, the technical specifications of the scanning sequences used should be optimized for the evaluation of the type and site of disease. The lesions should be measured on the same pulse sequence. Ideally, the same type of scanner should be used and the image acquisition protocol should be followed as closely as possible to prior scans. Body scans should be performed with breath-hold scanning techniques, if possible.

- **PET-CT:** If the site can document that the CT performed as part of a PET-CT is of identical diagnostic quality to a diagnostic CT (with IV and oral contrast), then the CT portion of the PET-CT can be used for RECIST measurements and can be used interchangeably with conventional CT in accurately measuring cancer lesions over time.
- **Chest X-ray:** Lesions on chest x-ray are acceptable as measurable lesions when they are clearly defined and surrounded by aerated lung. However, CT scans are preferable.
- **FDG-PET:** FDG-PET scanning is allowed to complement CT scanning in assessment of progressive disease [PD] and particularly possible 'new' disease. A 'positive' FDG-PET scanned lesion is defined as one which is FDG avid with an update greater than twice that of the surrounding tissue on the attenuation corrected image; otherwise, an FDG-PET scanned lesion is considered 'negative.' New lesions on the basis of FDG-PET imaging can be identified according to the following algorithm:

  a. Negative FDG-PET at baseline with a positive FDG-PET at follow-up is a sign of PD based on a new lesion.

  b. No FDG-PET at baseline and a positive FDG-PET at follow-up:

  1) If the positive FDG-PET at follow-up corresponds to a new site of disease confirmed by CT, this is PD.

  2) If the positive FDG-PET at follow-up is not confirmed as a new site of disease on CT at the same evaluation, additional follow-up CT scans (i.e., additional follow-up scans at least 4 weeks later) are needed to determine if there is truly progression occurring at that site. In this situation, the date of PD will be the date of the initial abnormal PDG-PET scan.

  3) If the positive FDG-PET at follow-up corresponds to a pre-existing site of disease on CT that is not progressing on the basis of the anatomic images, it is not classified as PD.

11.3.3 Measurement at Follow-up Evaluation:

- The cytological confirmation of the neoplastic origin of any effusion that appears or worsens during treatment when the measurable tumor has met criteria for response or stable disease is mandatory to differentiate between response or stable disease (an effusion may be a side effect of the treatment) and progressive disease.
- Cytologic and histologic techniques can be used to differentiate between PR and CR in rare cases (e.g., residual lesions in tumor types such as germ cell tumors, where known residual benign tumors can remain.)

11.4 Measurement of Treatment/Intervention Effect

11.4.1 Target Lesions & Target Lymph Nodes

- Measurable lesions (as defined in Section 11.2.1) up to a maximum of 5 lesions representative of all involved organs, should be identified as "Target Lesions" and recorded and measured at baseline. These lesions can be non-nodal or nodal (as defined in 11.2.1), where no more than 2 lesions are from the same organ and no more than 2 malignant nodal lesions are selected.

Note: If fewer than 5 target lesions and target lymph nodes are identified (as there often will be), there is no reason to perform additional studies beyond those specified in the protocol to discover new lesions.

- Target lesions and target lymph nodes should be selected on the basis of their size, be representative of all involved sites of disease, but in addition should be those that lend themselves to reproducible repeated measurements. It may be the case that, on occasion, the largest lesion (or malignant lymph node) does not lend itself to reproducible measurements in which circumstance the next largest lesion (or malignant lymph node) which can be measured reproducibly should be selected.
- **Baseline Sum of Dimensions (BSD):** A sum of the longest diameter for all target lesions plus the sum of the short axis of all the target lymph nodes will be calculated and reported as the baseline sum of dimensions (BSD). The BSD will be used as reference to further characterize any objective tumor response in the measurable dimension of the disease.
- **Post-Baseline Sum of the Dimensions (PBSD):** A sum of the longest diameter for all target lesions plus the sum of the short axis of all the target lymph nodes will be calculated and reported as the post-baseline sum of dimensions (PBSD). If the radiologist is able to provide an actual measure for the target lesion (or target lymph node), that should be recorded, even if it is below 0.5 cm. If the target lesion (or target lymph node) is believed to be present and is faintly seen but too small to measure, a default value of 0.5 cm should be assigned. If it is the opinion of the radiologist that the target lesion or target lymph node has likely disappeared, the measurement should be recorded as 0 cm.
- **The minimum sum of the dimensions (MSD)** is the minimum of the BSD and the PBSD.

11.4.2 Non-Target Lesions & Non-Target Lymph Nodes

Non-measurable sites of disease (Section 11.2.2) are classified as non- target lesions or non-target lymph nodes and should also be recorded at baseline. These lesions and lymph nodes should be followed in accord with Section 11.4.3.3.

11.4.3 Response Criteria

11.4.3.1 All target lesions and target lymph nodes followed by CT/MRI/PET-CT/Chest X-ray must be measured on re-evaluation. Specifically, a change in objective status to either a PR or CR cannot be done without re-measuring target lesions and target lymph nodes.

Note: Non-target lesions and non-target lymph nodes should be evaluated at each assessment, especially in the case of first response or confirmation of response. In selected circumstances, certain non-target organs may be evaluated less frequently. For example, bone scans may need to be repeated only when complete response is identified in target disease or when progression in bone is suspected.

11.4.3.2 Evaluation of Target Lesions

- Complete Response (CR): All of the following must be true:

a. Disappearance of all target lesions.

b. Each target lymph node must have reduction in short axis to < 1.0 cm.

- Partial Response (PR): At least a 30% decrease in PBSD (sum of the longest diameter for all target lesions plus the sum of the short axis of all the target lymph nodes at current evaluation) taking as reference the BSD (see Section 11.4.1).
- Progression (PD): At least one of the following must be true:

a. At least one new malignant lesion, which also includes any lymph node that was normal at baseline (< 1.0 cm short axis) and increased to ≥ 1.0 cm short axis during follow-up.

b. At least a 20% increase in PBSD (sum of the longest diameter for all target lesions plus the sum of the short axis of all the target lymph nodes at current evaluation) taking as reference the MSD (Section 11.4.1). In addition, the PBSD must also demonstrate an absolute increase of at least 0.5 cm from the MSD.

c. See Section 11.3.2 for details in regards to the requirements for PD via FDG-PET imaging.

- Stable Disease (SD): Neither sufficient shrinkage to qualify for PR, nor sufficient increase to qualify for PD taking as reference the MSD.

11.4.3.3 Evaluation of Non-Target Lesions & Non-target Lymph Nodes

- Complete Response (CR): All of the following must be true:

a. Disappearance of all non-target lesions.

b. Each non-target lymph node must have a reduction in short axis to <1.0 cm.

- Non-CR/Non-PD: Persistence of one or more non-target lesions or non-target lymph nodes.
- Progression (PD): At least one of the following must be true:

a. At least one new malignant lesion, which also includes any lymph node that was normal at baseline (< 1.0 cm short axis) and increased to ≥ 1.0 cm short axis during follow-up.

b. Unequivocal progression of existing non-target lesions and non-target lymph nodes. (NOTE: Unequivocal progression should not normally trump target lesion and target lymph node status. It must be representative of overall disease status change.)

c. See Section 11.3.2 for details in regards to the requirements for PD via FDG-PET imaging.

11.4.4 Overall Objective Status

The overall objective status for an evaluation is determined by combining the patient's status on target lesions, target lymph nodes, non-target lesions, non-target lymph nodes, and new disease as defined in the following tables:

For Patients with Measurable Disease

Target Lesions & Target Lymph Nodes	Non-Target Lesions & Non-Target Lymph Nodes	New Sites of Disease	Overall Objective Status
CR	CR	No	CR
CR	Non-CR/Non-PD	No	PR
PR	CR Non-CR/Non-PD	No	PR
CR/PR	Not All Evaluated*	No	PR**
SD	CR Non-CR/Non-PD Not All Evaluated*	No	SD
Not all Evaluated	CR Non-CR/Non-PD Not All Evaluated*	No	Not Evaluated (NE)
PD	Unequivocal PD CR Non-CR/Non-PD Not All Evaluated*	Yes or No	PD
CR/PR/SD/PD/Not all Evaluated	Unequivocal PD	Yes or No	PD
CR/PR/SD/PD/Not all Evaluated	CR Non-CR/Non-PD Not All Evaluated*	Yes	PD

* See Section 11.4.3.1

11.4.5 For those patients with non-measurable, bone-only disease, objective progression will be established if at least 1 of the following criteria is met:

- the appearance of 1 or more new lesions (in bone or outside of bone), or
- unequivocal progression of existing bone lesions.

The finding of a new lesion should be unequivocal and no attributable to findings thought to represent something other than tumor (for example, some 'new ributab lesions may be simply healing or flare of preexisting lesions). Pathologic fracture, new compression fracture, or complications of bone metastases will not be considered as evidence of disease progression, unless at least 1 of the above criteria is met.

11.4.6 Symptomatic Deterioration: Patients with global deterioration of health status requiring discontinuation of treatment without objective evidence of disease progression at that time, and not either related to study treatment or other medical conditions, should be reported as PD due to "symptomatic deterioration." Every effort should be made to document the objective progression even after discontinuation of treatment due to symptomatic deterioration.

12.0 TREATMENT/FOLLOW–UP DECISION AT EVALUATION OF PATIENT

12.1 No disease progression or Unacceptable Adverse Events

Patients who have not had disease progression and have not developed unacceptable toxicity will be eligible for re-treatment at their current dose level until disease progression, unacceptable toxicity, or refusal.

12.2 No disease Progression, Unacceptable Adverse Events

Those patients who have not had disease progression, but have experienced unacceptable toxicity may be eligible for retreatment at a lower dose (see Section 8.0).

12.3 Discontinuation of Protocol Therapy

Reasons why the protocol therapy may be discontinued:

- Tumor progression
- Request by the patient to withdraw
- Unacceptable toxicity
- Inter-current illness
- Administration of alternative treatment

Arm 1 patients:

Patients who discontinue treatment for any of the above reasons will proceed to event monitoring. Patients will be followed yearly for a maximum of 5 years from study registration. Once a patient has discontinued study treatment, future therapy is at the discretion of the treating physician.

Arm 2 patients:

Patients randomized to tamoxifen who progress during tamoxifen treatment may choose to switch to Z-Endoxifen HCl and continue on Z-Endoxifen HCl until disease progression is noted. After disease progression during Z-Endoxifen HCl treatment patient will proceed to event monitoring. Patients will be followed yearly for a maximum of 5 years from study registration. Once a patient has discontinued study treatment, future therapy is at the discretion of the treating physician.

Patients who discontinue treatment for any of the above reasons other than progression or patients who refuse to crossover to Z-Endoxifen HCl will proceed to event monitoring. Patients will be followed yearly for a maximum of 5 years from study registration/randomization. Once a patient has discontinued study treatment, future therapy is at the discretion of the treating physician.

12.4 Refuse to begin protocol treatment following randomization

If an eligible patient refuses to begin treatment following randomization (and is classified as a cancel by the Research Base), all on-study materials and the End of Active Treatment/Cancel Notification Form must be submitted. For those patients who also consent to the correlative studies, research biospecimens are not to be submitted. No further data submission is necessary. Future therapy is at the discretion of the treating physician.

12.5 Ineligible prior to the start of protocol treatment

If a patient is determined not to have satisfied each and every eligibility criteria for study entry after randomization but prior to the start of protocol treatment, on-study materials must be submitted. For those patients who also consent to the correlative studies, research biospecimens are not to be submitted. Bio-specimens should not be submitted. No further data submission is necessary. Future therapy is at the discretion of the treating physician.

12.6 Ineligible after to the start of protocol treatment

If a patient is determined not to have satisfied each and every eligibility criteria but has received some study treatment and is having some clinical benefit, the patient may be able to continue study treatment per protocol after consultation with the study chair. If the patient does continue on study treatment, study forms and bio-specimens should be submitted using the same schedule as eligible patients. If the patient does not continue study treatment, the patient will go into event monitoring phase for a maximum of 5 years post randomization. Future therapy is at the discretion of the treating physician.

12.7 Extraordinary Medical Circumstances

If, at any time the constraints of this protocol are detrimental to the patient's health and/or the patient no longer wishes to continue protocol therapy, protocol therapy shall be discontinued. In this event:

- Document the reason(s) for discontinuation of therapy on data forms.
- Follow the patient for protocol endpoints as required by the Study Calendar.

13.0 STATISTICAL CONSIDERATIONS

13.1 Study Design

A randomized phase II clinical trial design was chosen to assess whether Z-Endoxifen HCl shows promise in providing greater in antitumor activity (in terms of increasing progression-free survival) than tamoxifen in postmenopausal women with metastatic ER positive breast cancer that is endocrine resistant.

This study will use a dynamic allocation procedure to allocate an equal number of patients to each of the treatment arm. This procedure will balance the marginal distributions of the stratification factors between these treatment arms. The stratification factors that will be used are: prior palbociclib and/or everolimus, type of disease and degree of endocrine resistance (see Section 4.8).

All patients meeting the eligibility criteria who have signed a consent form and have begun treatment will be evaluable for assessment of all of the clinical endpoints. Patients will be included in the treatment group they were randomized to regardless of their actual treatment or duration of treatment. Summary statistics for patient and tumor characteristics, eligibility rates, length of follow‑up, and treatment acceptance rates will be calculated by assigned treatment arm.

13.1.2 Definition and documentation of primary endpoint.

The primary endpoint is progression-free survival (PFS) defined as the time from randomization to documentation of local, regional or distant disease progression or death without progression of disease.

13.1.3 Sample Size, Accrual Time and Study Duration

This trial was designed under the assumption that the median progression-free survival time among patients receiving tamoxifen is 3.0 months. Disease assessments in this trial are made at a frequency that is half the hypothesized median of the tamoxifen arm. Stone et al [41, 42] (2, 3) found that when disease assessments are made at a frequency that is half the control median, the difference in power between the log-rank test (with documented progression times) and generalized log-test with interval censored data was < 3%. As such we will determine the sample size assuming a log-rank test will be used with one-sided alpha=0.10 and power=95% so that the interval censored approach with the generalized log-rank test will have a power of > 90%.

With a sample size of 80 eligible patients (40 per treatment arm randomized in equal numbers) over a 15 month period and followed at least another 9 months after the close of enrollment before analysis is undertaken, a one-sided alpha=0.10 generalized log-rank test will have at least a 90% chance of detecting a 50% decrease in hazard of disease progression with Z-Endoxifen HCl relative to tamoxifen.

That corresponds to being able to detect an increase in the median PFS time from 3.0 months with tamoxifen to 6.0 months with Z-Endoxifen HCl.

An additional 14 patients will be screened to account for ineligible patients (tumors that are ER negative on biopsy) and those who cancel participation prior to starting treatment.

As of November 1, 2016, 24 (30%) of the 81patients enrolled on this trial were found to have ER negative and/or HER2 positive breast cancer, a second primary cancer, or benign disease, making them ineligible. Therefore, the number of patients needed to be screened to obtain 80 eligible patients will be increased from 94 to 115 patients.

The efficacy analysis will be conducted when at least 72 patients have provided documentation of local, regional or distant disease progression or death without progression of disease.

13.1.3.1 Study duration

It is anticipated that the enrollment period will be 15 months and the minimum follow-up period after the close of enrollment will 9 months, and an additional 3 months will be required to complete data submissions and curate the study data. Thus, it is anticipated that primary efficacy analysis will begin approximately 27 months after the trial is activated.

13.1.4 Interim efficacy/futility analysis plan

No formal interim efficacy/futility analysis is planned as the enrollment would be compete when 50% of the events will have occurred.

13.1.5 Analysis plan for primary efficacy endpoint

Progression-free survival estimates will be determined using nonparametric maximum likelihood estimation for interval censored data. The generalized log-rank test for interval censored data will be used to assess whether PFS differs between the treatment arms (1,2). A secondary analysis of the primary clinical endpoint will follow the approach proposed by Freidlin et al. using scan times of 3 and 6 months [43].

13.1.6 Secondary Clinical Endpoints

13.1.6.1 Adverse Events

Per CTEP Version 4.0 of the NCI CTCAE, an adverse event (AE) is any unfavorable and unintended sign (including an abnormal laboratory finding), symptom, or disease temporally associated with the use of a medical treatment or procedure that may or may not be considered related to the medical treatment or procedure. All grade 2, 3, 4 or 5 adverse events will be documented and assigned an attribute by treating clinician as to its relationship to treatment.

Note: Serious adverse events will be reported on CTEP-AERS using CTCAE v5.0.

For a given AE, the proportion of patients on each treatment arm who report developing a grade 2-5 of this AE will be determined.

13.1.6.2 Tumor Response Rate by study arm

The tumor response rate is defined as the 100% time the number of patients with a CR or PR (as defined by the RECIST criteria) on 2 consecutive evaluations at least 6 weeks apart divided by the total number of eligible patients who began study treatment. A 90% binomial confidence interval will be constructed for the true response rate.

13.1.6.3 Overall survival distribution by study arm

Overall survival time is defined as the time from randomization to death due to any cause. The distribution of survival times will be estimated using the method of Kaplan-Meier.

13.1.7 Monitoring

The study chair, the faculty statistician and the executive officer will review the trial data every 3 months to identify accrual, toxicity, and endpoint problems that might be developing. The faculty and secondary statistician will prepare a report containing accrual, adverse events, and efficacy data which will be submitted to the ALLIANCE Data and Safety Monitoring Board on a semi-annual basis.

Safety stopping rules will be applied to each treatment arm separately. If 3 or more of the first 10 patients randomized to a given treatment arm or 30% of more of patients randomized to that treatment arm thereafter develops a grade 4 hematologic or non-hematologic toxicity possibly, probably or definitely related to treatment, the enrollment to the trial will be temporarily suspended so that all AE data can be examined. The study chair and the faculty statistician will formulate a trial recommendation to present to the ALLIANCE DSMB and CTEP for approval.

The study chair and the faculty statistician will formulate a trial recommendation to present to the ALLIANCE DSMB and CTEP for approval.

13.1.8 Other Considerations

Toxicity, patterns of treatment failure observed in this study and scientific discoveries or changes in standard care will be taken into account in any decision to terminate these trials earlier than anticipated.

13.2 Evaluation of ESR1 alterations and markers of endocrine resistance in patients treated with either tamoxifen or Z-Endoxifen HCl (Alliance A011203-ST1)

13.2.1 Objectives

13.2.1.1 To examine whether DNA alterations as measured by Foundation medicine in all coding exons of 287 cancer-related genes as well as 78 polymorphisms in 34 ADME-related genes (assessed using a biopsy of metastatic disease obtained after progression on AI) are associated with longer PFS or higher response rates in the Z-Endoxifen HCl arm compared to the tamoxifen arm.

13.2.1.2 To examine whether SRC3 (AIB1) IHC expression levels (assessed using a biopsy of metastatic disease obtained after progression on AI) is associated with longer PFS or higher response rates in the Z-Endoxifen HCl compared to tamoxifen arm.

13.2.2 Analysis methods – DNA alterations

Data will be returned from Foundation Medicine as presence or absence of various DNA alterations, along with description of type and location of the alteration. The nature of alterations found in each pathway and gene will be described in tabular format. Association of presence or absence of DNA alterations with PFS will be assessed overall (presence/absence of any alteration), by pathway (presence/absence of alterations within a given pathway), and by gene (presence/absence of alterations within a given gene) via Cox regression with the goal of evaluating hazard ratio estimates and confidence intervals. Similarly, associations with response rates will be evaluated via logistic regression overall, by pathway, and by gene with the goal of evaluating odds ratio estimates and confidence intervals. Alterations will be considered together (polymorphisms, mutations, amplifications, indels, and fusions combined) as well as by type of alteration, e.g., for polymorphisms alone, mutations alone, etc. Model fit and stability will be evaluated, and exact methods used if needed. Due to sample size and expected prevalence rates, we expect the ability to adjust for covariates to be limited. These analyses will be performed in data from both treatment arms combined, as well as within treatment arm. This allows us to assess both prognostic and predictive ability in an exploratory fashion. The ER and PI3K pathways are of primary interest; other pathways will be examined as well. In addition, raw data will be received, and state-of-the-art bioinformatics algorithms utilized to assess the potential for genetic alterations not yet discovered.

13.2.3 Power – DNA alterations

The total trial sample size is 80 patients, with 40 for each treatment. Previous experience demonstrates successful assay rates range from 73% in ER+ Her2- patients [44] to 95% successful assay rate in a diverse set of cancers [45]. Based on these rates, the expected sample size per treatment arm is from 29 to 38. A previous study in this patient population found 96% of subjects to have at least one known somatic alteration, and 104 genes to have at least one known somatic alteration [44]. The same study found 56% of patients to have genetic alterations in the PI3K pathway and 44% to be wild type. Given the sample size and expected prevalence rates, these analyses are considered to be exploratory and hypothesis generating.

13.2.4 Analysis methods – SRC3 (AIB1) and PKCβ1 IHC expression levels

Approximately 25% of patients are anticipated to have metastatic SRC3-positive disease. Within each treatment arm, a point and interval estimate of the difference in tumor response rate between those with metastatic SRC3-positive disease and those with metastatic SRC3-negative disease will be constructed using the properties of the binomial distribution. Also, a point and interval estimate of the odds of disease progression among those with metastatic SRC3-positive disease relative to those with metastatic SRC3-negative disease will be ascertained from the parameter estimates of fitting a proportional hazard model to the progression data. The proportion of patients with metastatic PKCβ1 expressing breast cancer is unknown. Within each treatment arm, a point and interval estimate of the difference in tumor response rate between those with metastatic PKCβ1positive disease and those with metastatic PKCβ1-negative disease will be constructed using the properties of the binomial distribution. Also, a point and interval estimate of the odds of disease progression among those with metastatic PKCβ1-positive disease relative to those with metastatic PKCβ1negative disease will be ascertained from the parameter estimates of fitting a proportional hazard model to the progression data.

13.3 DNA alterations in patients treated with either tamoxifen or Z-Endoxifen HCl (Alliance A011203-ST2)

13.3.1. Objectives

13.3.1.1 To assess whether the molecular characteristics identified in the tumor biopsies are detectable in CTCs and/or cfDNA.

13.3.1.2 For each treatment arm, to examine ESR1 mutations or amplifications identified in CTCs or cfDNA change over time and explore the impact of these changes on PFS and response rates.

13.3.2 Analysis methods

For each patient, the genetic alterations seen in their tumor biopsy and their circulating tumor cells will be compared to ascertain which genetic alteration was seen in both tumor biopsy materials and CTCs, seen in only their tumor biopsy material, or seen in only their CTCs. For each alternation observed, we will tabulate the proportion of patients who had the genetic alternation seen in both tumor biopsy materials and CTCs, the proportion of patients who had the genetic alternation seen in only their tumor biopsy material, the proportion of patients who had the genetic alternation seen in only their CTCs, and the proportion of patients who did not have the genetic alternation in tumor biopsy materials or CTCs.

Toy et al. conducted a genetic analysis of tumors taken from individuals with metastatic ER-positive breast cancer whose tumors had grown or spread to new sites after at least 3 months with hormonal therapy. They found in 14 (17.5%) of 80 cases there were mutations in *ESR1* affecting the ligand-binding domain. The prevalence of mutations in this gene among the tumors from the patients who relapsed on hormonal treatment was were higher than that seen in The Cancer Genome Atlas (TCGA) invasive primary (untreated) breast cancer and luminal A and luminal B primary breast cancer cases, suggesting that these mutations may have a role in the development of acquired resistance to hormonal treatment.

For each treatment arm:

- a point and interval estimate of the proportion of patients with *ESR1*mutated CTCs prior to the start of treatment or is found to have *ESR1*mutated CTCs during treatment at or prior to progression will be determined using the properties of the binomial distribution
- a point and interval estimate of the proportion of patients with *ESR1*mutated CTCs prior to the start of treatment who progress within the first 4 months of treatment will be determined using the properties of the binomial distribution
- a point and interval estimate of the hazard of disease progression among the patients with *ESR1*mutated CTCs prior to the start of treatment or at end of the second cycle of treatment relative to patients who do not have *ESR1*mutated CTCs either prior to the start of treatment or at end of the second cycle of treatment will be constructed using the results of fitting a Cox model to time to progression data.

13.4 Biochemical markers of bone turnover (A011203-ST3)

For each of the biomarkers of bone formation (namely, bone-specific alkaline phosphatase, osteocalcin, P1NP) and bone resorption (namely, CTX-1, Trap5b and bone sialoprotein) the percent change after 2 cycles of treatment from pre-treatment levels will be determined.

A two sided alpha=0.01 z-test will be used to assess whether the percent change in a given bone absorption biomarker differs with respect to treatment.

Assuming the variability in percent change in a given bone absorption biomarker is similar between the treatment arms and at least 32 or more patients on each treatment arm have both a pre and Week 8 biomarker determination, then a two sided alpha=0.01 z-test will have a 90% chance of detecting whether the mean difference between the percent change in the biomarker of those treated with Z-Endoxifen HCl and that of those treated with tamoxifen is at least 1 standard deviation or more.

13.5 Z-Endoxifen HCl and Tamoxifen Pharmacokinetics and Pharmacogenetics (A011203-PP)

The pharmacokinetic, pharmacogenetic and pharmacodynamic data will be examined in an exploratory and hypothesis-generating fashion. A population PK will be developed using the software program NONMEM®, Version 7 (ICON Development Solutions, Ellicott City, MD, USA). The model will be parameterized in terms of AUC (the primary endpoint), as well as clearance and volume of distribution. Criteria for model selection will include the likelihood ratio test, shrinkage estimates, reasonableness of parameter estimates, as well as goodness-of-fit plots. Attempts will be made to identify the covariates that affect drug behavior or those that explain variability in this patient population.

The primary pharmacokinetic endpoint for exploring relationships with efficacy/adverse effects will be endoxifen AUC. Pharmacokinetic-Pharmacodynamic relationships will be explored for effects of endoxifen on efficacy-related, adverse events or laboratory parameters of clinical interest. Exploratory/graphical analyses will be conducted for PK/PD evaluations and may be followed by model-based analyses. The data may be pooled with data from other/future studies for additional population PK/PD analyses.

13.6 Inclusion of Women and Minorities

This study will be available to all eligible women regardless of race or ethnic group.

Men are excluded from this study the eligibility criteria requires all participants to have failed an aromatase inhibitor and aromatase inhibitors are not FDA approved for men.

DOMESTIC PLANNED ENROLLMENT REPORT					
Racial Categories	Ethnic Categories				Total
	Not Hispanic or Latino		Hispanic or Latino		
	Female	Male	Female	Male	
American Indian/ Alaska Native	0	0	0	0	0
Asian	0	0	3	0	3
Native Hawaiian or Other Pacific Islander	0	0	0	0	0
Black or African American	0	0	17	0	17
White	15	0	80	0	95
More Than One Race	0	0	0	0	0
Total	15	0	100	0	115

Ethnic Categories:

Hispanic or Latino – a person of Cuban, Mexican, Puerto Rico, South or Central American, or other Spanish culture or origin, regardless of race. The term "Spanish origin" can also be used in addition to "Hispanic or Latino."

Not Hispanic or Latino

Racial Categories:

American Indian or Alaskan Native – a person having origins in any of the original peoples of North, Central, or South America, and who maintains tribal affiliations or community attachment.

Asian – a person having origins in any of the original peoples of the Far East, Southeast Asia, or the Indian subcontinent including, for example, Cambodia, China, India, Japan, Korea, Malaysia, Pakistan, the Philippine Islands, Thailand, and Vietnam. (Note: Individuals from the Philippine Islands have been recorded as Pacific Islanders in previous data collection strategies.)

Black or African American – a person having origins in any of the black racial groups of Africa. Terms such as "Haitian" or "Negro" can be used in addition to "Black or African American."

Native Hawaiian or other Pacific Islander – a person having origins in any of the original peoples of Hawaii, Guam, Samoa, or other Pacific Islands.

White – a person having origins in any of the original peoples of Europe, the Middle East, or North Africa.

14.0 CORRELATIVE AND COMPANION STUDIES

There are 4 substudies within this protocol and all patients are encouraged to participate.

14.1 Evaluation of *ESR1* alterations and marker of endocrine resistance in patients treated with either tamoxifen or endoxifen (Alliance A011203-ST1)

14.1.1 Background

While protein levels of ER continue to be the most important factor determining response to endocrine therapy, an emerging area of research has demonstrated that genetic alterations in *ESR1*, either through mutations in the ligand binding domain, fusions, or the presence of *ESR1* amplification, are associated with endocrine resistance. Additionally, a key oncogenic co-activator, SRC3 (AIB1), known to be associated with tamoxifen and AI resistance is more likely to avidly bind ER in these settings, leading to potential resistance and/or greater agnostic effects of SERM. Therefore, new drug strategies are needed in patients with ESR1 alterations whose tumors continue to express the ER, but are resistant to standard hormonal therapies.

With regard to *ESR1* mutations, recent data suggest that these alterations are rare in newly diagnosed breast cancer, but reported in up to 20% of recurrent breast cancers [37]. These mutations lead to a conformational change in the LBD which mimics the conformation of activated ligand-bound receptor and constitutive, ligand-independent transcriptional activity resulting in and resistance to some forms of hormonal therapy. More specifically, preclinical studies suggest that cell lines that harbor some of these mutations, while insensitive to estrogen deprivation, retain sensitivity to higher doses of fulvestrant or potent SERMs such as endoxifen and 4HT. However, the concentrations of SERMS used in the study by Robinson et al [12] (1 micromolar) are neither observed nor achievable in tamoxifen treated humans [26]. Conversely, substantial endoxifen concentration can be achieved in humans (1-2 μM) and it is reasonable to hypothesize that these levels of endoxifen may be effective in patients with ESR1 mutations or amplification.

Additional data suggest that amplification of *ESR1*, observed more commonly in de novo resistant tamoxifen treated breast cancers and additionally observed in AI resistant breast cancers, may be an important marker of disease resistance. Finally, ESR1 fusions, as described by Li et al, appears additionally to confer resistance to hormonal therapy but are rare [38].

To compare the activity of endoxifen with tamoxifen, we performed an in vivo study comparing Z-Endx with letrozole and tamoxifen in an MCF7 aromatase expressing (MCF7/AC1) cell line. In that model, endoxifen was superior to both tamoxifen and letrozole, and importantly demonstrated substantially greater activity compared to tamoxifen in letrozole resistant MCF7/AC1. In this latter model, we have identified *ESR1* amplification in the letrozole resistant MCF7/AC1 cells (but not parental) consistent with other groups that have demonstrated *ESR1* amplification in MCF7 LTED cells [38]. Additionally, in the letrozole resistant MCF7/AC1 model, we identified substantial alterations in SRC3 after 4 weeks of either tamoxifen or endoxifen treatment (increase in SRC3 phosphorylation with tamoxifen while a decrease in SRC3 phosphorylation with endoxifen. Using the MCF7 letrozole resistant line, we identified that at 1 hour, TAM, 4-HT and estrogen activated SRC3, with additional downstream activation of IGF1R, PDK1, and AKT, as well as ERK and AP1 (c-Jun, c-Fos). Conversely, Z-Endox reduced SRC3 phosphorylation as well as IGF1R, PDK1, AKT, c-Jun and c-Fos (data not shown).

As noted previously, we have demonstrated that endoxifen inhibited PKCβ1 (IC50 350 nM) while the IC50 for TAM was substantially higher (>5 micromolar) and that inhibition of PKCβ1 led to reductions in SRC3 phosphorylation at 1 hour (Goetz *et al* data unpublished).These data suggest that this unique non-genomic effects of endoxifen on PKCβ1 may impart an advantage for endoxifen over tamoxifen, especially in hormonally resistant breast cancer that exhibits activated growth factor signaling.

To be eligible for enrollment in A011023, a biopsy of metastatic disease will be required, and those patients with ER levels > 10% (obtained in the local laboratory using ASCO CAP guidelines) will be eligible. Two additional research cores will be obtained on all patients, and a representative core will be sent to Foundation medicine to analyze all coding exons of 287 cancer-related genes including ESR1 alterations (mutations, amplification and fusions) as well as 78 polymorphisms in 34 ADME-related genes.

Fraser Symmans, MD leads a collaborative effort at M.D. Anderson Cancer Center that is focused on the development of predictive and treatment-prognostic gene expression signatures in breast cancer. The sensitivity to endocrine therapy (SET) index represents the genes with strongest correlation to expression of endocrine receptor gene expression. The SET index has been demonstrated to be prognostic for tamoxifen treatment of node-negative ER+ breast cancer and chemo-endocrine treatment of Stage II-III ER+ breast cancer [46], and also progression-free and overall survival for endocrine treatment of relapsed Stage IV ER+ breast cancer [47]. However, SET index was not prognostic for the natural history of node-negative ER+ breast cancer that did not receive any adjuvant systemic therapy [46]. The SET index is undergoing translation for use with formalin-fixed paraffin-embedded tumor samples and that assay will be applied to this study as a correlative science project.

14.1.2 Objectives

14.1.2.1 To examine whether *ESR1* alterations (defined as either ER activating mutations or ER amplification) (assessed using a biopsy of metastatic disease obtained after progression on AI) is associated with longer PFS or higher response rates in the Z-Endoxifen HCl arm compared to the tamoxifen arm.

14.1.2.2 To examine whether SRC3 (AIB1) or PKCβ1 IHC expression levels (assessed using a biopsy of metastatic disease obtained after progression on AI) is associated with longer PFS or higher response rates in the Z-Endoxifen HCl compared to tamoxifen arm.

14.1.2.3 To examine whether ERα alterations (defined as either ER activating mutations or ER amplification) are associated with longer PFS or higher response rates in the Z-Endoxifen HCl arm compared to the tamoxifen arm.

14.1.3 Methods

Patients are eligible for A011203 if their tumor is determined to be ERα positive. An additional 2-3 research cores will be obtained for the tissue translational studies for consenting patients. A biopsy of metastatic disease for patients that have progressed on prior hormonal therapy may be considered a standard of care, since multiple studies have demonstrated that ER and HER2 expression can change, and this change is associated with differences in DFS and OS [48]. Specifically, tamoxifen is not indicated for ER negative breast cancer. Furthermore, if the tumor is determined to be HER2+, patients would be eligible for anti-HER2 based therapy off study. Therefore, a biopsy at the time of pre-registration is required for all patients for confirmation of ER status. See Section

6.2.3 for details of specimen collection.

A portion of the tissue, corresponding to approximately 75% of the total from the biopsy as described in Section 6.2.3 will be fixed in formalin and sent to the Alliance Biorepository at Ohio State University for these analyses. There, the tumor block (s) will be used for nucleic acid extraction for the following translational objectives: DNA (Foundation medicine) and RNA (SET index) as well as sections cut for IHC analyses of PKCβ1and SRC3.

There, either one or two cores (depending on total volume) will be cut for an H&E (to confirm tumor) followed by 10 unstained sections (10 micron) for DNA (Foundation Medicine) and RNA extraction (SET index). Three 5 micron sections will additionally be cut for assessment of PKCβ1 [49] as well as total and phosphorylated SRC3 using the method previously described [50]. The Foundation Medicine DNA sequencing assay will be performed as previously described [37]. For the SET index, random primer reverse transcription will be performed using the Sensation Plus kit (Affymetrix) and labeled cDNA will be hybridized to Affymetrix U133 gene expression arrays. The SET index will be calculated from the normalized gene expression values using pre-defined methods. SET index will be compared to PFS between and across treatments as a continuous variable and as pre-defined categories of low and high SET index.

The remaining tissue any residual DNA/RNA will be stored in the Alliance Biorepository at Ohio State University. See Section 6.2.3 for shipping instructions.

14.2 DNA alterations in patients treated with either tamoxifen or Z-Endoxifen HCl (Alliance A011203-ST2): Circulating tumor cells and circulating cell free DNA

14.2.1 Background

Comprehensive noninvasive monitoring of genetic alterations over time should help guide use of molecularly targeted drug therapies, including endocrine therapy for breast cancer. If the driving genetic alterations of an individual patient's tumor are known, monitoring those alterations globally through serial blood sampling as opposed to repeated tumor biopsies of a single site would allow clinicians to respond directly to changes in genetic alterations and allow for truly tailored drug selection. This "liquid biopsy" approach is clearly favorable from the perspective of patient comfort, health economics, and maximizing therapeutic efficacy, although controversy remains as to whether circulating tumor cells or circulating cell free DNA is more representative of underlying tumor biology. We propose to evaluate the peripheral blood for specific *ESR1* alterations identified in tumor biopsies and determine whether or not serial changes in those alterations correlate with patient outcomes relative to treatment with either tamoxifen or endoxifen.

Circulating Tumor Cells (CTCs)

That CTCs have biologic relevance in breast cancer is clear, as their relative abundance in the peripheral blood has strong correlations with disease related outcomes in both early stage and advanced disease [51-54]. The development of CTCs as a "liquid biopsy" by which to perform ongoing tumor assessments in relation to treatment exposure is possible with recent technologic advances that allow for the capture of CTCs with epithelial, EMT-like, and/or stem cell like phenotypes, and for molecular characterization by immunocytochemistry, fluorescence in situ hybridization, RT-PCR, and next generation sequencing. Mayo Clinic Rochester has access to several platforms for CTC capture and analysis with the goal of studying the most clinically relevant population of CTCs for a given translational research question.

Circulating Cell Free DNA (cfDNA)

Several studies indicate that circulating cell free DNA (cfDNA) includes representation of key genetic alterations related to cancer progression or resistance to systemic therapy; these alterations include mutations of tumor suppressor genes (e.g., *TP53*) and oncogenes (e.g., *PIK3CA*, *KRAS* and *BRAF*). Commercially available platforms exist with a predefined set of genes and point mutations of interest for use across multiple malignancies. A more flexible system for individualized monitoring is needed. Mayo Clinic Rochester has developed an internal assay for this purpose.

14.2.2 Objectives

14.2.2.1 To assess whether the molecular characteristics identified in the tumor biopsies are detectable in CTCs and/or cfDNA.

14.2.2.2 For each treatment arm, to examine whether ESR1 mutations or amplifications identified in CTCs or cfDNA change over time and explore the impact of these changes on PFS and response rates.

14.2.3 Methods

Minetta Liu, MD leads a collaborative effort at Mayo Clinic Rochester that is focused on the optimization, validation, and implementation of CTC and cfDNA analyses in clinical practice. The platforms, technology, and expertise required to complete the proposed work are validated and available. To reduce patient discomfort and inconvenience, every effort will be made to collect the blood samples in conjunction with venous access for routine blood draws or chemotherapy administration.

Circulating Tumor Cell Capture and Analysis

Two 10 mL whole blood samples will be collected in a K3-EDTA tube at baseline (Cycle 1, Day 1 prior to the start of treatment), end of Cycle 2, (prior to the start of Cycle 3), end of Cycle 8, (prior to the start of Cycle 9) and at the time of disease progression. These samples will be processed within 96 hours of collection using the Cynvenio platform [55] [56] to isolate CTCs. ER expression will be assessed by immunofluorescence. Expression of other genes may also be assessed using RNA based assays. DNA will be extracted from the CTCs for the evaluation of prespecified gene mutations using the same techniques applied to cfDNA (below). See Sections 6.2.4 for shipping instructions.

For those patients with crossover from tamoxifen to Z-Endoxifen HCl, CTC samples will be collected as follows:

1) End of Cycle 2, (prior to the start of Cycle 3) on Z-Endoxifen HCl
2) End of Cycle 8, (prior to the start of Cycle 9) on Z-Endoxifen HCl
3) At the time of disease progression on Z-Endoxifen HCl

Circulating Cell Free DNA Isolation and Analysis

Two 10 mL whole blood samples will be collected in Streck Cell Free DNA BCT tubes at baseline (Cycle 1, Day 1 prior to the start of treatment), end of Cycle 2, (prior to the start of Cycle 3), end of Cycle 8, (prior to the start of Cycle 8) and at the time of disease progression. These samples will be processed within 7 days of collection for cfDNA extraction using the Qiagen QIAmp Circulating Nucleic Acid kit according to manufacturer's specifications. Evaluation for prespecified gene mutations will be performed using digital droplet PCR with the RainDrop platform (raindancetech.com). Circulating free RNA may also be measured. See Sections 6.2.4 for shipping instructions.

For those patients with crossover from tamoxifen to Z-Endoxifen HCl, cfDNA samples will be collected as follows:

1) End of Cycle 2, (prior to the start of Cycle 3) on Z-Endoxifen HCl
2) End of Cycle 8, (prior to the start of Cycle 9) on Z-Endoxifen HCl
3) At the time of disease progression on Z-Endoxifen HCl

14.3 Biochemical markers of bone turnover (Alliance A011203-ST3)

14.3.1 Background

In postmenopausal women, tamoxifen is known to increase bone mineral density and reduce the risk of fracture. Data from our group have now demonstrated that treatment of ovariectomized mice with an anti-cancer dose of endoxifen (50 mg/kg/day) results in substantial increases in bone mineral density and bone mineral content throughout the skeleton [57]. Additionally, we have demonstrated that a moderate dose of endoxifen (10 mg/kg/day) has similar effects in a pre-clinical ovariectomized rat model. Interestingly, this same dose of endoxifen resulted in increased bone mass in intact rats (unpublished data), an effect opposite of that reported for other selective estrogen receptor modulators including tamoxifen and raloxifene. In order to obtain data with regard to the effects of endoxifen on bone, and to directly compare this to the known effects of tamoxifen, we will assess serum procollagen type 1 amino-terminal propeptide (P1NP, a bone formation marker) and C-Telopeptide of Type I Collagen (CTX-1, a bone resorption marker) at baseline, following 6 weeks of treatment and following 6 months of treatment. Samples will be drawn fasting in the AM and the assays will be conducted using validated ELISAs in Dr. Hawse's laboratory.

14.3.2 Objective

For each treatment, to evaluate changes in markers of bone formation and rebsorption after 2 cycles of treatment.

14.3.3 Methods

10ml of whole blood will be collected in a Non-additive red top tube. Serum will be processed and aliquoted according to institutional procedures and subsequently frozen at -20□C or colder until ready to ship to the Alliance Biorepository at Ohio State University. See Section 6.2.5 for details of specimen collection and processing.

The specimens will be collected at the following time points:

1) Cycle 1, Day 1 prior to the start of treatment
2) End of Cycle 2, (prior to the start of Cycle 3)
3) End of Cycle 8, (prior to the start of Cycle 9)
4) At the time of disease progression

For those patients with crossover from tamoxifen to Z-Endoxifen HCl, PK samples will be collected as follows:

1) End of Cycle 2, (prior to the start of Cycle 3) on Z-Endoxifen HCl
2) End of Cycle 8, (prior to the start of Cycle 9) on Z-Endoxifen HCl
3) At the time of disease progression on Z-Endoxifen HCl

Alterations in the levels of bone formation (bone-specific alkaline phosphatase, osteocalcin and P1NP) and resorption (CTX-1, Trap5b and bone sialoprotein) markers will be determined t in duplicate using approximately 50 µL of serum through the use of

commercially available ELISA kits from Immunodiagnostic Systems following the manufacturers protocol.

14.4 Z-Endoxifen HCl and Tamoxifen Pharmacokinetics and Pharmacogenetics (Alliance A011203-PP1)

14.4.1 Background

Population variability of Z-Endoxifen HCl plasma concentrations will be lower following administration of Z-Endoxifen HCl than following administration of tamoxifen. In the ongoing Mayo Phase I trial, a daily dose of 40 mg/day has achieved sustained concentrations of >140 ng/ml Z-Endoxifen HCl after the first dose and >300 ng/ml at steady-state which is achieved 7 days after beginning treatment. In contrast, a daily dose of 100 mg/day has achieved sustained concentrations of > 450 ng/ml Z-Endoxifen HCl after the first dose and >1250 ng/ml at steady-state. However, the variability of steady-state Z-Endoxifen HCl levels has not been ascertained due to the small number of patients treated to date.

Population variability of Z-Endoxifen HCl and tamoxifen plasma concentrations are associated with genetic differences in drug metabolism and transport enzymes. Pharmacogenetic studies will focus on genotyping enzymes involved in the transport and metabolism of Z-Endoxifen HCl and tamoxifen.

The role of *CYP2D6* in the metabolism of tamoxifen to Z-Endoxifen HCL and 4-OH-Tam has been evaluated previously. *CYP2D6* genotyping will be performed to assess the presence/absence of the following variants:

CYP2D6 Allele	Nucleotide Change	Enzyme Metabolism
*1	None (wild type)	(Normal)
*3	2549A>del	No activity
*4	1846G>A	No activity
*5	Gene deletion	No activity
*6	1707T->del	No activity
*10	100C->T	Decreased activity
*17	1023C->T	Decreased activity
*41	-1584C, 2850T, and 988A	Decreased activity
Gene duplication		Depends on the allele duplicated (increased/decreased)

The glucuronidation of tamoxifen and its metabolites has previously been evaluated. The active metabolite, 4-OH-Tam, (but not Z-Endoxifen HCl) appears to undergo both N-glucuronidation and O-glucuronidation. For N-glucuronidation, only UGT1A4 appears to glucuronidate 4-OH-Tam. In contrast, all UGT isoforms were able to O-glucuronidate 4-OH-Tam. The most important enzyme responsible for the O-glucuronidation of the trans isomers of 4-OH-Tam and Z-Endoxifen HCl is UGT2B7. Notably, the rate of O-glucuronidation is significantly lower in human liver microsomal specimens appears known to carry functional UGT2B7 polymorphisms, suggesting that genetic variability in UGT2B7 may affect endoxifen pharmacokinetics.

Additionally, several transporters expressed in cancer cells play important roles in resistance to TAM, and therefore, possibly Z-Endoxifen HCl. Adenosine triphosphate–binding cassette (ABC) transporter B1 (ABCB1; also known as multidrug resistance 1 [MDR1]), ABCC2 (also known as multidrug resistance-associated protein 2 [MRP2]), and ABCG2 (also known as breast cancer resistance protein [BCRP]), may be involved in the transport of Z-Endoxifen HCl, and genetic variation may influence the individual difference in Z-Endoxifen HCl pharmacokinetics and efficacy. In one recently published study of patients receiving TAM in the adjuvant setting, a significant association was found at rs3740065 in ABCC2 (P = .00017; HR = 10.64; 95% CI, 1.44 to 78.88 in patients with AA v GG genotypes).31 Therefore, we will assess whether polymorphisms in ABCB1, ABCC2 and ABCG2 are associated with Z-Endoxifen HCl steady state concentrations and response.

Based on these data, we will develop assays to determine whether functional polymorphisms in UGT [e.g., UGT2B7(268Tyr)] are associated with steady state concentrations of Z-Endoxifen HCl as well as response. Genomic DNA extracted from peripheral blood will be used to assess for key polymorphisms in genes involved in the transport of Z-Endoxifen HCl including but not limited to rs2032582 (2677G>T/A) and rs3213619 (–129T>C) in ABCB1 and rs2273697 (1249G>A) in ABCC2. In addition, we may use the germ line DNA collected to consider other candidate SNPs involved in drug metabolism and transport.

Pharmacodynamic relationships with endoxifen pharmacogenetics and pharmacogenetics will be investigated in an exploratory analysis.

14.4.2 Objectives

To further characterize pharmacokinetics, pharmacogenetics and metabolism of Z-Endoxifen HCl and tamoxifen.

14.4.3 Methods

14.4.3.1 Blood samples for pharmacokinetics will be collected in all patients treated with Z-Endoxifen HCl or tamoxifen, who have consented, according to the following schedule:

- Cycle 1 Day 1: Prior to drug administration
- Cycle 1 Day 1: 2-4 hours after drug administration
- Cycle 1 Day 1: 4-6 hours after drug administration **Note:** Do not draw samples within 2 hours of each other.)
- Cycle 1 Day 2: Prior to drug administration
- End of Cycle 2, (prior to the start of Cycle 3)
- End of Cycle 8, (prior to the start of Cycle 9)
- At the time of disease progression

For those patients with crossover from tamoxifen to Z-Endoxifen HCl, PK samples will be collected as follows:

- End of Cycle 2, (prior to the start of Cycle 3) on Z-Endoxifen HCl
- End of Cycle 8, (prior to the start of Cycle 9) on Z-Endoxifen HCl
- At the time of disease progression on Z-Endoxifen HCl

NOTE: Samples are light sensitive. Use amber tubes or cover tubes with aluminum foil to prevent exposure to light.

Pharmacokinetics blood samples will be collected in 6 ml EDTA (purple top) tubes, wrapped in aluminum foil and immediately cooled in an ice water bath. Blood must be processed for plasma isolation within 20 minutes of collection.

Blood will be subjected to centrifugation (2,000 rpm for 10 minutes) in a refrigerated centrifuge kept at 4°C. Following centrifugation, the plasma will be transferred to polypropylene tubes, capped, wrapped in aluminum foil and immediately frozen at -70°C. Tube labels must contain the following data:

- Patient identification number
- Date and time (24-hour clock)) of collection

The Pharmacokinetic samples will be sent to BAP Freezer at the Mayo Clinic (see Section 6.2.7).

The plasma concentration-time data will be will be analyzed by non-linear mixed effects modeling using the program NONMEM (Pharsight Corp., Mountainview, CA) to determine clearance, volume of distribution and contributions of inter- and intra-subject variability.

14.4.3.2 All patients treated with Z-Endoxifen HCl or tamoxifen, who have consented, will provide a single blood sample for pharmacogenetic analysis.

Genomic DNA will be extracted by the Alliance Biorepository at CTSU from peripheral blood collected prior to treatment. Genotyping for key polymorphisms in genes involved in conjugation and transport of Z-Endoxifen HCl will be analyzed.

Genotyping assays primarily incorporate polymerase chain reactions (PCR) to amplify DNA regions of interest using sequence-specific primers. Polymorphic variations are detected by several methods, including DNA sequencing, fragment size analysis (with GeneScan) and/or fluorescent probe detection methods. Allelic variant assays are established with appropriate validation (using Corriel ethnic DNA panels, Hardy-Weinburg equilibrium and ethnic frequencies), and applied to DNA samples from protocol patients.

The Pharmacogenetic samples will be sent to Alliance Biorepository at Ohio State University.

15.0 GENERAL REGULATORY CONSIDERATIONS AND CREDENTIALING

There are no credentialing requirements for this study.

16.0 REFERENCES

1. Siegel, R., D. Naishadham, and A. Jemal, *Cancer statistics, 2013.* CA Cancer J Clin, 2013. **63**(1): p. 11-30.
2. Forbes, J.F., et al., *Effect of anastrozole and tamoxifen as adjuvant treatment for early-stage breast cancer: 100-month analysis of the ATAC trial.* The lancet oncology, 2008. **9**(1): p. 45-53.
3. Regan, M.M., et al., *Assessment of letrozole and tamoxifen alone and in sequence for postmenopausal women with steroid hormone receptor-positive breast cancer: the BIG 1-98 randomised clinical trial at 8.1 years median follow-up.* The lancet oncology, 2011. **12**(12): p. 1101-8.
4. Stearns, V., et al., *Active tamoxifen metabolite plasma concentrations after coadministration of tamoxifen and the selective serotonin reuptake inhibitor paroxetine.* J Natl Cancer Inst, 2003. **95**(23): p. 1758-64.
5. Murdter, T.E., et al., *Activity levels of tamoxifen metabolites at the estrogen receptor and the impact of genetic polymorphisms of phase I and II enzymes on their concentration levels in plasma.* Clinical pharmacology and therapeutics, 2011. **89**(5): p. 708-17.
6. Goetz, M.P., et al., *Pharmacogenetics of tamoxifen biotransformation is associated with clinical outcomes of efficacy and hot flashes.* J Clin Oncol, 2005. **23**(36): p. 9312-8.
7. Rae, J.M., et al., *CYP2D6 and UGT2B7 Genotype and Risk of Recurrence in Tamoxifen-Treated Breast Cancer Patients.* Journal of the National Cancer Institute, 2012. **104**(6): p. 452-460.
8. Regan, M.M., et al., *CYP2D6 Genotype and Tamoxifen Response in Postmenopausal Women with Endocrine-Responsive Breast Cancer: The Breast International Group 1-98 Trial.* Journal of the National Cancer Institute, 2012. **104**(6): p. 441-451.
9. Goetz, M.P., et al., *CYP2D6 metabolism and patient outcome in the Austrian Breast and Colorectal Cancer Study Group trial (ABCSG) 8.* Clinical cancer research : an official journal of the American Association for Cancer Research, 2013. **19**(2): p. 500-7.
10. Madlensky, L., et al., *Tamoxifen metabolite concentrations, CYP2D6 genotype, and breast cancer outcomes.* Clinical pharmacology and therapeutics, 2011. **89**(5): p. 718-25.
11. Karle, J., et al., *Influence of CYP2D6-genotype on tamoxifen efficacy in advanced breast cancer.* Breast cancer research and treatment, 2013. **139**(2): p. 553-60.
12. Robinson, D.R., et al., *Activating ESR1 mutations in hormone-resistant metastatic breast cancer.* Nature genetics, 2013. **45**(12): p. 1446-51.
13. Davies, C., et al., *Relevance of breast cancer hormone receptors and other factors to the efficacy of adjuvant tamoxifen: patient-level meta-analysis of randomised trials.* Lancet, 2011. **378**(9793): p. 771-84.
14. Davies, C., et al., *Long-term effects of continuing adjuvant tamoxifen to 10 years versus stopping at 5 years after diagnosis of oestrogen receptor-positive breast cancer: ATLAS, a randomised trial.* Lancet, 2013. **381**(9869): p. 805-16.
15. Burstein, H.J. and J.J. Griggs, *Deep time: the long and the short of adjuvant endocrine therapy for breast cancer.* Journal of clinical oncology : official journal of the American Society of Clinical Oncology, 2012. **30**(7): p. 684-6.
16. Xu, H.B., Y.J. Liu, and L. Li, *Aromatase inhibitor versus tamoxifen in postmenopausal woman with advanced breast cancer: a literature-based meta-analysis.* Clinical breast cancer, 2011. **11**(4): p. 246-51.
17. Howell, A., et al., *Comparison of fulvestrant versus tamoxifen for the treatment of advanced breast cancer in postmenopausal women previously untreated with endocrine therapy: a multinational, double-blind, randomized trial.* J Clin Oncol, 2004. **22**(9): p. 1605-13.
18. *Superiority of sequential methotrexate, fluorouracil, and leucovorin to fluorouracil alone in advanced symptomatic colorectal carcinoma: a randomized trial. The Nordic Gastrointestinal Tumor Adjuvant Therapy Group.* J Clin Oncol, 1989. **7**(10): p. 1437-46.

19. Di Leo, A., et al., *Results of the CONFIRM phase III trial comparing fulvestrant 250 mg with fulvestrant 500 mg in postmenopausal women with estrogen receptor-positive advanced breast cancer.* Journal of clinical oncology : official journal of the American Society of Clinical Oncology, 2010. **28**(30): p. 4594-600.
20. Bergh, J., et al., *FACT: an open-label randomized phase III study of fulvestrant and anastrozole in combination compared with anastrozole alone as first-line therapy for patients with receptor-positive postmenopausal breast cancer.* Journal of clinical oncology : official journal of the American Society of Clinical Oncology, 2012. **30**(16): p. 1919-25.
21. Johnston, S.R., et al., *Fulvestrant plus anastrozole or placebo versus exemestane alone after progression on non-steroidal aromatase inhibitors in postmenopausal patients with hormone-receptor-positive locally advanced or metastatic breast cancer (SoFEA): a composite, multicentre, phase 3 randomised trial.* The lancet oncology, 2013. **14**(10): p. 989-98.
22. Mehta, R.S., et al., *Combination anastrozole and fulvestrant in metastatic breast cancer.* The New England journal of medicine, 2012. **367**(5): p. 435-44.
23. Osborne, C.K., et al., *Tamoxifen and the isomers of 4-hydroxytamoxifen in tamoxifen-resistant tumors from breast cancer patients.* J Clin Oncol, 1992. **10**(2): p. 304-10.
24. Miller T, e.a., *Hyperactivation of phosphatidylinositol-3 kinase promotes escape from hormone dependence in estrogen receptor-positive human breast cancer.* J Clin Invest, 2010. **Jul 120**((7)): p. 2406-13.
25. Bachelot, T., et al., *Randomized Phase II Trial of Everolimus in Combination With Tamoxifen in Patients With Hormone Receptor-Positive, Human Epidermal Growth Factor Receptor 2-Negative Metastatic Breast Cancer With Prior Exposure to Aromatase Inhibitors: A GINECO Study.* Journal of clinical oncology : official journal of the American Society of Clinical Oncology, 2012.
26. Baselga, J., et al., *Everolimus in postmenopausal hormone-receptor-positive advanced breast cancer.* The New England journal of medicine, 2012. **366**(6): p. 520-9.
27. Wolff AC, e.a., *Randomized phase III placebo-controled trial of letrozole plus oral temsirolimus as first-line endocrine therapy in postmemopausal women with locally advanced or metastatic beast cancer.* J Clin Onc, 2013. **31**: p. 195-202.
28. Rugo, H.S., et al., *Incidence and time course of everolimus-related adverse events in postmenopausal women with hormone receptor-positive advanced breast cancer: insights from BOLERO-2.* Annals of oncology : official journal of the European Society for Medical Oncology / ESMO, 2014. **25**(4): p. 808-15.
29. Shou, J., et al., *Mechanisms of tamoxifen resistance: increased estrogen receptor-HER2/neu cross-talk in ER/HER2-positive breast cancer.* J Natl Cancer Inst, 2004. **96**(12): p. 926-35.
30. Osborne, C.K., et al., *Role of the estrogen receptor coactivator AIB1 (SRC-3) and HER-2/neu in tamoxifen resistance in breast cancer.* J Natl Cancer Inst, 2003. **95**(5): p. 353-61.
31. Smith, I.E., et al., *Neoadjuvant treatment of postmenopausal breast cancer with anastrozole, tamoxifen, or both in combination: the Immediate Preoperative Anastrozole, Tamoxifen, or Combined with Tamoxifen (IMPACT) multicenter double-blind randomized trial.* J Clin Oncol, 2005. **23**(22): p. 5108-16.
32. Britton, D.J., et al., *Bidirectional cross talk between ERalpha and EGFR signalling pathways regulates tamoxifen-resistant growth.* Breast Cancer Res Treat, 2006. **96**(2): p. 131-46.
33. Coutts, A.S. and L.C. Murphy, *Elevated mitogen-activated protein kinase activity in estrogen-nonresponsive human breast cancer cells.* Cancer Res, 1998. **58**(18): p. 4071-4.
34. Donovan, J.C., A. Milic, and J.M. Slingerland, *Constitutive MEK/MAPK activation leads to p27(Kip1) deregulation and antiestrogen resistance in human breast cancer cells.* J Biol Chem, 2001. **276**(44): p. 40888-95.
35. Jin, Y., et al., *CYP2D6 genotype, antidepressant use, and tamoxifen metabolism during adjuvant breast cancer treatment.* J Natl Cancer Inst, 2005. **97**(1): p. 30-9.

36. Long, B.J., et al., *Therapeutic strategies using the aromatase inhibitor letrozole and tamoxifen in a breast cancer model.* J Natl Cancer Inst, 2004. **96**(6): p. 456-65.
37. Jeselsohn, R., et al., *Emergence of constitutively active estrogen receptor-alpha mutations in pretreated advanced estrogen receptor positive breast cancer.* Clinical cancer research : an official journal of the American Association for Cancer Research, 2014.
38. Li, S., et al., *Endocrine-therapy-resistant ESR1 variants revealed by genomic characterization of breast-cancer-derived xenografts.* Cell reports, 2013. **4**(6): p. 1116-30.
39. Tan, A., Novotny,P, et al., *A patient-level meta-analytic investigation of the prognostic significance of baseline quality of life (QOL) for overall survival (OS) among 3,704 patients participating in 24 North Central Cancer Treatment Group (NCCTG) and Mayo Clinic Cancer Center (MC) oncology clinical trials." J Clin Oncol 26.; Sloan JA, Liu H, Sargent DJ, Satele D, Schaefer PL, Halyard MY, Grothey A, Garces YI, Brown PD, Loprinzi CL, Buckner JC. A patient-level pooled analysis of the prognostic significance of baseline fatigue for overall survival (OS) among 3,915 patients participating in 43 North Central Cancer Treatment Group (NCCTG) and Mayo Clinic Cancer Center (MC) oncology clinical trials. J Clin Oncol 2009; 27(507s)*2008.
40. al., S.J.e., *A patient-level pooled analysis of the prognostic significance of baseline fatigue for overall survival (OS) among 3,915 patients participating in 43 North Central Cancer Treatment Group (NCCTG) and Mayo Clinic Cancer Center (MC) oncology clinical trials.* J Clin Onc, 2009. **27**((507s)).
41. Stone AM, B.W., et al., *Research outcomes and recommendations for the assessment of progression in cancer clinical trials form a PhRMA working group.* Eu,, 2011. **47**: p. 1763-1771.
42. Stone A, W.C., Carroll K and Barge A., *Optimizing randomized phase II trials assessing tumor progression.* Contemporary Clinical Trials, 2007. **28**: p. 146-152.
43. Freidlin B, K.E.e.a., *Proposal for the use of progression-free survival in unblinded randomized trials. .* JCO, 2007. **25** ((50)): p. 2122-2126.
44. *BOLERO slides.* 2013.
45. Frampton GM, e.a., *Develompent and validation of a clinical cancer genomic profiling test based on mssively parallel DNA sequencing.* Nature Biotechnology, 2013. **31**: p. 1023-1031.
46. Symmans W, e.a., *Genomic indes of sensitivity to endocrien therapy for breast cancer.* JCO, 2010. **28**((27)): p. 4111-4119.
47. Symmans W, e.a., *Progression of genomic signatures in local and metastatic estrogen receptor-positive (ER+) breast cancer: Relevance to palliative treatment.* Proc ASCO, 2012.
48. Redmond AM, e.a., *Coassociations of estrogen receptor and p160 proteins predicts resistance to endocrine treatment; SRC-1 is an independent predictor of breast cancer recurrence. .* Clinical cancer research: an official journal of th American Association for Cancer Research, 2009. **15**: p. 2098-106.
49. Aaltonen, V., et al., *Heterogeneity of cellular proliferation within transitional cell carcinoma: correlation of protein kinase C alpha/betaI expression and activity.* The journal of histochemistry and cytochemistry : official journal of the Histochemistry Society, 2006. **54**(7): p. 795-806.
50. Spears, M., et al., *The p160 ER co-regulators predict outcome in ER negative breast cancer.* Breast cancer research and treatment, 2012. **131**(2): p. 463-72.
51. Cristofanilli M, B.G., Ellis MJ, et al., *Circulating tumor cells, disease progression, and survival in metastatic breast cancer.* New Engl JMed, 2004. **351**: p. 781-791.
52. Hayes DF, C.M., Budd GT, et al., *Circulating Tumor Cells at Each Follow-up Time Point during Therapy of Metastatic Breast Cancer Patients Predict Progression-Free and Overall Survival.* Clin Cancer Res, 2006. **12**: p. 4218-4224.
53. Liu MC, S.P., Warren RD, et al., *Circulating tumor cells: a useful predictor of treatment efficacy in metastatic breast cancer.* Clin Cancer Res, 2009. **27**: p. 5153-5159.

54. Bidard FC, P.D., Fehm T, et al, *Clinical validity of circulating tumour cells in patients with metastatic breast cancer: a pooled analysis of individual patient data.* Lancet Oncology, 2014. **15**: p. 406-14.
55. Winer-Jones J, e.a., *Circulating tumor cells: Clinically relavant molecular access based on a novel CTC flow cell.* PLOS One, 2014. **10.1371/journal.pone.0086717**.
56. Strauss WM, e.a., *Molecular platforms for mutation analysis from whole blood derived clinical samples by nextgen sequencing.* AACR Abstract #2846, 2014.
57. Gingery, A., et al., *The effects of a novel hormonal breast cancer therapy, endoxifen, on the mouse skeleton.* PLoS One, 2014. **9**(5): p. e98219.

APPENDIX I REGISTRATION FATIGUE/UNISCALE ASSESSMENTS

Registration Fatigue/Uniscale Assessments

At patient registration, this form is to be administered by a nurse/CRA, completed by the patient, and recorded on the Registration Fatigue/Uniscale Assessments Form (see Forms Packet).

If needed, this appendix can be adapted to use as a source document. A booklet containing this assessment does not exist – please do not order this booklet.

How would you describe:

Your level of fatigue, on the average in the past week including today?

0	1	2	3	4	5	6	7	8	9	10
No Fatigue										Fatigue as bad as it can be

Your overall quality of life in the past week including today?

0	1	2	3	4	5	6	7	8	9	10
As bad as it can be										As good as it can be

APPENDIX II: LIST OF POTENT CYP450 INDUCERS/INHIBITORS

Inhibitors				
1A2	**2C8**	**2C9**	**2D6**	**2A4,5,7**
Fluvoxamine Ciprofloxacin	Gemfibozil	Fluconazole	Buproion Fluoxetine Paroxetine Quindine	HIV Antivirals: Indinavir Nelfinavir Ritonavir Clarithromycin Itraconazole Ketoconazole Nefazodone Saquinavir Telithromycin

Inducers							
1A2	**2B6**	**2C8**	**2C9**	**2C19**	**2D6**	**2E**	**3A4,5,7**
Broccoli Brussel sprouts Insulin Methylcholanthrene Modafinil Nafcillin Beta-naphthoflavone Omeprazole Tabacco	Phenobarbital Rifampin	Rifampin	Rifanpin Secobarbital	Carbamazine Norethindrone Predisone Rifampin	Dexamethaxone Rifampin	Ethanol Isoniazid	HIV Anti-virals: Efavirenz Barbiturates Carbamzepine Glucocorticoids Modafinil Oxcarbazepine Phenobarbital Phenytoin Pioglitazone Rifabutin St. John's wort Troglitazone

The tables above do not contain comprehensive lists of potent CYP450 inducers and inhibitors. Refer to other credible information sources for up-to-date information

APPENDIX III: PATIENT MEDICATION LOGS

Z-Endoxifen HCL Medication Log (Arms 1 and Crossover Group)

Number of Capsules Given:_____ Capsule Bottle(s) returned: Circle Yes or No
Total Daily Dose:_____ Number of Capsules returned:_____

(To be completed by RN)

PLEASE FILL OUT AND BRING THIS SHEET TO ALL VISITS.

SPECIAL INSTRUCTIONS

1. Take _____, 40 mg Z-Endoxifen HCl capsules by mouth daily, at approximately the same time each day on an empty stomach, either 1 hour before or 2 hours after meals. The capsules should be swallowed whole and must not be crushed or broken.
2. If a dose is missed, please take the dose as soon as possible, but only if there are 12 or more hours remaining before the next dose.
    a. If the dose is due in less than 12 hours, skip the missed dose and take the next dose as scheduled.
    b. Remember to record missed doses.
3. If vomiting occurs after taking Z-Endoxifen HCl, do not take a replacement dose on that day. Resume at the next scheduled dose.
4. Z-Endoxifen capsules should be stored in the refrigerator.

CYCLE #: _____ # of WEEKS_____

DAY	Medication	DATE	TIME		Number of 40 mg capsules taken	Comments
Example	*Z-Endoxifen HCl*	*07/01/2012*	*9:00*	*AM/PM*	*2*	
1	Z-Endoxifen HCl			*AM/PM*		
2	Z-Endoxifen HCl			*AM/PM*		
3	Z-Endoxifen HCl			*AM/PM*		
4	Z-Endoxifen HCl			*AM/PM*		
5	Z-Endoxifen HCl			*AM/PM*		
6	Z-Endoxifen HCl			*AM/PM*		
7	Z-Endoxifen HCl			*AM/PM*		
8	Z-Endoxifen HCl			*AM/PM*		
9	Z-Endoxifen HCl			*AM/PM/*		
10	Z-Endoxifen HCl			*AM/PM*		
11	Z-Endoxifen HCl			*AM/PM*		
12	Z-Endoxifen HCl			*AM/PM*		
13	Z-Endoxifen HCl			*AM/PM*		
14	Z-Endoxifen HCl			*AM/PM*		
15	Z-Endoxifen HCl			*AM/PM*		
16	Z-Endoxifen HCl			*AM/PM*		
17	Z-Endoxifen HCl			*AM/PM*		
18	Z-Endoxifen HCl			*AM/PM*		
19	Z-Endoxifen HCl			*AM/PM*		
20	Z-Endoxifen HCl			*AM/PM*		
21	Z-Endoxifen HCl			*AM/PM*		

Patient Signature: _____Date:_____
Consenting Professional/Research RN Signature: _____Date:_____
Comments:_____

Tamoxifen Medication Log (Arm 2)

Number of Pills Given:_____
Total Daily Dose:_____

Pill Bottle(s) returned: Circle Yes or No
Number of Pills returned:_____

(To be completed by RN)

PLEASE FILL OUT AND BRING THIS SHEET TO ALL VISITS.

SPECIAL INSTRUCTIONS

1. Take one (1) 20 mg tablet of Tamoxifen by mouth each day without regard to meals. The tablets should be swallowed whole and must not be crushed or broken.
2. If a dose is missed, please take the dose as soon as possible, but only if there are 12 or more hours remaining before the next dose
    a. If the dose is due in less than 12 hours, skip the missed dose and take the next dose as scheduled
3. If vomiting occurs after taking Tamoxifen, do not take a replacement dose on that day. Resume at the next scheduled dose.
4. Tamoxifen tablets should be stored at room temperature.

CYCLE #: _____ # of WEEKS_____

DAY	Medication	DATE	TIME		Number of 20 mg tablets taken	Comments
Example	*Tamoxifen*	*07/01/2012*	*9:00*	*AM/PM*	*1*	
1	Tamoxifen			*AM/PM*		
2	Tamoxifen			*AM/PM*		
3	Tamoxifen			*AM/PM*		
4	Tamoxifen			*AM/PM*		
5	Tamoxifen			*AM/PM*		
6	Tamoxifen			*AM/PM*		
7	Tamoxifen			*AM/PM*		
8	Tamoxifen			*AM/PM*		
9	Tamoxifen			*AM/PM*		
10	Tamoxifen			*AM/PM*		
11	Tamoxifen			*AM/PM*		
12	Tamoxifen			*AM/PM*		
13	Tamoxifen			*AM/PM*		
14	Tamoxifen			*AM/PM*		
15	Tamoxifen			*AM/PM*		
16	Tamoxifen			*AM/PM*		
17	Tamoxifen			*AM/PM*		
18	Tamoxifen			*AM/PM*		
19	Tamoxifen			*AM/PM*		
20	Tamoxifen			*AM/PM*		
21	Tamoxifen			*AM/PM*		

Patient Signature: _____Date:_____
Consenting Professional/Research RN Signature: _____Date:_____
Comments:_____

APPENDIX IV: COLLABORATIVE AGREEMENT

The agent(s) supplied by CTEP, DCTD, NCI used in this protocol is/are provided to the NCI under a Collaborative Agreement (CRADA, CTA, CSA) between the Pharmaceutical Company(ies) (hereinafter referred to as "Collaborator(s)") and the NCI Division of Cancer Treatment and Diagnosis. Therefore, the following obligations/guidelines, in addition to the provisions in the "Intellectual Property Option to Collaborator" (http://ctep.cancer.gov/industryCollaborations2/intellectual_property.htm) contained within the terms of award, apply to the use of the Agent(s) in this study:

1. Agent(s) may not be used for any purpose outside the scope of this protocol, nor can Agent(s) be transferred or licensed to any party not participating in the clinical study. Collaborator(s) data for Agent(s) are confidential and proprietary to Collaborator(s) and shall be maintained as such by the investigators. The protocol documents for studies utilizing Agents contain confidential information and should not be shared or distributed without the permission of the NCI. If a copy of this protocol is requested by a patient or patient's family member participating on the study, the individual should sign a confidentiality agreement. A suitable model agreement can be downloaded from: http://ctep.cancer.gov.

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

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

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

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

3. Clinical Trial Data and Results and Raw Data developed under a Collaborative Agreement will be made available to Collaborator(s), the NCI, and the FDA, as appropriate and unless additional disclosure is required by law or court order as described in the IP Option to Collaborator (http://ctep.cancer.gov/industryCollaborations2/intellectual_property.htm). Additionally, all Clinical Data and Results and Raw Data will be collected, used and disclosed consistent with all applicable federal statutes and regulations for the protection of human subjects, including, if applicable, the *Standards for Privacy of Individually Identifiable Health Information* set forth in 45 C.F.R. Part 164.

4. When a Collaborator wishes to initiate a data request, the request should first be sent to the NCI, who will then notify the appropriate investigators (Group Chair for

Cooperative Group studies, or PI for other studies) of Collaborator's wish to contact them.

5. Any data provided to Collaborator(s) for Phase 3 studies must be in accordance with the guidelines and policies of the responsible Data Monitoring Committee (DMC), if there is a DMC for this clinical trial.

6. Any manuscripts reporting the results of this clinical trial must be provided to CTEP by the Group office for Cooperative Group studies or by the principal investigator for non-Cooperative Group studies for immediate delivery to Collaborator(s) for advisory review and comment prior to submission for publication. Collaborator(s) will have 30 days from the date of receipt for review. Collaborator shall have the right to request that publication be delayed for up to an additional 30 days in order to ensure that Collaborator's confidential and proprietary data, in addition to Collaborator(s)'s intellectual property rights, are protected. Copies of abstracts must be provided to CTEP for forwarding to Collaborator(s) for courtesy review as soon as possible and preferably at least three (3) days prior to submission, but in any case, prior to presentation at the meeting or publication in the proceedings. Press releases and other media presentations must also be forwarded to CTEP prior to release. Copies of any manuscript, abstract and/or press release/ media presentation should be sent to:

   Email: ncicteppubs@mail.nih.gov

   The Regulatory Affairs Branch will then distribute them to Collaborator(s). No publication, manuscript or other form of public disclosure shall contain any of Collaborator's confidential/ proprietary information.