A Single Arm Phase II Study of Palbociclib in Combination with Tamoxifen as First Line Therapy for Metastatic Hormone Receptor Positive Breast Cancer:

Big Ten Cancer Research Consortium BTCRC-BRE15-016

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I confirm I have read this protocol, I understand it, and I will work according to this protocol and to the ethical principles stated in the latest version of the Declaration of Helsinki, the applicable guidelines for good clinical practices, or the applicable laws and regulations of the country of the study site for which I am responsible, whichever provides the greater protection of the individual. I will accept the monitor's overseeing of the study. I will promptly submit the protocol to applicable ethical review board(s).

Instructions to the investigator: Please **SIGN** and **DATE** this signature page. **PRINT** your name and title, the name and location of the facility in which the study will be conducted, and the expected IRB approval date. Scan and email the completed form to BTCRC Administrative Headquarters and keep a record for your files.

_____ _____

Signature of Site Investigator Date

_____

Site Investigator Name (printed)

_____

Site Investigator Title

_____

Name of Facility

_____

Location of Facility (City and State)

_____ ❑ Not Submitting to IRB

Expected IRB Approval Date

PLEASE COMPLETE AND EMAIL TO BTCRC ADMINISTRATIVE HEADQUARTERS

STUDY SYNOPSIS

TITLE	A Single Arm Phase II Study of Palbociclib in Combination with Tamoxifen as First Line Therapy for Metastatic Hormone Receptor Positive Breast Cancer: Big Ten Cancer Research Consortium BTCRC-BRE15-016
PHASE	Phase II
OBJECTIVES	**Primary Objectives:** To estimate the activity of the combination of palbociclib and tamoxifen in first line therapy for subjects with HR(+)/HER2(-) advanced breast cancer who have not received prior systemic anti-cancer therapies for their advanced/metastatic disease, assessed by progression free survival (PFS) **Secondary Objective(s):** To evaluate: • safety and tolerability • response rates (complete or partial response) (RR) based on RECIST 1.1 or MDA Criteria (for patients with bone only disease) • clinical benefit rate (CBR; complete, partial response, or stable disease lasting 24 weeks or longer) based on RECIST 1.1 or MDA Criteria • 2-year overall survival (OS) **Correlative Objectives, if funding is secured:** • Perform proteomic analysis of plasma exosomes to identify potential mechanisms of primary and secondary resistance to tamoxifen/palbociclib. • Analyze tumor specimens for protein expression of candidate markers of primary resistance. • Compare difference in response for subjects presenting with de novo metastatic breast cancer to subjects that progressed while on aromatase inhibitors • Compare differences in response and toxicity profile in African American and Hispanic subjects to Caucasian subjects
STUDY DESIGN	Single arm phase II study
TOTAL NUMBER OF SUBJECTS	Up to 48 subjects
KEY ELIGIBILITY CRITERIA	1. Age ≥ 18 years 2. Locally advanced, locoregionally recurrent, or metastatic disease, not amenable to curative therapy 3. Histologically and/or cytologically confirmed diagnosis of ER positive and/or PR positive (ER>1%, PR>1%), Her2 negative breast cancer 4. No prior systemic anti-cancer therapy for advanced HR+ positive disease 5. Eastern Cooperative Oncology Group PS 0-2 6. Life expectancy greater than 4 months 7. Pre or postmenopausal women are eligible 8. Adequate hematologic function, as defined as meeting **all** three of the following criteria: a. Absolute neutrophil count (ANC) ≥ 1.5×10^9/L, and b. Platelets ≥ 100×10^9/L, and

	c. Hemoglobin (Hgb) ≥ 9.0 g/dL 9. Adequate renal function, as defined by either of the following criteria: a. Serum creatinine ≤ 1.5 × upper limit of normal (ULN), b. OR, if serum creatinine > 1.5 × ULN, estimated glomerular filtration rate (eGFR) ≥ 40 mL/min 10. Adequate hepatic function, as defined by all of the following: a. aspartate aminotransferase (AST) ≤ 2.5 × ULN or ≤ 5 × ULN for subjects with known hepatic metastases and b. alanine aminotransferase (ALT) ≤ 2.5 × ULN or ≤ 5 × ULN for subjects with known hepatic metastases and c. Total serum bilirubin ≤ 1.5 × ULN 11. Metastatic disease evaluable on imaging studies. Subject may have measurable disease as per RECIST 1.1 or bone-only disease as per MDA criteria. Bone-only subjects are also eligible if their disease can be documented/ evaluated by bone scans, PET or MRI 12. Negative pregnancy test for female subjects with reproductive potential, and agreement of all female subjects of reproductive potential to use a reliable form of contraception during the study and for 120 days after the last dose of study drug. 13. Willingness and ability to comply with scheduled visits, treatment plans, laboratory tests, and other study procedures. 14. Signed and dated informed consent document indicating that the subject (or legally acceptable representative) has been informed of all pertinent aspects of the trial prior to enrollment.
OUTCOME MEASURES	Progression free survival (PFS)
STATISTICAL CONSIDERATIONS	The primary endpoint is progression free survival (PFS) as assessed by RECIST v1.1 criteria Previous studies identified progression free survival with tamoxifen of 15 months in first line treatment for breast cancer. With assumptions of 80% power to detect median 25 months progression free survival in the combination treatment over historic data on single agent tamoxifen activity in metastatic breast cancer, the sample size will be 48. This sample size assumes 20% censoring rate due to loss to follow up, unevaluable, or drop out.
ENROLLMENT PERIOD	Estimated 24 months
TOTAL STUDY DURATION	Estimated 48 months

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SCHEMA

A Single Arm Phase II Study of Palbociclib in Combination with Tamoxifen as First Line Therapy for Metastatic Hormone Receptor Positive Breast Cancer
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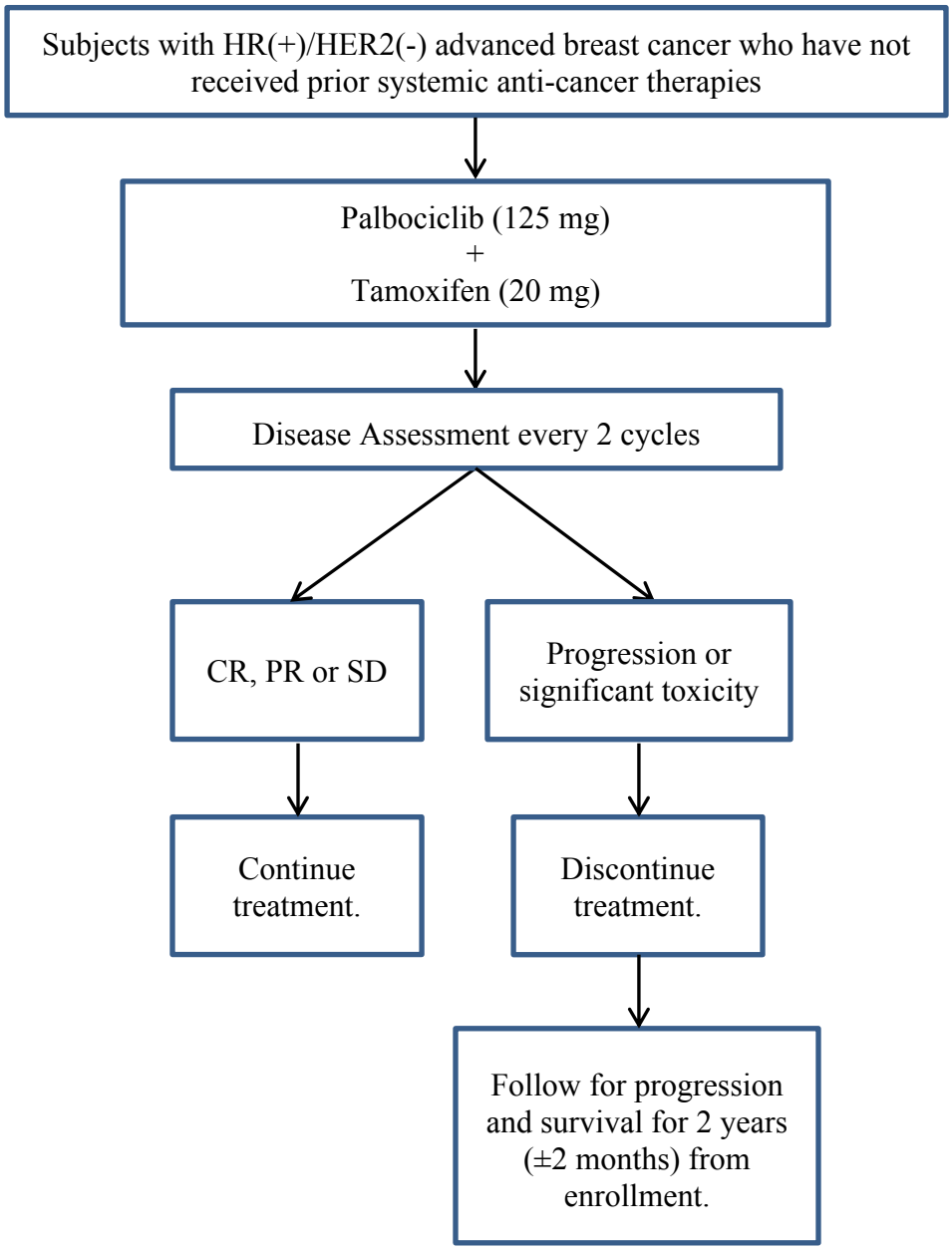

Subjects will be treated with palbociclib (po) at a 125 mg dose on days 1-21 of the 28-day cycle and with 20 mg tamoxifen (po) once a day continuously. Premenopausal subjects may also receive treatment with Goserelin (3.6 mg sc every 28 days or 10.8 mg sc every 3 months) or equivalent (e.g. Lupron) on day 1 of every treatment cycle.

ABBREVIATIONS

Abbreviation	Definition
AE	adverse event
AHQ	Administrative Headquarters
AI	aromatase inhibitor
ALT	alanine aminotransferase
ANC	absolute neutrophil count
ASCO	American Society of Clinical Oncology
AST	aspartate aminotransferase
ATE	arterial thromboembolic event
AUC	area under the curve
BMP	basic metabolic panel
BTCRC	Big Ten Cancer Research Consortium
CBC	complete blood cell count
CDK	cyclin-dependent kinase
CI	confidence interval
CLM	Correlative Laboratory Manual
cm	centimeter
CR	complete response
CT	computed tomography
CTC	cytotoxic T cell
CTCAE	(NCI) Common Terminology Criteria for Adverse Events
CTEP	Cancer Therapy Evaluation Program
CYP	cytochrome P450 enzyme
DCR	disease control rate
dL	deciliter
DLT	dose-limiting toxicity
DMBA	dimethylbenzanthracene
ECOG	Eastern Cooperative Oncology Group
eCRF	electronic case report form
EDC	electronic data capture
eGFR	Estimated Glomerular Filtration Rate
ER+	estrogen receptor positive
FDA	Food and Drug Administration
GI	gastrointestinal
GnRH	gonadotropin-releasing hormone
HER2-	human epidermal growth factor receptor 2 negative
Hgb	hemoglobin
HIPAA	Health Insurance Portability and Accountability Act
HIV	human immunodeficiency virus
hr	hour
HR	hazard ratio
HR+	hormone receptor positive

Abbreviation	Definition
ICH	International Conference on Harmonization
IEC	Independent Ethics Committee
IHC	immunohistochemistry
IND	Investigational New Drug
I/O	input/output
IRB	Institutional Review Board
IUD	intrauterine device
kg	kilogram
lb	pound
LMWH	low-molecular-weight heparin
mg	milligram
min	minute
mL	milliliter
mm^3	cubic millimeters
MRI	magnetic resonance imaging
NCI	National Cancer Institute
NDC	National Drug Code
OS	overall survival
PD	progressive disease
PIK3CA	phosphatidylinositol-4,5-bisphosphate 3-kinase, catalytic subunit alpha
PFS	progression-free survival
PR	partial response
PR	progesterone receptor
PS	performance status
Rb	retinoblastomaprotein
RECIST	Response Evaluation Criteria in Solid Tumors
RR	response rate
SAE	serious adverse event
SD	stable disease
SULT	sulfotransferase
T3	triiodothyronine
T4	thyroxine
TSH	thyroid-stimulating hormone
ULN	upper limit of normal
UPIRSO	unanticipated problems involving risk to subjects or others
US	United States
USP	United States Pharmacopeia
WHO	World Health Organization
WOCP	women of childbearing potential
wt	weight

1. BACKGROUND & RATIONALE

1.1. Introduction

The efficacy of endocrine therapy needs to be improved in patients with hormone receptor positive metastatic breast cancer.
Breast cancer is the most common cancer in women worldwide and more than 1.5 million new breast cancer cases are reported each year. Invasive breast cancer is classified based on the presence of estrogen receptor (ER), progesterone receptor (PR) and human epidermal growth factor receptor 2 (HER2) antigen. Hormone receptor positive (HR+) breast cancer is the most commonly diagnosed subset of breast cancer (60-65%), and affects thousands of patients every year. Hormone receptor positive subgroup is defined as estrogen receptor positive, progesterone receptor positive or both, and HER 2 negative. Endocrine therapy is highly effective for this subset of breast cancer, in both the adjuvant and metastatic settings. Despite advances in endocrine therapy, many patients have relapse during or after completing adjuvant therapy. Management of these patients remains challenging. Single agent treatment with aromatase inhibitors or tamoxifen has shown limited clinical benefit (1, 2). Tamoxifen works by blocking the binding of the estrogen receptor in the breast cancer cells and has an overall response rate of 25-30% when used as first-line therapy for advanced breast cancer (3). Even if HR+ metastatic breast cancer patients respond to tamoxifen initially, they eventually develop acquired resistance and their disease progresses, and transition to continuous lifelong treatment with chemotherapy. Therefore, improving the efficacy of endocrine therapy would benefit a large number of breast cancer patients, and is an unmet medical need. A variety of novel agents targeting critical pathways in cellular growth are in development to improve the efficacy of endocrine therapy against HR + breast cancer.

Palbociclib, its mechanism of action, and its efficacy in HR+ metastatic breast cancer
Palbociclib is a reversible, oral, small molecule inhibitor of cyclin dependent kinases 4 and 6 (CDK4/6) (4). CDK4 and CDK6 together with cyclin D have important roles in regulation of the G1/S transition via regulation of the phosphorylation state of retinoblastomaprotein (Rb). When phosphorylation of pRb occurs, it causes release of transcription factors that then allow the transition from G1 to S phase and progression of the cell cycle (5). The luminal A and B subtypes of breast cancer (85 % of which are HR+/Her2 negative) have high rates of cyclin D/CDK activation; in the luminal A and B subtypes, cyclin D1 (CCND1) amplifications were observed in 29% and 58% , and CDK4 amplifications were observed in 14% and 25% respectively (6). Luminal A subtype tumors also have loss of CDKN2A which encodes p16Ink4a, a CDK inhibitor (7). The luminal subtypes also maintain expression of Rb, which is essential for benefit from treatment with a CDK4/6 inhibitor. (8) Patients with HR + breast cancer exhibiting a gene expression signature of Rb loss had shorter recurrence-free survival following adjuvant tamoxifen (9). A tumor gene expression signature of E2F activation was associated with higher residual tumor cell proliferation following pre-surgical AI therapy. Therefore, activation of the CDK4/CDK6/E2F axis promotes endocrine resistance, and treatment with a CDK4/6 inhibitor or knockdown of CDK4 expression abrogates endocrine-resistant cell proliferation. Targeting the inhibition of CDK 4/6 pathway is an attractive therapeutic strategy.

The therapeutic effect of palbociclib in breast cancer was tested preclinically in a large panel of human breast cancer cell lines (10). In the clinical setting, palbociclib has been studied as monotherapy in phase I studies, as well as in combination with endocrine therapy. A prior phase II study suggested that single agent palbociclib induced responses in HR + breast cancer (11). In this trial, 7% of patients had a partial response (PR) and 50% had stable disease (SD). The overall clinical benefit was 21% among 33 ER + patients. Median progression free survival was 3.8 months for patients with ER+HER2- disease. A

randomized phase II trial of letrozole with or without palbociclib as first line treatment (PALOMA-1/TRIO-18) was conducted in postmenopausal women with HR+/ human epidermal growth factor receptor 2 negative (HER2-) metastatic breast cancer (12). At the time of the final analysis for progression-free survival (PFS) (median follow-up 29·6 months [95% CI 27·9–36·0] for the palbociclib plus letrozole group and 27·9 months [25·5–31·1] for the letrozole group), 41 PFS events had occurred in the palbociclib plus letrozole group and 59 in the letrozole group. Median PFS was 10·2 months (95% CI 5·7–12·6) for the letrozole group and 20·2 months (13·8–27·5) for the palbociclib plus letrozole group (HR 0·488, 95% CI 0·319–0·748; one-sided p=0·0004). The results of this trial led to FDA approval of palbociclib in combination with letrozole as first line therapy for patients with metastatic breast cancer. The recommended starting dose is 125 mg orally daily for 21 days followed by 7 days off in 28 days cycles in combination with letrozole 2.5 mg orally daily continuously. Most common adverse events from the phase II PALOMA-1 trial were neutropenia (48% grade 3), leukopenia (19% grade 3) and fatigue (36% grade 2) (12). Notably, despite the increased incidence of neutropenia, no neutropenic fever was reported. Dose delay was required in 45 % of patients in the combination arm and dose reduction was required in 40% of patients. Findings from the confirmatory trial PALOMA-2, a randomized phase III trial of palbociclib plus letrozole versus placebo plus letrozole for the first-line treatment of post-manopausal patients with ER+/HER2- advanced breast cancer is expected to be reported later this year. In second line setting, palbociclib plus fulvestrant demonstrated an improvement in PFS compared with placebo plus fulvestrant in the PALOMA-3 phase III trial (13). The median PFS was 9.2 months for the palbociclib-containing regimen and 3.8 months for the fulvestrant plus placebo arm.

Palbociclib may have synergistic anti-cancer effects with tamoxifen based on a phase I trial without either drug affecting each other's pharmacokinetic profile.
Preclinical data showed that in combination with tamoxifen, palbociclib had synergistic growth inhibitory activity as well as efficacy in a model of acquired tamoxifen resistance (11). Studies evaluating the role of cyclin D_1 in breast cancer support the observations of the activity of CDK4/6 inhibitor in luminal ER-positive breast cancer, its synergism with tamoxifen in cell lines that are sensitive to hormone manipulation, as well as the reversal of resistance of those that have acquired a resistant phenotype to anti-estrogen therapy. Estrogen effects on cell cycle progression are tightly linked to expression of cyclin D_1 (14). Cyclin D_1 amplification and/or overexpression has been more commonly associated with an ER-positive breast cancer subtype (15) and, is associated with tamoxifen resistance (16, 17).

Tamoxifen could affect the metabolism of co-administered drugs that are substrates for CYP3A4 since *in vitro* data indicate tamoxifen is a reversible and time-dependent inhibitor of CYP3A4 (18) as well as an inducer of CYP3A4 (19). Palbociclib is mainly metabolized by CYP3A4. A clinical drug interaction study of tamoxifen and palbociclib revealed that there were no significant changes in exposure in the presence of steady-state levels of tamoxifen when compared with exposure when given alone (20). Therefore based on this phase I study, tamoxifen is not expected to affect palbociclib pharmacokinetics due to CYP-mediated interactions.

1.2. Study Rationale

Combining palbociclib with tamoxifen in first line treatment of metastatic HR+ breast cancer offers an appealing alternative to other endocrine combinations. Tamoxifen remains an active endocrine therapy for pre- and post-menopausal women with hormone receptor (HR) positive breast cancers. In addition, most postmenopausal women with HR+ breast cancer receive aromatase inhibitors (AI) as adjuvant endocrine therapy. The group of women that progress on adjuvant therapy with AIs may not benefit from the addition of CDK 4/6 inhibition to their current endocrine therapy. Although fulvestrant could be considered in this setting, the cost and inconvenience of monthly injections remains a challenge. Thus, the combination of

tamoxifen with palbociclib will offer the option of continuing oral combination endocrine therapy with CDK 4/6 inhibition. The combination with tamoxifen could offer a different spectrum of side effects, and it may be better tolerated by subjects with osteoporosis or advanced arthritis. Accordingly, we will conduct a Phase II study to estimate the response rates of the combination of palbociclib and tamoxifen in first line therapy for subjects with HR(+)/HER2(-) advanced breast cancer who have not received prior systemic anti-cancer therapies for their advanced/metastatic disease.

2. OBJECTIVES AND ENDPOINTS

2.1. Study Objectives

2.1.1. Primary Objective

The primary objective of the Phase II study is to estimate the activity of the combination of palbociclib and tamoxifen in first line therapy for subjects with HR(+)/HER2(-) advanced breast cancer who have not received prior systemic anti-cancer therapies for their advanced/metastatic disease, assessed by progression free survival (PFS) per local assessment based on RECIST 1.1

2.1.2. Secondary Objectives

- Characterize safety and tolerability of palbociclib and tamoxifen in first line therapy for subjects with HR(+)/HER2(-) advanced breast cancer who have not received prior systemic anti-cancer therapies for their advanced/metastatic disease.
- Evaluate response rates (complete or partial response) (RR) per local assessment and RECIST 1.1 or MDA criteria in subjects with HR(+)/HER2(-) advanced breast cancer who have not received prior systemic anti-cancer therapies for their advanced/metastatic disease treated with palbociclib in combination with tamoxifen.
- Evaluate clinical benefit rate RR (complete, partial response, or stable disease, lasting 24 weeks or longer) based on RECIST 1.1 or MDA criteria in subjects with HR(+)/HER2(-) advanced breast cancer who have not received prior systemic anti-cancer therapies for their advanced/metastatic disease treated with palbociclib in combination with tamoxifen.
- Measure overall survival (OS) at 2 years in subjects with HR(+)/HER2(-) advanced breast cancer who have not received prior systemic anti-cancer therapies for their advanced/metastatic disease treated with palbociclib in combination with tamoxifen.

2.1.3. Correlative Objectives, if funding is secured

- Perform proteomic analysis of plasma exosomes to identify potential mechanisms of primary and secondary resistance to tamoxifen/palbociclib.
- Analyze tumor specimens for protein expression of candidate markers of primary resistance.
- Compare difference in response for subjects presenting with de novo metastatic breast cancer to subjects that progressed while on aromatase inhibitors.
- Compare differences in response and toxicity profile in African American and Hispanic subjects to Caucasian subjects.
- Compare differences in response and toxicity profile between pre- and post-menopausal women.

3. ELIGIBILITY CRITERIA

Study entry is open to subjects of any ethnic background. While there will be every effort to seek out and include minorities, the subject population is expected to be no different than that of other advanced solid tumor cancer studies at each participating institution.

3.1. Inclusion Criteria

Subjects must meet all of the following applicable inclusion criteria to participate in this study:

3.1.1 Male or female ≥ 18 years of age at time of consent.
NOTE: Both pre- and post-menopausal women are eligible.
Pre-menopausal status is defined as either:
-Last menstrual period within the last 12 months
-In case of therapy-induced amenorrhea, plasma estradiol and /or FSH is in the premenopausal range per local normal range.

3.1.2 Locally advanced, locoregionally recurrent, or metastatic disease, not amenable to curative therapy.
NOTE: Although not required as a protocol procedure, a patient with a new metastatic lesion should be considered for biopsy whenever possible to reassess ER/PR/HER2 status if clinically indicated. If a biopsy is prospectively done as part of standard of care, the study would like to store samples for correlative research.

3.1.3 Histologically and/or cytologically confirmed diagnosis of ER positive and/or PR positive (ER >1%, PR >1%), HER2 negative breast cancer.
NOTE: Subject has HER2-negative breast cancer (based on most recently analyzed biopsy) is defined as a negative in situ hybridization test or an IHC status of 0, 1+ or 2+. If IHC is 2+, a negative in situ hybridization (e.g. FISH, CISH, SISH, DISH, etc.) test is required by local laboratory testing.

3.1.4 Metastatic disease evaluable on imaging studies. Subjects may have measurable disease as per RECIST 1.1 or bone-only disease.
NOTE: Bone-only subjects are eligible if their disease can be documented/ evaluated by bone scans, CT or MRI. Their disease will be assessed using MDA criteria.
NOTE: Previously irradiated lesions are eligible as a target lesion only if there is documented progression of the lesion after irradiation.

3.1.5 No prior systemic anti-cancer therapy for advanced HR+ disease.
NOTE: Subjects receiving adjuvant treatment with aromatase inhibitors at time of recurrence are allowed to participate. There is no AI washout period required.

3.1.6 Eastern Cooperative Oncology Group (ECOG) Performance Status 0-2

3.1.7 Adequate hepatic function within 14 days prior to registration for protocol therapy defined as meeting **all** of the following criteria:

- aspartate aminotransferase (AST) ≤ 2.5 × ULN or ≤ 5 × ULN for subjects with known hepatic metastases and
- alanine aminotransferase (ALT) ≤ 2.5 × ULN or ≤ 5 × ULN for subjects with known hepatic metastases and

- total serum bilirubin ≤ 1.5 × ULN

3.1.8 Adequate renal function within 14 days prior to registration for protocol therapy defined by **either** of the following criteria:
- serum creatinine ≤ 1.5 × ULN
- **OR** if serum creatinine > 1.5 × ULN, estimated glomerular filtration rate (eGFR) ≥ 40 mL/min

3.1.9 Adequate hematologic function within 14 days prior to registration for protocol therapy defined as meeting **all** of the following criteria:
- hemoglobin ≥ 9 g/dL
- and absolute neutrophil count (ANC) ≥ 1.5×10^9/L
- and platelet count ≥ 100×10^9/L

3.1.10 Provided written informed consent and HIPAA authorization for release of personal health information, approved by an Institutional Review Board/Independent Ethics Committee (IRB/IEC).
NOTE: HIPAA authorization may be included in the informed consent or obtained separately.

3.1.11 Women of childbearing potential (WOCP) must not be pregnant or breast-feeding. A negative serum or urine pregnancy test is required within 72 hours of study registration from women of childbearing potential. If the urine test cannot be confirmed as negative, a serum pregnancy test will be required.

3.1.12 Women of childbearing potential (WOCP) must be willing to use two effective methods of birth control such as use of a double barrier method (condoms, sponge, diaphragm, or vaginal ring with spermicidal jellies or cream), or total abstinence for the course of the study until 120 days after the last dose of study drug. The use of hormonal contraceptives is discouraged.
NOTE: Women are considered to be of childbearing potential unless they are postmenopausal for at least 12 consecutive months or surgically sterile (bilateral tubal ligation, bilateral oophorectomy, or hysterectomy).

3.1.13 Male subjects capable of fathering a child must agree to use adequate contraception (see section 5.6) or total abstinence for the course of the study until 120 days after the last dose of the study drug.
NOTE: Male subjects will be considered as capable of fathering a child unless they have azoospermia (whether due to having had a vasectomy or due to an underlying medical condition).

3.1.14 Willingness and ability to comply with scheduled visits, treatment plans, laboratory tests, and other study procedures.

3.1.15 Co-enrollment in an imaging biomarker study or other non-therapeutic study is allowed.

3.2. Exclusion Criteria

Subjects meeting any of the criteria below may not participate in the study:

3.2.1 Prior treatment with any CDK 4/6 inhibitor.

3.2.2 Confirmed diagnosis of HER2 positive disease.

3.2.3 Known uncontrolled or symptomatic CNS metastases. Subjects with known brain metastasis will only be eligible after their tumors have been treated with definitive resection and /or radiotherapy and they are neurologically stable for at least 1 month off steroids

3.2.4 Advanced, symptomatic, visceral spread with a life expectancy less than 4 months.

3.2.5 Prior (neo)adjuvant treatment with tamoxifen within the 12 months before study entry.

3.2.6 Prior history of blood clots, pulmonary embolism or deep vein thrombosis.

3.2.7 Impairment of gastrointestinal (GI) function or GI disease that may significantly alter the absorption of the study drugs (e.g., ulcerative diseases, uncontrolled nausea, vomiting, diarrhea, malabsorption syndrome, or small bowel resection).

3.2.8 Concurrent malignancy or malignancy within 3 years of randomization, with the exception of adequately treated basal cell carcinoma, squamous cell skin carcinoma, non-melanomatous skin cancer or curatively resected cervical cancer.

3.2.9 Any other concurrent severe and/or uncontrolled medical condition that would, in the investigator's judgment, contraindicate subject participation in the clinical study

3.2.10 Currently receiving any of the following substances and cannot be discontinued 7 days prior to study registration:

- Known strong inducers or inhibitors of CYP3A4/5, including grapefruit, grapefruit hybrids, pomelos, star-fruit, and Seville oranges.
- Medications that have a narrow therapeutic window and are predominantly metabolized through CYP3A4/5.
- Known strong inducers or inhibitors of CYP2D6.

3.2.11 Major surgery within 14 days prior to study registration or has not recovered from major side effects of surgery.

3.2.12 Known history of human immunodeficiency virus [(HIV) HIV 1/2 antibodies].

3.2.13 Known active Hepatitis B (e.g., HBsAg reactive) or Hepatitis C (e.g., HCV RNA [qualitative] is detected) (testing not mandatory)

3.2.14 Any clinically significant infection defined as any acute viral, bacterial, or fungal infection that requires specific treatment.
NOTE: Anti-infective treatment must be completed ≥ 7 days prior to study registration.

3.2.15 Known allergy to palbociclib or any of its excipients

3.2.16 Presence of any non-healing wound, fracture, or ulcer within 28 days prior to study registration. NOTE: if fracture is at a metastatic site, is chronic, and no surgical treatment is planned, the subject can be enrolled.

3.2.17 Any condition that, in the opinion of the investigator, might jeopardize the safety of the subject or interfere with protocol compliance.

3.2.18 Any mental or medical condition that prevents the subject from giving informed consent or participating in the trial.

3.2.19 Treatment with any therapeutic investigational agent within 28 days prior to registration for protocol therapy. The subject must have recovered from the acute toxic effects of the regimen.

4. SUBJECT REGISTRATION

All subjects must be registered through the Big Ten Cancer Research Consortium (BTCRC) Administrative Headquarters' (AHQ) electronic data capture (EDC) system OnCore®. A subject is considered registered when an 'On Study' date is entered into OnCore®.

Subjects must be registered after signing consent but prior to starting protocol therapy. Protocol therapy must begin therapy within 5 business days of registration.

Subjects Who Do Not Begin Study Treatment

If subject signs consent, is registered to the study, and later is not able to begin the planned study treatment, for whatever reason, the subject will be removed from study and treated at the physician's discretion. The subject will be considered a screen/baseline failure and be replaced. The reason for removal from study will be clearly indicated in EDC system.

If a subject begins treatment, and then is discontinued for whatever reason, the subject must be followed per section 7.5.

5. TREATMENT PLAN

5.1. Overall Design and Study Plan

This is a non-randomized, open-label, single-arm, multicenter, phase II study of palbociclib in combination with tamoxifen in subjects with HR(+)/HER2(-) advanced breast cancer who have not received prior systemic anti-cancer therapies for their advanced/metastatic disease. The primary objective of the Phase II trial is to determine progression free survival (PFS) based on RECIST 1.1 Treatment will continue until disease progression, unacceptable toxicity, subject refusal, or subject death either from progression of disease, the therapy itself, or from other causes. Subjects, who voluntarily stop the study, have progressive disease, or unacceptable toxicities will be followed for a total of 24 months after discontinuation of study drug.

If funding is secured, correlative research analyses includes correlating alterations in circulating tumor DNA at the time of progression, correlating molecular alterations of genes associated with ER+ breast cancer in tumor tissue samples with clinical outcome and at the time of progression, comparing difference in response for subjects presenting with de novo metastatic breast cancer to subjects that progressed while on aromatase inhibitors, and comparing differences in response and toxicity profile in African American and Hispanic subjects to Caucasian subjects.

5.2. Pre-medication

Pre-medication is not required but may be administered per physician discretion.

5.3. Drug Administration

5.3.1. Palbociclib + Tamoxifen Administration

The palbociclib dose will be 125 mg administered orally once daily on days 1-21 of each 28-day cycle. Subjects will not take palbociclib on days 22-28. The tamoxifen dose will be 20 mg administered orally once daily for every day of the 28-day cycle (i.e., continuously). It is encouraged but not mandatory that premenopausal subjects will also receive treatment with Goserelin or equivalent (e.g. Lupron) given as an injectable subcutaneous implant on day 1 of every 28 days cycle or every 3 months.

Drug	Dose	Route	Schedule	Cycle Length
Palbociclib	125mg	Oral	Days 1-21	28 days
Tamoxifen	20mg	Oral	Continuous	

Palbociclib should be taken with food in combination with Tamoxifen. Subjects should be encouraged to take their dose at approximately the same time each day.

5.3.2. Missed Doses:

Missed doses may be taken up to 6 hours of the time that the dose was scheduled. If the timing is beyond 6 hours, the subject will skip the dose and take the next dose as scheduled.

If emesis occurs after study medication ingestion and whole capsule(s) are visible in the vomitus, replacement capsule(s) should be taken; otherwise, the dose will not be re-administered and subjects should simply adhere to the dosing schedule and resume dosing at the next scheduled time with the prescribed dosage. Subjects should record the time of the emesis in their dosing diary (see Documents/Info tab of the EDC). Under no circumstance should a subject repeat a dose or double-up doses.

5.4. Supportive Care

Optimal patient care is to be given to all subjects.

Subjects should receive full supportive care during the study, including transfusion of blood and blood products, treatment with antibiotics, analgesics, erythropoietin, or bisphosphonates (or denosumab), when appropriate.

Subjects should receive appropriate supportive care measures as deemed necessary by the treating investigator including but not limited to the items outlined below:

- Nausea/vomiting: Nausea and vomiting should be treated aggressively, and consideration should be given in subsequent cycles to the administration of prophylactic antiemetic therapy according to standard institutional practice. If prophylactic antiemetic therapy is needed, 5-HT3 receptor antagonists (without corticosteroids) should be tried first. Due to the potential of benzodiazepines to cause sedation, the use of benzodiazepines for antiemetic prophylaxis should be reserved for subjects who cannot be satisfactorily managed otherwise. Subjects should be strongly encouraged to maintain liberal oral fluid intake.
- Anti-infectives: Subjects with a documented infectious complication should receive oral or IV antibiotics or other anti-infective agents as considered appropriate by the treating investigator for a given infectious condition, according to standard institutional practice.

- Prophylactic myeloid growth factors are not permitted for prevention of neutropenia and are only permitted if clinically indicated in response to a clinically significant episode of neutropenia.

5.5. Concomitant Medications

Medications specifically prohibited in the exclusion criteria are not allowed during the ongoing trial. If there is a clinical indication for one of these or other medications specifically prohibited during the trial, discontinuation from trial therapy may be required. The site investigator should discuss any questions regarding this with the sponsor-investigator by contacting the BTCRC Project Manager. The final decision on any supportive therapy rests with the investigator and/or the subject's primary physician. However, the decision to continue the subject on trial therapy requires the mutual agreement of the local Investigator, the sponsor-investigator, and the subject.

Permitted Concomitant Medications and Procedures

All treatments the investigator considers necessary for a subject's welfare may be administered at the discretion of the investigator in keeping with the community standards of medical care. All concomitant medications will be recorded on the electronic case report form (eCRF).

Palliative radiation is permitted but palbociclib must be withheld throughout the course of radiotherapy. Palliative RT should not be delivered to a target lesion and it should not encompass more than 25% of the irradiated bone marrow. The reason for radiotherapy must be clearly documented and progression as per RECIST 1.1 must be ruled out. No dose modification of study treatment is mandated after completion palliative radiotherapy.

If elective surgery is needed during study, it is recommended the surgery be scheduled between treatment cycles. Palbociclib should be withheld 14 days prior to surgery. The next cycle of treatment may be delayed up to 4 weeks to allow for additional recovery at the discretion of the investigator.

All concomitant medications received within 28 days before the first dose of trial treatment and 30 days after the last dose of study drug should be recorded. Concomitant medications administered beyond 30 days after the last dose of trial treatment should be recorded for SAEs as defined in Section 11.

Antiemetic agents may be administered at the discretion of the investigator but are not commonly required as a prophylactic agent. All other manifestations of the subject's malignancy should be treated at the discretion of the investigator.

All other medical conditions should be treated at the discretion of the investigator in accordance with local community standards of medical care.

The Effects of Other Drugs on Palbociclib

Palbociclib is primarily metabolized by CYP3A and sulfotransferase (SULT) enzyme SULT2A1.

The use of strong CYP3A inhibitors (e.g., clarithromycin, indinavir, itraconazole, ketoconazole, lopinavir/ritonavir, nefazodone, nelfinavir, posaconazole, ritonavir, saquinavir, telaprevir, telithromycin, verapamil, and voriconazole) should be avoided as these drugs could increase plasma levels of palbociclib.

The use of strong CYP3A inducers (e.g., phenytoin, rifampin, carbamazepine and St John's Wort) or moderate CYP3A inducers (e.g., bosentan, efavirenz, etravirine, modafinil, and nafcillin) should be avoided as these drugs could decrease plasma levels of palbociclib.

The use of strong inhibitors or inducers of CYP2D6 (Bupropion, dacomitinib, fluoxetine, paroxetine, quinidine) should be avoided

The Effects of Palbociclib on Other Drugs
In vivo, palbociclib is a time-dependent inhibitor of CYP3A.

Coadministration of midazolam with multiple doses of palbociclib increased the midazolam plasma exposure by 61%, in healthy subjects, compared with administration of midazolam alone. The dose of the sensitive CYP3A substrate with a narrow therapeutic index (e.g., alfentanil, cyclosporine, dihydroergotamine, ergotamine, everolimus, fentanyl, pimozide, quinidine, sirolimus and tacrolimus) may need to be reduced as palbociclib may increase their exposure.

Prohibited Concomitant Medications
Subjects are prohibited from receiving the following therapies during the Screening and Treatment Phase of this trial:

- Anti-cancer systemic chemotherapy or biological therapy not specified in this protocol
- Other investigational and antineoplastic therapies

Subjects who, in the assessment by the investigator, require the use of any of the aforementioned treatments for clinical management should be removed from the trial.

There are no prohibited therapies during the Post-Treatment Follow-up Phase.

5.6. Diet/Activity/Other Considerations

Diet
Subjects should maintain a normal diet unless modifications are required to manage an AE such as diarrhea, nausea, or vomiting.

Contraception
Palbociclib may have adverse effects on a fetus in utero. Non-pregnant, non-breast-feeding women may be enrolled if they are willing to use 2 methods of birth control or are considered highly unlikely to conceive. Highly unlikely to conceive is defined as 1) surgically sterilized, or 2) postmenopausal (a woman who is ≥45 years of age and has not had menses for greater than 1 year will be considered postmenopausal), or 3) not heterosexually active for the duration of the study.

It is encouraged but not mandatory that premenopausal subjects will also receive treatment with goserelin or equivalent (e.g. Lupron) given as an injectable subcutaneous implant on day 1 of every 28 days cycle or every 3 months.

The following are considered adequate barrier methods of contraception: diaphragm, condom (by the partner), copper intrauterine device, sponge, or spermicide. The use of hormonal contraceptives is discouraged.

Subjects should be informed that taking the study medication may involve unknown risks to the fetus (unborn baby) if pregnancy were to occur during the study. In order to participate in the study they must adhere to the contraception requirements described above for the duration of the study and at least 120 days following the last dose of study drug. If there is any question that a subject will not reliably comply with the requirements for contraception, that subject should not be entered into the study.

Use in Pregnancy
If a subject becomes pregnant while on treatment with palbociclib, the subject will immediately be removed from the study therapy. All pregnancies must be reported to BTCRC AHQ within 1 business day using the Pregnancy Report form (See Documents/Info tab of the EDC).

The study investigator will make every effort to obtain permission to follow the outcome of the pregnancy and report the condition of the fetus or newborn to BTCRC AHQ. The site should make every effort to contact the subject at least monthly and document the subject's status until the pregnancy has been completed or terminated. The outcome of the pregnancy will be reported to BTCRC AHQ as soon as the information is available. If the outcome is a serious adverse event (e.g., death, abortion, congenital anomaly, or other disabling or life-threatening complication to the mother or newborn), it will be reported to BTCRC AHQ within 1 business day.

Use in Nursing Women
It is unknown whether palbociclib is excreted in human milk. Since many drugs are excreted in human milk, and because of the potential for serious adverse reactions in the nursing infant, subjects who are breast-feeding are not eligible for enrollment.

6. DOSE DELAYS

The NCI Common Terminology Criteria for Adverse Events (CTCAE) Version 4 will be used to grade adverse events.

Subjects enrolled in this study will be evaluated clinically and with standard laboratory tests before and at regular intervals during their participation in this study as specified in Section 7.

Subjects will be evaluated for adverse events (all grades), serious adverse events, and adverse events requiring protocol therapy interruption or discontinuation at each study visit for the duration of their participation in the study.

Subjects discontinued from the study drug for any reason will be evaluated within 30 days (± 7) of the last dose of study drug.

6.1. Dose Modifications

6.1.1. Palbociclib Dose Modifications

Table 1. Dose Modifications for Adverse Reactions

Dose Level	Dose
Recommended starting dose	125 mg/day
First dose reduction	100 mg/day
Second dose reduction	75 mg/day *
*If further dose reduction below 75 mg/day is required, the subject will be discontinued from study treatment.	

Palbociclib will be withheld for drug-related ≥Grade 3 hematologic toxicities, non-hematological toxicity ≥Grade 3 including laboratory abnormalities and severe or life-threatening AEs as per Table 2 below.

Table 2: Dose modification guidelines for drug-related adverse events.

Toxicity	Grade	Hold Treatment (Y/N)	Timing for restarting treatment	Dose/Schedule for restarting treatment	Discontinue Subject
Hematological Toxicity	1, 2	No	N/A	N/A	N/A
	3* *Except lymphopenia (unless associated with clinical events, e.g.., opportunistic infections)	Withhold initiation of next cycle. Repeated hematologic monitoring one week later	Toxicity resolves to Grade ≤2	No dose adjustment is required	Toxicity does not resolve within 4 weeks of last study drug administration *Permanent discontinuation should be considered for any severe or life-threatening event*
	Grade 3 ANC (<1000 to 500/mm^3) + Fever>38.5° C and/or infection Grade 3 Plt count (<50000 to 25000/ mm^3) with clinically significant bleeding	Yes	Toxicity resolves to Grade ≤2 (ANC>1000/ mm^3, platelets > 50000/mm^3)	Resume at next lower dose	
	4* *Except lymphopenia (unless associated with clinical events, e.g.., opportunistic infections)	Yes	Toxicity resolves to Grade ≤2	Resume at next lower dose	
Non-hematological toxicity† Note: Exception to be treated similar to grade 1 toxicity • Grade 2 alopecia • Grade 2 fatigue	1,2	No	N/A	N/A	N/A
	3,4 (if persisting despite medical treatment)	Yes	Toxicity resolves to: • Grade ≤1; • Grade ≤2 (if not considered a safety risk for the subject)	Resume at next lower dose	Toxicity does not resolve within 4 weeks of last study drug administration *Permanent discontinuation should be considered for any severe or life-threatening event*
Footnotes: †Concurrent elevation of bilirubin > 2x ULN and ALT > 3 x ULN in absence of an evident alternative explanation (such as liver mets) will lead to discontinuation of the study drugs					

- Complete blood count will be monitored prior to start of study treatment and at the beginning of each cycle, as well as on Day 15 of the first two cycles, and as clinically indicated.

6.1.2. Tamoxifen Dose Modifications

The established clinical dose of tamoxifen (20 mg/day) will be used and no dose modification of tamoxifen is planned in this study.

For information on tamoxifen and management of tamoxifen related adverse events refer to the Tamoxifen SmPC or Prescribing Information.

6.1.3. Goserelin (or equivalent) Dose Modifications

The established clinical dose of goserelin (3.6 mg subcutaneous injection every 28 days or 10.8 mg subcutaneous injection every 12 weeks) may be used and no dose modification of goserelin is planned in this study.

For information on goserelin and management of goserelin related adverse events refer to the Zoladex® SmPC or Prescribing Information.

The established clinical dose of leuprolide (3.75 mg intramuscular injection every 28 days or 11.25 mg intramuscular injection every 12 weeks) may be used and no dose modification of leuprolide is planned in this study.

For information on leuprolide and management of leuprolide related adverse events refer to the Lupron® SmPC or Prescribing Information.

7. STUDY CALENDAR & EVALUATIONS

[Cycle = 28 days]

Study Day	Screening		Cycle 1	Cycle 2	Cycle 3-5	Every 2 cycles	Every 3 cycles	Safety follow up	Long-Term follow up
	-28 days	-14 days	Day 1[1]	Day 1 (±3)	Day 1 (±3)	(± 7)	(± 7)	30 days (±7) post last study drug	Every 3 months (±14) for 2 years from registration
REQUIRED ASSESSMENTS									
Informed Consent/ HIPAA auth.	X								
Medical hx including prior therapies; pathology	X								
Physical examination	X		X	X	X	C7+		X	
Vital Signs, weight, height (screen only)	X		X	X	X	C7+		X	
ECOG status	X		X	X	X	C7+		X	
Baseline signs/ symptoms; AE assessment	X		X	X	X	C7+		X[5]	
Concomitant medications	X		X	X	X	C7+		X	
Survival									X[6]
LABORATORY TESTS									
Blood Chemistries[2]		X	X	X	X	C7+			
Platelets, ANC & Hgb[3]		X	D1, D15	D1, D15	X	C7+			
Pregnancy test for WOCP[4]		-72h	X	X	X	C7+			
DISEASE ASSESSMENT[7]						C3-19	C22+		
CT/MRI chest, abdomen/pelvis	X					X[8]	X[8]		X
CT or MRI Brain, if clinically indicated	X								
Whole body bone scan	X					X[8,9]	X[8,9]		
Bone x-ray, CT, or MRI[10]	X					X[8]	X[8]		
Skin color photography, if applicable	X					X[8]	X[8]		
TREATMENT EXPOSURE									
Palbociclib (Day 1-21)			X	X	X[16]	C7+[16]			
Tamoxifen (Continuous)			X →			C7+			
CORRELATIVE STUDIES (SPECIMEN COLLECTION)							C8, 11, etc.		
Tumor tissue (unstained slides)[11]			X					X[11]	
Blood for circulating tumor DNA[12]			X		C5		X[12]	X[12]	
BANKING SAMPLES (SPECIMEN COLLECTION)									
Whole Blood[13]			X						
Unstained Slides[14] (archival, if available)			X						
Serum and Plasma[15]			X					X	

Footnotes:

1: For cycle 1 only: labs do not need to be repeated if done within 7 days of day 1.
2: Blood Chemistries to include: sodium, potassium, serum creatinine (or GFR; see 3.1.8), calcium, albumin, ALT, AST, bilirubin, alkaline phosphatase.
3: For Cycles 1 and 2 only, hematology assessments done on Day 1 and also on Day 15
4: A negative serum or urine pregnancy test is required within 72 hours of study registration and at the beginning of each cycle, if clinically indicated. If the urine test cannot be confirmed as negative, a serum pregnancy test will be required.
5: For subjects with unresolved treatment-related toxicity, follow as medically appropriate until resolution or stabilization
6: Survival assessment to be recorded every 3 months until 2 years from registration
7: Appropriate scans to assess disease status will be obtained within 28 days of study enrollment including CT chest, abdomen/pelvis. PET/CT: The CT portion of PET/CT is usually of lower quality and should typically not be used in place of dedicated diagnostic CT. However, if the CT portion is of diagnostic quality, it may be used.
8: Disease assessment will be performed on Day 1 of every 2 cycles starting at cycle 3 (ex. Day 1 of cycle 3, 5, 7, etc.) for the first 19 cycles (18 months). Disease assessment will be performed on Day 1 of every three cycles starting at cycle 22 (ex. Day 1 of cycle 22, 25, etc.) until discontinuation of study drug.
9: Bone scan will be repeated as per footnote 8 above only if positive for bone mets at the time of screening or if clinically indicated.
10: If bone lesion(s) detected from whole body bone scan, perform bone x-ray, CT or MRI of the largest bone metastatic area.
11: Histological confirmation of breast cancer diagnosis is required (Section 3.1.3). Although not required as a protocol procedure, a patient with a new metastatic lesion should be considered for biopsy whenever possible to reassess ER/PR/HER2 status, if clinically indicated. Also, biopsy at the time of progression should be considered as clinically indicated, but it is not required as a protocol procedure. If a biopsy is prospectively done as standard of care, the study would like to collect samples for correlative research. Unstained slides from either the fresh biopsy or from an archival tumor specimen (metastatic lesion) may be submitted at baseline.
12: Blood for circulating tumor DNA will be collected cycle 1 day 1, cycle 5 day 1 and day 1 of every subsequent 3rd cycle (e.g. cycle 8, 11, etc.), and at time of progression.
13: Submission of whole blood for banking is to be collected at Pre-Treatment Cycle 1 Day 1. See CLM for collection, processing, labeling and shipping instructions.
14: Submission of unstained slides for banking from an archived FFPE tumor block (if available). See CLM for collection, labeling, and shipping instructions.
15: Submission of serum and plasma for banking are to be collected at Pre-Treatment Cycle 1 Day 1 and at the 30 Day Safety Follow up visit. See CLM for collection, labeling, processing, and shipping instructions.
16: After cycle 5, subjects will be assessed every 2 cycles (cycle 7, 9, 11, etc.). Two cycles of palbociclib may be dispensed from cycle 5 onward.

7.1. Screening

7.1.1. Within 28 days prior to registration for protocol therapy

- Informed consent, HIPAA authorization
- Medical history including prior therapies and pathology
- Physical exam, height, weight
- Vital signs (blood pressure, heart rate, temperature)
- ECOG performance status
- Concomitant medications
- CT/MRI chest, abdomen, pelvis
- CT or MRI of brain, if clinically indicated
- Whole body bone scan
- If bone lesion detected from whole body bone scan, bone X-ray, CT, or MRI of the largest bone lesion
- If skin lesion detected by physical exam, skin color photography

7.1.2. Within 14 days prior to registration for protocol therapy

- Blood chemistries (sodium, potassium, serum creatinine, calcium, albumin, ALT, AST, bilirubin, alkaline phosphatase,)
- Platelets, ANC and hemoglobin

7.1.3. Within 72 hours prior to registration for protocol therapy

- Pregnancy test for women of childbearing potential (WOCP). A negative serum or urine pregnancy test is required within 72 hours of study registration. If the urine test is positive or cannot be confirmed as negative, a serum pregnancy test will be required.

7.2. On Treatment

7.2.1. Day 1 of Cycle 1

Note: Cycle 1 Day 1 lab testing need not be repeated if completed within 7 days of starting protocol therapy.

- Physical exam, weight
- Vital signs
- ECOG performance status
- Blood chemistries (sodium, potassium, serum creatinine, calcium, albumin, ALT, AST, bilirubin, alkaline phosphatase)
- Platelets, ANC and hemoglobin
- Pregnancy test for women of childbearing potential (WOCP), if clinically indicated. If the urine test is positive or cannot be confirmed as negative, a serum pregnancy test will be required.
- Assess adverse events (AE)
- Concomitant medications
- Dispense Palbociclib
- Prescribe Tamoxifen (commercial supply)

- Correlative and Banking samples. See CLM for collection, labeling, processing, and shipping instructions.

7.2.2. Day 15 of Cycle 1 (±3 days)

- Platelets, ANC and hemoglobin

7.2.3. Day 1 of Cycle 2 (±3 days)

- Physical exam, weight
- Vital signs
- ECOG performance status
- Blood chemistries (sodium, potassium, serum creatinine calcium, albumin, ALT, AST, bilirubin, alkaline phosphatase)
- Platelets, ANC and hemoglobin
- Pregnancy test for women of childbearing potential (WOCP), if clinically indicated. If the urine test is positive or cannot be confirmed as negative, a serum pregnancy test will be required.
- Assess adverse events
- Concomitant medications
- Dispense Palbociclib
- Prescribe Tamoxifen (commercial supply)

7.2.4. Day 15 of Cycle 2 (±3 days)

- Platelets, ANC and hemoglobin

7.2.5. Day 1 of Cycle 3-5 (±3 days)

- Physical exam, weight
- Vital signs
- ECOG performance status
- Blood chemistries (sodium, potassium, serum creatinine calcium, albumin, ALT, AST, bilirubin, alkaline phosphatase)
- Platelets, ANC and hemoglobin
- Pregnancy test for women of childbearing potential (WOCP), if clinically indicated. If the urine test is positive or cannot be confirmed as negative, a serum pregnancy test will be required.
- Assess adverse events
- Concomitant medications
- Dispense Palbociclib (dispense 2 cycles at cycle 5)
- Prescribe Tamoxifen (commercial supply)
- Correlative blood sample: cycle 5 day 1

7.3. Every two cycles (±7 days)

- Disease assessment will be performed every 2 cycles starting at cycle 3 (cycle 3, 5, 7, etc.) for the first 19 cycles (18 months):
  - CT/MRI chest, abdomen, pelvis (±7 days).
  - Whole body bone scan if positive for mets at screening or if clinically indicated.

- If bone lesion detected from whole body bone scan, repeat bone scan, bone X-ray, CT, or MRI of the largest skeletal lesion identified.
- If skin lesion detected by physical exam at screening, skin color photography.

- Subjects will be assessed every 2 cycles starting after cycle 5 (cycle 7, 9, 11, etc.):
  - Physical exam, weight
  - Vital signs
  - ECOG performance status
  - Blood chemistries (sodium, potassium, serum creatinine calcium, albumin, ALT, AST, bilirubin, alkaline phosphatase)
  - Platelets, ANC and hemoglobin
  - Pregnancy test for women of childbearing potential (WOCP), if clinically indicated. If the urine test is positive or cannot be confirmed as negative, a serum pregnancy test will be required.
  - Assess adverse events
  - Concomitant medications
  - Dispense 2 cycles of Palbociclib
  - Prescribe Tamoxifen (commercial supply)

7.4. Every three cycles (±7 days)

- Disease assessment will be performed every 3 cycles starting at cycle 22 (cycle 22, 25, etc.) until discontinuation of study drug:
  - CT/MRI chest, abdomen, pelvis (±7 days).
  - Whole body bone scan if positive for mets at screening or if clinically indicated.
  - If bone lesion detected from whole body bone scan, repeat bone scan, bone X-ray, CT, or MRI of the largest skeletal lesion identified.
  - If skin lesion detected by physical exam at screening, skin color photography.
- Correlative blood sample: day 1 of every 3rd cycle starting at cycle 8 (cycle 8, 11, etc.)

7.5. Off Treatment

7.5.1. Protocol therapy discontinuation

A subject will be discontinued from the protocol therapy under the following circumstances:

- If there is evidence of disease progression
- If the treating physician thinks a change of therapy would be in the best interest of the subject
- If the subject requests to discontinue protocol therapy
- If the protocol therapy exhibits unacceptable toxicity
- If a female subject becomes pregnant

Subjects can stop study participation at any time. However, if they decide to stop, subjects will continue to be followed for survival for 24 months after discontinuation.

7.6. Safety Follow-up Evaluations

Subjects discontinued from the treatment phase of the study for any reason will be evaluated within 30 days (± 7) of the last dose of study drug.

- Physical exam, weight
- Vital signs
- ECOG performance status
- Assess adverse events
- Concomitant medications
- Correlative blood samples at the time of progression
- Optional standard biopsy at the time of progression, with extra passes for research.
- Banking samples. See Correlative Laboratory Manual for specific instructions.

7.7. Long Term Follow-up Evaluations (±14 days)

Subjects will continue to be followed for survival every 3 months for 24 months after registration.

8. CRITERIA FOR DISEASE EVALUATION

Response assessments for subjects with visceral/ visceral plus bone disease will be made using RECIST v1.1. Response assessments for subjects with bone only disease will be made using MDA criteria.

8.1. Definitions Associated with Response Evaluation Criteria in Solid Tumors (RECIST) version 1.1

8.1.1. Measurable disease: The presence of at least one measurable lesion. If the measurable disease is restricted to a solitary lesion, its neoplastic nature should be confirmed by cytology/histology.

8.1.1.1. ***Measurable lesions:*** Measurable lesions are defined as those that can be accurately measured in at least one dimension (longest diameter to be recorded) as ≥20 mm by chest x-ray, as ≥10 mm with CT scan, or ≥10 mm with calipers by clinical exam. All tumor measurements must be recorded in millimeters (or decimal fractions of centimeters).

8.1.1.2. ***Non-measurable lesions:*** All other lesions (or sites of disease), including small lesions (longest diameter <10 mm or pathological lymph nodes with ≥ 10 to <15 mm short axis), are considered non-measurable disease. Bone lesions, leptomeningeal disease, ascites, pleural/pericardial effusions, lymphangitis cutis/pulmonitis, inflammatory breast disease, and abdominal masses (not followed by CT or MRI), are considered as non-measurable.

Note: Cystic lesions that meet the criteria for radiographically defined simple cysts should not be considered as malignant lesions (neither measurable nor non-measurable) since they are, by definition, simple cysts.

'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 subject, these are preferred for selection as target lesions.

8.1.1.3. ***Malignant lymph nodes***. To be considered pathologically enlarged and measurable, a lymph node must be ≥15 mm in short axis when assessed by CT scan (CT scan slice thickness recommended to be no greater than 5 mm). At baseline and in follow-up, only the short axis will be measured and followed.

8.1.1.4. ***Baseline documentation of "Target" and "Non-Target" lesions:***

Target lesions. All measurable lesions up to a maximum of 2 lesions per organ and 5 lesions in total, representative of all involved organs, should be identified as **target lesions** and recorded and measured at baseline. Target lesions should be selected on the basis of their size (lesions with the longest diameter), be representative of all involved organs, but in addition should be those that lend themselves to reproducible repeated measurements. It may be the case that, on occasion, the largest lesion does not lend itself to reproducible measurement in which circumstance the next largest lesion which can be measured reproducibly should be selected. A sum of the diameters (longest for non-nodal lesions, short axis for nodal lesions) for all target lesions will be calculated and reported as the baseline sum diameters. If lymph nodes are to be included in the sum, then only the short axis is added into the sum. The baseline sum diameters will be used as reference to further characterize any objective tumor regression in the measurable dimension of the disease.

Non-target lesions. All other lesions (or sites of disease) including any measurable lesions over and above the 5 target lesions should be identified as **non-target lesions** and should also be recorded at baseline. Measurements of these lesions are not required, but the presence, absence, or in rare cases unequivocal progression of each should be noted throughout follow-up.

8.2. Response Criteria for Target Lesions –RECIST version 1.1

8.2.1. Evaluation of target lesions

Complete Response (CR)	Disappearance of all target lesions. Any pathological lymph nodes (whether target or non-target) must have reduction in short axis to <10 mm.
Partial Response (PR)	At least a 30% decrease in the sum of the diameters of target lesions, taking as reference the baseline sum diameters
Progressive Disease (PD)	At least a 20% increase in the sum of the diameters of target lesions, taking as reference the smallest sum on study (this includes the baseline sum if that is the smallest on study). In addition to the relative increase of 20%, the sum must also demonstrate an absolute increase of at least 5 mm. (Note: the appearance of one or more new lesions is also considered progressions).

Stable Disease (SD)	Neither sufficient shrinkage to qualify for PR nor sufficient increase to qualify for PD, taking as reference the smallest sum diameters while on study

8.2.2. Evaluation of non-target lesions

Response Criteria	**Evaluation of non-target lesions**
* Complete Response (CR)	Disappearance of all non-target lesions and normalization of tumor marker level
* Incomplete Response/ Stable Disease (SD)	Persistence of one or more non-target lesion(s) or/and maintenance of tumor marker level above the normal limits
* Progressive Disease (PD)	Appearance of one or more new lesions and/or unequivocal progression of existing non-target lesions*

* Although a clear progression of "non-target" lesions only is exceptional, the opinion of the treating physician should prevail in such circumstances, and the progression status should be confirmed at a later time by the Sponsor-Investigator.

8.3. Evaluation of best overall response –RECIST version 1.1

The best overall response is the best response recorded from the start of the treatment until disease progression/recurrence (taking as reference for PD the smallest measurements recorded since the treatment started). In general, the subject's best response assignment will depend on the achievement of both measurement and confirmation criteria.

Target Lesions	**Non-Target Lesions**	**New Lesions**	**Overall Response**
CR	CR	No	CR
CR	Non-CR/ Non-PD	No	PR
CR	Not evaluated	No	PR
PR	Non-PD/ or not all evaluated	No	PR
SD	Non-PD or not all evaluated	No	SD
Not all evaluated	Non-PD	No	Non-evaluable
PD	Any	Yes or No	PD
Any	PD*	Yes or No	PD
Any	Any	Yes	PD
*In exceptional circumstances, unequivocal progression in non-target lesions may be accepted as disease progression.			

Subjects with a global deterioration of health status requiring discontinuation of treatment without objective evidence of disease progression at that time should be classified as having "symptomatic deterioration." Every effort should be made to document the objective progression even after discontinuation of treatment.

In some circumstances it may be difficult to distinguish residual disease from normal tissue. When the evaluation of complete response depends on this determination, it is recommended

that the residual lesion be investigated (fine needle aspirate/biopsy) to confirm the complete response status.

8.4. Definitions for Response Evaluation –RECIST version 1.1

8.4.1. First Documentation of Response:

The time between initiation of therapy and first documentation of PR or CR.

8.4.2. Confirmation of Response:

To be assigned a status of complete or partial response, changes in tumor measurements must be confirmed by repeat assessments performed no less than four weeks after the criteria for response are first met.

8.4.3. Duration of Response:

Duration of overall response—the period measured from the time that measurement criteria are met for complete or partial response (whichever status is recorded first) until the date that recurrent or progressive disease is objectively documented (taking as reference for progressive disease the smallest measurements recorded since treatment started).

8.4.4. Duration of Overall Complete Response:

The period measured from the time that measurement criteria are met for complete response until the first date that progressive disease is objectively documented.

8.4.5. Objective response rate:

The objective response rate is the proportion of all subjects with confirmed PR or CR according to RECIST v1.1 from the start of treatment until disease progression/recurrence (taking as reference for progressive disease the smallest measurements recorded since the start of treatment).

8.4.6. Progression-Free Survival:

Progression-free survival is defined as the time the treatment is initiated until to the first documented progression of disease, as determined per RECIST 1.1 criteria, or death from any causes, whichever occurs first. Subjects who have not progressed will be right-censored at the date of the last disease evaluation.

8.5. Methods of Measurement –RECIST version 1.1

All measurements should be taken and recorded in metric notation using a ruler or calipers. All baseline evaluations should be performed as closely as possible to the beginning of treatment and never more than 28 days before the beginning of the treatment.

Imaging-based evaluation is preferred to evaluation by clinical examination unless the lesion(s) being followed cannot be imaged but are assessable by clinical exam. The same imaging modality must be used throughout the study to measure disease.

8.5.1. CT and MRI:

CT and MRI are the best currently available and most reproducible methods for measuring target lesions. 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. MRI is also acceptable in certain situations (e.g. for body scans).

Use of MRI remains a complex issue. MRI has excellent contrast, spatial, and temporal resolution; however, there are many image acquisition variables involved in MRI, which greatly impact image quality, lesion conspicuity, and measurement. Furthermore, the availability of MRI is variable globally. 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. Furthermore, as with CT, the modality used at follow-up should be the same as was used at baseline and the lesions should be measured/assessed on the same pulse sequence. It is beyond the scope of the RECIST guidelines to prescribe specific MRI pulse sequence parameters for all scanners, body parts, and diseases. 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. At present, the low dose or attenuation correction CT portion of a combined PET-CT is not always of optimal diagnostic CT quality for use with RECIST measurements. However, 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. Note, however, that the PET portion of the CT introduces additional data which may bias an investigator if it is not routinely or serially performed.

8.5.2. Chest X-Ray:

Lesions on chest x-ray are acceptable as measurable lesions when they are clearly defined and surrounded by an aerated lung (CT is preferable).

8.5.3. Clinical Examination:

Clinically detected lesions will only be considered measurable when they are superficial (e.g. skin nodules and palpable lymph nodes). For skin lesions, documentation by color photography, including a ruler to estimate size of the lesion, is recommended. Photographs should be retained at the institution.

8.5.4. Cytology and Histology:

Cytologic and histologic techniques can be used to differentiate between complete and partial responses in rare cases (e.g. after treatment to differentiate residual benign lesions and residual malignant lesions in germ cell tumors). Cytologic confirmation of the neoplastic nature of any effusion that appears or worsens during treatment is required when the measurable tumor has met response or stable disease criteria.

8.6. Response Criteria for MD Anderson (MDA) Criteria (21)

Complete Response (CR)	• Complete sclerotic fill-in of lytic lesions on XR or CT • Normalization of bone density on XR or CT • Normalization of signal intensity on MRI • Normalization of tracer uptake on SS
Partial Response (PR)	• Development of a sclerotic rim or partial sclerotic fill-in of lytic lesions on XR or CT • Osteoblastic flare - Interval visualization of lesions with sclerotic rims or new sclerotic lesions in the setting of other signs of PR and absence of progressive bony disease • ≥ 50% decrease in measurable lesions on XR, CT, or MRI • ≥ 50% subjective decrease in the size of ill-defined lesions on XR, CT, or MRI • ≥ 50% subjective decrease in tracer uptake on SS
Progressive Disease (PD)	• > 25% increase in size of measurable lesions on XR, CT, or MRI • > 25% subjective increase in the size of ill-defined lesions on XR, CT, or MRI • > 25% subjective increase in tracer uptake on SS • New bone metastases
Stable Disease (SD)	• No change • < 25% increase or < 50% decrease in size of measurable lesions • < 25% subjective increase or < 50% subjective decrease in size of ill-defined lesions • No new bone metastases
Abbreviations: XR: radiography; CT: computed tomography; SS: skeletal scintigraphy; MRI: magnetic resonance imaging.	

Measurements are based on the sum of a perpendicular, bi-dimensional measurement of the greatest diameters of each individual lesion.

9. BIOLOGICAL CORRELATIVES

The goal of the correlative objectives are to identify candidate biological mechanisms underlying primary and secondary resistance to endocrine therapy combined with CDK 4/6 inhibitors (CDK 4/6i) using liquid biopsy approaches. Previous work from the PALOMA-3 trial (22) shows that approximately 30% of patients treated with endocrine therapy plus palbociclib acquire a driver mutation during treatment that may contribute to disease progression, including *RB1* aberrations, amplification of *FGFR* and mutations in *ESR1* and *PIK3CA* in circulating tumor DNA (ctDNA). However, the majority of tumors do not demonstrate emergence of a characteristic driver mutation at the time of progression that is driving resistance to therapy. Therefore, there is a need for studies exploring other mechanisms of resistance in clinical specimens. We will conduct a multi-omics analysis of circulating plasma exosomes isolated from blood samples collected at baseline and at the time of disease progression in order to identify candidate mechanisms of resistance to the combination of tamoxifen plus palbociclib.

The release of membrane-bound extracellular vesicles (EVs) from cancer cells into body fluids like plasma is a tightly regulated mechanism of intracellular communication that involves cell-to-cell transfer of lipids, proteins, and nucleic acids. This process has been implicated in several aspects of cancer-related pathology, including tumor growth, invasion, angiogenesis, and metastasis. Emerging evidence suggests that oncogenic mutations in tumors impact EV-mediated cell-cell communication, and that EVs shed from tumors contain oncogenic macromolecules (23, 24). Thus, analyzing EV cargo can shed light on biologic processes ongoing in tumors in response to treatment. Exosomes are an abundant form of EVs that are ideally suited for serial analyses in patients participating clinical trials since exosomes and their cargo are highly stable in plasma for prolonged periods of time and can be readily isolated from frozen plasma samples (25), unlike circulating tumor cells. Importantly, analysis of exosomes provides a dynamic and functional read-out of biological pathways that are activated in cancer cells since exosome cargo includes proteins and RNA. This is a significant advantage compared to analysis of ctDNA. For all of these reasons, a multi-omics analysis of plasma exosomes isolated from study participants is a promising liquid biopsy approach for identifying candidate mechanisms of primary and secondary resistance to tamoxifen and palbociclib.

We collected blood samples (6 mL) in Streck tubes on cycle 1 day 1, cycle 5 day 1, and at the time of progression. Samples were shipped overnight on the day of collection to the Laboratory of Genomic Medicine at the University of Illinois at Chicago. Plasma was isolated immediately on arrival and was frozen at -80C° with 48 hours of collection. Plasma exosomes will be isolated from frozen specimens using the ExoQuick Exosome Isolation and RNA Purification for future RNAseq experiments if additional funding is obtained, and the ExoQuick-ULTRA® for proteomic experiments as described below (System Biosciences, Palo Alto, CA).

9.1. Perform proteomic analysis of plasma exosomes to identify potential mechanisms of primary and secondary resistance to tamoxifen/palbociclib.

A collaborator (Y. Gao, PhD, UIC) pioneered single-cell mass spectrometry for proteomic analysis of sub-populations of circulating tumor cells. The methods developed in the Gao lab have shown that sub-populations of cells could be differentiated at single-cell resolution (26). We will not perform single-cell proteomics, but rather we will use this enabling technology to perform ultra-sensitive mass spectrometry on the small amount of protein typically isolated from exosomes. The single-cell proteomics method developed in the Gao lab combines

chemical derivatization, isotopologue labeling, advanced statistical modeling and optimized sample preparation to sample ultra-low protein concentrations in a sensitive and quantitative manner. This newly developed chemical derivatization method is able to enhance peptide ionization by 10-20 fold. The isotopologue labeling approach allows analysis of multiple samples in parallel. Statistical sampling allows further extension of the resolving power beyond instrument limitations. Finally, optimized sample preparation ensures minimal sample loss.

To compare the protein contents of exosomes, total exosome will be first extracted from each plasma sample as previously described (27, 28). Once separated, the exosome from each sample will be labeled with Tandem Mass Tag (TMT) isobaric tags for quantitative mass spectrometry (MS) analysis. Exosomes obtained from each patient prior to treatment will be measured three times in three independent experiments as technical replicates. To improve the detection and quantitation of low abundant exosome proteins, we will use existing exosome proteomics data and ExoCarta (http://www.exocarta.org/) to guide our analysis. Specifically, an inclusion list will be generated to include all the known exosome protein-derived peptides, as well as proteins that belong to candidate resistance mechanisms (e.g. Cdk2, cyclin E, Rb, and other cell cycle regulatory proteins and proteins involved in ER signaling). We will also guide the mass spectrometer to aim for these ions as specific retention time points. This type of experiment is similar to multiple reaction monitoring (MRM), which is highly sensitive and reproducible. After mass spectrometry analysis, all the data will be analyzed and quantified using ProLuCID (29) and Census (30) to generate a complete exosome protein identification and quantitation data set. We will then analyze the data to identify key proteins and pathways that contribute to the differences between responders and non-responders in pre-treatment samples. **This will provide insight into possible mechanisms of primary resistance.** For the responders, we will also compare the exosome proteome at baseline vs. at the time of progression**. This will provide insight into potential mechanisms responsible for secondary (i.e. acquired) resistance to CDK 4/6i**. For both analyses, we will, in addition, correlate with the primary outcome PFS.

Protein clusters that persistently change together will be subject to pathway analysis using STRING database (http://www.string-db.org/). With both the proteomics data and transcriptomics data, we will perform a multi-omics data integration to find the correlation between the proteome and the transcriptome. We have previously shown that such analysis could greatly improve the quality and confidence of the results (26).

9.2. Analyze tumor specimens for protein expression of candidate markers of primary resistance.

In order to confirm potential biomarkers of primary resistance identified with the multi-omics analysis of plasma exosomes, we will analyze pre-treatment tumor specimens using a multiplex immunofluorescence (IF) tissue imaging system. We will use a multiplex immunofluorescence (IF) staining assay and automated digital image analysis with the Vectra 3® automated quantitative pathology imaging system (Perkin-Elmer, Waltham, MA) to assess the extent of protein staining in tumor cells for up to 5 proteins of interest. Optimization of antibody staining with commercially available antibodies, tumor staining, image analysis and quantification of protein staining will be conducted by the Research Histology and Tissue Imaging Core (RHTIC) in the Research Resources Center (RRC) at the

University of Illinois at Chicago in collaboration with the study pathologist. The multiplex antibody panel will include a pan-cytokeratin antibody that will allow us to distinguish tumor cells from the stromal compartment, and DAPI to identify membrane staining. Stains will be optimized using Opal® reagents (PerkinElmer). Slides will be scanned on a Vectra 3® multispectral imaging system for whole-slide image analysis. Machine learning algorithms will isolate epithelium and stroma, and all images will be reviewed by the study pathologist (Wiley) to confirm the accuracy of cellular compartment segmentation. Quantification of protein staining in digitized images will be accomplished using algorithms that generate a normalized, log2 transformed staining intensity for each protein. T-tests will compare staining intensity in responders vs. non-responders. Cox model will be used to correlate staining intensity with PFS.

9.3. Compare differences in response for subjects presenting with de novo metastatic breast cancer to subjects that progressed while on aromatase inhibitors.

There will be two main subject populations in this trial: subjects that present with de novo metastatic disease and subjects that will progress while on adjuvant endocrine therapy with aromatase inhibitors. Looking at differences in response between these 2 groups could provide further information about the mechanism for endocrine resistance.

9.4. Compare differences in response and toxicity profile in African American and Hispanic subjects to Caucasian subjects.

Minorities are underrepresented in clinical trials. Obtaining more information about possible differences in response to therapy is important to better the biology. Many African American subjects will have a baseline low neutrophil count. Observing the toxicity profile of palbociclib in this population may provide guidance for further monitoring while on therapy and supportive care.

9.5. Banking of Leftover Biospecimens

Subject consent will be obtained to bank any leftover samples that were collected for study-specific correlative research. Hoosier Cancer Research Network (HCRN), as Administrative Headquarters for the Big Ten CRC, will manage the banked samples. Samples will be banked indefinitely in the Hoosier Cancer Research Network Biorepository and used for future unspecified cancer-related research.

9.6. Banking Samples for Future Unspecified Research

Subject consent will be obtained for additional samples collected for future Big Ten Cancer Research Consortium studies. HCRN will manage the banked samples. Samples will be banked indefinitely in the HCRN Biorepository.

This includes:

- Whole blood: Whole blood will be collected prior to treatment on Cycle 1 Day 1.
- Pre- and Post-treatment plasma: Whole blood for plasma will be collected prior to treatment on Cycle 1 Day 1 and at End of Treatment.
- Pre- and Post-treatment serum: Whole blood for serum will be collected prior to treatment on Cycle 1 Day 1 and at End of Treatment.
- Unstained slides: Unstained slides will be obtained from the subject's archived formalin fixed paraffin embedded tumor sample.

Please refer to the Correlative Laboratory Manual for all sample collection, processing, labeling, and shipping instructions.

10. DRUG INFORMATION

10.1. Drug Name

IBRANCE® (palbociclib)

Classification

Kinase inhibitor.

Mechanism of Action

Palbociclib is an inhibitor of cyclin-dependent kinase (CDK) 4 and 6. Cyclin D1 and CDK4/6 are downstream of signaling pathways which lead to cellular proliferation. In vitro, palbociclib reduced cellular proliferation of estrogen receptor (ER)-positive breast cancer cell lines by blocking progression of the cell from G1 into S phase of the cell cycle.

How Supplied

Palbociclib is supplied in the bottles of 21 capsules of the following strengths: 125 mg (NDC 0069-0189-21), 100 mg (NDC 0069-0188-21), and 75 mg (NDC 0069-0187-21).

Availability

Pfizer will supply palbociclib at no charge to subjects participating in this clinical trial.

The investigator shall take responsibility for and shall take all steps to maintain appropriate records and ensure appropriate supply, storage, handling, distribution, and usage of investigational product in accordance with the protocol and any applicable laws and regulations.

Storage, Handling, and Accountability

Store at 20 °C to 25 °C (68 °F to 77 °F); excursions permitted between 15 °C to 30 °C (59 °F to 86 °F).

Description

125 mg capsules: opaque hard gelatin capsules, size 0, with caramel cap and body, printed with white ink "Pfizer" on the cap, "PBC 125" on the body. 100 mg capsules: opaque hard gelatin capsules, size 1, with caramel cap and light orange body, printed with white ink "Pfizer" on the cap, "PBC 100" on the body. 75 mg capsules: opaque hard gelatin capsules, size 2, with light orange cap and body, printed with white ink "Pfizer" on the cap, "PBC 75" on the body.

Administration

Palbociclib should be taken with food in combination with tamoxifen on days 1-21 of the 28-day cycle once daily. Subjects should be encouraged to take their dose at approximately the same time each day. Palbociclib capsules should be swallowed whole (do not chew, crush or open them prior to swallowing). No capsule should be ingested if it is broken, cracked, or otherwise not intact. See Section 5.3.2 for missed doses.

Risks

Most common adverse reactions (incidence ≥10%) were neutropenia, leukopenia, fatigue, anemia, upper respiratory infection, nausea, stomatitis, alopecia, diarrhea, thrombocytopenia, decreased appetite, vomiting, asthenia, peripheral neuropathy, and epistaxis.

Warnings and Precautions

Please refer to the package insert or Investigator's Brochure for a complete list of adverse events.

Neutropenia

Neutropenia was the most frequently reported adverse reaction in Study 1 (PALOMA-2) with an incidence of 80% and Study 2 (PALOMA-3) with an incidence of 83%. A Grade ≥3 decrease in neutrophil counts was reported in 66% of patients receiving palbociclib plus letrozole in Study 1 and 66% of patients receiving palbociclib plus fulvestrant in Study 2. In Study 1 and 2, the median time to first episode of any grade neutropenia was 15 days and the median duration of Grade ≥3 neutropenia was 7 days.

Febrile neutropenia has been reported in 1.8% of patients exposed to palbociclib across Studies 1 and 2. One death due to neutropenic sepsis was observed in Study 2. Physicians should inform patients to promptly report any episodes of fever.

Pulmonary Embolism

Pulmonary embolism was reported in 15/872 (1.7%) of palbociclib treated patients in Study A5481003, A5481008, and A5481023, and in 5/471 (1.1%) of patients treated in the comparator arms of these studies. These incidences are consistent with epidemiological data on the frequency of pulmonary embolism in the advanced breast cancer population, and the higher incidence for pulmonary embolism in the palbociclib treated patients may be explained by the increased median treatment duration on the palbociclib arms of these studies. Pulmonary embolism and other venous thromboembolic events will continue to be monitored but are not currently considered to be adverse drug reactions of palbociclib.

Effects on the Gastrointestinal Tract

Non-adverse palbociclib-related gastrointestinal (GI) tract findings were noted in nonclinical toxicity studies. Treatment-related GI tract events have been commonly observed in patients who receive palbociclib as a single agent or in combination with other anticancer therapies. The most frequent gastrointestinal treatment-related events were Nausea, Vomiting, Diarrhea, and Decreased appetite. Most occurrences were mild (Grade 1).

Embryo-Fetal Toxicity

Based on findings in animals and mechanism of action, palbociclib can cause fetal harm. Palbociclib caused embryo-fetal toxicities in rats and rabbits at maternal exposures that were greater than or equal to 4 times the human clinical exposure based on area under the curve (AUC). Advise females of reproductive potential to use effective contraception during therapy with palbociclib and for at least 120 days after the last dose of study drug.

Effects on Testes
Palbociclib caused testicular degeneration in rats and dogs. The incidence and severity was dose related and correlated with decreases in testicular weight in the rat. Reversibility of the testicular degeneration was demonstrated following a 12-week non-dosing period in rats and dogs (also following a 4-week recovery period in rats). Patients should consider sperm preservation prior to beginning therapy with palbociclib.

Male patients must be surgically sterile or must agree to use effective contraception during the therapy and for at least 120 days after the last dose of study drug.

10.2. Drug Name

Tamoxifen (Nolvadex®)

Classification

Nonsteroidal antiestrogen.

Mechanism of Action

Tamoxifen citrate is a nonsteroidal agent that has demonstrated potent antiestrogenic properties in animal test systems. The antiestrogenic effects may be related to its ability to compete with estrogen for binding sites in target tissues such as breast. Tamoxifen inhibits the induction of rat mammary carcinoma induced by dimethylbenzanthracene (DMBA) and causes the regression of already established DMBA-induced tumors. In this rat model, tamoxifen appears to exert its antitumor effects by binding the estrogen receptors.
In cytosols derived from human breast adenocarcinomas, tamoxifen competes with estradiol for estrogen receptor protein.

How Supplied

20 mg Tamoxifen Citrate Tablets, USP are available containing tamoxifen as the citrate in an amount equivalent to 20 mg of tamoxifen. They are available as follows: bottles of 30 tablets (NDC 0378-0274-93), bottles of 100 tablets (NDC 0378-0274-01), and bottles of 250 tablets (NDC 0378-0274-25).

Availability

Commercial supplies of tamoxifen will be used in this study and billed to third party payers or the subject.

Storage, Handling, and Accountability

Store at 20° to 25°C (68° to 77°F). [See USP Controlled Room Temperature.] Avoid excessive heat (over 104°F/40°C). Protect from light. Dispense in a tight, light-resistant container as defined in the USP using a child-resistant closure

Description

Twenty- mg tamoxifen tablets are white to off-white, unscored, round tablets debossed with **M** on one side of the tablet and **274** on the other side.

Administration

Tamoxifen can be taken with or without food on each day of the 28-day cycle once daily. Subjects should be encouraged to take their dose at approximately the same time each day.

Risks:

General

In general, hot flashes, nausea, and vomiting have been the most commonly reported adverse effects, occurring in up to 25% of subjects.

Less common but serious side effects are:

- Risk of blood clots, especially in the lungs and legs

- Stroke
- Cataracts
- Endometrial and uterine cancers
- Bone loss in premenopausal women
- Mood swings, depression, and loss of libido

10.3. Drug Name

Zoladex® (Goserelin acetate implant)

Classification

Luteinizing hormone releasing hormone (LHRH) agonist

Mechanism of Action

Goserelin is a synthetic decapeptide analogue of GnRH. Goserelin acts as an inhibitor of pituitary gonadotropin secretion when administered in the biodegradable formulation. In animal and in vitro studies, administration of goserelin resulted in the regression or inhibition of growth of the hormonally sensitive dimethylbenzanthracene (DMBA)-induced rat mammary tumor and Dunning R3327 prostate tumor.

How Supplied

Goserelin is supplied as a sterile and totally biodegradable D,L-lactic and glycolic acids copolymer (13.3-14.3 mg/dose) impregnated with goserelin acetate equivalent to 3.6 mg of goserelin in a disposable syringe device fitted with a 16-gauge x 36 +/- 0.5 mm siliconized hypodermic needle with protective needle sleeve [SafeSystem™ Syringe] (NDC 0310-0950-36). The unit is sterile and comes in a sealed, light- and moisture-proof, aluminum foil laminate pouch containing a desiccant capsule.

Availability

Commercial supplies of goserelin will be used in this study and billed to third party payers or the subject.

Storage, Handling, and Accountability

Store at room temperature (do not exceed 25°C [77°F])

Administration

Goserelin, at a dose of 3.6 mg, should be administered subcutaneously on day 1 of each cycle into the anterior abdominal wall below the navel line using an aseptic technique by qualified medical personnel.

Risks

Hot flashes (flushing), dizziness, headache, increased sweating, decreased libido, trouble sleeping, nausea, change in breast size, hair loss, or mental/mood changes (such as depression, mood swings, hallucinations) may occur. Pain, bruising, bleeding, redness, or swelling at the injection site may also occur. In women, vaginal dryness may also occur.

10.4. Drug Name

Lupron® (leuprolide acetate)

Classification

Luteinizing hormone releasing hormone (LHRH) agonist

Mechanism of Action

Leuprolide acetate is a synthetic nonapeptide analogue of GnRH. Leuprolide acetate is a long-acting GnRH analog. A single monthly injection of LUPRON DEPOT 3.75 mg results in an initial stimulation followed by a prolonged suppression of pituitary gonadotropins.

How Supplied

Each syringe contains sterile lyophilized microspheres, which is leuprolide incorporated in a biodegradable copolymer of lactic and glycolic acids.

Availability

Commercial supplies of leuprolide will be used in this study and billed to third party payers or the subject.

Storage, Handling, and Accountability

Store at 25°C (77°F); excursions permitted to 15-30°C (59-86°F)

Administration

Leuprolide, at a dose of 3.75 mg, should be administered intramuscularly on day 1 of each cycle into the gluteal area, anterior thigh, or deltoid; injection sites should be alternated. using an aseptic technique by qualified medical personnel.

Risks

Estradiol levels may increase during the first weeks following the initial injection of leuprolide, but then decline to menopausal levels. This transient increase in estradiol can be associated with a temporary worsening of signs and symptoms.

As would be expected with a drug that lowers serum estradiol levels, the most frequently reported adverse reactions were those related to hypoestrogenism. Adverse events occurring in clinical studies with LUPRON DEPOT that are associated with hypoestrogenism include: hot flashes, headaches, emotional lability, decreased libido, acne, myalgia, reduction in breast size, and vaginal dryness. Patients should be counseled on the possibility of the development or worsening of depression and the occurrence of memory disorders.

The induced hypoestrogenic state also results in a loss in bone density over the course of treatment, some of which may not be reversible.

Pain, bruising, bleeding, redness, or swelling at the injection site may also occur.

11. ADVERSE EVENTS

11.1. Definitions of Adverse Events

11.1.1. Adverse Event (AE):

An AE is any unfavorable and unintended medical occurrence during the course of the study whether or not considered related to the study therapy. The following are examples of AEs:

- A sign (including an abnormal laboratory finding) or symptom
- A disease temporally associated with participation in an investigational study
- An intercurrent illness or injury that impairs the well-being of the subject

Hospitalization for elective surgery or routine clinical procedures that are not the result of an AE (e.g., surgical insertion of central line) should not be recorded as an AE. Disease progression should not be recorded as an AE, unless it is attributable by the site investigator to the study therapy.

11.1.2. Serious Adverse Event (SAE):

A SAE is an adverse event that:

- Results in death. NOTE: Death due to disease progression should not be reported as a SAE, unless it is attributable by the site investigator to the study drug(s)
- Is life-threatening (defined as an event in which the subject was at risk of death at the time of the event; it does not refer to an event which hypothetically might have caused death if it were more severe)
- Requires inpatient hospitalization for >24 hours or prolongation of existing hospitalization. **NOTE:** Hospitalization for anticipated or protocol specified procedures such as administration of chemotherapy, central line insertion, metastasis interventional therapy, resection of primary tumor, or elective surgery, will not be considered serious adverse events.
- Results in persistent or significant disability/incapacity
- Is a congenital anomaly or birth defect
- Is an important medical event (defined as a medical event(s) that may not be immediately life-threatening or result in death or hospitalization but, based upon appropriate medical and scientific judgment, may jeopardize the subject or may require intervention (e.g., medical, surgical) to prevent one of the other serious outcomes listed in the definition above). Examples of such events include, but are not limited to, intensive treatment in an emergency room or at home for allergic bronchospasm; blood dyscrasias or convulsions not resulting in hospitalization; or the development of drug dependency or drug abuse.
    - Palbociclib overdose. For purposes of this trial, an overdose will be defined as any dose exceeding the prescribed dose for palbociclib by 20% over the prescribed dose.

11.1.3. Unexpected Adverse Event:

For this study, an AE is considered unexpected when it varies in nature, intensity or frequency from information provided in the current Investigator's Brochure (IB),

prescribing information or when it is not included in the informed consent document as a potential risk. Unexpected also refers to AEs that are mentioned in the IB as occurring with a class of drugs or are anticipated from the pharmacological properties of the drug, but are not specifically mentioned as occurring with the particular drug under investigation.

11.1.4. Causality

AEs will be categorized according to the likelihood that they are related to the study drug(s). Specifically, they will be categorized using the following terms:

Unrelated	The Adverse Event is ***clearly not related*** to the drug(s)
Unlikely	The Adverse Event is ***doubtfully related*** to the drug(s)
Possible	The Adverse Event ***may be related*** to the drug(s)
Probable	The Adverse Event is ***likely related*** to the drug(s)
Definite	The Adverse Event is ***clearly related*** to the drug(s)

11.2. Adverse Event (AE) Reporting

- Adverse events (AEs) will be recorded from the time of consent and for at least 30 days after last dose of study drug.
- AEs will be recorded regardless of whether or not the event(s) are considered related to trial medications.
- All AEs considered related to trial medication will be followed until resolution, return to baseline or ≤ Grade 1, deemed clinically insignificant, and/or until a new anti-cancer treatment starts, whichever is earlier

11.3. Reporting of Pregnancy

Although pregnancy is not considered an adverse event, it is the responsibility of investigators or their designees to report any pregnancy in a subject (spontaneously reported to them) that occurs during the trial or within 120 days of last dose of study drug. All subjects who become pregnant must be followed to the completion/termination of the pregnancy. All pregnancies must be reported within 1 business day to BTCRC AHQ on the Pregnancy Report form (See Documents/Info tab of the EDC).

Pregnancy outcomes of spontaneous abortion, missed abortion, benign hydatidiform mole, blighted ovum, fetal death, intrauterine death, miscarriage, and stillbirth must be reported as serious events (Important Medical Events). If the pregnancy continues to term, the outcome (health of infant) must also be reported. Such events must be reported within 1 business day to BTCRC AHQ. BTCRC AHQ will report the event within 1 business day to Pfizer Global Safety (Attn: Worldwide Product Safety; FAX 1-866-997-8322).

11.4. Serious Adverse Event (SAE) Reporting

11.4.1. Study Center (Site) Requirements for Reporting SAEs:

- SAEs caused by a protocol-mandated intervention occurring after informed consent but prior to initiation of study therapy will be recorded (e.g. SAEs related to invasive procedures such as biopsies or medication washout).

- SAEs that occur on or after initiation of study therapy until 90 days after last dose of study therapy or until a new anti-cancer treatment starts, whichever occurs first, will be reported **within 1 business day** of discovery of the event.
- SAEs include events related and unrelated to the study therapy.
- All SAEs regardless of relation to study drug will be followed until resolution to ≤ Grade 1 or baseline and/or deemed clinically insignificant and/or until a new anti-cancer treatment starts, whichever occurs first.
- For any pregnancy SAE, the site investigator will follow the female subject until completion of the pregnancy, and must document the outcome of the pregnancy (either normal or abnormal outcome). If the outcome of the pregnancy was abnormal (e.g., spontaneous or therapeutic abortion), the site investigator should report the abnormal outcome as an AE. If the abnormal outcome meets any of the serious criteria, it must be reported as an SAE. This information will be reported on the Pregnancy Form.

The completed SAE Submission Form (see Documents/Info tab of the EDC) must be sent to Big Ten Cancer Research Consortium (BTCRC) Administrative Headquarters (AHQ) within 1 business day of discovery of the event. The form must be sent electronically to safety@hoosiercancer.org. The site investigator is responsible for informing the IRB and/or other local regulatory bodies of the SAE as per local requirements.

The original copy of the SAE Submission Form and the e-mail correspondence must be kept within the study file at the study site.

Once the SAE has resolved (see resolution guidelines listed in 11.2.2.1), sites must submit a follow up SAE Submission Form within a reasonable timeframe to BTCRC AHQ at safety@hoosiercancer.org.

11.4.2. BTCRC AHQ Requirements for Reporting SAEs to Pfizer:

BTCRC AHQ will submit all SAEs (e.g. SAEs, overdose, pregnancy, etc.) received from sites to Pfizer **within one business day** of receipt of the SAE Submission Form. BTCRC AHQ will provide follow-up information to Pfizer as it is received from participating site or within one business day.

BTCRC AHQ will fax SAE Submission Form for all SAE reports and any other relevant safety information to Pfizer Global Safety (Attn: Worldwide Product Safety) at:

Facsimile number: 1-866-997-8322

11.4.3. Sponsor-Investigator Responsibilities

BTCRC AHQ will send a SAE summary to the sponsor-investigator **within 1 business day** of receipt of SAE Submission Form from a site. The sponsor-investigator will promptly review the SAE summary and assess for expectedness and relatedness.

11.4.4. BTCRC AHQ Responsibilities to FDA

The FDA has concluded this protocol is exempt from the requirements of an IND. BTCRC AHQ will continue to facilitate compliance of applicable requirements for the sponsor-

investigator in relation to this study. This includes but is not limited to 21 CFR 50.20 informed consent, 21 CFR Part 56 IRB, and pertinent sections of the Public Health Service Act and FDAAA.

IND Safety Reports Unrelated to this Trial

Pfizer will send IND safety reports from external studies that involve the study drug to BTCRC AHQ at (safety@hoosiercancer.org). BTCRC AHQ will forward safety reports to the sponsor-investigator who will review these reports and determine if revisions are needed to the protocol or consent. BTCRC AHQ will forward these reports to participating sites within 1 business day of receiving the sponsor-investigator's review. Based on the sponsor-investigator's review, applicable changes will be made to the protocol and informed consent document (if required). All IND safety reports will also be made available to sites via OnCore®.

Upon receipt from BTCRC AHQ, site investigators (or designees) are responsible for submitting these safety reports to their respective IRBs, as per their IRB policies.

12. STATISTICAL CONSIDERATIONS

12.1. Study Design

This is a non-randomized, open-label, multicenter, phase II study of palbociclib in combination with tamoxifen in subjects with HR(+)/HER2(-) advanced breast cancer who have not received prior systemic anti-cancer therapies for their advanced/metastatic disease. Palbociclib is taken orally with food at a dose of 125 mg on days 1-21 of each 28-day cycle. Tamoxifen is taken orally at a dose of 20 mg on days 1-28 of the 28-day cycle (i.e., continuously). Treatment will continue until disease progression, unacceptable toxicity, or subject refusal.

It is planned that the data from all centers participating in the trial will be combined, so that an adequate number of subject are available for analysis.

12.2. Analysis Datasets

Methods of Statistical Analysis

The definitions of the study populations are listed below. The appropriate study population for each analysis should be chosen and defined in the protocol.

Population	Definition
Enrolled	This will comprise all subjects who meet the eligibility criteria and are registered onto the study.
Evaluable	This will comprise all subjects who receive at least one cycle of trial drug and either undergo at least one post-baseline assessment or die before any evaluation.
Safety	This will comprise all subjects who received at least a single dose of study drug.

12.3. Sample Size

Study endpoint is progression free survival (PFS) as assessed by RECIST v1.1 criteria). Previous studies (31, 32) identified progression free survival with tamoxifen of 13 months in pre-menopausal women and 14.5 months in post-menopausal women in first line treatment for breast cancer. We use a null hypothesis of median PFS 15 months as a conservative choice and the alternative hypothesis of median PFS 25 months (31, 32). Controlling for a probability of one-sided Type I error of 0.05, the proposed sample size will be 48 to achieve an 80% statistical power for detecting this difference, assuming 20% censoring rate due to unevaluable, drop-out, and other lost-to-follow-up. The sample size analysis was conducted using the software PASS 14 (NCSS, Kaysville, Utah, USA.). The test was based on an exponential distribution assumption for single arm studies. The accrual period was assumed to be 24 months and the maximum follow-up 24 months too.

12.4. Analysis of Primary Objectives/Aims

The primary objective of the Phase II study is to estimate the activity of the combination of palbociclib and tamoxifen in first line therapy for subjects with HR(+)/HER2(-) advanced breast cancer who have not received prior systemic anti-cancer therapies for their advanced/metastatic disease, evaluated by progression-free survival (PFS) using RECIST 1.1 in subjects with HR(+)/HER2(-) advanced breast cancer who have not received prior systemic anti-cancer therapies for their advanced/metastatic disease treated with palbociclib in combination with tamoxifen. Median PFS times will be computed, and PFS rate will be calculated with associated 95% confidence intervals. Kaplan-Meier curves will be plotted. Data will be analyzed using the PROC FREQ and PROC LIFETEST in SAS.

12.5. Analysis of Secondary Endpoints

Characterize safety and tolerability of palbociclib and tamoxifen in first line therapy for subjects with HR(+)/HER2(-) advanced breast cancer who have not received prior systemic anti-cancer therapies for their advanced/metastatic disease.

- Proportion of subjects with each grade of adverse events as defined by CTCAE v4 will be computed along with 95% confidence intervals, and reported in a tabular and descriptive manner.

Assess response rate based on RECIST 1.1 or MDA Criteria. The response rate (complete or partial response) and 95% confidence interval for the response rate will be computed using PROC FREQ in SAS (Cary, NC).

Evaluate clinical benefit rate RR (complete, partial response, or stable disease) based on RECIST 1.1 or MDA Criteria in subjects with HR(+)/HER2(-) advanced breast cancer who have not received prior systemic anti-cancer therapies for their advanced/metastatic disease treated with palbociclib in combination with tamoxifen.

- Clinical benefit rate (complete, partial response, or stable disease) will be assessed every 8 weeks +/- 1 week while on study treatment using RECIST 1.1 (section 8) or MDA Criteria (for patients with bone only disease). Response will be recorded as disease control rate (DCR), or stable disease plus partial response plus complete response, and reported via waterfall plot. The response rate and 95% confidence interval for the response rate will be computed using PROC FREQ in SAS (Cary, NC).

Measure 2-year overall survival (OS) in subjects with HR(+)/HER2(-) advanced breast cancer who have not received prior systemic anti-cancer therapies for their advanced/metastatic disease treated with palbociclib in combination with tamoxifen.

- Median OS times will be computed, and OS rate will be calculated with associated 95% confidence intervals. Kaplan-Meier curves will be plotted. Data will be analyzed using the PROC FREQ and PROC LIFETEST in SAS.

12.6. Analysis of Correlative Endpoints, if funding is secured

- Perform proteomic analysis of plasma exosomes to identify potential mechanisms of primary and secondary resistance to tamoxifen/palbociclib.
- Analyze tumor specimens for protein expression of candidate markers of primary resistance.
- Compare difference in response for subjects presenting with de novo metastatic breast cancer to subjects that progressed while on aromatase inhibitors
- Compare differences in response and toxicity profile in African American and Hispanic subjects to Caucasian subjects
- Compare differences in response and toxicity profile between pre- and post-menopausal women.

13. TRIAL MANAGEMENT

13.1. Study Monitoring

Monitoring visits to the trial sites will be made periodically during the trial to ensure key aspects of the protocol are followed. For-cause visits may occur as necessary. Source documents will be reviewed for verification of agreement with data entered into OnCore®. It is important for the site investigator and their relevant personnel to be available for a sufficient amount of time during the monitoring visits or audit, if applicable. The site investigator and institution guarantee access to source documents by BTCRC AHQ or its designee.

Remote validation of Oncore® data will be completed on a continual basis throughout the life cycle of the study. Automated edit check listings will be used to generate queries in the EDC system and transmitted to the site to address in a timely fashion. Corrections will be made by the study site personnel.

The trial site may also be subject to quality assurance audit by Pfizer or its designee as well as inspection by appropriate regulatory agencies.

13.2. Data and Safety Monitoring Plan

The study will be conducted in accordance with the University of Illinois Cancer Center's Data and Safety Monitoring Plan. The University of Illinois Cancer Center Data Safety Monitoring Committee (DSMC) will review and make recommendations on this trial. BTCRC AHQ will provide the University of Illinois Cancer Center DSMC with periodic data reports to comply with the UICC DSMC review requirements.

In addition, BTCRC AHQ data and safety monitoring activities include:

- Review of all adverse events requiring expedited reporting as defined in the protocol
- Notify participating sites of adverse events requiring expedited reporting
- Provide the Sponsor Investigator with trial accrual progress and safety information
- Provide data summary reports to the sponsor-investigator
- Provide data summary reports to the lead institution's Data Safety Monitoring Committee for review as per their DSMP.

Data and Safety Monitoring and Reporting Guidelines
BTCRC AHQ will compile data summary reports for this trial and submit these reports monthly to the Sponsor Investigator. BTCRC AHQ will submit data summary reports at minimum twice per year for review by the University of Illinois Cancer Center Data Safety Monitoring Committee (DSMC).

13.3. Amendments

If it is necessary for the study protocol to be amended and/or the informed consent revised, the amendment or a new version of the study protocol (amended protocol) and/or the revised informed consent will be generated by BTCRC AHQ and must be approved by the sponsor-investigator, Pfizer (if required by the contract), the FDA (if applicable), and each site's IRB.

The site investigator is responsible for the distribution of these documents to his or her IRB, and to the staff at his or her center.

13.4. Compliance with Trial Registration and Results Posting Requirements

Under the terms of the Food and Drug Administration Modernization Act (FDAMA) and the Food and Drug Administration Amendments Act (FDAAA), the sponsor-investigator of the trial is solely responsible for determining whether the trial and its results are subject to the requirements for submission to the Clinical Trials Data Bank, http://www.clinicaltrials.gov. The sponsor-investigator has delegated responsibility to BTCRC AHQ for registering the trial and posting the results on clinicaltrials.gov. Information posted will allow subjects to identify potentially appropriate trials for their disease conditions and pursue participation by calling a central contact number for further information on appropriate trial locations and study site contact information.

14. DATA HANDLING AND RECORD KEEPING

14.1. Data Management

BTCRC AHQ will serve as the Clinical Research Office for this trial. Data will be collected through a web based clinical research platform, OnCore®, a system compliant with Good Clinical Practices and Federal Rules and Regulations. BTCRC AHQ personnel will coordinate and manage data for quality control assurance and integrity. All data will be collected and entered into OnCore® by study site personnel from participating institutions.

14.2. Case Report Forms and Submission

Generally, clinical data will be electronically captured in OnCore® and correlative results will be captured in OnCore® or other secure database(s). If procedures on the study calendar are performed for standard of care, at minimum, that data will be captured in the source document. Select standard of care data will also be captured in OnCore®, according to study-specific objectives. Please see the Data and Safety Oversight Process (DSOP) guidelines for further details.

The completed dataset is housed at BTCRC AHQ and is the sole property of the sponsor-investigator's institution. It should not be made available in any form to third parties, except for authorized representatives of appropriate Health/Regulatory Authorities, without written permission from the sponsor-investigator and BTCRC AHQ. After the initial publication, the complete data set will be available to all BTCRC institutions.

14.3. Record Retention

To enable evaluations and/or audits from Health Authorities/BTCRC AHQ, the investigator agrees to keep records, including the identity of all participating subjects (sufficient information to link records; e.g., hospital records), all original signed informed consent forms, copies of all source documents, and detailed records of drug disposition. All source documents are to remain in the subject's file and retained by the site investigator in compliance with local and federal regulations.

14.4. Confidentiality

There is a slight risk of loss of confidentiality of subject information. All records identifying the subject will be kept confidential and, to the extent permitted by the applicable laws and/or regulations, will not be made publicly available. Information collected will be maintained on secure, password protected electronic systems. Paper files that contain personal information will be kept in locked and secure locations only accessible to the study site personnel.

Samples that are collected will be identified by a subject's study number assigned at the time of registration to the trial. Any material issued to collaborating researchers will be anonymized and only identified by the subject study number.

Subjects will be informed in writing that some organizations including the sponsor-investigator and his/her research associates, BTCRC AHQ, Pfizer, IRB, or government agencies, like the FDA, may inspect their medical records to verify the information

collected, and that all personal information made available for inspection will be handled in strictest confidence and in accordance with local data protection laws.

If the results of the study are published, the subject's identity will remain confidential.

15. ETHICS

15.1. Institutional Review Board (IRB) Approval

The final study protocol, including the final version of the Written Informed Consent Form, must be approved or given a favorable opinion in writing by an IRB. The investigator must submit written approval to the BTCRC AHQ office before he or she can enroll any subject into the study.

The site investigator is responsible for informing the IRB of any amendment to the protocol in accordance with local requirements. In addition, the IRB must approve all advertising used to recruit subjects for the study. The protocol must be re-approved by the IRB annually, as local regulations require.

Progress reports and notifications of serious and unexpected adverse events will be provided to the IRB according to local regulations and guidelines.

15.2. Ethical Conduct of the Study

The study will be performed in accordance with ethical principles originating from the Declaration of Helsinki, which are consistent with ICH Good Clinical Practice, and applicable regulatory requirements.

15.3. Informed Consent Process

The site investigator will ensure the subject is given full and adequate oral and written information about the nature, purpose, possible risks and benefits of the study. Subjects must also be notified they are free to discontinue from the study at any time. The subject should be given the opportunity to ask questions and allowed time to consider the information provided.

The subject's signed and dated informed consent must be obtained before conducting any procedure specifically for the study. The investigator must store the original, signed informed consent form. A copy of the signed informed consent form must be given to the subject.

16. REFERENCES

1. Baselga J, Campone M, Piccard M et al. Everolimus in postmenopausal hormone-receptor-positive advanced breast cancer. N Engl J Med 2012; 366: 520-9.
2. Klijn JG, Beex LV, Mauriac L, et al. Combined treatment with buserelin and tamoxifen in premenopausal metastatic breast cancer. N Engl J Med 2012; 366: 520-9
3. Rose C, Mouridsen HT. Endocrine therapy of advanced breast cancer. Acta Oncologica 1988;27:721-8.
4. Fry DW, Harvey PJ, Keller PR, et al. Specific inhibition of cyclin-dependent kinase 4/6 by PD 0332991 and associated antitumor activity in human tumor xenografts. Mol Cancer Ther 2004; 3:1427–38.
5. Choi YJ, Anders L. Signaling through cyclin D-dependent kinases. Oncogene 2014; 33:1890–903.
6. Holm et al 2012; The Cancer Genome Atlas Network 2012
7. Beroukhim R, Mermel CH, Porter D, et al. (2010) The landscape of somatic copy-number alteration across human cancers. Nature. 463(7283):899-905.
8. Thangavel C, Dean JL, Ertel A, et al. (2011) Therapeutically activating RB: reestablishing cell cycle control in endocrine therapy-resistant breast cancer. Endocr Relat Cancer. 18(3):333-45
9. Bosco EE, Wang Y, Xu H, et al. (2007) The retinoblastoma tumor suppressor modifies the therapeutic response of breast cancer. J Clin Invest. 117:218-28.
10. Finn RS, Dering J, Conklin D, et al. PD 0332991, a selective cyclin D kinase 4/6 inhibitor, preferentially inhibits proliferation of luminal estrogen receptor-positive human breast cancer cell lines in vitro. Breast Cancer Res 2009;11:R77.
11. DeMichele A et al. CDK 4/6 inhibitor palbociclib (PD0332991) in Rb+ advanced breast cancer: phase II activity, safety and predictive biomarker assessment. Clin Cancer Res 2015;21: 995-1001.
12. Finn RS et al. The cyclin-dependent kinase 4/6 inhibitor palbociclib in combination with letrozole versus letrozole alone as first-line treatment of oestrogen receptor-positive, HER2-negative, advanced breast cancer (PALOMA-1/TRIO-18): a randomised phase 2 study. Lancet Oncol 2015; 16:25-35
13. Turner NC et al. Palbociclib in Hormone-Receptor-Positive Advanced Breast Cancer. NEJM 2015;373:209-19
14. Butt AJ et al. Downstream targets of growth factor and oestrogen signalling and endocrine resistance: the potential roles of c-Myc, cyclin D1 and cyclin E. Endocr Relat Cancer. 2005;12 Suppl 1:S47-59.
15. Zukerberg LR et al. Cyclin D1 (PRAD1) protein expression in breast cancer: approximately one-third of infiltrating mammary carcinomas show overexpression of the cyclin D1 oncogene. Mod Pathol. 1995; 8:560-7
16. Rudas M et al. Cyclin D1 expression in breast cancer patients receiving adjuvant tamoxifen-based therapy. Austrian Breast and Colorectal Cancer Study Group Clin Cancer Res. 2008;14:1767-74
17. Jirström K et al. Adverse effect of adjuvant tamoxifen in premenopausal breast cancer with cyclin D1 gene amplification. Cancer Res. 2005; 65:8009-16.
18. Zhao et al. Reversible and irreversible inhibition of CYP3A enzymes by tamoxifen and metabolites. Xenobiotica 2002;32 : 863-78
19. Desai PB et al. Induction of cytochrome P450 3A4 in primary human hepatocytes and activation of the human Pregnane X receptor and 4-hydroxytamoxifen. Drug Metabolism and Disposition 2002;30 :608-12

20. Hoffman JT et al. A phase I open-label fixed-sequence two-period crossover study of the effect of multiple doses of tamoxifen on palbociclib (PD-0332991) pharmacokinetics in healthy male volunteers. Ann Onc. 2014;25: i27
21. Costelloe CM et al. Cancer Response Criteria and Bone Metastases: RECIST 1.1, MDA and PERCIST. J Cancer 2010;1:80-92.
22. O'Leary B, et al. The genetic landscape and clonal evolution of breast cancer resistance to palbociclib plus fulvestrant in the PALOMA-3 trial. Cancer Discov 2018;8(11); 1390–403.
23. Mills J, et al. Cancer-Derived Extracellular Vesicle-Associated MicroRNAs in Intercellular Communication: One Cell's Trash Is Another Cell's Treasure. Int. J. Mol. Sci. 2019;20:6109
24. Turchinovich A, Drapkina O and Tonevitsky A. Transcriptome of Extracellular Vesicles: State-of-the-Art. Front. Immunol. 2019;10:202.
25. Mitchell PS, Parkin RK, Kroh EM, et al. Circulating microRNAs as stable blood-based markers for cancer detection. Proc Natl Acad Sci U S A. 2008;105(30):10513–8.
26. Gao, Y et al. Multi-Omics Integration Reveals Short and Long-Term Effects of Gestational Hypoxia on the Heart Development. Cells 2019;8(12):E1608.
27. Sharma P, Mesci P, Carromeu C, et al. Exosomes regulate neurogenesis and circuit assembly. Proc Natl Acad Sci U S A. 2019;116(32):16086–16094.
28. Smolarz M, Pietrowska, M, Matysiak N, Mielańczyk Ł, Widłak P. Proteome Profiling of Exosomes Purified from a Small Amount of Human Serum: The Problem of Co-Purified Serum Components. Proteomes. 2019;7(2):18.
29. Xu T, Park SK, Venable JD, et al. ProLuCID: An improved SEQUEST-like algorithm with enhanced sensitivity and specificity. J. Proteomics. 2015;129:16–24.
30. Park SK, Venable JD, Xu T, Yates JR 3rd. A quantitative analysis software tool for mass spectrometry-based proteomics. Nat. Methods. 2008;5(4):319–322.
31. Finn, RS et al. Palbociclib and letrozole in advanced breast cancer. N Engl J Med 2016;375:1925-1936.
32. Tripathy, D et al. Ribociclib plus endocrine therapy for premenopausal women with hormone-receptor-positive, advanced breast cancer (MONALEESA-7): a randomised phase 3 trial. Lancet Oncol 2018; 19:904-915.